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About the Journal

Overview
Pertanika Journal of Science & Technology (JST) is the official journal of Universiti Putra Malaysia published by UPM Press. It is an open-access online scientific journal which is free of charge. It publishes the scientific outputs. It neither accepts nor commissions third party content.

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JST is a quarterly (January, April, July and October) periodical that considers for publication original articles as per its scope. The journal publishes in English and it is open to authors around the world regardless of the nationality.

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The Introduction explains the scope and objective of the study in the light of current knowledge on the subject; the Materials and Methods describes how the study was conducted; the Results section reports what was found in the study; and the Discussion section explains meaning and significance of the results and provides suggestions for future directions of research. The manuscript must be prepared according to the Journal’s Instructions to Authors.

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Foreword

Welcome to the Second Issue of 2019 for the Journal of Science and Technology (JST)!

JST is an open-access journal for studies in Science and Technology published by Universiti Putra Malaysia Press. It is independently owned and managed by the university for the benefit of the world-wide science community.

This issue contains 30 articles; 3 are review articles, 1 case study and the rest are regular articles. The authors of these articles come from different countries namely Algeria, Bangladesh, France, India, Iran, Iraq, Malaysia, Nigeria, Philippines, Thailand, Turkey and United Arab Emirates.

Articles submitted in this issue cover various scopes of Science and Technology including environmental sciences, engineering sciences, information, computer and communication technologies, mathematical sciences, applied sciences and technologies, medical and health sciences, chemical sciences, earth sciences, environmental sciences and material sciences.

Selected from the scope of environmental science is an article entitled “FeNO as a Biomarker for Airway Inflammation Due to Exposure to Air Pollutants among School Children nearby Industrial Areas in Terengganu” by Anis Syafiqah Kamaruddin, Juliana Jalaludin, Titi Rahmawati Hamedon and Nur Hazirah Hisamuddin, fellow researchers from Universiti Putra Malaysia, Malaysia. They conducted a cross-sectional comparative study among Malay primary school children in Kemaman, Terengganu and assessed indoor air quality in each primary school and home using indoor air monitoring equipment. Fractional exhaled nitric oxide (FeNO) was measured using an NIOX MINO device. The researchers found a significant difference between concentrations of PM10, PM2.5, NO2, SO2, and VOCs in different classrooms from selected schools and homes of exposed and comparative groups. Statistical analysis revealed that the FeNO level was significantly higher among the exposed group compared to the comparative group. This study suggested that the exposure to industrial air pollutants would increase the risk of getting respiratory inflammation among primary school children living near industrial areas. Details of the article are available on page 589.

Selected from the scope of engineering science is an article entitled “Experimental Study of Tsunami Bore Induced Forces on Vertical Seawall” by Zaty Aktar Mokhtar, Badronnisa Yusuf and Saiful Bahri Hamzah from Malaysia; and Thamer Ahmad Mohammed from Iraq. The study performed a sequence of laboratory experiments using dam-break waves to simulate the interactions between the tsunami-like bore flow and vertical seawall as well as to measure the bore-induced pressures and to estimate forces exerted on the vertical seawall model. The experimental result revealed that the maximum pressure (approximately 8 kPa) exerted on the vertical seawall was measured at the lowest pressure sensor location. This study used the experimental data to re-examine the relevant empirical formulae found in the literature. The results obtained by this study could be useful for calibrating mathematical and numerical models as well as for future research concerning the design of tsunami barriers. Details of the article is available on page 673.
Selected from the scope of information, computer and communication technologies is an article entitled “Structural and Statistical Similarity Measure based Approach for Effective Eye Blink Recognition” by Kapil Juneja and Chhavi Rana, fellow researchers from Maharshi Dayanand University, India. The study presented a three-stage model to detect the eye blinks accurately. The proposed model was applied on real time, web-collected and the NRC-IIT dataset videos which were associated to the indoor and outdoor environments. The study analysed news reading and other complex video sequences and found out that the proposed model had reduced the possible generated errors and provided the accurate detection of eye blinks. Details of the article is available on page 779.

Selected from the scope of material science is an article entitled “Fabrication of Scaffold in Tissue Engineering using Selective Laser Sintering Process” by Gajanan Nanasaheb Thokal and Chandrakant Ramesh Patil from India. This article described an experimental investigation of bone scaffold to measure the porosity using gas porosimeter and suggested an alternative to bone scaffold. The researchers made a prototype using additive manufacturing of selective laser sintering technique, using synthetic polymer PA12. The behaviour of actual bone and prototype had been observed under compressive load of fixed interval loading condition. Mechanical properties of polymer had been evolved and compared with actual bone. The results revealed that the prototype showed more deformation before ultimate load and suggested further experiment to get required strength in the polymer, as an alternative to bone scaffold. Details of the article is available on page 1013.

We anticipate that you will find the evidence presented in this issue to be intriguing, thought-provoking and useful in reaching new milestones in your own research. Please recommend the journal to your colleagues and students to make this endeavour meaningful.

All the papers published in this edition underwent Pertanika’s stringent peer-review process involving a minimum of two reviewers comprising internal as well as external referees. This was to ensure that the quality of the papers justified the high ranking of the journal, which is renowned as a heavily-cited journal not only by authors and researchers in Malaysia but by those in other countries around the world as well.

We would also like to express our gratitude to all the contributors, namely the authors, reviewers and editors, who have made this issue possible.

JST is currently accepting manuscripts for upcoming issues based on original qualitative or quantitative research that opens new areas of inquiry and investigation.

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Review Article

An Overview on Biodegradation of Carbamate Pesticides by Soil Bacteria

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ABSTRACT

Carbamates are poisonous pesticides which have been used widely in agriculture production for decades. Unlike other pesticides such as organophosphate, carbamate pesticides are not persistent in the environment however, their degradation is crucial due to their toxicity to living system. The World Health Organization, categorized carbamate pesticide as toxic, hazardous and restricted for use. Example of carbamates pesticides include Carbaryl, Aldicarb, Methomyl, Carbofuran, and Propoxur. They are extensively used to control many insect and pests of crops. Presently, there is significant awareness regarding the negative effects of pesticides due to their ability to pollute soil and water bodies. Most pesticides are readily degraded or metabolized by microbes. Carbamate pesticide degradation by microorganisms relies not only on the availability of microbes with suitable biodegradative enzymes, but also on the various ecological factors. This review-article outlines the present development in biodegradation of carbamate pesticides, their toxicity and enzymatic degradation as well as their degradative pathways.

Keywords: Biodegradation, carbamates, soil bacteria, toxicity
INTRODUCTION

Pesticide is a substance or formulation of substances used in controlling, destroying and preventing any pests, that include rodents, insects, weeds, birds, nematodes, microorganisms or mammals that interfere or destroy things. Apart from their toxic effect, pesticides are also beneficial to humans in many ways. Pesticides may be categorized by chemical structure, physical form or their target species. They can also be categorized as inorganic, synthetic or biological (bio pesticides). Furthermore, pesticides may be grouped into chemical families. The most common pesticide families include the organophosphate, pyrethroid, organochlorine and carbamate pesticides which are further categorized as rodenticides, weedicides, fungicide, herbicide, and insecticide. Carbamate consists of a wide spectrum of biologically active pesticides used worldwide to control insects and nematodes. Carbamate pesticides are generally categorized into N-methyl-carbamate insecticides and N-allyl-carbamate herbicides in view of their chemical structures and biological actions (Ozturk et al., 2016; Parks et al., 1987). Carbamates are closely linked with organophosphorus compounds in mode of action and resistance meaning they are inhibitors of acetyl cholinesterase causing very similar symptoms. They have high polarity, soluble in water and thermally unstable. They are poisonous to living organisms due to their acute toxicity (Alvarez et al., 2017). The use of carbamate has received great concern not only because of their widespread use, but also due to its higher toxicity to plants and animals (Chin-Pampillo et al., 2015). Carbamates pesticides are usually converted into several products through different paths such as oxidation, biotransformation, hydrolysis, bio-augmentation, photolysis, biodegradation as well as metabolic reactions in living organisms (Cai et al., 2015). Many scientists have reported biodegradation of several pesticides by bacteria under different physiological conditions. Soil bacteria that are constantly exposed with synthetic toxic chemicals may develop new capabilities to degrade such chemicals (Wu et al., 2014). Microbial populations in soil have the ability of metabolizing (hydrolyzing) carbamate pesticide and can easily adapt themselves to various form of carbamates. However, carbamates pesticide and their metabolites can affect the micro flora and soil productivity (Gupta et al., 2016). Even though carbamate pesticides do not persist longer in aquatic environment and are very stable under aquatic conditions, the application of these toxic compounds may cause a major reduction of non-target organisms. Therefore degradation of carbamate using microbes is of great interest since many conventional approaches of removing pesticides contamination proved to be ineffective and expensive. Hence an effective and inexpensive techniques is required for treating such pollutant. Table 1, below gives details of carbamate type, chemical formula year of production and the rat LD50 in mg/kg values for some carbamate insecticides. It also notes their half-lives in soil.
Table 1

History, toxicity and half-life of some Carbamates pesticides

<table>
<thead>
<tr>
<th>Name</th>
<th>Trade Name</th>
<th>Type</th>
<th>Formula</th>
<th>Year of introduction</th>
<th>Rat LD50 (mg/kg)</th>
<th>Half-life soil (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbofuran</td>
<td>Furadan</td>
<td>Insecticides</td>
<td>C₉H₁₂NO₃</td>
<td>1967</td>
<td>8–14</td>
<td>3-60</td>
</tr>
<tr>
<td>Cabaryl</td>
<td>Prokoz® and Sevin®</td>
<td>Insecticides</td>
<td>C₉H₁₁NO₂</td>
<td>1958</td>
<td>250 to 850</td>
<td>4-72</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>Temik®</td>
<td>Insecticides</td>
<td>C₃H₄N₂O₂S</td>
<td>1965</td>
<td>0.9 - 1.0</td>
<td>1-15</td>
</tr>
<tr>
<td>Propoxur</td>
<td>Baygon®</td>
<td>Insecticides</td>
<td>C₉H₁₀NO₃</td>
<td>1959</td>
<td>80 -191</td>
<td>80 - 210</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>Vydate®</td>
<td>Nematicide</td>
<td>C₃H₁₀N₂O₃S</td>
<td>1974</td>
<td>2.5 - 3.1</td>
<td>4 - 20</td>
</tr>
<tr>
<td>Methomyl</td>
<td>Lannate®</td>
<td>Insecticides</td>
<td>C₄H₈N₂O₂S</td>
<td>1966</td>
<td>12-48</td>
<td>30-45</td>
</tr>
<tr>
<td>Methiocarb</td>
<td>Mesurol®</td>
<td>Molluscide</td>
<td>C₃H₁₁N₂O₂S</td>
<td>1960</td>
<td>23-140</td>
<td>15 to 50</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>Mecarzole,Bavistin</td>
<td>Fungicide</td>
<td>C₃H₁₄N₂O₂</td>
<td>1972</td>
<td>&gt;2000</td>
<td>42 to 175</td>
</tr>
<tr>
<td>Primicarb</td>
<td>Pirimor, Abol</td>
<td>Insecticides</td>
<td>C₃H₁₃N₂O₂</td>
<td>1970</td>
<td>68-221</td>
<td>53</td>
</tr>
<tr>
<td>Carbosulfan</td>
<td>Alachlor. Lasso, Pillarzo</td>
<td>Insecticides</td>
<td>C₃H₁₄N₂O₃S</td>
<td>1979</td>
<td>90 to 250</td>
<td>1.4</td>
</tr>
<tr>
<td>Fenoxycarb</td>
<td>omply, Insegar, Logic, Pictyl.</td>
<td>Growth</td>
<td>C₃H₁₄NO₄</td>
<td>1987</td>
<td>&gt;16,800</td>
<td>14-45</td>
</tr>
</tbody>
</table>
STRUCTURES AND TOXICITY OF CARBAMATE PESTICIDES

Carbamates are acetylcholinesterase inhibiting compounds, and they are used worldwide and are responsible for poisoning and prevalent occurrence of an infectious diseases in many developing countries (Anguiano et al., 2017). Carbamate pesticides, have high acute toxicity. Chemically, carbamate insecticides remain as esters of carbamates as well as organic components derived from N-methyl carbamic acid (C$_2$H$_5$NO) which take weeks or months to break down in soil (McDonald et al., 2005). Carbamates are insecticides, herbicides, and fungicides that act like organophosphates pesticides for insecticides and nematocides by inhibiting acetyl-cholinesterase, except that the action is reversible. The toxicity is generally low, with the except for compounds such as aldicarb. Carbamate pesticides play a vital role in agriculture because of their broad spectrum nature as well as varied degree of compounds, degradable in soil and usually have small amount of toxicity on living organisms (Chapalamadugu & Chaudhry, 1992; Cycon et al., 2017). Carbamates are inhibitors of acetylcholinesterase (AChE) which become acetylcholine in synapses and neuromuscular junctions and eventually produce serious disorder of central nervous system (Berman et al., 2017; Vidair, 2004). The inhibition of the hydrolysis response of AChE results in the accumulation of ACh, which brings about many symptoms, such as hyper salivation, sweating and seizure (Guo et al., 2017). Carbamate pesticides are less hazardous with respect to human exposure than organophosphorus (Hernandez et al., 2013). The use of pesticides has brought a chain of uninterrupted effect to the environment and health of rural workers in Brazil rural environment (Soares et al., 2003). The clinical effects of pesticide carbamate usually depend upon the type carbamate involved, the dose and route of exposure (Lamb et al., 2016). Carbamate pesticides toxicity are responsible for many cases of poisonings in the rural environment (Gupta, 1994). Carbamates pesticides are absorbed via oral or dermal route, even though the last has a tendency to be the less toxic route (Lamb et al., 2016). For instance, carbofuran insecticide has a rat oral LD$_{50}$ of 5-6 mg/kg, compared to rat dermal LD$_{50}$ of 120 mg/kg, making the oral absorption nearly 24 times more toxic when consumed (Li et al., 2009; Yu et al., 2009). LD$_{50}$ is the way of measuring toxicity. The dosage has to be considered as compounds with high LD$_{50}$ may yield lethal indications if high dose is ingested (Purushothaman & Kuttan, 2017). Carbamate toxicity is likely to be of shorter in duration compared with organophosphate toxicity due to the reversibility of the (AChE).

The clinical effects of carbamate pesticides depend on the type of carbamate pesticide used and the dose and mode of exposure (Ahmad et al., 2004). The study on biodegradation of toxic insecticides is useful, because the reactions that discharges poisonous insecticides transform most of the insecticide residues in the environment to harmless and less toxic metabolites (Cycon et al., 2017). Figure 1 shows the different structures of carbamates pesticides. However, the toxicity of the carbamate compounds differs in terms of molecular
structure, but generally the AChE inhibition is of shorter time than other forms of pesticides. Figures 2, 3 and 4 show some of their degradative pathways.

**Figure 1.** Structures of some carbamate pesticides

**Figure 2.** Proposed Biodegradation pathway of carbofuran by spingomonas sp. (Satish et al., 2017)

**Figure 3.** Degradation pathway of carbendazim by Bacterial strain (Fang et al., 2010)

**Figure 4.** Generalized degradation pathway of carbaryl (Jones et al., 2003)

**SOURCES, TRANSPORT AND DISTRIBUTION OF CARBAMATE**

Production of chemical pesticides and marketing have continued to increase from 1950s (Munnecke, 1979). Fungicidal chemical, Benzimidazole was made available in market around 1970. Carbamate pesticides are derived from carbamic acid. Generally, carbamate is characterized by low vapour pressure at room temperature, they have low evaporation or sublimation rate, which resulted in volatilization from soil. Nevertheless, dispersal through air will be a less significant factor. Carbamates are characterized by light absorbance, thereby contributing to its photolytic decomposition in water. Therefore, persistent hazardous contamination of carbamates is small. The insecticide are mostly applied to plants, which will subsequently reach the soil, however, herbicidal and nematicidal carbamates are directly applied to the soil (Kazano et al., 1972). Factors such as soil type, soil moistness, adsorption, soil pH, volatility and photo-decomposition affect the biodegradation of carbamates pesticides in the soil (Wang et al., 2009). Some carbamates may decompose easily, while some are strongly absorbed in the soil (Ozturk et al., 2016). Soil type and solubility in aqueous medium are very vital in these processes. However, it should be noted parent compound should not be the only consideration, but also the breakdown products or metabolites (Sahoo et al., 1990).
The initial stage in the metabolic break down of insecticide carbamates in soil is hydrolysis. Several factors influence the fate and transport of pesticides which includes, their adsorption, absorption, Run-off, microbial degradation, volatilization, photochemical decomposition uptake by plant and chemical degradation. The products of this process would then metabolize further in the soil plant system (Tien et al., 2013). Plants metabolize carbamates in which aryl-hydroxylation and conjugation or hydrolytic decomposition are the important pathways of the detoxification. Many studies suggested that carbamates pesticides were entirely dispersed through the apoplectic system in plants (Miyamoto, 1975). They are also broken down by microbes, florae and faunae in water and soil environment.

**METABOLISM OF CARBAMATE PESTICIDE**

The metabolic product of carbamates pesticide is generally similar in mammals, insects, and plants. Carbamates pesticides are commonly absorbed simply via respiratory tract, dermal contact and mucous membrane. Generally, carbamate metabolites are less toxic as compared with their parent compounds. In some circumstances, the metabolites are even more toxic than the original compound (Cycon et al., 2017). Environmental circumstances that favor the growth of microbial community equally favor the degradation of pesticides carbamates. The primary phase in metabolic degradation of carbamate pesticide in soil is the hydrolysis leading to carbamic acid, then breakdown to carbon dioxide (CO$_2$) as well as similar amine (Chanika et al., 2011). The mechanisms of hydrolysis is not the same for N-methyl carbamate and N-dimethyl derivatives. The N-methyl carbamates pass via an isocyanate metabolites, while in the hydrolysis of N–dimethyl carbamates, a product containing hydroxyl ion is formed resulting in alcohol and N-dimethyl replaced acid (Rosman et al., 2009). Apart from hydrolysis, oxidation also happen which consists of: hydroxylation of the aromatic ring, N-dealkylation, oxidation of aliphatic side chains, O-dealkylation, N-methyl hydroxylation, and sulfoxidation to the corresponding sulfone (Chaudhry & Ali, 1988; Otieno et al., 2010).

**MICROBIAL DEGRADATION OF CARBAMATE PESTICIDES**

Pesticides biodegradation by microorganism is not new. Microorganisms simply supply all the required energy source for simple chemical reactions to take place (Pandey et al., 2010). Numerous factors are involved in pesticide biodegradation such as physicochemical mechanism, photochemical mechanism, microbial degradation and bioremediation. The biodegradation of carbamates by diverse microbial consortia that metabolize the pesticides has been studied extensively (Onunga et al., 2015; Satish et al., 2017). Mostly, the studies did not undermine the involvement of abiotic processes in the microbial degradation (Rangasamy et al., 2017). The use of pesticides over decades, has caused numerous
microbes to develop mechanism to degrade toxic compounds, including pesticides, by means of different mechanisms, approaches and enzymatic pathways. Organophosphate degrading bacterial strain was first isolated in Philippines in 1973 in a paddy field area. Subsequently, many phylogenetically diverse strain capable of metabolizing pesticides by co-metabolism, and use them as nutrient and carbon sources, have been isolated by many researchers from different geographical locations (Das et al., 2005; Talwar et al., 2014). Pesticides are mainly used to protect damages of crops and fruits while, these chemicals are applied directly to soil. When these pesticides are used in soil they undergo different process such as volatilization, degradation, sorption, or surface transport to other places (Li et al., 2016). Studies showed that soils with a history of pesticide application mostly have shorter half-lives compared with soil that has no history of pesticide application (Cycon et al., 2017). Moreover, the chemicals considered as non-degradable become biodegradable after several years in soil. Thus, the soil is a major source of microorganisms that can degrade pesticides. Other microbial sources include; microbes from pesticide industry wastewater and sediment, activated sludge, sewage slurry, surface waters and their sediments, as well as areas closed pesticide manufacturing industries. Many groups of microorganisms characterized by growth and degradation ability of pesticides (Ishag et al., 2016). Isolation and characterization of microbes for pesticides degradation bring about new tools to restore environments polluted with pesticides. Several microbial species capable of degrading pesticide such as Pseudomonas, Flavobacterium, Achromobacterium sp., Sphingomonas sp., Arthrobacter and Bacillus species have been isolated and characterized in effort to know their mechanism to remove pesticides. Degradation of Carbofuran was first carried out through degradation to carbofuran phenol and subsequently degraded to 2-hydroxy-3-(3-methylpropan-2-ol) phenol by Sphingomonas sp. and Arthrobacter sp. strains (Chaudhry & Ali, 1988; Kim et al., 2004). Carbaryl is another carbamate that is widely used and poisonous to living organism (Anguiano et al., 2017). Bacterial identified as Aeromonas, Pseudomonas, Bacillus and Morganella genera isolated from Gaza Strip utilized carbaryl as source of nutrient. Bacillus and Morganella degraded carbaryl up to 94.6% and 87.3% degradation rate (Hamada et al., 2015). In another study, Pseudomonas sp. C5pp isolated from soil was described to have mineralize carbaryl by means of 1-naphthol, salicylate and gentisate (Trivedi et al., 2016). The genomic investigation of C5pp strain shows that carbaryl pesticides catabolic genes are arranged into three operons; middle, lower and upper. The study mentioned the role of horizontal gene transfer events in the evolution of carbaryl degradative pathway in C5pp isolate. Table 2 shows the summary of microbial species used for biodegradation of carbamate pesticides in which different bacterial and fungal strains from different sources were studied by different scholars.

Microorganisms have the capability to degrade multiplicity of environmental pollutants, including carbamate pesticides. It is highly significant to understand the biochemical
bases for the development of new degradative capacities of microorganisms involve in pesticide degradation (Sogorb & Vilanova, 2002). Enzyme-based degradation method are potentially capable of qualitatively faster action than traditional microbial remediation methods (Nguyen et al., 2014). Several enzymes that hydrolyzed carbamates compound are either esterases or amidases. The hydrolysis of carbamate compound is influence by the chemical structure of the side chains and substrate (Lei et al., 2017). The parent compound is often detoxified by these hydrolytic process. Carbamate insecticide enzymes mediated hydrolysis typically yield an alcohol and methylamine as well as carbondioxide (Lei et al., 2017). Several carbamate hydrolyzed enzymes responsible for degradation have been isolated and also carboxylesterases from various bacteria were previously reported (Ufarte et al., 2017; Wang et al., 2017).

Table 2

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Microbial species</th>
<th>Environmental media</th>
<th>Mechanism involve</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbofuran</td>
<td>Consortia</td>
<td>Soil</td>
<td>Degradation</td>
<td>Tien et al. (2017)</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas sp.</td>
<td>Soil</td>
<td>Degradation</td>
<td>Devi et al. (2017)</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas and Alcaligenes</td>
<td>Soil</td>
<td>Degradation</td>
<td>Omolo et al. (2012)</td>
</tr>
<tr>
<td></td>
<td><em>Enterobacter cloacae strain TA7</em></td>
<td>Soil</td>
<td>Degradation</td>
<td>Fareed et al. (2017)</td>
</tr>
<tr>
<td></td>
<td><em>Mucor ramannianus</em></td>
<td>Soil</td>
<td>Degradation</td>
<td>Seo et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Novosphingobium sp</td>
<td>Soil</td>
<td>Degradation</td>
<td>Nguyen et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>Aspergillus sp.</td>
<td>Soil</td>
<td>Degradation</td>
<td>Devi et al. (2017)</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>Bacillus, Morganella, Pseudomonas, Aeromonas, Corynebacterium sp.</td>
<td>Soil</td>
<td>Degradation</td>
<td>Hamada et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>Micrococcus sp.</td>
<td>Soil</td>
<td>Degradation</td>
<td>Doddamani et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Enterobacter cloacae sp. Paeclomyces</td>
<td>Soil</td>
<td>Degradation</td>
<td>Fareed et al. (2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chan-Cupul et al. (2016)</td>
</tr>
<tr>
<td>Propoxur</td>
<td><em>Corynebacterium kutscheri, Staphylococcus aureus, Bacillus pasteurii and Aeromonas sp.</em></td>
<td>MSW</td>
<td>Degradation</td>
<td>Anusha et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>Consortia</td>
<td>Sediment</td>
<td>Degradation</td>
<td>Dewi et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas sp.</td>
<td>Soil</td>
<td>Degradation</td>
<td>Kamanavalli et al. (2000)</td>
</tr>
</tbody>
</table>
Microorganisms genetically engineered for bioremediation would be an environmentally friendly and economical alternate for the removal of contaminants in polluted areas (Li et al., 2007). Diverse categories of engineered microorganisms have been developed through recombinant DNA and RNA technologies, which have been used for the remediation of several pollutants from polluted environments (Jiang et al., 2005). Genetic manipulation bids a means for engineered microbes to deal with contaminant, such as pesticides in polluted sites. The strategy is to prolong the degradative abilities of already present metabolic pathways in an organism by introducing extra enzymes from other organisms or by modifying the specificity of the catabolic genes previously present (Lan et al., 2014). Microbial genes were aimed to form new metabolic pathways so as to increase degradative processes. GM microorganisms can be favorite tool for biodegradation because of the special features of their metabolic pathways. Recent researches have shown the way that lead to development of degradation pathways and the organization of catabolic genes, hence making it simpler to produce genetically modified microorganisms (GMO) for the degradation of organic compounds such as carbamates (Liu et al., 2016; Zhang et al., 2016). Biodegradation is an environmentally friendly, economical, highly efficient approach.
compared to the conventional techniques which were typically costly and has negative effect on the environment. The genetically engineered microbes have ample ability to degrade organic compounds such as pesticide as they discharge several specific enzymes that contain specific catabolic gene into plasmids. The studies of recombinant DNA provide the means to develop DNA and RNA examinations for the purpose of identifying microorganisms from varied populaces with unique capability to degrade pesticides (Jiang et al., 2005).

CONCLUSION

The continuous application of pesticides in agricultural field, has a negative effect on the environment. Bioremediation using microbes is an emerging technology and useful phenomenon for the degradation of toxic pesticides in soil. Resistant microbes from the soils with a history of pesticide application shows the existence of pesticide degrading bacteria in the soil. Alterations in the structure and diversity of microbial populations after exposure to pesticides may result from toxic effects of pesticides on some communities of microbes. Some microorganisms have the ability of using pesticides as nutrients and energy source and may benefit from these pesticide exposure. The isolation of novel microbes for biodegradation of toxic compounds has long been a key challenge, but the emergent knowledge of the genetic and biochemical bases of the metabolism of substances has increased. Various species of bacterial and fungal species having capability to degrade carbamate pesticides have been identified by many scientists. The application of molecular-based approaches in the remediation of pollutants is being increasingly used and has provided valuable evidence for improving biodegradation methods. Moreover, environmental meta-genomic information from soil and sea can be an important source of genes. The viability of the remediation technique has to be assessed bearing in mind its real applicability, its possible limitations and shortcomings as well as its benefits. Furthermore, a great deal of effort is essential to resolutely establish comprehensive knowledge of the molecular basis for catabolic sequences, to limit inactivation of enzymes at high threshold concentration of toxic organic contaminants to increase the compounds bio-availability in the environments. These species of microorganisms are enabling us to suggest an alternative pathway for pesticide biodegradation. In order to fully understand the mechanism of carbamate degradation, a detail explanation of the mechanisms involved in the degradation of pesticides should be conducted as this study is about the carbamates toxicity and their degradation pattern by microbes.

FUTURE PROSPECTS

Microorganisms have the ability to degrade different compounds, including carbamate pesticides in the field or under laboratory conditions. Nevertheless, effective methodologies have yet to be established to remove residues and recalcitrant pesticides from soil and water...
bodies. Advances in several aspect of contaminants degradation by microbes, have further explained the mechanisms, and pathways for their degradation as well as developing viable remediation approaches for different polluting compounds. The system of bio molecular engineering should be exploited more to improve the capabilities of degradative enzymes. The advance of genetically engineered microbes with enhanced abilities to degrade diverse types of pesticides under field conditions should be made. Future research should explore towards faster and economic restoration of pesticide polluted soil in order to protect the soil quality and agricultural produce for the health of human beings.

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An Overview on Biodegradation of Carbamate Pesticides by Soil Bacteria


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An Overview on Biodegradation of Carbamate Pesticides by Soil Bacteria


Water Quality Characteristic of the National Hydraulic Research Institute of Malaysia (NAHRIM) Lake Undergoing Remediation by the Constructed Wetlands: A Baseline Study

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ABSTRACT

This study was conducted to determine the baseline water quality characteristics of a contaminated NAHRIM lake undergoing remediation by the constructed wetlands, based on the physico-chemical and biological parameters. The sampling was conducted for six months (May-October) in 2016 from 5 stations of the lake and analysed using APHA standard methods for water and wastewater analysis, while Malaysian water quality index (WQI) was used to calculate quality of the lake. The results showed that, the Conductivity, Dissolved Oxygen, NO3-N, NO2-N, PO4, Temperature, Turbidity, TDS, TSS, and Zn were under class I, while pH, B and COD were categorized under class II. NH3-N, BOD, Fe, Escherichia coli, Total coliform and Mn were categorized as class III. Moreover, Al was not given any classification under NWQS but their concentration did not exceed EPA guidelines. Furthermore, as compared to the water samples from the constructed wetlands that reported a class III WQI, the lake was observed to show an overall class II WQI. This is suggestive of the retaining and remedial role of the constructed wetlands being the first point
of contact for the contaminants going to the lake. Thus the lake is suitable for recreational activities.

*Keywords:* Biological and physicochemical parameters, constructed wetlands, lake, water quality

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**INTRODUCTION**

Water is one of the important natural resources that have consistently given support to man in different ways. The application of water is found in diverse human activities that cut across the industrial, agricultural and other activities. In the past decades, the persistent use of water resources for various activities by man contributes to the pressure on their availability (UN Water, 2017; Ramakrishnaiah et al., 2009) and degradation in the water quality (Juhair et al., 2011; Jackson et al., 2012). The quality of a water sources is usually identified in relation to their physical, chemical, and biological contents (Loukas, 2010). The degradation in water quality has been a disturbing issues in the developing tropical countries, where the treatment of effluents before being discharged into the water bodies is of low priorities (Konnerup et al., 2011).

Lakes are important type of water sources that are designated as places of recreation, reflection and as storm water retention pond; these activities subjects them to an increasing pressure and stress by contaminants resulting in most of them being degraded (EPA, 2009). Various authors have conducted studies into Malaysian lake with the aim of determining their water quality characteristics. Ismail et al. (2012), in their study of Tasik Chini for its hydrological water quality characteristic found the lake to be under NWQS class II which was suitable for recreational activities. Another study was conducted at Ampang Hilir lake, in Selangor, Peninsular Malaysia. The results classified the water quality as class II which allowed for the body contact for recreation as well as for fishing activities. The concentrations of the metals were lower than the stipulated values (Said et al., 2012a).

Said et al. (2012b) reported a similar study on the water quality parameters in Titiwangsa Lake, Selangor Peninsular Malaysia. Selected parameters were analysed *in-situ* using Hydrolab data sonde 4 and surveyor 4, a water quality multi probe (USA), while the metals were determined using Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The findings identified the water quality to be appropriate only for recreational activities (class II); the metal concentrations found in the lake were reported to be lower than the standard stipulated in international indexes and as such presenting non-toxic effects to the aquatic organisms in the lake. Akinbile et al. (2013) evaluated Bukit Merah lake water for the level of pollution detection which would lead to suggestions of the possible treatment required before the usage of the water. Standard laboratory procedures were employed to assess the concentration of water quality parameters and trace metals. Their findings
showed Bukit Merah Lake to be slightly contaminated with WQI value of 75.63 which placed the status of the lake in class III.

Although, considerable efforts have been expended on understanding the condition of Malaysian lakes water quality, the focus has always been on the natural lakes with bigger sizes while fewer studies have been conducted on the other man-made lakes with respect to discussing the remediation efforts that may enhance their quality.

Constructed wetlands have been shown to have the capability to trap and remove toxic materials through sedimentation, filtration, microbial interactions and uptake or transformation by helophytes to cause an improvement in the water quality (Kadlec and Wallace, 2008; Martins et al., 2013). Moreover, very little information has been reported on the deployment of CWs as contaminant treatment media in the developing countries despite its relative acceptance in the other part of the world (Bojcevska & Tonderski, 2007).

The National Hydraulic Research Institute of Malaysia (NAHRIM) have an artificial lake that serve as the storm water retention ponds and also designed to serve the recreational needs of the staffs. The water quality in this pond was affected by the pollution from point sources and non-point sources (sewerage treatment plant, sullage water from the administrative office, hydraulic laboratory and runoff from the impervious area) surrounding the lake which is making the water unfit for human contact.

The constructed wetlands serving as the remediation media were built with sole purpose of serving as the first point of contact for the discharges and runoff coming from the sewerage treatment plant, drainage system and impervious areas to the Lake. The constructed wetlands were designed as a surface flow type based on the type of the contaminants they were set up to treat. Nine aquatic plants species (Cyperus papyrus, Echinodorus cordifolius, Nymphaea capensis, Nymphaea nouchali, Nymphaea lotus, Sagittaria lancifolia, Thalia sp., Nelumbo nucifera, Typha angustifolia and Victoria amazonica) were planted in the CWs, only four become firmly rooted in the wetland; and Cyperus papyrus being the most dominant plant species. Based on the preliminary study of the wastes that is being discharged into the lake prior to the construction of the wetlands, the discharge rate of the contaminants of the NAHRIM lake is 163.82m³/day and the estimated pollution load contribution from the STP and other effluents sources ranges between 2.785kg/day to 3.457kg/day for the parameters studied (NAHRIM, 2012).

This was supported by the class III water quality status reported for the water sampled from the wetland cells. However, the assessments of the NAHRIM lake water quality improvement have not been carried out since the construction of the wetlands. Thus, the objective of this study is to evaluate the water quality characteristic of the NAHRIM Lake through the analysis of water physicochemical, biological and elemental parameters in order to understand the remedial effects of the constructed wetlands on the lake.
MATERIALS AND METHODS

Study Area and Sampling Stations

The National Hydraulic Research Institute of Malaysia (NAHRIM) is located along Jalan Putra Permai (Lat.3°00’03.88”N and Long.101°41’05.11”E) in Seri Kembangan, Selangor State, Malaysia. The location of the study area is characterised by climate of substantially high but uniform average daily temperatures of 21°C to 32°C. The average daily humidity levels of the area is above 80% with the mean annual rainfall of around 2,500 mm (Abdullah et al., 2012). The Institute has in its compound a drainage system that comprises of three retention ponds named Pond A, Pond B and the lake. The surface area of the lake is approximately 3600m² and with a depth of 3-4 meters at the deepest site. NAHRIM lake covers the entire watershed which is made up of the Institute head office, the Staff quarters, Water quality and Environmental research center, Pond A, Pond B, Hydraulic laboratory, Sewerage treatment plants and the impervious area surrounding the lake are shown in Figure 1.

In this study, five sampling stations were selected along the Lake, from the upstream to downstream (NL1-NL5), and identified by the GARMIN handheld GPS (GARMIN Handheld GPS, GARMIN, Olathe, Kansas, USA). This is to enable the gauging of the overall water quality of the lake. The sampling points selected were described in Table 1.

Sampling Methods and Analytical Procedures

The water samples collection from the NAHRIM Lake was conducted according procedures in the standard method for water and wastewater treatment (APHA, 2005). Duplicates water samples were collected from five sampling sites (NL1-NL5) of the NAHRIM Lake accessed with the aid of a hand paddled boat. Water samples were collected for the
duration of six months (May-October, 2016). Prior to the sampling, Polyethene sampling bottles which were pre-condition with 5% nitric acid and rinsed thoroughly with distilled de-ionized water were used for the collection of the water samples. Water samples were collected using Van Dorn automatic sampler. About 1 L of the water samples was taken at each of the sampling site. Water samples collected from the lake were placed in an ice box and transported to the laboratory. The water samples with colloidal particles were filtrated using 0.45µm membrane filter (Whatman Milipores, Clifton NJ). This was done to avoid clogging which may interfere with the spectrometry during analysis (APHA, 2005). The water samples that were used for heavy metals were preserved with concentrated HNO₃ to pH<2 to prevent precipitation of metal oxides and hydroxides as well as to stop any biological activities (Radojevic & Bashkin, 2007). The samples were then kept at -10 °C until further analysis.

In-situ water quality parameters such as temperature, turbidity, DO, conductivity, pH and TDS of the water were measured at each sampling point using multiparameters water quality sonde YSI6600 V2 sonde (YSI incorporated, Yellow Spring OH, USA). Ex-situ water parameters such as the anions and heavy metals were determined in accordance with the APHA standard method for water and wastewater analysis (APHA, 2005). Biochemical oxygen demand was determined using a modified 5-Day BOD test procedure (method 5210B) (APHA, 2005), Chemical oxygen demand was determined using colorimetric method 5220D of APHA (APHA, 2005). Total suspended solids was determined by using a modified direct HACH Method No. 8006. The determination of NH₃-N concentration was conducted using the APHA standard method 4500-B&C, while Nitrites (NO₂-N) concentration in the water samples was determined by USEPA Diazotization methods (HACH method 8507). Cadmium reduction method 8192 was used to determine the

Table 1
The description of the sampling points of the NAHRIM Lake

<table>
<thead>
<tr>
<th>NAHRIM Lake sampling points</th>
<th>Locations</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL1</td>
<td>N 3°.0'0.36&quot;, 101°.41'6.65'E</td>
<td>Located besides the wetland A (downstream of STP discharge)</td>
</tr>
<tr>
<td>NL2</td>
<td>N 3°.0'0.38&quot;, 101°.41'5.95'E</td>
<td>Middle of the NAHRIM Lake</td>
</tr>
<tr>
<td>NL3</td>
<td>N2°.59'59.50&quot;, 101°.41'4.92'E</td>
<td>Close to Overflow point</td>
</tr>
<tr>
<td>NL4</td>
<td>N 3°.0'0.36&quot;, 101°.41'4.70'E</td>
<td>Downstream of the wetland B (Canteen discharge area)</td>
</tr>
<tr>
<td>NL5</td>
<td>N2°.59'58.84&quot;, 101°.41'5.40'E</td>
<td>Located besides the fence downward of NL3.</td>
</tr>
</tbody>
</table>
concentration of Nitrates (NO$_3$-N) in the water samples, and the concentration of Total phosphorus (PO$_4$$_3$) in the water samples was achieved using the modified APHA method 4500-P E.

The determination of the *Escherichia coli* and Total coliform in the water samples was carried out using the USEPA method 9223B (Colilert-18/ Quanti-Tray 2000). The analysis of Heavy metals were carried out by Inductively Coupled Plasma-Optical Electron Spectroscopy. The samples were digested according to APHA standard methods for the examination of water and wastewater (APHA, 2005) and analysed.

The Water Quality Index (WQI) of NAHRIM Lake was calculated using the method of Department of Environment Malaysia (DOE, 2008; Rosli et al., 2010). This index has been in use for quite some time now. The WQI is a product of opinion-poll formulated by the experts on the choice of water quality parameters to choose, and the weightage to be assigned to each of the parameters. Thus the parameters chosen were Dissolved oxygen (DO), Biological oxygen demand (BOD), Chemical oxygen demand (COD), Suspended solid (SS), pH value, and Ammonical nitrogen (Khuan et al., 2002). The resultant equation as approved by the DOE was calculated based on the above six parameters with the weighing factors of 0.22, 0.19, 0.16, 0.16, 0.15 and 0.12 assigned to DO, BOD, COD, SS, AN and pH respectively. The overall WQI equation was then turned into sub-indices calculated according to the best-fit relations as illustrated in equation 1 below.

\[
\text{WQI}=0.22\text{SIDO}+0.19\text{SIBOD}+0.16\text{SICOD}+0.16\text{SISS}+0.15\text{SIAN}+0.12\text{SIpH} \quad (\text{Eqn 1})
\]

Where, WQI = Water quality index; SIDO = Sub-index of DO; SIBOD = Sub-index of BOD; SICOD = Sub-index of COD; SIAN = Sub-index of AN; SISS = Sub-index of TSS; SIpH = Sub-index of pH.

**Statistical Analysis**

Data generated were subjected to statistical analyses using IBM SPSS (Version 22.0, IBM Corp., Chicago, IL, USA). Basic descriptive statistic reported as mean ± Standard deviation was performed. One way ANOVA and a post-hoc Tukey pair wise comparison were used to test for significant difference of the each of the studied parameters among the sites for each of the months. Statistical significant difference was set at confidence level of 95% (alpha = 0.05). Pearson correlation was use to combine correlated variables in the data set.

**RESULTS**

**Physical (*In-situ*) Water Quality Parameters of the NAHRIM Lake**

Figure 2 (a-f) illustrated the concentration of the analysed *in-situ* parameters of the NAHRIM lake. The parameters were reported as mean ± SD. The average temperature value recorded was 29.10°C and ranged from 28.33±0.06°C to 29.90±0.0°C. The highest
Figure 2. (a) Temperature (b) Conductivity (c) pH (d) Turbidity (e) Dissolved Oxygen and (f) Total Dissolved Solids distribution in water samples of NAHRIM lake in the sites for the months. Bars are expressed as mean ± standard deviation. Bars with the same superscript letters are not significantly different in their concentration at \( p<0.05 \) across the sites in each of the months. The dotted line in red is the National Water Quality Standard limit for water bodies stipulated for recreational contact.
and the lowest temperature values were observed at sites NL4 and NL1 in the months of May respectively. Significant differences \((p<0.05)\) in temperature values for sites were recorded in all of the studied months (Figure 2a).

The illustration of conductivity was depicted in Figure 2b. The average conductivity values recorded for the lake water samples was 159.10\(\mu\)S/cm and this ranged from 152\(\pm\)7.07 to 169.5\(\pm\)5\(\mu\)S/cm. The highest value was recorded in October at site NL4, while the lowest value was recorded in August at site NL3. The significant differences \((p<0.05)\) in conductivity values was observed across the sites in each of the months.

The pH (Figure 2c) average value recorded for the NAHRIM Lake was 7.20; the range concentration is from 6.72\(\pm\)0.04 to 7.82\(\pm\)0.21. The highest and lowest concentrations were observed at sites NL1 and NL2 in October and May respectively. There is a significant difference \((p<0.05)\) in the concentration among the studied sites for months except for the months of September.

The mean turbidity concentration was illustrated in Figure 2d, with the overall mean of 112.80 NTU which ranged from 8.40\(\pm\)0.00 to 17.5\(\pm\)0.85NTU. The highest turbidity was recorded at site NL2 in May and the lowest at site NL3 in August. The sites were not significantly different \((p<0.05)\) in concentration of turbidity across the months.

The average dissolved oxygen concentration recorded for the studied water samples of the NAHRIM Lake was 6.77mgL\(^{-1}\). The highest was in May and June \((9.2\pm0.014\text{ mgL}^{-1})\) while the lowest \((4.67\pm0.35\text{ mgL}^{-1})\) concentration were recorded at site NL5 in August respectively (Figure 2e). The concentration in the studied sites differ significantly \((p<0.05)\) across the months.

The mean concentration of total dissolved solids in the water samples of NAHRIM Lake was 0.079gL\(^{-1}\) and this ranged from 0.05\(\pm\)0.00 to 0.11\(\pm\)0.01gL\(^{-1}\). The highest concentration was recorded at sites NL3 and NL4 in the month of June, while the lowest was recorded at sites NL5 and NL3 in the months of August and September respectively. The studied sites exhibited a significant difference \((p<0.05)\) in the concentration in each of the months (Figure 2f).

**Laboratory \((Ex-situ)\) Water Quality Parameters of the NAHRIM lake**

The overall descriptive statistics of the laboratory water quality parameters was illustrated in Figures 3(a-e). The illustration of chemical oxygen demand was presented in Figure 3a. The mean chemical oxygen demand recorded for the water samples was 10.13mgL\(^{-1}\), ranging from 8.61\(\pm\)0.04 to 13.00\(\pm\)1.41mgL\(^{-1}\). The highest \((13.00\pm1.41\text{ mgL}^{-1})\) COD concentration was recorded at site NL3 in June and the lowest \((8.61\pm0.04\text{ mgL}^{-1})\) was observed at site NL4 in May. The concentration of COD differed significantly \((p<0.05)\) in studied sites across the six months.
The biochemical oxygen demand (BOD) (Figure 3b) concentration of the water samples of NAHRIM lake ranged from 2.97±0.05 to 5.86±0.09mgL⁻¹. The average BOD concentration was 4.41mgL⁻¹ and the highest and lowest BOD concentrations were recorded at sites NL5 and NL3 in October. The concentrations in sites for the month of May show no differences (p>0.05), while other months present a significant differences (p<0.05) across the sites.

The concentration of ammoniacal nitrogen (NH₃-N) in the water samples of NAHRIM lake was illustrated in Figure 3c. The mean concentration is 0.31 mgL⁻¹, with the range of 0.17±0.014 to 0.4±0.021mgL⁻¹. The highest and lowest concentrations were recorded at sites NL1 and NL4 respectively in June. All the sites show a significant difference (p<0.05) in their ammoniacal nitrogen concentrations across the months.

The nitrites (NO₂⁻N) concentration in the water samples was depicted in Figure 3d, and ranged from 0.000 to 0.009±0.002 mgL⁻¹. The average concentration was 0.0054mgL⁻¹. The highest (0.008±0.002mgL⁻¹) and lowest (0.000mgL⁻¹) concentrations recorded were at sites NL2 and NL1 in October and August respectively. The concentrations in the sites differ significantly (p<0.05) from each other across the months.

Figure 3e elucidated the concentration of nitrates (NO₃⁻N) in the water samples of NAHRIM lake. The mean concentration of nitrates (NO₃⁻N) was 0.035mgL⁻¹ ranging from 0.015±0.007 to 0.06±0.014mg L⁻¹. The highest (0.06±0.014mgL⁻¹) and lowest

![Figure 3.](image-url)
Figure 3. (c) Ammoniacal Nitrogen (d) Nitrites and (e) Nitrates distribution in water samples of NAHRIM lake in the sites for the months. Bars are expressed as mean ± standard deviation. Bars with the same superscript letters are not significantly different in their concentration at p<0.05 across the sites in each of the months. The dotted line in red is the National Water Quality Standard limit for water bodies stipulated for recreational contact for the analysed parameter.

(0.015±0.007mgL⁻¹) concentrations were recorded in May at sites NL1 and NL5 respectively. The sites in each of the months differs significantly (p<0.05) in the concentration of nitrates. The total phosphorus (PO₄) recorded mean was 0.056mgL⁻¹ and this ranged from 0.046±0.0042 to 0.065±0.0042mgL⁻¹. The highest (0.065±0.0042mgL⁻¹) and the lowest (0.046±0.0042mgL⁻¹) concentrations were recorded in May at sites NL5 and NL2 respectively. The analysed concentration indicated a significant differences (p<0.05) in the sites across the months (Figure 4a).

The total suspended solid concentration in the water samples of NAHRIM Lake was presented in Figure 4b. The mean concentration was 12.89 mg L⁻¹. The range was from...
5.84±0.23 to 27.06±0.08 mg L⁻¹. Site NL4 in the month of September recorded the highest concentration (27.06±0.08 mg L⁻¹) while site NL5 in August had the lowest (5.84±0.23 mg L⁻¹) concentration. The concentration in the sites for October statistically differs significantly (p<0.05) from each other for the studied months.

The analysed data for *Escherichia coli* and Total coliform are presented as a logarithmical transformation of the original data (Figure 4c and 4d). The mean log₁₀ *E. coli* CFU/100mL was 2.91. The range was from 2.47±0.21 to 3.52±0.12. The highest (3.52±0.12) was recorded at September in site NL2 and the lowest (2.47±0.21) in July at site NL1. The significant difference (p<0.05) in the colony forming units of the *E.coli* in the studied sites was exhibited in July and September, while other months presented a non-significant difference (p>0.05) in the *E.coli* counts for the studied sites. The total

![Graphs showing water quality parameters](image)

*Figure 4.* (a)Phosphorus (b) Total Suspended Solids (c) *Escherichia coli* and (d) Total coliform distribution in water samples of NAHRIM lake in the sites for the months. Bars are expressed as mean ± standard deviation. Bars with the same superscript letters are not significantly different in their concentration at p<0.05 across the sites in each of the months. The dotted line in red is the National Water Quality Standard limit for water bodies stipulated for recreational contact for the analysed parameter.
coliform mean log10 CFU/100ml in the water samples of NAHRIM Lake was 3.93 and ranged from 3.72±0.04 to 4.14±0.12. The highest (4.14±0.12) and lowest (3.72±0.04) total coliform were recorded at site NL4 in October and August respectively. The month of August and October also show a significant difference in the counts of total coliform in the studied sites, while other months did not indicate any significant difference in the total coliform counts among the studied sites.

**Heavy Metals in the Water Samples of the NAHRIM Lake**

The descriptive statistic of the concentration of the five detected heavy metals in NAHRIM Lake is given in Figures 5(a-e). The average Aluminium (Al) concentration was 0.19 mgL⁻¹. The range concentration was from 0.07±0.01 to 0.37±0.02 mg L⁻¹. The highest Aluminium (Al) concentration (0.37±0.02 mgL⁻¹) was detected at site NL4 in August and the lowest

![Figures 5](image)

*Figure 5.* (a) Aluminium (b) Iron (c) Manganese (d) Zinc distribution in water samples of NAHRIM lake in the sites for the month. Bars are expressed as mean ± standard deviation. Bars with the same superscript letters are not significantly different in their concentration at p<0.05 across the sites in each of the months. The dotted line in red is the National Water Quality Standard limit for water bodies stipulated for recreational contact for the analysed parameter.
The water quality of the National Hydraulic Research Institute of Malaysia (NAHRIM) Lake was monitored from May to October 2018. The aluminium concentration in the water samples of NAHRIM Lake was measured to be (0.07±0.01mgL\(^{-1}\)) at site NL5 in September. All the sites across the months show significant difference (\(p<0.05\)) in their aluminium concentration (Figure 5a).

Figure 5b shows the distribution trend of the iron in the water samples of NAHRIM Lake. The mean iron (Fe) concentration in the water samples of NAHRIM Lake was 1.33 mgL\(^{-1}\), ranging from 0.20 ±0.03 to 2.22±0.03mgL\(^{-1}\). The detected highest iron concentration (2.22±0.03mg L\(^{-1}\)) was at site NL1 in June, while the lowest concentration (0.20 ±0.03mg L\(^{-1}\)) was recorded at site NL5 in September. Each of the studied sites across the months indicates a significant difference (\(p<0.05\)) from the other.

The variation in the manganese (Mn) concentration of the water samples of NAHRIM Lake (Figure 5c) was from 0.12±0.03 to 0.52±0.01mgL\(^{-1}\) and presented in Fig. 4.7c. The mean concentration was 0.26 mgL\(^{-1}\). The highest manganese concentration was 0.52±0.01mgL\(^{-1}\) recorded at site NL4 in July and lowest concentration of 0.12±0.03mg L\(^{-1}\) was recorded at site NL1 in May. The significant differences (\(p<0.05\)) in the concentration level of manganese in sites was observed in each of the reported months. The zinc (Zn) concentration detected in the water samples was highest (0.05±0.007mgL\(^{-1}\)) at site NL5 in October and lowest (0.01±0.00mgL\(^{-1}\)) at site NL3 in June. The overall zinc contents varied from 0.01±0.00 to 0.05±0.007mgL\(^{-1}\). The sites in the months exhibited a significant difference (\(p<0.05\)) in the level of zinc present in (Figure 5d).

The average boron (B) concentration trend (Figure 5e) observed for the water samples of NAHRIM Lake was 0.046mgL\(^{-1}\), and this ranged from 0.02±0.014 to 0.08±0.007mgL\(^{-1}\). The highest (0.08±0.007mgL\(^{-1}\)) and lowest (0.02±0.014mgL\(^{-1}\)) concentration were recorded at sites NL4 and NL2 in May and October respectively. The sites show any significant differences (\(p<0.05\)) in the boron concentration across the months.

Figure 5. (e) Boron distribution in water samples of NAHRIM lake in the sites for the month. Bars are expressed as mean ± standard deviation. Bars with the same superscript letters are not significantly different in their concentration at \(p<0.05\) across the sites in each of the months. The dotted line in red is the National Water Quality Standard limit for water bodies stipulated for recreational contact for the analysed parameter.
Correlation Analysis of the Studied Water Quality Parameters of the NAHRIM Lake

The statistical analysis of the Pearson correlation of the studied water quality parameters of the NAHRIM Lake is presented in Table 2. The correlation analysis was employed to show how the concentrations of the water quality parameters impacted on each other. This usually helps in explaining the role such parameters plays in the maintaining the status of such water quality and how they impacted the general ecosystem. The observed correlation varies from moderate negatively to strong positively correlations. There was however one very strong positive relationship (r = 0.930*) between turbidity and electrical conductivity; a strong positive correlation between ammoniacal nitrogen and COD(r = 0.746*) and a moderate positive relationship between turbidity and total coliform (r = 0.570), ammoniacal nitrogen and conductivity(r = -0.542*), Fe and B(r = 0.408*), manganese and calcium (r = 0.546**) as well as a moderate negative relationship between DO and turbidity (r = -0.538*), DO and Manganese (r = -0.484*) and between total coliform and iron (r = -0.420**).

Table 2
Pearson correlation analysis of studied water quality parameter of the NAHRIM Lake

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r-value</th>
<th>Parameters</th>
<th>r-value</th>
<th>Parameters</th>
<th>r-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp x B</td>
<td>0.326*</td>
<td>DO x Turb</td>
<td>-0.538*</td>
<td>TDS x EC</td>
<td>0.243*</td>
</tr>
<tr>
<td>EC x Ph</td>
<td>0.311*</td>
<td>DOxNO₂</td>
<td>0.348**</td>
<td>TDS x Mn</td>
<td>0.383*</td>
</tr>
<tr>
<td>ECxNH₃</td>
<td>0.542*</td>
<td>DO x Fe</td>
<td>-0.349**</td>
<td>COD x NH₃</td>
<td>0.746*</td>
</tr>
<tr>
<td>ECxNO₂</td>
<td>0.255*</td>
<td>DO x TCO</td>
<td>0.335*</td>
<td>NO₂ x NH₃</td>
<td>0.330**</td>
</tr>
<tr>
<td>pH x DO</td>
<td>-0.284*</td>
<td>DO x Mn</td>
<td>-0.484**</td>
<td>NO₂ x Mn</td>
<td>0.296*</td>
</tr>
<tr>
<td>pH x TDS</td>
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<td>DO x Ca</td>
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<td>NO₂ x TCO</td>
<td>0.319*</td>
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<tr>
<td>pH x NO₂</td>
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<td>DO x Mg</td>
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<td>NO₂ x TCO</td>
<td>0.32*</td>
</tr>
<tr>
<td>pH x Mn</td>
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<td>-0.314*</td>
<td>NO₂ x Mn</td>
<td>-0.279*</td>
</tr>
<tr>
<td>pH x B</td>
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<td>Turb x EC</td>
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<td>NH₃ x Fe</td>
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</tr>
<tr>
<td>pH x Mg</td>
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<td>Turb x TCO</td>
<td>0.570*</td>
<td>TCO x Fe</td>
<td>-0.420**</td>
</tr>
<tr>
<td>pH x K</td>
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<td>Turb x COD</td>
<td>-0.306*</td>
<td>Al x Mn</td>
<td>0.261*</td>
</tr>
<tr>
<td>NO₂ x B</td>
<td>-0.294*</td>
<td>Fe x B</td>
<td>0.408*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Temp., Temperature; EC, Electrical Conductivity; B, Boron; NO₂, Nitrites; NO₃, Nitrates; NH₃, Ammoniacal Nitrogen; PO₄, Phosphorus; Mn, Manganese; TDS, Total dissolved solids; DO, Dissolved oxygen; Turb., Turbidity; Fe, Iron; COD, Chemical oxygen demand; BOD, Biochemical oxygen demand; ECO, Escherichia coli; TCO, Total coliform; Al, Aluminum.

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)
Water Quality Index (WQI) of the water samples from the NAHRIM Lake and Constructed wetlands

The mean calculated water quality index for the NAHRIM lake sampling sites varies from 84.27 to 87.19; with the highest and the lowest water quality index recorded at site NL4 and site NL2 respectively. The highest WQI of the water samples from the wetlands was recorded at the sites CW3 (66.32) and the lowest at the CW2 (61.99) (Table 3).

Table 3
The mean Water Quality Index (WQI) for the sampling sites of NAHRIM lake and the constructed wetlands

<table>
<thead>
<tr>
<th>Study sites</th>
<th>Sampling points</th>
<th>DOE-WQI</th>
<th>Class</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAHRIM Lake</td>
<td>NL1</td>
<td>86.93</td>
<td>II</td>
<td>Clean</td>
</tr>
<tr>
<td></td>
<td>NL2</td>
<td>87.19</td>
<td>II</td>
<td>Clean</td>
</tr>
<tr>
<td></td>
<td>NL3</td>
<td>86.44</td>
<td>II</td>
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</tr>
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<tr>
<td></td>
<td>CW5</td>
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</tr>
<tr>
<td></td>
<td>CW6</td>
<td>65.23</td>
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</tbody>
</table>

DISCUSSION

In-situ Water Quality Parameters

The temperature value reported in this study varies among the sites in the studied months. The mean temperature value of 29.10°C is lower than the NWQS threshold for class IIB (normal ±2) but slightly higher than the temperature (29.02°C) reported by Said et al. (2012b) for Titiwangsa lake with related recreational activities. The slight differences in the temperature may be due to the stagnant nature of the lake that allows for the retention of the heat during low precipitation periods. In an event of an increase in temperature, which is not the case with the NAHRIM Lake, there will be a shoot up in metabolic activities of the living components which in turn will lead to a reduction in dissolved oxygen content of the lake and may impact negatively on fish and other organisms.

The conductivity of the water samples exhibited slight spatial and temporal differences. However, the total mean conductivity value of 159.10µs/cm was far below the stipulated level for class IIB of NWQS and can be categorised under class I. The conductivity was higher than those reported by Gasim et al. (2015) and those reported by Al-Badaii
et al. (2013) for Cempaka Lake and Semenyih river respectively. The highest detected conductivity was at NL4 which was located at the downstream of the constructed wetland B support the facts that conductivity is affected by wide range of organic chemicals and a failed sewerage system. And a very strong significant positive correlation of conductivity with Turbidity ($r = 0.93^*$) lends credence to the impact of particles that contributes to the turbid water which is a contributing factor to higher conductivity (Bhateria & Jain, 2016).

The overall mean pH value was 7.20 which is within the class IIB (pH= 9). However, the slight variation in pH concentration from weak acidic to basic is an indication of decomposition of some organic matter in a complex photosynthetic process by plants and algae that utilised carbondioxide released in the water (Gandaseca et al., 2011). The pH contaminant sources are from the discharges of canteen and laboratory activities. This is clearly supported by the low to moderate positive significant relationship of pH with NO$_2$-N ($r = 0.383^{**}$), Mg ($r = 0.273^*$) and Mn ($r = 0.289^*$) that may be the content of the discharges stated above and responsible for the pH. The pH recorded in this study is similar to those reported by Ismail et al. (2012) in their study of hydrological and water quality characteristics of Chini Lake but contrasted the findings of Said et al. (2012b) that reported a higher pH value for Lake Titiwangsa.

There is spatial distribution in dissolved oxygen concentration for the NAHRIM water samples. Dissolved oxygen is one of the critical parameter in water quality assessment that present an insight into the biological and physical processes occurring in the water (Dirican, 2015). The dissolution of oxygen in water occurs from the atmospheric diffusion and aquatic vegetations photosynthetic processes. The total mean concentration of dissolved oxygen of 6.77mg L$^{-1}$ was below the NWQS class IIB threshold of 7 mg L$^{-1}$ but on the monthly basis, the majority of sites present DO concentration level higher than the class IIB threshold and fell under class I, which is similar to the one reported for water quality studies of Temmengor lake, Perak by Khalik & Abdullahi, (2012) and Gasim et al. (2015) for Cempaka lake in Selangor. The reported DO in this study can effectively support the physiological activities of various organism especially the planktons since the deleterious effect of DO can only manifest at a DO level of below 5 mgL$^{-1}$ and critical for fishes at 2mgL$^{-1}$ (Said et al., 2012b).

The mean turbidity concentration (8.40 NTU) is less than NWQS class 1 threshold of 1000 NTU, and appropriate for NAHRIM lake recreational activities since the NWQS does not have a defined level for turbidity. Turbidity occurred from atmospheric deposition that leads to erosion taking place at the NAHRIM lake watershed, with most of the effluents coming off the wash down of the materials from the impervious areas. However, the turbidity value recorded in this study did not agree with the turbidity findings of Ismail et al. (2012) reported for Chini Lake. High turbid water may have injurious effect to the fishes and other organisms and this may lead to the movement of the fishes away from a very turbid water (Said et al., 2012b).

The total dissolved solids concentration among the sites in the studied months did not highlight any spatio-temporal variation. The average TDS value of 0.079gL$^{-1}$ categorised
the lake on water quality basis of class 1. Although there is no stipulated level of TDS for the freshwaters meant for recreational activities, it high presence in water can lead to clarity issues which may have devastating effect on the photosynthetic organism. In addition to the runoff from the roads and impervious areas during weathering processes, organic substances from the waste treatment and agricultural chemicals like fertilizers contributes to the level of total dissolved solids in the lake (Bhateria & Jain, 2016). The high increment in TDS especially those with salt content disrupts aquatic life in the water since the salt enhances the dehydration of the skin of most of the animals. The minimal TDS recorded in this study may be due to less land use for anthropogenic activities at NAHRIM Lake but more of runoff with large suspended particles from the impervious area. In comparison, the TDS value reported in this study is slightly higher than the one reported for by Ismail et al. (2012) for Chini Lake; Rostom et al. (2017) for Mariut Lake but slightly lower than that reported for Siling reservoir by Naveedullah et al. (2016).

**Ex-situ Water Quality Parameters**

The chemical oxygen demand (COD) concentration pattern across the sites in the months exhibited a slight spatial and temporal variation. However, the mean COD (10.15 mg L\(^{-1}\)) recorded for the study lies between the NWQS class 1 and II which is suitable for recreational body contact. This identifies the organic pollution of the lake water to be at minimal level. COD examination is usually used to determine the oxygen required for the decomposition of organic matter and oxidation of inorganic chemicals to occur. This implies that theoretically, a high COD concentration is an indication of a polluted water (Amneera et al., 2013). The COD concentration reported in this study is in agreement with those reported for Temenggor lake and Tasik Chini (Ismail et al., 2012; Khalik & Abdullah, 2012) but contrasted the higher findings of Mood et al. (2017) in their study of effectiveness of Lake remediation towards water quality: application in varsity lake as well as that of Sener et al. (2017) for Aksu lake in Turkey.

The biochemical oxygen demand (BOD) together with COD are the two important water quality parameters that gives an indication of the organic pollutant content in the water bodies. The biochemical oxygen demand (BOD) distribution trend recorded in this study does not shows high differences among the study sites, however, the mean BOD of 4.41 mgL\(^{-1}\) recorded is above the recommended threshold for the surface water meant for recreational activities (3.0 mgL\(^{-1}\)) and fell in NWQS class III. This finding is similar to the findings reported by Sujaul et al. (2012) but deviates from the findings of Sabri et al. (2016) in their study of water quality monitoring of Tasik Cempaka, Bangi, where a lower BOD was reported.
The ammoniacal nitrogen which ranged from 0.17 to 0.38 mgL$^{-1}$ (0.34 mg L$^{-1}$) is slightly above the class IIB but still suitable for the recreational contact. The ammoniacal nitrogen concentrations in the surface water is associated with the biological decay of organic matter discharged from the watershed of the lakes or released from the bottom sediment of the lake. Farming and domestic sewage discharge were also contributing agent to ammoniacal nitrogen contamination in the water (Naveedullah et al., 2016). This scenario is true for the NAHRIM Lake since discharges occurs from the laboratory and canteen area as well as sewerage treatment plant with a pollution load that ranged between 2.785kg/day to 3.457kg/day (NAHRIM, 2012). The result of this study is partially different from the one reported by Al-Badaii et al. (2013) for Semenyih river and Sujaul et al. (2012) in their study of surface water pollution in peninsular Malaysia. Ammoniacal nitrogen, nitrates, nitrate and phosphorus have a role in the eutrophication of the lake (Orderud & Vogt, 2013).

The nitrites (NO$_2^-$-N) and nitrates (NO$_3^-$-N) concentrations in the NAHRIM Lake were far below the stipulated level of 0.4 and 7 mg L$^{-1}$ by the NWQS. They can be categorised as class I. This implies that, the agricultural and canteen sources in the lake watershed that should be responsible for their contamination is having minimal effects on the water quality of the lake. The findings deviated from the one reported by Gasim et al., (2015) for Cempaka lake.

The mean phosphorus concentration (0.056mgL$^{-1}$) recorded for the NAHRIM Lake were below the recommended level for surface water and hence categorised under class I. Although phosphorus contamination results from the domestic discharges containing detergents, fertilizers and wastes from treatment plants which are present in NAHRIM, the discharges from NAHRIM phosphorus sources are having a minimal effect on the lake water pollution. The concentration of phosphorus above 0.025mg L$^{-1}$ usually triggers algae and other aquatic plant growth (Yasir et al., 2017). However the concentration recorded here is lower than those reported by Gasim et al. (2015) and Yasir et al. (2017) in their respective studies of water quality of Cempaka lake and Chini lake respectively.

TSS is an important parameter for water quality study because the oxygen will easily dissolve in low suspended solids water. The concentration of the total suspended solids of the NAHRIM Lake falls within the range of class I to II of the NWQS. The average TSS for the lake effectively categorised the lake in Class I. This occurrence is associated with less consistent land use within the lake general area as well the erosive contribution of the rain that washed down the particles from the impervious areas that is less covered by the constructed wetlands. The finding of TSS is higher than those reported by the Gasim et al. (2015) and Ismail et al. (2012) for Cempaka and Chini lakes respectively but consistent with the findings of Sabri et al. (2016) for the same Cempaka lake.

The *Escherichia coli* and total coliform content of the lake were way above the stipulated level of Class II (5000CFU/100mL) and fell in the class III category of NWQS.
This occurrence is attributed to the high amounts of discharge containing substances that will aid the growth of the microbes. This effluents majorly from the sewerage treatment plant of the Institute with a contribution from drainage system that connects the laboratory, canteen and workshop to the lake. This findings is consistent with similar one reported for Cempaka Lake by Gasim et al. (2015) and Al-Badaii et al. (2013) for Semenyih river. Bacterial counts evaluation of the water is usually utilised to determine the pathogenic strains of organisms that may be related to the feacal microbes.

**Heavy Metals**

Heavy metals are important part of the earth crust that are needed for various developmental activities by the organisms. However, at certain concentrations they induce toxicity. Hence analysing their concentration in the water bodies meant for human contact is important. The detected heavy metal concentrations in the water samples of the NAHRIM lake followed a decreasing order of Fe (1.33±0.62) > B (0.46±0.01) > Mn (0.26±0.09) > Al (0.19±0.08) > Zn (0.026±0.01). The threshold level was not stipulated for aluminium for surface water with recreational purpose but the mean concentration can be categorised under class I. Meanwhile, the lowest concentration of aluminium recorded at sites in September is an indication of less discharge of effluents for that particular month. Iron threshold was set at 1mg L\(^{-1}\) and this implies that, the finding reported here is slightly higher (class III) than the stipulated level. Similar lower iron concentration was also observed for September.

The distribution trend observed for manganese follows the pattern observed for iron. Different trend was observed for zinc that recorded a lower concentration as compared to the class IIB level of 0.2mg L\(^{-1}\). These findings are similar to those reported by Said et al. (2011) for related heavy metals in Cempaka Lake, and for Lake Titiwangsa and Tasik Chini (Shuhaimi-Othman et al., 2008; Said et al., 2012b). Thus, the elemental concentrations of the lake water can be said to be at the natural level. The lower concentration of the elements detected may be attributed to their higher accumulation first by the components of constructed wetlands such as the sediments and plants.

**Water Quality Index (WQI)**

The calculated water quality index for the lake varies from a strong class II to a very strong class II for each of the sites in the studied months. This shows that, with respect to NWQS standard, the lake is suitable for the recreational contact and also for the survival of the organisms. The contribution of each of the parameters in the index varies. Similar findings of class II status have also been reported for other Malaysian lakes such as Tasik Chini, Ampang Hilir lake and Titiwangsa lake (Ismail et al., 2012; Yasir et al., 2017; Said et al., 2012a, Said et al., 2012b) while a class III was reported for Bukit Merah lake by Akinbile et al. (2013).
The analysis of the water samples taken from the constructed wetlands indicated higher concentrations for majority of the parameters as compared to the water sampled from the lake with majority of the parameters being categorised as class III. This may be attributed to the filtration process of the wetland which leads to the reduction in the concentration of the same parameters in the lake. This finding is in agreement with earlier researches (Sim et al., 2008; Zhai et al., 2011; Mburu et al., 2012) that reported the effective role of the wetlands in improving the water quality from the contaminants of different of matrices.

CONCLUSION

The water quality characteristics of NAHRIM Lake was determined. The analysis of the individual water quality parameters shows that most of the water quality parameters fell between the NWQS class I and II. However, the bacterial counts (class III) recorded in the water samples of the lake is above the stipulated threshold. The cumulative National Water Quality Standard of Malaysia water quality index categorised the lake under Class II which makes it suitable for the recreational activities. Generally, the anthropogenic activities observed in the study area is the major factors that deteriorate the water quality of the NAHRIM lake, however the remediation process by the constructed wetlands acting as the first point of contacts limits their impact which is supported by the WQI of class III reported for the water samples from the wetlands. Thus, it is recommended that constructed wetland can be deployed as a medium of the improvement of water quality in other urban lakes with similar contamination problems.

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REFERENCES


FeNO as a Biomarker for Airway Inflammation Due to Exposure to Air Pollutants among School Children Nearby Industrial Areas in Terengganu

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ABSTRACT

Exposure to industrial air pollutants is a public health concern particularly in children due to their immaturity of respiratory systems. The distance between their school and home from the industrial area will elevate the risk of airway inflammation among children. This study aims to determine the exposure of Industrial Air Pollutants (PM₁₀, PM₂.₅, NO₂, SO₂, and VOCs) and its association with airway inflammation (FeNO) among primary school children in industrial and non-industrial areas in Kemaman, Terengganu. A cross-sectional comparative study was conducted among Malay primary school children in Kemaman, Terengganu. A validated questionnaire was randomly distributed to children to get the background information, respiratory symptoms and exposure history of the children. The assessment of indoor air quality was carried out in each primary school and home using indoor air monitoring equipment. Fractional exhaled nitric oxide (FeNO) was measured using an NIOX MINO device. The results showed a significant difference between concentrations of PM₁₀, PM₂.₅, NO₂, SO₂, and VOCs in different classrooms from selected schools and homes of exposed and comparative groups, P<0.05. Statistical analysis revealed that the FeNO level was significantly higher among the exposed group compared to the comparative group.
(Z = -9.442, p = 0.001). The research suggests that the exposure to industrial air pollutants will increase the risk of getting respiratory inflammation among primary school children living near industrial areas.

**Keywords:** FeNO, NO$\textsubscript{2}$, PM$\textsubscript{10}$, PM$\textsubscript{2.5}$, respiratory inflammation, SO$\textsubscript{2}$, VOCs,

**INTRODUCTION**

Airway inflammation is a complex response of the immune system to a threat such as allergens, irritants and infectious agents in the respiratory tract. Numerous of respiratory diseases such as cystic fibrosis, bronchiectasis, chronic bronchitis and asthma are associated with airway inflammation (Alving & Malinovshi, 2010). FeNO is defined as a non-invasive, well-tolerated, and reproducible marker of airway inflammation (Dweik et al., 2011). This online and direct method will be done to measure airway inflammation, and is usually used in asthma patients. It makes asthma monitoring and treatment easier to be conducted routinely in a clinical practice (Yates, 2001).

Exposure to air pollution is a public health concern especially among children as their respiratory system is still not fully developed. Generally children are more vulnerable to the health effects of air pollution than adults as they inhale and exhale more air in proportion to their weight. In addition, children might be more likely to be affected by inflammation by air pollutants, as they have high physical activity and ventilation rate as well as smaller diameter of airways which contributes to greater inhalation dose of air pollutant (Sánchez-Guerra et al., 2012). Thus, the evaluation of airway inflammation in children is vital to understand the underlying mechanisms of respiratory illnesses and preventing children from getting the diseases.

This study was conducted to determine the association between particulate matter of less than 10 micrometers (PM$\textsubscript{10}$) and 2.5 micrometers aerodynamic diameter (PM$\textsubscript{2.5}$), nitrogen dioxide (NO$\textsubscript{2}$), sulphur dioxide (SO$\textsubscript{2}$), and volatile organic compounds (VOCs) with airway inflammation (FeNO) among primary school children in industrial areas in Kemaman, Terengganu due to recent expansion and development of industrial activities, which are petrochemical, iron and steel industries that have the potential to contribute adverse effects to community health especially children. Besides, there are significant findings of association between industrial air pollutants with the prevalence of airway inflammation by previous studies (Noor Hassim & Jalaludin, 2014). The importance of this study will either indirectly or directly help to increase the awareness of the community regarding the risk of exposure towards industrial air pollutants, which can later cause airway inflammation. This is also expected to provide supportive information or data to help developers or any related agencies to prepare a better development plan or control measure if there is a threat towards people nearby industrial areas.
MATERIALS AND METHODS

Study Design and Location

A cross sectional comparative study was conducted among male and female primary school children with the aim to determine the airway inflammation (FeNO) among primary school children in primary schools located near the industrial area under the exposures of PM\textsubscript{10}, PM\textsubscript{2.5}, NO\textsubscript{2}, SO\textsubscript{2}, and VOCs. The parameter involved in airway inflammation was FeNO level. Exposed schools (E1, E2, E3) were schools located within 5 km radius to the nearest boundary of an industrial site, whereas comparative Schools (C1, C2) were located more than 5 km radius from the nearest boundary of an industrial site with less traffic.

![Study locations](Image)

**Figure 1.** Study locations (E1) and (C1) within and outside a 5 km radius from industrial areas in Kemaman, Terengganu
*Source: Image generated by Google Maps, 2015*

**Figure 2.** Study locations (E1), (E2) and (C2) within and outside a 5 km radius from industrial areas in Kemaman, Terengganu
*Source: Image generated by Google Maps, 2015*
Study Sample
A total of 204 primary school children aged 11 years old were selected from five primary schools located in Kemaman, Terengganu. Exposed populations were selected among boys and girls who attended primary school located within a 5 km radius of the industrial area and for comparative group was selected among boys and girls who attended primary school located outside the 5 km radius from the industrial area in Kemaman. Random sampling method was employed to recruit the respondents with several inclusive criteria; only primary school children aged 11 years old, healthy, Malay, and free from any respiratory diseases. The name list of children was obtained from the teachers. The list of schools was obtained from the Ministry of Education before being divided into two groups; exposed and comparative group.

Monitoring in School and Residences
The measurement of indoor air quality was carried out in primary school using indoor air monitoring equipment. The air sampling was conducted for at least 5 or 6 hours during primary school normal activities and 24 hours in homes. There were 5 primary schools and 162 residences selected for the study. The indoor air monitoring equipment used were DustTrak DRX Aerosol Monitor 8534, Air Sampling Pump, PbbRAE Portable VOC Monitor (pbbRAE 3000), LaMotte Air Sampling Pump, Q-TrakPlus Model 8554 Monitor and TSI Velocicalc Plus Model 8386. The equipment were placed at a height of 0.6 to 1.5 metres above the floor, to simulate the location of breathing zone and was not closer than 1 metre to a door, window and wall. Whenever possible, all the equipment were placed at the back of the classroom to ensure no sound disruption from equipment during learning sessions and to avoid the children from being distracted by them. Meanwhile, the measurements in residences were performed by Gillian Air Sampling Pumps. This pump comprises a filter membrane cassette for PM$_{2.5}$ and PM$_{10}$, portable air sampling pump and cyclone. Using the volumetric flow control method, the pump under a fully charged battery draws ambient air at 1.7 litre/minute for 24 hours. The filter paper used was Poly Vinyl Chloride (PVC) filters. The method used was in accordance with MDHS 14/3.

Fractional Exhaled Nitric Oxide (FeNO)
The respondents from each study group were recruited randomly to undergo a single-breath FeNO analysis. This FeNO measurement was done concurrently during measurements of indoor air pollutant. Exhaled nitric oxide (eNO) was measured online following the guidelines adopted from ATS/ERS using chemo-luminescence analyser Niox. (Aerocrine, Inc., Stockholm, Sweden). The measurement was carried out at least one hour after heavy physical activity (if any) while consumption of nitrate-rich food and caffeine among respondents was discouraged. The test began with each respondent required to stand up
quietly and comfortably. First, the respondent’s nose was clipped, and the respondent was required to fully expel air from the lungs by doing a full exhalation through the mouth, after which the respondent inserted the mouthpiece of the NIOX system and inhaled NO-free air calmly to the total lung capacity over a period of 2-3 seconds. The respondent then exhaled steadily for 6 seconds, maintaining a constant air speed in which the machine will emit a continuous humming sound if they were breathing correctly. Each of the respondent was provided with a disposable mouthpiece. As an error prevention measure in maintaining constant air speed while exhaling the air, a visual display of how strong the respondent is exhaling during the test was displayed on the screen of a computer. The visual display was shown to them so that they can control the speed of the air that was being exhaled. However, no repetition of analysis was made among the preschool children. The measured value of 20 ppb or more was indicated as occurrence of eosinophilic inflammation causing less likely of responsiveness to corticosteroids in children less than 12 years old (ATS/ERS, 2005).

Statistical Analysis

Statistical analysis was performed using SPSS version 20.0. Mann-Whitney U test and Chi square test were employed to determine the association and differences between indoor air pollutant concentrations and the FeNO level. The multiple regression test was performed to identify the main factors that influenced the FeNO levels of the children.

RESULTS AND DISCUSSION

The study was conducted to determine the associations of exposure to industrial air pollutants (PM_{10}, PM_{2.5}, NO_{2}, SO_{2}, and VOCs) with FeNO among primary school children in Kemaman, Terengganu. There were 48 boys (47.1%) and 54 girls (52.9%) from exposed area and 54 boys (52.9%) and 48 girls (47.1%) from comparative area who took part in this study. The children underwent the airway inflammation test for indicating the FeNO levels which was done concurrently with indoor air monitoring in schools.

Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Exposed Group Median (IQR)</th>
<th>Comparative Group Median (IQR)</th>
<th>Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM_{10} (µg/m³)</td>
<td>116.00 (39.00)</td>
<td>53.00 (24.00)</td>
<td>-11.333</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PM_{2.5} (µg/m³)</td>
<td>94.00 (39.00)</td>
<td>48.00 (22.00)</td>
<td>-11.762</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SO_{2} (µg/m³)</td>
<td>331.46 (100.49)</td>
<td>123.79 (129.60)</td>
<td>-12.344</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>NO_{2} (µg/m³)</td>
<td>220.04 (56.61)</td>
<td>22.60 (22.45)</td>
<td>-12.357</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>VOCs (ppm)</td>
<td>0.25 (0.53)</td>
<td>0.07 (0.32)</td>
<td>-3.168</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Note. N = 204, Mann-Whitney U test; *Significant at p<0.05
Tables 1 and 2 show the concentration of air pollutants in schools and residences between exposed and comparative areas. The concentrations of PM$_{10}$, PM$_{2.5}$, NO$_2$, and SO$_2$ were monitored based on the Malaysian Air Quality Guidelines (Ambient Standards) at a threshold of 150 μg/m$^3$, 75 μg/m$^3$, 75 μg/m$^3$, and 105 μg/m$^3$ for 24 hours average. It was concluded that the exposure of the exposed group from the concentrations of PM$_{2.5}$, NO$_2$, and SO$_2$ in schools and residences were higher compared to the comparative group and the Malaysian standard. The concentrations of VOCs were monitored based on the Industrial Code of Practice (ICOP), in which the recommended threshold level of TVOC should not exceed 3 ppm over an 8 hour time weighted average airborne concentration (DOSH, 2010). Both VOCs concentration in the exposed and comparative areas in this study were within the recommended levels.

Based on Table 3, the results showed that the FeNO levels were significantly higher among the exposed group compared to the comparative group ($Z = -9.442$, $p = 0.001$). The mean (S.D) for the exposed group [21.57(6.70)] was higher compared to the comparative group [12.80(3.95)]. According to the study conducted by Noor Hassim and Jalaludin (2014), the results showed a significant difference between the FeNO levels with the study groups (exposed and comparative) with $p$-value of 0.001. The mean for FeNO values among those who were in the exposed area (11.43 ppb) was higher than those in the comparative area (8.17 ppb). Similarly, Yusoff et al. (2016) also reported a significant difference in FeNO values among school children of studied and comparative groups at $p<0.001$, suggesting that the school location might have influenced the FeNO levels among...
study respondents. The results could be due to the higher degree of exposure experienced by those in the exposed area as compared to those in the comparative area (Ayuni et al., 2014). Another study conducted by Aida et al. (2014) revealed that the mean concentrations of FeNO at both areas (urban = 11.3 ppb, rural = 9.5 ppb) were lower than the standards (20 ppb to 35 ppb for children) based on the ATS clinical practice guidelines for interpretation of FeNO. At a low FeNO value (<20 ppb), eosinophilic airway inflammation is unlikely to happen; whereas at a high FeNO (>20) level, eosinophilic airway is more likely to happen. Besides, several factors such as individual and environmental were highly related to the increase of FeNO values and they are needed to be investigated further in the multivariate analysis (Franklin, 2007). Individual factors influencing FeNO levels among children are gender, active lifestyle, nitrate-rich diet, airway calibre, any infectious disease, and medication while for environmental factors can be described as exposure to tobacco smoke and environmental pollutants such as $\text{SO}_x$ and $\text{NO}_x$ (ATS/ERS, 2005; Boqqs & Dokmeci, 2012). Thus, the findings support the hypothesis that the FeNO levels of the primary school in exposed areas are significantly higher than the primary school in the comparative area.

Table 4
The association of exposure between industrial air pollutants concentration in schools and FeNO levels among study groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>FeNO</th>
<th>$\chi^2$</th>
<th>p-value</th>
<th>PR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM$_{10}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (≥87 μg/m$^3$)</td>
<td>62 (30.40)</td>
<td>40 (19.61)</td>
<td>7.84</td>
<td>0.001*</td>
<td>0.32</td>
</tr>
<tr>
<td>Low (&lt;87 μg/m$^3$)</td>
<td>42 (20.58)</td>
<td>60 (29.41)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (≥81 μg/m$^3$)</td>
<td>65 (31.86)</td>
<td>29 (14.22)</td>
<td>12.44</td>
<td>0.001*</td>
<td>0.17</td>
</tr>
<tr>
<td>Low (&lt;81 μg/m$^3$)</td>
<td>49 (24.02)</td>
<td>61 (29.90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (≥170 μg/m$^3$)</td>
<td>68 (33.33)</td>
<td>28 (13.73)</td>
<td>10.82</td>
<td>0.043*</td>
<td>2.93*</td>
</tr>
<tr>
<td>Low (&lt;170μg/m$^3$)</td>
<td>48 (23.53)</td>
<td>60 (29.41)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SO$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (≥243 μg/m$^3$)</td>
<td>72 (35.29)</td>
<td>30 (14.71)</td>
<td>25.68</td>
<td>0.005*</td>
<td>4.64*</td>
</tr>
<tr>
<td>Low (&lt;243μg/m$^3$)</td>
<td>31 (15.20)</td>
<td>71 (34.80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (≥0.084ppm)</td>
<td>48 (23.53)</td>
<td>60 (29.41)</td>
<td>18.70</td>
<td>0.001*</td>
<td>0.11</td>
</tr>
<tr>
<td>Low (&lt;0.084ppm)</td>
<td>70 (34.31)</td>
<td>26 (12.74)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. N= 204, Chi- Square Test; *Significant at p< 0.05; Significant PR at 95% CI>1
Table 4 shows the association between the industrial air pollutant concentrations of PM$_{10}$, PM$_{2.5}$, NO$_2$, SO$_2$, and VOCs with airway FeNO values among exposed and comparative groups. Based on the median value, the level of PM$_{10}$ concentration in schools was categorised into two groups; high level ($\geq 87 \mu g \text{ m}^{-3}$) and low level ($< 87 \mu g \text{ m}^{-3}$), PM$_{2.5}$ concentration; high level ($\geq 81 \mu g \text{ m}^{-3}$) and low level ($< 81 \mu g \text{ m}^{-3}$), NO$_2$ concentration; high level ($\geq 170 \mu g \text{ m}^{-3}$) and low level ($< 170 \mu g \text{ m}^{-3}$), SO$_2$ concentration; high level ($\geq 243 \mu g \text{ m}^{-3}$) and low level ($< 243 \mu g \text{ m}^{-3}$) and VOCs concentration; high level ($\geq 0.084$ ppm) and low level ($< 0.084$ ppm).

Based on Table 4, there was a significant association found between NO$_2$ and SO$_2$ with the FeNO value as $p<0.005$. The children who were exposed to a high concentration of NO$_2$ were 2 times more likely to have a higher FeNO level ($PR= 2.93, 95\%\ CI=1.02-8.45$) while the children who were exposed to a high concentration of SO$_2$ were 4 times more likely to have a higher FeNO level ($PR= 4.64, 95\%\ CI=1.56-13.81$), depicting higher airway inflammation.

Table 5 shows the association of exposure between industrial air pollutant concentrations in residences and FeNO levels among study groups. Based on the median value, the level of PM$_{10}$ concentration in residences were categorised into two groups; high level

<table>
<thead>
<tr>
<th>Variables</th>
<th>FeNO</th>
<th>χ$^2$</th>
<th>p-value</th>
<th>PR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unlikely ($&lt;20$ ppb)</td>
<td>Present ($\geq 20$ ppb)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>Total (%)</td>
<td>Total (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High ($\geq 74 \mu g/\text{m}^3$)</td>
<td>40 (24.69)</td>
<td>42 (25.93)</td>
<td>11.78</td>
<td>0.001*</td>
<td>0.317</td>
</tr>
<tr>
<td>Low ($&lt; 74 \mu g/\text{m}^3$)</td>
<td>60 (37.04)</td>
<td>20 (12.34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>($\geq 61 \mu g/\text{m}^3$)</td>
<td>39 (24.07)</td>
<td>49 (30.25)</td>
<td>24.72</td>
<td>0.001*</td>
</tr>
<tr>
<td>($&lt; 61 \mu g/\text{m}^3$)</td>
<td>61 (37.65)</td>
<td>13 (8.03)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_2$</td>
<td>($\geq 59 \mu g/\text{m}^3$)</td>
<td>27 (16.67)</td>
<td>48 (29.63)</td>
<td>39.13</td>
<td>0.001*</td>
</tr>
<tr>
<td>($&lt; 59 \mu g/\text{m}^3$)</td>
<td>73 (45.06)</td>
<td>14 (8.64)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SO$_2$</td>
<td>($\geq 84 \mu g/\text{m}^3$)</td>
<td>33 (20.37)</td>
<td>48 (29.63)</td>
<td>30.21</td>
<td>0.001*</td>
</tr>
<tr>
<td>($&lt; 84 \mu g/\text{m}^3$)</td>
<td>67 (41.36)</td>
<td>14 (8.64)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOCs</td>
<td>($\geq 0.070$ ppm)</td>
<td>16 (9.87)</td>
<td>60 (37.04)</td>
<td>5.800</td>
<td>0.016*</td>
</tr>
<tr>
<td>($&lt; 0.070$ ppm)</td>
<td>75 (46.30)</td>
<td>11 (6.79)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. N= 162, Chi-Square Test; *Significant at $p<0.05$; Significant PR at 95% CI>1

Table 5 shows the association of exposure between industrial air pollutant concentrations in residences and the FeNO levels among study groups. Based on the median value, the level of PM$_{10}$ concentration in residences were categorised into two groups; high level
Industrial Air Pollutant and Respiratory Inflammation in Children

(≥74 µgm⁻³) and low level (<74 µgm⁻³), PM₂.₅ concentration; high level (≥61 µgm⁻³) and low level (<61 µgm⁻³), NO₂ concentration; high level (≥59 µgm⁻³) and low level (<59 µgm⁻³), SO₂ concentration; high level (≥84 µgm⁻³) and low level (<84 µgm⁻³) and VOCs concentration; high level (≥0.070 ppm) and low level (<0.070 ppm).

Based on Table 5, children who were exposed to high industrial air pollutants in residences had high FeNO levels [PM₁₀ (25.93%), PM₂.₅ (30.25%), NO₂ (29.63%), SO₂ (29.63%), and VOCs (37.04%)]. As shown in Table 5, there was a significant association found between PM₂.₅, NO₂, and SO₂ with the FeNO value as p< 0.005. The children who were exposed to high concentrations of PM₂.₅ were 5 times more likely to have higher FeNO levels (PR= 5.024, 95% CI=2.58- 9.79). The children who were exposed to high concentrations of NO₂ were 5 times more likely to have higher FeNO levels (PR= 5.497, 95% CI=3.02- 10.01) while the children who were exposed to high concentration of SO₂ were 4 times more likely to have higher FeNO levels (PR= 4.598, 95% CI=2.55- 8.30), depicting higher airway inflammation.

Previous local studies had shown that the concentrations of indoor air pollutants in schools and residences nearby the industrial area were higher compared to the comparative group (Jamil et al., 2015; Suhaimi et al., 2016). A local study by Aziz et al. (2014) found that only NO₂ showed a significant association with the elevated levels of FeNO (p<0.05) in rural areas. However, the exposure to NO₂ in this study was lower in both areas based on the measurements carried out. When the data of these two study areas were combined, the results showed a significant association between NO₂ and FeNO. However, the relationship was not strong (coefficient of 1). This result may be related to individual susceptibility (nutrition status and genetics) instead of pollutant exposure (Stefania et al., 2012). Franklin et al. (2007) previously explained that the FeNO value in children increased until the age of 13. Thus, the findings support the hypothesis that there is a significant association of industrial air pollutant concentrations between FeNO levels in schools and residences.

Table 6
Multiple linear regressions for association between PM₁₀, PM₂.₅, SO₂, NO₂ and VOCs in school with airway inflammation after controlling the confounders

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>B (SE)</th>
<th>p-value</th>
<th>PR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.76 (0.23)</td>
<td>0.001</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>PM₁₀ (µg/m³)</td>
<td>1.67 (1.31)</td>
<td>0.203</td>
<td>5.30</td>
<td>0.41- 69.26</td>
</tr>
<tr>
<td>PM₂.₅ (µg/m³)</td>
<td>-1.54 (1.07)</td>
<td>0.151</td>
<td>0.21</td>
<td>0.03- 1.76</td>
</tr>
<tr>
<td>NO₂ (µg/m³)</td>
<td>1.11 (0.49)</td>
<td>0.023</td>
<td>3.02*</td>
<td>1.16- 7.82</td>
</tr>
<tr>
<td>SO₂ (µg/m³)</td>
<td>0.54 (0.74)</td>
<td>0.466</td>
<td>1.72</td>
<td>0.40- 7.39</td>
</tr>
<tr>
<td>VOCs (ppm)</td>
<td>0.50 (0.54)</td>
<td>0.326</td>
<td>1.66</td>
<td>0.57- 4.80</td>
</tr>
</tbody>
</table>

Note. N=204, Method = Stepwise; *Significant at p<0.05; Adjusted R² = 0.42795%; CI= 95% Confidence Interval; B= Regression Coefficient; S.E= Standard Error
It is known that air pollutants in schools and residences are influenced by different activities; indoors or outdoors. Table 6 shows five variables to represent factors at schools which were significantly associated with airway inflammation among school children when they were statistically analysed at univariate level. Statistical analysis showed that NO$_2$ was the most significant factor in schools that influenced airway inflammation among primary school children. There was a significant direct linear relationship between NO$_2$ in schools and FeNO levels (p<0.05). With an increase of 1 µg/m$^3$ in NO$_2$ in schools, FeNO levels among school children increased by 0.001%. A study in the Netherlands found that changes in NO$_2$ concentrations were associated with evidences of acute airway inflammation (FeNO value) and impaired lung function (Strak et al., 2012). These findings were consistent with findings of Noor Hassim and Jalaludin (2014) whereby indoor NO$_2$ was found to have stronger associations with FeNO levels than PM$_{2.5}$. Other than that, a study done in London by Kharitonov et al. (1994) revealed that the level of FeNO increased in atopic patients than in non-atopic asthma patients. In another study in Italy conducted by Van den Toorn et al. (2001) on randomised clinical trial evaluating two groups of asthmatic children; one of them was assessed for FeNO measurements. After six months of follow-ups, they found that there was a significant reduction of FeNO levels, asthma exacerbation and clinical symptoms in the FeNO group after 6 months of therapy. Based on the study, it revealed that FeNO was potentially capable in evaluating the role of airways inflammation.

CONCLUSION

In summary, the research indicates that children exposed to industrial air pollutants might have increased risk of getting airway inflammation. This study also suggests that exposure and specific explanation about the risk of getting airway inflammation due to poor air quality inside classrooms and residences should be given to the public, primary school management, and parents. Further studies are required to confirm the observed association between industrial air pollutant concentrations and respiratory health among primary school children in the industrial and non-industrial areas. Thus, FeNO can be suggested as a non-invasive biomarker for airway inflammation among children.

ETHICAL CONCERN

Ethical approvals for this study were obtained from the Research Ethics Committee of Universiti Putra Malaysia (Reference no. FPSK EXP15 P148). The objectives of this study were explained to the parents in getting their approval from them in order for their children to be the respondents for this study. All respondents participated on a voluntary basis and may leave at any time without penalty. All the information obtained remains confidential.
ACKNOWLEDGEMENT

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Study of Dissolved Nutrient Condition at Pulau Perhentian, Terengganu

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ABSTRACT

This study compares the distribution of dissolved nutrients (NO$_3^-$ and PO$_4^{3-}$) between two seasons (pre-monsoon and post-monsoon) in Pulau Perhentian, Terengganu. The concentration of dissolved PO$_4^{3-}$ was found to be 16 to 83 times higher during the post-monsoon period (April 2015) compared to the pre-monsoon period (October 2014). On the other hand, the concentration of dissolved NO$_3^-$ was two (2) to three (3) times higher during the post-monsoon period (April 2015) compared to the pre-monsoon period (October 2014). These nutrients' inputs were converted from P limitation condition during the pre-monsoon period to N limitation condition during the post-monsoon period at our study area. The results of this study suggest that the Northeast monsoon plays an important role in influencing the distribution of dissolved nutrients between seasons in Pulau Perhentian. It is thought that during the post-monsoon period, a considerable input of nutrients from bottom water is responsible for increasing dissolved nutrients in surface water, in particular PO$_4^{3-}$.

Keywords: Dissolved nutrients, Monsoon, N-limitation, P-limitation, seawater

INTRODUCTION

Dissolved nutrients in seawater are essential for phytoplankton growth (Malone et al., 1996) and are considered as one of the most important parameters in the ocean environment (Mohamed & Amil, 2015), as well as for reproduction and metabolic activities of living beings. Knowledge of...
seasonal growth-rate responses of coastal phytoplankton communities to increasing nutrient loading offers insights into the potential effects of eutrophication on energy transfer within the ecosystem. It also provides a tool for establishing ecologically relevant management strategies (Olson et al., 2001). High rates of nutrient supply to these environments frequently enhance phytoplankton growth and biomass, and increase the rate of organic matter loading (Smith & Bennett, 1999). The rate of nutrient supply is subject to many factors, including human activities, which add to the variability and uncertainty of the nutrient budget of its zone (Rabouille et al., 2001).

The relative concentrations of N and P have been used to estimate which of these nutrients is limiting the growth of phytoplankton in aquatic systems. The approach is simple and easy to use provided that data exists on N and P concentrations. However, interpretation of the results should be undertaken with caution as the N:P ratio may not correctly indicate the limiting nutrient of the system. Redfield (1934, 1958) argued that marine phytoplankton contains a molecular C:N:P ratio of 106:16:1 (50:7:1 by weight), and application of the ratios has become widespread not only in marine, but also in freshwater phytoplankton studies. A departure from this ratio has been assumed to imply nutrient deficiency. In such a case, there is not only sub-optimal growth of phytoplankton, but also sub-standard food resources for primary consumers of phytoplankton.

It is known that the nutrient condition is not constant but varies according to seasonal and environmental conditions. Previous studies reported that a mass N:P ratio above 17 indicates P limitation while a ratio below 10 is N limitation. Values between 10 and 17 indicate that either of the nutrients may be limiting (Forsberg & Ryding, 1980; Hellström, 1996). A recent study by Adiana et al. (2014) concerning the South China Sea off peninsular Malaysia suggested that the climatic changes between Northeast monsoons had a major influence on metal partitioning as well as affecting the metal’s distribution in the waters off the southern Terengganu coast. It drives the mixing and transport that determine upper ocean structure; where metals are carried to the surface by water mixing. These phenomena may supply nutrients into surface water to support the growth of phytoplankton in the area. In coastal areas where water mixing is seasonal or intermittent, the nutrient content of the surface waters may show marked fluctuations and may actually increase during the season of monsoon events.

In addition, a previous study by Mohamed and Amil (2015) found a lack of dissolved nutrients during the pre-monsoon event (October 2014) at Pulau Perhentian. A dramatic growth of phytoplankton biomass in the incubation bottles was recorded when enriched with a combination of nitrogen (N), phosphorus (P) and carbon (C) as well as exposure to sunlight for three days. These results suggested that during the pre-monsoon period, phytoplankton were living in a low-nutrient condition which could possibly be due to a monsoon event. However, this study was only based on nutrient enrichment incubation
analysis. Further comprehensive study is needed to evaluate the impact of a Northeast monsoon event upon the condition of nutrients in Pulau Perhentian. For that reason, this study aims to observe and examine the temporal and spatial distribution of dissolved NO$_3^-$ and PO$_4^{3-}$ in Pulau Perhentian during pre- and post-monsoon events. Findings from this study are important in order to understand a possible relationship between dissolved nutrient condition and phytoplankton growth in the area.

MATERIALS AND METHODS

Seawater Sampling

Samplings were carried out in October 2014 (pre-monsoon) and April 2015 (post-monsoon) at Pulau Perhentian, Terengganu (Figure 1). Seawater profile samples were collected by using Van Dorm water sampler and filled into 1.0 L low-density polyethylene (LDPE) bottles. The 1.0 L seawater samples were collected at five to six different depths from each station for nutrients and trace element analysis after filtration and acidifying (trace element analysis) procedures. Seawater samples of 10.0 L in the surface layer (3 m depth) were collected at Station 1 for nutrient enrichment incubation analysis.

Figure 1. Map showing the location for each of the selected stations during pre-monsoon and post-monsoon sampling at Pulau Perhentian
In-situ Measurements

In-situ parameters for each seawater sample were measured by using: SCT YSI Model 30; YSI Model 54 conductivity meter; Thermo Orion AQ 4500 turbidity meter; and Thermo Orion 230A Plus pH meter. All samples (1.0 L) were filtered with 0.45µm pore size of filter paper, for further nutrient analysis in the laboratory field.

Nutrients Analysis

Nutrient analysis was performed with the USEPA method. The concentration of dissolved nitrate ($\text{NO}_3^-$) and phosphate ($\text{PO}_4^{3-}$) were measured by adding NitraVer 5 (range of detection: 0.3-30.00 mg/L) and PhosVer 3 (range of detection: 0.02-2.50 mg/L) Reagent Powder Pillow (HACH) into 10.0 mL seawater samples. The measurement was conducted using an SHIMADZU HACH DR 2800 spectrophotometer. Distilled water was used as a blank solution.

RESULTS AND DISCUSSION

The vertical profile for in-situ parameters such as temperature, dissolved oxygen, salinity, pH and turbidity during pre-monsoon and post-monsoon events are shown in Tables 1 and 2, respectively. All the parameters’ horizontal and vertical profiles showed variations in their spatial distribution in the water column (Figure 2).

Table 1

<table>
<thead>
<tr>
<th>St.</th>
<th>Depth (m)</th>
<th>pH</th>
<th>Temp. (°C)</th>
<th>Cond. (µS/cm)</th>
<th>Sal. (ppt)</th>
<th>DO (mg/L)</th>
<th>Turb. (NTU)</th>
<th>$\text{PO}_4^{3-}$ (mg/L)</th>
<th>$\text{NO}_3^-$ (mg/L)</th>
<th>N:P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>7.19</td>
<td>29.90</td>
<td>52.30</td>
<td>31.50</td>
<td>7.53</td>
<td>0.10</td>
<td>0.02 ± 0.00</td>
<td>1.52 ± 0.10</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.84</td>
<td>29.80</td>
<td>52.50</td>
<td>31.60</td>
<td>6.38</td>
<td>0.02</td>
<td>0.12 ± 0.00</td>
<td>2.94 ± 0.12</td>
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</tr>
<tr>
<td></td>
<td>15</td>
<td>7.80</td>
<td>29.90</td>
<td>53.10</td>
<td>31.90</td>
<td>7.46</td>
<td>0.00</td>
<td>0.11 ± 0.02</td>
<td>2.83 ± 0.00</td>
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</tr>
<tr>
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<td>7.84</td>
<td>29.80</td>
<td>53.20</td>
<td>31.90</td>
<td>6.66</td>
<td>0.00</td>
<td>0.14 ± 0.00</td>
<td>2.51 ± 0.17</td>
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<td>30</td>
<td>7.84</td>
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<td>0.11 ± 0.00</td>
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<tr>
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<td>7.86</td>
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<td>31.50</td>
<td>8.68</td>
<td>0.05</td>
<td>0.06 ± 0.02</td>
<td>2.23 ± 0.10</td>
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<tr>
<td></td>
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<td>7.94</td>
<td>29.60</td>
<td>53.30</td>
<td>31.90</td>
<td>8.30</td>
<td>0.00</td>
<td>0.04 ± 0.01</td>
<td>2.26 ± 0.38</td>
<td>57</td>
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<td>29.60</td>
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<td>8.34</td>
<td>0.00</td>
<td>0.04 ± 0.00</td>
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<td>29.60</td>
<td>53.50</td>
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<tr>
<td>3</td>
<td>3</td>
<td>7.68</td>
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<td>31.50</td>
<td>6.89</td>
<td>0.04</td>
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<td>1.04 ± 0.60</td>
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<tr>
<td></td>
<td>10</td>
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<td>29.50</td>
<td>53.30</td>
<td>31.90</td>
<td>7.34</td>
<td>0.04</td>
<td>0.04 ± 0.00</td>
<td>1.34 ± 0.00</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7.65</td>
<td>29.60</td>
<td>53.40</td>
<td>32.00</td>
<td>7.75</td>
<td>0.03</td>
<td>0.03 ± 0.00</td>
<td>1.19 ± 0.06</td>
<td>40</td>
</tr>
</tbody>
</table>
Surface Physicochemical Characteristics (3 m Depth)

During pre-monsoon sampling activity, the surface water temperature was found to vary between 29.1°C and 29.9°C as highlighted in Table 1 and Figure 2b. However, it was found to be slightly warmer during the post-monsoon sampling activity with a temperature range
between 29.0°C and 33.0°C (Table 2). The low temperature readings recorded during the pre-monsoon period could be attributed to strong land sea breezes and precipitation as suggested by Govindasamy et al. (2000).

Figure 2(c) shows that the surface seawater salinity levels between the stations were constant during pre-monsoon sampling (31.5 to 31.7 ppt) as opposed to a high variability of salinity recorded during a post-monsoon event (30.8 to 31.3 ppt). However, maximum salinity reading was recorded during the pre-monsoon period while minimum reading was recorded during the post-monsoon period. According to Saravanakumar et al. (2008), a high salinity value during the pre-monsoon period might be due to: low rainfall; the absence of river discharge; tidal mixing; and dominance of neritic water from the open sea.

The pH of surface seawater was found to be between 7.19 and 7.91 as shown in in Table 2 and Figure 2(a) during the pre-monsoon period, and between 8.25 and 8.32 during a post–monsoon event. It was discovered that the surface seawater at the stations remained alkaline during both sampling activities. The different in pH between both seasons can be attributed to several factors such as: removal of CO$_2$ by photosynthesis through bicarbonate degradation; reduction in salinity and temperature; and decomposition of organic matter (Rajasegar, 2003). Maximum pH was observed during the post-monsoon period, and the minimum was observed during the pre-monsoon season. It followed a trend similar to that of surface temperature. Statistical analysis revealed that pH has a highly significant negative correlation with rainfall and a positive correlation with water temperature, but dissolved oxygen has an inverse relationship with pH (Anitha & Kumar, 2013).

The distribution of surface dissolved oxygen showed as being higher during the pre-monsoon event than the post-monsoon event. The range of dissolved oxygen was 5.90-8.68 mg/L (Table 1, Figure 2(d)) and 4.37-6.37 mg/L (Table 2, Figure 2(d)) during pre-monsoon and post-monsoon sampling, respectively. In aquatic systems, oxygenation is the result of an imbalance between the process of photosynthesis, degradation of organic matter (Granier et al., 2000), and physicochemical properties of water (Aston, 1980).

Turbidity values varied from 0.00-0.10 NTU (Table 1, Figure 2(e)) and 0.04-0.22 NTU (Table 2, Figure 2(e)) during pre-monsoon and post–monsoon events, respectively. High turbidity was recorded during the post-monsoon period and minimum during the pre-monsoon period. This could be attributed to an increase of wave action during the post-monsoon event due to the northerly wind and the current prior to the onset of the Northeast monsoon. This would result in turbulent conditions in the coastal waters favoring the resuspension of the bottom sediment due to stirring action that causes low water transparency (Nixon, 1988).
Nutrient distribution at surface water (3 m depth). The distributions of dissolved NO$_3^-$ and PO$_4^{3-}$ in surface water at Pulau Perhentian during both monsoon events are shown in Figure 3. During the pre-monsoon event, surface-dissolved PO$_4^{3-}$ concentration was found to decrease with increasing distance from the Pulau Perhentian.

The highest concentration was recorded at St. 4 (0.19±0.00 mg/L) (Table 1) while the lowest concentration was recorded at St. 1 (1.52±0.10 mg/L) (Table 1). On the other hand, during the post-monsoon event, the surface distribution of dissolved PO$_4^{3-}$ was higher at
St. 1 (1.67±0.29 mg/L) (Table 2) and St. 2 (1.00±0.00 (Table 2) compared to St. 4 and 5. Interestingly, the surface dissolved NO$_3^-$ was found to be higher than dissolved PO$_4^{3-}$ for both seasons. During the pre-monsoon event, as opposed to the trend observed for dissolved PO$_4^{3-}$ mentioned earlier, surface dissolved NO$_3^-$ was found to increase with increasing distance from the Pulau Perhentian. The highest surface concentration of dissolved NO$_3^-$ was recorded at St. 2 (2.23±0.10 mg/L) (Table 1) while the lowest concentration of dissolved NO$_3^-$ was recorded at St. 5 (0.73±0.00 mg/L) (Table 1).

The surface distribution of dissolved NO$_3^-$ and PO$_4^{3-}$ was observed to change significantly during the post-monsoon period. In contrast to pre-monsoon trend distribution of dissolved PO$_4^{3-}$, the post-monsoon distribution of dissolved PO$_4^{3-}$ was found to increase with increasing distance from the Pulau Perhentian. The highest concentration of dissolved PO$_4^{3-}$ was recorded at St. 1 (1.67±0.29 mg/L) (Table 1) while the lowest concentration of dissolved PO$_4^{3-}$ was recorded at St. 3, 4 and 5 (0.50±0.00 mg/L) (Table 1). Conversely, the post-monsoon surface distribution of dissolved NO$_3^-$ was found to decrease with increasing distance from the Pulau Perhentian, as opposed to observations made earlier during the post-monsoon period. The highest concentration of dissolved NO$_3^-$ was recorded at St. 4 (8.00±0.00 mg/L) (Table 2) while the lowest concentration of dissolved NO$_3^-$ was recorded at St. 1 (4.33±0.23 mg/L) (Table 2).

These results suggested that both concentrations of dissolved NO$_3^-$ and PO$_4^{3-}$ were found to be higher during the post-monsoon compared to the pre-monsoon event. For example, the concentrations of dissolved PO$_4^{3-}$ were 83 times higher during the post-monsoon period than during the pre-monsoon period at St. 1. Similarly, the concentrations of dissolved PO$_4^{3-}$ were 16 times higher during the post-monsoon period than during the pre-monsoon period at St. 2. On the other hand, the rate of change for concentrations of dissolved NO$_3^-$ is not as high as that of dissolved PO$_4^{3-}$. For example, the concentrations of dissolved NO$_3^-$ were twice as high during the post-monsoon period than during the pre-monsoon period at St.1. Similarly, the concentrations of dissolved NO$_3^-$ were three times higher during the post-monsoon period than during the pre-monsoon period at St.1.
higher during the post-monsoon period than during the pre-monsoon period at St.2. This observation highlighted the influence of the Northeast monsoon on dissolved NO$_3^-$ and PO$_4^{3-}$ concentration in surface water. The seasonal Northeast monsoon brings a huge input of dissolved PO$_4^{3-}$ into surface water at Pulau Perhentian.

**Nutrients Distribution Throughout Water Column**

The vertical profiles for dissolved NO$_3^-$ and PO$_4^{3-}$ during both monsoon events at all stations are highlighted in Table 1 and Figure 4. In general, it can be observed that the concentration of dissolved NO$_3^-$ was higher than dissolved PO$_4^{3-}$ along the water column. Furthermore, both concentrations of dissolved NO$_3^-$ and PO$_4^{3-}$ are also recorded as being higher during the post-monsoon period than during the pre-monsoon period. For example, the concentrations of dissolved NO$_3^-$ were between 1.67±0.29 and 1.00±0.00 mg/L (Table 1) while the concentrations of dissolved PO$_4^{3-}$ were between 1.52±0.10 to 2.54±0.00 mg/L (Table 1) at St. 1 during the pre-monsoon event. During the post-monsoon event, the concentrations of dissolved NO$_3^-$ decreased to between 1.00±0.00 and 3.00±0.02 mg/L (Table 2) while the concentrations of dissolved PO$_4^{3-}$ increased to between 4.00±0.00 and 10.83±2.89 mg/L (Table 2) at the same station.

In addition, the profile for both dissolved NO$_3^-$ and PO$_4^{3-}$ shows a nutrient-like profile during the pre-monsoon event throughout the water column as shown in Figures 4(a) and 4(b) below. Both figures highlight the depletion of nutrients at surface layer (3 m depth) and maximum concentration at 6 m depth. Below 6 m depth, their concentrations converged to constant values with column during the pre-monsoon event (Oct. 2014) and the post-monsoon (April 2015) event at Pulau Perhentian.

**Figure 4.** Profile concentration of: (a) phosphate (PO$_4^{3-}$), October 2015; (b) nitrate (NO$_3^-$), October 2014; (c) phosphate (PO$_4^{3-}$), April 2015; and (d) nitrate (NO$_3^-$), April 2015 in water
For example, at St.1, the concentration of dissolved PO$_4^{3-}$ was 0.02±0.00 mg/L (Table 1) at surface layer and increasing to 0.12±0.00 mg/L (Table 1) at 6 m depth before it decreased to a constant concentration below 30 m depth. Similarly, at the same station, the concentration of dissolved NO$_3^-$ was 1.52±0.10 mg/L (Table 1) at surface layer and increased to a maximum concentration 2.94±0.12 mg/L (Table 1) at 6 m depth before decreasing to a constant concentration below 30 m depth. A similar profile between both micronutrients throughout the water column suggested a similar process that influences their distributions in the water column during the pre-monsoon period. This indicated the active dissolved nutrients’ uptake by phytoplankton (Bruland & Lohan, 2004) and resulted in the depletion of dissolved PO$_4^{3-}$ and NO$_3^-$ at the surface layer. Nitrate and phosphate concentrations increase with depth, as remineralisation of sinking particulate matter. This remineralisation process (which is the process of degradation of the dead phytoplankton) sinks to the deeper layer and undergoes nitrification, which returns the N in the form of particulate into dissolved nitrate again (Sigman et al., 2009).

In contrast with the pre-monsoon event, the vertical profile for both micronutrients shows unclear profiles and higher concentration throughout the water column during the post-monsoon event as highlighted in Figures 4(c) and 3(d). The concentration of dissolved PO$_4^{3-}$ profile at station 1 was 1.67±0.29 mg/L (Table 2) in the surface layer before decreasing to 1.00±0.00 mg/L (Table 2) at 6 m depth and increasing again to maximum concentration 3.00±0.02 mg/L (Table 2) at 15 m depth. Following this, it then decreased again to minimum concentration 1.00±0.00 mg/L (Table 2) at 30 m depth. An inconsistent profile was also observed for dissolved NO$_3^-$, which recorded a maximum concentration 10.83±2.89 mg/L (Table 2) at 30 m depth as shown in Fig. 4d). These results seem to indicate the existence of water mixing in the water column during the post-monsoon period.

It is a known fact that the east coast of peninsular Malaysia is strongly influenced by seasonal monsoon winds. These winds provide energy for vertical mixing and force large-scale resuspension of surface sediment during the Northeast monsoon. Recorded measurement of the turbidity, salinity and conductivity profile during both monsoon events at St. 1 as depicted in Figure 5 could explain the condition for the water column at Pulau Perhentian between both seasons. As can be observed in the figure, the measured parameters showed an unclear profile throughout the water column during the post-monsoon period, as opposed to pre-monsoon distributions which were more stable with clear trends. For example, during the pre-monsoon period, turbidity was found to be higher in surface water (0.10 NTU) before decreasing with depth to a constant turbidity (0.00 - 0.02 NTU). In contrast, during the post-monsoon period, the turbidity was highest at the surface 0.08 NTU before reducing to 0.00 NTU at 15 m depth and subsequently increasing again to a constant turbidity at 30 m.
These results suggested that the influence of the Northeast monsoon into Pulau Perhentian not only affected the distribution of dissolved nutrients in surface water but also throughout the water column. The distribution of dissolved nutrients during the pre-monsoon period was affected by an uptake process at surface layer and remineralization in a deeper layer. However, during the post-monsoon period, their concentration was more affected by vertical mixing in the water column. Consequently, an input of nutrients to the surface water at St. 1 and St. 2 during the post-monsoon event might be the result of mixing and forcing arge-scale resuspension of surface sediment rich in N and P, as suggested by Broecker and Peng (1982). A huge input of nutrients into surface water might be issued with a limiting factor (Capone et al., 1997; Falkowski, 1997) that controls primary production.

The effect of input to the production is an essential study in order to understand the condition of nutrients between both seasons.

**Nutrients’ Condition**

The ratio of N:P at surface layer (3 m depth) showed the different pattern of ratio distribution between the pre-monsoon and post-monsoon events as highlighted in Figure 6(a). During the pre-monsoon event, the wide range of N:P ratio was observed, between 3 recorded at St. 5 and 76 recorded at St. 1. This means the ratio increased as distance from the island increased. However, during the post-monsoon event, the ratio was more consistent where its ratio ranged only between 3 recorded at St. 1 and 16 recorded at St. 4, which was the highest ratio recorded.
According to Hecky and Kilham (1988), ecosystems with N:P molar ratios less than the average required cellular ratio of 16:1 were generally N-limited and those with ratios >16:1 were P-limited. Therefore, it could be suggested that, during the pre-monsoon event, low concentrations of dissolved PO$_4^{3-}$ and NO$_3^-$ in surface water resulted in the existence of P-limitation condition at stations further away from Pulau Perhentian, St. 1, 2 and 3. Conversely, N-limitation condition was recorded at stations nearer to the island (St. 4 and 5) during the pre-monsoon period and at all of the stations during the post-monsoon period. This indicated that St. 1, 2 and 3 were in P-limitation condition during the pre-monsoon period and changed to N-limitation condition during the post-monsoon period. The transformation between both conditions was reported previously by McComb et al. (1981) in Western Australia.

The presence of P-limitation condition (N:P >16) at St. 1, 2 and 3 during the pre-monsoon event might be explained by low concentrations of dissolved PO$_4^{3-}$ due to active biological uptake by phytoplankton. In fact, P-limitation has also been observed along:
the upper Chesapeake Bay (Boynton et al., 1982); the Peel-Harvey estuaries (McComb et al., 1981); the Mediterranean Sea (Bonin et al., 1989); and in Norwegian waters (Paasche & Erga, 1988). According to Harrison et al. (1986), the P-limitation could be attributed to the growth of phytoplankton (mainly diatoms) which caused phosphate concentrations to drop, before nitrate concentration in seawater. In addition, due to the mode of $\text{PO}_4^{3-}$ input into stations St. 1 to St. 5 (most likely from the island) it might be sedimented more rapidly in closer proximity to the island (Smith & Veeh, 1989). The condition could lead to frequent limitation of production by P than N, which was similarly noted in Howarth et al. (1995). P supply rate was equally limiting during this period (pre-monsoon) and the addition of P to this environment condition will elicit phytoplankton biomass stimulation (Fisher et al., 1992).

The vertical profile of N:P ratio was also less than 16 throughout the water column at St. 4 and 5 during the pre-monsoon period as shown in Figure 6(b). Its ratio was ranged between 5 and 6, and between 3 and 9 at St. 4 and 5, respectively. Moreover, the high N:P ratio (>16) was recorded throughout the water column at St. 1, 2 and 3 during the pre-monsoon period. This vertical profile of N:P ratio (>16) could indicate the existence of P-limitation not only at surface water, but also throughout the water column at St. 1, 2 and 3 during the pre-monsoon event. On the other hand, there was an N-limitation condition at most of the stations during the post-monsoon event, due to less N:P ratio (<16) at surface water and throughout the water column resulting from a huge input of $\text{PO}_4^{3-}$.

The existence of a P-limitation condition at St. 1, 2 and 3 during the pre-monsoon period was converted to N-limitation condition during the post-monsoon event. This conversion was also observed in previous studies (D’Elia et al., 1986; Fisher et al., 1992) that could explain the strong influence of the Northeast monsoon on $\text{PO}_4^{3-}$ distribution rather than $\text{NO}_3^-$ distribution at these stations. The addition input of nutrients (with a high concentration of $\text{PO}_4^{3-}$) during the post-monsoon period was brought up from bottom water to surface layer by water mixing due to high turbulence throughout the water column. This additional supply especially for dissolved $\text{PO}_4^{3-}$ was lower down the N:P ratio at surface layer and throughout the water column.

In this study, the seasonal Northeast monsoon has shifted the nutrient condition from P to N limitation condition at St.1, St.2 and St.3 in Pulau Perhentian. In fact, most scientists have put their efforts into determining why this apparent shift from P limitation to N limitation occurs. Some of the more obvious reasons include: the widely-observed more efficient recycling of P; the high losses of fixed N to the atmosphere due to denitrification in coastal waters (Nixon, 1981); and the role of sulfate in recycling P in coastal sediments (Caraco et al., 1989). Based on our present data, we would suggest that this transformation of P limitation to N limitation was more likely to have been influenced by the recycling of P. This included biological uptake by phtoplankton (diatom) at surface water and water mixing throughout the water column.
CONCLUSION
This study was an initial study on nutrient condition at Pulau Perhentian, Terengganu. Our present data has suggested the existence of P limitation condition (N:P ratio >16) during the pre-monsoon period due to active biological uptake by phytoplankton. However, during the post-monsoon period, this nutrient condition was converted to the N limitation condition (N:P ratio <16) due to a huge input of dissolved nutrients, especially dissolved PO$_4^{3-}$ from bottom water resulting from water mixing. However, future comprehensive studies are needed in order to understand the spatial and temporal nutrient condition around Pulau Perhentian, as well as to formulate the long-term strategic plan for Marine Park sustainability.

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Study of Nutrient Condition in Seawater


Sustainable Approach using *Carica papaya* Stem for *in vitro* Propagation of *Clinacanthus nutans*

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**ABSTRACT**

*Clinacanthus nutans*, known to the locals as *Belalai Gajah*, is a medicinal plant widely used by Malaysians in the belief to cure various diseases including cancer. However, the production of *Belalai Gajah* hardly fulfils the market demand since the growing techniques of stem cutting and tissue culture are inefficient and expensive. Therefore, improvements in tissue culture techniques and materials application are required. Hence, this study tested *Carica papaya* (locally known as papaya) stem, an agricultural waste, to enhance the tissue culture of *C. nutans*. The effects of *C. papaya* stem powder and extract on the shoot proliferation of *C. nutans* parameters were investigated. The average number of shoot, leaves and length of leaves were observed. Phytochemicals screening was also conducted. Overall, *C. papaya* stem extract showed positive performance and 1% extract was found to be the optimum concentration to enhance the shoot proliferation. Meanwhile, *C. papaya* stem powder inhibited the shoot proliferation. It was also found that flavonoids, glycosides, steroid and terpenoid contributed in the shoot proliferation. In conclusion, the papaya stem extract is a potential *in-vitro* supplement for tissue culture studies. This study gained insights in sustainable green economy and showed that...
zero waste can be attained by converting local papaya stem waste to valuable product. In addition, sustainable technology in pharmaceutical approach could be addressed through in-vitro low cost large-scale production of Belalai Gajah medicinal plant.

Keywords: Clinacanthus nutans, papaya stem waste, phytochemicals, shoot proliferation, supplement, tissue culture

INTRODUCTION
The Clinacanthus nutans or ‘Belalai Gajah’ plant is useful as herbal medicine in Malaysia. This is due to its active biological and phytochemicals contents (Alam et al., 2016; Bertrand et al., 2014). Belalai Gajah is traditionally used to assist cancer, gout, kidney syndrome, uterine fibroid, skin inflammations and lesion (Ali et al., 2014; Arullappan et al., 2014; Fong et al., 2014; Shahzad et al., 2015; Tu et al., 2014). C. nutans products are commercially available in the market in the forms of cream, lotions, capsule, tablet, herbal tea, concentrated extract. However, manufacturers are not able to fulfil the market demands due to the inefficient stem cuttings growth method. Thus a new growth method needs to be addressed.

Plant tissue culture technique has been reported to have produced high quality plantlets rapidly (Asghari & Ahmadvand, 2018; Chen et al., 2015; Kataria et al., 2013). Plantlets produced through plant tissue culture have high resistance to diseases and stress when transferred to soil (Loyola-Vargas & Ochoa-Alejo, 2012; Hussain et al., 2012; Deepthi & Satheeshkumar, 2017; Muda & Awal, 2017; Rathore et al., 2014; Syaiful et al., 2009). However, this method requires application of expensive synthetic growth hormone, 6-benzylaminopurine (BAP). Thus low-cost agricultural waste should be extensively explored as a substitute to enhance shoot proliferation and reduce cost (Rattana & Sangchanjiradet, 2017; Tay et al., 2016).

In another perspective, various tissue culture studies had reported successful application of agricultural waste of Carica papaya (locally know as papaya) juice (Daud et al., 2011), banana Musa acuminate peel (Daud et al., 2011; Molnár et al., 2011; Swamy et al., 2014), coconut Cocos nucifera husk (Deb & Pongener, 2013; Gnasekaran et al., 2010) leaf litter and sugarcane Saccharum officinarum bagasse (Basirat, 2011) as a substitute of growth hormone.

Hence, agricultural waste is a promising tissue culture supplement because it contains natural phytochemicals (Anjusha & Gangaprasad, 2016; Jamal et al., 2017). Phytochemicals in plants include alkaloids (Aravind et al., 2013), flavonoids (Milind & Gurditta, 2011), saponin, tannin, glycoside, steroid, terpenoid, carotenoids (Aravind et al., 2013), minerals and vitamins (Ward et al., 2017). The phytochemicals play important roles in shoot proliferation, plant defence against pathogens and nutrient absorption.
Previous study on *C. nutans* tissue culture demonstrated that spent mushroom compost (Tay et al., 2016) and corn *Zea mays* stem (Tay et al., 2017) had enhanced shoot proliferation of the plant. For application of *C. papaya* as supplement, it is noted that the studies were only limited to papaya fruit juice. The papaya fruit juice served as a supplement in tissue culture, where it enhanced the production of *Celosia* sp. (Daud et al., 2011) and orchid *Doritaenopsis* (Rahman et al., 2004). Since the application of the *C. papaya* stem powder and extract on *C. nutans* is yet to be addressed, this study focuses on *C. papaya* stem as a potential supplement to induce the tissue culture of *C. nutans*.

The objective of this study was to determine the performance of the in-vitro *C. nutans* shoot proliferation using papaya stem powder and extract. The phytochemicals compounds of the papaya stem were investigated to gauge its ability in enhancing the *C. nutans* shoot proliferation.

**MATERIALS AND METHODS**

*C. papaya* Stem Powder and Extract Preparation

Papaya stems at aged of 2 with size 5 inches long from top to bottom of the main stem were collected from the UiTM Perlis farm. It was dried, ground and sieved through a 710 µm size sieve. The powder was also used to prepare the *C. papaya* stem extract. A water extraction method was conducted to obtain the extract. An approximate 10 g of *C. papaya* stem powder was mixed with 50 ml of distilled water. The mixture was incubated in an incubator shaker (Infors HT Ecoatron) at 200 rpm and 25 ± 2°C for 2 hours. Then, the mixture was centrifuged at 5000 rpm (4458 × g) for 10 minutes to obtain the supernatant. The separated supernatant (extract) was then stored at 4°C.

Preparation of the Culture Medium

Distilled water was mixed with 3% β-D-Fructofuranosyl-α-D-glucopyranoside (sucrose) powder (Sigma Aldrich™), 0.4% Murashige and Skoog (MS) powder (Duchefa Biochemie™), 0.01% 1,2,3,4,5,6-Hexahydroxycyclohexane (myo-inositol) powder (Duchefa Biochemie™), 0.005% of prepared 0.1 µM 6-benzylaminopurine (BAP) (Duchefa Biochemie™) solution. After that, the pH of the MS solution was adjusted to 5.8. The prepared MS solution was heated using a hot plate and 0.3% polysaccharide (gelrite) powder (Duchefa Biochemie™) was added. Then the *C. papaya* stem powder or extract was added followed by polysaccharide powder. The completed mixture (MS solution) was poured into jam jars and the medium were left to cool. The jam jars containing MS solution were autoclaved at 15 lbs psi and 121°C for 15 minutes (Das et al., 2015; Tay et al., 2017).
Surface Sterilisation
The autoclavable apparatus was autoclaved and the non-autoclavable materials were cleaned with 70% ethanol. The nodal segments of the \( C. \) nutans were excised and washed with tap water for five minutes to remove particles that might lead to contamination. All apparatus and materials were put in a laminar flow. Then the nodal segments of \( C. \) nutans were soaked in 70% ethanol for three minutes, followed by 30% sodium hypochlorite (Sigma Aldrich™) for three minutes, 0.1% mercuric chloride (Sigma Aldrich™) and sterile distilled water. Lastly, the nodal segments were air dried before the shoot induction.

Shoot Induction
The nodal segments of \( C. \) nutans were sub-cultured on a fresh MS medium. One nodal segment was placed in each jam jar. The culture was incubated in an incubator at 25 ± 2°C under 16 hours of cool white fluorescent light and 8 hours dark photoperiod.

The nodal segments of \( C. \) nutans from a sterile explants stock were subcultured in three conditions. The first condition only contained an MS medium, which served as a control. The second condition contained the \( C. \) papaya stem powder and the third, had the \( C. \) papaya stem extract.

Then 1% of the (m/v) powder, 2% (m/v) powder, 4% (m/v) powder, 1% (v/v) extract, 2% (v/v) extract and 4% (v/v) extract were added into each of the MS medium (Tay et al., 2017). The average number of shoot and leaves, and the average length of leaves were observed and recorded for 6 consecutive weeks. The samples were duplicated for each condition.

Phytochemicals Screening
The aqueous extract of \( C. \) papaya stem was prepared using an amount of 4 g of \( C. \) papaya stem powder in 100 ml distilled water agitated in an incubator shaker at 200 rpm at 25 ± 2°C for 24 hours. After 15 minutes of evaporation through a rotary evaporator, approximately 2 mL the \( C. \) papaya stem extract was obtained. These steps were repeated seven times. The extract was subjected to different phytochemicals tests including alkaloid test using mercuric iodide reagent (Ikeyi et al., 2013), sodium hydroxide test for flavonoids (Usman et al., 2009), saponins test (Ikeyi et al., 2013), ferric chloride test for tannins (Ikeyi et al., 2013), Keller-Killani test for glycosides (Emmanuel et al., 2014), steroids test (Njoku & Obi, 2009) and terpenoids test (Njoku & Obi, 2009).

Statistical Analysis
The recorded data were analysed using Microsoft Excel 2010. The obtained results were expressed in mean ± standard deviation (n=2). Data then were further analysed using one-way analysis of variance (ANOVA) with 95% level of confidence.
This study was conducted in 2017 at the Faculty of Applied Sciences, UiTM, Arau, Malaysia. To note, University Teknologi MARA, (UiTM), Malaysia, has its branch campuses in all states of Malaysia, including at Arau, the state of Perlis.

RESULT AND DISCUSSION

Effects of the *C. papaya* Stem Powder on Shoot Proliferation of the *C. nutans*

Figures 1(a), (b) and (c) show the inhibition of *C. nutans* shoot proliferation compared to the control. At the end of week 6, the control showed 50% higher number of shoots, number of leaves and length of leaves (p>0.05), compared to the samples treated with the...
powder. An increase of the powder concentration significantly decreased the measured parameters thus inhibiting the number of shoots and length of leaves. However, 2% (m/v) of the powder treatment recorded the lowest inhibition, which is 50% for the number of leaves compared to 1% and 4% (m/v).

It was seen that a 100% inhibition had occurred in the first few weeks in the *C. papaya* stem powder treatment. This inhibition occurred because the *C. papaya* powder had absorbed the nutrients from the surrounding environment and therefore limiting the nutrients for *C. nutans*. This then led to the inhibition of the shoot proliferation. The shoot proliferation occurred at the later weeks as the *C. papaya* stem powder released its nutrients to the surrounding environment due to the concentration gradient. As a result, the plant utilised the nutrients and the shoot proliferation started. Mercy and Jenifer (2014) also reported a similar observation and explanation that the application of *Punica granatum* pomegranate peel powder inhibited the shoot proliferation of the *Vinca rosea*.

It was also observed that the shoot proliferation in the control samples had increased in the early weeks but decreased later. Such condition implied that the senescence stage of the *C. nutans* and the depletion of nutrients and growth hormone in the medium had occurred. Hence, this resulted in the loss of leaves of the *C. nutans*. Zwack and Rashotte (2013) supported this study by explaining that the senescence of leaves occurred due to the depletion of the cytokinin growth hormone in medium.

**Effects of *C. Papaya* Stem Extract on Shoot Proliferation of the *C. nutans***

The effects of the papaya stem extract on the *C. nutans* shoot proliferation are shown in Figures 2(a), (b) and (c). The percentage of shoot proliferation growth, ranging from 32% to 400%, was recorded at the end of week 6. Statistically, when the extract concentration increases, it significantly decreases the measured shoot proliferation parameters (p<0.05).

Only 1% (v/v) of the extract exhibited positive and the most efficient shoot proliferation. This was due to the *C. papaya* stem extract containing high soluble nutrients in aqueous liquid form, which easily absorbed and utilised by the *C. nutans*. Contrary, the shoot proliferation of the *C. nutans* in the 2% and 4% extract were lower than the control in the early weeks. Such observation occurred due to addition of high concentration of nutrients causing excessive growth nutrients and osmotic pressure in the plants, thus turning the plant dry and brown (Al-Khateeb, 2008). Daud et al. (2011) and Swamy et al. (2014) also reported similar results with our finding, where lowest concentration of papaya fruit extract efficiently promoted shoot proliferation of the *Celosia* sp. and *Pogestemon cablin*. 
Comparison between The Effects of C. Papaya Stem Powder and Extract on Shoot Proliferation of the C. nutans

Table 1 shows that 1% (v/v) of C. papaya stem extract was most effective to promote the C. nutans shoot proliferation. Comparison of the C. papaya stem powder and extract on the C. nutans shoot proliferation over time is illustrated in Figures 3(a), (b) and (c). Statistical analysis shows there was significant difference for all treatments (p < 0.05).
All parameters showed a similar pattern where in earlier weeks, the *C. nutans* shoot proliferation in the medium with the extract and control had increased. Meanwhile, there was no growth in the medium with the *C. papaya* stem powder. This was due to the soluble nutrients in the liquid extract that enhanced the shoot proliferation. However, the solid *C. papaya* stem powder absorbed the nutrients required for the *C. nutans* shoot proliferation, thus inhibiting its growth.

Table 2 shows the comparison of plant-based extract on various plant growth performances in tissue culture. The plant extracts were the *Carica papaya*, banana *Musa acuminata*, corn *Zea mays*, tomato *Solanum lycopersium*, carrot *Daucus carota* and coconut *Cocos nucifera*. A low concentrations of 5% to 30% of plant extracts was optimum for plants growth. The optimum concentration of water-based extracts was dependent on the sources of extracts and types of plant (Daud et al., 2011; Swamy et al., 2014; Gnasekaran et al., 2010; Molnár et al., 2011; Chukwuka et al., 2014; Deb & Pongener, 2013).

Table 1
*The most effective treatment using C. papaya stem for shoot proliferation of the C. nutans*

<table>
<thead>
<tr>
<th>Parameters of Shoot Proliferation</th>
<th>Optimum Condition</th>
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<tbody>
<tr>
<td>Average Number of Shoots</td>
<td>1% (v/v) extract</td>
</tr>
<tr>
<td>Average Number of Leaves</td>
<td>1% (v/v) extract</td>
</tr>
<tr>
<td>Average Length of Leaves</td>
<td>1% (v/v) extract</td>
</tr>
</tbody>
</table>

Table 2
*Comparison of plant-based extracts on various plant growth performances in tissue culture*

<table>
<thead>
<tr>
<th>Extract source</th>
<th>Type of extract</th>
<th>Optimum concentration</th>
<th>Plant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papaya <em>Carica papaya</em> fruit</td>
<td>Juice/water extract</td>
<td>2% (2%, 3%, 5%, 7%)</td>
<td><em>Celosia sp.</em></td>
<td>Daud et al., 2011</td>
</tr>
<tr>
<td>Banana <em>Musa acuminata</em></td>
<td>Juice/water extract</td>
<td>5% (2%, 3%, 5%, 7%)</td>
<td><em>Celosia sp.</em></td>
<td>Daud et al., 2011</td>
</tr>
<tr>
<td></td>
<td>MS liquid extract</td>
<td>10% (5%, 10%, 20%)</td>
<td><em>Pogostemon cablin</em></td>
<td>Swamy et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Water extract</td>
<td>5% (0%, 5%, 10%, 20%, 30%)</td>
<td><em>Phalenopsis violacea</em></td>
<td>Gnasekaran et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Water extract</td>
<td>Not mentioned</td>
<td><em>Pisum sativum, Nicotiana tabacum</em></td>
<td>Molnár et al., 2011</td>
</tr>
<tr>
<td>Tithonia diversifolia</td>
<td>Water extract</td>
<td>50% (50%, 100%)</td>
<td><em>Corn Zea mays</em></td>
<td>Chukwuka et al., 2014</td>
</tr>
<tr>
<td>Tomato <em>Solanum lycopersium</em></td>
<td>Water extract</td>
<td>5% (2%, 3%, 5% and 7%)</td>
<td><em>Celosia sp.</em></td>
<td>Daud et al., 2011</td>
</tr>
<tr>
<td></td>
<td>Extract with MS liquid</td>
<td>10% (5%, 10%, 20%)</td>
<td><em>Pogostemon cablin</em></td>
<td>Swamy et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Water extract</td>
<td>10% (0%, 5%, 10%, 20%, 30%)</td>
<td><em>Phalenopsis violacea</em></td>
<td>Gnasekaran et al., 2010</td>
</tr>
</tbody>
</table>
Table 2 (continue)

<table>
<thead>
<tr>
<th>Extract source</th>
<th>Type of extract</th>
<th>Optimum concentration</th>
<th>Plant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot <em>Daucus carota</em></td>
<td>Extract with MS liquid</td>
<td>10% (5%, 10%, 20%)</td>
<td><em>Pogostemon cablin</em></td>
<td>Swamy et al., 2014</td>
</tr>
<tr>
<td>Coconut <em>Cocos nucifera</em></td>
<td>Water extract</td>
<td>7% (2%, 3%, 5% and 7%)</td>
<td><em>Celosia sp.</em></td>
<td>Daud et al., 2011</td>
</tr>
<tr>
<td></td>
<td>Water extract</td>
<td>10% (0%, 5%, 10%, 20%, 30%)</td>
<td><em>Phalenopsis violacea</em></td>
<td>Gnasekaran et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td><em>Cymbidium iridioides</em></td>
<td>Deb and Pongener, 2013</td>
</tr>
<tr>
<td></td>
<td>Water extract</td>
<td>Not mentioned</td>
<td><em>Pisum sativum, Nicotiana tabacum</em></td>
<td>Molnár et al., 2011</td>
</tr>
<tr>
<td>Papaya <em>C. papaya</em></td>
<td>Water extract</td>
<td>1% (1%, 2% and 4%)</td>
<td><em>C. nutans</em></td>
<td>This study</td>
</tr>
</tbody>
</table>

Figure 3. Comparison of the effects of *C. papaya* stem powder and extract on shoot proliferation of the *C. nutans* (a) average number of shoots, (b) average number of leaves, and (c) average length of leaves.
Phytochemicals Screening of *C. papaya* Stem

The phytochemicals screening of the *C. papaya* stem showed a presence of flavonoids, glycoside, steroid and terpenoid (Table 3). Researchers explained that the flavonoids functioned as plant defence system against ecological and physiological pressures such as pathogen and insect attack (Khoddami et al., 2013). Grabkowska et al. (2014) reported that the glycoside was able to enhance *Harpagophytum procumbens* shoot proliferation. Meanwhile, a study conducted by Kandelinskaya et al. (2007) recorded that steroid had increased the protein content in various types of lupine plants and resulted in better light signaling. Hence, presence of steroid in the *C. papaya* stem increased the protein content in the *C. nutans* and thus promoting its shoot proliferation. An application of terpenoid for *in vitro* culture had also enhanced the multiplication of shoots and root (Khan et al., 2016). The root multiplication had increased the nutrients absorption and produced higher number of shoots. The identified phytochemicals in *C. papaya* stem are important where phytochemicals contributed to *C. nutans* shoot proliferation.

Table 3
*The phytochemicals screening result of the C. papaya stem*

<table>
<thead>
<tr>
<th>Phytochemicals parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
</tr>
</tbody>
</table>

CONCLUSION

This study concluded that agricultural waste, *C. papaya* stem extract has potential to be a sustainable supplement for *C. nutans* tissue culture. *C. papaya* stem is an agriculture waste and easily procured, thus the application is cost-effective for commercial utilisation. The 1% extract of the *C. papaya* stem resulted in the highest average number of shoot, leaves and average length of leaves. The one-way ANOVA demonstrated significant difference (p<0.05) among all conditions of the powder and extract treatments. Meanwhile, the *C. papaya* stem powder inhibited the *C. nutans* shoot proliferation up to the fourth week. It contains flavonoids, glycoside, steroid and terpenoid, showing that phytochemicals support *C. nutans* shoot proliferation. This study had also provided vital and fundamental information that supports future application of *papaya* stem extract in the *in-vivo* growth of *C. nutans*. In this case, it is concluded that the *C. papaya* stem extract can be a sustainable supplement for *C. nutans* tissue culture. Therefore, this study not only shed light into waste-to-product and zero waste concepts, but also contributed to the green economy. The papaya stem, an agriculture waste is easily procured in many tropical countries, hence can
be viable for commercial production. Through application of agricultural waste as for tissue culture supplement, the waste is recycled. At the same time, the demand for plant-based drug is fulfilled and environmental pollution is minimised.

**ACKNOWLEDGEMENTS**

This work was supported and funded by the Universiti Teknologi MARA grant, 600-RMI/IRAGS 5/3(13/2015). The authors would like to thank Associate Professor Dr. Megawati Omar for language editing of this paper.

**REFERENCES**


Case Study

Characterization of Landfill Leachates and Its Impact to Groundwater and River Water Quality: A Case Study in Beris Lalang Waste Dumpsite, Kelantan

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ABSTRACT

Solid waste management in developing countries including Malaysia is dominated by waste dumpsites which have a high possibility of transporting organic and inorganic pollutants to the underlying groundwater and surface water within the surrounding area. The objective of this study is to characterise the landfill leachates and its surrounding groundwater and river water quality and metals concentrations, namely arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) from Beris Lalang, Kelantan. Nine sampling points were collected within the dumpsite for analysis of in-situ: dissolved oxygen (DO), pH, total dissolved solids (TDS), conductivity, turbidity and ex-situ; Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Suspended Solids (SS) and the results were compared with permissible limits. As and Pb in groundwater samples exceeded the standard of Ministry of Health. Cr, Cu and Pb in leachates exceeded Environmental Quality Act (EQA, 1974)’s standard, whereas Ni, Pb and Zn in surface water of Gali River exceeded the limit of class III National Water Quality Standard (NWQS). Exceeding metals concentration in leachates and its surrounding groundwater and river water relative to their respective standards points out toward potential leachate migration to the waterbodies within the surrounding vicinity of Beris...
Lalang dumpsite. This study provides the initial baseline data and preliminary monitoring assessments as a first step towards improving water security and waste management in Kelantan.

**Keywords:** Dumpsites, groundwater, Kelantan, leachates, metals, surface water, waste

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**INTRODUCTION**

Solid waste management issues are critical in developing countries due to illegal dumping, an excessive amount of waste generated, and the lack of waste collection services provided (Aiman et al., 2016). Consequently, pollutants accumulate, leach or flow through the surrounding dumpsite before affecting the surrounding environment and health. Thus, majority of solid waste management practised in tropical countries, such as Kenya, Ghana, Nigeria (Oyelami et al., 2013) and Pakistan (Ali et al., 2014; Alam et al., 2017), were carried out without adequate lining materials and leachate treatment plants, which induced contamination through the soil, subsequently leached to the groundwater and eventually to the nearest river.

In Malaysia, only 13 out of 300 existing landfills are operating sanitary landfills. Particularly, in Kelantan, the existing active landfills are open waste dumpsites (Jabatan Pengurusan Sisa Pepejal Negara [JPSPN], 2015). Open waste dumpsites are piled with unattended wastes exposed to physical, chemical and biological processes simultaneously accelerating the waste decomposition together with the generation of leachate and landfill gases (Oketola & Akpotu, 2015). The situation further aggravates due to the high temperature and humid conditions of the tropical countries such as Malaysia (Suleman et al., 2015). Subsequently, these wastes gradually degrade and accumulate substantial amounts of dissolved organics, xenobiotic organic compounds, inorganic salts, ammonia, metals and other toxicants (Kanmani & Gandhimathi, 2013).

Household products of electronic waste, painting waste and used batteries contain metals such as Pb, Cd, mercury, As, Cu and Zn. These products are among the waste discovered in waste dumpsites, which cannot be biodegraded. Thus, solid waste disposals (open dumps, landfills, sanitary landfills or incinerators) represent a significant source of metals into the environment (Kanmani & Gandhimathi, 2013).

Dumpsite leachates contain mixtures of both dissolved and suspended materials based on their method of burying, climate, rainfall, moisture content, the geological nature of the site, the age of the landfill and the waste composition (Arunbabu et al., 2017). Therefore, the quality of leachates is site-specific. Studies about leachates in waste dumpsites (sanitary and non-sanitary landfill, open waste dumpsites) are not new (Ishak et al., 2016; Kamaruddin et al., 2017; Moody & Townsend, 2017). The growing interest in leachate characterisation emerges from the potential migration impacts of leachates to the surrounding environmental receptors may increase the likelihood of hydrogeological and water pollution. Hence, this...
study is conducted to evaluate the environmental contamination from the leachates and its potential effect to the groundwater and surface water nearby Beris Lalang dumpsite area of 8 years. This baseline monitoring may assist in analysing the extent of risk to water security and planning for future sanitary landfill in Kelantan.

MATERIALS AND METHODS

Study Site and Field Sampling

The sampling was collected at Beris Lalang, the largest active waste dumpsite covering Kota Bharu district in Kelantan. Beris Lalang is a peat swamp area surrounded by palm oil plantations and consists of a small stream leading to Gali River, which eventually flows to Kandis Beach, Bachok. It receives approximately 350 tonnes/day of daily waste (Kamaruddin et al., 2016). Nine locations were selected as the sampling points within Beris Lalang (Figure 1). Two of which were groundwater samples collected from the surface water of dug grounds (GW1 and GW2), another five sampling points representing leachate samples (L1-L5) and the other two sampling points were surface waters (SW1 and SW2). SW1 was located from a stream flowing out of the leachate collection pond before it is released to Gali River whereas the second surface water samples were collected from Gali River within 500 m from SW1 (SW2). Table 1 sets out the coordinate of sampling locations.

![Figure 1. Nine sampling points of environmental constituents sampling at Beris Lalang dumpsite, Kelantan](image)
Table 1
Coordinates of sampling locations from Beris Lalang dumpsite, Kelantan

<table>
<thead>
<tr>
<th>Station</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GW1</td>
<td>N 05°55.780'</td>
<td>E102°24.729'</td>
<td>Groundwater used for ablution by landfill workers and waste collectors</td>
</tr>
<tr>
<td>GW2</td>
<td>N 05°55.796'</td>
<td>E102°24.741'</td>
<td>Groundwater occasionally used for a dip by locals</td>
</tr>
<tr>
<td>L1</td>
<td>N 05°55.576'</td>
<td>E102°24.915'</td>
<td>Fresh leachates near lorry unloading the waste</td>
</tr>
<tr>
<td>L2</td>
<td>N 05°55.615'</td>
<td>E102°24.853'</td>
<td>The passageway of lorry unloading waste</td>
</tr>
<tr>
<td>L3</td>
<td>N 05°55.740'</td>
<td>E102°24.673'</td>
<td>Near MPKB’s landfill supervisor office</td>
</tr>
<tr>
<td>L4</td>
<td>N 05°55.566'</td>
<td>E102°24.620'</td>
<td>Between the dumpsite’s entrance and leachate collection pond</td>
</tr>
<tr>
<td>L5</td>
<td>N 05°55.654'</td>
<td>E102°24.640'</td>
<td>Exit-entry before flowing to Gali River, a small stream</td>
</tr>
<tr>
<td>SW1</td>
<td>N 05°55.571'</td>
<td>E102°24.598'</td>
<td>After leachate discharge to Gali River</td>
</tr>
<tr>
<td>SW2</td>
<td>N 05°55.649'</td>
<td>E102°24.635'</td>
<td>Gali River opposite L4</td>
</tr>
</tbody>
</table>

Beris Lalang dumpsite has a size of 30.5 hectares. It is located 400 m away from the nearest house, 1 km away from two schools, namely Sekolah Kebangsaan Beris Lalang and Sekolah Menengah Ugama Darul Iman and 7 km from Tok Bali, a popular tourist spot. The sampling of groundwater, leachates and surface water within the vicinity of the dumpsite were conducted to assess the degree to which the environmental constituents were contaminated in the study area. In this study, surface water samples from groundwater and river water samples were collected about 10cm below the surface water by employing a water sampler or a pail attached to a rope. Within 24 hours prior to samples collection, the pre-washed HDPE bottles were soaked with 10% concentrated nitric acid.

On-site measurements of water pH, DO, temperature, TDS and Electrical Conductivity (EC) were performed by using YSI 556 MPS (Multi-Probe System) Multiparameter. On the other hand, turbidity was measured by utilised a HACH Portable Turbidimeter Model 2100P.

Laboratory Analysis
Biochemical Oxygen Demand (BOD₅) and Chemical Oxygen Demand (COD) were analysed according to the standard method of APHA at Occupational Safety and Health Laboratory, Universiti Sains Malaysia Health Campus. On the other hand, Ammoniacal Nitrogen (AN) was analysed by utilising HACH DR 2010 spectrophotometer. Furthermore, Suspended solids (SS) was analysed by using the USEPA standard method. The water samples were filtered with 0.45µm membrane filter for analysis of metals. The samples were subsequently transferred into 15 mL vials and a total of 7 metals were analysed by employing Inductively Coupled Plasma Mass Spectrometry (ICPMS) model Agilent 7700. Leachate samples were filtered twice by using Whatman 0.45-mm filter paper. Raw leachate...
samples were diluted 25 times before transferred into 50 ml polypropylene tube. Agilent multi-element standard solutions were adopted to prepare the calibration curve and the calibration curves with $R^2 > 0.999$ were accepted for concentration calculation. Precision analysis from the triplicate measurements of each sample for metals concentration indicates a % relative standard deviation of less than 10% per cent recovery for all metals that were within the range of 92-104%.

**Statistical Analysis**

Data were analysed by using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 7 (GraphPad Software Inc, San Diego, USA). The statistical analyses were adopted to reveal the significant value of each element in the 95 % confidence level ($a=0.05$). Data were presented as average and standard deviation for water quality parameters and average and standard error for the metals. Statistical differences between the means of groundwater and river water samples were compared by utilising t-test, whereas using one-way ANOVA for leachate samples at p-value <0.05. National Guidelines for Raw Drinking Water Quality, Ministry of Health (MOH, 2011) was adopted for groundwater samples whereas Class III NWQS was used for river water to monitor the guidelines of selected water quality and metals analysis in Beris Lalang waste dumpsite. Leachates was compared with Second Schedule (Acceptable Conditions for Discharge of Leachate) of the Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009, Environmental Quality Act 1974 [Act 127] (Department of Environment [DOE], 2009).

**RESULTS AND DISCUSSION**

**Physicochemical Characteristics and Metal Concentrations of groundwater, River Water and Leachates from Beris Lalang Waste Dumpsites**

Tables 2-4 indicate the water quality results of groundwater, river water and leachate at Beris Lalang dumpsite. In the groundwater, DO was low in GW1 (1.41 ±0.16) and 0.9 ±0.05 in GW2. DO is generally low in groundwater samples as reported from a study conducted by Rahim et al. (2010). The parameters were compared with groundwater standards established by MOH (2011) and DOE (2011). The groundwater qualities were within the standards for all parameters measured except for pH in GW2 (Table 2). pH in GW2 was discovered to be below the recommended standard and was very acidic (3.88±0.02). The acidic condition of GW2 may expose the workers and scavengers of Beris Lalang waste dumpsite to the possibility of skin irritation if they were in frequent contact with the water. The acidification might have been caused by oxidation of sulfide minerals within the original peat conditions of the dug grounds (Appleyard et al., 2004). Another implication of using
acidic groundwater is the corrosion of plumbing materials if being used (Ugwoha & Emete, 2015). Low pH may also cause metals, such as cadmium, to dissolve and encourage high iron concentrations (MacDonald et al., 2002).

All groundwater samples were significantly different between GW1 and GW2 (t-test, p<0.05). EC values were lower in GW1 (0.049± 0.005 mS/cm) compared to GW2 (0.373± 0.010 mS/cm), indicating that GW1 contained less soluble salts than GW2. EC is a measurement of the ability of water to conduct electric current which is largely influenced by dissolved salts such as sodium chloride and potassium chloride (Isah et al., 2015). Based on observations, the colour of both groundwaters was also different. For instance, GW2 had a turquoise blue-like colour, whereas GW1 was with a turbid colour. This might explain the differences in the TDS and turbidity readings. Higher BOD₅, COD and AN was reported in Yusoff et al. (2013), reflecting site-specific influences and affecting the groundwater samples (Table 2). The BOD₅ and COD values of the groundwater samples in GW1 are 10.88 ± .076 and 8.33± 1.53, respectively, indicate the presence of insignificant biologically and chemically oxidizable organic contaminants in the groundwater. This implies that the groundwater is safe to be used for ablation by the workers and scavengers in that area. The values of BOD₅ and COD in both GW1 and GW2 indicate that there is no organic contamination from the leachate to the groundwater surrounding the site. This is supported by Hassan and Ramadan (2005), who assessed the effects of sanitary landfill leachate on the groundwater and discovered that no organic contamination of piezometer wells around the active cells of the landfill. Nevertheless, GW1 is more suitable to be used with skin contact considering the acidity of GW2.

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groundwater</th>
<th>Standard</th>
<th>Other studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>GW1</td>
<td>GW2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO</td>
<td>1.41±0.16</td>
<td>0.9±0.05</td>
<td>0.11-0.82ᵇ</td>
</tr>
<tr>
<td>pH</td>
<td>6.77±0.09</td>
<td>3.88±0.02</td>
<td>5.5-9.0</td>
</tr>
<tr>
<td>EC (mS/cm)</td>
<td>0.049± 0.005</td>
<td>0.373± 0.010</td>
<td>*0.3</td>
</tr>
<tr>
<td>Temp (℃)</td>
<td>28.44± 0.036</td>
<td>29.71±0.145</td>
<td>-</td>
</tr>
<tr>
<td>TDS</td>
<td>0.029±0.004</td>
<td>0.224±0.004</td>
<td>1500</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>38.27±5.52</td>
<td>4.00±1.20</td>
<td>1000</td>
</tr>
<tr>
<td>BOD₅</td>
<td>10.88± 0.076</td>
<td>2.58± 0.06</td>
<td>6</td>
</tr>
<tr>
<td>COD</td>
<td>8.33± 1.53</td>
<td>ND</td>
<td>10</td>
</tr>
<tr>
<td>AN</td>
<td>0.29± 0.006</td>
<td>1.34±0.006</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Note. ND-not detected; Standard refers to Ministry of Health Malaysia (MOH, 2011) except the ones marked with asterisk* refers to World Health Organization (WHO, 2011); ᵃChakraborty and Kumar (2016); ᵃRahim et al. (2010); ᵃAkinbile (2012); ᵃYusoff et al. (2013)
Cr, Zn and Pb are significantly different between GW1 and GW2 (t-test, p<0.05). Zn has the highest concentrations for GW2 relative to other metals in the groundwater samples of the dug grounds (Figure 2). As and Pb were discovered to exceed the permissible limits of MOH (2011) in both GW1 and GW2. As is categorized as poisonous despite it is in small quantity. Therefore, the usage of the groundwater by waste workers in Beris Lalang may increase the exposure to health issues related to respiratory illnesses, lung cancer and cardiopulmonary associated with As contamination (Farooqi et al., 2007; Muhammad & Zhonghua, 2014). Apart from that, Pb is toxic to human and could lead to health implications such as mental deficiency to the waste collectors and waste workers at the dumpsite (Al Sabahi et al., 2009). Pb is also carcinogenic, which may increase the potential of having cancer (Rousseau et al., 2007).

Out of all leachates, leachates in the L1 exhibit the highest concentration of EC, SS, turbidity, BOD₅ and COD (Table 2). The pH of L1 was also very acidic, suggesting young leachate with a pH of less than 6.5 (Foo & Hameed, 2009). Leachate collected from L1 was gathered near the new wastes dumped by the disposal lorries from Kota Bharu Municipal Councils, Bachok Municipal Councils and Pasir Puteh Municipal Councils and were considered reflecting fresh leachates composition. The wide range of pH found in each study corresponds to the different stages of waste stabilization and evolution, from the transition aerobic, acid, methane and stabilization phase (Kamaruddin et al., 2017; Mukherjee et al., 2015). Typical leachate samples are reported to have a pH range between 4.5 and 9 (Kawai et al., 2012; Muhammad Umar et al., 2010). Low pH in the early phases of the landfill is associated with high concentrations of volatile fatty acids during the solubilisation phase of the organic acids (Kamaruddin et al., 2017; Muhammad Umar et al., 2010).

Figure 2. Metals concentration in groundwater samples from dug grounds within the vicinity of the waste dumpsite in Beris Lalang, Kelantan reported as mean ±SEM, n=3. * denotes exceeding MOH (2011), + significant difference (t-test, p<0.05)
Umar et al., 2010). Nevertheless, pH in other leachates collected at L2-L5 indicates pH ranges between 7.52 and 8.41, reflecting old and stabilised leachates associated with the conversion of organic acids into gaseous phase (Kamaruddin et al., 2017; Muhammad Umar et al., 2010). Other studies reported in the literature has shown pH ranges between 7.0 and 7.8 (El-Salam & Abu Zuid, 2015), 6.7 (Bahaa-Eldin et al., 2008) and an average of 7.6 and 8 (Zin et al., 2013).

The black colour and high EC and SS in L1 also suggested the presence of high suspended matters and high dissolved organic matters in the waste stream (Ishak et al., 2016). The black colouration of leachate was associated with the oxidation of ferrous to a ferric form and the formation of ferric hydroxide colloids and complexes with humic substances (Chu et al., 1994; Ishak et al., 2016). The results from this study were also compared the leachate characteristics from other landfills or waste dumpsites (Table 3). The data were within the range of other reported studies and some parameters were discovered to be higher than the others, specifically for L1. On the other hand, the findings from other studies indicated that the leachates characteristics are site-specific and based on the nature of the waste and source location (Bahaa-Eldin et al., 2008; Ishak et al., 2016; Rahim et al., 2010)

Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Leachates</th>
<th>Standard</th>
<th>Other studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
<td>L2</td>
<td>L3</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>0.70 ± 0.15</td>
<td>0.76 ± 0.078</td>
<td>1.59 ± 0.017</td>
</tr>
<tr>
<td>pH</td>
<td>2.03 ± 0.02</td>
<td>8.31 ± 0.076</td>
<td>8.41 ± 0.012</td>
</tr>
<tr>
<td>EC (mS/cm)</td>
<td>22.56 ± 0.83</td>
<td>10.92 ± 0.096</td>
<td>7.16 ± 0.05</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>32.30 ± 0.16</td>
<td>32.94 ± 0.09</td>
<td>33.87 ± 0.71</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>13.48 ± 0.14</td>
<td>6.13 ± 0.064</td>
<td>3.91 ± 0.05</td>
</tr>
<tr>
<td>SS (mg/L)</td>
<td>4013 ± 0.18</td>
<td>381 ± 0.125</td>
<td>240 ± 1.25</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>4505 ± 29.44</td>
<td>1483 ± 0.28</td>
<td>416 ± 5.55</td>
</tr>
<tr>
<td>BOD5</td>
<td>1965 ± 2.28</td>
<td>798 ± 0.86</td>
<td>202 ± 0.71</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>1640 ± 11.14</td>
<td>328 ± 19</td>
<td>163 ± 10</td>
</tr>
</tbody>
</table>

Note: Standard used: Second schedule (Acceptable Conditions for Discharge of Leachate) of the Environmental Quality Act 1974 [Act 127] (EQA, 1974); Chakraborty & Kumar (2016); Rahim et al. (2010); Matejczyk et al. (2011); Ishak et al. (2016); Oketola & Akpotu (2015).
Cu concentrations were the highest at L1, followed by Zn and Pb (Figure 3). The presence of higher Cu, Zn and Pb in L1 could be related to the low pH condition in L1, which has higher metal solubility caused by the production of organic acids during the acetogenic phase (Kamaruddin et al., 2017; Muhammad Umar et al., 2010). Cr, Cu and Pb concentrations at L1 have exceeded the standard of EQA (1974). Pb is carcinogenic whereas Cu and Cr are non-biodegradable, which may consequently be accumulated in the food chain, causing ecotoxicological consequences (Kjeldsen et al., 2002; Langston 1990). Metals concentrations (Cr, Ni, Cu, Zn, As, Cd and Pb) were significantly different based on the sampling locations (one way ANOVA, p<0.05). This indicates that the variations of metals contaminations are strongly affected by locations, especially leachate samples collected from the locations nearest to the active activity of unloading waste conducted by the lorries of the municipal council and scavenging of wastes by waste collectors.

Surface water was compared to class III NWQS. Table 4 indicates the physical-chemical analysis of surface water after the leachate collection pond that flows into Gali River. The data showed that most parameters for surface water from the Gali River were higher (worse) than the Class III NWQS standard. Hence, it can be categorized as Class IV, indicating the water can be utilised only for irrigation (DOE, 2012).
Ni was discovered to be the highest in the surface water of Gali River, followed by Zn and Pb concentrations (Figure 4). All metals were significantly different from one another in the river water except Cu (Figure 4, t-test; p<0.05). Comparison with NWQS showed

Table 4
The physical-chemical analysis of surface water after the leachate collection pond that flows into Gali River

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Surface river water</th>
<th>Standard</th>
<th>Other studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SW1</td>
<td>SW2</td>
<td></td>
</tr>
<tr>
<td>DO</td>
<td>1.15±0.06</td>
<td>1.23±0.06</td>
<td>3-5</td>
</tr>
<tr>
<td>pH</td>
<td>6.71 ±0.09</td>
<td>3.93±0.06</td>
<td>5-9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC (mS/cm)</td>
<td>1.24± 0.11</td>
<td>1.21± 0.04</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>32.47 ±0.11</td>
<td>29.86±0.04</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDS</td>
<td>0.89±0.06</td>
<td>0.73±0.03</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>119.33±2.52</td>
<td>111±1</td>
<td>50</td>
</tr>
<tr>
<td>BOD₅</td>
<td>20.52 ± 0.09</td>
<td>10.08 ± 0.06</td>
<td>6</td>
</tr>
<tr>
<td>COD</td>
<td>50 ± 5</td>
<td>30 ± 5.56</td>
<td>50</td>
</tr>
<tr>
<td>AN</td>
<td>0.29±0.006</td>
<td>1.34±0.006</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Note: Standard used: Class III National Water Quality Standard for Malaysia (DOE, 2012); aIshak et al. (2016); bYusoff et al. (2013)

Figure 4. Metals concentration in surface water of Gali River within the vicinity of Beris Lalang waste dumpsite. Data are reported as mean ±SEM, n=3.SW1-surface water 1, SW2-surface water 2; *denotes exceeding class III NWQS, + significant differences between sampling stations (t-test, p<0.05)
that Ni, Pb and Zn exceeded class III of NWQS. Thus, the surface water could be classified under class IV, which is only suitable for irrigation. These exceeding metals concentration could be the consequences of potential leachate migration from the leachate collection pond that flows to the river.

CONCLUSION

Based on the baseline monitoring conducted, the pH levels detected at the groundwater of GW2 posed a risk of skin condition to the waste collectors who occasionally used the groundwater to freshen up after working under the sun during a hot afternoon. As and Pb concentrations in groundwater also exceeded the standard, exposing the users to the risk of carcinogenic and health effects. Leachate samples exceeded EQA (1974) for toxic metals, such as Pb and Cr, which increased the risk of health implications, such as mental deficiency. The surface water of Gali River contains exceeding levels of Ni and Zn compared to class III NWQS. Thus, the water is inappropriate for any recreational or fishing activities. This shows the potential migration of leachates to the surrounding environmental constituents considering the exceeding metals concentration in the groundwater and surface water samples. Hence, full characterisation of the waste dumpsite is crucially required to evaluate the potential environmental risks and to identify suitable remediation options.

ACKNOWLEDGEMENTS

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REFERENCES


Design and Fabrication of an Alarm System to Prevent Tractor Overturning

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ABSTRACT

Serious agriculture-related accidents are increasing and tractor overturning is one of the most important factors leading to death in farms in Iran. In fact, the interplays between tractor operator and environment cause such damages. There are several reasons involved in tractors overturn that the recent method in tractor design is nearly to improve with using the electronics instrumentation that gives the operator key guides related to the tractor’s stability as it is operated. In this study a new system was developed. To measure a steep slope land electromagnetic gauge was constructed. The working principles of the slope gauge, based on the magnetic field were changed by changing the slope of the ground. The embedded inclinometer within the pendulum inclinometers with their period are used to altering the magnetic field. The output of inclinometer analog and the voltage range was (47/2-32/0) V. This information was then processed by a microprocessor, and an assessment of rollover potential was reported to the display device.

Keywords: Accident prevention, agricultural tractors, tractor overturn, safety

INTRODUCTION

According to the statistics from the National Safety Council (1983), the deaths caused by tractors overturn, are estimated around 49% (about 300 deaths). The main goal of this research is to know when and why tractors overturn. So if we can predict the possible over turn, a device or procedure can avoid it; otherwise we can control or cut off the factors that lead to overturn.

Tractors are involved in more than 45% of the farm victims and 13.7% of all confinements. According to Javadi and Rostami (2007), the widest percentage of vehicles injuries was reported to be roll overs and run overs and also tractor reversal.
was the prominent cause of agriculture machine-related deaths in the US (Sanderson et al., 2006). However, it is proven that the losses of rollover protective structure (ROPS) and seat belt play major roles in these fatalities.

A research in Finland proved that running around or walking around in the field are two important factors involved in tractors overturning (Rissanen & Taattola, 2003). In addition construction work and animal husbandry and forest work are the other factors that lead to deaths in the farms.

Two major etiologies for tractor overturn are habitual and unique. In habitual one, the operator makes the same mistakes repeatedly without knowing; then with a slight change in the land and position like having high speed or sharper turning radius an accident may happen. But if a driver knows and realizes about the danger of uphill and how to control it, the outcome may change. Thus learning is a useful Factor. However, driving badly in a steep slope or with wrong shutting the loads, the tractor becomes unstable and with a little shake or if the clutch works faster, then the tractor gets closer to reversal (Sommer et al., 2006).

Consequently minor terrain changes (erosion, rodent burrow), slightly faster ground speed or sharper turning radius can now lead to overturn. In particular, a sudden uphill turn to avoid an unforeseen obstacle on a marginally safe but familiar slope is a potential habituated overturn that could be avoided by learning that this slope is truly dangerous. Habituated rear overturns may be caused by repeatedly climbing very steep slopes or by repeated improper hitching of loads that create operating conditions very near instability. Consequently, if an attached implement encounters an obstacle or if the clutch is engaged faster than usual, tractor operation that was previously on the borderline of instability can now become a fatal incident.

On the other hand, unique overturn is very hard to forecast and usually happens when farmers try a new operation in the farm such as loading a large hay bale. Then for this type, operator education is more efficient than on-the-job learning by disabling the electronic clutch or releasing the throttle (gas valve); thus preventing the tractor from overturning doesn’t seem impossible. Actually informed operator intervention is the most reliable way to safe guide the tractor.

Mechanical and operator intermediation while active intervention to prevent side overturns would require massive lateral actuators for outriggers or counterweights, electronic clutch deactivation and/or throttle release holds promise to prevent both rear overturn. Operator intervention addresses the root cause of habituated overturn by helping tractor operators learn to avoid hazard condition. To this end, one must be able to monitor tractor stability and detect how close the tractor is to an unstable condition or even to predict that an unseen future event might possibly cause an overturn. Then, the operator can predict and prevent overturn. Informed operator intervention is the most effective way to prevent tractor overturn events.
According to Nichol et al. (2005), studies and researchers claimed that by using cheap microelectronic machine system (MEMS) sensors and two axes accelerometer, they could manage and control tractors condition. In addition, they had designed a colour (LCD) visual display that helped the operator to be aware of the stability condition of the tractor.

A study has shown that a tractor may arrive in position in 3/4 of a second, but it takes more time for the operator to react and brake. However they are a lot of tractors operating condition in which the responding time is even less than 3/4 of a second to observe and react to the possible overturn (Agriculture Safety, Deere and Company, Inc., 2007). Imagine a tractor in a deep hole or travelling up a steep slope, in that case the space between tractors CG1 and back constant baseline will achieve the “point of no return”. Figure 1 discusses this situation.

![Figure 1](image)

*Figure 1. The "point of no return" during a rear turnover may be reached in 3/4 of a second. Source: Agricultural Safety, Deere and Company, Inc.*

In this paper the new inclinometer was developed to measure the slope of land and a new dynamometer was fabricated in order to measure the draft forces, because draft forces in a tractor were critical when a tractor was pulling an agricultural implement that might cause to tractor overturning. In this paper in order to predict side and overturning, two inclinometers were used. In order to record and analyse the input data, Micro controller AT Mega 32 and a LCD were used. This paper discusses the effective ways to warn tractor operators about possible hazards.

**MATERIALS AND METHODS**

In this study a 4WD Mitsubishi tractor with 25 hp was used. This tractor was a case study to this research. In order to sense the draft forces and slope of land, a micro electronic circuit was designed. Following diagram was used to sense the output of inclinometer and dynamometer. Because the outputs of these sensors were very low, two amplifiers were used. Microcontroller AT Mega 32 was used to analyse outputs of the sensors. Finally a
monitor was used in order to show the slope and the draft force to the driver. With this monitor, a driver could see the critical operation of tractor and decided correct and safe operation (Figure 2).

![Diagram of the sensing device]

**Figure 2. Block diagram of the sensing device**

**Center of Gravity**

The center of gravity is a place where the total weights of tractor are located. Its position depends on the various types of tractor. In addition, we need to design the position of the center of gravity for analyzing the chassis of the tractor. Its common location is near to the rear axle (Point G in figure 3) (Macmillan, 2002).

**Longitudinal Location.** By measuring the weight of front \(W_f\) and rear \(W_r\) wheel, we are able to locate the center of gravity in linear (X) Direction. In the force equilibrium condition, the tractor weight \(W\):

\[
W = W_f + W_r \quad (1)
\]

Also, consider the longitudinal axis \(X_r\) in the moment equilibrium condition as shown in Figure (3).

For the tractor take moments around point O:

\[
W \cdot x_r = W_f \cdot x \quad (2)
\]

\[
x_r = \frac{W_f}{W} \cdot x \quad (3)
\]

The wheel base (X) between the back and front axle is considered in the manufacturer’s specification, if not, they can be calculated easily.

For most rear wheel drive tractors, \(X_r\) is nearly 30% of \(x\), also this is the percentage of the static tractor weight on the front wheel.

**Vertical Location.** Assigning the position of center of gravity in this type is very difficult. So by lifting the front or rear of tractor (as shown in Figure 3), we can measure the weight
on the front wheel in the raised condition. Similarly the vertical position ($Y_g$) is achieved by moment balance (as shown in Figure 3, section c) (Barger et al., 1952). For the tractor take moments around point O:

$$X'_r = \frac{W'_f}{W} x''$$  \hspace{1cm} (4)

The geometry of the positions of the center of gravity (Figure 3(c)) gives:

$$Z = \frac{X'_r}{\cos \beta}$$  \hspace{1cm} (5)

$$y'_r = \frac{x'_r - \frac{X'_r}{\cos \beta}}{\tan \beta}$$  \hspace{1cm} (6)

Substituting for (z) gives

$$y'_r = \frac{x'_r - \frac{X'_r}{\cos \beta}}{\tan \beta}$$  \hspace{1cm} (7)

Figure 3. Center of gravity. (a) Distribution of tractor weight on front and rear tires; (b) Rise the front of tractor to measure the $Y_g$; (c) Free diagram of section (b); and (d) real life photo
Where $x'_r$, is as calculated from equation 4 above. And

$$\beta = \beta_1 + \beta_2$$

(8)

$$\tan \theta = \frac{x'_r - x'_r}{x} + \frac{y' - r}{x}$$

(9)

Examination of equation (7) proves that if the difference between $x'_r$ and $x'_r / \cos \beta$ calculated correct, $\beta$ should be relatively large or accurately specified.

The angle of slope, $\alpha_s$, is the angle that causes the tractor to tip as a hard body on the ground. Contact points under static conditions for example, no drawbar pull condition are calculated from equation (10).

$$\frac{x_r}{r + y_g} = \tan \alpha = \tan \alpha_s$$

(10)

The critical angle for tractor that was used in this research was calculated as follows:

$$\tan \theta_{critical} = \frac{x_r}{r_r + y_g} = \frac{69}{55 + 25.55} = 0.88 \rightarrow \theta_{critical} = 41$$

(11)

This critical angle ($\theta_{critical} = 41^\circ$) is inserted in microcontroller.

**Inclinometer**

In order to measure the slope, an electro-magnetic inclinometer was developed (Figure 4). The inclinometer included a Mira pendulum that had been changed by changing the magnetic field when the slope of land changed. Pendulum consisted of a plastic page on both sides of it. The permanent magnets were installed under magnetic brass weights installed to cause centre of gravity of pendulum to be moved down. In order to measure the angle of slope the kmz41 and uzz9000 sensor were used (Figure 5). A capacitor with 470 nF was used to decrease the noise. The inclinometer was installed on the chassis of the tractor according to Figure 6.

**Dynamometer**

In this project a three point hitch dynamometer with the weight of 49 kg and U-shaped frame chassis from the class 0/I was made which could use the PTO simultaneously. The draft forces in each link were measurable beside the vertical forces on the lower link. The dynamometer system was made of 3 parts: The chassis, sensor element and recorder system. This dynamometer was built to measure the resistance pull of engaged soil and critical force that led to possible rear overturn. Note that the main purpose of this dynamometer was to
Printed circuit to measure the magnetic field angle

Figure 4. Inclinometer

Figure 5. The output of KMZ41 versus the external magnet
Source: Data sheet of the kmz41 and uzz9000 sensor

Figure 6. Inclinometer installed on chassis
calculate one or even more bottom tillage tools. The calculations related to dynamometer chassis were based on tractors design parameters and maximum horizontal force.

The resultant force $P$, which was applied by the tractor, was divided into 3 parts: horizontal ($F_x$), vertical ($F_y$) and side ($F_s$). $F_s$ was the element over the lower link arms and $F_x$ and $F_y$ were elements over the upper link arms. Among the elements of draft force, $F_s$ was worthless, then it could be omitted from the calculations and just the horizontal force $F_x$, was measured in upper link arm. As a result, the only force led to the reverse is $F_x$. As mentioned, the chassis of the dynamometer was made in involved U – shaped frame that provided a condition to use the PTO at the same time. Because this dynamometer had been allocated to the small size tractors, then it should be as light as possible. The typical mast design was used instead of connecting clips. For measuring the impact and dynamic force the chassis was joined to the tractor by force converters sensing pins from one side and mast members from the other side.

**Force Transducer**

Converter is a device that changes mechanical to electrical signals (Niari, 2002). In this research three (3) force converters were used on the dynamometer; one of them was for measuring the force on the upper link and the other two were used to measure the forces on the lower links (Figure 7). Converters contain 2 parts: elastic member (called sensing pin) and Wheatstone bridge (called full bridge). This structure increased the converter’s sensitivity as well as compensation for temperature. It should be noted that the strain caused by exerting the force on the elastic member must not exceed the allowable strain of the strain gauge. (Alimardani, 1997).

Equation (12) is used for calculating the elastic strain.

$$\varepsilon_{total} = \varepsilon_s + \varepsilon_p = \frac{F_s}{AE} + \frac{MC}{IE}$$

Figure 7. The Dynamometer
Where
(εₐ) is side strains
(εₚ) is vertical strains
(Fₚ) is considered as the side force
(A) is the cross section of elastic member
(M) is bending moment (it is caused by vertical force)
(C & I) are radius and inertia moment of the elastic member.

Cross section and total (ε) in maximum tension resulted from vertical and side forces, which lead to the standard design for the elastic element.

Note that the strain yielded (compound of both vertical and side force) was considerably less than total (ε). If assigning the allowable strain of all strain gauges located on the elastic member is needed, so the following equation can be used:

\[ \varepsilon_{SG} = \left( \frac{\Delta R}{R} \right) S_g \]  

Where (εᵦ) is the allowable strain caused by resistance change of strain gauge, Sₔ is the measurement factor and R is the tensile strength.

**Rear Overturning**

The function of stability of tractor in rear overturning is shown in Figure 8. When rear overturning occurs, the forces on front wheels are zero. When the tractor is driven on sloped land, if the slope increases and rises until critical angle, tractor will be close to rear overturning. Also the critical draft force can cause rear over turning.

The following equations are the stability function of tractor. Nᵢ in this function is the reaction force on front tyres of the tractor.

![Figure 8. The Free Body Diagram of tractor in rear overturning](image-url)
When $N_f = 0$

\[ \frac{1}{x_b + (e_f - e_r)} \left[ m_t g (x_b - x_c - e_r) \cos(\theta) - y_c m_t g \sin(\theta) - y_f F \cos(\beta) \right] - (x_f + e_r)F \sin(\theta) \]  

When $N_f = 0$

\[ F = \frac{m_t g (x_b - x_c - e_r) \cos(\theta) - y_c m_t g \sin(\theta)}{y_f F \cos(\beta) - (x_f + e_r)F \sin(\theta)} \]  

And,

\[ \theta = \tan^{-1} \left[ \frac{x_f + e_r}{y_f} \right] \]  

Therefore the critical draft force can be calculated from equation (20). This force is measured with a dynamometer and input into the microcontroller. When the draft force increased up to a critical position the alarm system started and showed to operator that the tractor was close to critical operation. Alarm system includes two LED (yellow and red) and a loudspeaker. When the draft force increased up to 10000 N (for this type of tractor), the yellow LED turned on and showed that operation was next to critical situation and operator should be careful and when the draft force becomes 15000 N the red LED and loudspeaker turned on and in this position operator must stop the tractor.

**Side Overturning**

The equations for side overturning are shown below.

\[ W = F_r + F_1 \]  

\[ F_1 = W \left( \frac{a'}{a + b} \right) \]  

\[ a' = c. \cos(\alpha + \varphi) \]
The angular \( \varphi \) was calculated with the dimension of tractor. The value of this parameter was 53 degrees. Therefore if \( \alpha \) becomes 37 degrees the value of \( F_1 \) becomes zero:

\[
\cos (\alpha + \varphi) = 0 \quad \rightarrow \quad F_1 = 0 \quad (26)
\]

When the \( F_1 \) becomes zero, side overturning accrues. In this study the side overturning had been predicted, too. Monitoring system and alarm system were developed the same way the rear overturning was developed. The Figure 9 shows the free diagram of side overturning.

**RESULTS AND DISCUSSION**

In this research a new mechanism of tractor overturning alarm system was developed. 4WD Mitsubishi tractor with 25hp was used and according to experimental study and mathematical calculation the critical angle of this type of tractor (41 degrees) was obtained. The critical draft force of this tractor (15000N) was obtained. The experimental research of alarm system for tractor overturning was done in the laboratory in the University of Tehran. Results show that there is good relationship between output of inclinometer and dynamometer for measurement of the slope of land and draft force, respectively.

**CONCLUSION**

The following are suggestions in order to avoid tractor overturning and can be useful for operators:

1. Tractors should be equipped with rollover protective equipment such as ROPS (Roll over Protective Structure) and seat belts.
2. Reduce speed on rough ground, on slopes, when turning, or when driving onto roads
3. Avoid sudden turns, especially on sloping ground. Avoid uphill turns, and turning too fast with a load.
4. If the tractor has ROPS and the tractor starts to roll, do not jump off of the tractor. Stay with it until the machine comes to rest.
5. If the tractor is traversing a slope or travelling on the shoulder of the road with a sharp pavement incline, do not turn up slope. Always turn down slope.
6. When working on sloping land, add weight to the front and widen the wheel base of the tractor. This adds stability to the machine.
7. Do not “pop” the clutch or give a sudden jerk when pulling out stuck vehicles or stumps, or when pulling any machinery.

REFERENCES
Analysis of Oil Palm Leaf Phyllotaxis towards Development of Models to Determine the Fresh Fruit Bunch (FFB) Maturity Stages, Yield and Site-Specific Harvesting

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ABSTRACT

In order to ensure the optimum quality of palm oil, oil palm fruit needs to be harvested at the optimum maturity to avoid free fatty acid (FFA) accumulation. The high content of FFA not only reduces the quality of palm oil but also increases the refining cost. Optimum maturity based on plantation standard operating procedure (SOP) is determined by identifying the tree with loose fruit on the ground. The matured bunch is further identified based on the colour of the bunch. This paper presents a research work on formulation and validation of mathematical equations to estimate the maturity stages (age in weeks) of fresh fruit bunch (FFB) based on FFB position in oil palm phyllotaxis (spiral leaves arrangement) as an additional feature to confirm the maturity level of oil palm FFB. Regression analysis showed that the proposed method was able to estimate the maturity stages of oil palm FFB with the coefficient of determination \( R^2 = 0.9 \) and a root mean square error (RMSE) of 1.58 weeks. The FFB yield estimation model and harvesting can be created based on the extracted data using the formula which will help in the planning of harvesting operation. Plantation manager can use this information to generate yield variability map and estimate the appropriate number of workers and machine. Planned harvesting operation can save a significant amount of time in site harvesting operation.

Keywords: Oil palm maturity stages, oil palm harvesting model, oil palm phyllotaxis
INTRODUCTION

Malaysia is known as the second world largest producer of oil palm after Indonesia followed by Nigeria, Thailand, and Colombia (Ali et al., 2013). Oil palm is the major agriculture product in Malaysia which covers up to 5 million hectares of Malaysia’s land (Hazir et al., 2012). High demand for oil palm product has encouraged researchers to study possible ways to increase the productivity of the oil palm yield production (Corley, 2009). Oil palm can produce two types of oil which are palm oil and palm kernel oil. Palm oil is the oil extracted from the flesh of oil palm FFB, whereas palm kernel oil is the oil extracted from the seed of the oil palm fruitlet. Oil palm products such as vegetable oil, cosmetic, pharmaceutical and biofuel are important in our daily life. In order to ensure optimum quality of palm oil, oil palm FFB should be harvested at its optimum maturity to avoid high FFA content (Ishak & Hudzari, 2010; Rajanaidu et al., 1988).

Oil palm FFB harvesting and collecting is a very laborious operation. These operations are carried out within 10 - 12 days’ intervals. A common tool used to harvest FFB is using a sickle attached to a bamboo or aluminum pole for oil palm tree higher than 12 m, or a chisel for shorter oil palm tree (Azhar et al., 2012). The first process in harvesting is searching for the ripe FFB. Harvester carries the harvesting tool and moves from one tree to another without prior information on the exact location of the tree that has the ripe bunch. This process can be accelerated if the harvester is equipped with a harvesting map. A harvesting map requires information of the maturity levels of the FFB on each tree. The second process of harvesting operation is to determine the maturity level of the bunch; this process is simply observing the colour of the FFB and the number of loose fruits that fell under the oil palm tree. However, because of the harvesting cycle of every 10 – 12 days on particular site, harvester tends to harvest over-ripe FFB causing lots of loose fruits to fall on the ground. Plantation manager does not have a proper tool to rectify this problem. The FFB needs to be sorted at the mill level by a human expert before processing. Payment deduction will be imposed to the batch of FFB delivered to the mill if the bunch does not meet the maturity standard.

Time-consuming process in searching for matured bunches, tedious harvesting task and sorting process at mill, have encouraged researchers to study method of detecting matured FFB such as on oil palm maturity sensor (Saeed et al., 2012), motorized harvesting tool (Jelani et al., 2008) and integrated harvesting, collecting and transporting machine (Lin, 2011). The result from the research findings could lead to improvement in oil palm plantation productivity, increased machine efficiency, and satisfied economic return. Development of machines should be reliable to agronomic aspect in order to solve the laborious process.
Harvesting a high quality bunch is the ultimate goal of the plantation in order to generate profitable investment out of all input costs for plantation management. Hence, a reliable harvesting support system that can provide sufficient and relevant information needs to be developed to improve harvesting operation. The support system must be able to feed information on the number of available FFB on a particular tree, maturity level of each bunch on a single tree, location of the tree, estimated number of FFB to be harvested at each harvesting cycle, number of workers to be allocated and estimated time to complete harvesting operation.

A common way for researchers to develop a method to determine oil palm FFB maturity stages is by analyzing the FFB colour features. Various image based systems have been developed to determine the maturity levels of FFB (Razali et al., 2011). Determining the maturity stage of oil palm FFB by colour still has some drawbacks due to the variation of biological conditions between individual palms and between geographical areas (Kassim et al., 2012). According to Abdullah et al. (2001), an accurate estimation of oil palm FFB maturity based on red, green and blue (RGB) colour index requires more than 100 samples per class. The growth model and colour recognition system developed by Kassim et al. (2014), was able to sort oil palm FFB into several maturity classes based on RGB and Hue colour space. Junkwon et al. (2009) and Groß et al. (2016) had successfully determined oil palm FFB maturity stages using hyperspectral and multispectral imaging methods. However, these methods are costly and meant for harvested bunch.

Therefore, alternative feature needs to be explored to determine the maturity of oil palm FFB in order to support the decision making based on the colour feature. The additional feature proposed in this research is the formulation and validation of mathematical equations to estimate the maturity stages (age in weeks) of FFB based on FFB position in oil palm phyllotaxis (spiral leaves arrangement). GIS coordinate system is a useful feature to create the real time harvesting map with the actual location of the targeted oil palm tree (Mat et al., 2016).

**Oil Palm Phyllotaxy**

An oil palm tree normally produces two new leaves per month, but young palm trees (below seven years old) produce three new leaves per month (Corley & Tinker, 2003). An oil palm tree has a phyllotaxy leaf type. There are two types of phyllotaxy spiral arrangements, namely right-handed spiral and left-handed spiral. The difference between the right and left-handed arrangements was the clockwise and counter-clockwise arrangements. There are five spirals and each spiral has eight leaves. Figure 1(a) shows the left-spiral arrangement and Figure 1(b) shows the right-spiral arrangement.
The leaves spiral starts with leaf number 1 which is characterised by the feature of having fully opened leaflets. An oil palm FFB can be found in the axil of its leaf and subtends onto the leaf in a lower whirl. The leaf axil has an equal potential to produce flowers in between leaf 17 to 20 depending on the varieties (Corley & Tinker, 2003). The development of FFB to ripeness takes six months from the anthesis stage. Oil palm FFB is supported by a leaf below it during the growth period. The leaf that subtends by the oil palm FFB is cut together with the bunch during harvesting process.

Matured oil palm FFB can be determined with the presence of loose fruits. Loose fruits scattered on the ground is a sign of a ripened bunch. As low as one detached loose fruit is enough to decide that the bunch is matured (Kassim et al., 2014; Ghani et al., 2004). Once the oil palm bunch is matured, the number of loose fruits detaching from the bunch will increase. At the same time, FFA content will gradually increase in the fruitlets. An under-ripe bunch appears blackish purple whereas a ripe bunch appears reddish orange (Ismail & Razali, 2012).

This research introduces FFB maturity determination by identifying FFB’s position in oil palm phyllotaxis. The harvesting model formula is derived using the rate of leaf production per month, duration of FFB to mature, and position of FFB inflorescences during anthesis. The actual harvesting data were compared with the predicted harvesting date using this formula. Based on this information, FFB yield model and harvesting plan route model can be developed.

Figure 1. Graphical representation of oil palm spiral. (a) Left-handed spiral (b) Right-handed spiral (Fairhust, 1998)
Harvesting matured bunches involves cutting the bunch from the tree and allowing it to fall to the ground by gravity. An overripe bunch will release many fruitlets due to the impact when the bunch falls to the ground. Those loose fruits which have a high oil content are often left uncollected which contributes to the losses in Malaysia oil palm industries. It is clearly important to harvest oil palm FFB at its optimum ripeness to avoid this problem.

MATERIALS AND METHODS

Data collection process was performed at Ladang 15 (2.979524 latitudes, 101.728368 longitudes), Universiti Putra Malaysia, Serdang, Selangor. Thirty oil palm trees aged 9 years old were monitored. The location of each monitored tree was taken using Trimble JUNO handheld GPS unit for mapping purposes. Each available FFB on the tree was recorded together with its position in the leaf spiral. This process requires a skillful worker to identify the position of the leaf in the spiral.

Oil Palm Tree Leaf Spiral Arrangement

Figure 2 shows an oil palm leaf spiral with its position number. This 3D oil palm tree was created by using cloud point data acquired by using Terrestrial Lidar Scanner (TLS). The first step in the process of labelling the leaf position is identifying the position of the first leaf (the first fully-opened leaf) around the centre of the leaves spiral. A non-fully opened leaf is considered as leaf number 0 (also known as spear leaf). The remaining leaf number is identified using the graphical representation of oil palm leaf spirals as shown in Figure 1. Figure 3 shows the oil palm leaf spiral number arrangement starts with center spear leaf.
Oil Palm FFB Data Collection

Each oil palm FFB observed for this research was given a specific identification name (ID). Figure 4 shows an example of the ID tag for a particular sample bunch. Tree identification, P2, shown in the figure 4 indicates that it was the second tree from the sample. FFB identification, A, shows the first sample of oil palm FFB for a particular tree. L23 indicates that the bunch was located at leaf number 23 in the leaf spiral. Lastly, date of data acquired (DODA) was also recorded.

Figure 3. Oil palm leaf number starts with centre spear leaf (Thomas et al., 1969)

Figure 4. Example of oil palm FFB sample tagging
Tagged FFB sample was monitored until they were harvested based on the harvester judgment. The cycle of harvesting for this research site was once a week. Farmers identify the presence of matured bunch by observing the loose fruits on the ground. This was the standard operating procedure (SOP) that is used by most of the oil palm plantations in Malaysia during the harvesting operation. Figure 5 shows the loose fruits indicating mature bunch. The date of harvest by farmers of each FFB sample was recorded as actual harvesting date.

Figure 5. Loose fruits indicating mature bunch

**Formulation of FFB Maturity Model**

Once the positions of all the leaves have been identified, the information on the position of FFB in leaf spirals can be obtained. Based on the biological information of the oil palm tree (rate of leaf production per month, duration of FFB development to mature, and position of FFB at anthesis), together with the position of FFB in leaf spirals, a formula is derived to calculate the estimated oil palm FFB’s age in weeks. Equation 1 is the derived formula to estimate the age of FFB in term of the weeks.

\[
W_{HM} = 4 \left[ \frac{FFBPLS - FFBALS}{RLP} \right] \quad \text{--- Equation 1}
\]

where

- \(W_{HM}\) = Harvesting model estimated age of FFB (weeks)
- \(FFBPLS\) = FFB position in leaf spirals (\(x^{th}\) leaf)
- \(FFBALS\) = FFB at anthesis in leaf spirals (20\(^{th}\) leaf)
- \(RLP\) = Rate of leaf production (2 leaves per month)
- 4 = Constant (number of weeks in a month)
The rate of leaf production per month \((RLP)\) was taken as two leaves per month for mature palm since the age of oil palm tree is above seven years old. The position of FFB in leaf spirals can be determined using the graphical representation of the oil palm leaf spirals (Figure 1) as a guide. In order to determine the age of FFB \((W_{HM})\), the position of FFB in leaf spiral \((FFBPLS)\) was subtracted with a constant value 20 \((FFBALS, 20^{th} \text{ leaf})\) which is the position of inflorescences at anthesis in leaf spiral. The result was divided by the rate of leaf production per month \((RLP)\), which is two leaves, per month and multiplied by 4 (number of weeks per month).

Based on the information of the FFB development from anthesis to the matured stage \((DOFFB)\), date of FFB data acquisition \((DODA)\) and estimated age of FFB \((W_{HM})\), Equation 2 was derived to calculate the estimated harvesting date for oil palm FFB \((DOH_{HM})\).

\[
DOH_{HM} = DODA + [DOFBB - (W_{HM} \times 7)] \rightarrow \text{Equation 2}
\]

where

- \(DOH_{HM}\) = Harvesting model date of Harvest (DD/MM/YYYY)
- \(DODA\) = Date of Data Acquired (DD/MM/YYYY)
- \(DOFBB\) = Days of FFB Development (6 months = 180 days)
- \(W_{HM}\) = Predicted age of FFB (weeks)
- \((W_{HM} \times 7)\) = Predicted age of FFB (days)

The estimated age of FFB \((W_{HM})\) obtained from Equation 1 was substituted into Equation 2. The duration of FFB development \((DOFFB)\) is six months, which is 180 days. \(DODA\) is the date of data acquired for each oil palm FFB sample. The date of harvest \((DOH_{HM})\) can be calculated by adding \(DODA\) with the days left to be harvested, \([DOFFB - (W_{HM} \times 7)]\) which is the subtraction of the duration of FFB development (180 Days) with an estimated age of FFB (Days).

**RESULTS AND DISCUSSION**

Table 1 presents a sample of the calculated data for tree ID P4, for oil palm FFB A, B, and C. The information in Table 1 is divided into two sections, in which one is the estimated data based on the proposed harvesting model method and the other one is the actual in-field data.

The estimated harvesting date based on the proposed harvesting model method consists of the position of FFB in leaf spiral, estimated age of FFB in weeks \((W_{HM})\), estimated harvesting date \((DOH_{HM})\). Below is an example of the calculation to estimate the harvesting date of FFB P4-A using equations 1 and 2.

\[
W_{HM} = 4[(FFBPLS - FFBALS)/RLP] \rightarrow \text{Equation 1}
\]

\[
W_{HM} = 4 \text{ week/month } [(25 \text{ leaf} - 20 \text{ leaf}) / (2 \text{ leaf/month})]
\]

\[
W_{HM} = 10 \text{ week}
\]
DOH_{HM} = DODA + [DOFB - (W_{HM} \times 7)] \quad \text{Equation 2}

DOH_{HM} = 9 \text{ Oct 2015} + [180 \text{ day} - (10 \text{ week} \times 7 \text{ day/week})]

DOH_{HM} = 9 \text{ Oct 2015} + [110 \text{ day}]

= 27 \text{ January 2016}

Estimated harvesting week left to be harvested is calculated by subtracting DOH_{HM} with DODA and dividing by 7 days per week. For example, the FFB sample of P4-A; Subtraction of 27-Jan-2016 and 9-Oct-2015 is equal to 110 days. 110 days is then divided by 7 days per week to equal 15.7 weeks to harvest. In-field data consist of actual in-field harvesting date (DOH_{IF}) and the actual age of harvesting in weeks is calculated as the subtraction of DOH_{IF} from DODA and dividing by 7 days per week. For P4-A sample actual age of harvest (week) is calculated as subtraction of 29-Jan-2016 from 9-Oct-2015 equal to 102 days. 102 days is then divided by 7 days per week to equal 16 weeks.

The estimated week left to be harvested was compared with the actual in-field week left to be harvested. Figure 6 shows a scatter plot of the data; this plot shows that the proposed method was able to predict the harvesting week of oil palm FFB sample with a coefficient of determination, $R^2=0.90$, and root mean square error, RMSE=1.58 weeks. Therefore the proposed method to determine maturity stages of oil palm FFB based on position in leaf spiral can be used to estimate harvesting week of oil palm FFB.

Single Tree Data Analysis

Figure 7 shows the scatter plot for a single tree. Oil palm tree sample ID P4, P11, P13, P15, P16, and P18 was chosen for this analysis because it has more than 4 bunches per tree during the date of data acquisition. The result shows the value of the coefficient of determination $R^2=0.94, 0.98, 0.97, 0.88$ and 0.93 respectively. The result shows that the proposed method was able to estimate the maturity of oil palm FFB.

FFB Yield Data

From the estimation of harvesting week based on the proposed method, FFB yield model can be developed to determine the number of the readily harvested mature bunch at every harvesting schedule (1 week Interval). Figure 8 shows a bar chart number of mature FFB. The data shows a high number of mature bunches in December 2015 and January 2016. Based on this information a yield variability map can be created. Such yield model will be useful for plantation or farmers to plan their workers and harvesting machine for harvesting operation. Further investigation on the course of yield variability in relation to crop management and environmental factors can be carried out. For a cycle of FFB data collection, the result of the analysis is valid for six months considering the development of FFB from anthesis to mature stage is six months.
Table 1
Recorded and calculated data using the leaves spiral equation

<table>
<thead>
<tr>
<th>Tree FFB</th>
<th>Date of data acquired, $DODA$</th>
<th>Position of FFB in leaf spiral</th>
<th>Estimated age of FFB in weeks, $W_{est}$</th>
<th>Estimated harvesting date, $DOH_{est}$</th>
<th>Estimated week left to be harvested, $DOH_{est}-DODA$ (7 day/week)</th>
<th>Actual In-field harvesting date, $DOH_{IF}$</th>
<th>Actual Week left to be harvested, $DOH_{IF}-DODA$ (7 day/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4-A</td>
<td>9-Oct-15</td>
<td>25</td>
<td>10</td>
<td>27-Jan-16</td>
<td>15.7</td>
<td>29-Jan-16</td>
<td>16</td>
</tr>
<tr>
<td>P4-B</td>
<td>9-Oct-15</td>
<td>29</td>
<td>18</td>
<td>2-Dec-15</td>
<td>7.7</td>
<td>4-Dec-15</td>
<td>8</td>
</tr>
<tr>
<td>P4-C</td>
<td>9-Oct-15</td>
<td>27</td>
<td>14</td>
<td>30-Dec-15</td>
<td>11.7</td>
<td>30-Dec-15</td>
<td>11.7</td>
</tr>
</tbody>
</table>

Figure 6. Scatter plots of in-field and estimated harvesting week of oil palm FFB

$y = 0.9616x + 0.3132$

$R^2 = 0.9021$
Figure 7. Scatter plots of in-field and estimated harvesting weeks for single tree analysis

Figure 8. Expected number of matured between October 2015 to April 2016
FFB Harvesting Map and Route

A harvesting route provides information on the tree with mature bunches based on the collected data and GPS coordinate. Figure 9(a) shows the proposed harvesting route based on the information of specific trees with mature bunches; whereas Figure 9(b) shows the typical/standard harvesting pattern without information of trees with mature bunches. Typically, a harvester will stop at each tree to assess the presence of mature bunches. A planned harvesting route is clearly the better option because harvester only needs to stop at the targeted tree, hence saving time and energy during the harvesting operation.

CONCLUSION

This paper proposes and describes a new method of determining maturity stages of oil palm FFB based on its position in leaf spiral using harvesting model equation. The coefficient of determination of the proposed method is $R^2=0.9$ and RMSE = 1.58 weeks. FFB yield model and harvesting route can be made by combining the result from the harvesting model and the GPS coordinate of the oil palm tree. Planned harvesting route is clearly the better option to save time and energy during the harvesting operation. Farmers only need to stop at the targeted oil palm tree rather than go through all of the trees in the field. In future studies,
the data collection process for this research can be improved by using current technology. The technology of rapid imaging, 360 imaging, and 3D point cloud can be used to identify the position of the FFB in leaf spiral rather than manual identification used in this research. Further improvement can be done to save time on data collection process and to be cover a larger area of oil palm field.

Harvesting route with the actual geographical coordinate of the oil palm tree could lead to the robotic application in the oil palm industry. The robotic application is useful for data collection such as bunch counting, tree monitoring, and can determine the location of oil palm tree containing matured FFB. This technology and information on oil palm site is an introduction to the futuristic autonomous vehicle and operation in oil palm industry.

REFERENCES


Experimental Study of Tsunami Bore Induced Forces on Vertical Seawall

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ABSTRACT

Field surveys of the 2011 Tohoku Tsunami reported massive failures of many seawalls and coastal barriers. The massive damages are vivid evidence that there are flaws in the design of seawalls and barriers. With this as the background, a sequence of laboratory experiments using dam-break waves was performed to simulate the interactions between the tsunami-like bore flow and vertical seawall as well as to measure the bore-induced pressures and to estimate forces exerted on the vertical seawall model. The experimental result revealed that the maximum pressure (approximately 8 kPa) exerted on the vertical seawall was measured at the lowest pressure sensor location. Experimental data were used to re-examine the relevant empirical formulae found in the literature. The obtained results could be useful for calibrating mathematical and numerical models as well as for future research concerning the design of tsunami barriers.

Keywords: Dam-break wave, force, physical model, tsunami bore, vertical seawall
INTRODUCTION

In some coastal areas, seawalls are intended to protect the coasts from potentially massive hazards such as storm surges and tsunamis. Thus, most critical counter measures for instance seawalls, breakwaters and dykes are crucial and become one of the mitigating measures for protection of life and property against such extreme waves.

However, a catastrophic damage of many sea defence structures was observed along the shoreline areas after the occurrence of the 2011 Tohoku tsunami. The mechanisms behind the failure of many seawalls and coastal barriers due to the tsunami are still being investigated and have not yet been fully elucidated thus far. Evidence from the 2011 Tohoku Tsunami video footages shown that a visible white foam strip coming towards the shore, which is known to be tsunami bores.

Over the past few decades, there have been several studies on the interaction of tsunami-like solitary wave with seawall and their impacts (Hamzah et al., 2000; Kato et al., 2006; Hsiao & Lin, 2010; Lin et al., 2012). Nevertheless, forces study resulting from tsunami bores hitting onshore seawalls using the dam-break flow to simulate a tsunami bore is less explored. Chanson (2006) demonstrated that a dam-break flow could exhibit a reasonable simulation of the tsunami-induced bore.

Cross (1967) carried out an experimental study to investigate the properties of incident bores and surges advancing into still water and dry bed as well as their impact forces on a vertical wall. He proposed the tsunami force exerted on the vertical wall could be estimated using the following equation:

$$ F = \frac{1}{2} \rho g h_b^2 + \rho h_b u_b^2 \quad [1] $$

where $\rho$ is the mass density, $g$ is the gravitational acceleration, $h_b$ is the height of the bore from the ground level (bore depth), $u_b$ is the bore velocity.

Asakura et al. (2000) measured forces exerted on an onshore vertical structure that was located on a dry bed and proposed the following empirical equation to estimate the force exerted on the vertical wall:

$$ F = \frac{1}{2} \rho g (3h)^2 \quad [2] $$

where $h$ is the bore height (height of the bore from the still water). Fujima et al. (2009) performed experimental studies to investigate wave force exerted on rectangular onshore structures subjected to tsunami breaking bore. A measured total force obtained from their experiments was used to formulate the tsunami force estimation equation that was based on the maximum inundation depth and the distance of structure from the shoreline. The equation is expressed as follows:
The Overseas Coastal Area Development Institute (OCDI) of the Ports and Harbours Bureau of Japan (2009) suggested that the tsunami force per unit width on an upright wall (when the tsunami is a bore-type) could be estimated using the following equation:

\[ F = 1.3\rho hu_b^2 \]  \hspace{1cm} [3]

Robertson et al. (2013) conducted large-scale hydraulic tests to study the tsunami-induced forces and pressures exerted on a vertical wall. They investigated the impact forces of tsunami bore advancing over both dry and wet bed reefs which generated from a broken solitary wave and proposed the following force estimation equation on the vertical wall:

\[ F = 3.3\rho gh^2 + 2.2 \rho ghd_s \]  \hspace{1cm} [4]

In this study, forces exerted on vertical seawall were calculated from the measured pressures obtained by Zaty et al. (2019). The bore that was generated using a dam-break method propagated over the wet flume bed before hitting the structure model.

RESEARCH METHOD

Experimental Setup
The experiments were performed at the Hydraulics Laboratory of the National Hydraulic Research Institute of Malaysia (NAHRIM), Selangor. The test flume was 100 m x 1.5 m x 2.0 m (length x width x height). The concrete flume was partitioned into two sections in order to create an upstream reservoir area of 44 m long and the downstream test area as shown in Figure 1. In this study, the dam-break flow was used to simulate tsunami bore. A steel sluice gate was installed between the upstream reservoir and the test section area. The gate was lifted rapidly to release the water from the reservoir to generate tsunami-like bore in the downstream flume test area. The simulated tsunami bore travelled on the wet flume bed with a still water depth \(d_s\) of 0.05 m in all experimental cases.

Model Details
A model scale of 1:10 was adopted in this study. The test model used in this research is a vertical-front type seawall model (hereafter VW). The seawall model was installed on the horizontal flume bed at 9.0 m downstream from the gate in the flume test area and rigidly bolted to the floor. The 0.5 m high seawall model structure was constructed from 10 mm thick acrylic sheet. The transverse length of the seawall was extended across the entire
width of the flume. To provide sufficient rigidity to the seawall model during bore impacts, steel frames were fixed at the rear of the model as a support.

![Figure 1](image-url)

**Figure 1.** Plan and elevation views showing the location of VW seawall, wave gauges (H1-H5) and velocimeter (V1) in the test flume. All dimensions are in meters and not to scale.

**Instrumentation**

Five resistance wave gauges (H1 to H5) were installed along the downstream flume test area to measure the bore elevations during the experiments. The wave gauges were mounted onto a frame located on top of the flume which consisted of several steel pipes installed across the longitudinal axis of the flume. Each wave gauge was located at 1.0 m apart. An Acoustic Doppler Velocimeter (ADV, Nortek), V1 was used to measure the bore velocity that was co-located with wave gauge H5 in this study. Both V1 and H5 were located at 1.0 m upstream from the seawall model and 8.0 m downstream from the gate. In this study, three pressure sensors (Keller® PR-25Y) were used and were fixed flush against the upstream face of the seawall model at six different locations to record the time histories of pressures (Figure 2). However, due to the limited number of pressure sensors available during the tests, similar experiments were repeated several times by moving the sensors to different sensor hole positions on the front face of the VW model. The sensors (P1 to P6) were installed vertically at 70 mm intervals. The location of the pressure sensor ports on the seawall model is shown in Figure 3. The pressure sensors were logged at 1000 Hz, while the measured flow depths and velocity data were digitised with a sampling rate of 50 Hz. The flow depths and pressure data measurements were using the same data logging system (HR Wallingford Data AcQuisition software) and were synchronised to the same time. In this research, two digital cameras and a high-speed digital video camera that operated at a frame rate of 240 fps were utilised to film and qualitatively document the bore impacts processes.
Experimental Conditions

In this study, five different combinations of impoundment water depths \( (h_d) \) and gate opening heights \( (GO) \) were used to produce five experimental bore cases with different bore depths \( (h_b) \) and bore velocities \( (u_b) \) as listed in Table 1. Each test was repeated at least three times to verify repeatability and to ensure the reliability of the results. The repeatability of all tests was less than 5% between repetitions and demonstrated good repeatability.

RESULTS AND DISCUSSION

Bore Depth–Velocity Relationship

In this study, various tsunami bore heights and velocities (Table 1) were generated in the
flume from a dam-break method (Chanson, 2006). A typical time history of bore depth and bore velocity for the bore generated from 0.7 m impoundment water depth measured at 1.0 m upstream from the seawall model is depicted in Figure 4.

As can be seen in Figure 4, it is observed that the water level rose rapidly at approximately $t = 5.0$ s. Measurement at H5 showed rapid increases in water level due to the bore reflection from the seawall, while at the same time the velocity decreases to negative values which indicated that the velocity was in the same direction as the reflected bore (negative x-direction).

Figure 5 shows snapshots of the bore impacting the VW model taken from the side of the wave flume during the experiment. The bore front (with air bubbles plumes can be seen trapped at the bore front) hit the 0.5 m seawall model structure that was installed in the downstream flume test area and then the wave began to surge up on the front face of the VW model with a high water velocity together with a splash-up.

<table>
<thead>
<tr>
<th>Case</th>
<th>Impoundment water depth ($h_d$), m</th>
<th>Gate Opening ($GO$), m</th>
<th>Bore depth ($h_b$), m</th>
<th>Bore velocity ($u_b$), m/s</th>
<th>Maximum Bore Force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>0.55</td>
<td>0.3</td>
<td>0.218</td>
<td>1.98</td>
<td>1726.0</td>
</tr>
<tr>
<td>Case 2</td>
<td>0.60</td>
<td>0.5</td>
<td>0.226</td>
<td>2.29</td>
<td>1952.3</td>
</tr>
<tr>
<td>Case 3</td>
<td>0.65</td>
<td>0.3</td>
<td>0.243</td>
<td>2.39</td>
<td>2305.4</td>
</tr>
<tr>
<td>Case 4</td>
<td>0.70</td>
<td>0.5</td>
<td>0.257</td>
<td>2.45</td>
<td>2533.1</td>
</tr>
<tr>
<td>Case 5</td>
<td>0.75</td>
<td>0.3</td>
<td>0.274</td>
<td>2.51</td>
<td>2744.0</td>
</tr>
</tbody>
</table>

*Figure 4. Example of the time histories record of changes in bore depth and velocity upstream from the model location for case 4*
Bore-Induced Pressures and Forces Distribution on Seawall

The experiments were also performed to quantify the characteristics of bore-induced forces on the upstream face of the VW model and bore-induced pressures exerted on the wall. Figure 6 illustrates the variation of maximum wave pressures with respect to impoundment water depths recorded at each pressure sensor. From the figure, it can be concluded that there is a linear relationship between the maximum recorded pressure and impoundment depths. For all cases, it is noted that the highest pressure was observed at the lowest-located pressure sensor (P1), while the lowest pressures were measured at the highest-located pressure sensor (P6).

Figure 7 shows time histories of bore pressures recorded by different pressure sensor P1 to P6 located on the upstream face of the VW model (Case 4). High impulsive pressures (usually characterized by a short-duration pressure) were recorded by the lower-located pressure sensors (P1 and P2). It is found that the maximum pressure measured by the sensor nearest to the flume bed (at \( z = 0.07 \) m) was approximately 7 kPa. A similar trend was observed in the previous studies by Al-Faesly (2016), Kihara et al. (2015) and Nouri et al. (2010) where high impulsive pressures were measured at the lowest-located pressure sensor in their experimental case studies.

The vertical distribution of maximum bore pressures exerted on the VW model for all experimental cases are shown in Figure 8. As can be seen from the figure, the pressure measured by the sensors typically increased as bore height increased. It is also found that the maximum pressures decreased with height for each case in this study.

Figure 9 shows the calculated force from the integration of the measured pressure exerted on the VW model from bore generated by three impounding water depths of 0.75 m, 0.65 m and 0.55 m. For this analysis, the force acting on the VW model was obtained...
by integrating pressure data along the seawall surface. The pressure integration method was previously used by Hsiao et al. (2010), Robertson et al. (2013) and Shafiei et al. (2016). The horizontal seawall surface was divided into strips and the exerted pressure was assumed to be constant.

In this study, a typical time history of bore-induced forces exerted on the seawall model are characterized by four phases: (1) impulsive force that occurs when bore front impacts the seawall (the force durations were in the range of 0.01 s - 0.05 s); (2) run-up force that occurs when bore begins to run-up the wall and bore depth increases in front of the wall; (3) as the water starts accumulating in front of the wall (since the wall extending across the entire of the flume width), the overflow occurs and a gradual decrease in the force is observed in the time-history; and finally (4) when the force distribution on the wall is approximately constant over a longer period during the quasi-steady phase. The second phase was usually characterised by significant pressure oscillations due to the high turbulence of bore flow, and the maximum force was recorded during this phase in the time history recording. Findings in this study were consistent with the previous study by Al-Faesly (2016).

![Image](image_url)

*Figure 6. Variation of maximum wave pressures with respect to impoundment depths*

**Tsunami Force Estimation Equations**

The comparison has been made between previous empirical formulas and the experimental data obtained in the present study to estimate bore impact force on the vertical wall. Figure 10 graphically shows the comparison of the maximum calculated bore impact force obtained from the present study with that calculated using previous formulas of Equations [1], [2], [3], [4] and [5]. From the results, the calculated forces from the integration of the measured pressure in the present study exhibit a similar inclination with Equation [2].
Figure 7. Bore pressure-time histories induced on the seawall model from bore generated by 0.7 m impounding water depth recorded at each pressure sensor

Figure 8. Vertical distribution of maximum bore-induced pressure on the upstream face of VW model for all cases
although the proposed equation underestimated the force. It is also noted that Equation [5] tends to overestimate the estimated bore force using the data of the present study. Figure 10 reveals that the models by Cross (1967), Fujima et al. (2009), and OCDI (2009) appeared in agreement with the model proposed in the present study. The high correlation coefficients determined from the regression analysis confirm the linear relationship between the maximum forces and bore depth.

Figure 9. Computation of the bore force on the VW seawall model from bore generated by 0.75 m, 0.65 m and 0.55 m impounding water depths

Figure 10. Comparison of maximum force of previous studies with the present study
DISCUSSION AND CONCLUSIONS

The 2004 Indian Ocean Tsunami, the 2011 Tohoku Tsunami and the recent 2018 Palu Sulawesi Tsunami have resulted in large number of casualties and huge economical loss. The occurrence of these events necessitates reviewing the designs of all costal defence structures including the design of seawalls. The failures of many seawalls during the 2011 Tohoku tsunami were practical cases indicated that seawalls cannot take the dynamic force due to tsunami bore. The present study was focused on conducting experiments on vertical seawall model that was subjected to dynamic force due to tsunami bore. In this study, the investigation on the characteristics of bore pressures and forces exerted on the vertical seawall was carried out via a series of experimental measurements. The scale of vertical seawall model was selected by considering the available dimensions of some existing seawalls. The trend of force distribution exerted on the seawall model due to the impact of tsunami bores are characterized by four distinct phases: (1) bore front impact phase, (2) bore run-up the wall phase, (3) redirected or overflow phase, and (4) the quasi-steady phase. In this study, the maximum pressure exerted on the seawall model was recorded at the lowest-located pressure sensor with the value approximately at 8 kPa (Case 5). The maximum force obtained in this study was 2.74 kN (Case 5). The present data also exhibit that the maximum forces exerted on the seawall for all bore depths produced in this study and the empirical formulas by Cross (1967), Fujima et al. (2009), and OCDI (2009) models are quite reliable and the values calculated using these equations came very close to the dataset of the present study.

However, further studies using higher tsunami bore height cases and utilising the entire seawall surface model instrumented with pressure sensors are highly recommended for accurate estimation of the pressures and forces on the seawall subjected to a tsunami.

ACKNOWLEDGEMENTS

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Development of Information System for Efficient Use of Nectar Resources and Increase Honey Yield per Colony

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ABSTRACT

In this study, for 5.1 million bee colonies and nearly 42 thousand migratory beekeepers in Turkey, an information system is recommended that determines the areas where the honey season will pass taking into account the flowering periods of plants. Migratory beekeepers produce honey by following the flowering periods of nectar sources. Bee colonies should be placed in the optimum number in areas with nectar sources. Less colony settlement has a negative impact on agricultural production. Colony condensation also adversely affects the honey yield of bee colonies per hive. In this study focuses on the optimal number of colonies in the nectar region. In the first stage, 81 provinces in Turkey were analyzed in terms of nectar resources and meteorological conditions which are the major sources of honey production. This evaluation used fuzzy cognitive maps. As a result of the evaluation, 33 provinces were identified as the most suitable provinces in terms of nectar sources and meteorological conditions. In the second phase of the study, a new approach has been proposed for migratory beekeepers to pass the nectar flow season at maximum efficiency and to use nectar resources at maximum level. This approach is based on the placement of bee colonies, considering the potential of the bee farming of the regions and the number of bee colonies subjected to migratory beekeeping.

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One of the advantages of this approach is that it will maximize honey yield per colony for migratory beekeepers. Another advantage of this system is that the distribution of bee colonies according to the number of plants in the region will be positive in terms of quality and quantity of agricultural production.

Keywords: Fuzzy cognitive maps, information system, migratory beekeeping, nectar flow

INTRODUCTION

Beekeeping is carried out as an important agricultural activity in Turkey as well as all over the world. The number of beekeepers in Turkey is around 57,897 according to official records. These beekeepers have a total of 6.8 million bee colonies (Kekeçoğlu et al., 2007). Approximately 75% (42,000) of beekeepers in our country are migratory beekeepers. Approximately 75% of the bee colonies are subjected to migratory apiculture, which corresponds to about 5.1 million colonies. (Fıratlı et al., 2010). According to the Food and Agriculture Organization of the United Nations (FAO) in 2012, a total of 37,863,019 bee colonies and 1,636,398,98 tons of honey are produced in the world (Albayrak et al., 2018). The yield per colony is 43.21 kg. Turkey ranks second in terms of total number of colonies in the world (6.8 million bee colonies). Honey yield per colony is approximately 15 kg in Turkey. Referring to Turkey’s average yield per colony was 32% lower than the world average (Kekeçoğlu et al., 2007). Beekeepers in Turkey, according to the colonies of bees, carry nectar sources flowering period. Following this flowering period, this kind of beekeeper is called migratory beekeeper. Beekeeper can produce more honey by migratory beekeeping (Şahinler & Cengiz, 2010).

Beekeeping is the lifestyle of honey bees and it is one of the animal dependent activities most dependent on nature because of the natural gathering of the raw materials of their products. This dependence makes the beekeeping more sensitive to the environment. As a matter of fact, the result of environmental changes can change the yield of honey per hive. It is known that 85% of the differences between the colonies in terms of honey yield are due to environmental conditions and 15% is due to genotype difference (Kekeçoğlu et al., 2007; Fıratlı et al., 2010).

Honey production is a main goal of beekeeping. For honey production, a variety of conditions must be appropriate, both inside and outside the hive. Another parameter of success in honey production is the beekeeper itself. It positively contributes to the maintenance and management of colonies, where the beekeeper has sufficient knowledge and experience. Inside elements affecting honey production include: bee numbers and health status in the colonies, the age and fertility of the queen bee in the colonies, race and morphological conformity of the bees in the colon. The non-hive elements can be given
as the number of agricultural crops near the area where the colonies are located, and if so, the density of pesticides, the number and density of nectar plants in the area where the colonies are located and the suitability of meteorological conditions (Semerci, 2007; Sudarsan et al., 2012).

In beekeeping, literature on information systems (IS) or decision support systems (DSS), researchers have focused more on the genetic makeup of bees in colonies and on the improvement of these genetic constructs. Thus, beekeepers that yield higher yields could be obtained. One of the most important factors affecting the yield of honey is the disease, harmful and parasites that occur in the colon. These are bacterial and viral diseases. BeeAWARE is an expert system developed for the diagnosis and treatment of honey bee diseases, pests, parasites and bee colon which require on-site inspection and analysis. Many studies like this expert system have been reported in the literature (Zacepins et al., 2015). These studies are mostly the monitoring of colonies and the early diagnosis and treatment of developing diseases, monitoring of the spawning capacity of the queen bee, management of the swarming and control of the deaths in the colonies. IS/DSS in beekeeping is a system that can process different information such as video, weight, temperature and sound measurements of the column (Zacepins et al., 2015). New methods for determining the status of bee colonies can be developed by creating intuitive approaches through the interdisciplinary collaboration of bee specialists and engineers, physicists, mathematicians and information technology experts. IS/DSS operations are likely to remain limited soon, as only a small part of bee farming operations can be automated (Zacepins et al., 2015).

Fuzzy cognitive map (FCM) is a method used for modelling complex systems using existing knowledge and human experience. FCM is used to predict the behavior of a system, to test the influence of parameters and to analysis and simulate the system. FCM performs modelling using a connection matrix in the light of experience (Albayrak et al., 2018). In this study, FCM is used to digitize expert beekeeper knowledge and model the problem.

There is not much work in the literature on the efficient use of nectar resources and the placement of honey bee colonies. The works done is limited to a certain extent. Some studies focus only on mathematical modeling, while others are limited to certain regions. This study focuses on colony settlements for the whole country, considering the external factors affecting beekeeping. It focuses on maximizing efficiency and maximum pooling per colony. How many bee colonies a region can take can be due to the fulfillment of these two criteria.

“The efficient use of the nectar resources in the area where the colonies will be placed.” The area in which the colonies are to be placed should not have more or less bee populations. In the case of a bee colony, nectar resources in the region may not be available. As a result, less honey will be produced. At the same time, the pollen of the flowers in the region will not be able to be pollinated enough, so the crop will be less and less productive. If more
bees are placed in the area, all the nectar resources can be collected, but the bee colonies placed will compete. This means that the bees in the region at that time will not reach the expected amount of honey. Especially for migratory beekeepers this is extremely difficult.

“In the region, a sufficient number of bee colonies.” As in the first criterion, it is known that the number of colonies to be placed in a region is related to the nectar resources of that region. For sustainable agriculture, bee colonies should be placed in an appropriate number of bees as both beekeepers and when considering the fruits and vegetables that the bees pollute.

In beekeeping, IS/DSS studies where nectar resources are assessed are limited in the literature. In a study conducted by Nuru Adgaba in Saudi Arabia from these studies, pictures were taken by remote measurement method. These pictures were classified by the Hopfield neural network and the number of trees was obtained in hectare. Trees and shrubs are a great source of nectar in the region. Thus, practical information has been obtained for beekeepers. An information system has been proposed to show where beekeeping can be done with the highest efficiency in terms of time and space (Agbaba et al., 2017).

In the study conducted by Zoccali et al. (2017) to determine suitability of bee farming in southern Italy, the data were classified by the Analytic Hierarchy Process (AHP) and fuzzy logic. As a result, Calabria region in southern Italy was found to be the best region (47.76%) in terms of its suitability for bee farming. Olive trees were found in this area as 38.12%, oak forests as 15.16% and annual plants with high and medium honey yield as 16.22% (Zoccali et al., 2017).

In Egypt, according to the study of beekeeping analysis, Egypt map is divided into three categories. These categories are classified as inappropriate, appropriate and more appropriate. While most areas are classified as eligible or more appropriate, areas with very severe climatic conditions are classified as inadequate (Abou-Shaara, 2015). In Iran, Amiri and her colleagues selected criteria under three headings in their study of beekeeping planning. These are environmental factors, nutrients and water resources. As environmental factors, proximity to traffic and road areas, temperature values, altitude and soil type were chosen. The number of nectar plants in the region selected as food sources has been considered (Amiri et al., 2011). In Turkey, the whereabouts of nectar sources and intensity information is available. Considering the nectar resources, nomadic bee growers can be directed. In this study, as a result of the evaluation of nectar and pollen sources and meteorological conditions, a system has been developed to provide the most efficient use of flower sources of migrant beekeepers in our country.

The aim of this study is to develop an information system that will ensure maximum use of nectar resources and at the same time ensure maximum efficiency per hive. The aim of this study is to develop an information system that will ensure maximum use of nectar and resources and at the same time ensure maximum efficiency. This study consists
of problem description, determination of nectar source potential of provinces with fuzzy cognitive maps, determination of nectar flow periods and result sections.

MATERIALS AND METHODS
Recommended System

In this study, bee farming potential (BFP) was first calculated for each province by FCM. Then, with Equation 3, nectar flow time (NFT) periods were calculated for each province. The coefficient (BFP) obtained by FCM and the nectar flow seasons (NFT) were multiplied when the number of colonies (NC) that each province could receive. The values obtained from this process are collected. Total value of the results obtained, gives the total nectar source for bees in Turkey. This represents the total amount of seasonal food for the bees. This value is distributed among the migratory bee colonies in Turkey. The block diagram of the proposed system is given in Figure 1.

As shown in Figure 1, in the last step migratory bee colonies are distributed according to the nectar densities of the illusions. Non-migratory bee colonies were not considered in this study. The Beekeeping Association and Agricultural Credit Cooperatives, which are of interest to this study topic, were selected as stakeholders. The Beekeeping Association (TBA) is an association where all beekeepers are members. The Agricultural Credit Cooperatives (ACC) is also working with migratory beekeeper. The city where ACC is located calculates and supervises how many bee colonies should come. Migratory beekeepers are required to obtain permission from ACC when they plant their colonies. The city in which ACC is located uses very non-scientific methods to determine how many bee colonies to place. The calculations made while determining the number of bee colonies the city can take do not satisfy most migratory beekeepers.
Bee Farming Potential Assessment with FCM

FCM is a tool used for modeling complex systems with existing knowledge and human experiences. It is used to estimate the behavior of the FCM system, to test the effect of the parameters, to analyze and simulate the system (Papageorgiou et al., 2013). FCM developed a decision support system in 2014 with a user interface for renewable energy projects in Greece. The system was used locally in planning energy investments and determining profitability levels (Kyriakarakos et al., 2014). FCM has been successfully used in the prediction of apple harvest. Six concepts and prediction models were designed (Papageorgiou et al., 2013).

FCM is a combination of fuzzy logic and cognitive mapping. FCM includes concept/node factors. The causal relationships between the factors and the connections are given. Direct links are labeled with [0,1] or [-1,1] with fuzzy values, which represent the power of influence between concepts. The fuzzy part allows us to define the degree of causality of the connections between these diagrams and concepts (Papageorgiou et al., 2013).

Fuzzy cognitive maps can model the relationship between concepts defined in problem space with the help of weight matrix. Factors affecting honey production were considered when selecting concepts for FCM. While these factors were chosen, expert beekeeper knowledge was utilized. The concepts used in this study are given in Table 1 respectively.

<table>
<thead>
<tr>
<th>Concepts</th>
<th>Number of Membership Function</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1: Nectar producing plants</td>
<td>Three membership functions</td>
<td>%</td>
</tr>
<tr>
<td>C2: Pollen producing plants</td>
<td>Three membership functions</td>
<td>%</td>
</tr>
<tr>
<td>C3: Average temperature</td>
<td>Three membership functions</td>
<td>◦C</td>
</tr>
<tr>
<td>C4: Sunshine duration</td>
<td>Three membership functions</td>
<td>Hour</td>
</tr>
<tr>
<td>C5: Number of rainy days</td>
<td>Three membership functions</td>
<td>Day</td>
</tr>
<tr>
<td>C6: Bee farming potential</td>
<td>Five membership functions</td>
<td>%</td>
</tr>
</tbody>
</table>

Concepts are expressed as state vectors within the FCM (Ai). The state vector provides information on the behavior of the system (Papageorgiou et al., 2013). Since the concepts C3, C4, C5 are meteorological data, this data is taken from the state meteorology department. This is the last fifty years (1968-2018). The concept of C6 (Bee Farming Potential) constitutes the honey production potential that they possess. These membership functions, which are defined as Very Low, Low, Medium, High and Very High, determine how much honey can be produced (Bee Farming Potential). Questionnaires were conducted with experts in the field of beekeeping to determine the relationship of the concepts created.
with each other. Survey questions “C1 concept, how much influence the C3 concept” is formed. The answer to each question consists of 11 selective answers. Each of these answers is defined by a different type (triangle, trapezoidal) membership function. Thus, expert opinion is added to the system as a numerical value. The weight matrix obtained from the questionnaire is given in Table 2.

Table 2
Survey result weight matrix (Aij)

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0</td>
<td>0</td>
<td>0.135</td>
<td>0.475</td>
<td>0.16</td>
<td>0.012</td>
</tr>
<tr>
<td>C2</td>
<td>0</td>
<td>0.475</td>
<td>0.52</td>
<td>0.11</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>0.3</td>
<td>1</td>
<td>0.215</td>
<td>0.475</td>
<td>-0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

The result of this questionnaire is the weight matrix to hold the causal connections between concepts (Wij). There is no data in the empty spaces in Table 2. These gaps also mean that there is no interaction between the concepts in the column and the line. The values obtained for each concept connection are refined with the sum of the centers of gravity according to Equation 1.

\[
W_{ij} = \frac{\int y \mu(y) dy}{\int y \mu(y) dy}
\]  

(1)

In Equation 1, Wij [-1,1] is the weight matrix, y is the membership function, and \(\mu(y)\) is the membership function. The FCM state vector is updated with the connection value between the concepts. The update operation is the product of the Wij values in the weight matrix, which is associated with the state vector Ai. In addition, the state vector is added to the previous state. This equation is given in Equation 2.

\[
A^{(k)} = f(A^{(k-1)} + \sum A^{(k-1), W})
\]  

(2)

In Equation 2, A (k-1) represents the old state of the state vector and W is the weight matrix. The obtained values must be passed through the threshold function while being transferred to the new state vector A (k) (Kannappan et al., 2011). Since the state vector contains positive values [0,1], the sigmoid threshold function is preferred in this study. After stakeholders have identified the concepts, which membership functions and concepts
should be represented and the limit values of the concepts to be used in membership functions have been determined (Papageorgiou & Laspidou, 2015). The limit values of the concepts are given in Table 3.

Table 3

<table>
<thead>
<tr>
<th>Limit values of concepts</th>
<th>Nectar producing plants (%)</th>
<th>Pollen producing plants (%)</th>
<th>Average temperature (°C)</th>
<th>sunshine duration (hour)</th>
<th>Number of rainy days (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0-30</td>
<td>0-30</td>
<td>0-10</td>
<td>0-3</td>
<td>0-15</td>
</tr>
<tr>
<td>Medium</td>
<td>30-60</td>
<td>25-50</td>
<td>10-20</td>
<td>3-6</td>
<td>15-25</td>
</tr>
<tr>
<td>High</td>
<td>60-100</td>
<td>50-100</td>
<td>&gt;20</td>
<td>&gt;6</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Bee Farming Potential (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Low</td>
<td>0-20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>20-40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>40-60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>60-80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very High</td>
<td>80-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

With experts from stakeholders, it has been decided that the type and location of the membership function will be used to represent the concepts. In Figure 2, the membership functions and settlements representing the concepts are given.

*Figure 2. Membership functions of concepts*
The fuzzy cognitive map developed in Figure 3 is given. As can be seen from Figure 3, meteorological conditions affect both nectar-pollen bearing plants and honey production. It emphasizes the importance of meteorological conditions for the area where these bee colonies will be placed.

![Fuzzy cognitive map](image)

*Figure 3. Fuzzy cognitive map*

It is necessary to train the weight matrix which holds the relations between concepts that the fuzzy cognitive map has. To stop the simulation, it must be \( A_k = A_{k-1} \) or \( A_k - A_{k-1} \). Here \( e \) represents the acceptable error rate (\( e = 0.001 \)). Training is terminated when the acceptable error rate is reached. Immediately after updating the state vector, the weight matrix is updated with the Non-Linear Hebbian learning algorithm. The training algorithm of the weight matrix of FCM is as follows:

1. **Step 1:** Read the state vector \( A_0 \) and the weight matrix \( W_{ij} \)
2. **Step 2:** Repeat for each iteration step
   2.1: Calculate the state vector according to Equation 2.
   2.2: Calculate the weight matrix according to the Hebbian algorithm.
   2.3: Calculate the stopping criterion
3. **Step 3:** Repeat step 2 until you reach the stopping criterion.
4. **Step 4:** Show the latest weight matrix and state vector to the user.

When the above algorithm is run for 81 provinces, it can be said that 33 provinces are more efficient in honey production. These provinces are given in Table 4.
Determination of Nectar Flow Periods of Provinces

To be successful in beekeeping; it depends on the richness of the nectar resources and the use of honey bees with eothoped genotypes adhered to the region. The plants with the most effect in honey production are nectar-harvested plants. In a study conducted by the General Directorate of Forestry in Turkey, pollen and nectar in the amount of 100 grams of honey has been removed. Nectar production and pollen production classification according to the quantities measured (General Directorate of Forestry, 2012; Sorkun, 2007; Davis, 1988). This classification is given in Table 4.

Table 4
Classification within 100 g honey

<table>
<thead>
<tr>
<th>Classification name</th>
<th>Quantity (gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOMINANT</td>
<td>&gt;=45</td>
</tr>
<tr>
<td>Secondary</td>
<td>=16-45</td>
</tr>
<tr>
<td>Minor</td>
<td>=3-15</td>
</tr>
<tr>
<td>Trace</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

As the number of plants carrying nectar-pollen increases, the resources that bees can visit also increase. To be able to produce honey at the desired level, elevation and flowering periods of the plants which are dominant nectar-pollen potential should be considered. In this study, periods in which each nectar flow is calculated by Equation 3. In Equation 3 Ni i. Nectar is the ratio of nectar that nectar has, and Nic is the coefficient expressing the effect of nectar on honey formation. Pi i. how much pollen is possessed by the plant having the pollen potency, and Pic gives the constant value-coefficient expressing the effect of the pollen on honey formation. These coefficients were determined because of questionnaires conducted with selected experts from the stakeholders of the study. The effect of nectar on honey formation was found to be 0.8, and the effect of pollen on honey formation was found to be 0.2 (Bayır & Albayrak, 2016; Albayrak et al., 2018). Because of operating the equation for cities, the nectar flow density coefficient (NFDC) is obtained for each province.

\[ NFDC = \sum_{i=0}^{t=n} (Ni * Nic + Pi * Pic) \]  

Here n is the number of plants carrying pollen each nectar has. In this study, the NFDC coefficient is primarily scaled and then limited as migratory beekeepers are considered. At the end of the scaling, the NFDC coefficient is defined between zero and one hundred. As migratory beekeepers were considered, the numbers below the NFDC coefficient value
of thirty were not considered (Bayır & Albayrak, 2016). At the same time as Table 4, the nectar flow periods of the provinces recommended for the traveler bee growers are given. The value of bee farming potential (BFP) obtained because of FCM can vary for each province. In this study, provinces with BFP value less than 45% were not included in the evaluation because migratory beekeepers were considered.

RESULTS AND DISCUSSION

After the determination of beekeeping breeding potential (Table 5), ArcGIS from GIS software was used for visualization. In the literature, the use of GIS in the field of beekeeping is recommended to be used as a technology to allow new discoveries (Rogers & Staub, 2013). Turkey on a thematic map in ArcGIS environment, independent of spatial information, the data is processed. With the processing of the data, a total of 12 maps were produced, giving nectar flow periods for the whole country. One of these maps is given in Figure 4.

*Figure 4. Potential map of beekeeping.*
Table 5
Assessment of the provinces selected for FCM (bee farming potential) and nectar periods

<table>
<thead>
<tr>
<th>Province</th>
<th>Bee Farming Potential (FCM) (%)</th>
<th>Nectar Flow Time (Month) (Equation 3)</th>
<th>Nectar Flow Time (Month) (Equation 3)</th>
<th>Total Area (km²)</th>
<th>Areas where bee farming can be done! (km²)</th>
<th>Areas where bee farming can be done! (%)</th>
<th>Number of Colonies (x1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93.75</td>
<td>5,6,7,8</td>
<td>14,03</td>
<td>8,2777</td>
<td>59%</td>
<td></td>
<td>375</td>
</tr>
<tr>
<td>2</td>
<td>46.875</td>
<td>1</td>
<td>7</td>
<td>7,436</td>
<td>41%</td>
<td></td>
<td>46.875</td>
</tr>
<tr>
<td>3</td>
<td>90.625</td>
<td>5</td>
<td>4,5,6,7,8,9</td>
<td>7,943</td>
<td>36%</td>
<td></td>
<td>453.125</td>
</tr>
<tr>
<td>4</td>
<td>53.125</td>
<td>2</td>
<td>7,8</td>
<td>14,927</td>
<td>44%</td>
<td></td>
<td>106.25</td>
</tr>
<tr>
<td>5</td>
<td>78.125</td>
<td>4</td>
<td>5,6,7,8</td>
<td>20,909</td>
<td>30%</td>
<td></td>
<td>312.5</td>
</tr>
<tr>
<td>6</td>
<td>84.375</td>
<td>3</td>
<td>6,7,8</td>
<td>14,299</td>
<td>45%</td>
<td></td>
<td>253.125</td>
</tr>
<tr>
<td>7</td>
<td>71.875</td>
<td>4</td>
<td>5,6,7,8</td>
<td>10,882</td>
<td>7%</td>
<td></td>
<td>287.5</td>
</tr>
<tr>
<td>8</td>
<td>65.625</td>
<td>2</td>
<td>7,8</td>
<td>8,253</td>
<td>60,40%</td>
<td></td>
<td>131.25</td>
</tr>
<tr>
<td>9</td>
<td>53.125</td>
<td>4</td>
<td>5,6,7,8</td>
<td>9,933</td>
<td>44%</td>
<td></td>
<td>212.5</td>
</tr>
<tr>
<td>10</td>
<td>87.5</td>
<td>4</td>
<td>5,6,7,8</td>
<td>12,134</td>
<td>43%</td>
<td></td>
<td>350</td>
</tr>
<tr>
<td>11</td>
<td>62.5</td>
<td>3</td>
<td>6,7,8</td>
<td>15,272</td>
<td>32%</td>
<td></td>
<td>187.5</td>
</tr>
<tr>
<td>12</td>
<td>78.125</td>
<td>4</td>
<td>5,6,7,8</td>
<td>6,098</td>
<td>70%</td>
<td></td>
<td>312.5</td>
</tr>
<tr>
<td>13</td>
<td>53.125</td>
<td>3</td>
<td>6,7,8</td>
<td>9,383</td>
<td>35%</td>
<td></td>
<td>159.375</td>
</tr>
<tr>
<td>14</td>
<td>95</td>
<td>3</td>
<td>7,8,9</td>
<td>25,355</td>
<td>22%</td>
<td></td>
<td>300</td>
</tr>
<tr>
<td>15</td>
<td>81.25</td>
<td>3</td>
<td>7,8,9</td>
<td>11,903</td>
<td>30%</td>
<td></td>
<td>243.75</td>
</tr>
<tr>
<td>16</td>
<td>78.125</td>
<td>3</td>
<td>7,8,9</td>
<td>6,934</td>
<td>30,40%</td>
<td></td>
<td>234.375</td>
</tr>
<tr>
<td>17</td>
<td>81.25</td>
<td>5</td>
<td>5,6,7,8,9</td>
<td>5,524</td>
<td>43%</td>
<td></td>
<td>406.25</td>
</tr>
<tr>
<td>18</td>
<td>81.25</td>
<td>2</td>
<td>8,9</td>
<td>9,521</td>
<td>78%</td>
<td></td>
<td>162.5</td>
</tr>
<tr>
<td>19</td>
<td>87.5</td>
<td>4</td>
<td>6,7,8,9</td>
<td>15,62</td>
<td>44%</td>
<td></td>
<td>350</td>
</tr>
<tr>
<td>Province</td>
<td>Bee Farming Potential (FCM) (%)</td>
<td>Nectar Flow Time (Month) (Equation 3)</td>
<td>Nectar Flow Time (Month) (Equation 3)</td>
<td>Total Area (km²)</td>
<td>Areas where bee farming can be done! (km²)</td>
<td>Areas where bee farming can be done! (%)</td>
<td>Number of Colonies (x1000)</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------</td>
<td>----------------------------------</td>
<td>---------------------------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>20 İzmir</td>
<td>90.625</td>
<td>4</td>
<td>5,6,7,8</td>
<td>12,007</td>
<td>5,88343</td>
<td>49%</td>
<td>362.5</td>
</tr>
<tr>
<td>21 Kars</td>
<td>62.5</td>
<td>3</td>
<td>7,8,9</td>
<td>2,347</td>
<td>1,50208</td>
<td>64%</td>
<td>187.5</td>
</tr>
<tr>
<td>22 Kayseri</td>
<td>50</td>
<td>3</td>
<td>7,8</td>
<td>17,17</td>
<td>5,151</td>
<td>30%</td>
<td>150</td>
</tr>
<tr>
<td>23 Kırklareli</td>
<td>75</td>
<td>3</td>
<td>6,7,8</td>
<td>6,55</td>
<td>3,7335</td>
<td>57%</td>
<td>225</td>
</tr>
<tr>
<td>24 Manisa</td>
<td>75</td>
<td>3</td>
<td>6,7,8</td>
<td>13,269</td>
<td>4,737033</td>
<td>35.70%</td>
<td>225</td>
</tr>
<tr>
<td>25 Muğla</td>
<td>98</td>
<td>7</td>
<td>4,5,6,7,8,9,10</td>
<td>12,974</td>
<td>6,22752</td>
<td>48%</td>
<td>700</td>
</tr>
<tr>
<td>26 Ordu</td>
<td>84.375</td>
<td>3</td>
<td>6,7,8</td>
<td>5,952</td>
<td>1,01184</td>
<td>17%</td>
<td>253.125</td>
</tr>
<tr>
<td>27 Sakarya</td>
<td>46.875</td>
<td>3</td>
<td>6,7,8</td>
<td>4,878</td>
<td>1,65852</td>
<td>34%</td>
<td>140.625</td>
</tr>
<tr>
<td>28 Samsun</td>
<td>65.625</td>
<td>4</td>
<td>6,7,8</td>
<td>9,352</td>
<td>3,92784</td>
<td>42%</td>
<td>262.5</td>
</tr>
<tr>
<td>29 Sivas</td>
<td>90.625</td>
<td>3</td>
<td>7,8,9</td>
<td>28,488</td>
<td>5,12784</td>
<td>18%</td>
<td>271.875</td>
</tr>
<tr>
<td>30 Siirt</td>
<td>81.25</td>
<td>2</td>
<td>8,9</td>
<td>6,182</td>
<td>0,9273</td>
<td>15%</td>
<td>162.5</td>
</tr>
<tr>
<td>31 Şanlıurfa</td>
<td>68.75</td>
<td>4</td>
<td>5,6,7,8</td>
<td>19,451</td>
<td>5,44628</td>
<td>28%</td>
<td>275</td>
</tr>
<tr>
<td>32 Trabzon</td>
<td>59.375</td>
<td>1</td>
<td>7</td>
<td>4,662</td>
<td>0,55944</td>
<td>12%</td>
<td>59.375</td>
</tr>
<tr>
<td>33 Van</td>
<td>62.5</td>
<td>3</td>
<td>7,8,9</td>
<td>21,334</td>
<td>3,41344</td>
<td>16%</td>
<td>187.5</td>
</tr>
</tbody>
</table>

Total Colony: 8,346,875
The potential value of beekeeping for Adana province is 93.75. This means that the honey yield per colony in Adana province is 93.75%. Of course, this yield will be achieved if the meteorological conditions are appropriate, the colony health is good, the hives are modern hives, the bees are suitable for the region and the beekeepers are sufficiently experienced in beekeeping. It is seen that the nectar flow period for Adana province is 4 months. It does not mean that there is no nectar flow season for the remaining 8 months of the year. It only means that there is a flow of nectar at maximum level in the 4-month period. This four-month period is 5 (May), 6 (June), 7 (July) and 8 (August) for Adana province are months. Migratory beekeepers are predominantly coming to the city of Adana during these periods.

Figure 4 gives a map for the month of June. How many hives can be placed in Adana province can be determined by subtracting settlements, industrial zones, bare mountains, steppes, agricultural land and pesticides from the total surface area of the province. The total area of Adana is 14,030 km\(^2\). The area where beekeeping can be done covers 59% of this area. This area is 8,277 km\(^2\). The number of colonies subjected to migratory beekeeping was equal to the number of beekeeping potentials multiplied by the nectar flow periods. In this way, bee colonies subjected to migratory beekeeping during the nectar flow season can be distributed to the zones depending on the nectar flow density.

With this study, while the pollination of the plants is achieved at the maximum level, the maximum efficiency is obtained from the bee colonies on the other hand. More colonies are accepted during periods of intense nectar flow and pollination is not missed. Due to the density of nectar flow, the number of bee colonies accepted by each province varies dynamically. As such, this study can be considered as sustainable agricultural practice. Figure 5 shows the software screen used by stakeholders. The software used by the stakeholders was developed using the C# programming language on the Microsoft .NET platform.

Stakeholders are logged into the system with username and password. Meteorological data are automatically updated by the system. This information is obtained through web services following the publication of the State Meteorology Department. ACC administrators instantly enter into the system the number of hives that come into their province. Thus, migratory beekeepers can determine their route by following the number of colony the provinces they will go to simultaneously. The maps give the potential for bee farming up to date and at the same time contain the knowledge of how many bee colonies it can take. The introduction of a new bee feed into the system and the updating or deletion of the existing plant in terms of nectar and pollen have been left to the ACC administrators.
Testing the System with Migratory Beekeeper Information

We tested the proposed information system with information from the migratory beekeeper. The head of the Beekeepers Association of Ordu Province, who is a migratory beekeeper, has 300 bee colonies. This person spent the year 2017 hosting three bee colonies in three different places. He was in Mersin / Tarsus from 15 January 2017 until 15 May 2017. Then from 15 May 2017 until 15 September 2017, the bee colonies were hosted in Erzurum / Hınıs. This period is the period of nectar flow for that region. The bee colonies produced the actual honey production during this period. It is a high plateau near the Van district of Hınıs district of Erzurum. Table 6 gives the amount of honey produced per colony in these three periods.

Table 6
Migratory beekeeper yield per colony

<table>
<thead>
<tr>
<th>Accommodation Periods</th>
<th>Mersin</th>
<th>Tarsus</th>
<th>Erzurum</th>
<th>Hınıs</th>
<th>Samsun</th>
<th>Çarşamba</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 Jan - 15 May</td>
<td>~7.1 kg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15 May - 15 Sep</td>
<td>-</td>
<td>~47 kg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15 Sep – 15 Jan</td>
<td>-</td>
<td>-</td>
<td>~6.4 kg</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
The migratory beekeeper finally housed bee colonies from September 15, 2017 until the end of the year on Samsun / Çarşamba. In this period, bee colonies were able to produce only winter honey. As shown in Table 5, the migratory beekeeper produced the actual honey production in Erzurum / Hınıs and between 15 May and 15 September. In this section, the migratory beekeeper information (for 2017) and the proposed system were compared.

The apiculture potential of Erzurum province was determined as 95% (Table 5). The migratory beekeeper was able to produce 47 kg of honey per colony in this region. This value is significantly high as honey yield per colony obtained in Turkey. The highest yield was measured to be about 51 kg per colony in Turkey (Kekeçoğlu et al., 2007). When compared with this value, it is seen that the system gives correct results with 92%. Similarly, the period of nectar flow for Erzurum province was determined as 3 months. The migratory beekeeper remained in the region for 4 months. Migratory beekeepers can stay longer in the regions where they go to avoid the period, especially during nectar flow periods.

CONCLUSION

In this study, the number of colonies that can be taken by the nectar flow density is calculated. These numbers are for migratory beekeepers. Migratory beekeepers can enter the system, choose the appropriate ones for themselves and make annual migration plans. In determining the number of colonies based on the density of nectar flow, it is possible for the nectar flow seasons not to be missed (all nectar can be collected) and the bees can reach the maximum honey yield per colony without entering the stratum without being in the race.

In Turkey, TBA coordination and cooperation between the ACC is not sufficient. Due to the inadequacy of cooperation, there can be major problems from time to time. This study is important because it is a transparent application that will minimize the problems among the relevant stakeholders. While our country is in a very favorable position for beekeeping as plant diversity, the world average of honey per colony has not yet been caught. Developing an application that agricultural farming bees for Turkey within the country must be sustainable. GIS-supported remote sensing methods can be used for more stable and real-time operation of the system.

REFERENCES


Evaluating the Surrounding Physical Habitat for *Thynnichthys thynnoides*'s Spawning Areas using a Visual-Based Habitat Assessment at Rui River, Perak

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ABSTRACT

This study was conducted to evaluate the physical habitat of spawning areas for *Thynnichthys thynnoides* (*T. thynnoides*) in the Rui River, Gerik, Perak. Five sampling sites of Rui River’s floodplain were chosen. Sampling was conducted between May and October 2015 by using a visual-based habitat assessment developed for Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish. This study showed that *T. thynnoides* still migrated to the upper stream of Rui River during spawning season mainly in August. Habitat assessment scoring indicated that the physical habitat structure of Rui River fell into a suboptimal category, which was most likely able to support fish populations and thus providing a suitable habitat for *T. thynnoides* during the spawning season. Conclusively, it was observed that the *T. thynnoides* population was dependent on environmental conditions.

Keywords: Fisheries, habitat, riverine, Rui River, spawning, *Thynnichthys thynnoides*

INTRODUCTION

*Thynnichthys thynnoides* (Bleeker 1852) or Lomah (Malay common name) was categorized on the IUCN Red List as a species of least concern (Ambak et al., 2010; Vidthayanon, 2012). However, Chong et al. (2010) classified *T. thynnoides* as being under medium threat due to several factors such as overfishing, habitat degradation
and pollution (Dolasoh, 2014). It is anticipated that fishing pressure on *T. thynnoides* will increase as this species is selectively targeted during the spawning season (“Loam fish threatened”, 2015). Although there are no high threats recorded at present, habitat degradation and overexploitation could be a concern in the future (Casazza et al., 2016). Alteration of the river habitat is becoming an alarmingly serious threat to fish populations, especially to *T. thynnoides* in the Rui River. Turbidity and sedimentation caused by tin mining, which is operated at the upper stream of the Rui River, may worsen the situation. In a recent study, the mean seston value in the Rui River was $9.97 \pm 12.59$ mg/L; this was significantly greater compared to before $(0.25 \pm 0.05$ mg/L) and after $(1.40 \pm 0.52$ mg/L) its convergence with the main stem of the Perak River (Zarul, 2013). Survival of *T. thynnoides* is mostly influenced by movement from one habitat to another. During flood inundation, *T. thynnoides* use floodplain habitats for various purposes such as shelter, breeding, nursery and feeding. The timing and duration of flooding are highly variable, and greatly affect the growth and survival of fish populations. *T. thynnoides* has been subject to special consideration as they are particularly vulnerable to changes in river ecology that is induced by human activities. This vulnerability shows that this fish is inter-connected with its habitat and surrounding land use. Consequently, the establishment and maintenance of suitable spawning habitats, and the recruitment and maturation of fish stocks, are crucial in sustaining and protecting these riverine fisheries.

Therefore, this study aims to assess physical structures of riverine habitat that influence *T. thynnoides*’s spawning activity. This study contributes to the body of knowledge regarding freshwater fish species in Malaysia. Information on the surrounding environment of *T. thynnoides* from this study benefits a conservation-conscious society and researchers generally. The research outcome from this study is expected to inform various governmental agencies, namely, the Department of Fisheries (DOF), Department of Environment (DOE), district councils, and other stakeholders regarding *T. thynnoides* species and its environmental status in the Rui River. This study provides relevant information in assisting the stakeholders’ decision-making process while they constitute regulation or governance matter on the Rui River, especially for *T. thynnoides*. Inevitably, decisions made by these stakeholders directly or indirectly affect local communities that depend on fisheries as their source of income. Besides that, future generations would benefit from sustainable fisheries with a continuous effort to protect the environment, maintain source of income, and conserve life quality in the sense of the economic and aesthetic value of the Rui River. Sustainable fisheries could provide improved and sustained of the local communities’ quality life.
MATERIALS AND METHODS

Site Description

This study was conducted in the Rui River, which is one of the Perak River tributaries in Hulu Perak District (Figure 1). The Rui River is about 38.48 km long, with a catchment area of 868 km² (Figure 1). It flows from the Banjaran Bintang watershed and heads north towards Intan before flowing southeast to Kampung Lalong (Lalang village) and finally merges with the main stem of the Perak River. The mouth of the Rui River is located about 4-km below Bersia Dam. The flow then heads to Kenering Reservoir, which is about 40 km further downstream.

Figure 1. Location of the Rui River in Perak, Malaysia, shown in circle
The Rui River is surrounded by several forests of unknown status and certain compartments of the respective forest have been cleared for oil palm plantation. In the upstream area, a small impoundment of unknown size and status exists. The Rui River is used extensively for small scale fishing, agricultural irrigation, and recreational uses (Table 1). Some of the local villagers still use ‘Lei’, which is a traditional method to catch migrating fishes, such as *T. thynnoides* and *Osteochilus hasselti*, at the Rui River. A summary of each sampling point is described in Table 1.

**Table 1**

*Summary of descriptions and coordinates of sampling points on the Rui and Perak rivers*

<table>
<thead>
<tr>
<th>Sites</th>
<th>Descriptions</th>
</tr>
</thead>
</table>
| S1 Kampung Alai | • Coordinate: N 05.62 1394° E 101.08 3572°  
• River bank substrates are mainly sand, rock, mud and silt.  
• Sand bar visible during low water level.  
• Rubber tree plantations along both sides of the river.  
• Locals use the river for small scale fishing and agriculture. |
| S2 Kampung Bharu | • Coordinate: N 05.586 96° E 101.08 48°  
• River bank substrates are mainly sand, rock, mud and silt.  
• Secondary forest along both sides of the river.  
• Locals use the river for small scale fishing during rainy season.  
• S2 was approximately 5.3 km from S1. |
| S3 Kampung Plang | • Coordinate: N 05.57 987° E 101.09 438°  
• River bank substrates are mainly sand, rock and mud.  
• Big boulders reduced the water path and increased the water flow.  
• Secondary forest along both sides of the river.  
• Locals used the river for small scale fishing during rainy season.  
• S3 was approximately 1.7 km from S2. |
| S4 Kampung Kerunai | • Coordinate: N 05.51 4777° E 101.13 0459°  
• River bank substrates are mainly sand, mud and silt.  
• Oil palm tree plantation along both sides of the river.  
• S4 was approximately 12.5 km from S3. |
| S5 Kuala Rui | • Coordinate: N 05.45 468° E 101.18 043°  
• River bank substrates are mainly sand, mud and silt.  
• Oil palm and rubber tree plantations along both sides of the river.  
• Dataran loma with concrete river bank located here.  
• Locals used the river for fishing during rainy season.  
• S5 was approximately 13.5 km from S4. |
| S6 Kampung Perah | • Coordinate: N 05.44435° E 101.17 500°  
• River bank substrates are mainly sand, mud and silt.  
• Rubber tree plantation along both sides of the river.  
• Locals used the river for fishing during rainy season.  
• Two small jetties used by local fishermen to land fish.  
• Hectic boat transportation from dawn to dusk.  
• S6 was approximately 2.55 km from S5. |
| S7 Air Ganda | • Coordinate: N 05.44 966° E101.19 086°  
• River bank substrates are mainly sand and mud.  
• Oil palm and rubber tree plantations along both sides of the river. |
Habitat Assessment

For this study, a visual-based habitat assessment was used based on the modified parameter checklist from the Rapid Biological Assessment Protocol (Barbour et al., 1999). For streams, an encompassing approach to assessing the structure of the habitat, which includes an evaluation of the variety and quality of the substrate, channel morphology, bank structure, and riparian vegetation. Habitat parameters pertinent to the assessment of habitat quality cover those that characterize the stream in to a “micro scale” habitat (e.g., estimation of embeddedness), the “macro scale” features (e.g., channel morphology), and the riparian and bank structure features that are most often influential in affecting the other parameters. Measurements of these parameters or characteristics serve to stratify and place streams into distinct classifications.

The primary data collection was conducted from May 2015 to October 2015. The selection of five sampling points (from S1 to S5) for this assessment was based on the spawning areas of *T. thynnoides* (N = 5 sampling points × 3 replicates × 3 observers = 45 samples/river). The location of this fish in May 2015 was believed to be further downstream of Perak River, and the fish were spotted moving upstream from S7 and S8 to S5 in August (Mohamad Radhi, 2017). In September and October 2015, *T. thynnoides* started to migrate further to the upper stream of the Rui River at S2 and S3 (Figure 2).

Based on the Rapid Biological Assessment Protocol guideline (Barbour et al., 1999), this habitat assessment consists of 10 parameters, which are (1) substrate, (2) habitat complexity, (3) velocity-depth combination, (4) bank stability, (5) bank conservation, (6) vegetation cover, (7) vegetation diversity, (8) intensity of human activities, (9) water cognition, and (10) riverside land use. For parameters one to seven (as previously listed), each parameter was evaluated at four different levels of condition: optimal (score of 16-20), sub-optimal (score of 11-15), marginal (score 6-10), and poor (score of 1-5), according to the guideline. For parameters eight to ten, each bank was evaluated separately from 0 to 10, and the scores for both right and left banks were then cumulated (refer Barbour et al., 1999). Scores increase as habitat quality increases (refer to Appendix A). The sum of
10 parameter scores was calculated then the habitat assessment scores were obtained by using the formula for Physical Characterisation score (Barbour et al., 1999):

\[
\text{Total Physical Characterisation Score} = \frac{\text{Sum score of ten parameters}}{10}
\] (1)

Figure 2. Migration movement of *Thynnichthys thynnoides* towards spawning areas during spawning season (May to October 2015). Spawning areas are predicted at S2 and S3 as no migration was observed at S1 during spawning season.
The total scores were then totaled and compared to a reference condition to provide a final habitat total scoring. A brief set of decision criteria is given for each parameter corresponding to each of the four categories reflecting a continuum of conditions (optimal, suboptimal, marginal, and poor) (Table 2). All calculation and procedures for habitat assessment followed Rapid Bioassessment Protocols (Barbour et al., 1999).

Table 2  
*Integrated scores for habitat suitability conditions*

<table>
<thead>
<tr>
<th>Condition Categories</th>
<th>Total Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>16-20</td>
</tr>
<tr>
<td>Suboptimal</td>
<td>11-15</td>
</tr>
<tr>
<td>Marginal</td>
<td>6-10</td>
</tr>
<tr>
<td>Poor</td>
<td>0-5</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

This study revealed that the habitat along the Rui River was in good condition. The habitat score ranged from 11.6 to 18.8. Sampling sites of S2, S3, and S4 were categorized as optimal while S1 and S5 were considered as suboptimal (Table 3).

Station 2 had the highest average scores for condition category (18.8) as compared to other sampling sites (Table 3). Station 2 supported favorable substrate for epifaunal colonization and fish cover because S2 was located upstream of the Rui River and there were only local housing areas established nearby, especially at the riparian zones. The intact forest of the river habitat protects the river from exposure to direct light and shades the stream (Rohasliney & Jackson, 2008; Rohasliney & Jackson, 2009; Rohasliney, 2010). This is because riparian forests provide a wide range of key ecosystem function and services (da Silva et al., 2017) that can serve the needs of *T. thynnoides* during spawning season and its juvenile at the nursery grounds. The stream channel consists of a mixture of substrate materials, gravel and sand, root mats, and submerged vegetation. This area was also full of deep pools with a distinctive stream. Station 3 was categorized as optimal, which had similar habitat characteristics with S2. Both sites had deeper pools and strong water flow after the stream section. There were minor sediment depositions in the area. An optimized river has little or no enlargement of islands or point bars, and less than 5% of the bottom is affected by the river. Water channel flow status at S2 and S3 districts were considered good, where water reached the base of both lower banks, and only a minimal amount of channel substrates were exposed. The mixture of four different velocities (slow-deep, slow-shallow, fast-deep and fast-shallow) were observed at the sampling sites. Channel alterations were observed at S2 and S3 with normal pattern streams and the bends in the area were stable. The surrounding areas were still protected by more than 90% of the stream bank surfaces, and an immediate riparian zone was covered by native vegetation;
including trees, understory shrubs, or woody macrophytes. Thus, S2 and S3 theoretically were considered to be the most attainable habitat for fish spawning areas and nursery grounds for the juveniles.

S1 and S5 were categorized as suboptimal (11-15) (Table 3). The average habitat condition in S1 and S5 areas scored 12.0 and 11.6, respectively. The area for S1 was mainly dominated by agricultural activity, and S5 was located near a small-town area where schools, shops, and oil palm plantations were. The substrate was either disturbed or removed. Mud or sand could be observed in the bottom of the river with no root mat submerged vegetation attached. The shallow pools and deep pools were found in similar numbers. A previous channelization was present in the areas of bridge abutments. The most obvious establishment built at S5 was the Dataran Loma (Loma Square). This area was built to facilitate the locals and outsiders capture *T. thynnoides*. The pickled fish (ikan pekasam) traders were found at S5 and bought captured *T. thynnoides* in bulk during spawning season. S5 may not be the spawning areas for the fish, but S5 is the path for migration towards its spawning areas. Conservation and preservation of this stream channel from habitat degradation are crucial.

Urbanization in this area had caused river bank erosion and the accumulation of sediment in the stream. A major flood in 2014 was also one factor that caused significant erosion at S5. The fast-current flow from the upper stream of the Rui River and overflowed water from the main stem (Perak River) entered S5 and caused erosion. Sediment deposition of both sampling points showed that the river bottom was affected by a mixture of gravel, sand, and fine sediment form bars. The local fishermen stated that they had difficulty maneuvering their boats to S5 during intermediate season, as the sediment deposition lowers the water level. Only a few tracks remained accessible to S5 from the main stem (Perak River). Similar issues occurred in Morobe, Papua New Guinea, as there were discernible changes in water quality at Nauti due to a mining operation upstream of the Watut River. Suspended solids were reported to have increased about 100-fold in Watut River throughout the 16 years of observation (Roche & Mudd, 2014).

S4 revealed an optimal condition with a score of 15.3 (Table 3).

<table>
<thead>
<tr>
<th>Sampling points</th>
<th>Total score of habitat parameter</th>
<th>Condition score</th>
<th>Condition category</th>
<th>Average condition category for Rui River</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>120</td>
<td>12.0</td>
<td>Suboptimal</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>188</td>
<td>18.8</td>
<td>Optimal</td>
<td>Suboptimal (15.3)</td>
</tr>
<tr>
<td>S3</td>
<td>186</td>
<td>18.6</td>
<td>Optimal</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>153</td>
<td>15.3</td>
<td>Optimal</td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>116</td>
<td>11.6</td>
<td>Suboptimal</td>
<td></td>
</tr>
</tbody>
</table>

Table 3

*Condition category of habitat assessment in the Rui River*
This sampling point was an area of local housing and oil palm plantation within the riparian zone (Figure 3).

Deposits of fine materials increased the bar development and less than 5% of the bottom changed frequently. As for the channel flow status, there were different velocities with small streams. The channel stream had been routed to build a bridge, but the bank seemed to be stable with vegetation cover and trees. For vegetative protection, 90% of the stream bank surfaces were estimated to be covered by vegetation, and it was obvious that disruption occurred. The width of the riparian zone was observed to be more than 18 meters, and human activities had little impact on the zone. Statistically, the habitat condition scores of S2 and S3 (upper stream) differed from the score of S5 (downstream of the Rui River). Results of Kruskal-Wallis analysis showed that the score differed among sampling
sites \( r^2/n (4, N = 273 = 538,7236, P < 0.05) \). S4 had the highest mean rank of 11,051, as compared to other sampling points. The lowest mean ranks with a significant difference value of \( U = 305.5, P < 0.05 \), was S3 (rank sum = 1995.5) and S1 (rank sum = 1085.5). The highest mean rank with a significant difference value of \( U = 3879.5, P < 0.05 \), was between S5 (rank sum = 5285.5) and S4 (rank sum = 6960.5).

The habitat result for this study is supported by Chong et al. (2010) in their evaluation on habitat status for *T. thynnoides*. The conservation status provided in their study included habitat degradation, overharvesting, and pollution. From this study, the habitat in the Rui River is potentially at threat from the existing sources of pollution, namely tin mining and sand mining, at the upper stream of the river. Based on observations, the tailings were spotted along the Rui River, deposited on river banks and sand banks. The impact from tin mining, such as heavy sedimentation, sand bank formation, and changes in water quality, also occur at other metal mining sites such as Selangor River, Selangor (Nurhidayu & Azhar, 2015), Chini Lake, Pahang (Ahmad et al., 2008), Natchez Trace Parkway, Mississippi, USA (Rohasliney & Jackson, 2007), Morobe, Papua New Guinea (Roche & Mudd, 2014), and Tanshui River, Taiwan (Young et al., 2014). This situation, if not mitigated well by stakeholders, could greatly affect the population and spawning area of *T. thynnoides* species at the Rui River. Based on the assessment of this study, S2 and S3 are the most suitable spawning sites for *T. thynnoides*, as they have high habitat scores (Figure 3). The existing cover from trees, and natural channels with abundant river bank vegetation, may promote spawning activity for the species.

**CONCLUSION**

In conclusion, it is apparent that habitat degradation due to land use and water pollution inhibit replenishment of the fish species that inhabit the Rui River. Being a unique fish species, which migrates from downstream to upper stream to locate a suitable spawning area, *T. thynnoides* needs the best attainable habitat in order to survive, grow and reproduce. This study revealed that the habitat along the Rui River is in good condition with overall suboptimal status from the physical habitat assessment score. Thus, the Rui River provides suitable habitat for a spawning site for *T. thynnoides*.

**REFERENCES**


An Android-Based Ubiquitous Notification Application for Bukidnon State University

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ABSTRACT

Events are the most important bits of information that should be delivered timely wherever the person is. It is but equally important that a person must have the ability to keep abreast with the recent trends of technology in order to be notified of the important events that need to be addressed or responded immediately. Sometimes, notification of events is given in a short period of time, which sometimes leads to failure in attending or responding such events. In today’s generation, smartphones are always and almost available to every individual, with which one of its most important features is notification. It is under this premise that prompted the researchers to develop an Android-based ubiquitous notification app for Bukidnon State University (BukSU) to speed up the dissemination of information about the upcoming events and activities of the university. The University Administrators will act as an admin in order to input and update the list of activities or events for the entire academic year which is either planned, unplanned, routine or emergency in nature. The faculty, staff, students and other stakeholders will act as a user and will be notified of the academic activities. A Waterfall Model was used in the design process and Android studio in the development of the application. As a result, the system was tested in terms of its functionality, applicability, relevance, and usability. The overall result has a mean of
4.736 which indicates that the respondents are very satisfied with the mobile application. A ubiquitous notification app is currently being utilized by the Bukidnon State University Administrators in order to notify the students, faculty, staff, and alumni in notifying them of the event before it takes place.

**Keywords:** Android app for notification, Bukidnon State University Notification App, BukSU Notification App, information system, mobile apps, notification app, notification system, Ubiquitous Notification App

**INTRODUCTION**

A notification is something that gives official information to someone through mobile phones. According to El-Gazzar et al. (2010), events are the most important bits of information that should be delivered timely wherever the user is. Information and communications technology can help people stay updated with events in the world by Vastenburg et al. (2009). As it is mentioned by Shirazi et al. (2014), notifications are a core feature of mobile phones because they inform users about a variety of events. Osunade et al. (2014) also stated that people were involved in many activities that were planned, unplanned, routine and emergency in nature.

According to the study of DeVoe (2008), in his article about the “Evolution of Notification Systems”, most of the American generally favored the phone and e-mail notification system. Even with the benefits gained from choosing the latest and popular technologies, people remain comfortable with radio and TV broadcasts for sending information or announcement from schools. The study shows that only 39% of these respondents say that their school is “very strong” or “good” in transmitting emergency information. Thus, the researchers felt the need to introduce mobile-based notification system as the latest and more popular tool for reaching out to the stakeholders.

Gulum and Murray (2009) conducted a study on the evaluation of the effectiveness of a mass emergency notification system. These systems being studied were used to deliver information about critical events. The survey results showed that even though students recognized a mass emergency notification system, their belief and lack of mindfulness in the systems might be the reasons behind the very small confirmation rates. Thus, the researchers introduced a new technology where students could enjoy the benefits of using their mobile phones and be notified easily with the latest updates of their school. Emphasizing the importance of the notification system for Bukidnon State University, the researchers joined the school during University-wide student and stakeholder’s forum and orientation to introduce the newest app.

A study was conducted by Voos et al. (2013) on the notification system for the medical event using SMS over the cellular network. The study aimed to determine the implementation of the mobile-based technology which guarantees to reach all recipients even in remote locations. Though SMS services are promising due to the massive adoption of phones, the researchers realized the financial implication it requires when engaging with
the Telecommunications Company for the mass sending of information. The proposed system has the advantage of low-cost development and implementation since the mobile application can be downloaded for free.

In 2013, Campus Announcement Platform- Email and Voice Alert, developed an ideal campus alert system for colleges and universities that needed to communicate quickly and effectively to all personnel, volunteers, students, media, first responders, media and others. This study gives the researchers an overview of the different techniques used by authors in developing mobile applications specifically for notification applications. It also shows factors to consider when to develop mobile applications.

Moreover, the researchers intended to develop a new platform for the school using the Desktop and Mobile Application Alert System as an effective tool for information dissemination. Figure 1 illustrates the conceptual framework of Ubiquitous Notification App of Bukidnon State University. The university administrator inputs the list of activities or events for the entire school year using the application. The administrator is the only person who is authorized to make updates whenever there are changes in the calendar. These inputted lists of activities are then be processed through Android-based ubiquitous notification app.

This study generally aims to develop an Android-based ubiquitous notification app for Bukidnon State University. Specifically, it aims to:

1. Design an Android-based ubiquitous notification app that can speed up the dissemination of information about upcoming events and activities of the university.

![Conceptual framework of Ubiquitous Notification App](image_url)
2. Develop a user-friendly and secured Android-based ubiquitous notification app

3. Implement a downloadable Android-based ubiquitous notification app mobile application which will be accessible by users through Google Play.

4. Evaluate the Android-based ubiquitous notification app in terms of its functionality and usability.

The Ubiquitous Notification App will be a great help to assist the BukSUan’s (name assigned to students and other stakeholders of BukSU) to be notified of the different events and activities of the university. Through the Ubiquitous Notification App, they will no longer go to the nearest bulletin boards or ask anybody with regard to information that they need to know.

METHODS

The researchers used the Waterfall Model (see Figure 2) and Android studio in the development of an Android-based ubiquitous notification app. The study began with a data gathering. Survey questions were given to students and instructors to identify the common problems encountered in the course of disseminating information. Results of such survey were used as a guide in developing a more effective requirements’ specification for the application. After specifying the requirements, the researchers made use of the Android studio in application development.

![Figure 2. Waterfall model](image)
Research Design

Logical Design. Figure 3 shows the Entity Relationship diagram of the system which consists of 11 tables namely: accounts, profiles, job status, colleges, venues, year levels, departments, attendee, category, announcements, and events. The profiles have five connected foreign keys, and table events have eight connected foreign keys. The use of those foreign keys is to have a cascade update and restricted deletion of the data. Use of the foreign keys can secure the data when there is an unofficial adding and updating of the database.

Figure 4 shows the Use-Case model applied to the ubiquitous notification system showing different sections and its corresponding events.

Figure 5 shows the actual flowchart of the ubiquitous notification system for users in the Bukidnon State University.

Figure 6 illustrates the organized summary of all model elements of the project. This shows the relationship between diagrams, how they are related and how each depends on with regard to what they are representing in the overall functionality of the application. The Client Side Mobile Application depends on the Server Side Application because the client side receives notifications coming from the server side. Model elements coming from the Server Side Application are required and are considered dependent on the Client Side Mobile Application. The Server-Side Application is also dependent on the Server Side Model which describes the model being applied to the Server Side Application’s functionality. All other packages are dependent on the Database Model, which is the basis of the organization and collection of data which is represented by events.

Figure 3. Entity Relationship diagram
Figure 4. Use-Case model

Figure 5. Flowchart for Ubiquitous Notification Application for user
Development Tools

The system uses Java and Android SDK as software development tool in developing a mobile-based application program under Android Studio Platform. On the web development aspect, PHP and JavaScript were used for adding creativity to Web sites and asynchronous to Web applications. MySQL and SQLite are used as an open source SQL database to store files on a device. These tools were used in order to come up with a Notification System for Bukidnon State University in all of its academic and non-academic undertakings, planned or unplanned. The researchers deployed the system to the user of the application with admin functionality which is the Vice President for Administration, Planning, Development (VPAPD) and the Vice President for Academic Affairs (VPAA) of Bukidnon State University.

RESULTS AND DISCUSSION

Table 1 shows the number of samples who participated in the evaluation of the application. There are forty (40) BukSUan’s or users who evaluated the Android-based Ubiquitous Notification App during the Stakeholder’s Forum. Using random sampling method, 5 participated from each college: College of Arts and Sciences (CAS), College of Education (COE), College of Business (COB), College of Social Development and Technology (CSDT), College of Nursing (CON) and College of Law (COL). A total of 30 students joined during beta testing. Other evaluators include 5 teaching personnel and 5 non-teaching personnel of the University. The number of samples was determined during the said activity organized by the Office of the Vice President of the Administration, Planning, and Development (VPAPD) where the group introduced and demonstrated the mobile application intended for Bukidnon State University. The application is evaluated in terms
of its usability, functionality, applicability, and relevance. The system assessment is as follows: 5- very satisfied, 4-somewhat satisfied, 3-neutral, 2-somewhat dissatisfied and 1-very dissatisfied. Summary of the evaluation is shown in Table 1.

Table 1
Number of samples

<table>
<thead>
<tr>
<th>Unit/College</th>
<th>Number of Evaluators</th>
</tr>
</thead>
<tbody>
<tr>
<td>College of Arts and Sciences (CAS) Students</td>
<td>5</td>
</tr>
<tr>
<td>College of Education (COE) Students</td>
<td>5</td>
</tr>
<tr>
<td>College of Business (COB) Students</td>
<td>5</td>
</tr>
<tr>
<td>College of Social Development and Technology (CSDT) Students</td>
<td>5</td>
</tr>
<tr>
<td>College of Nursing (CON) Students</td>
<td>5</td>
</tr>
<tr>
<td>College of Law (COL) Students</td>
<td>5</td>
</tr>
<tr>
<td>Teaching Personnel</td>
<td>5</td>
</tr>
<tr>
<td>Non-Teaching Personnel</td>
<td>5</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>40</strong></td>
</tr>
</tbody>
</table>

Table 2 shows the summary of system evaluation. The overall result has a mean of 4.736 which indicates that the respondents are very satisfied with the Android-based ubiquitous notification app. The usability which covers the user interface and security has a mean of 4.6875 and 4.73125 which indicates that the respondents are very satisfied. In terms of functionality, it has a mean of 4.69 which also indicates that the respondents are also very satisfied. With regard to the applicability, it has a mean of 4.825 which indicates that the respondents are again very satisfied application. The overall relevance has a mean of 4.768 that indicates that the respondents which are the users are very satisfied.

The data presented shows that 95% of the students from different colleges noted that the Android-based ubiquitous notification app was very applicable and easy to use. 90% of the students were notified based on the survey. However, 40% of students said that the application loaded quickly and does not crash. The result of the survey shows that 17 or 85% of 20 students surveyed received notifications on time. The survey conducted helped the researchers in the modification of the application of the better performance of the system.

Currently, the system is being maintained by the Information and Communications Technology-Service Unit (ICT-SU) of the Bukidnon State University thru the Office of the Data Center. Regular preventive, corrective and adaptive maintenance activities are being conducted to make sure that the system is fully operational. At present, the use of a private network with a dedicated connection to the web which works only for the app is being highly considered and will be integrated into the Strategic Planning of the said office in order to serve better the students and other stakeholders.
Table 2  
Summary of evaluation

<table>
<thead>
<tr>
<th></th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>Weighed Mean</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Usability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>User Interface</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. The design of the application is functional and visually stimulating</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.75</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>2. The content of the application meets customers needs</td>
<td>27</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4.65</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>3. The application delivers messages or warnings to the users whenever there are confirmation or permission to perform</td>
<td>31</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.775</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>4. The application provides useful feedback</td>
<td>29</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4.7</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>5. Readability of fonts and texts on the screen</td>
<td>29</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4.675</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>6. Overall Colour-Scheming</td>
<td>24</td>
<td>15</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4.575</td>
<td></td>
</tr>
<tr>
<td><strong>Security</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Password Encryption</td>
<td>29</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.725</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>2. Safety of user accounts and information</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.75</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>3. Availability of log information and history</td>
<td>29</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4.7</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>4. Access control to all account</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.75</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td><strong>Functionality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Availability to provide updated and accurate information</td>
<td>36</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4.875</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>2. Application loads quickly and does not crash</td>
<td>18</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.45</td>
<td>Satisfied</td>
</tr>
<tr>
<td>3. Visibility of all features of the application.</td>
<td>29</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.725</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>4. Users can exit the application at any time</td>
<td>29</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.725</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>5. Dynamic viewing of school activities</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.75</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>6. Available on the internet or Wireless LAN</td>
<td>25</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.625</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td><strong>Applicability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Actual response time of the application</td>
<td>36</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.9</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>2. The application is very easy to learn and the instructions are clear and simple to follow.</td>
<td>34</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4.825</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>3. The application notifies on time.</td>
<td>29</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.725</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>4. The instructions included are helpful</td>
<td>35</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.875</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>5. Responsiveness of the interface relative to the device being used</td>
<td>32</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td><strong>Relevance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Significance of the system in general</td>
<td>32</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>2. Significance of the study</td>
<td>29</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4.7</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>3. Ability of students to get notifications</td>
<td>33</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.825</td>
<td>Very Satisfied</td>
</tr>
<tr>
<td>4. Sustainability of the user’s notification application</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.75</td>
<td>Very Satisfied</td>
</tr>
</tbody>
</table>
On the other hand, features such as rates or comments will be integrated into the application program in order to solicit feedback from the end users as the basis for improvement. Table 3 shows the summary of comments and suggestions given by the students during software evaluation. Thirteen of the respondents said that the notification system at Bukidnon State University provided them real-time alerts. Four also agreed that the app was very useful especially for sending information which was critical or emergency in nature. Two respondents also commended the University and the developer for coming up with an alternative solution to inform the students and various stakeholders using the latest technology.

Table 3
*Summary of comments and suggestions*

<table>
<thead>
<tr>
<th>Comments/Suggestions</th>
<th>Number of Times Mentioned</th>
</tr>
</thead>
<tbody>
<tr>
<td>This notification system solution of Bukidnon State University is</td>
<td>4</td>
</tr>
<tr>
<td>useful for sending information which are emergency in nature</td>
<td></td>
</tr>
<tr>
<td>Please provide prompt notification for unread announcements/events upon log-in</td>
<td>2</td>
</tr>
<tr>
<td>Separate read and unread notifications</td>
<td>2</td>
</tr>
<tr>
<td>It does alert me real-time</td>
<td>13</td>
</tr>
<tr>
<td>No specific office that provides the announcement</td>
<td>1</td>
</tr>
<tr>
<td>Kudos to BukSU for introducing latest technology</td>
<td>2</td>
</tr>
</tbody>
</table>

Meanwhile, there are two respondents who suggested having better notification scheme to prompt the user for any unread notifications upon log into the system, as well as, a separate tab for a read and unread notifications. Moreover, one respondent mentioned about lacking information of the specific office/unit which announced the event or activity.

Figure 7 shows the process of administrator’s logging into the system. The system will navigate the administrator to the login page wherein the system will ask for the administrator’s username and password. After filling up the username and password, the administrator should click the login button. If the login is successful, the system will redirect the administrator to the home/main page.

Figure 8 shows the home page of the system wherein the administration can view the two important updates. They need to know first the number of announcements and events posted. Figure 9 illustrates the process of viewing the announcement section. After logging on to the system, the administrator can view the announcement list. Clicking the announcement section tab will show a drop-down list of announcements.

Figure 10 depicts the procedure of adding an event by clicking the event section tab. In the event section tab, a drop-down list of menus will show the Calendar of Activities, Add Event, and Event List tab. The administrator can view the Calendar of Activities by navigating to My Calendar Tab. The administrator is capable to add, edit and delete events.
Figure 7. Activity Diagram for Administrator’s Login

Figure 8. Activity Diagram for Administrator’s Home Section

Figure 9. Activity Diagram for Administrator’s Announcement Section
Figure 11 illustrates the process of navigating to the account setting section. By clicking the account setting sections on the home page, a drop-down list will appear which consist of Edit Account and View Accounts tab. In the edit account tab, the administrator can modify his account. He can also look at the list of registered users who can browse announcements and events through their Android devices by clicking the View Accounts tab. The administrator is responsible for registering the user to the System.

Figure 12 shows the interaction of the Administrator to the system upon logging in. The administrator will input the username and password to access the system. Figure 13 illustrates the interaction of the administrator of the system in viewing announcements. The administrator will navigate to the announcement section and view in the announcement list. The administrator can also search for a specific announcement.

Figure 14 shows the interaction between the Administrators to the System when navigating to Event Section. By clicking the Event Section, a drop-down list of tabs will appear containing Calendar of Activities. The second tab, which is the Add Event tab, can
An Android-based Ubiquitous Notification Application for Bukidnon State University

Figure 11. Activity Diagram for Administrator’s Account Setting Section

Figure 12. Sequence Diagram for Administrator’s Login
Figure 13. Sequence Diagram for Administrator’s Announcement Section Setting

Figure 14. Sequence Diagram for Administrator’s Event Section Setting
add event information. Last is the Event List wherein administrator can search for a specific event. The administrators can View Event, Edit and Update Events.

Figure 15 shows the interaction between the Administrators to the system. By clicking the Account Setting Section, a drop-down list will appear containing the Edit Account and View Account tab wherein the Administrator can make any changes to its account. In the View Accounts Tab, the administrator can see the list of registered users.

Figure 16 shows the interaction of the user with the application. Users need to have its own username and password by logging in to the system. The user can view announcement, calendar, view events, and update notifications.

Figure 17 illustrates the process of logging in to the system. The users may opt to use a 2-step verification process. The first step requires the user to enter its username and password. The second step requires the user to enter his registered mobile number. The mobile phone will receive an SMS message bearing the 4-digit code. The user will enter the code into the application and it will redirect the user to the Home/Main page of the application.

Figure 18 illustrates the process of navigating to the announcement tab. By simply clicking the announcement tab, the application will produce a list of announcements wherein user can also view specific event.

Figure 19 illustrates the process of navigating to the Calendar which will display the calendar of activities. The user has to click the date that holds an event in order to see the details.

![Sequence Diagram for Administrator’s Account Setting Section](image-url)
Figure 16. Sequence Diagram for Ubiquitous Notification Application for User
An Android-based Ubiquitous Notification Application for Bukidnon State University

Figure 17. Activity Diagram for User Login Using 2-Step Verification Process

Figure 18. Activity Diagram for User’s Announcement Section

Figure 19. Activity Diagram for User’s Calendar of Activities
Figure 20 illustrates the process of navigating to the View Events Tab. By clicking the view event, the application will display the details of the events.

Figure 21 shows the User’s Login Activity. To access the application, the user should provide a username and password. Once the user is successfully logged in, it will directly go to the home page, and the user can view all the information and details of the application.

Figure 22 shows the Home Page for the user. Once the user successfully logs in to the application, it will proceed to the home page which accesses various tabs.

Figure 23 shows the new List of Tabs for User. The page has four tabs which are Announcements, My Calendar, View Events and Logout. Under the new app version, the Update Now Tab which allows the user to refresh on the number of announcements and posted events has been removed due to automatic notification feature of the application. The Announcement Tab is used by the administrator to post a public statement, declaration, notification, report or any action of making a formal statement. The My Calendar Tab shows a chart of events organized according to days, weeks, and months of a year. The View Events Tab shows a summary of events and activities of the University organized in reverse chronological order. The Logout Tab ends the login session and exits the application.

Figure 24 shows the Announcement List page. If the user wants to view all the events created, they have to click the Announcement list tab. This page provides the user to view all the events.

Figure 25 shows the Calendar of Activities. To view the entire event that is posted from the Vice President for Academic Affairs (VPAA) or Vice President for Administration, Planning, and Development (VPAPD), the user must click the Calendar of Activities Tab and the system automatically displays the details of a selected event.
Figure 23. List of Tab for User

Figure 24. View Announcement List for User.

Figure 25. View Calendar for User.

Figure 26 shows the View Events List page. The View Events List page will display all the events and details about the events. Once the users click the event, it will automatically display the event details. Figure 27 depicts the View Event Details. Once the user clicks the selected event, the application will automatically display the details of the said event.

Figure 28 illustrates the Administrator’s Login Page of the Ubiquitous Notification System. The Administrator has to input username and password. The system will then redirect the administrator to the Home Page.

Figure 29 shows the Home or Main Page of the system. There are five options or list of menus in the tab: Announcement Section, Event Section, Account Setting Section, and Logout Section.

Figure 30 illustrates the Announcement Section. It is where the Administrators can view announcements. Whenever there are changes in the events, the updates will first appear in the announcement list. The administrator can also search for a specific announcement or event through the Search Box. There are 3 columns in the table which includes the Events, Contents, and the date the event was posted.

Figure 31 shows the Calendar of Activities under the Event Section. In order to see all the events that are posted by the administrator, the user must click the Calendar of Activities Tab, and automatically the system will display the calendar with all the events listed.

Figure 32 illustrates the viewing of the events listed. By clicking the event section tab, a drop-down list of events will appear. When the event list is clicked, the system will let the administrator view the list of events. The following data will appear in the search results: event name, category, attendee, and action.

Figure 33 illustrates the viewing of registered accounts and adding of user accounts under the account section tab.
Figure 31. Administrator Event Section (Calendar of Activities)

Figure 32. Administrator Event Section (Event List)

Figure 33. Administrator Account Setting Section
CONCLUSION
One most important feature of the smartphones is notification. The smartphone is a mobile phone with an advanced mobile operating system. With smartphones, users may enjoy the variety of features such as mobile apps. A notification is something that gives official information to someone through mobile phones. An Android-based ubiquitous notification application notifies the users of the events and activities in the university. It is concluded that of this application is useful to the students, faculty, staff and other stakeholders, wherein they can be notified of the information by using the application. Hence, by setting up and installing this application, they are now updated with all the events and activities of the university which can be planned, unplanned, routine or emergency in nature.

Recommendations
The researchers highly recommend performing external testing and evaluation of the system. The researchers also recommend that the school should provide an exclusive internet connection to the system.

REFERENCES
Real and Complex Wavelet Transform Approaches for Malaysian Speaker and Accent Recognition

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²Department of Biomedical-Engineering, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur 603 203 Kancheepuram District, Tamil Nadu, India
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ABSTRACT
A new approach for speaker and accent recognition based on wavelets, namely Discrete Wavelet Packet (DWPT), Dual Tree Complex Wavelet Packet Transform (DT-CWPT) and Wavelet Packet Transform (WPT) based non-linear features are investigated. The results are compared with conventional MFCC and LPC features. k-Nearest Neighbors (k-NN), Support Vector Machine (SVM) and Extreme Learning Machine (ELM) classifier are used to quantify the speaker and accent recognition rate. The database for the research was developed using English digits (0~9) and Malay words. The highest accuracy for speaker recognition obtained is 93.54% while for accent recognition; it is 95.86% using Malay words. Combination of features for speaker recognition is obtained from ELM classifier is 98.68 % and for accent recognition is 98.75 % using Malay words.

Keywords: Accent recognition, Discrete Wavelet Packet (DWPT), Dual Tree- Complex Wavelet Packet Transform (DT-CWPT), Speaker recognition, Wavelet Packet Transform (WPT)

INTRODUCTION
Biometric refers to the identification of humans by their characteristic or traits. It can be categorized into physiological versus
behavioural characteristic. Common physical characteristics are fingerprints, face and palm print. Typing rhythm, gait and voice are examples of behavioural characteristics which are often related to behaviour of a person. Some of the characteristics are unique to every individual as it varies from one person to another, even if they are twins (Jain & Sharma, 2013; Kinnunen & Li, 2010; Anand et al., 2012).

Many studies have investigated speech/speaker and accent recognition using voice signals. There are various well-known feature extraction techniques for extracting useful features from voice such as MFCC, LPC, Linear Predictive Cepstral Coefficients (LPCC). MFCC and LPC features are widely preferred for studies on speech, speaker and accents recognition.

MFCC and LPC methods transform the speech signal from time-based to frequency-based domain. In transforming the signals, time information is lost. Wavelet based analysis does not use a time-frequency region, but rather a time-scale region. The wavelets work by scaling properties. They are localized in time and frequency, permitting a closer connection between function being represented and their coefficients (Lee & Yamamoto, 1994).

Wavelets approaches have proven to be one of the promising techniques in applications such as infant cry classification, speech signals processing for pathological detection and voice access system to name a few (Oung et al., 2018; Lim et al., 2016; Johari et al., 2011). Although there are many studies on Malay speech/speaker and accent recognition using wavelets, the difference in characteristics between different types of wavelet transforms are less explored (Yusnita et al., 2012; Almaadeed et al., 2015; Yadav & Bhalke, 2015; Pandiaraj & Kumar, 2015; Lei & Kun, 2017). Therefore, this study undertakes the task of evaluating the performance of non-linear features derived from the various wavelet based approach (DWPT, DT-CWPT and WPT) in predicting speaker and accent recognition. Conventional LPC and MFCC parameters are also derived, and combination of these features with non-linear entropies are also evaluated in an effort to identify new parameters that can contribute to overall best prediction rate for speaker and accent recognition. A new speech database consists of Malay words uttered by Malaysian speakers from three major races, namely Malay, Chinese and Indian were used. Since we are using the Malay words, it will not only give the advantages for Malaysian who speak the language but also for people who speak this language in the South- east Asia such as Indonesia, south Thailand and south Philippines (Hanifa et al., 2017).

RELATED WORKS

This section describes a previous research works in speech/speaker and accent recognition area using wavelets. Yusnita et al. (2012) studied hybrid Discrete Wavelet Transform (DWT) feature space using uniform and dyadic extraction of Linear Predictive Coding (LPC) for accent classification using k-Nearest Neighbors (k-NN). The best classification
rate was 93.25 % with 32- and 21- dimension space for uniform and dyadic manner. The dyadic type DWT-LPC yielded an increase in classification rate by 9.28 % with respect to conventional LPC method. Almaadeed et al. (2015) proposed speaker identification using multimodal neural networks and wavelet analysis. This approach used multiple Neural Network (Probabilistic Neural Network (PNN), Radial Basis Function NN (RBF-NN) and General Regressive NN (GRNN) using wavelet-based selection method. The proposed system obtained 97.5% accurate with a 50 ms identification time. Performance tests conducted using the GRID database corpora had shown that this approach had faster identification time and greater accuracy, compared to traditional approaches, and it was applicable to real-time, text-independent speaker identification systems.

According to Yadav and Bhalke (2015), speaker identification system based on the wavelet transform which is DWT based MFCC and Traditional MFCC are used as a feature for speaker identification system. MFCC based DWT results show 85% accuracy & Traditional MFCC results show 80% accuracy.

Pandiaraj and Kumar (2015) discovered speaker identification system using DWT and Gaussian Mixture Model (GMM) used for classification. Daubechies wavelets were used and analysed using 8 levels of decomposition. The maximum accuracy of 83.3 % was achieved for the proposed method.

Lei and Kun (2017) researched on speaker recognition using Wavelet Packet Entropy (WPE), I- Vector and Cosine Distance Scoring (CDS) in noisy environment. Experimental results showed that WPE-I-CDS was robust in noisy environment compared with MFCC-I-CDS and Fused MFCC (FMFCC)-I-CDS. Based on the 94.36% of accuracy obtained, it was concluded that WPE using i-vector and CDS classifier was best suited for speaker recognition.

Rathor and Jadon (2017) proposed text independent speaker recognition using Wavelet Cepstral Coefficient (WCC) and Butter worth filter. The Wavelet Transform was used to find the frequency spectrum while WCC was used to capture the characteristic of the signal. The proposed method obtained 98.5% accuracy by using Butter worth filter. The authors concluded that the proposed method achieved a good performance in noisy environment.

Chelali and Djeradil (2017) had developed text dependent speaker recognition applied for Algerian Berber language using MFCC, delta MFCC, delta-delta MFCC, LPC and DWT. Identification rate for MFCC varied from 83% for the word “Tazalit” to 100 % for the word “Attas”. LPC technique combined with DWT improved the efficiency of the system. The speaker recognition system improved the identification by 10 % compared with the classical MFCC and reduced identification time since the length was less than MFCC.

Motivated by previous studies, this study was undertaken to improve recognition rate of speaker and accent recognition using MFCC, LPC, DWPT, DT-CWPT and WPT based combined features. New database that contained English digits (0-9) and Malay words from
the major races in Malaysia; Malay, Chinese and Indian was constructed. The aims of this study are: (i) to compare performances of feature extraction methods for English digits and Malay words using speaker and accent database; and (ii) to study the performance of combined feature extraction methods using different classifiers.

METHODOLOGY

Database
The speech corpus was created from 39 male and female undergraduate students of University Malaysia Perlis from different races. Each speaker pronounced the English digits (0~9) and Malay words for 15 sessions. Every session consisted of predefined digit and Malay word organized randomly. The Malay words selected represented the six vowels of a, e, i, o, u, e’ and had a combination of consonants and vowels in monosyllable and bi-syllable structure. The total speech samples were 12285 files. Table 1 and 2 summarize the database.

Experiment Setup
The experiment was conducted based on the methodology as presented in Figure 1. Speech signals were recorded with a sampling rate of 44 kHz and down-sampled to 16 kHz. Based on the Shannon sampling theorem, the 16 kHz sampling was enough to reconstruct an 8 kHz bandwidth signal (telephony speech bandwidth) maximum frequency (Gruhn et al., 2011). In Pre-processing stage, the speech signal was normalized and filtered, so that, only useful speech information was retained. In feature extraction process, MFCC, LPC, DWPT, DT-CWPT and WPT based features were extracted from the sampled speech signals. At this stage, all of the information necessary to distinguish speaker and accent was preserved. Configurable feature combination block selected which of the features to be used for accuracy calculation. It supported single feature or combined features extraction output. The accuracy was investigated for individual and combined features using k-NN, SVM and ELM classifier for Malaysian speaker and accent recognition. This process involved classifying the speech signal to determine whether the input speech matched any of learnt speech.

Feature Extraction

Mel Frequency Cepstral Coefficients (MFCC). The MFCC was introduced by Davis and Mermelstein (Saksamudre & Deshmukh, 2015) which was based on human hearing perceptions and cannot perceive frequencies over 1Khz. It is linear frequency spacing below 1000Hz and a logarithmic spacing above 1000Hz. This can be represented mathematically as:
\[ M(f) = 1125 \times \log_2\left(1 + \frac{f}{700}\right) \]  

(1)

In this work, MFCC is used to extract feature from input signal. Frame size for the analysis was set to 512 sample points, such that the time period of the signal is \( \frac{512}{16000} = 32 \text{ms} \). This is because a short period of time (20 – 40msec) speech signals are known to exhibit quasi-stationary behavior (Jain & Sharma, 2013). Hamming window was used to smooth the signal and make it more amendable for spectral analysis. Fast Fourier Transform (FFT) was applied to convert each frame of 256 samples from time domain into frequency domain. The Mel scale is based on pitch perception and triangular-shaped filter was used. Discrete Cosine Transform (DCT) is used to convert the log mel spectrum into time domain. The result of conversion is called MFCC and the set of coefficients is acoustic vector. Thirteen acoustic vectors were used to represent and recognize the voice characteristic of the speaker for this study.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Malay word syllable structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Word</td>
<td>Phoneme Sequence</td>
</tr>
<tr>
<td>Jam</td>
<td>/Jam/</td>
</tr>
<tr>
<td>Pas</td>
<td>/Pas/</td>
</tr>
<tr>
<td>Cap</td>
<td>/Cap/</td>
</tr>
<tr>
<td>Tol</td>
<td>/Tol/</td>
</tr>
<tr>
<td>Sen</td>
<td>/Sen/</td>
</tr>
<tr>
<td>Aku</td>
<td>/A-ku/</td>
</tr>
<tr>
<td>Basi</td>
<td>/Ba-si/</td>
</tr>
<tr>
<td>Pulau</td>
<td>/Pu-lau/</td>
</tr>
<tr>
<td>Rabu</td>
<td>/Ra-bu/</td>
</tr>
<tr>
<td>Jalan</td>
<td>/Ja-lan/</td>
</tr>
<tr>
<td>Muka</td>
<td>/Mu-ka/</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Database details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>Description</td>
</tr>
<tr>
<td>Speakers</td>
<td>39</td>
</tr>
<tr>
<td>Session /speaker</td>
<td>15 times</td>
</tr>
<tr>
<td>Wordlist</td>
<td>1. Digit English (0–9)</td>
</tr>
<tr>
<td></td>
<td>2. Malay word (Jam, Pas, Cap, Tol, Sen, Aku, Basi, Pulau, Rabu, Jalan, Muka)</td>
</tr>
</tbody>
</table>
Linear Predictive Coding (LPC). LPC analysis models the speech signal as a p-order autoregressive (AR) system which is a special case of all-pole IIR filter. The current value of the real-valued time series, is predicted based on past samples by minimizing the prediction error in the least squares sense (Paulraj et al., 2008). All the speaker data can be approximated to be a linear combination of past samples given by:

\[ \hat{s}(n) = \sum_{k=1}^{p} a_k s(n - k) \]  \hspace{1cm} (2)

where \( \hat{s}(n) \) is the estimated sample, \( p \) is the order of the model, \( a_k \) is the linear predictive coefficients and \( s(n-k) \) is the previous speech sample. \( p \) orders range between 8-20 gives...

Table 2 (Continue)

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>19- 24 years old</td>
</tr>
<tr>
<td>Race</td>
<td>Malay, Chinese, Indian</td>
</tr>
<tr>
<td>Microphone</td>
<td>Stereo microphone</td>
</tr>
<tr>
<td>Types of room</td>
<td>Controlled environment</td>
</tr>
<tr>
<td>Sampling frequency</td>
<td>16kHz</td>
</tr>
<tr>
<td>Audio file format</td>
<td>Wav</td>
</tr>
</tbody>
</table>

Figure 1. Flow chart of proposed methodology
Real and Complex Wavelet Transform Approaches

good performances for recognition system. Prasanna et al., (2006) and Yusnita et al., (2011) found that 16 orders gave a good result. Thus, in this study we set the orders of p as 16.

**Discrete Wavelet Packet Transform (DWPT).** DWPT is an extension of DWT, whereby all nodes in the tree structure are allowed to split further at each level of decomposition. In the DWT, each level is calculated by passing only the previous wavelet approximation coefficients through discrete-time low and high pass quadrature mirror filters. However, in the DWPT, both the detail and approximation coefficients are decomposed to create the full binary tree. Therefore, features can be generated based on approximation and detail coefficients at different levels to obtain more information (Zhang et al., 2015).

**Dual Tree Complex Wavelet Packet Transform (DT-CWPT).** DT-CWPT’s shift invariance and directional selectivity provides an accurate measure of spectral energy at a particular location in space, scale and orientation (Lim et al., 2016). The DT-CWPT consisted of two DWPT operating in parallel on an input signal. The second wavelet packet filter bank was obtained by replacing the first stage filter $h_i^{(1)}(n)$ by $h_i^{(1)}(n-1)$ and by replacing by $h_i^{(1)}(n)$ for $i \in \{0,1\}$.

For the research, input speech signals were decomposed into 5 levels using DT-CWPT. Non-linear entropy features were extracted from each sub-band for the analysis, which produced 124 feature vectors.

**Wavelet Packet Transform (WPT).** Wavelet Packet Transform (WPT) is an extension of Discrete Wavelet Transform and can be obtained by a generalization of the fast-pyramidal algorithm. For DWT decomposition procedure, signal is decomposed into lower frequency band (approximation coefficients) and higher frequency band (detail coefficients). For further decomposition, low frequency band is used and hence, DWT gives a left recursive binary tree structure. However, in Wavelet Packet Transform, lower and higher frequency bands are decomposed into two sub-bands. Therefore, wavelet packet gives a balanced binary tree structure (Johari et al., 2011). Forth order Daubechies wavelets were used for the analysis based on observations from works by (Lei & Kun, 2017; Bong et al., 2017) that demonstrated that this particular wavelet family was best suited for analysis of speech signals. Daubechies wavelet are found to be time invariant, computationally fast and has sharp filter transition bands (Cohen et al., 2006).

**Classifier**

**k-Nearest Neighbor (k-NN).** k-NN classifier is used to classify the English digits and Malay words. Due to its simple implementation and flexibility to feature/distance choices, k-NN is considered in this works. The k-NN classification system is a simple, supervised algorithm that employs lazy learning (Hariharan et al., 2012). The test samples are classified
based on majority of k-Nearest Neighbor’s category. An object being classified to the class most common amongst its k nearest neighbors where k is a positive integer. The Euclidean Distance measure is used to calculate the closest members of the training set to test class being examined.

From this k-NN category, class label is determined by applying majority voting. Euclidean Distance is shown in Equation 3.

\[ d_E [x, y] = \sum_{i=1}^{N} \sqrt{x_i^2 - y_i^2} \]  

Normally, larger values of \( k \) can cause boundaries classes to be less distinct and will reduce the effect of noise on the classification (Liu et al., 2010). However, a lot of neighbors means neighbors that are far apart are also counted, which are irrelevant. Therefore, in this study, \( k \) values were varied between 1 and 10.

**Support Vector Machine (SVM).** For data classification, SVM is a supervised algorithm that can be used for two classes and multiclass recognition. It is based on principle of Structural Risk Minimization (SRM). It searches the best compromise between complexity of model and learning ability on the basis of limited sample information to obtain the best generalization ability. SVM generates an excellent performance which comes out from the fact that, SVM apply a linear algorithm to the data in a high dimensional space (Amami et al., 2015).

The parameters of the best C (Cost) and gamma (G) were optimized using Lib SVM Tool (Chang & Lin, 2011). SVM was chosen since it has a better generalization (less overfitting) and robust to noise.

**Extreme Learning Machine (ELM).** A new learning of single hidden layer feedforward networks (SLFNs), proposed by Huang et al (Cao et al., 2015). It has been used in various applications to overcome the slow training speed and overfitting problems of the conventional neural network learning algorithms (Sangeetha & Radha, 2013). The idea in ELM is that the weight of the hidden nodes and output nodes are randomly selected and analytically determined. ELM was chosen for having a better performance in learning efficiency and universal approximation capability. Moreover, it is a fast learning speed and good generalization performance. In this study the best value of the regularization coefficients of ELM classifier was found between -10 and 10.
RESULTS AND DISCUSSIONS

Table 3-6 show the results of MFCC, LPC, DT-CWPT, DWPT and WPT features for Malaysian speaker and accent recognition. The maximum recognition accuracies are highlighted in Table 3. From the highlighted results in Table 3, the highest accuracies for speaker recognition using English digits was 92.16 % and for Malay words was 93.54 % achieved using ELM classifier. It was found that the highest accuracies from speaker recognition was obtained from DT-CWPT features. This indicates that the percentage of recognition accuracies was improved using Wavelets from DT-CWPT features. Table 4 shows accuracy of accent recognition using English digits and Malay words. SVM classifier and MFCC achieved maximum accuracy rate of 94.48 % for accent recognition using English digits and for Malay words was 95.86%. The percentage shows that the recognition accuracies using MFCC, LPC, DT-CWPT, DWPT and WPT features are comparable.

Table 5 shows accuracy of speaker recognition using English digits and Malay words by combining the features. ELM has yielded higher recognition rate of about 98.09 % for English digits and 98.68 % for Malay words. Table 6 contains the results of accent recognition using English Digits and Malay words. It is observed that the maximum accuracy achieved from ELM classifier was 98.15% for English digits and for Malay words was 98.75 %.

From table 3-4, it can be summarized that in the accuracy results for MFCC, LPC and Wavelet based approach (DWPT, DT-CWPT and WPT), SVM performed better than ELM. Meanwhile for the combined features in Table 5 and 6, ELM gave better result. This is because combined features generate bigger data sets. ELM is well suited for solving big data and their solution is so rapidly obtained (Akusok et al., 2015). SVM with a greater number of samples will start to drop in terms of performance (Li & Yu, 2014). SVM has high algorithmic complexity and extensive memory requirements due to the use of a quadratic programming (Valyon & Horváth, 2003). From Table 3-4, in every experiment for speaker and accent recognition, ELM and SVM classifier showed a better performance than k-NN which was run separately. However, for combined features as can be seen in Table 5-6, ELM gave a slightly better result than SVM. From the results displayed in Tables 3-6, higher recognition rate was obtained using Malay words compared to English digits. It is because of vowels in the words have significantly more energy than consonants (Mohd Yusof et al., 2008). From a previous work in speaker/speech and accent identification/recognition, the accent identification researched by Yusnita et al., (2012) using hybrid DWT-LPC features and k-NN showed promising accuracy. The classification rate was 93.25 % compared than the conventional LPC while retaining the feature size. Adam et al (2013) reported an improved feature extraction method using Wavelet Cepstral Coefficients (WCC) recognized 26 English alphabets. The authors had found that WCCS showed comparable result with MFCCs. The best recognition was found from WCCs at level 5 of the DWT decomposition with a small difference of 1.19 % and 3.21 % when compared to MFCCs. Meanwhile Islam et al., (2016) proposed a new speaker identification system using 2-D neurograms constructed...
from the responses of a physiologically-based computational model of the auditory. The identification score was found to be 93.5 % (40 dB), 93.5 % (60 dB) and 96.5 % (90 dB). Soon et al (2017) researched on speech recognition system for spoken English and Malay words from a group of Malay native speakers using DWT. Surface electromyogram (sEMG) employed to capture the speech and feature extraction was done in both temporal and time-frequency domains. The classification result showed that the Malay words (‘satu’, ‘dua’, ‘tiga’, ‘empat’, ‘lima’) gave a promising accuracy than English words (‘one’, ‘two’, ‘three’, ‘four’, ‘five’).

In this work, the accuracy for individual features was able to achieve 93.54 % (speaker) and 95.86 % (accent) while for the combined features, the result obtained were 98.68 % (speaker) and 98.75 % (accent). It is observed that the results are slightly better with previous works. This is because a variety of information inside features in DWPT, DT-CWPT and WPT contributes the promising accuracy. The results prove that proposed feature extraction and classifier help to improve Malaysian speaker and accent recognition.

Table 3

<table>
<thead>
<tr>
<th>Features Extraction Method (no of coeff)</th>
<th>Accuracy (%) ±SD</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KNN</td>
<td>SVM</td>
<td>ELM</td>
<td>KNN</td>
<td>SVM</td>
<td>ELM</td>
</tr>
<tr>
<td>MFCC (13)</td>
<td>88.44</td>
<td>91.49</td>
<td>89.57</td>
<td>89.91</td>
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<td>±0.09</td>
<td>±0.13</td>
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<td>LPC (16)</td>
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<td>90.41</td>
<td>87.89</td>
<td>87.82</td>
<td>92.21</td>
<td>89.85</td>
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<td>±0.16</td>
<td>±0.09</td>
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<tr>
<td>DWPT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy Entropy (62)</td>
<td>84.08</td>
<td>90.49</td>
<td>90.79</td>
<td>84.55</td>
<td>92.35</td>
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<td>±0.15</td>
<td>±0.21</td>
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<tr>
<td>Renyi Entropy (62)</td>
<td>83.91</td>
<td>90.79</td>
<td>90.74</td>
<td>84.49</td>
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<td>±0.17</td>
<td>±0.17</td>
<td>±0.13</td>
<td>±0.20</td>
<td>±0.17</td>
<td>±0.08</td>
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<tr>
<td>Shannon Entropy (62)</td>
<td>79.64</td>
<td>89.11</td>
<td>86.46</td>
<td>80.59</td>
<td>90.69</td>
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<td>±0.22</td>
<td>±0.18</td>
<td>±0.18</td>
<td>±0.19</td>
<td>±0.12</td>
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Table 3 (Continue)

<table>
<thead>
<tr>
<th>Features Extraction Method (no of coeff)</th>
<th>Accuracy (%) ±SD</th>
<th>Accuracy (%) ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Speaker (Digits)</td>
<td>Speaker (Malay words)</td>
</tr>
<tr>
<td></td>
<td>KNN</td>
<td>SVM</td>
</tr>
<tr>
<td>DT-CWPT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy Entropy (124)</td>
<td>84.51 ±0.22</td>
<td>90.86 ±0.11</td>
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<tr>
<td>Renyi Entropy (124)</td>
<td>84.38 ±0.13</td>
<td>91.24 ±0.15</td>
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<tr>
<td>Shannon Entropy (124)</td>
<td>80.37 ±0.17</td>
<td>90.34 ±0.23</td>
</tr>
<tr>
<td>WPT</td>
<td></td>
<td></td>
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<tr>
<td>Energy Entropy (62)</td>
<td>80.12 ±0.26</td>
<td>88.07 ±0.16</td>
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<tr>
<td>Renyi Entropy (62)</td>
<td>80.21 ±0.16</td>
<td>87.79 ±0.10</td>
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<tr>
<td>Shannon Entropy (62)</td>
<td>73.61 ±0.32</td>
<td>86.15 ±0.21</td>
</tr>
</tbody>
</table>

Table 4

Accuracy of accent recognition using English digits and Malay words

<table>
<thead>
<tr>
<th>Features Extraction Method</th>
<th>Accuracy (%) ± SD</th>
<th>Accuracy (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Accent (Digits)</td>
<td>Accent (Malay words)</td>
</tr>
<tr>
<td></td>
<td>KNN</td>
<td>SVM</td>
</tr>
<tr>
<td>MFCC (13)</td>
<td>93.30 ±0.17</td>
<td>94.48 ±0.14</td>
</tr>
<tr>
<td>LPC (16)</td>
<td>92.05 ±0.10</td>
<td>93.56 ±0.08</td>
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<tr>
<td>Energy Entropy (62)</td>
<td>90.94 ±0.17</td>
<td>93.61 ±0.17</td>
</tr>
<tr>
<td>DWPT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renyi Entropy (62)</td>
<td>90.82 ±0.11</td>
<td>93.85 ±0.18</td>
</tr>
<tr>
<td>Shannon Entropy (62)</td>
<td>87.42 ±0.16</td>
<td>91.03 ±0.22</td>
</tr>
</tbody>
</table>
Table 4 (Continue)

<table>
<thead>
<tr>
<th>Features Extraction Method</th>
<th>Accuracy (%) ± SD</th>
<th>Accuracy (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Speaker (digits)</td>
<td>Speaker (Malay words)</td>
</tr>
<tr>
<td></td>
<td>KNN</td>
<td>SVM</td>
</tr>
<tr>
<td>KNN</td>
<td>91.12 ±0.17</td>
<td>93.75 ±0.14</td>
</tr>
<tr>
<td>SVM</td>
<td>90.74 ±0.13</td>
<td>94.03 ±0.11</td>
</tr>
<tr>
<td>ELM</td>
<td>87.94 ±0.21</td>
<td>91.84 ±0.20</td>
</tr>
<tr>
<td>DT-CWPT</td>
<td>Energy</td>
<td>88.76 ±0.14</td>
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<td></td>
<td>Entropy (124)</td>
<td>90.74 ±0.13</td>
</tr>
<tr>
<td></td>
<td>Renyi</td>
<td>87.94 ±0.21</td>
</tr>
<tr>
<td></td>
<td>Entropy (124)</td>
<td>88.76 ±0.14</td>
</tr>
<tr>
<td></td>
<td>Shannon</td>
<td>88.73 ±0.21</td>
</tr>
<tr>
<td></td>
<td>Entropy (62)</td>
<td>83.86 ±0.18</td>
</tr>
</tbody>
</table>

Table 5

Accuracy of speaker recognition English digits and Malay words with combination features

<table>
<thead>
<tr>
<th>Features Extraction Method (no of coeff)</th>
<th>Accuracy (%) ± SD Speaker (digits)</th>
<th>Accuracy (%) ± SD Speaker (Malay words)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNN</td>
<td>SVM</td>
<td>ELM</td>
</tr>
<tr>
<td>MFCC + LPC + DWPT + DT-CWPT + WPT-773</td>
<td>89.18 ±0.18</td>
<td>95.72 ±0.15</td>
</tr>
</tbody>
</table>

Table 6

Accuracy of accent recognition English digits and Malay words with combination features

<table>
<thead>
<tr>
<th>Features Extraction Method (no of coeff)</th>
<th>Accuracy (%) ± SD Accent (digits)</th>
<th>Accuracy (%) ± SD Accent (Malay words)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNN</td>
<td>SVM</td>
<td>ELM</td>
</tr>
<tr>
<td>MFCC + LPC + DWPT + DT-CWPT + WPT-773</td>
<td>94.02 ±0.15</td>
<td>96.85 ±0.16</td>
</tr>
</tbody>
</table>
CONCLUSION

This paper investigates the use of MFCC, LPC, WPT and DT-CWPT (first wavelet packet FB and second wavelet packet FB) based feature for speaker and accent recognition. SVM, KNN and ELM were used to measure the effectiveness of recognition of speaker and accent to identify speaker and accent. The accuracy is calculated for individual and combined features.

The result of this work shows best performance on accent recognition with 96.08 % for single feature extraction method and 98.98 % for the combination of the method. For speaker recognition, best performance achieved is 93.54 % for single feature extraction method and 98.92 % for the combination of features.

The result of feature extraction clearly outperforms the previous works even though we were using a new bigger features database. It is also found that accent identification gives better result for single feature extraction and combined features compared to speaker identification. The results of this study can be extended by using a bigger database with polysyllabic in Malay words to improve the Malaysian speaker and accent recognition. In the future work, feature reduction algorithms such as Principal Components Analysis (PCA), will be applied to reduce feature dimension. This will be developed to improve the results. It would be interesting to include experimenting, with different numbers of coefficients and other wavelet families, to observe the recognition result.

The study has many potential and useful in applications such as access control to computers, smart mobile attendance system, telephone banking, electronic commerce and forensic.

ACKNOWLEDGMENT

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REFERENCES


A Novel Multi Stego-image based Data Hiding Method for Gray Scale Image

Aditya Kumar Sahu* and Gandharba Swain

ABSTRACT

In this paper, we present a novel multi stego-image based data hiding method using the principle of the modified least significant bit (LSB) matching to improve the embedding capacity (EC) as well as image quality. Initially, each original pixel produces four new pixels. The secret data is hidden in all the four produced pixels. Then the pixels are readjusted to improve the quality of the stego-images. There are four separate stego-images developed from the four different readjusted pixels. Each stego-image hides one bit per pixel. The average peak signal-to-noise ratios (PSNR) for the stego-images are 36.06 dB, 37.88 dB, 39.60 dB and 41.00 dB respectively. Furthermore, the proposed method successfully withstand against RS-steganalysis.

Keywords: Data hiding, LSB Matching, RS-analysis, steganography

INTRODUCTION

Over the years, steganography has emerged as an elementary and conducive choice to transmit digital data (Cheddad et al., 2010). Steganography is the art of covert communication (Johnson & Jajodia, 1998). Here the data transmission accomplishes through various cover mediums such as image, audio, video, and text. Image steganography use image to carry the information through the public channel...
Image steganography is achieved in 2 ways (1) reversible (2) irreversible (Subhedar & Mankar, 2014). The reversible approach ensures the retrieval of secret data as well as the original image at the receiver side. Whereas the irreversible approaches focus only on the successful retrieval of secret data. Our proposed work is based on the irreversible approach. Irreversible methods such as LSB, LSB matching, pixel value differencing (PVD), exploiting modification direction (EMD) and modulus function (MF) are some of the prominent methods in the field of image steganography (Hussain et al., 2018).

The image quality and EC are the two image steganographic parameters to gauge the efficacy of a data hiding method. The image quality depends on the distortion of the stego-image. Various image quality assessment (IQA) metrics such as; (i) mean square error (MSE), (ii) peak signal-to-noise ratio (PSNR), (iii) root mean square error (RMSE), (iv) Weighted PSNR (WPSNR) (v) The universal image quality index (Q), and (vi) structural similarity (SSIM) index exists in literature (Pradhan et al., 2016). PSNR measures the visual quality (Bong & Khoo, 2015) of a stego-image. The high PSNR is an indication of lower distortion and vice versa. The MSE compares the original and stego-image to measure the quality of stego-image. It should be as low as possible (Bong & Khoo, 2014). Further, the WPSNR uses MSE and Noise Visibility Function (NVF) to gauge the quality of the stego-image. Similarly, Q and SSIM are also used to measure the stego-image quality (Wang, et al., 2004). The Q should always be at upper side i.e. approximately 1 for the better quality of stego-image. The EC is the number of secret data bits the image can conceal without noticeable artifacts (Hussain et al., 2018).

The simplicity and straightforwardness of the least significant bit (LSB) image steganography methods made it convenient for information hiding. Johnson and Jajodia (1998) concealed the secret data by replacing the LSB of the original image pixel. This method was susceptible to the intruder as by accessing the LSBs, the data can be easily accessed. In recent years, voluminous articles have been proposed using LSB methods (Wu & Hwang, 2017; Wang et al., 2001; Chan & Cheng, 2004; Yang et al., 2009; Sahu & Swain, 2016, Sahu & Swain, 2017, Sahu & Swain, 2018, Sahu et al., 2018). Sharp (2001) proposed the LSB matching to lower the distortion of the stego-image by randomly performing +1 or -1 to the original pixel values in case if the secret data did not match with the LSB. This method limits the embedding capacity to one bit per pixel. Mielikainen (2006) came with an alteration to the LSB method called LSB matching revisited. Here the secret bits are concealed with the help of the binary function and four embedding rules. The suggested method also produces the same embedding capacity as produced by Sharp (2001) but it modifies fewer bits in the original image.
Though LSB approaches greatly enhance the embedding capacity, it is exposed to RS-analysis (Fridrich & Goljan, 2002). With the motive to increase the capacity and lowering the distortion to the stego-image, Wu and Tsai (2003) proposed pixel values differencing (PVD) method. The pixels are bifurcated into blocks with 2 pixels each and then the difference value (d) between the two pixels is computed. The value d is mapped to the specified range table in order to identify the number secret data bits to be embedded inside a block. Wang et al. (2008) found the solution for the falling-off boundary problem (FOBP) which existed in Wu and Tsai (2003) by bringing together the PVD and modulus function. Wang et al. (2008) method enhanced the PSNR value as compared to Wu and Tsai (2003). To expel the restriction of capacity in original PVD, Chang et al. (2008) introduced Tri-way pixel value differencing (TPVD) method. It finds the difference value d in three directions such as horizontal, vertical and diagonal by choosing a reference point. Swain (2016) proposed contemporary adaptive directional PVD method which contributed by significant upgradation in the embedding capacity as well as retaining the image quality. There are large number of articles in literature utilizing the advantage of PVD methods (Shen et al., 2015; Lee et al., 2012; Hameed et al., 2017; Lu et al., 2006; Hussain et al., 2017).

LSB substitution methods offer larger capacity whereas PVD methods attain better imperceptibility. Methods such as LSB and PVD when combined they outperform others in terms of capacity and imperceptibility (Khodaei & Faez, 2012; Hussain et al., 2016; Wu et al., 2005; Jung, 2018; Swain, 2018).

The existing image steganographic methods produce single stego-image from the original image. The restriction of single stego-image limits the EC. Various methods in literature made an attempt to increase the EC, but at the same time, the image quality reduced (Jung, 2018; Khodaei & Faez, 2012). In this paper, we present a novel multi stego-image based method to conceal the secret data. The proposed method produces four stego images from one original image. Each pixel of the produced stego-images hides one bit.

RELATED WORK

Wu and Hwang’s (2017) Method

Wu and Hwang (2017) proposed a novel LSB substitution method to reduce the distortion to of the stego-image. It conceals 3 bits in a group of 3 pixels with maximum modification of +1 or -1 to each pixel. The expected number of modifications per pixel (ENMPP) is reduced compared to the conventional LSB substitution methods. The reduction in the ENMPP results in an enhanced image quality. However, the capacity remains the same i.e. 1 bit per pixel. The step by step explanation of Wu and Hwang’s (2017) method is explained in this section, followed by an example shown in Figure 1.
Step 1: At first take 3 pixels $P_1$, $P_2$ and $P_3$ from the original image horizontally.
Step 2: Now, represent $P_1$, $P_2$ and $P_3$ to its corresponding binary such as $P_1 = a_1a_2a_3a_4a_5a_6a_7a_8$, $P_2 = b_1b_2b_3b_4b_5b_6b_7b_8$ and $P_3 = c_1c_2c_3c_4c_5c_6c_7c_8$.

Now, compute $X$, $Y$, $Z$ values using equation (1-3).

$$X = a_8 \oplus a_7 \oplus b_8 \quad (1)$$
$$Y = b_8 \oplus b_7 \oplus c_8 \quad (2)$$
$$Z = c_8 \oplus c_7 \oplus a_8 \quad (3)$$

Step 3: Let, $s_1$, $s_2$ and $s_3$ be the 3 secret bits.
Step 4: Now, update the value of, $P_1$, $P_2$ and $P_3$ using the given condition below. Here $==$ is the bit comparison operator and $\&\&$ is logical AND operator.

```c
if (X == s_1) \&\& (Y == s_2) \&\& (Z == s_3)
    then, P_1 = P_1, P_2 = P_2 and P_3 = P_3
else if (X = s_1) \&\& (Y = s_2) \&\& (Z = s_3)
    if $P_2 \mod 2 = 0$, then $P_2 = P_2 - 1$.
    else $P_2 = P_2 + 1$.
end
else if (X == s_1) \&\& (Y = s_2) \&\& (Z = s_3)
    if $P_3 \mod 2 = 0$, then $P_3 = P_3 - 1$.
    else $P_3 = P_3 + 1$.
end
else if (X == s_1) \&\& (Y == s_2) \&\& (Z = s_3)
    if $P_1 \mod 2 = 0$, then $P_1 = P_1 - 1$.
    else $P_1 = P_1 + 1$.
end
else if (X = s_1) \&\& (Y = s_2) \&\& (Z = s_3)
    if $P_2 \mod 2 = 0$, then $P_2 = P_2 + 1$.
    else $P_2 = P_2 - 1$.
end
else if (X == s_1) \&\& (Y = s_2) \&\& (Z = s_3)
    if $P_3 \mod 2 = 0$, then $P_3 = P_3 + 1$.
    else $P_3 = P_3 - 1$.
end
```

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else if \((X = s_1) \&\& (Y = s_2) \&\& (Z = s_3)\)
  if \(P_2 \mod 2 = 0\), then \(P_3 = P_3 - 1\).
  else \(P_3 = P_3 + 1\).
  end
else if \((X \neq s_1) \&\& (Y \neq s_2) \&\& (Z \neq s_3)\)
  if \(P_2 \mod 2 = 0\), then \(P_3 = P_3 + 1\).
  else \(P_3 = P_3 - 1\).
  end
if \(P_2 \mod 2 = 0\), then \(P_3 = P_3 + 1\).
else \(P_3 = P_3 - 1\).
end

Step 2: Obtain the stego-pixels as \(P_1^* = P_1\), \(P_2^*\) and \(P_3^* = P_3\).
Step 3: At the receiver side, retrieve the stego-pixels as \(P_1^*, P_2^*\) and \(P_3^*\). Now find the secret
data \(s_1, s_2\) and \(s_3\) from the stego-pixels using the same equations (1), (2) and (3), assuming
\(s_1 = X, s_2 = Y\) and \(s_3 = Z\). Figure 1 shows the embedding and extraction example for Wu
and Hwang’s (2017) method.

![Figure 1. Example for Wu and Hwang’s (2017) method](image)

**PROPOSED METHOD**

The step by step embedding and extraction procedure for the proposed method is presented in
this section. The pixels of an image are processed in raster scan order to conceal the
secret data. Let \(P_i\) be the \(i\)th pixel.

**Embedding Steps**

Step 1: Obtain \(P_{i1}, P_{i2}, P_{i3}\) and \(P_{i4}\) from \(P_i\) using equation (4)
\[ P_{i1} = \left[ \frac{P_1}{4} \right], P_{i2} = \left[ \frac{P_2}{4} \right], P_{i3} = \left[ \frac{P_3}{4} \right] \text{ and } P_{i4} = \left[ \frac{P_4}{4} \right] \]  

Step 2: Find the remainder (rmd) using equation (5) and update \( P_{i1}, P_{i2}, P_{i3} \) and \( P_{i4} \) using equation (6-9).

\[
\begin{align*}
\text{rmd} & = P_i \mod 4 \quad \text{(5)} \\
P_{i1} & = \begin{cases} 
P_{i1} + 1, & \text{if } \text{rmd} = 3 \text{ or } 2 \text{ or } 1 \\
P_{i1}, & \text{if } \text{rmd} = 0
\end{cases} \\
P_{i2} & = \begin{cases} 
P_{i2} + 1, & \text{if } \text{rmd} = 3 \text{ or } 2 \\
P_{i2}, & \text{if } \text{rmd} = 0 \text{ or } 1
\end{cases} \\
P_{i3} & = \begin{cases} 
P_{i3} + 1, & \text{if } \text{rmd} = 3 \\
P_{i3}, & \text{if } \text{rmd} = 0 \text{ or } 1 \text{ or } 2
\end{cases} \\
P_{i4} & = \begin{cases} 
P_{i4}, & \text{if } \text{rmd} = 0 \text{ or } 1 \text{ or } 2 \text{ or } 3
\end{cases}
\end{align*}
\]

Step 3: Let \( s_1, s_2, s_3 \) and \( s_4 \) be the secret data in binary.

Step 4: Obtain the binary values of \( P_{i1}, P_{i2}, P_{i3} \) and \( P_{i4} \) and store in \( \text{bin}_1, \text{bin}_2, \text{bin}_3 \) and \( \text{bin}_4 \) respectively.

Step 5: if \( \text{LSB} (\text{bin}_1) = s_1 \)
then \( P_{i1} = P_{i1} \)
else \( P_{i1} = P_{i1} + 1 \)

Step 6: Find \( \text{sum}_1 \) using equation (10).

\[ \text{sum}_1 = \left[ \frac{P_{i1}}{2} \right] + P_{i2} \quad \text{(10)} \]

Let \( \text{bin}_{\text{sum}_1} \) is the corresponding binary of \( \text{sum}_1 \).

Step 7: if \( \text{LSB} (\text{bin}_{\text{sum}_1}) = s_2 \)
then \( P_{i2} = P_{i2} \)
else \( P_{i2} = P_{i2} + 1 \)

Step 8: if \( \text{LSB} (\text{bin}_3) = s_3 \)
then \( P_{i3} = P_{i3} \)
else \( P_{i3} = P_{i3} + 1 \)

Step 9: Find \( \text{sum}_2 \) using equation (11).

\[ \text{sum}_2 = \left[ \frac{P_{i3}}{2} \right] + P_{i4} \quad \text{(11)} \]

Let \( \text{bin}_{\text{sum}_2} \) is the corresponding binary of \( \text{sum}_2 \).

If \( \text{LSB} (\text{bin}_{\text{sum}_2}) = s_4 \)
then \( P_{i4} = P_{i4} \)
else \( P_{i4} = P_{i4} + 1 \)
Step 10: Obtain the four stego pixels for the pixels $p_{11}$, $p_{12}$, $p_{13}$ and $p_{14}$ using equation (12)

\[
\begin{align*}
    p_{11}' &= p_{11} \times 4 \\
    p_{12}' &= p_{12} \times 4 \\
    p_{13}' &= p_{13} \times 4 \\
    p_{14}' &= p_{14} \times 4
\end{align*}
\]  

Step 11: Create four stego-images using $p_{11}'$, $p_{12}'$, $p_{13}'$ and $p_{14}'$.

Step 12: The embedding is done.

**Extraction Steps**

Step 1: Suppose the $i$th stego-pixels of the 4 stego-images are $p_{11}^{**}$, $p_{12}^{**}$, $p_{13}^{**}$ and $p_{14}^{**}$. Compute $p_{11}^{***}$, $p_{12}^{***}$, $p_{13}^{***}$ and $p_{14}^{***}$ using equation (13)

\[
\begin{align*}
    p_{11}^{***} &= \frac{p_{11}^{**}}{4} \\
    p_{12}^{***} &= \frac{p_{12}^{**}}{4} \\
    p_{13}^{***} &= \frac{p_{13}^{**}}{4} \\
    p_{14}^{***} &= \frac{p_{14}^{**}}{4}
\end{align*}
\]  

Step 2: Let $\text{bin}_1$ and $\text{bin}_3$ be the eight bit binary for $p_{11}^{***}$ and $p_{13}^{***}$.

Step 3: Compute $\text{sum}_{s1}$ using equation (14)

\[
\text{sum}_{s1} = \left\lfloor \frac{p_{11}^{***}}{2} \right\rfloor + p_{12}^{***}
\]

Represent $\text{sum}_{s1}$ to its eight binary bits and store in $\text{bin}_2$.

Step 4: Compute $\text{sum}_{s2}$ using equation (15)

\[
\text{sum}_{s2} = \left\lfloor \frac{p_{13}^{***}}{2} \right\rfloor + p_{14}^{***}
\]

Represent $\text{sum}_{s2}$ to its eight binary bits and store in $\text{bin}_4$.

Step 5: Obtain $s_1$, $s_2$, $s_3$, $s_4$ using equation (16)

\[
\begin{align*}
    s_1 &= \text{LSB} (\text{bin}_1) \\
    s_2 &= \text{LSB} (\text{bin}_2) \\
    s_3 &= \text{LSB} (\text{bin}_3) \\
    s_4 &= \text{LSB} (\text{bin}_4)
\end{align*}
\]

Step 6: $s_1$, $s_2$, $s_3$, $s_4$ are the 4 extracted bits.

Step 7: The extraction is done.
EXAMPLE FOR THE PROPOSED METHOD

Embedding Steps

Step 1: Let the original pixel be $R_1 = 123$ and the secret bits in binary are 0010.

Step 2: Now find $P_{11} = 30$, $P_{12} = 30$, $P_{13} = 30$, $P_{14} = 30$ using equation (4).

Step 3: Obtain the remainder, $rmd = 3$ using equation (5) and compute $P_{11} = 31$, $P_{12} = 31$, $P_{13} = 31$, and $P_{14} = 30$ using equations (6), (7), (8) and (9) respectively.

Step 4: Now obtain the corresponding eight bit binary for $P_{11}$, $P_{12}$, $P_{13}$ and $P_{14}$ as $bin_1 = 00011111$, $bin_2 = 00011111$, $bin_3 = 00011111$ and $bin_4 = 00011110$.

Step 5: As $LSB(bin_1) \neq 0$, so $P_{11} = 32$.

Step 6: Obtain $sum_1 = \left\lfloor \frac{P_{11}}{2} \right\rfloor + P_{12} = 47$ using equation (10) and $bin_{sum_1} = 00101111_2$.

Step 7: As $LSB(bin_{sum_1}) \neq 0$, so, $P_{12} = P_{12} + 1 = 32$.

Step 8: Again $LSB(bin_3) = 1$, so, $P_{13} = 31$.

Step 9: Now $sum_2 = \left\lfloor \frac{P_{14}}{2} \right\rfloor + P_{14} = 45$ equation (11) and $LSB(bin_{sum_2}) \neq 0$, hence $P_{14} = 31$.

Step 10: The final stego-pixels are $P_{11}^{*} = 128$, $P_{12}^{*} = 128$, $P_{13}^{*} = 124$, and $P_{14}^{*} = 124$ computed using equation (12).

Step 11: The embedding is done.

Extraction Steps

Step 1: The four stego-pixels are $P_{11}^{*} = 128$, $P_{12}^{*} = 128$, $P_{13}^{*} = 124$ and $P_{14}^{*} = 124$.

Step 2: Obtain the values of $P_{11}^{**} = 32$, $P_{12}^{**} = 32$, $P_{13}^{**} = 31$, and $P_{14}^{**} = 31$ using equation (13).

Step 3: Obtain $bin_1^* = 00100000$ and $bin_3^* = 00011111$.

Step 4: Compute $sum_{s1} = 48$ using equation (14). So, $bin_{s1}^* = 00110000$.

Step 5: Compute $sum_{s4} = 46$ using equation (15). So, $bin_{s4}^* = 00101110$.

Step 6: Obtain $s_1$, $s_2$, $s_3$, $s_4$ using equation (16) as $s_1 = 0$, $s_2 = 0$, $s_3 = 1$ and $s_4 = 0$.

Step 7: The extraction is done.
RESULTS AND DISCUSSION

The experiment had been conducted using Matlab tool. The images were taken from USC–SIPi and CV online databases and three of them are shown in Figure 2. The proposed method had been compared with existing methods (Wu & Hwang, 2017, Wu & Tsai, 2003, Khodaei & Faez, 2012 and Jung, 2018) and 1-LSB, 2-LSB and 3-LSB substitution with respect to the image steganographic parameters such as embedding capacity and PSNR. The PSNR can be computed using equation (17).

$$\text{PSNR} = 10 \times \log_{10} \frac{255 \times 255}{\text{MSE}}$$  \hspace{1cm} [17]

Where MSE is the mean square error and can be found using equation (18).

$$\text{MSE} = \frac{1}{m \times n} \sum_{i=1}^{m} \sum_{j=1}^{n} (x_{ij} - y_{ij})^2$$  \hspace{1cm} [18]

Where, $x_{ij}$ and $y_{ij}$ are the pixel values for the original and stego-image at position (i, j) respectively.

![Original images](image1.jpg)

*Figure 2. Original images (a) Lighthouse, (b) Boat and (c) Zelda*

The proposed method produces four different stego-images from one original image. The modified LSB matching method hides one bit per each produced stego-image. So, for four stego-images the total bits hidden are four times of 262144 bits. Again, due to hiding one bit per pixel, the distortion caused to the image is significantly reduced. The results of PSNR and EC for the proposed method and the other existing methods are presented in Tables 1, 2, 3, 4, 5 and 6. The average PSNR for the stego-images 1 to 4 are 36.06 dB, 37.88 dB, 39.60 dB and 41.00 dB respectively. The PSNR for Wu and Hwang (2017) is 51.69 dB but its capacity is limited to 262144 bits only. The embedding capacity of Jung (2018) is 916317 bits with a significant reduction in image quality i.e. with PSNR of 31.17 dB only. Further, the PSNR for Wu & Tsai, (2003) and Khodaei & Faez (2012) are 40.27 dB, 37.45 dB with EC having 407125 and 794816 bits respectively. Hence from the above analysis, we conclude that the proposed method is superior in terms of embedding capacity and PSNR.
Table 1

*PSNR for the proposed method*

<table>
<thead>
<tr>
<th>Images 512×512</th>
<th>Stego-image 1</th>
<th>Stego-image 2</th>
<th>Stego-image 3</th>
<th>Stego-image 4</th>
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Table 2

*PSNR for 1-LSB, 2-LSB, 3-LSB*

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Table 3
PSNR Wu & Hwang, 2017b, Wu & Tsai, 2003a, Khodaei & Faez, 2012 and Jung, 2018

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Table 4
EC for the proposed method

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Table 5
EC for 1-LSB, 2-LSB and 3-LSB

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Table 6
EC for Wu & Hwang, 2017b, Wu & Tsai, 2003a, Khodaei & Faez, 2012 and Jung, 2018

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</tbody>
</table>

**RS-analysis**

The RS-analysis is based on the dual statistical method. The pixels are categorized into 3 groups such as (1) the regular group with $R_M$ and $R_M$, (2) the singular group with $S_M$ and, $S_M$ and (3) the unusable group. The discrimination function (DF) finds the value of $R_M$ and $R_M$, $S_M$ and $S_M$. 

---

Multi Stego-image Based Data Hiding Method

Figure 3. RS-plot for (a) 1-LSB, (b) 2-LSB, (c) 3-LSB and (d) Jung (2018)

Figure 4. RS-plot for the proposed method (a) stego-image 1, (b) stego-image 2, (c) stego-image 3 and (d) stego-image 4
In the resulting RS-plot, the x-axis represents the percentage of hiding capacity and the y-axis represents the percentage of regular or singular groups at the various level of embedding. From the obtained plot If the condition, $R_M \approx R_M - S_M \approx S_M$ holds then it implies that the said technique has successfully passed the RS-analysis. Otherwise, if the condition $R_M - S_M > R_M - S_M$ holds then the technique is exposed to RS-analysis. The RS-plots for the 1-LSB, 2-LSB, 3-LSB substitutions and Jung’s (2018) method are shown in Figure 3, it is clearly observed that the condition $R_M - S_M > R_M - S_M$ is satisfying and hence these methods are caught by RS-analysis. Whereas RS-plots for the proposed method for the boat image with four stego-images are shown in Figure 4, it can be observed the condition $R_M \approx R_M > S_M \approx S_M$ holds for the proposed method. Hence from the above analysis it is found that the proposed method is resistant to RS-analysis.

**CONCLUSION AND FUTURE SCOPE**

This paper proposes a novel way of data hiding using multi stego-images to achieve high capacity with low distortion. First, each pixel of the original image produces four pixels. The secret data is hidden on each of the produced pixels using the modified LSB matching method. Then pixels are readjusted to reduce the distortion. In this way, the original image produces four different stego-images and each stego-image hides 1 bit per pixel. Further, the proposed method resists RS-analysis. For further improvement, the authors aim to develop a reversible steganography by improving the EC for the individual stego-images by combining LSB matching with PVD.

**ACKNOWLEDGMENTS**

We are thankful to the editor and the anonymous reviewers for their suggestions and comments. We declare this work is an independent work and no financial assistance has been received for this work.

**REFERENCES**


Simplified APP Computation of High Order Constellations Combined with Non-Binary LDPC Decoders

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\(^{2}\)RCAM Laboratory, University of Djillali Liabès, Sidi Bel-Abbès, Algeria
\(^{3}\)IEMN UMR CNRS 8520, Polytechnic University of Hauts-de-France, Valenciennes, France

ABSTRACT

Combining non-binary LDPC (Low-Density Parity-Check) codes and high order constellations, such as Quadrature Amplitude Modulations (QAM), is an effective way to improve the bandwidth efficiency. Since, the message exchanged in the LDPC decoder can be measured by the APP (A Priori Probability) or the LLR (Log-Likelihood Ratio), depending on the decoding algorithm type, the message at the decoder input that computed by the QAM must be using the same calculation. However, the number of operations performed by the QAM increases with the constellation order, and the calculation changes with the channel type. In this paper, we use simplified LLR computations, introduced for binary codes, to simplify the APP calculation of square-QAM-Gray demapping for APP-based non-binary LDPC decoders. Under Gaussian channel, the simplified APP calculation achieves the same performance that obtained with the exact APP computation. The same simplified APP calculation used for a Gaussian channel can be applied, with minor operations added, for Rayleigh channel, and it shows a small performance loss with respect to the exact APP computation. These simplifications simplify the combination of non-binary LDPC codes with QAM. With this method, it is easy to change a decoding algorithm based on the APP to an algorithm based on the LLR.
INTRODUCTION

Given the increasing number of applications requiring high-speed transmission without increasing the bandwidth of the transmission channel; this is the reason for the use of high order constellations. When the constellation order is higher than eight, one uses a Quadrature Amplitude Modulation (QAM) rather than Phase Shift Keying (PSK). Therefore, the QAM is highly recommended as a high order constellation. However, communication systems using QAM require a high signal to noise ratio. To overcome this disadvantage, it is interesting to combine high error correction codes such as LDPC codes with QAM.

Binary LDPC codes that are linear block codes, can approach the Shannon limit (Shannon, 1948). They are proposed by Gallager (1962 & 1963) and rediscovered by Mackay (1999). Unfortunately, binary LDPC code shows performance degradation when the code size is small or moderate, and higher order modulations are used for transmission. Non-binary LDPC code, designed in high order Galois Fields $GF(Q)$ where $Q$ is the cardinality of the Galois field, is investigated by Davey and Mackay (2002) to avoid this weakness.

The LDPC decoding is done according to the principle of iterative decoding. One class of algorithms used to decode LDPC codes is the commonly known message propagation algorithms (Johnson, 2010). Message propagation algorithms are also known as iterative decoding algorithms. The first practical iterative decoding algorithm is the Sum-Product algorithm (SPA) (Gallager, 1963), also known as the belief propagation algorithm is an optimal iterative decoding algorithm but with a high computational complexity. Several algorithms have been proposed to reduce the complexity of the SPA, each one with a particular performance-complexity tradeoff.

The messages exchanged in the SPA and its versions can be measured by the APP or the LLR depending on the decoding algorithm type. Therefore, the input message of the decoder, performed by the QAM, must be computed with the same calculation that used in the decoder. However, the number of operations to calculate the LLR or the APP that introduced to the decoder, increases with the constellation order. Also, the calculation changes with the considered channel.

Several algorithms have been introduced in order to simplify the exact calculation of the LLR. The pragmatic algorithm, introduced in Le Goff et al., (1994) & Le Goff, (2000), attempts to simplify the calculation assuming that the likelihood values are Gaussian variables. The max-log-MAP (max-log Maximum A Posteriori) algorithm is the most popular simplifying the exact algorithm (Liu & Kosakowski, 2015). The simplified LLR calculations are used only for binary decoders based on LLR. This simplifications are
applied for binary LDPC codes and non-binary LDPC codes (Mostari & Taleb-Ahmed, 2017 & Mostari et al., 2015) respectively. While for decoder-based on APP, we need to calculate the APP, and this latter is complex. Simplified APP computations are introduced only in Mostari et al. (2018).

The authors in Mostari et al. (2018) proposed a method for applying the simplifications of the LLR and adapted them to the binary LDPC decoder based on the APP, and even to simplify the calculation of the APP. In fact, it is easy to change a decoding algorithm based on LLR to an algorithm based on APP, while keeping unchanged the simplified calculation of LLR. The proposed method is programmed to adapt as perfectly as possible the transmission system to the channel type. Therefore, the proposed method allowing to simplify the APP calculation leads to simplify the implementation of the transmission system. In this work, we use this method for non-binary LDPC decoder based on the APP.

In this paper, we restrict our description of combining non-binary LDPC code, decoded by FFT-SPA (Fast Fourier Transform SPA) that uses APP calculations, with square Gray-QAM constellations, over Gaussian channel for satellite transmission and Rayleigh Channel for radio transmission (Barnault & Declercq, 2003; Carrasco & Johnston, 2008). Note that square QAM constellations and the others QAM constellations have different simplifications.

The rest of the paper is organized as follows: the exact APP computation, under Gaussian and Rayleigh channels, for square QAM constellations, the proposed diagram on the simplified APP calculations, the simplified APP calculation for non-binary LDPC code is given, the simulation results, discussion and concluding remarks.

**EXACT APP CALCULATIONS FOR QAM WITH SQUARE CONSTELLATIONS OVER GAUSSIAN AND RAYLEIGH CHANNELS**

2\(^m\)-QAM transmit, at each instant \(nT\), \(m\) bits \(\{u_{n,i}\}, \ i \in \{1, \ldots, m\}\) that is represented by \(\alpha_n + jb_n\), where \(\alpha_n\) and \(b_n\) are \(\{\pm 1, \pm 3, \pm 5, \ldots, m \pm 1\}\). Binary symbols \(\{u_{n,i}\}\) are obtained by the conversion of non-binary symbols to binary symbols at the output of LDPC encoder.

The simplest diagram of a digital transmission system as part of the association of an LDPC code and a 2\(^m\)-QAM, is given in Figure 1.

After passing through the transmission channel, the observation relating to the couple \((a_n, b_n)\) is represented by a couple \((a'_n, b'_n)\). In the case of Rayleigh channel \(a'_n\) and \(b'_n\) are given by:

\[
\begin{align*}
a'_n &= \alpha_n a_n + z_n \\
b'_n &= \alpha_n b_n + z_n
\end{align*}
\]

where \(z_n\) is a Gaussian noise, centered, with variance \(\sigma^2\) and \(\alpha_n\) is a variable characterizes the attenuation of the transmitted signal. In the case of a Gaussian channel \(\alpha_n = 1\).
At the reception, we treat the couples \((a'_n, b'_n)\) in order to extract \(m\) samples \(\{\hat{u}_{n,i}\}, i \in \{1, \ldots, m\}\), that are calculated by \(\text{LLR}(u_{n,i})\) or \(\text{APP}(u_{n,i})\).

\[
\text{APP}(u_{n,i}) = \begin{cases} 0 & \text{if } \text{Pr}[(a'_n, b'_n) / u_{n,i} = 0] = \text{Pr}[(a'_n, b'_n) / u_{n,i} = 1] \text{,} \\
1 & \text{if } \text{Pr}[(a'_n, b'_n) / u_{n,i} = 0] = \text{Pr}[(a'_n, b'_n) / u_{n,i} = 1] \text{.} 
\end{cases}
\]

\[
\text{APP}(u_{n,i}) = 1 - \text{APP}(u_{n,i} = 0)
\]

where \(\text{Pr}[(a'_n, b'_n) / u_{n,i} = w]\) is the probability that the available couple is \((a'_n, b'_n)\); knowing the binary symbol \(u_{n,i}\) is equal to \(w\).

\[
\text{LLR}(u_{n,i}) = \log \left[ \frac{\text{Pr}[(a'_n, b'_n) / u_{n,i} = 1]}{\text{Pr}[(a'_n, b'_n) / u_{n,i} = 0]} \right]
\]

This equation is the exact calculation of \(\text{LLR}\), it is the optimal calculation that represents the log-MAP algorithm (Maximum A Posteriori). However, it involves complicated operations. Several algorithms have been introduced in order to simplify the exact calculation of the \(\text{LLR}\) such as: max-log-MAP algorithm and pragmatic algorithm.

In the case of \(m=2p\), \(2^{2p}\)-QAM modulation uses a square constellation (case of 16-QAM, 64-QAM and 256-QAM). Such modulation has the particularity to be reduced to two amplitude modulations with \(2^p\) states independently acting on two carriers in phase and quadrature. In our work, we use a square constellation.

According to the above property of a square constellation, \(p\) expressions in the phase of the APP, for a Gaussian channel, are the following:

\[
\text{APP}(u_{n,i} = 0) = \frac{\Sigma_{j=1}^{2p} \exp \left[ \frac{1}{2 \sigma^2} (a'_n - a_n a'_j)^2 \right] + \Sigma_{j=1}^{2p} \exp \left[ \frac{1}{2 \sigma^2} (a'_n - a_n a'_j)^2 \right]}{\Sigma_{j=1}^{2p} \exp \left[ \frac{1}{2 \sigma^2} (a'_n - a_n a'_j)^2 \right] + \Sigma_{j=1}^{2p} \exp \left[ \frac{1}{2 \sigma^2} (a'_n - a_n a'_j)^2 \right]}, i \in \{1, \ldots, p\}
\]

\[
\text{APP}(u_{n,i} = 1) = 1 - \text{APP}(u_{n,i} = 0), \quad i \in \{1, \ldots, p\}
\]

Similarly, the \(p\) relations in the quadrature path of the APP eventually lead to the following expressions:

\[
\text{APP}(u_{n,i} = 0) = \frac{\Sigma_{j=1}^{2p} \exp \left[ \frac{1}{2 \sigma^2} (b'_n - a_n b'_j)^2 \right] + \Sigma_{j=1}^{2p} \exp \left[ \frac{1}{2 \sigma^2} (b'_n - a_n b'_j)^2 \right]}{\Sigma_{j=1}^{2p} \exp \left[ \frac{1}{2 \sigma^2} (b'_n - a_n b'_j)^2 \right] + \Sigma_{j=1}^{2p} \exp \left[ \frac{1}{2 \sigma^2} (b'_n - a_n b'_j)^2 \right]}, i \in \{p + 1, \ldots, 2p\}
\]

\[
\text{APP}(u_{n,i} = 1) = 1 - \text{APP}(u_{n,i} = 0), \quad i \in \{p + 1, \ldots, 2p\}
\]

In the following section, we show the proposed method, that we have introduced for binary LDPC codes presented in other paper, to simplify the APP calculation.
PROPOSED BLOCK DIAGRAM OF THE SIMPLIFIED APP CALCULATIONS

In order to simplify the APP calculation, we apply the simplified LLR computation, for Gaussian and Rayleigh channels, that used for a decoder based on LLR. The simplified LLR algorithm got on a Gaussian channel can be reused efficiently on a Rayleigh channel (Figure 1), this provided to insert an additional block to accommodate, each time $nT$, the channel attenuation $\alpha_n$ (Le Goff, 1995).

Then, we insert an additional module to make the conversion from the LLR to the APP, as shown in Figure 2. Indeed, it is easy to change a decoding algorithm based on LLR to an algorithm based on APP, while keeping unchanged the simplified LLR calculation. Therefore, the proposed method leads to simplify the system implementation that is well shown in figure 1.

Figure 2. Principle of simplified APP calculation

Following the Figure 2, the simplification of the APP is obtained after two operations: the first is the simplification of the LLR calculation, and the second is the derivation of the APP from the LLR (Lee et al., 2005). Therefore, the derivation of the APP from the simplified LLR, leads to the simplified equations of the APP:

\[
APP(u_{ni} = 0) = \frac{1}{1 + \exp[\text{LLR}(u_{ni})]}, \quad i \in \{1, ..., 2p\}
\]

\[
APP(u_{ni} = 1) = 1 - APP(u_{ni} = 0), \quad i \in \{1, ..., 2p\}
\]

where $\text{LLR}(u_{ni})$ represents the simplified calculation of the LLR. In our work, we use max-log-MAP algorithm and pragmatic algorithm :

Max-log-MAP Algorithm

The Max-log-MAP algorithm, introduced in Liu and Kosakowski (2015) shows that the $p$ relations in the phase and $p$ relations in the quadrature are given respectively by the following equations:
The pragmatic algorithm introduced in LeGoff et al. (1994) shows that the $p$ relations in the phase and $p$ relations in the quadrature, are given respectively by the following equations:

$$
LLR(u_{n,1}) = a'_n
$$

$$
LLR(u_{n,2}) = -|LLR(u_{n,1})| + 2^{p-1}
$$

$$
\vdots
$$

$$
LLR(u_{n,i}) = -|LLR(u_{n,i-1})| + 2^{p-i+1}
$$

$$
\vdots
$$

$$
LLR(u_{n,p}) = -|LLR(u_{n,p-1})| + 2
$$

And

$$
LLR(u_{n,p+1}) = b'_n
$$

$$
LLR(u_{n,p+2}) = -|LLR(u_{n,p+1})| + 2^{p-1}
$$

$$
\vdots
$$

$$
LLR(u_{n,p+i}) = -|LLR(u_{n,p+i-1})| + 2^{p-i+1}
$$

$$
\vdots
$$

$$
LLR(u_{n,2p}) = -|LLR(u_{n,2p-1})| + 2
$$

Therefore, it is remarkable that the simplified APP calculation are less number of operations that the exact APP calculation.

**SIMPLIFIED APP CALCULATION FOR NON-BINARY LDPC CODES**

For non-binary LDPC decoding algorithms based on APP, defined in a Galois Field $GF(2^q)$, the exchanged message is the APP calculated on non-binary symbols $a$, $a \in GF(2^q)$. $\text{APP}(a)$, that is calculated as follows:

$$\text{APP}(a = v) = Pr\{a = v/(a'_n, b'_n)\}$$

Where $Pr\{a = v/(a'_n, b'_n)\}$ represents the probability that the symbol $a$ transmitted has a value $v$, $v \in GF(2^q)$, knowing that the available couple at the channel output is $(a'_n, b'_n)$.

Each non-binary symbol $a$, $a \in \{0, 1, \ldots, 2^q - 1\}$, corresponds to the binary sequence $\{u_{n,1}, u_{n,2}, \ldots, u_{n,q}\}$ in $GF(2)$. Therefore, the equation (22) becomes:

$$\text{APP}(a = v) = \prod_{i=1}^{q} Pr\{u_{n,i} = w/(a'_n, b'_n)\}$$
where the binary value $w$ is associated to the value $v$.

Therefore, using Bayes rule, the precedent expression becomes:

$$APP(a = v) = \prod_{i=1}^{q} APP(u_{n,i} = w)$$

**SIMULATION RESULTS**

In this section, we will show the effect of the simplified calculation of the APP on the performance of non-binary LDPC codes constructed on GF(4) of rate equals to 1/2 and a parity check matrix of size $512 \times 1024$ (4-ary (512, 1024) LDPC Code).

A decoding algorithm using the FFT-SPA where the number of iterations is four. The transmission chain for which we evaluated the Binary Errors Rate (BER) after the decoding used a non-binary LDPC code, attached to two square constellations: 16-QAM and 64-QAM, using Gray mapping, and two transmission channels: Gaussian and Rayleigh.

Results, obtained by computer simulations, are given in terms of Bit Error Rate (BER) versus $E_b/N_0$, where $E_b$ is the energy per information and $N_0$ is the spectral density noise.

Figure 3 shows performance comparisons, on a Gaussian channel, between a non-binary LDPC code using the exact calculation of the APP, a non-binary LDPC code using the simplified calculation by applying the pragmatic algorithm and a non-binary LDPC code using the simplified calculation by applying the max-log-MAP algorithm.

In order to study the influence of the simplified calculation on the performance of a non-binary LDPC code on a Rayleigh channel, the same performance comparison of figure 3 are performed on a Rayleigh channel, in Figures 4 and 5.
Figure 4. Performance comparisons, under a Rayleigh channel, of 4-ary (512, 1024) LDPC code decoded by FFT-SPA using exact APP and simplified APP algorithms, using pragmatic algorithm, of 16-QAM and 64-QAM

Figure 5. Performance comparisons, under a Rayleigh channel, of 4-ary (512, 1024) LDPC code decoded by FFT-SPA using exact APP and simplified APP algorithms, using max-log-MAP algorithm, of 16-QAM and 64-QAM

DISCUSSION

Figure 3 shows that the simplified calculation of the APP, on a Gaussian channel, using a max-log-MAP algorithm and a pragmatic algorithm, has no effect on the performance of a non-binary LDPC code.

Under Rayleigh channel, as seen in Figures 4 and 5 respectively, in comparison with the exact APP computation of 16-QAM and 64-QAM associated with non-binary LDPC codes, the simplification of the APP computation, with pragmatic algorithm, has a small
performance loss, and the simplification of the APP computation, with max-log-MAP algorithm, has a very small performance loss at high $E_b/N_0$.

The results of our simulation are generalized with small or high order square-QAM constellations. As a result, the simplification of the APP calculation can achieve a good performance with a simple computation. It leads to simplify the system implementation.

CONCLUSION

In this work, we used the simplified LLR calculation, introduced for binary code, to facilitate the APP calculation for non-binary LDPC codes. The proposed method for making these simplifications, puts a system combining a non-binary LDPC code and a high order constellation simple to implement. It is programmed to adapt as perfectly as possible the system to the type of channel in question and to the type of decoding algorithm. Also, it ensures an efficient decoding regardless of the channel type. Therefore, since LDPC codes are selected as candidate for 5th generation wireless communications (5G), it is essential to develop a new technique allowing the simplification of the transmission system for 5G and Satellite communication systems.

REFERENCES


Structural and Statistical Similarity Measure based Approach for Effective Eye Blink Recognition

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ABSTRACT

Eyeblinks are having the significance to analyze the attention, fatigue, behaviour and emotion of an individual. Eyeblink recognition is adopted by many medical and surveillance applications to identify the person’s state. The eye blink recognition on videos requires tracking the eye region and to count the number of eye blinks. In this paper, a three-stage model is presented to detect the eye blinks accurately. In the first stage, the frame similarity analysis, background separation, positional and mathematical filters are applied collectively to identify the effective eye region on unique frames. In the second stage, the similarity analysis using wavelet decomposition and statistical filters are applied on the segmented eye region. The filtered evaluation is performed to identify the change on the eye region of continuous segmented frames. At the final stage, distance driven map on structural and statistical features is applied to remove the invalid frame changes and to obtain the accurate eye blink count. The proposed model is applied on real time, web-collected and the NRC-IIT dataset videos. These complex videos are associated to the indoor and outdoor environments. The news reading and other complex video sequences are analyzed in this research. The observations identified that the proposed model has reduced the possible generated errors and provided the accurate detection of eye blinks.

Keywords: Eyeblinks, statistical, structural, video sequences

INTRODUCTION

The Eye and eye-blink tracking has gained its scope in various real-time applications in
recent time (Choi et al., 2011; Ryu et al., 2013; Junjea, 2015). The static eye has already proven as individual biometric or part of multi-model systems to recognize an individual. Eye direction, movement and blink are also recorded as the advancement to generate more interactive observations. The confidence or attention of a student or employee can be observed within the class or conference using these measures. The fatigue or doziness of a driver or athlete or worker can be recognized by characterizing the eye behaviour. The quality of these eye tracking systems depends on the continuous recording of face and eye-region. Earlier, the distance cameras were used to record the eye movement. Such cameras were not effective and sensitive to face movement, pose and occlusion. But in recent times, various wearable devices are available with specialized cameras to record each and minor movement clearly. The smart eye-glasses and head-movement sensitive cameras are available to acquire more focused and continuous tracking of eye-region (Rihana et al., 2012; Choi et al., 2011; Abe et al., 2014). This kind of tracking is able to resolve many of the real time deficiencies that can affect the accuracy of such predictive systems. Even, the distance camera based eye-tracking is also used through surveillance cameras to observe the group of people. The eye blink tracking and recognition are having several challenges and characteristics that are required to resolve and measure for improving the accuracy of the decision system. These characteristics and relative functional behaviour are provided in table 1.

Table 1

<table>
<thead>
<tr>
<th>Feature</th>
<th>Role</th>
<th>Usage/Scope</th>
<th>User</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupil</td>
<td>Position and direction of Pupil</td>
<td>Attention Recognition</td>
<td>Student, Employee</td>
</tr>
<tr>
<td>Blink</td>
<td>Frequency, transition duration</td>
<td>Doziness, Fatigue estimation</td>
<td>Driver, Athlete, Patient</td>
</tr>
<tr>
<td>Eye</td>
<td>Biometric Feature</td>
<td>Biometric and Multimodal Authentication</td>
<td>Online Users</td>
</tr>
</tbody>
</table>

The applications and the functional behaviour of eye blink are dependent on various physiological and environmental factors recognition (Chen et al., 2015; Bacivarov et al., 2008; Choi et al., 2011). The head position or the pose of the individual can obscure the acquired information or features. The geometric misalignment and out-of-focus situation can drop the recognition rate. The camera alignment and positioning can also be the reason of such kind of disruption. The environmental situation such as lighting, fog and camera quality can also affect the visualized features of the eye and iris. The real time eye tracking is affected by all such disruptions and can affect the quality of eye blink recognition.

In this paper, a statistical and mathematical filter based model is provided to recognize the eye blink count accurately. The model is defined to process on real time videos. The
Structure & Similarity Analysis for Eye Blink Recognition

video frame processing, eye region processing and blink characterization are included as intermediate work stages of this model. The similarity measures are implied at each stage based on mathematical, structural or statistical filters. In this “INTRODUCTION” section, the characterization and scope of eye blink recognition are discussed. The applications and the behaviour of eye and eye-blink tracking are provided in this section. In “RELATED WORK” section, the algorithms and models adopted by the researchers for improving the eye-blink tracking are provided. In “RESEARCH METHODOLOGY” section, the proposed algorithmic framework is provided with the functional description of each inclusive stage. In section “RESULTS AND DISCUSSION”, the results are generated for sample videos taken in different environments. The graphical evaluation is provided to verify the significance of the proposed model. In section “CONCLUSION”, the conclusion of the proposed eye-blink recognition model is provided.

RELATED WORK

Eyes are considered as an effective biometric feature to recognize the individual. But in the recent years, the application and phenomenon of eye processing are extended. Now, eye movement, eye blink, iris region is processed separately or collectively to identify disease, fatigue, attention-level and behaviour of an individual. The camera position, quality and application increase the complexities to eye processing. The researchers have provided the methods to handle these real time challenges as well as to improve the processing behaviour of each integrated stage. In this section, the contribution of researchers in terms of stage, challenge and application specific methods is discussed.

The eye tracking and blink detection are challenging in real environment because of scene, head movement and low-resolution cameras. Effective segmentation methods are required to track the eyes and to count the eye-blink. Pauly and Sankar (2015b) processed the HOG (Histogram of Oriented Gradients) features with SVM (Support Vector Machine) classifier to detect the eye blink accurately and gained the accuracy over 92%. A wavelet transformation adaptive Neuro-fuzzy system was provided to count the fast eye-blinking accurately (Azar & Akhbardeh, 2007). Author improved the performance of eye-blink detection while handling the overlapped eye-blink problem. The template matching with similarity measure was proposed to reduce the false detection of eye-blink in the changing background scene (Awais et al., 2013). The correlation score evaluation based eye blink detection method achieved the higher accuracy for different experimental conditions. The Eigen-eye approach was employed to recognize the close-eyes with smart glasses (Le et al., 2013). Author combined the non-maximum suppression algorithm with Gradient Boosting algorithm to improve the robustness and accuracy of eye-blink recognition. The RBF (Radial Basis Function) classifier was applied on acquired statistical features to classify the eye-blink (Rihana et al., 2012). The color information surrounding eye-
region was analyzed to predict the eyelid movement (Panning et al., 2011). The adaboost learning and grouping method provided by Choi et al. (2011) to provide outlier robust detection of eye and eye-blink (Bacivarov et al., 2008) used the statistical features to encode the variations caused by blinking. The model was also robust to head-pose and gaze variations. SIFT (Scale-Invariant Feature Transform) feature processing with affine transformation was proposed to achieve pose robust eye-blink tracking (Lalonde et al., 2007). The constraint adaptive blob region was trained to determine the eye-blink length and sequence. The lighting, reflection and illumination challenges were dealt by Chen et al. (2015) for robust iris detection. Author used the polynomial interpolation and inter-class similarity for eye, gaze and eye-blink detection. Ryu et al. (2013) had proposed the Local ternary pattern and SVM (Support Vector Machine) based composite algorithm for real time eye blink detection and integrated it to the smart devices. The composition of peak detection algorithm and ICA (Independent Component Analysis) algorithm was provided to detect the eye-blink (Gao et al., 2010).

The accuracy and effectiveness of eye-blink recognition depends on the quality and mechanism of video capturing. Various devices, camera types, camera-integrations and sensors integrated devices are designed by the researchers to getting more informative and robust eye-blink recognition. Smart glasses camera was used with low-energy imaging and effective computation capabilities to improve the accuracy of eye-blink detection (Le et al., 2013). The Doppler sensors were used by Kim (2015) for noise and pose robust eye and eye-blink tracking. A camera based integrated system was designed to monitor the head, eye and eye-blinks (Pullano et al., 2016). The ultrasonic transmitter integrated network was designed to validate the system reliability in the real environment.

The eye-blink processing and analysis is having its effect in various real-time applications including attention identification, fatigue determination and driver drowsiness has evaluated the level of attentiveness of a person to determine the fatigue of person (Haq & Hasan, 2016). The addressed application can be employed in vehicles to warn the driver by observing the fatigue and the number of accidents can be reduced. The vigilance driving was detected using iris processing to improve the road safety (Nacer et al., 2014). The doze state of an individual was observed using Blink burst and isolated-blink detection (Naito et al., 2012). The consciousness level cases for fatigue, lack of sleep and repetition of task were identified by the author. Involuntary and voluntary blinks were isolated by to monitor the Human’s fatigue (Kurylyak et al., 2012). Pander et al. (2008) also designed a fatigue indicator by observing the spontaneous eye blink action. An evaluation on drowsiness condition of driver was observed to reduce the car crashes (Cristiani et al., 2010). The algorithm observed the face and eye under drowsiness constraints. The HOG features integrated SVM classifier was applied by Pauly and Sankar (2015a) to detect the
drowsiness of drivers. A multi-featured probabilistic model was provided to identify the driving disability of an individual because of fatigue or drowsiness (Junjea, 2015).

RESEARCH METHODOLOGY

Eyeblinks can be monitored and considered as a decisive phenomenon to identify the drowsiness and fatigue of individuals. The accurate estimation eyeblink count improves its significance in various medical and surveillance applications. This paper provides a mathematical, structural and statistical aspect based model to identify the eyeblink count accurately. The model has accepts the real-time facial video as input and extracts the effective frames and eye-region in the earlier stage. At the earlier stage, the mathematical filters are applied to identify the effective frames by observing the frame dissimilarity. The identified effective frames are analyzed under positional and mathematical filters to segment the eye region. The redial filter is applied on the eye region to segment the continuous frames. This extracted eye region is now acquired for significant individual frames to generate the effective features. A wavelet decomposition based block map is applied to acquire the low pass features. The content feature evaluation is implied on these block regions to describe the effective eye region. In the final stage, a distance driven map is applied of generated features to the open and close eye feature datasets. The maximum match based decision is considered to identify the eyeblink. The proposed structural and statistical feature based eyeblink detection model is provided in figure 1.

![Figure 1. Structural and Statistical Feature adaptive Model](image-url)
The proposed eyeblink model shown in Figure 1 is divided into three functional stages. In the first stage, the mathematical and positional filters are applied to identify the qualified frames and eye-region. The distinctive frames were identified by applying the frame-similarity evaluation. The identified frames were processed by mathematical morphological operators to separate the background and to identify the eye region. The positional estimation is also combined to identify the relevant and decisive eye region. To acquire the features, the low pass filtration is applied on effective eye region using wavelet decomposition. The block segmentation is applied on low featured region. The statistical and structural features are extracted on each block featured segment. This structural and statistical featured mapped eye is compared with open and close eye-image dataset using the Euclidian distance measure. The proposed model is defined as a generic model can be later applied for medical application, fatigue estimation, attention identification and driver drowsiness identification. The model is implemented on the larger face focused video sets with complex and diverse scenes. The implementation results are provided and discussed in “RESULTS AND DISCUSSION” section.

**Effective Eye Region Extraction**

The face-focused video is taken from diverse scene is accepted as the input to the eye-region extraction stage. The mathematical and position based evaluation is performed to identify the contributing frames and the eye region. The Distinctive frame detection, background separation and eye-region identification have defined the functional description of eye-region extraction.

**Distinct frame Extraction**

The video file is transformed to frame-images by applying the Matlab tool. The frame-rate and file format are defined while extracting the frames. The distance-based analysis is performed between the consecutive frames. The n-dimensional transition and feature vector generation is done in terms of energy (En) and mean coefficient (MC) evaluation. The distance estimation between these two vectors is done on consecutive frames as dist(frame_i,frame_{i+1}). Where each frame is described by frame_i=(En,MC). The distance evaluation is done based on Euclidean distance (Ed) estimation. The formulations of feature vector and distance estimator are provided below:

$$En = \frac{1}{N^2} \sum_{i=1}^{N} Frame(i)^2$$ (1)

Where, N is the length of vector, i is the block index of vector.

The energy is the high pass information vector that that generates the derivation ratio based absolute value. The content driven analysis is provided by this energy vector. The
mean-coefficient evaluation is provided in equation (2). It computes the average intensity of each frame block.

\[
MC = \frac{1}{N} \sum_{i=1}^{N} Frame(i) \quad (2)
\]

The Energy (En) and Mean-Coefficient (MC) vector based frames were compared on consecutive frames using Euclidean distance measure shown in equation (3). The similar frames are neglected and the non-similar frames are considered as an effective frame to take the decision on eye-blink.

\[
EDist(\text{Frame}_i, \text{Frame}_{i+1}) = \sqrt{\sum_{i=1}^{N} (\text{Frame}_i - \text{Frame}_{i+1})^2} \quad (3)
\]

**Background Separation**

The eyeblink detection is focused on the facial and eye region (Choi et al., 2011; Kim, 2015). The background separation is applied to avoid the scene complexity and to process only the relevant region. The skin region adaptive mean filter evaluation is performed on YUV (Luminance (Y), blue–luminance (U), red–luminance (V)) converted frames. This color model is more robust to noise and illumination change. The RGB (Red-Green-Blue) to YUV color model transition is shown in equation (4)

\[
\begin{pmatrix}
Y \\
U \\
V
\end{pmatrix} =
\begin{pmatrix}
+0.0247 & +0.504 & +0.098 \\
-0.148 & -0.291 & +0.439 \\
+0.0439 & -0.368 & -0.071
\end{pmatrix}
\begin{pmatrix}
R \\
G \\
B
\end{pmatrix} =
\begin{pmatrix}
16 \\
128 \\
128
\end{pmatrix} \quad (4)
\]

The threshold adaptive morphological operators are applied on color transited image to identify the effective region. The log transition function adopted for region extraction is provided in equation (5)

\[
ERegion(x, y) = a + \frac{log(EFrame(x, y) + 1)}{b \times log(c)} \quad (5)
\]

Where, EFrame is the effective frame; a, b and c are the illumination controlled constraints.

The threshold limits are applied through a, b and c values to identify the effective region (ERegion) using this log transformation. The morphological and convolutional filters are also applied respective to mean pixel value to clean the smaller chunks and to identify the effective skin region over the image. The stage has removed the background and the non-relevant region from the effective frames and identified the effective region (ERegion).
Eye-Region Detection

The effective facial region (ERegion) is processed using positional parameters to identify the eye region (Nacer et al., 2014). The ratio adaptive geometric analysis is performed to identify the eye region. Once the face region is captured, the facial components can be identified by applying the geometric ratio evaluation. The horizontal and vertical analysis can be applied for different facial components. The estimation on eye position, distance between eye-nose and between-eyes is done to extract the eye region. The work is successful implementation of facial component extraction. Same work is applied to extract the effective eye region in this work.

Eye-Blink Detection

In the second stage of this model, the eye blink detection process is accomplished by applying the structural and statistical feature evaluation. It is a composite process in which, the extracted eye region is first decomposed using the low-pass filtration. This filtration stage has identified the relevant content information from the image. Now the block segmentation is applied on this extracted featured region. For each block, the structural and statistical features are extracted to represent the eye region as processing feature set. In this Eye-Blink Detection stage, each of the integrated process stage is described.

Wavelet Decomposition

Haar wavelet transformation method is applied in this research for low-pass filtration and to reduce the dimension of processing information. In this filter, the average of two adjacent pixels is taken to take the decision on the low-pass filtration. Two-level wavelet decomposition is applied independently on eye-region columns and then rows. The sub-bands are generated at each level of this decomposition. The low frequency subband is processed to acquire the adaptive and reduced resolution features. The extracted features are adaptive to the energy so that the information contents are extracted using this decomposition stage. The approximation coefficient is taken at each level of this decomposition. This decomposed energy features are processed by block adaptive feature generation stage to generate the processing featureset.

Block Segment based Feature Extraction

After extracting the relevant content driven eye region, the block segmentation is applied to generate the information and region adaptive featureset. The rectangular blocks of 4x4 are generated over the segmented region. On each block, the statistical and structural features such as Energy (En), Inertia (In), Standard Deviation (SD), Entropy (E) parameters. These are the content-driven parameters which are able to identify the intensity and variation
driven analysis within the block (Blk). Each of the block pixels is processed for these features to generate the content adaptive wider feature set. These equations are these content features are provided:

\[
En = \frac{1}{N^2} \sum_{i=1}^{N} Blk(i)^2
\]  

(6)

Energy (En) identifies the derivation adaptive content information to represent the eye-region effectively. Equation (6) represents the energy feature evaluation based on content specific ratio value. Another content driven evaluation is performed by Inertia (In) parameter described through equation (7). It identifies the intensity level distribution within the block. In these equations, N is the size the length of block and w, h are the weight and height of block.

\[
In = \sum_{x=1}^{w} \sum_{y=1}^{h} (x - y)^2 Blk(x, y)
\]  

(7)

The intensity and content driven variation exist within the block is evaluated using Standard Deviation (sD) and Entropy (E) features provided in equation (8) and (9). The variations exist within the block and the degree of heterogeneity based on pixel intensity is evaluated by these features.

\[
sD = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} Blk(i)^2}
\]  

(8)

\[
E = -\sum_{i=1}^{N} Blk(i) \log Blk(i)
\]  

(9)

These structural and content based features are generated for each block over the effective eye-region. The wider feature set of eye-region is compared with feature transformed open and close eyes. The maximum matched eye-status is considered as the status of that particular eye-frame. The distance-based comparison is done using Euclidean distance measure described in the “RESULTS AND DISCUSSION” section.

**Euclidean Distance Matching**

Once the eye-region of input video-frames and the eye images of training datasets are transformed to statistical and structural features, the Euclidean distance based match is applied to identify the eye status. The feature adaptive vector set is analyzed using the Euclidean distance (EuD) based pattern measure shown in equation (10).

\[
EuD = \sqrt{\sum_{i=1}^{M} [InputEye_i - TrainingEye_i]^2}
\]  

(10)

Where, M is the length of feature vector.
The feature vector of extracted eye region is compared with open-eye and close-eye training set images separately. The identified eye image with maximum match is considered as the status of the eye. Once the eye statuses are identified, the sequence of change of eye status from open-to-close and close-to-open are identified to estimate the eye-blink count. The proposed eye-blink detection model is implemented on self captured and the videos collected through random web sources. The implementation results and the description of dataset are provided in the next section.

RESULTS AND DISCUSSION

In this paper, the mathematical and statistical filters are applied at different levels to count the eye blinks. The proposed model has acquired the eye region effectively from complex scenes of video frames. The mathematical filters are applied to recognize the contributing frames and the eye-region. The statistical measures such as Energy (En), Inertia (In), Standard Deviation (sD) and Entropy (E) are applied on block segmented region of each selected-frame to represent the decisive features. These features are compared to the open and close eye datasets using Euclidean distance measure to recognize the frame class. The frame sequence is finally mapped respective to eye status to identify the blink-count. In this section, the comparative evaluation of the proposed structural and statistical measure adaptive model is applied on multiple video collected randomly from Google search (Youtube.com, 2018), self-captured real time videos and the videos taken from NRC-IIT (Videorecognition.com, 2018) dataset of facial videos. The description of these videosets used in this research is provided in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Characterization of eye-blink tracking</th>
<th>Real Time Videos</th>
<th>External Web Sources (Youtube.com, 2018)</th>
<th>NRC-IIT Dataset (Videorecognition.com, 2018)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Videos</td>
<td>30</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Type</td>
<td>Color</td>
<td>Color</td>
<td>Color</td>
</tr>
<tr>
<td>Category</td>
<td>Indoor, Outdoor Driving</td>
<td>Indoor, Outdoor Driving, Drunken Videos</td>
<td>Indoor Office Video</td>
</tr>
<tr>
<td>Format</td>
<td>3gp</td>
<td>AVI</td>
<td>AVI</td>
</tr>
<tr>
<td>Resolution</td>
<td>176x144</td>
<td>Multiple</td>
<td>160x120</td>
</tr>
<tr>
<td>Environment</td>
<td>Indoor, Outdoor</td>
<td>Indoor, Outdoor</td>
<td>Indoor</td>
</tr>
</tbody>
</table>

These videos are categorized as new reader videos, drunken driver videos, person real-time videos and outdoor scene based videos. These all videos are collected in versatile environments with individual focusing with different camera distances. The
personal real time videos are self collected videos in indoor and outdoor environment as family video dataset. These videos are collected in different formats such as AVI, WMV, MP4 and converted to standard AVI format. The evaluation is performed by identifying the actual eyeblinks identified by monitoring the video file manually and the number of eyeblinks identified by the model. To verify the significance of the proposed model, the implementation of existing-threshold based, dynamic-threshold based approaches is also provided. Junjea (2015) is the work already done by me to identify the eye-blink count for driver disability evaluation. These three existing and proposed statistical measure adaptive method are implemented in the matlab environment on more than 50 videos of diverse lengths and environment which are taken from various sources shown in Table 2. The detailed evaluation of nine videos is provided in Table 3. These videos are taken randomly from the video pool of different lengths and categories. The table contains the actual number of eye-blinks in each video and the eyeblinks identified by each of existing and proposed approach.

<table>
<thead>
<tr>
<th>Video Files</th>
<th>Total Eye Blinks</th>
<th>Threshold based Approach</th>
<th>Dynamic Threshold</th>
<th>(Junjea, 2015)</th>
<th>Proposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Video1.avi</td>
<td>18</td>
<td>11</td>
<td>14</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Video2.avi</td>
<td>24</td>
<td>16</td>
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</tr>
<tr>
<td>Video3.avi</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Video4.avi</td>
<td>14</td>
<td>9</td>
<td>11</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Video5.avi</td>
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<td>7</td>
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<td>7</td>
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<tr>
<td>Video6.avi</td>
<td>19</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Video7.avi</td>
<td>31</td>
<td>24</td>
<td>27</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Video8.avi</td>
<td>27</td>
<td>19</td>
<td>22</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Video9.avi</td>
<td>11</td>
<td>7</td>
<td>8</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

The analytical observations collected for a random set of videos is provided in Table 3. The video files names with actual eye blink count are listed in the table. The existing threshold based method has considered the single static value for taking the decision on eyeblink detection. As the method performed the lesser dynamic computation, the detected eyeblink count is very less in this method. The dynamic threshold based method has performed the intensity and region density specific evaluation to identify the eyeblinks. The geometric and color model analysis was performed by Junjea (2015) for eye region extraction. The probabilistic estimation on correlation and content similarity analysis was
performed for identification of eye status. This method also provided the comparatively better results than threshold and dynamic threshold based method. The proposed structural and statistical measure based evaluation method has used multiple measures to take the concrete decision to track the eye status. The results identified that the proposed model

![Figure 2. Accuracy ratio analysis of eyeblinks (sample videos)](image)

has identified the eyeblinks effectively for variant scenes. All the eyeblinks are identified in many of the sample videos by this proposed model. The accuracy based evaluation estimated from the statistics of this table is depicted in Figure 2.

The accuracy ratio of eye blink detection on each sample video is provided in this figure for existing and proposed methods. The bar of threshold-based approach observed the minimum accuracy of 61.11% and maximum of 77.42% for eyeblink detection. The Dynamic threshold taken the decision based on the scene strength by setting up the dynamic decisive values. In this method, the minimum accuracy obtained is 72.73%, whereas all other videos achieved the accuracy over 75%. The maximum accuracy achieved for this method is 100% for video5.avi. Junjea (2015) defined the probabilistic estimation based on structural and content specific evaluation. In this method, on an average 90% accuracy rate is achieved. The maximum accuracy achieved for this method is 100% for 3 videos. The proposed structural and statistical feature based evaluation method achieved the significant gain in accuracy of eyeblink detection. In this method, all the videos achieved the accuracy over 90% and on an average 96.8% accuracy is achieved. Out of nine videos, the eyeblink detection on five videos is done with 100% accuracy rate.

The robustness of the proposed structural and statistical feature evaluation model is verified by applying it on the larger set of videos. These videos are captured personally through mobile camera or collected from video sites through Google search. All these videos are categorized based on the scene or the environment in which the video is captured.
The collected videos are divided in four categories named News reader videos, drunken driver videos, Personal videos and outdoor videos. Each of the video categories is defined with the different number of video instances. The evaluation provided in figure 3 is having 12 instances of New-reader categories, 8 videos for drunken drivers, 14 videos of random outdoor scenes and 18 personal videos. Each of the video in the sample set is processed manually and by using each of existing and proposed method. The comparative evaluation of accuracy rate of eye-blink detection methods is provided in Figure 3.

![Figure 3. Accuracy analysis of eyeblink detection (video categories)](image)

Figure 3 has provided the comparative evaluation in terms of existing and proposed methods for each video category. The results show that the threshold has achieved the minimum accuracy rate and the proposed method achieved the maximum accuracy rate for each video category. The new-reader videos are more face-focused with stable camera position so that the maximum accuracy is achieved for these videos respective to each eyeblink detection method. The minimum accuracy rate achieved for new-reader videos is 87.8% by using the threshold based method and the maximum accuracy rate achieved is 97.39% for proposed method. The drunken videos are generally captured in the night through mobile cameras. The minimum accuracy rate achieved for these videos is 75.17% and the maximum accuracy rate is 94.63%. The outdoor and personalized videos provided the mixed results based on the camera and video quality.

Other than the real time and web-collected videos, the NRC-IIT (avi) dataset. The eye blink analysis was conducted on five random videos of videos taken from same dataset and captured from the real environment. These videos were also used by Juneja (2015). The analysis was conducted in terms number of eyeblinks and eye movement count against the actual number of eyeblinks and eye-movement exist in the videos.
Figure 4. Actual Vs. computed eyeblinks analysis (RealTime DB)

Figure 4 provides the comparative analysis on eyeblinks count against the number of actual eyeblinks and the eyeblinks detected by (Junjea, 2015). The figure shows that the number of eyeblinks detected by this proposed method are higher than for video I, Video III and video IV (Junjea, 2015). It shows that the proposed approach has performed effectively well then existing Junjea (2015) approach.

Figure 5. Actual Vs. computed eyemovement analysis (RealTime DB)

The evaluation on eye-movement detection for real-time videos is provided in Figure 5. The comparative results show that the proposed approach has performed better than Junjea (2015) approach. In case of Junjea (2015) method the minimum eye-movement detection rate achieved was 86.96%, whereas, in proposed approach the minimum accuracy detected for eye-movement recognition is 91.3%. The average accuracy of eye-movement detection in Junjea (2015) and proposed approaches are 92.4% and 94.80% respectively. These results verify a significant gain is achieved for these real-time videos using proposed approach.
Figure 6 provides the analysis results of eye blink detection on five sample videos taken from NRC-IIT DB dataset. The comparative evaluation was conducted respective to the number of actual eyeblinks existed in the videos. The analysis was conducted against Junjea (2015) approach. The comparative results show all the eye blinks are detected for Video I, Video II and Video IV using proposed and Junjea (2015) approaches. The Junjea (2015) method also provided the significant results except Video 5. The proposed approach has detected all the eyeblinks even for this video. It shows that the proposed approach has performed effectively well for videos taken from NRC-IIT DB videos.

The eye-movement detection was another evaluation performed on NRC-IIT dataset to verify the significance of proposed work. The analysis was performed on five sample videos taken from the dataset. The comparative results against the number of eye-movements are provided in Figure 7. The bar graph clearly identifies that the proposed approach has improved the accuracy of eye-movement detection over Junjea (2015) approach. The average rate of eye-movement recognition in Junjea (2015) approach is 82.3%, whereas, in proposed approach the average accuracy of eye-movement detection is 89.67%. It shows that the proposed approach has significantly improved the accuracy of eye-movement recognition.
CONCLUSION

In this paper, the structural and statistical feature adaptive model is provided for eye-blink detection for real time complex videos. The normalized AVI video frames are taken as input to the model. In the earlier stage, the statistical evaluation on consecutive frames is done using Energy (En) and Mean Coefficient (MC) parameters. The effective frames identified in first stage are processed under color adaptive mathematical and convolutional filter to identify the effective face region. The positional and geometric evaluation is performed on face region to extract the eye region. These extracted eye regions are gone through the next stage to transform the frame to effective features. The two-level wavelet decomposition is applied to identify the content features. Now to transform the eye region to adaptive structural and content based feature vector, the block segmentation is applied. The energy, pixel deviation and content specific features are generated for input and training set eye regions. In the final stage, the generated feature vectors of input eye-regions are compared on training set images to identify the eye status. The proposed model is applied on complex scene based videos captured in real time or collected through google search and NRC-IIT datasets. The evaluation is conducted for detection of eye-blinks and eye-movements in the videos. The comparative results are generated against threshold based, dynamic threshold based and Junjea (2015) methods. The results identify the significant improvement of 2% to 5% in accuracy rate for videos taken in different environments.
REFERENCES


A Functional Data Approach to the Estimation of Mortality and Life Expectancy at Birth in Developing Countries

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ABSTRACT

The functional data model has recently received increasing attention particularly in their application to mortality forecasting. The advantage of this method over the well-known Lee-Carter model is the ability to treat the underlying process as functional, and provide estimations that are robust to outliers. This research investigates the accuracy of functional data approach in estimating the mortality rates and life expectancy at births in developing countries including Malaysia, Indonesia, Thailand and Singapore. The functional data method was applied to these countries’ mortality data, and the out-sample forecast errors showed that, in terms of overall, the functional data model was more accurate than that of the original Lee-Carter model for males and females. The results provide evidence that the functional model is accurate to forecast the life expectancy at births for developing countries.

Keywords: Functional data model, Lee-Carter model, life expectancy at births, mortality forecasts

INTRODUCTION

Throughout the second half of the 20th century, majority of developing countries experienced a continuation of declining mortality rates, following the trends that had occurred in many developed nations in the past. This mortality decline had resulted in an increase in the average human’s survival. There is no sign that the average human life span will approach a certain limit. Hence, life expectancy is expected to continue to increase in the future. As a result, an accurate mortality forecasting model which accounts for changes in mortality trends is important for insurance companies as well as government agencies. For illustration, the
calculation of the expected present values of benefits in pricing requires accurate mortality projections to avoid underestimation of future costs. Moreover, accurate estimation of deaths is necessary for government planning particularly to ensure the social security benefits and medical care costs are sufficient for a growing number of elderly.

Actuaries and demographers put extensive efforts to develop methods to estimate mortality rates. Initially, several human mortality and survival functions were proposed including DeMoivers, Gompertz and Makeham laws (Bowers et al., 1997). Nonetheless, these functions do not include the change in mortality over time. The understanding of mortality improvement over time is important in more recent decades, in particular for social policy planning. Thus, the development of stochastic approaches based on time series methods received an increasing recognition. Example works are from McNown and Rogers (1989, 1992), Bell and Monsell (1991), and Lee and Carter (1992). The Lee and Carter model became one of the most influential stochastic method in the field of mortality forecasts. The model involves two parameters which are known as age-component and time-component. In order to estimate the two parameters, singular value decomposition (SVD) method is applied to decompose the log of age and year specific mortality rates matrices. It is noteworthy that, only the time-component is forecasted using a non-stationary time series model. Due to its parsimony in research, the Lee-Carter model received recognition and has been applied worldwide.

In recent years, we have seen a significant development of the Lee-Carter model purposely to improve the accuracy of forecast values. These extensions often use the original approach as a reference with some additional statistical techniques included in the models. Booth et al. (2005) provided reviews on some of these extensions. One of the Lee-Carter extensions is the functional data model from Hyndman and Ullah (2007). This model combines the idea from the functional data analysis, non-parametric smoothing and robust statistics to model the age specific mortality rates. It was proven that the functional model was more accurate than that of the original Lee-Carter model when forecasting both, mortality rates and life expectancy at births of fourteen selected developed countries (Shang et al., 2011). Moreover, the functional data model had successfully enhanced the accuracy of the age-mortality predictions for breast cancer patients in United States and England-Wales (Erbas et al., 2010). In addition, the model’s applications were extended to other demographic components in Australia including fertility and migration (Hyndman & Booth, 2008).

Nonetheless, the application of the functional data model to developing countries is rather limited. Although a few researches showed the functional data model outperformed the Lee-Carter model for Malaysian mortality rates (Shair et al., 2017; Husin et al., 2015), the performance of the model in other developing countries is unknown. Thus, it is of interest to this research to extend the application of the functional data model to other developing countries.
This research aims to evaluate two mortality forecasting models: the Lee-Carter and its extended version, the functional data. Each method is applied to mortality data by age and gender in four developing countries including Malaysia, Indonesia, Thailand and Singapore. The evaluation of the models involves fitting different methods to data from 1960 to 2001, then forecast within the period of 2002 to 2015. To evaluate the forecast accuracies of both models, the forecast values of mortality rates and life expectancies were then compared with observations.

This paper is organized as follows. Section 2 describes data that we use in this study and explains the Lee-Carter and the functional data models and error measurements for evaluation. Section 3 reports the out-sample forecast errors of both models. Finally, concluding comments with suggestions for future works are presented in Section 4.

MATERIALS AND METHODS

Data

We utilized mortality data by age and gender in developing countries including Malaysia, Indonesia, Thailand and Singapore. The length of data is consistent for each country, from 1960 to 2015 (56-year data). For Malaysia, data were collected from the Department of Statistics Malaysia (DoSM), whereas for other developing countries, data were obtained from the United Nation (2015). The oldest age groups were excluded due to high mortality variations and some missing values, giving the age range was from 0 to 85 years old. It should be noted that, from the collected mortality data, the observed life expectancy at birth was calculated using the standard actuarial life table approach.

Figure 1. Age-specific mortality rates of Malaysia Indonesia, Thailand and Singapore males from year 1960 (---) to 2015 (----)
To have an idea of mortality evolution in developing countries, Figures 1 and 2 display the decreasing trends in log mortality rates from 1960 (solid lines) to 2015 (dashed lines), for males and females respectively. In comparison to other age groups, infants and children who were below five years old experienced remarkable decreases in mortality over the years for both genders. This trend is shown by the biggest gaps between the two lines for each country and gender. Factors contributing the declines in infant deaths among developing countries were the decrease in the number of children who were malnourished and poor also the improvement in mother’s education background (Rutstein, 2000).

Conversely, it is noticeable that the gap between the two lines for people aged fifty-five years and above is diminutive for Malaysia, Thailand and Indonesia, indicating the curative medical technologies in recent years had little effects on reducing deaths of old-age people of these countries. This trend however is not applicable to Singapore as both figures show that the gaps for older male and female Singaporean are wider than that of other three countries and they are consistent for all age groups. This result suggests that healthcare services in Singapore has successfully reduced deaths among the elderly. According to Tan (2017), Singapore has adopted one of the best health care system so called the hybrid health care model that combines government subsidies with patient co-payments.

![Graphs showing age-specific mortality rates of Malaysia, Indonesia, Thailand and Singapore females from year 1960 to 2015.](image-url)

*Figure 2. Age-specific mortality rates of Malaysia Indonesia, Thailand and Singapore females from year 1960 (---) to 2015 (----)*
Due to a decline in mortality over time, life expectancy at births of all four developing countries have increased significantly for both genders. From the Figure 3, it can be seen that the differences in life expectancy between genders are generally wider over the years. The sex differentials in mortality and life expectancy are normally attributed to trends in behavioral and social risk factors such as cigarette smoking, heavy drinking, violence and occupational hazards (Villegas & Haberman, 2014).

The increase in life expectancy has occurred unevenly between countries. It is reported in the Table 1 that although life expectancy at births of Indonesian are the lowest compared to other countries, the rate of increase from 1960 to 2015 is the highest, 39.03%. On the other hand, Malaysian males’ life expectancy at births are ranked in the second highest among male populations, however the rate of increase is the slowest which is 21.48% over the same period. There are many factors that could affect the rate of improvement in life expectancy such as lifestyles, accident rates, government policies etc. It is also noteworthy that, the life expectancy at births of developing countries has increased at a substantially more rapid rate compared to majority of developed countries. This issue becomes a main concern to policy makers recently. Efforts should be done to ensure retirement benefits and other social benefits which are sufficient for the growing number of elderly.

Figure 3. Life expectancy at births for Malaysia, Indonesia, Thailand and Singapore females (---) and males (—) from 1960 to 2015
The Lee and Carter Model

The Lee-Carter model is one the most popular model for projecting future mortality rates. Although there are various extensions and modifications suggested by many authors, the Lee and Carter model is often used as the performance benchmark. The Lee and Carter model has the following form:

\[ \ln(m_{x,t}) = a_x + b_xk_t + \xi_{x,t} \]

where \( m_{x,t} \) is the central mortality rate for persons age \( x \) in year \( t \). The \( a_x \) variable represents the average age-pattern of mortality rates. The \( k_t \) variable is the time-varying parameter which is known as mortality index, describes the level of mortality at time \( t \) and the \( b_x \) variable is the age-specific component, measures the change of mortality at each age \( x \).

In order to estimates \( k_t \) and \( b_x \) parameters, following the Lee and Carter (1992) research, we use the singular value decomposition (SVD) that assumes the errors are homoscedastic. Constraints are imposed such that \( \sum_x b_x = 1 \) and \( \sum_t k_t = 0 \). Under these assumptions, the \( a_x \) parameter is estimated by averaging \( \ln(m_{x,t}) \) over time. The Lee-Carter model also assumes that the \( k_t \) values portray a linearly decreasing pattern hence a random walk with drift is an appropriate model to forecast the values. The random walk with drift model is described as the following:

\[ k_t = k_{t-1} + d + e_t \]

where \( d \) is the average annual change in \( k_t \) values, and \( e_t \) are its respective errors. It is noteworthy that only variable \( k_t \) is forecasted. Hence, the equation of age specific mortality rate forecasts for males and females in each country are calculated using the forecasted \( \hat{k}_t \) values and the estimated \( a_x \) and \( b_x \) as follows:

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
Year & Males & & & Females & & & & \\
\hline & IND & THA & MAL & SIG & IND & THA & MAL & SIG \\
\hline 1960 & 48.91 & 53.74 & 60.09 & 63.41 & 51.57 & 58.52 & 61.76 & 70.04 \\
1970 & 54.84 & 58.18 & 64.24 & 65.96 & 57.09 & 63.32 & 66.76 & 72.76 \\
1980 & 59.53 & 62.81 & 67.24 & 68.44 & 61.85 & 68.88 & 70.46 & 75.67 \\
1990 & 62.68 & 66.79 & 69.49 & 74.75 & 65.68 & 73.67 & 73.25 & 79.22 \\
2000 & 64.88 & 67.71 & 71.26 & 76.69 & 68.54 & 75.17 & 75.42 & 81.74 \\
2010 & 66.62 & 70.84 & 72.21 & 79.58 & 70.70 & 77.81 & 76.87 & 85.59 \\
2015 & 67.43 & 71.83 & 73.00 & 80.62 & 71.70 & 78.68 & 77.67 & 86.63 \\
\hline
(Δ%) & 37.87 & 33.66 & 21.48 & 27.14 & 39.03 & 34.45 & 25.76 & 23.69 \\
\hline
\end{tabular}
\caption{Life expectancy at births from 1960 to 2015 for males and females in four developing countries (MAL=Malaysia, IND=Indonesia, THA=Thailand and SIG=Singapore)}
\end{table}
\[ \ln(\mu_{x,t}) = a_x + b_x \bar{k}_t + \varepsilon_{x,t} \] (3)

From the equation (3), the values of the fitted parameters \(a_x\) and \(b_x\) for each country (Malaysia, Indonesia, Thailand and Singapore) and gender (male and females) can be referred to Table 2 and Table 3. Also, the respective forecasted mortality index \(\bar{k}_t\) are listed in Table 4.

### Table 2
The estimated \((a_x)\) of the Lee-Carter model for males and females in developing countries including Malaysia (MAL), Indonesia (IND), Thailand (THA) and Singapore (SIN)

<table>
<thead>
<tr>
<th>Age ((x))</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAL ((a_x))</td>
<td>IND ((a_x))</td>
</tr>
<tr>
<td>25</td>
<td>-6.048</td>
<td>-5.918</td>
</tr>
<tr>
<td>30</td>
<td>-5.863</td>
<td>-5.840</td>
</tr>
<tr>
<td>35</td>
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<tr>
<td>40</td>
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<tr>
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<tr>
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<tr>
<td>80</td>
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<tr>
<td>85</td>
<td>-1.359</td>
<td>-1.230</td>
</tr>
</tbody>
</table>

### Table 3
The estimated age-component \((b_x)\) of the Lee-Carter model for males and females in developing countries including Malaysia (MAL), Indonesia (IND), Thailand (THA) and Singapore (SIN)

<table>
<thead>
<tr>
<th>Age ((x))</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAL ((b_x))</td>
<td>IND ((b_x))</td>
</tr>
<tr>
<td>0</td>
<td>0.150</td>
<td>0.116</td>
</tr>
<tr>
<td>1</td>
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<td>0.179</td>
</tr>
<tr>
<td>5</td>
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<td>0.126</td>
</tr>
<tr>
<td>10</td>
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<td>0.100</td>
</tr>
<tr>
<td>15</td>
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<td>0.067</td>
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</table>
### Table 3 (continue)

<table>
<thead>
<tr>
<th>Year (t)</th>
<th>MAL ($b_x$)</th>
<th>IND ($b_x$)</th>
<th>THA ($b_x$)</th>
<th>SIN ($b_x$)</th>
<th>MAL ($b_x$)</th>
<th>IND ($b_x$)</th>
<th>THA ($b_x$)</th>
<th>SIN ($b_x$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.052</td>
<td>0.064</td>
<td>0.013</td>
<td>0.040</td>
<td>0.076</td>
<td>0.064</td>
<td>0.062</td>
<td>0.051</td>
</tr>
<tr>
<td>25</td>
<td>0.051</td>
<td>0.060</td>
<td>-0.019</td>
<td>0.051</td>
<td>0.071</td>
<td>0.063</td>
<td>0.047</td>
<td>0.056</td>
</tr>
<tr>
<td>30</td>
<td>0.042</td>
<td>0.054</td>
<td>-0.013</td>
<td>0.047</td>
<td>0.065</td>
<td>0.060</td>
<td>0.055</td>
<td>0.064</td>
</tr>
<tr>
<td>35</td>
<td>0.040</td>
<td>0.048</td>
<td>0.013</td>
<td>0.048</td>
<td>0.059</td>
<td>0.054</td>
<td>0.061</td>
<td>0.054</td>
</tr>
<tr>
<td>40</td>
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<td>0.035</td>
<td>0.049</td>
<td>0.054</td>
<td>0.047</td>
<td>0.071</td>
<td>0.060</td>
</tr>
<tr>
<td>45</td>
<td>0.038</td>
<td>0.030</td>
<td>0.046</td>
<td>0.048</td>
<td>0.047</td>
<td>0.037</td>
<td>0.046</td>
<td>0.049</td>
</tr>
<tr>
<td>50</td>
<td>0.034</td>
<td>0.022</td>
<td>0.049</td>
<td>0.048</td>
<td>0.042</td>
<td>0.033</td>
<td>0.040</td>
<td>0.043</td>
</tr>
<tr>
<td>55</td>
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<td>0.048</td>
<td>0.045</td>
<td>0.038</td>
<td>0.029</td>
<td>0.033</td>
<td>0.047</td>
</tr>
<tr>
<td>60</td>
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<td>0.014</td>
<td>0.051</td>
<td>0.043</td>
<td>0.032</td>
<td>0.031</td>
<td>0.032</td>
<td>0.041</td>
</tr>
<tr>
<td>65</td>
<td>0.021</td>
<td>0.013</td>
<td>0.042</td>
<td>0.039</td>
<td>0.028</td>
<td>0.028</td>
<td>0.025</td>
<td>0.036</td>
</tr>
<tr>
<td>70</td>
<td>0.017</td>
<td>0.015</td>
<td>0.044</td>
<td>0.043</td>
<td>0.023</td>
<td>0.027</td>
<td>0.027</td>
<td>0.039</td>
</tr>
<tr>
<td>75</td>
<td>0.015</td>
<td>0.014</td>
<td>0.038</td>
<td>0.040</td>
<td>0.016</td>
<td>0.022</td>
<td>0.021</td>
<td>0.032</td>
</tr>
<tr>
<td>80</td>
<td>0.014</td>
<td>0.013</td>
<td>0.019</td>
<td>0.044</td>
<td>0.011</td>
<td>0.017</td>
<td>0.017</td>
<td>0.034</td>
</tr>
<tr>
<td>85</td>
<td>0.012</td>
<td>0.009</td>
<td>0.012</td>
<td>0.034</td>
<td>0.005</td>
<td>0.008</td>
<td>0.006</td>
<td>0.024</td>
</tr>
</tbody>
</table>

### Table 4

The out-sample forecasted mortality index ($\hat{k}_t$) of the Lee-Carter model for males and females in developing countries including Malaysia (MAL), Indonesia (IND), Thailand (THA) and Singapore (SIN)

<table>
<thead>
<tr>
<th>Year (t)</th>
<th>MAL ($\hat{k}_t$)</th>
<th>IND ($\hat{k}_t$)</th>
<th>THA ($\hat{k}_t$)</th>
<th>SIN ($\hat{k}_t$)</th>
<th>MAL ($\hat{k}_t$)</th>
<th>IND ($\hat{k}_t$)</th>
<th>THA ($\hat{k}_t$)</th>
<th>SIN ($\hat{k}_t$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>-0.401</td>
<td>-0.305</td>
<td>-0.355</td>
<td>-0.543</td>
<td>-0.580</td>
<td>-0.414</td>
<td>-0.528</td>
<td>-0.548</td>
</tr>
<tr>
<td>2003</td>
<td>-0.801</td>
<td>-0.610</td>
<td>-0.710</td>
<td>-1.086</td>
<td>-1.160</td>
<td>-0.828</td>
<td>-1.055</td>
<td>-1.097</td>
</tr>
<tr>
<td>2004</td>
<td>-1.202</td>
<td>-0.915</td>
<td>-1.065</td>
<td>-1.629</td>
<td>-1.740</td>
<td>-1.242</td>
<td>-1.583</td>
<td>-1.645</td>
</tr>
<tr>
<td>2006</td>
<td>-2.004</td>
<td>-1.525</td>
<td>-1.775</td>
<td>-2.715</td>
<td>-2.899</td>
<td>-2.069</td>
<td>-2.638</td>
<td>-2.742</td>
</tr>
<tr>
<td>2011</td>
<td>-4.007</td>
<td>-3.049</td>
<td>-3.550</td>
<td>-5.431</td>
<td>-5.799</td>
<td>-4.139</td>
<td>-5.275</td>
<td>-5.484</td>
</tr>
</tbody>
</table>
The Functional Data Model
The model of Hyndman and Ullah (2007) uses the functional data to forecast the age specific mortality rates. The fundamental principle of the functional data analysis is to transform discrete data into a function or curve (Ramsay et al., 2009; Shaadan et al., 2014). In order to estimate mortality rates, the functional data model from Hyndman and Ullah (2007) extended the Lee-Carter approach to include the following additional statistical procedures:

1. The model assumes there is underlying smooth function of age for each year, \( f_t(x) \) in which the rates are smoothed using a non-parametric method such that:

\[
y_t(x) = f_t(x) + \sigma_t(x) \varepsilon_{t,x}
\]

where \( y_t(x) \) is observation rate for person age \( x = 0, 1, \ldots, 85 \) in year \( t = 1, 2, \ldots, n \). The \( f_t(x) \) is the corresponding smoothed rate, \( \varepsilon_{t,x} \) are iid standard normal error in year \( t \) with and \( \sigma_t(x) \) allows the error to vary by age.

2. The model used a robust principal component analysis to estimate more than one set of mortality index, \( k_t \) and age component, \( b_x \). Otherwise, the Lee-Carter model only estimated one set of the two variables using the SVD method. Multiple sets of \( (k_t, b_x) \) are important to comprise higher percentage of data variations. The number of set of parameters \( (H) \) are estimated such that it minimizes the mean integrated squared errors.

3. In order to forecast mortality indices, the model uses more general univariate time series methods, \( ARIMA(p,d,q) \) than random walk with drift. Therefore, the functional data model is mathematically expressed as follows.

\[
f_t(x) = \mu(x) + \sum_{h=1}^{H} b_h(x) K_{t,h} + e_t(x) + \sigma_t(x) \varepsilon_{t,x}
\]

where \( f_t(x) \) is the smoothed mortality rates across ages in each year. Following Hyndman and Ullah (2007), the smoothed data is estimated using a one-dimensional (function of age only) non-parametric approach, based on weighted penalized regression splines with a monotonicity increasing applies after age \( c \). In this research, we set \( c = 40 \) as the log mortality plot in Figure 1 and Figure 2 show that the rates constantly increase after age 40 for all countries.

According to Dokumentov and Hyndman (2013), the weighted penalized regression spline involves calculating a vector \( \beta \) which minimizes the following expression:

\[
||\omega(y-X\beta)||^2 + \lambda^2 \beta^T D \beta
\]

where \( y \) is a vector of observations, \( X \) is a matrix representing linear spline bases, \( D \) is a diagonal matrix, \( \omega \) is a vector of weights and \( \lambda \) is a parameter. In the case of mortality rates, observations in year \( t \) are given by \( y = m_{x,t} \) the weights \( \omega \) are taken as the inverse.
of the estimated variances of $y$ in which $y$ is assumed to have a binomially distribution. For details of this smoothing technique, see Hyndman and Ullah (2007). In this research, the weighted penalized regression spline smoothing procedure is performed using the demography package for R developed by Hyndman (2013).

The $\mu(x)$ from equation (5) refers to the average of mortality rates for each age across years. The $b_h(x)$ and $K_{t,h}$ are the $h$th age component and mortality index respectively. The optimum number $H$ is determined using the mean integrated squared errors (MISE):

$$MISE = \frac{1}{n} \sum_{t=1}^{n} \int e_t(x)^2 \, dx$$  \hspace{1cm} (7)

The $b_h(x)$ and $K_{t,h}$ variables are estimated by decomposing the matrix of $|f_t(x) - \mu(x)|$ using the principal component analysis. The $e_t(x)$ is the error term assumed to be homoscedastic such that $e_t(x) \sim (0, v(x))$. Next is to forecast the variables $K_{t,h}$ using the fitted univariate time series models to get $\tilde{R}_{t,h}$. Finally, the forecasts of $f_t(x)$ is obtained as the following:

$$\hat{f}_t(x) = \mu(x) + \sum_{h=1}^{6} b_h(x)\tilde{R}_{t,h} + e_t(x)$$  \hspace{1cm} (8)

From the equation (8), the estimated age parameters or bases of principle components for Malaysian males: $\mu(x)$, $b_1(x)$, $b_2(x)$, $b_3(x)$, $b_4(x)$, $b_5(x)$ and $b_6(x)$ are summarized in Table 5 whereas the respective forecasted coefficient values: $\tilde{R}_{1,1}$, $\tilde{R}_{1,2}$, $\tilde{R}_{1,3}$, $\tilde{R}_{1,4}$, $\tilde{R}_{1,5}$, and $\tilde{R}_{1,6}$ re presented in Table 6. It should be noted that, we provide the parameter values only for Malaysian males. The parameter values for the remaining seven sub-populations including Malaysian females, Indonesian males, Indonesian females, Thailand males, Thailand females, Singaporean males and Singaporean females are not included in this paper, however they are available by contacting the correspondence author of this paper.

### Table 5
The estimated of principle component bases of the functional data model for Malaysian males

<table>
<thead>
<tr>
<th>$x$</th>
<th>$\mu(x)$</th>
<th>$b_1(x)$</th>
<th>$b_2(x)$</th>
<th>$b_3(x)$</th>
<th>$b_4(x)$</th>
<th>$b_5(x)$</th>
<th>$b_6(x)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-3.823</td>
<td>0.390</td>
<td>0.214</td>
<td>-0.176</td>
<td>-0.069</td>
<td>0.393</td>
<td>-0.948</td>
</tr>
<tr>
<td>1</td>
<td>-5.842</td>
<td>0.199</td>
<td>-0.304</td>
<td>-0.011</td>
<td>-0.416</td>
<td>-0.058</td>
<td>-0.088</td>
</tr>
<tr>
<td>5</td>
<td>-6.689</td>
<td>0.123</td>
<td>-0.295</td>
<td>0.013</td>
<td>0.054</td>
<td>0.042</td>
<td>0.054</td>
</tr>
<tr>
<td>10</td>
<td>-6.700</td>
<td>0.121</td>
<td>-0.111</td>
<td>0.009</td>
<td>0.234</td>
<td>-0.045</td>
<td>-0.009</td>
</tr>
<tr>
<td>15</td>
<td>-6.512</td>
<td>0.131</td>
<td>0.026</td>
<td>0.011</td>
<td>0.163</td>
<td>-0.177</td>
<td>-0.033</td>
</tr>
<tr>
<td>20</td>
<td>-6.304</td>
<td>0.135</td>
<td>0.092</td>
<td>0.023</td>
<td>0.022</td>
<td>-0.191</td>
<td>-0.012</td>
</tr>
<tr>
<td>25</td>
<td>-6.100</td>
<td>0.131</td>
<td>0.107</td>
<td>0.023</td>
<td>-0.116</td>
<td>-0.133</td>
<td>0.036</td>
</tr>
<tr>
<td>30</td>
<td>-5.899</td>
<td>0.123</td>
<td>0.090</td>
<td>0.009</td>
<td>-0.120</td>
<td>-0.002</td>
<td>0.062</td>
</tr>
<tr>
<td>35</td>
<td>-5.646</td>
<td>0.113</td>
<td>0.072</td>
<td>-0.042</td>
<td>-0.104</td>
<td>0.039</td>
<td>0.072</td>
</tr>
<tr>
<td>40</td>
<td>-5.371</td>
<td>0.105</td>
<td>0.048</td>
<td>-0.079</td>
<td>0.032</td>
<td>0.139</td>
<td>0.048</td>
</tr>
</tbody>
</table>
A Functional Data Approach for Mortality Forecasting

Table 5 (continue)

<table>
<thead>
<tr>
<th>x</th>
<th>( \mu(x) )</th>
<th>( b_1(x) )</th>
<th>( b_2(x) )</th>
<th>( b_3(x) )</th>
<th>( b_4(x) )</th>
<th>( b_5(x) )</th>
<th>( b_6(x) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>-5.026</td>
<td>0.096</td>
<td>0.037</td>
<td>-0.116</td>
<td>0.089</td>
<td>0.156</td>
<td>0.030</td>
</tr>
<tr>
<td>50</td>
<td>-4.620</td>
<td>0.086</td>
<td>0.042</td>
<td>-0.114</td>
<td>0.034</td>
<td>0.064</td>
<td>0.027</td>
</tr>
<tr>
<td>55</td>
<td>-4.203</td>
<td>0.076</td>
<td>0.041</td>
<td>-0.047</td>
<td>0.026</td>
<td>0.038</td>
<td>0.015</td>
</tr>
<tr>
<td>60</td>
<td>-3.765</td>
<td>0.065</td>
<td>0.033</td>
<td>0.020</td>
<td>0.039</td>
<td>0.077</td>
<td>0.004</td>
</tr>
<tr>
<td>65</td>
<td>-3.306</td>
<td>0.053</td>
<td>0.029</td>
<td>0.061</td>
<td>0.003</td>
<td>0.080</td>
<td>0.008</td>
</tr>
<tr>
<td>70</td>
<td>-2.858</td>
<td>0.045</td>
<td>0.027</td>
<td>0.117</td>
<td>-0.020</td>
<td>0.061</td>
<td>0.010</td>
</tr>
<tr>
<td>75</td>
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<td>0.040</td>
<td>0.024</td>
<td>0.188</td>
<td>0.009</td>
<td>0.065</td>
<td>0.002</td>
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<tr>
<td>80</td>
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<td>0.020</td>
<td>0.228</td>
<td>0.031</td>
<td>0.071</td>
<td>-0.004</td>
</tr>
<tr>
<td>85</td>
<td>-1.360</td>
<td>0.031</td>
<td>0.018</td>
<td>0.225</td>
<td>0.012</td>
<td>0.058</td>
<td>-0.001</td>
</tr>
</tbody>
</table>

Table 6
The out-sample forecast values of principle component coefficients of the functional data model for Malaysian males

<table>
<thead>
<tr>
<th>( l )</th>
<th>( \hat{R}_{l,1} )</th>
<th>( \hat{R}_{l,2} )</th>
<th>( \hat{R}_{l,3} )</th>
<th>( \hat{R}_{l,4} )</th>
<th>( \hat{R}_{l,5} )</th>
<th>( \hat{R}_{l,6} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>-2.451</td>
<td>-0.547</td>
<td>-0.058</td>
<td>-0.059</td>
<td>-0.044</td>
<td>0.066</td>
</tr>
<tr>
<td>2003</td>
<td>-2.536</td>
<td>-0.476</td>
<td>-0.058</td>
<td>-0.062</td>
<td>-0.046</td>
<td>0.068</td>
</tr>
<tr>
<td>2004</td>
<td>-2.623</td>
<td>-0.405</td>
<td>-0.058</td>
<td>-0.064</td>
<td>-0.049</td>
<td>0.070</td>
</tr>
<tr>
<td>2005</td>
<td>-2.709</td>
<td>-0.334</td>
<td>-0.058</td>
<td>-0.067</td>
<td>-0.051</td>
<td>0.072</td>
</tr>
<tr>
<td>2006</td>
<td>-2.795</td>
<td>-0.263</td>
<td>-0.058</td>
<td>-0.070</td>
<td>-0.054</td>
<td>0.074</td>
</tr>
<tr>
<td>2007</td>
<td>-2.881</td>
<td>-0.192</td>
<td>-0.058</td>
<td>-0.072</td>
<td>-0.056</td>
<td>0.076</td>
</tr>
<tr>
<td>2008</td>
<td>-2.967</td>
<td>-0.121</td>
<td>-0.058</td>
<td>-0.075</td>
<td>-0.059</td>
<td>0.078</td>
</tr>
<tr>
<td>2009</td>
<td>-3.054</td>
<td>-0.050</td>
<td>-0.058</td>
<td>-0.077</td>
<td>-0.061</td>
<td>0.080</td>
</tr>
<tr>
<td>2010</td>
<td>-3.140</td>
<td>0.021</td>
<td>-0.058</td>
<td>-0.080</td>
<td>-0.064</td>
<td>0.082</td>
</tr>
<tr>
<td>2011</td>
<td>-3.226</td>
<td>0.092</td>
<td>-0.058</td>
<td>-0.083</td>
<td>-0.066</td>
<td>0.084</td>
</tr>
<tr>
<td>2012</td>
<td>-3.312</td>
<td>0.163</td>
<td>-0.058</td>
<td>-0.085</td>
<td>-0.069</td>
<td>0.086</td>
</tr>
<tr>
<td>2013</td>
<td>-3.398</td>
<td>0.234</td>
<td>-0.058</td>
<td>-0.088</td>
<td>-0.071</td>
<td>0.089</td>
</tr>
<tr>
<td>2014</td>
<td>-3.484</td>
<td>0.305</td>
<td>-0.058</td>
<td>-0.091</td>
<td>-0.074</td>
<td>0.091</td>
</tr>
<tr>
<td>2015</td>
<td>-3.570</td>
<td>0.375</td>
<td>-0.058</td>
<td>-0.093</td>
<td>-0.076</td>
<td>0.093</td>
</tr>
</tbody>
</table>

The Forecast Error Measurements

In order to evaluate the performance of the Lee-Carter model and the functional data model, data from 1960 to 2001 (42 years) were fitted into the models and then forecasted the mortality rates and life expectancy at births over the 2002 and 2015 period (14 years). On the issue of the length of data series for estimation and evaluation processes, there is no clear indication on the appropriate length of data for each process. However, Lazim (2012) suggested that at least one-quarter of the full length of data was appropriate to be saved for evaluation. Furthermore, Hyndman and Athanasopoulos (2014) stated that the size of test set was typically 20% of the total data. The difference between the forecasted values of mortality rates and life expectancy at births from 1990 to 2015, and its respective actual
rates in the same period are defined as the out-sample forecast errors. In this research, the out-sample forecast errors are estimated using two error measurements which are the Root Mean Squared Forecast Error (RMSFE) for mortality and the Mean Absolute Forecast Error (MAFE) for life expectancy at births.

The RMSFE and MAFE are commonly used scale-dependent measure which have been applied in many fields and the formula for each sub-population are given as follows:

\[
RMSFE = \sqrt{\frac{\sum_{n=1}^{N} \sum_{j=1}^{P} (F_{j,n} - y_{j,n})^2}{N \times P}}
\]

\[
MAFE = \frac{\sum_{n=1}^{N} |F_{n} - y_{n}|}{N}
\]

Where
- \(F_{j,n}\) is the forecast value (mortality rate) for a person age \(j\) in year \(n\)
- \(y_{j,n}\) is the observed value (mortality rate) for a person age \(j\) in year \(n\)
- \(F_{n}\) is the forecast value (life expectancy at birth) in year \(n\)
- \(y_{n}\) is the observed value (life expectancy at birth) in year \(n\)
- \(P\) is the maximum age
- \(N\) is the latest observation year

RESULTS AND DISCUSSIONS

In this section we report the forecast errors of log age and gender specific mortality rates and life expectancy at births for two different mortality forecasting models, the Lee-Carter and functional data in four developing countries including Malaysia, Indonesia, Thailand and Singapore.

Table 7 summarizes the RMSFE of log mortality rates segregated by genders and countries from both models. For males, the functional model is more accurate than that of the Lee-Carter model in all four countries including Malaysia, Indonesia, Thailand and Singapore, hence outperforming in terms of overall error (taking the average over countries). For females, although the functional model performs better than the Lee-Carter model only for two out of four countries, Indonesia and Thailand, the overall error indicates that the functional model is more accurate than the Lee-Carter model.

Figure 4 and 5 display the root mean square forecast errors of log mortality rates by age and country from two models: the Lee-Carter (solid lines) and the functional data (dashed line). Figure 4 clearly shows that the Lee-Carter model provides larger errors than the functional data model for males in certain age groups such as infants and 40 to 60 in
Malaysia, 0 to 50 in Indonesia and 0 to 19, 45 to 55 and 65 to 75 in Thailand. The forecast errors from both models are almost the same in Singapore. Furthermore, Figure 5 shows that the Lee-Carter model provides considerably large errors in 0 to 50 age groups for Indonesian females and in 10 to 35 for Thailand females, resulting the functional model out-performing the Lee-Carter model for females in these two countries. These results are consistent with the previous outcomes in the Table 7. In addition, it is noteworthy that both models successfully produce accurate results for older males and females age 50 and above as the forecast errors fluctuate approximately around zero in all countries.

Table 7
The out-sample Root Mean Squared Forecast Errors (RMSFEs) of mortality rates for males and females in four developing countries using two methods (LC=Lee-Carter and FD= Functional Data)

<table>
<thead>
<tr>
<th>Country</th>
<th>Males</th>
<th></th>
<th></th>
<th>Females</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC</td>
<td>FD</td>
<td></td>
<td>LC</td>
<td>FD</td>
<td></td>
</tr>
<tr>
<td>Malaysia</td>
<td>0.15256</td>
<td>0.12681</td>
<td></td>
<td>0.20340</td>
<td>0.23593</td>
<td></td>
</tr>
<tr>
<td>Indonesia</td>
<td>0.16304</td>
<td>0.05492</td>
<td></td>
<td>0.24213</td>
<td>0.06628</td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td>0.21023</td>
<td>0.13055</td>
<td></td>
<td>0.13359</td>
<td>0.08826</td>
<td></td>
</tr>
<tr>
<td>Singapore</td>
<td>0.21797</td>
<td>0.21599</td>
<td></td>
<td>0.19623</td>
<td>0.22453</td>
<td></td>
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<tr>
<td>Overall</td>
<td>0.18595</td>
<td>0.13207</td>
<td></td>
<td>0.19383</td>
<td>0.15377</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Root Mean Square Forecast Errors (RMSFEs) of mortality rates by age and methods: Lee-Carter (——) and Functional data (----), for males in four developing countries
In addition to mortality rates, we include another outcome measure which is life expectancy at births, for models’ evaluation. The Table 8 summarizes the out-sample forecast errors of life expectancy at births segregated by genders and countries. Promising results can be concluded from the Table 8 in which the functional data model significantly out-performs the Lee-Carter model for females in all countries and provides better forecasts for males in three out of four countries, Malaysia, Indonesia and Thailand. For example, as we can see from the Table 8, the functional model substantially reduces the forecast error of Indonesian males from 1.00015 to 0.38785 (by 61%) and Indonesian females from 1.23932 to 0.22426 (by 82%).

The superior performance of the functional data model to forecast life expectancy at births arises for several reasons. Firstly, the model allows more complex dynamics than the original Lee-Carter model by setting K>1, thus allowing higher order terms to be included for estimation. Secondly, the additional statistical procedures adopted in the functional model such as smoothing technique allows the observational error to be treated separately from the time series forecast hence reducing the forecast errors. Thirdly, the use of robust method minimizing the risk of outlying years or data (Hyndman et al., 2007).

Figure 5. Root Mean Square Forecast Errors (RMSFEs) of mortality rates by age and methods: Lee-Carter (——) and Functional data (----), for females in four developing countries
It is noteworthy that the out-sample forecast errors are not consistent between mortality and life expectancy at birth. For example, although the functional data model is less accurate than that of the Lee-Carter model to forecast the mortality of Malaysian and Singaporean females (Table 7), the model performs better than the Lee-Carter model to forecast life expectancy at births for these sub-populations (Table 8). This issue was highlighted previously with evidence that the most accurate model for mortality is not essentially the best model for life expectancy, vice versa (Booth et al., 2005).

The life expectancy at birth forecast values from different models and its respective observations are presented in the Figure 6. It can be seen clearly from the figure that both models successfully provide accurate results for Malaysian females as almost all forecasted values lie closely to the observation lines. There is no major structural change in the Malaysian females’ life expectancy in the past hence simplifying predictions. In addition, the Lee-Carter model significantly overestimates the life expectancy of Indonesian males and females such that the Lee-Carter model forecast values (dashed lines) lie above the observations (full lines). The significant overestimation of the Lee-Carter model for Indonesian life expectancies maybe due to the model does not impose weight on the structural changes in the life expectancy that have occurred just before the forecast period, after 1990. On the contrary, the functional data model provides better forecasts than the Lee-Carter model for Indonesian females also for Thailand males and females. These results can be seen clearly from the Figure 6 that the forecast values from the functional model (dotted lines) lie closer to observations (full lines) than the forecast values from the Lee-Carter model (dashed lines).
CONCLUSION

This research extends the application of two mortality forecasting models: the Lee-Carter model and its extended version, the functional data model to four developing countries including Malaysia, Indonesia, Thailand and Singapore. In overall, the out-sample mortality forecast values show that the functional model is more accurate than that of the original Lee-Carter model for males and females. Moreover, the evaluation both models using life expectancy at birth as the outcome measure indicated that the functional model significantly out-performed the Lee-Carter model for females in all countries and males in three out of four countries. It can be concluded that the functional model has the potential to accurately forecast age and gender specific mortality rates and the life expectancies of developing countries provided there is no major structural changes in recent years. It is recommended for future works to include other Lee-Carter extensions to determine most accurate model for developing countries.

Figure 6. The observed and 14-year forecasts of life expectancy at births for males and females in four developing countries
REFERENCES


Vibrating Particle System Algorithm for Hard Clustering Problems

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2Department of Computer Science and Engineering, Northcap University, Gurugram, 122017 India

ABSTRACT

In the field of data analysis, clustering is an unsupervised technique that can be used to find identical sets of data. But, it is tough task to find the optimal centroid for a given dataset, especially in hard clustering problems. Recently, a vibrating particle system (VPS) algorithm was developed for solving the optimization problems. This algorithm is based on the concept of free vibration and forced vibration. This algorithm provides more effective and optimal solutions for constrained optimization problems. In this work, the performance of VPS algorithm is evaluated for solving hard clustering problems. The objective of this algorithm is to compute optimal centroid for hard clustering problems. The efficiency of the proposed algorithm is measured on well known clustering datasets and compared with some popular clustering algorithms. The simulation results demonstrate that the VPS algorithm obtains effective results as compared to other algorithms.

Keywords: Clustering, K-means, meta-heuristic algorithm, vibrating particle system

INTRODUCTION

Clustering is an unsupervised method to arrange the data into different clusters using distance measure. The data within a cluster is more similar in nature than the other clusters. This method is also used to understand the organisation of data. In clustering, data is partitioned into several clusters based on similarity factor. The similarity factor is based on distance function. The clustering techniques have wide importance in diverse fields such as data analysis, stock market, pattern identification and machine learning paradigms. In literature, different objective functions are reported to find identical group...
of data such as Euclidean distance, Manhattan distance, City block distance and Hamming distance. But, it is seen that most of work is reported with Euclidean distance as an objective function. Moreover, clustering is divided into two categories - partitional clustering and hierarchical clustering. In partitional clustering, a fitness function is considered to partition the data into $k$ clusters. While in hierarchical clustering, data are merged and split on the basis of objective function. Further, a tree structure is designed to represent clustering results. It is noticed that clustering methods are further categorized into different subcategories based on the nature of data and representations. Some of these are density based clustering, spectral clustering, graph clustering, model based clustering (Hahsler & Bolanos, 2016; Kannan et al., 2004; Murphy & Murphy, 2017; Schaeffer, 2007). Graph clustering is a method in which vertices are grouped together and having minimum number of edges in between clusters. While, model based clustering based on the finite mixture model. This clustering method is implemented on the resulted variable, not on the related covariates. In density based clustering method, clusters are described as maximum set of density connected points. The spectral clustering methods determine the similarity matrix such that all objects lie in singular vector of matrix. But, it is observed that every clustering method requires some similarity measures to find the closeness between data.

Due to technological evolution and wide application area of clustering, many researchers have developed numerous algorithms for solving clustering problems. Large number of meta-heuristic algorithms, swarm based algorithms, evolutionary algorithms and approximation algorithms have been reported in literature. But, none of these algorithms can give exact solution. Hence, according to No Free Lunch Theorem, there is a scope to develop a new algorithm for solving optimization problems that can provide more accurate results. Recently, VPS algorithm is developed for solving the constrained optimization problems (Kaveh & Ghazaan, 2017a). It is seen that this algorithm gives more efficient and optimized results for solving constrained optimization problems. The aim of this work is to examine the effectiveness and performance of the VPS algorithm for solving hard partitional clustering problems. The VPS algorithm is applied to determine the optimal centroid from a given dataset. The performance of the VPS algorithm is evaluated on five real and two artificial datasets. It is seen that the VPS algorithm provides state of art results in comparison to the same class of algorithms.

Related Work

This section describes recent works reported on the clustering problems. Kumar et al. (2016) proposed magnetic charged system search (MCSS) algorithm to handle problem of optimal cluster centers. The proposed algorithm formulates the behavior of charged particles. In the proposed algorithm, Newton’s second law of motion is applied for global search. In this study, two artificial and eight real data sets are considered to compute the
performance of the MCSS algorithm. It is reported that the proposed algorithm gives effective and efficient clustering results in comparison to $K$-means, genetic algorithm (GA), particle swarm optimization (PSO), and ant colony optimization (ACO) algorithms. A hybrid algorithm based on cat swarm optimization (CSO) and $K$-harmonic means is developed for solving clustering problem (Kumar & Sahoo, 2015a). The CSO algorithm is integrated with $K$-harmonic means to overcome the drawback of local optima. In this work, seven data sets are considered to evaluate the performance of proposed improved CSO (ICS0) algorithm. The performance of the proposed algorithm is compared with existing algorithms and it is concluded that the proposed algorithm resolves the problem of local optima. Moreover, this algorithm also improves convergence speed of CSO algorithm. To handle partitional clustering problems in effective manner, a MCSS-PSO based clustering algorithm is presented (Kumar & Sahoo, 2015b). The performance of the proposed algorithm is evaluated on two artificial and eight real data sets. From simulation results, it is stated that the proposed algorithm is more effective and efficient for handling partitional data clustering problems. An improved version of CSO algorithm is also developed to resolve local optima problem and also improve convergence speed of CSO (Kumar & Sahoo, 2017a). The Cauchy mutation operator is used to prevent local optima problem. The performance of the improved CSO algorithm is evaluated using two artificial and four real data sets. It is seen that the proposed algorithm is more effective and successfully overcome above mentioned problems. A hybrid clustering algorithm based on Monte Carlo equation and Gaussian probability distribution function is also reported to handle local optima problem (Kumar & Sahoo, 2017b). In this work, few benchmark datasets are considered to compute the performance of the proposed algorithm. It is claimed that the proposed algorithm is more effective and robust in order to tackle clustering problems. Han et al. (2017) introduced an enhanced version of gravitational search algorithm, known as bird flock gravitational search algorithm for data clustering. This algorithm is inspired from collective behaviors of birds. In this work, thirteen data sets are considered to measure the performance of the proposed algorithm. The simulation results are compared with several other popular data clustering algorithms. It is seen that the proposed algorithm is one of effective and efficient algorithm for solving clustering problems. Tsai et al. (2017) proposed a clustering framework for cloud data analytics. In this study, eleven data sets are considered to compute the performance of the proposed algorithm. It is observed that the proposed algorithm is more suitable to determine better clustering results in cloud environment. Ozbakır and Turna (2017) presented two novel meta-heuristic algorithms for clustering problems. These algorithms are weighted superposition attraction algorithm and Ion Motion Optimization algorithm. Prior to apply, these algorithms are integrated with the Deb’s rule to overcome infeasible solutions problem. It is seen that the proposed algorithm generates more competitive solutions than other algorithms. Kushwaha et al.
(2017) reported a novel clustering algorithm for partitional data clustering. It is based on the concept of magnetic force. The eleven data sets are considered to evaluate the performance of the proposed algorithm. It is observed that the proposed algorithm generates more accurate and robust results as compared to other algorithms. Boushaki et al. (2018), developed an enhanced version of cuckoo search algorithm, known as quantum chaotic cuckoo search algorithm for clustering. The quantum concept is integrated into cuckoo algorithm to resolve local optima problem. In this work, six real life datasets are considered to compute the performance of the proposed algorithm. It is revealed that the proposed algorithm provides better results as compared to other well-known algorithms in terms of internal and external clustering quality. Kumar and Singh (2017) presented an enhanced CSO (ECSO) algorithm for clustering. Further, a local search technique is also incorporated for enhancing the quality of clusters. In this study, five datasets are considered to evaluate the performance of the proposed algorithm. The simulation results are compared with the other existing clustering algorithms. It is reported that ECSO algorithm provides better and enhanced results.

Vibrating Particle System

VPS algorithm is based on the concept of vibration. The term vibration is described using two types- free vibration and forced vibration. In free vibration, the restoring forces are only responsible to maintain the motion. While in forced vibration, a force is applied at certain intervals of time. The frictional effects can be neglected in vibrating system due to undamped vibration. However, due to frictional forces, these vibrations are damped up to some extent. These frictional forces are incepted due to friction between the rigid bodies, dry friction, fluid friction and inter molecular friction (Kaveh & Ghazaan, 2017a; Kaveh & Ghazaan, 2017b). Kaveh and Ghazaan (2017a) presented VPS algorithm based on the concept of free vibration with viscous damping to find global or near global solutions. It is claimed that the proposed algorithm is more convenient and robust in nature. It is a population based meta-heuristic algorithm. Like other meta-heuristic algorithms, the balance between diversification and intensification in VPS is also maintained using particle current population and historical best position. The optimal solution is represented using particle positions. In this algorithm, three equilibrium positions are mentioned with different weights. Further, these positions are updated in each successive generation due to previous best position of the population, known as historically best (HB), good particle (GP) and bad particle (BP). To determine the GP and BP from population, the entire population is sorted according to the objective function value. Further, a threshold limit is defined for GP and BP and finally, GP and BP are selected randomly from population pool. The particles are initialized in $d$-dimensional search space using Equation (1).
\[ X^j_i = x_{\text{min}} + r \times (x^j_{\text{max}} - x^j_{\text{min}}) \]  

(1)

where \( X^j_i \) is the \( j^{th} \) variable of the particle i.e. \( x_{\text{min}} \) represents lower bound vectors, \( x_{\text{max}} \) denotes upper bound vectors, \( r \) is random function in the range of \([0, 1]\). To control the effect of the damping level, a decreasing function is also proposed in VPS model. This function is defined in Equation (2). Figure 1 illustrates the vibration motion of a particle with mass. Further, to control the damping effect in vibration, Equation (2) is adopted. This equation can decreases the damping effect in vibration.

\[ D = \left( \frac{\text{iteration}}{\text{iteration}_{\text{max}}} \right)^\beta \]  

(2)

In Equation (2), \( D \) represents the damping, \( \text{iteration} \) is current iteration number, \( \text{iteration}_{\text{max}} \) is maximum number of iteration used for optimization and \( \beta \) is a constant. Further, it is stated that every particle has three equilibrium positions i.e. \( HB, GP \) and \( BP \) and these positions are updated in each generation. To select good particle or the bad particle for each generation is to sort current population using objective function. Further, a random selection of the \( GP \) and \( BP \) is to be made in first and second half respectively. The population of the VPS algorithm is updated using Equations (3-5).
\[ x'_i = w_1 [D.A.r_1 + HB^j] + w_2 [D.A.r_2 + GP^j] + w_3 [D.A.r_3 + BP^j] \]  \tag{3}

\[ A = [w_1.(HB^j - x'_i)] + [w_2.(GP^j - x'_i)] + [w_3.(BP^j - x'_i)] \]  \tag{4}

\[ w_1 + w_2 + w_3 = 1 \]  \tag{5}

Here, \( w_1 \) is the parameter to measure \( HB \), \( w_2 \) is the parameter to measure \( GP \) and \( w_3 \) is the parameter to measure \( BP \). \( r_1 \), \( r_2 \) and \( r_3 \) are the uniformly distributed random numbers in the range of 0 and 1 and \( r_1 \neq r_2 \neq r_3 \).

**MATERIALS AND METHODS**

**Proposed VPS Clustering Algorithm**

This section describes VPS based clustering algorithm for solving real world clustering problems. The clustering problems are \( NP \)-hard problem especially when clusters are more than three. The motive of the VPS algorithm is to determine the optimal cluster centroid for hard clustering problems. In clustering problems, the optimal set of clusters is computed using Euclidean distance. In this work, Euclidean distance can be considered as the objective function. It is described using distance between data objects and cluster centres. This function is evaluated for each cluster centres and data objects. Further, the data are associated with the different clusters using minimum Euclidean distance. Euclidean distance is described in Equation (6).

\[
\minimize F(X,C) = \sum_{k=1}^{K} \sum_{x \in D_i} \min \| X_i - C_k \|^2 \tag{6}
\]

In Equation (6), \( X_i \) denotes the \( i^{th} \) data object, \( C_k \) represents the \( k^{th} \), and data objects are assigned to clusters according to the minimum distance. A fitness function is also associated with each cluster centres. The fitness function describes the goodness of the clusters. When the data are assigned to clusters, then the value of the function is computed for each cluster centre. In this work, sum of square error (SSE) based function is considered to measure the goodness of each cluster centre. This function is defined in Equation (7).

\[
F(C_k) = \frac{\sum_{k=1}^{K} \frac{SSE(C_k)}{\sum_{k=1}^{K} SSE(C_k)}}{\sum_{k=1}^{K} SSE(C_k)} \tag{7}
\]

**Steps of the VPS Clustering Algorithm.** VPS algorithm is a recently developed algorithm, inspired through the behaviour of vibrations. In VPS algorithm, population is represented using particles which are randomly distributed in \( d \)-dimensional space. In case of clustering
problems, generally the population is described in terms of number of clusters presented in a dataset. The population of VPS algorithm is defined in terms of number of clusters (K) present in a dataset. Further, it is noted that the population should be lie within the boundary constraint (Kaveh & Talatahari, 2010). If boundary condition is violated, then harmony search based approach is adopted to generate the new population within boundary. For clustering problems, the boundary constraints are denoted using minimum and maximum value of each attributes. The main steps of VPS clustering algorithm are listed in Algorithm 1.

### Algorithm 1: VPS Clustering Algorithm for Hard Partitional Problems

**Step 1**: Set up the initial parameters of the VPS algorithm and initialize the initial locations (populations) in an arbitrarily (random) manner.

**Step 2**: Compute the objective function values using Equation (6) and also determined the previously best position i.e., HB and also compute the fitness function associated with each population.

**Step 3**: To determine the good and bad particles from the population according the fitness function.

**Step 4**: For every particle, compute the values of $w_2$ and $w_3$ using random function and satisfied Equation (5).

**Step 5**: Determine the next locations with the help of Equation (3).

**Step 6**: The violated positions are updated using harmony search based mechanism and compute the objective function values for new locations.

**Step 7**: Until the termination condition is reached, repeat steps 3-7

**Step 8**: Obtain final solution generated for hard partitional problems.

### RESULTS AND DISCUSSION

This section describes the experimental results of VPS clustering algorithm. To investigate the efficiency of the VPS clustering algorithm, some benchmark datasets are considered. These are the well defined datasets and the performance of the newly developed algorithms is tested on these datasets. Further, the effectiveness and efficiency of the algorithm are evaluated using intra cluster distance and standard deviation parameters. The quality of clusters is measured using intra cluster distance parameter and it is measured in terms of best, average and worst. The parameters setting of the proposed VPS clustering algorithm are illustrated in Table 1. The proposed algorithm is implemented in Matlab environment. The experimental results of the VPS algorithm are compared with some other clustering algorithms reported in literature (İnkaya et al., 2015; Kanungo et al., 2016; MacQueen, 1967; Zhan et al., 2009).
Performance Matrices

**Intra Cluster Distance.** The distance between data objects and their respective cluster centres is known as intra cluster distance. It is used to determine the clustering quality and the results are presented in terms of best, average and worst.

**Standard Deviation.** Standard deviation is used to determine the information regarding dispersion of data within a cluster. If the value of the standard deviation is low it shows data objects are dispersed near to cluster centres and if the value is high, data is away from centroid.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population Size</td>
<td>No. of clusters (K)</td>
</tr>
<tr>
<td>β</td>
<td>0.05</td>
</tr>
<tr>
<td>w₁</td>
<td>0.3</td>
</tr>
<tr>
<td>w₂</td>
<td>0.3</td>
</tr>
<tr>
<td>Number of iterations</td>
<td>200</td>
</tr>
</tbody>
</table>

This section describes the results of the proposed algorithm and other meta-heuristic algorithms. Table 2 illustrates the results of the VPS and other clustering algorithms using artificial datasets i.e. ART1 and ART2.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Parameters</th>
<th>K-means</th>
<th>PSO</th>
<th>ACO</th>
<th>CSO</th>
<th>TLBO</th>
<th>Proposed VPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART1</td>
<td>Best</td>
<td>157.12</td>
<td>155.46</td>
<td>153.21</td>
<td>153.34</td>
<td>153.96</td>
<td>150.24</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>161.12</td>
<td>159.78</td>
<td>157.45</td>
<td>156.54</td>
<td>158.42</td>
<td>156.51</td>
</tr>
<tr>
<td></td>
<td>Worst</td>
<td>166.08</td>
<td>165.34</td>
<td>162.48</td>
<td>160.04</td>
<td>163.07</td>
<td>160.73</td>
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<tr>
<td></td>
<td>SD</td>
<td>0.846</td>
<td>0.681</td>
<td>0.523</td>
<td>0.689</td>
<td>0.572</td>
<td>0.438</td>
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<td></td>
<td>F-Measure</td>
<td>99.14</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>ART2</td>
<td>Best</td>
<td>743</td>
<td>742.26</td>
<td>743.49</td>
<td>738.46</td>
<td>736.12</td>
<td>727.16</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>749.83</td>
<td>746.52</td>
<td>747.84</td>
<td>745.17</td>
<td>744.08</td>
<td>741.91</td>
</tr>
<tr>
<td></td>
<td>Worst</td>
<td>754.28</td>
<td>751.03</td>
<td>752.29</td>
<td>750.24</td>
<td>748.59</td>
<td>149.63</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.726</td>
<td>0.567</td>
<td>0.714</td>
<td>0.498</td>
<td>0.514</td>
<td>0.396</td>
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<tr>
<td></td>
<td>F-Measure</td>
<td>98.94</td>
<td>99.17</td>
<td>99.08</td>
<td>99.14</td>
<td>99.43</td>
<td>99.48</td>
</tr>
</tbody>
</table>

It is seen that the proposed algorithm gives better results in comparison to other algorithms using all datasets. Further, it is also noticed that the proposed algorithm having minimum intra cluster distance among all other clustering algorithms. The average
intra cluster distance of the proposed algorithm is also better than other algorithms. On the analysis on standard deviation parameter, it is observed that VPS algorithm obtains minimum SD values for all datasets.

Table 3
Simulation results of the proposed VPS and other clustering results

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Parameters</th>
<th>Algoritms</th>
<th>K-means</th>
<th>PSO</th>
<th>ACO</th>
<th>CSO</th>
<th>TLBO</th>
<th>Proposed VPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iris</td>
<td>Best Case</td>
<td>97.43</td>
<td>96.48</td>
<td>96.89</td>
<td>96.94</td>
<td>96.56</td>
<td>95.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Avg. Case</td>
<td>113.08</td>
<td>98.56</td>
<td>98.28</td>
<td>97.86</td>
<td>96.84</td>
<td>95.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worst Case</td>
<td>124.21</td>
<td>99.67</td>
<td>99.34</td>
<td>98.58</td>
<td>98.08</td>
<td>97.79</td>
<td></td>
</tr>
<tr>
<td>Iris</td>
<td>SD</td>
<td>16.26</td>
<td>0.467</td>
<td>0.426</td>
<td>0.392</td>
<td>0.546</td>
<td>0.214</td>
<td></td>
</tr>
<tr>
<td>Iris</td>
<td>F-Measure</td>
<td>0.782</td>
<td>0.78</td>
<td>0.778</td>
<td>0.781</td>
<td>0.782</td>
<td>0.785</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>Best Case</td>
<td>2991.64</td>
<td>2972.28</td>
<td>2989.12</td>
<td>2978.38</td>
<td>2865.71</td>
<td>2843.41</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>Avg. Case</td>
<td>3243.50</td>
<td>3124.09</td>
<td>3184.54</td>
<td>3129.43</td>
<td>3091.44</td>
<td>3045.92</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>Worst Case</td>
<td>3614.24</td>
<td>3367.58</td>
<td>3308.17</td>
<td>3456.18</td>
<td>3246.65</td>
<td>3158.64</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>SD</td>
<td>256.58</td>
<td>107.14</td>
<td>93.45</td>
<td>128.46</td>
<td>42.11</td>
<td>58.15</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>F-Measure</td>
<td>0.832</td>
<td>0.826</td>
<td>0.829</td>
<td>0.831</td>
<td>0.834</td>
<td>0.836</td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>Best Case</td>
<td>5813.29</td>
<td>5786.81</td>
<td>5746.23</td>
<td>5718.78</td>
<td>5778.61</td>
<td>5648.23</td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>Avg. Case</td>
<td>5914.46</td>
<td>5837.72</td>
<td>5828.42</td>
<td>5804.52</td>
<td>5836.25</td>
<td>5761.28</td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>Worst Case</td>
<td>5992.33</td>
<td>5949.47</td>
<td>5941.14</td>
<td>5921.28</td>
<td>5921.32</td>
<td>5873.42</td>
<td></td>
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<tr>
<td>CMC</td>
<td>SD</td>
<td>49.62</td>
<td>48.86</td>
<td>44.34</td>
<td>43.29</td>
<td>38.96</td>
<td>34.56</td>
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<tr>
<td>CMC</td>
<td>F-Measure</td>
<td>0.337</td>
<td>0.333</td>
<td>0.332</td>
<td>0.334</td>
<td>0.331</td>
<td>0.335</td>
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<tr>
<td>Wine</td>
<td>Best Case</td>
<td>16768.18</td>
<td>16483.61</td>
<td>16448.35</td>
<td>16431.76</td>
<td>16578.42</td>
<td>16106.42</td>
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<tr>
<td>Wine</td>
<td>Avg. Case</td>
<td>18061.24</td>
<td>16417.47</td>
<td>16530.53</td>
<td>16395.18</td>
<td>16360.04</td>
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<tr>
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<td>Worst Case</td>
<td>18764.49</td>
<td>16594.26</td>
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<tr>
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<td>796.13</td>
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<td>56.14</td>
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<tr>
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<td>0.516</td>
<td>0.522</td>
<td>0.521</td>
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<tr>
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<td>Best Case</td>
<td>222.43</td>
<td>264.56</td>
<td>273.22</td>
<td>256.53</td>
<td>246.89</td>
<td>229.22</td>
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<tr>
<td>Glass</td>
<td>Avg. Case</td>
<td>246.51</td>
<td>278.71</td>
<td>281.46</td>
<td>264.44</td>
<td>256.44</td>
<td>246.38</td>
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<tr>
<td>Glass</td>
<td>Worst Case</td>
<td>258.38</td>
<td>283.52</td>
<td>286.08</td>
<td>282.27</td>
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<td>SD</td>
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<tr>
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<td>0.402</td>
<td>0.416</td>
<td>0.422</td>
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</table>

Table 3 demonstrates the experimental results of VPS and other state of art clustering algorithms using real life datasets. In this study, five datasets are taken into consideration. These datasets are iris, cancer, CMC, wine and glass. It is seen that proposed VPS clustering algorithm obtains minimum intra cluster distance among all other algorithms. On the analysis of F-measure parameter, it is also stated that the proposed algorithm gave better performance as compared to other algorithms. On the behalf of simulation results, it can be concluded that in VPS algorithm, objects within clusters are tightly bound than other algorithms being compared.
Figure 2 shows the distribution of the objects in wine dataset using malic acid and alcohol attributes. While, Figure 3 shows the clustering of objects in wine dataset into different clusters using VPS clustering algorithm. Figure 4 depicts the distribution of wine dataset using malic acid, alcohol, and ash attributes. Figure 5 depicts the clustering results of VPS algorithm. Figure 6 illustrates the convergence behaviour of proposed VPS, ACO, K-Means and PSO clustering algorithms using wine dataset. It can be concluded that convergence rate of the proposed algorithm is better than other algorithms. Finally, it is concluded that the proposed algorithm is more capable and efficient than other algorithm being compared.
Figure 4. Distribution of data objects in Wine dataset (3D View)

Figure 5. Clustering results of VPS algorithm using Wine dataset (3D View)

Figure 6. Convergence results of VPS algorithm using Wine dataset
CONCLUSION

In this work, VPS algorithm is proposed for solving hard clustering problem. This algorithm is based on the concept of free vibration. The VPS algorithm is adopted to determine optimal cluster centroid and also minimize intra cluster distance for hard clustering problem. The efficacy of the proposed algorithm is tested on benchmark clustering datasets. Further, intra cluster distance and standard deviation parameters are used as performance parameters. The simulation results showed that VPS algorithm obtains better results than other existing cluttering algorithms. It is concluded that the proposed algorithm is more effective, efficient and robust for solving hard clustering problem.

REFERENCES


Hahsler, M., & Bolaños, M. (2016). Clustering data streams based on shared density between micro-clusters. IEEE Transactions on Knowledge and Data Engineering, 28(6), 1449-1461.


Fuzzy and Entropy based approach for Feature Extraction from Digital Image

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ABSTRACT
This paper presents texture feature extraction scheme with the help of extended version of Fuzzy local binary pattern (FLBP) to get more efficient feature from the input image. The proposed scheme extends (FLBP) technique employing Intuitionistic fuzzy set and it is known as Intuitionistic fuzzy local binary pattern (IFLBP). Moreover, IFLBP provides an additional bin in the distribution of IFLBP values. Additionally, it can be used as the feature vector of the image and can be apply in diverse fields of image processing. The proposed algorithm has used various medical as well as image processing images of different sizes for result analysis. It clearly shows that the obtained results are better than the existing techniques and its extracted feature are more informative than the reported methods.

Keywords: Entropy, fuzzy local binary pattern, feature extraction, intuitionistic fuzzy local binary pattern, histogram, intuitionistic fuzzy sets

INTRODUCTION
There are diverse research areas of image processing where features extraction is the primary step. Most of the researchers employed their algorithm after extracting features from the image because it reduces the dimension as well as time. Further, it is a process of extracting the compact as well as crucial information against an image. Moreover, the prime purpose of feature
extraction is to locate the most important information against original as well as raw data. Additionally, when the input image and raw data of an algorithm are terribly huge to be handled also it is hypothetical to be redundant (diminutive information, bulky data). The input data is going to be converted into a drastically compact dimension demonstration as well as a set of features (acknowledged as features vector). Further, these extracted feature vectors are going to represent the complete image. Moreover, if extracted features are vigilantly chosen then it is likely to get the features set which will excerpt important informative data in order to carry out the chosen task with this compact demonstration in the place of input image as well as full data. Lastly, the excerpt features vectors have been employed in diverse area of image processing as well as signal processing namely image forensics, remote sensing, bio medical image processing, visual inspection, image classification, character recognition, document verification, terrain delimitation, script recognition, pattern recognition, reading bank deposit slips and object discrimination. (Ansari & Ghrera, 2016; 2017; 2018a; 2018b; Castellano et al., 2004; Tsymbal et al., 2005; Li et al., 2003; Wang et al., 2008; Chow et al., 2007; Kumar et al., 2013; Kaur et al., 2018; Singh & Gupta, 2018; Ansari et al. 2014; 2016; 2017).

The main features are existing in the image consist of color, texture as well as shape. Further, feature extraction is primarily reliant on these kind of features. Additionally, performance of every preferred task is also reliant on these extracted features. In general, feature illustration techniques are classified into three types namely global based, block-based and region-based, features (Tian, 2013; Chow et al., 2007). Further, a smaller amount work been done in the field of feature extraction interconnected to a significant research on annotation as well as retrieval model itself designed. This article mainly focus on texture feature extraction methods. Lastly, developed technique is applied on diverse medical images such as X-Ray, Thyroid, Brain CT scan, image processing images namely Lena image as well as JUIT logo.

Various techniques are developed for textural feature extraction method (Wagner, 1999; Petrou & Sevilla, 2006). The local binary pattern (LBP) technique (Ojala et al., 1996) is giving his efforts on the concept of binary patterns for demonstration of texture. Further, it is comprehensively accepted because LBP is simple and effectual in depicting the local spatial construction of an image. Moreover, the LBP technique were intensified as well as accompanied with diverse methods. In outcome, the extensive variety of texture revelation idea is suitable for various image analysis tasks. The classic examples consist of LBP extensions rotation invariance (Fehr, 2007), fusion of micro LBP and macro Gabor features (Li & Staunton, 2008) merger of inter as well as intra spatial structure of the LBP patterns (Wu et al., 2008) and featuring scale invariance (Chan et al., 2007).
BACKGROUND

The primary research on visual inspection (Iivarinen, 2000) has indicated with the aim of the LBP features was proficiently applied in surface defect detection. Additionally, BP based features are also applied in wood quality discrimination (Silven et al., 2003). Lately, such features have been used in automatic defect detection (Hadizadeh et al., 2008) as well as in remote sensing (Grabner et al., 2008). Various investigation have indicated with the purpose of the BP based feature eradication techniques are advisable for content based image retrieval (CBIR) (Jiang et al., 2004) while currently the LBP based techniques have been utilized in discriminative model for image ranking against text queries (Grangier & Bengio, 2008). Furthermore, in the field of face recognition LBP is identified as a highly proficient texture illustration technique. Additionally, it is efficiently applied in invariant face recognition (Li et al., 2007; Zhang et al., 2008), recognition of facial expressions (Shan et al., 2009) and face authentication (Destrero et al., 2009). Fantastic outcomes are accomplished from its diverse application of biomedical imaging together with classification of protein images (Nanni & Lumini, 2008), video endoscopy (Iakovidis et al. 2006), computer aided neuroblastoma prognosis system (Sertel et al., 2009).

Finally, it is also fruitfully employed in diverse field of motion analysis namely underwater image matching (Garcia et al., 2001), object tracking (Petrovic et al., 2008), modeling and detection of moving objects (Heikkila & Pietikainen, 2006).

The research society has a lot of attention on LBP texture demonstration, various techniques have been developed based on the BP model. Futher, the approach was designed for the estimation of local contrast measure (Ojala et al., 1996). Afterward, the LBP/C technique was developed with the help of joint distribution of LBP codes and local contrast measures. Moreover, it is also applied to get better discrimination ability of the original LBP technique (Ojala et al., 1996). Additionally, other edition of LBP is local edge patterns (LEP) approach which was proposed for image segmentation (Yao et al., 2003). This method depicts the spatial formation of local texture throughout the spatial orientation of edge pixels. Identical technique median binary pattern (MBP) was designed by Hafiane et al., (2007).

Fuzzy sets offer a compliant structure for handling indeterminacy characterizing real world systems, originating primarily from the imprecise as well as imperfect nature of information. Moreover, the fuzzy sets do not cope the hesitancy (intuitionistic index) in a images originated out of diverse aspects, in which the preponderance are caused by inherent weaknesses of the acquirement as well as imaging mechanisms. Further, distortions occur as a result of the limitations of acquirement chain, like quantization noise, the suppression of dynamic range, or the non-linear behavior of mapping system, affect our certainty regarding the “brightness” or “edginess” of a pixel and therefore introduce a degree of hesitancy associated with the corresponding pixel.
Fuzzy sets theory introduced by Zadeh, (1965) on the extraction of texture spectrum features (Barcelo et al., 2007) and their competent successors. Furthermore, the LBP features are capable to enhance their robustness to noise (Ahonen & Pietikäinen, 2007; Iakovidis et al., 2008; Keramidas et al., 2008; Keramidas et al., 2011). Though, the above studies can only be considered as elementary since they incorporate only a constrained experimental estimation. Keramidas et al. (2011) and Iakovidis et al. (2008) proposed a generic, uncertainty aware approach for the derivation of fuzzy local binary pattern (FLBP) texture replicas. Furthermore, intuitionistic fuzzy set theory developed by Atanassov (1986; 1989) provides a compliant mathematical structure to cope uncertainty with the hesitancy arising from imperfect as well as imprecise information. Additionally, a prominent characteristic of IFS is that it appoints to every element a membership degree as well as a non membership degree with assertive amount of hesitation degree. Finally, this manuscript presents intuitionistic fuzzy local binary pattern (IFLBP) for texture demonstration with the help of the Atanassov’s intuitionistic fuzzy sets.

**PROPOSED METHOD AND ALGORITHM**

To simplify the FLBP approach, this method is capable of managing the uncertainty through hesitancy arising as of either imperfect as well as imprecise information. Moreover, this segment describes the proposed intuitionistic fuzzy local binary pattern (IF-LBP) for texture representation by using IFSs as follows:

Let \( V = \{0, 1, \ldots, n-1\} \) be the universal set for \( n \)-pixels neighborhood. Then a IFSs \( R \) on the universe of discourse \( V \) is defined as given below:

\[
R = \left\{ \left( p_x, \mu_R(p_x), \nu_R(p_x) \right) \mid p_x \in V \right\},
\]

such that \( \mu_R \) belong to \( U[0,1] \) and \( p_x \) belong to \( U[0,1] \) are membership and non-membership function of IFS \( R \) to which a member \( p_x \) has less grey or greater than \( I_x \) in \( R \), respectively, under \( 0 \leq P_x + (p_x) + \nu_R(p_x) \leq 1 \). The indeterminacy degree \( \mu_R(p_x) = 1 - \mu_R(p_x) - P_x(p_x), \) \( p_x \in U, \) where \( 0 \leq P_x(p_x) \leq 1 \) is measurement of ambiguity such that \( p_x \) belongs to \( R \) or not \( \forall \ p_x \in V \), where \( 0 \leq \mu_R(p_x) \leq 1 \) is measurement of ambiguity such that \( p_x \) belongs to \( R \) or not.

The membership and non-membership functions of IFS \( R \) are defined as follows:

\[
\mu_x(x) = \begin{cases} 
0, & \text{if } I_x < I_x - S, \\
0.5(1+\eta)
\left(1 + \frac{I_x - L_x}{S}\right), & \text{if } I_x \in [I_x - S, L_x], S \neq 0, \\
0.5\left[\frac{1+L_x-I_x}{S} + \eta\left(\frac{L_x-I_x}{S} - 1\right)\right], & \text{if } I_x \in [L_x, I_x + S], S \neq 0, \\
1, & \text{if } I_x \geq I_x + S.
\end{cases}
\]

\[
(2)
\]
For neighbourhood pixel \((n\times n)\), in a single bin of IFLBP histogram, the contribution \(IFLBP_C(\alpha, \beta, j)\) of each pattern code is given by

\[
IFLBP_C(\alpha, \beta, j) = \prod_{k=0}^{\xi-1} \left[ b_{j}^k (j) (\alpha) + (1 - b_{j}^k (j)) \nu_{R} (\alpha) \right],
\]

where \(\nu \in [0, n]\), \((\alpha, \beta)\) and \(b_{j}^k (j) \in \{0, 1\}\) represent the number of neighbouring pixels, the coordinates of a pixel and calculated value of \(l^{th}\) bit of binary representation of bin \(j\), respectively, and complete IFLBP histogram is given by

\[
IFLBP_H (j) = \sum_{\alpha, \beta} IFLBP_C(\alpha, \beta, j), \quad j = 0, 1, ..., 2^{\xi} - 1.
\]

We can examine that employing the crisp LBP operator, in which each \(n\times n\) pixel adjacent invariably gives one bin of the histogram. Moreover, FLBP as well as IF-LBP histogram of each \(n\times n\) pixel adjacent mostly provides more than one bin of the histogram. Though, the sum of contribution of each \(n\times n\) pixel adjacent (IF-LBP) to the bins of IF-LBP histogram is equal to 1, given as follows:

\[
\sum_{j=0}^{2^{\xi}-1} IFLBP_C(\alpha, \beta, j) = 1.
\]

An example of IF-LBP computation scheme for \(3\times3\) pixel surroundings is shown in Figure 1.

We proposed a new technique for texture feature extraction from the image as shown in Figure 2. These extracted features can be used in various image processing areas. Firstly, the image is taken as input and converted into grey scale which is resized to \(512\times512\). Furthermore, \(3\times3\) Local neighbourhoods have chosen for each pixel from input image and then fix the threshold (Ts) to get the IFLBP code of each block. Additionally the IFLBP code of each block is combined. Lastly the histograms have been plotted for FLBP code as shown in Figure 3-7. To measure the information in the image, the entropy value has been calculated depicted in Table 1-5.

**Remarks 1:** When \(S \neq 0\), \(h = 0\), the resulting Intuitionistic fuzzy membership and non-membership functions given by the Equation 2 and 3 are almost equivalent to the fuzzy membership function \(\mu_A(x)\), the distinctness being that \(\mu_A(x) = \nu_A(x) = 0.5\) where as \(\mu_A(x)=1\) when \(I = I_c\).
Remarks 2: When S=0, the resulting Intuitionistic fuzzy membership and non-membership functions are equivalent to the crisp thresholding function $X_A(x)$.

PERFORMANCE METRICS: ENTROPY OF IFLBP FEATURES

The information content of an image is frequently computed by calculating the uncertainty or entropy of an image. Moreover, as the amount of entropy increases, more information...
is associated with the image. Furthermore, the entropy measures the average as well as the
global information content of an image in terms of average bits per pixel.

The entropy measure for probability distribution is defined as:

\[ e = - \sum_{j=0}^{255} x_j \log_2 x_j \] (7)

This is Shannon entropy (Shannon, 1948) and where \( x_j \) is jth pattern probability.

It is worthiness to comment that the logarithmic entropic measure (7) that as \( x_j \) tend
to 0 its analogous self-information of this event, \( I(x_j) = \log x_j \) tends to infinity \( I(x_j = 1) = \log x_j \) tends to 0. Thus, we see that information gain for an event is neither bounded at both ends nor defined at all points. In practice, the gain in information for an event, either highly probable or uncertain, is estimated to lie between two definite limits. i.e., as maximum pixels of an image are analyzed, the information gain increases such that all the pixels are examined the gain achieves its maximum value, irrespective of the essence of an image.

In Shannon’s entropy, which is vastly acclaim, the self-information of an event with
\( x_j \) is chosen as \( \log x_j \) which is a decreasing function of \( x_j \) The similar characteristic may be preserved by considering it as a function of \( 1 - x_j \) instead of \( 1/x_j \). These deliberations imply the self-information as an exponential function of \( 1 - x_j \) rather of logarithmic behavior. This is also suitable while taking into account the idea of information gain in an image. Lastly, Pal and Pal entropy (Pal & Pal, 1989) is defined by

\[ e_p = \sum_{j=0}^{255} x_j \exp(1 - x_j) \] (8)

RESULT AND DISCUSSION

It is recognized that Keramidas’s method (Keramidas et al., 2011) and Ansari’s method
(Ansari & Ghrera, 2018) have zero values for some bins out of 255 bins. Though, developed technique histograms do not have bins with zero values and there are more spikes, though limited in magnitude. This indicates that Ansari’s method is more informative than Keramidas method and proposed method is more informative than existing techniques.

The more diversified signal illustrates the higher entropy and more actual information gain. Normally, if all the bins of histogram are having equal possibility, then the maximum entropy will be obtained. Ostensibly, for the fixed threshold \( S \), developed technique’s histograms always provide better entropy than existing method’s histograms. However, Ansari’s method, histograms for the same threshold and using hesitation threshold values \( h \in [0, 1] \) gives greater entropy than

Keramidas’s technique histograms. Hence, IFLBP histogram gains more information than other reported methods histograms.
Furthermore, we have employed IFLBP approach on diverse images as depicted in Figures 3-7 of size 256×256 to determine the histograms feature vector for various threshold values as well as varying hesitation \( h \in [0,1] \) and measure the entropies from these histograms, lastly the results are depicted in Tables 1 to 5. Moreover, we can observe from these tables that the maximum entropy achieved in Table 1 for the S=6, S=10 are at S=0.3, for S=2, S=14, S=20, S=12 are at \( h = 0.1 \). Similarly, we have followed the same process for other images; the results are depicted in Tables 2-5 and Figures 3-7 correspondingly. Thus, the entropies achieved by developed technique are invariably better than the entropies achieved by existing techniques. Further, we have plotted the histograms of IFLBP codes of each image where we have obtained the highest entropy as depicted in Figures 3-7. With these histograms we can examine that the IFLBP histograms do not have bins with zero values. Additionally, the IFLBP features of all images are more informative than existing features.

Table 1

The entropy values at diverse threshold and hesitation for x-ray image

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Hesitancy degree ( (h) )</th>
<th>Threshold (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Keramidas et al., 2011</td>
<td>0.0</td>
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<td></td>
<td>0.1</td>
<td>1.85</td>
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<td>Ansari &amp; Ghrera, 2018</td>
<td>0.3</td>
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<td></td>
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<td>1.79</td>
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<td>1.75</td>
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<td>1.0</td>
<td>1.67</td>
</tr>
<tr>
<td>Proposed Method</td>
<td>0.1</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2.68</td>
</tr>
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<td></td>
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<td>2.67</td>
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<tr>
<td></td>
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<td>2.61</td>
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Table 2

The entropy values at diverse threshold and hesitation for thyroid image

<table>
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<tr>
<th>Techniques</th>
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<tr>
<td></td>
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<td>6</td>
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<tr>
<td>Keramidas et al., 2011</td>
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</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.74</td>
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<tr>
<td>Ansari &amp; Ghrera, 2018</td>
<td>0.3</td>
<td>1.83</td>
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### Table 2 (Continue)

<table>
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<tbody>
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<td>6</td>
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<tr>
<td>Ansari &amp; Ghrera, 2018</td>
<td>0.5</td>
<td>1.74</td>
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<tr>
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<td>1.69</td>
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<tr>
<td></td>
<td>1.0</td>
<td>1.35</td>
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<tr>
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<td>2.68</td>
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<tr>
<td>Proposed Method</td>
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</tr>
<tr>
<td></td>
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<td>2.66</td>
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<td></td>
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### Table 3

*The entropy values at diverse threshold and hesitation for Brain CT Scan image*

<table>
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<td>6</td>
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<tr>
<td>Keramidas et al., 2011</td>
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<td></td>
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<td>Proposed Method</td>
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### Table 4

*The entropy values at diverse threshold and hesitation for Lena image*

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Table 5

The entropy values at diverse threshold and hesitance for JUIT Logo

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Fuzzy and Entropy based Approach for Feature Extraction

Figure 3. (a) Original image (b) Histogram Plot of IFLBP Code for X-Ray Image

Figure 4. (a) Original image of IFLBP Code for Thyroid Image
Figure 4. (b) Histogram Plot of IFLBP Code for Thyroid Image

Figure 5. (a) Original image (b) Histogram Plot of IFLBP Code for Brain CT Scan Image
Figure 6. (a) Original image (b) Histogram Plot of IFLBP Code for Lena Image
CONCLUSION AND FUTURE WORK

A novel and efficient technique for extracting texture features from digital images have been developed using Intuitionistic fuzzy local binary pattern with the help Intuitionistic fuzzy set theory. The proposed scheme extends (FLBP) technique employing Intuitionistic fuzzy set theory and it is known as Intuitionistic fuzzy local binary pattern (IFLBP). Moreover, IFLBP provides additional bin in the distribution of IFLBP values. Further, it can be used as the feature vector of the image and can be apply in diverse fields of image processing. The developed method is experimentally executed on various medical as well as image processing images of different sizes. Moreover, the obtained result (Tables 1-5) clearly shows that the entropy value of developed technique is always greater than the reported methods. The extracted features are more informative than the existing ones. Additionally, IFLBP features can be employed in different research fields of image processing as well as medical image processing like face recognition, pattern recognition, image de-noising, image segmentation, image forgery detection, image and pattern classifications etc.

REFERENCES


A Side-sensitive Modified Group Runs Control Chart with Auxiliary Information to Detect Process Mean Shifts

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ABSTRACT

This study aims to develop a side-sensitive modified group runs control chart using auxiliary information (SSMGR-AI) to enhance the speed of detecting mean shifts in a process. The average run length (ARL) and expected average run length (EARL) criteria are adopted as performance measures of the proposed chart. The performance of the proposed chart is compared to the exponentially weighted moving average chart with AI (EWMA-AI) and the run sum chart with AI (RS-AI), in terms of the ARL and EARL criteria. The results reveal that the optimal SSMGR-AI chart generally outperforms all charts under comparison for detecting shifts in the process mean. An application with numerical data is presented to elaborate the implementation of the SSMGR-AI chart.

Keywords: Auxiliary information (AI), average run length (ARL), expected average run length (EARL), side-sensitivity, side-sensitive modified group runs (SSMGR)

INTRODUCTION

Control charts are a well-known process used to maintain the quality of production in modern industries and manufacturing sectors. The main purpose of the control chart is to monitor infrequent changes in manufacturing and industrial processes. In 1924, Shewhart first developed the Shewhart control chart which depended on a single characteristic to monitor the process mean.
of product quality (Montgomery, 2009). Later, enormous research on control charts has resulted in different dimensions of the basic chart used to detect dissimilarities in production parameters such as mean, variation or both. The concept of rational sub-grouping is employed to detect unusual patterns in the production process. Efficient estimators of the desired parameters are computed from each sub-group and integrated into the Shewhart, EWMA or CUSUM control chart to detect shifts in the mean, variance or both. Bourke (1991) suggested that the conforming run length (CRL) chart could be used to detect shifts in the fraction of non-conforming items in a production process. Wu and Spedding (2000) developed a synthetic chart which combined Shewhart $X$ chart and CRL chart to monitor shifts in the process mean. To enhance the performance of synthetic chart, Gadre and Rattihalli (2004) proposed group runs (GR) control chart to detect shifts in the process mean. By modifying the GR chart, Gadre and Rattihalli (2006) further presented a modified group runs (MGR) control chart which was useful to identify increases in the fraction of non-conforming items and detect shifts in the process mean.

Davis and Woodall (2002) highlighted that side-sensitivity feature could be used to improve the chart performance and proposed the side-sensitive synthetic chart. A side-sensitive group runs (SSGR) chart investigated by Gadre and Rattihalli (2007) had surpassed the Shewhart, synthetic and GR charts. Furthermore, Gadre et al. (2010) proposed a side-sensitive modified group runs (SSMGR) chart, where it was shown that this chart performed better than the Shewhart, synthetic, GR, side-sensitive group runs (SSGR) and MGR charts. To extend the work of Garde et al. (2010), Saha et al. (2018a) proposed the SSMGR double sampling (SSMGRDS) chart and the latter was found to outperform its basic counterparts.

In the last decade, the use of auxiliary information in Statistical Process Control has gained the attention of researchers. If a variable is known for every unit of the population but it is not a variable of interest, then the said variable can be used as an auxiliary variable, where information from the auxiliary variable, called auxiliary information, along with the main variable of interest, enhances the level of precision of the control charting statistic. Riaz (2008) proposed a new Shewhart type chart to monitor the process mean with auxiliary information along with a regression estimator. Riaz et al. (2013) highlighted that in the presence of auxiliary information, a control chart could perform efficiently under normality and non-normality assumptions with estimation effects. Abbas et al. (2014) showed that the exponentially-weighted moving average chart with single auxiliary information (EWMA-AI) performed better than its univariate and bivariate competitors to detect small and moderate shifts. Abbasi and Riaz (2016) found that the use of auxiliary information in-control charts enabled the charts to detect shifts more efficiently and quickly than the charts without auxiliary information.
Haq and Khoo (2016) showed that a new synthetic control chart based on both study and auxiliary variable had performed more effectively than the classical synthetic chart and also its univariate and bivariate competitors. Saha et al. (2018b) developed a variable sample size and sampling interval (VSSI) control chart using auxiliary information (VSSI AI) to monitor the process mean, while the average time to signal (ATS) and expected time to signal (EATS) criteria were adopted as performance measures. A run sum chart for the mean based on auxiliary characteristics (RS-AI) was proposed by Ng et al. (2018) and compared with the Shewhart AI, synthetic AI, and EWMA-AI charts.

In this research, the auxiliary information procedure is incorporated into the existing SSMGR chart, in order to propose the side-sensitive modified group runs chart with auxiliary information (SSMGR-AI) to detect process shifts. The ARL and EARL criteria are applied to measure how quickly the proposed chart can detect infrequent changes in production. An optimal design is conducted to compute optimal parameters of the SSMGR-AI chart by minimizing the out-of-control ARL and EARL values for different mean shifts and shift intervals, respectively. After that, the SSMGR-AI chart is compared with the EWMA-AI and RS-AI charts. Finally, a numerical example, based on generated data, is given to explain the implementation of the proposed SSMGR-AI chart.

**Existing Control Charting Method: SSMGR Chart**

Gadre et al. (2010) assimilated both the $X$ sub-chart and the CRL sub-chart into the SSMGR chart. The upper control limit ($UCL_{X}$) and lower control limit ($LCL_{X}$) of the $X$ sub-chart is calculated as

$$UCL_{X} = \mu_0 + k_{X} \frac{\sigma_0}{\sqrt{n}}$$  \[1\]

$$LCL_{X} = \mu_0 - k_{X} \frac{\sigma_0}{\sqrt{n}}$$  \[2\]

where, $k_{X}$ is the width constant controlling the width of the $X$ sub-chart to satisfy the desire in-control performance. When the sample mean $\bar{X}$ falls within $(LCL_{X}, UCL_{X})$, the sample is called conforming; otherwise, the sample is considered as non-conforming. The conforming run length (CRL) is defined as the number of conforming samples inspected between the $(q-1)^{th}$ and $q^{th}$ non-conforming samples, including the $q^{th}$ non-conforming sample. The value of CRL$_q$ at the $q^{th}$ non-conforming sample is defined as $Y_q$ throughout the paper. The procedure of the SSMGR $\bar{X}$ chart is described by the following steps:

Step 1. Draw $n$ successive products and compute sample mean $\bar{X}$ from a process of following $N(\mu_i, \sigma^2)$ distribution with the in-control mean $\mu_i$ and standard deviation $\sigma$. Here $\mu_i = \mu_0 \pm \delta \sigma$, and when the process is in-control, $\delta = 0$; otherwise, the process is out-of-control.
Step 2. When $\bar{X}$ falls between the limits $UCL_{\bar{X}}$ and $LCL_{\bar{X}}$, the sample is declared as conforming, otherwise, it is considered as non-conforming.

Step 3. If a sample is conforming, return to Step 1. Otherwise, compute $Y_q$, for $q = 1, 2, \ldots$.

Step 4. If $Y_1 \leq W_2$ or for $q > 1$, $Y_q \leq W_1$, and $Y_{q+1} \leq W_2$, declare the process as out-of-control, where the $q^{th}$ and $(q+1)^{th}$ non-conforming sample means lie on the same side of the target value $\mu_0$, while $W_l (l = 1, 2)$ is the lower limit of the CRL sub-chart. Otherwise, return to Step 1.

**New Control Charting Method: SSMGR-AI Chart**

In this research, information from the primary variable ($S$) and auxiliary variable ($M$) are considered to develop the SSMGR-AI chart by incorporating the SSMGR charting approach. The chart statistic is designed in such a way that it detects only the shifts in the process mean of primary variable $S$.

Assume a joint distribution between the two bivariate normal variates $(S, M)$ with parameters $\mu_S$, $\mu_M$, $\sigma_S^2$, $\sigma_M^2$, and $\rho$. Here, $\mu_S$ and $\sigma_S^2$ indicate the population mean and variance of the primary variable $S$, while $\mu_M$ and $\sigma_M^2$ are the population mean and variance of the auxiliary variable $M$. $\rho$ is the correlation coefficient between $S$ and $M$. The joint distribution of $(S, M)$ can be expressed as

$$ (S,M) \sim N_2(\mu_{S0} + \delta \sigma_S \mu_M, \sigma_S^2, \sigma_M^2, \rho). \tag{3} $$

where $\mu_S = \mu_{S0} + \delta \sigma_S \mu_M$. Here, $\delta$ is the size of the standardized mean shift of variable $S$. Let $(S_j, M_j)$ for $j = 1, 2, \ldots, n$, denote the $i^{th}$ random sample from a bivariate normal distribution. According to Riaz (2008), an unbiased estimator of $\mu_S$ is given as

$$ \hat{\mu}_S^* = \hat{\mu}_S + \beta (\mu_M - \hat{\mu}_M), \tag{4} $$

where $\hat{\mu}_S$ and $\hat{\mu}_M$ are the $i^{th}$ sample means of $S$ and $M$, respectively. Here, $\hat{\mu}_S = \frac{\sum_{j=1}^{n} S_j}{n}$ and $\hat{\mu}_M = \frac{\sum_{j=1}^{n} M_j}{n}$, and $\beta = \rho (\sigma_S / \sigma_M)$. It is noted that $\hat{\mu}_S$ is a special case of $\hat{\mu}_S$ when $\rho = 0$ (see Equation [4]).

$(S, M)$ follows the bivariate normal distribution and the random variable $\hat{\mu}_S^*$ follows a normal distribution and can be defined as (Riaz, 2008):

$$ \hat{\mu}_S^* \sim N(\mu_{S0} + \delta \sigma_S (\sigma_S^2 / n)(1 - \rho^2)). \tag{5} $$

Here, $\hat{\mu}_S^*$ (for $i = 1, 2, \ldots$) are the quality characteristics monitored by a process. If the process is in-control, the target mean of $\hat{\mu}_S^*$ is $\mu_{S0}$.

The control limits of the SSMGR-AI chart based on $\hat{\mu}_S^*$ are obtained by
SSMGR-AI Chart

\[ UCL = \mu_{\bar{y}0} + k \frac{\sigma}{\sqrt{n}} \sqrt{1 - \rho^2} \]  \[ 6 \]

\[ LCL = \mu_{\bar{y}0} - k \frac{\sigma}{\sqrt{n}} \sqrt{1 - \rho^2} \]  \[ 7 \]

where, \( k \) is the control limit coefficient of the SSMGR-AI chart which depends on the desired in-control performance.

The implementation procedures for the SSMGR-AI chart is the same as that of the SSMGR \( \bar{X} \) chart of Gadre et al. (2010) which were discussed in above Section. The probability of a non-conforming sample when the process mean has shifted by \( \delta \) standard deviation is defined as

\[ P(\delta) = 1 - \Phi \left( k - \delta \sqrt{\frac{n}{(1 - \rho^2)}} \right) + \Phi \left( -k - \delta \sqrt{\frac{n}{(1 - \rho^2)}} \right). \]

The ARL for the SSMGR-AI chart is formulated as

\[ \text{ARL}(\delta) = \frac{1}{P(\delta)} \times 1 + C_1 - C_2 - 2C_1C_2(1 - \alpha) \]

\[ \frac{C_1}{C_2} \{1 - 2\alpha(1 - \alpha)\}, \]  \[ 8 \]

where \( C_l = 1 - (1 - P(\delta))^l \), for \( l = 1, 2 \), and

\[ \alpha = \frac{1 - \Phi \left( k - \delta \sqrt{\frac{n}{(1 - \rho^2)}} \right)}{P(\delta)}. \]  \[ 9 \]

Here, \( \alpha \) is the probability of the non-conforming sample having an upward shift with a shift size of \( \delta \), and \( \Phi(\cdot) \) represents the cumulative distribution function of a standard normal random variable.

The expected average run length (EARL) to signal which considers an overall range of shifts \( (\delta_{\text{min}}, \delta_{\text{max}}) \) is computed as

\[ \text{EARL} = \int_{\delta_{\text{min}}}^{\delta_{\text{max}}} \text{ARL}(\delta) f(\delta) d\delta, \]  \[ 10 \]

where \( \text{ARL}(\delta) \) is the value of ARL in Equation [8] for the shift \( \delta \) and \( f(\delta) \) are the probability density function (pdf) of the shift \( \delta \). The probability that a mean shift will occur in the range \( \delta_{\text{min}} \leq \delta \leq \delta_{\text{max}} \) is considered equal. Here, \( \delta \) is assumed to be uniformly distributed (Sparks, 2000), i.e. \( \delta \sim U(\delta_{\text{min}}, \delta_{\text{max}}) \). It can be written from Equation [9] as follows

\[ \text{EARL}(\delta_{\text{min}}, \delta_{\text{max}}) = \frac{1}{\delta_{\text{max}} - \delta_{\text{min}}} \int_{\delta_{\text{min}}}^{\delta_{\text{max}}} \text{ARL}(\delta) d\delta. \]  \[ 11 \]

**Optimization Method of the SSMGR-AI Chart**

The purpose of an optimal design is to compute the optimal parameters \( (k, W_1, W_2) \) in such a way that minimizes the ARL(\( \delta \)) or ARL \( (\delta_{\text{min}}, \delta_{\text{max}}) \) criterion, for a given \( \rho \) and an exact
shift size $\delta$ or shift interval ($\delta_{\text{min}}, \delta_{\text{max}}$). The algorithm to compute the optimal parameters of the SSMGR-AI chart in minimizing $\text{ARL}(\delta)$ consists of the following eight steps:

Step 1. Specify the in-control $\text{ARL}$ ($\text{ARL}_0$), sample size ($n$) and shift size ($\delta$). Then let $W_1 = 0$ and $W_2 = 0$. Additionally, initialize $\text{ARL}_{\text{min}} = \infty$ and $\text{ARL}_{\text{opt}} = \infty$. Here, $\text{ARL}_0$ is the target value of $\text{ARL}(\delta)$ when $\delta = 0$.

Step 2. Start with $W_1 = W_1 + 1$.

Step 3. Set $W_2 = W_2 + 1$.

Step 4. Determine the value of $k$ by solving Equation [8], so that $\text{ARL}(0) = \text{ARL}_0$.

Step 5. Calculate $\text{ARL}(\delta)$, for the shift size $\delta$, using Equation [8] and the current values of $k$, $W_1$ and $W_2$.

Step 6. If $\text{ARL}(\delta) < \text{ARL}_{\text{min}}$, then let $\text{ARL}_{\text{min}} = \text{ARL}(\delta)$ and return to Step 3. Otherwise, proceed to the next step.

Step 7. If $\text{ARL}_{\text{min}} < \text{ARL}_{\text{opt}}$ then $\text{ARL}_{\text{opt}} = \text{ARL}_{\text{min}}$. Reset $W_2 = 0$ and return to Step 2. Otherwise, proceed to the next step.

Step 8. $\text{ARL}_{\text{opt}}$ is recorded as the minimum $\text{ARL}(\delta)$ value and the corresponding $k$, $W_1$ and $W_2$ values are taken as the optimal parameters of the SSMGR-AI chart which satisfies $\text{ARL}(0) = \text{ARL}_0$.

This eight steps algorithm is also presented in a flowchart in Figure 1, in order to facilitate a better understanding of the aforementioned algorithm.

By following the same algorithm as in Steps 1 – 8, the optimal parameters of the SSMGR-AI chart are also obtained by minimising the $\text{EARL}(\delta_{\text{min}}, \delta_{\text{max}})$ value for the shift interval ($\delta_{\text{min}}, \delta_{\text{max}}$). The only differences are

(i) the shift interval ($\delta_{\text{min}}, \delta_{\text{max}}$) is used instead of an exact shift size $\delta$, and

(ii) Equation [11] is used to compute $\text{EARL}(\delta_{\text{min}}, \delta_{\text{max}})$, instead of using Equation [8] for computing $\text{ARL}(\delta)$.

The proposed chart is designed to minimise $\text{EARL}(\delta_{\text{min}}, \delta_{\text{max}})$ in such a way that the $\text{EARL}(0) = \text{ARL}_0$. Note that, when $\rho = 0$ the SSMGR-AI chart is like the basic SSMGR chart.

Using the steps from 1 to 8, an optimization MATLAB program is written to compute the optimal parameters, as well as the minimum $\text{ARL}(\delta)$ and $\text{EARL}(\delta_{\text{min}}, \delta_{\text{max}})$ values. Tables 1 to 2 and Tables 3 to 4 report the optimal chart parameters ($k$, $W_1$, $W_2$), for different $n \in \{5, 7\}$, $\delta \in \{0.1, 0.3, 0.5, 0.7, 1, 1.5, 2\}$ and $\rho \in \{0, 0.25, 0.5, 0.75, 0.95\}$ that minimize the $\text{ARL}(\delta)$ and $\text{EARL}(\delta_{\text{min}}, \delta_{\text{max}})$ values, respectively of the SSMGR-AI chart. For example, when SSMGR-AI chart is optimally set to minimize $\text{ARL}(0.7)$, i.e. $\delta = 0.7$, $\rho = 0.5$ and $n = 5$ are specified, the value of the optimal parameters are ($k$, $W_1$, $W_2$) $\in (1.5694, 1, 5)$ (see Table 1). Similarly, to minimize the $\text{EARL}(\delta_{\text{min}}, \delta_{\text{max}})$ values, the values of the parameters are ($k$, $W_1$, $W_2$) $\in (2.0690, 1, 56)$ for the optimal SSMGR-AI chart, when ($\delta_{\text{min}}$, $\delta_{\text{max}}$) $\in (0.5, 1.0)$, $\rho = 0.25$, $n = 7$ and in-control $\text{ARL}_0 = 200$ are specified (see Table 3).
RESULTS AND PERFORMANCE EVALUATION

Figure 1. A flowchart showing the algorithm in computing the optimal parameters of the SSMGR-AI chart in minimizing $ARL(\delta)$

Table 1
Optimal parameters of the SSMGR-AI chart for minimizing the $ARL(\delta)$ when $ARL_0 = 200$

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## Table 2

*Optimal parameters of the SSMGR-AI chart for minimizing the ARL ($\delta$) when $\text{ARL}_0 = 370$*

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Table 3

Optimal parameters of the SSMGR-AI chart for minimizing the EARL \( (\delta_{\text{min}}, \delta_{\text{max}}) \) values when \( \text{ARL}_0 = 200 \)

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The performance of any control chart is measured by how rapidly the chart can detect a process shift. If an in-hand chart is faster than its competing charts in spotting a process shift after setting all charts in a similar in-control performance, then the in-hand chart has a better performance than the other charts. In this study, the ARL ($\delta$) and EARL ($\delta_{\text{min}}$, $\delta_{\text{max}}$) criteria are used to measure the performance of the proposed SSMGR-AI chart, and compared to that of the EWMA-AI and the RS-AI charts (see Table 5-8). Abbas et al. (2014) used the simulation approach to study for ARL performance of the EWMA-AI chart. In this study, the researchers considered the optimal EWMA-AI chart in computing the ARL and EARL values. Ng et al. (2018) developed the RS-AI chart and showed that the RS AI chart outperformed the existing Synthetic-AI chart, and the performance of the seven-region RS-AI chart was better than that of the four-region RS-AI chart. Thus, this study considered only the seven-region RS-AI chart.

It is observed from Tables 5 and 6 that when $\delta = 0.1$ and $\rho \in (0, 0.25, 0.5, 0.75)$,

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**Table 4**

*Optimal parameters of the SSMGR-AI chart for minimizing the EARL ($\delta_{\text{min}}$, $\delta_{\text{max}}$) values when $\text{ARL}_0 = 370$*

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the EWMA-AI chart performs better than the SSMGR-AI chart and RS-AI chart but when $\rho > 0.75$, the performance of the proposed SSMGR-AI chart is significantly better compared to the EWMA-AI chart. For example, when $\delta = 0.1$, $\rho = 0.75$ and $n = 0.5$ the ARL (0.1) for EWMA-AI chart is 36.41 which is smaller than that of the SSMGR-AI and the RS-AI charts, i.e. 50.31 and 66.39, respectively, while for $\rho = 0.95$, the ARL (0.1) of the SSMGR-AI chart is 10.94, which is smaller than that of the EWAM-AI and the RS-AI chart, i.e. 13.42 and 13.40, respectively (see Table 5). As the shift increases, the performance of the proposed chart is shown to be more outstanding compared to the EWMA-AI chart and the proposed chart outperforms the RS-AI chart for all size of mean shifts and correlation coefficients. When $\delta = 0.5$, $\rho = 0.75$, $n = 5$, and ARL$_0$ = 200, the ARL ($\delta$) value of the SSMGR-AI chart is 1.92, but the corresponding values of the EWMA-AI chart and RS-AI chart are 3.85 and 4.02, respectively (see Table 5), which are greater than that of the SSMGR-AI chart. It is also observed from Table 5 that the performance of the proposed chart is better as $n$ increases. It is also apparent in Table 5 that the performance of the charts considered improve as $\rho$ increases.

In Table 6, for $\delta = 1$, $\rho = 0.5$, $n = 5$, ARL$_0$ = 370, the average run length values of the SSMGR-AI, EWMA-AI and RS-AI charts are 1.19, 2.26, 1.57, respectively, i.e. ARL ($\delta$)$_{SSMGR-AI} < $ ARL ($\delta$)$_{RS-AI} < $ ARL ($\delta$)$_{EWMA-AI}$. A similar trend is also noticeable when $n = 7$ (see Table 6). On the other hand, in Tables 7 and 8, it is found that for any ($\delta_{min}$, $\delta_{max}$) combination, for $n = 5$ or 7, the EARL values of the SSMGR-AI chart are less than that of the EWMA-AI and RS-AI charts, except for the combination ($\delta_{min}$, $\delta_{max}$) = (0.1, 0.5) and $\rho \leq 0.5$. When ($\delta_{min}$, $\delta_{max}$) = (0.1, 0.5) and $\rho \leq 0.5$, the EWMA-AI chart shows a better performance than the other charts when ARL$_0$s are set as 200 and 370 (see Tables 7 and 8).

The speed in which the SSMGR-AI chart is quicker in detecting a process shift compared with the existing EWMA-AI and RS-AI charts is also shown in parentheses in Tables 5 – 8, in terms of percentages. A positive (negative) percentage for a certain chart means that the SSMGR-AI chart is quicker (slower) than the said chart in the detection of a shift. For example, in Table 5, when ARL$_0$ = 200, $n = 5$, $\delta = 0.5$ and $\rho = 0.25$, the ARL(0.5) values of the EWMA-AI, RS-AI and SSMGR-AI charts are 7.68 and 3.91, respectively, where these ARL(0.5) values indicate that the SSMGR-AI chart is 72.6% and 96.4% quicker than the EWMA-AI and RS-AI charts, respectively, in detecting the shift $\delta = 0.5$. Additionally, consider Table 8, where ARL$_0$ = 370, $n = 7$, ($\delta_{min}$, $\delta_{max}$) = (0.5, 1) and $\rho = 0.5$. Here, EARL($\delta_{min}$, $\delta_{max}$) = 3, 3.03 and 1.5, for the EWMA-AI, RS-AI and SSMGR-AI charts, respectively, and these EARL($\delta_{min}$, $\delta_{max}$) values show that the SSMGR-AI chart is 100% quicker than the EWMA-AI and RS-AI charts, in detecting shifts in the interval ($\delta_{min}$, $\delta_{max}$) = (0.5, 1).

The findings reveal that the proposed SSMGR-AI chart generally prevails over existing competing charts to detect process mean shifts, in terms of the ARL and EARL performance criteria. However, in the case of detecting small shifts ($\delta = 0.1$) and $\rho \leq 0.75$, the EWMA-AI chart outperforms the SSMGR-AI chart.
Table 5

ARL ($\delta$) values for the EWMA-AI, RS-AI and SSMGR-AI charts and the percentage (in parenthesis) in which the SSMGR-AI chart is quicker (positive %) or slower (negative %) than the EWMA-AI and RS-AI charts, in detecting shifts $\delta$, when $\text{ARL}_0=200$

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Table 6

ARL (δ) values for the EWMA-AI, RS-AI and SSMGR-AI charts and the percentage (in parenthesis) in which the SSMGR-AI chart is quicker (positive %) or slower (negative %) than the EWMA-AI and RS-AI charts, in detecting shifts, when ARL₀ = 370

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<th>n = 7</th>
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<td>SSMGR-AI</td>
<td>EWMA-AI</td>
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### Table 7

EARL ($\delta_{\text{min}}$,$\delta_{\text{max}}$) values for the EWMA-AI, RS-AI and SSMGR-AI charts and the percentage (in parenthesis) in which the SSMGR-AI chart is quicker (positive %) or slower (negative %) than the EWMA-AI and RS-AI charts, in detecting shifts, when $\text{ARL}_0=200$

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<td>SSMGR-AI</td>
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</table>

### Table 8

EARL ($\delta_{\min}$,$\delta_{\max}$) values for the EWMA-AI, RS-AI and SSMGR-AI charts and the percentage (in parenthesis) in which the SSMGR-AI chart is quicker (positive %) or slower (negative %) than the EWMA-AI and RS-AI charts, in detecting shifts ($\delta_{\min}$,$\delta_{\max}$), when $\text{ARL}_0=370$

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<td>0.75</td>
<td>13.99 (-6.6%)</td>
<td>20.96 (39.9%)</td>
<td>14.98</td>
<td>10.99 (6.6%)</td>
</tr>
<tr>
<td>0.95</td>
<td>4.71 (64.1%)</td>
<td>3.68 (28.2%)</td>
<td>2.87</td>
<td>3.69 (74.9%)</td>
</tr>
</tbody>
</table>
In this section, a numerical example is given for a clear understanding on how the proposed SSMGR-AI chart can be employed. For this purpose, Statistical Analysis software (SAS) is used to generate one hundred (100) in-control observations \((S, M)\) from a bivariate normal distribution, i.e.

\[
\begin{align*}
S & \sim N(\mu_S, \sigma_S^2) \\
M & \sim N(\mu_M, \sigma_M^2)
\end{align*}
\]

and followed by one hundred (100) out-of-control observations \((S, M)\) are generated from same underlying distribution, where \(\delta = 0.5\), \(\rho = 0.25\), \(\mu_S = \mu_M = 0\) and \(\sigma_S^2 = \sigma_M^2 = 1\) are considered. Table 9 shows that among the 27 bivariate samples, the first 15 samples are grouped from 100 in-control observations and the next 12 samples are grouped from 100 out-of-control observations. Here, each sample contains 5 observations. It is assumed that the SSMGR-AI chart is optimally designed by considering \(ARL_0 = 200\), \(\delta = 0.5\), \(n = 5\) and the corresponding optimal parameters \((k, W_1, W_2) = (1.7273, 1, 11)\) are chosen from Table 1.

From Table 9, it is seen that for the first sample, i.e. when \(i = 1\), \(\hat{\mu}_S = 0.089\), which is computed using Equation [4]. Since \(\hat{\mu}_S \in [LCL, UCL] = [-0.748, 0.748]\) the first sample is conforming. In a similar manner, the procedure is repeated for samples 2 – 11 (see Table 9). At sample number 12, as \(\hat{\mu}_S = 0.878\) \(\notin [-0.748, 0.748]\), the sample is known as non-conforming and hence, \(Y_1 = 12\) is obtained. Since \(Y_1 > W_2\), the process is considered as non-conforming.
in-control. Other non-conforming samples are detected at samples number \( i = 18, 22, 25, 26 \) and 27. It follows that \( Y_2 = 6, Y_3 = 4, Y_4 = 3 \) and \( Y_5 = Y_6 = Y_1 \). Figure 2 shows that the first out-of-control signal appears at sample number 27 as \( Y_5 \leq 1 \) and \( Y_6 \leq 11 \) (see Figure 2) and the control chart’s statistics \( \hat{\mu}_{S_{26}}^* \) and \( \hat{\mu}_{S_{27}}^* \) both fall on the same side of the target mean value of the SSMGR-AI chart. Therefore, corrective actions should be taken to bring the out-of-control process back into the in-control situation. Examples of corrective actions include removal of assignable causes, such as inferior materials, operator errors and faulty parts.

![SSMG-AI chart for the numerical example](image)

**Figure 2**. SSMGR-AI chart for the numerical example

<table>
<thead>
<tr>
<th>Sample number, ( i )</th>
<th>( S_1 )</th>
<th>( S_2 )</th>
<th>( S_3 )</th>
<th>( S_4 )</th>
<th>( S_5 )</th>
<th>( M_1 )</th>
<th>( M_2 )</th>
<th>( M_3 )</th>
<th>( M_4 )</th>
<th>( \hat{\mu}_S )</th>
<th>( \hat{\mu}_M )</th>
<th>( \hat{\mu}<em>{S</em>{26}}^* )</th>
<th>( \hat{\mu}<em>{S</em>{27}}^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.182</td>
<td>0.278</td>
<td>1.215</td>
<td>-1.399</td>
<td>0.316</td>
<td>1.228</td>
<td>1.215</td>
<td>-0.834</td>
<td>-1.276</td>
<td>-1.204</td>
<td>0.046</td>
<td>-0.174</td>
<td>0.089</td>
</tr>
<tr>
<td>2</td>
<td>0.159</td>
<td>-0.012</td>
<td>1.502</td>
<td>-0.095</td>
<td>0.465</td>
<td>0.536</td>
<td>-0.042</td>
<td>-0.871</td>
<td>0.195</td>
<td>1.516</td>
<td>0.404</td>
<td>0.267</td>
<td>0.337</td>
</tr>
<tr>
<td>3</td>
<td>0.162</td>
<td>0.089</td>
<td>-0.698</td>
<td>-0.295</td>
<td>1.980</td>
<td>1.630</td>
<td>-0.863</td>
<td>0.761</td>
<td>0.478</td>
<td>-0.109</td>
<td>0.248</td>
<td>0.379</td>
<td>0.153</td>
</tr>
<tr>
<td>4</td>
<td>-0.187</td>
<td>0.595</td>
<td>-0.617</td>
<td>-0.832</td>
<td>-0.271</td>
<td>-0.721</td>
<td>1.938</td>
<td>-1.529</td>
<td>-0.025</td>
<td>0.464</td>
<td>-0.262</td>
<td>0.026</td>
<td>-0.269</td>
</tr>
<tr>
<td>5</td>
<td>-0.367</td>
<td>1.302</td>
<td>0.216</td>
<td>0.986</td>
<td>0.860</td>
<td>-0.893</td>
<td>-0.524</td>
<td>0.619</td>
<td>0.762</td>
<td>0.261</td>
<td>0.599</td>
<td>0.045</td>
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Table 9 (Continued)

<table>
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<th>$S_3$</th>
<th>$S_4$</th>
<th>$M_1$</th>
<th>$M_2$</th>
<th>$M_3$</th>
<th>$M_4$</th>
<th>$\hat{\mu}_{S_1}$</th>
<th>$\hat{\mu}_{M_1}$</th>
<th>$\hat{\mu}_{S_2}$</th>
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<td>6</td>
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<td>-1.783</td>
<td>-0.893</td>
<td>0.902</td>
<td>-1.681</td>
<td>-0.476</td>
<td>-0.572</td>
<td>-1.845</td>
<td>0.425</td>
<td>-0.338</td>
</tr>
<tr>
<td>7</td>
<td>-0.867</td>
<td>1.071</td>
<td>1.074</td>
<td>-0.109</td>
<td>0.378</td>
<td>-0.047</td>
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<td>-0.378</td>
<td>-0.011</td>
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<td>0.101</td>
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<tr>
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<td>-0.470</td>
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<td>0.270</td>
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<td>-0.779</td>
<td>-0.915</td>
<td>-0.009</td>
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</tr>
<tr>
<td>11</td>
<td>0.833</td>
<td>1.304</td>
<td>-0.879</td>
<td>1.194</td>
<td>-0.234</td>
<td>2.473</td>
<td>1.216</td>
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<td>-0.840</td>
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<td>1.696</td>
<td>1.718</td>
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<td>1.414</td>
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<td>0.536</td>
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<td>-0.142</td>
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<td>1.776</td>
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<td>0.193</td>
<td>0.046</td>
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<td>-0.230</td>
<td>-0.196</td>
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<td>-1.281</td>
<td>2.459</td>
<td>0.597</td>
<td>1.829</td>
<td>-0.940</td>
<td>-0.591</td>
<td>1.388</td>
<td>0.350</td>
<td>1.327</td>
</tr>
<tr>
<td>19</td>
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<td>0.559</td>
<td>1.491</td>
<td>0.624</td>
<td>0.745</td>
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<td>0.557</td>
<td>-0.264</td>
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<td>0.000</td>
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<td>1.733</td>
<td>-0.556</td>
<td>0.238</td>
<td>-1.150</td>
<td>-0.633</td>
<td>-0.875</td>
<td>0.781</td>
<td>-1.261</td>
</tr>
<tr>
<td>26</td>
<td>1.545</td>
<td>1.094</td>
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<td>1.738</td>
<td>1.462</td>
<td>-2.174</td>
<td>0.166</td>
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<td>1.518</td>
<td>2.028</td>
</tr>
<tr>
<td>27</td>
<td>1.245</td>
<td>-0.007</td>
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<td>2.439</td>
<td>0.781</td>
<td>-0.390</td>
<td>-1.631</td>
<td>-0.230</td>
<td>0.477</td>
<td>1.519</td>
</tr>
</tbody>
</table>

CONCLUSION

This research has proposed the SSMGR-AI chart which is based on the SSMGR charting concept of Gadre et al. (2010) to detect shifts in the process mean by using auxiliary information. Information from the study and auxiliary variables is used to derive the charting statistics of the SSMGR-AI chart, in order to monitor the mean shifts effectively. Results show that information from both these variables improves the sensitivity of the chart in detecting process mean shifts. The SSMGR-AI chart reduces to the standard SSMGR chart when $\rho = 0$. The construction procedure, optimal design, performance evaluation and implementation of the chart are elaborated in this study. Additionally, we have presented the methodology and tables of optimal parameter combinations of the SSMGR-AI chart.
The optimization algorithm developed enables the optimal SSMGR-AI chart in minimizing the ARL$_1$ and EARL$_1$ values, for known and unknown shift sizes, respectively.

A numerical example is also given to illustrate the construction and implementation of the proposed chart. In terms of the ARL and EARL criteria, the SSMGR-AI chart is shown to perform significantly better than the existing RS-AI chart in detecting all sizes of mean shifts, while the EWMA-AI chart performs better in detecting small shifts. However, for moderate and large shifts, the SSMGR-AI chart outperforms the EWMA-AI chart as the former has lower ARL$_1$ and EARL$_1$ values than the latter. Thus, the SSMGR-AI chart is deemed as an effective AI chart among existing AI charts, for monitoring the process mean.

As this study is based on the univariate SSMGR-AI chart, future research can be done on the construction of a multivariate SSMGR-AI chart for detecting shifts in the process mean vector. Furthermore, the proposed control charting concept can be extended to the monitoring of process variability or a simultaneous monitoring of the process mean and variance.

ACKNOWLEDGEMENTS
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REFERENCES


Review Article

Fire Test and Effects of Fire Retardant on the Natural Ability of Timber: A Review

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ABSTRACT

Timber is one of the most sustainable and renewable raw materials available. Globally, it has been increasingly used for the manufacture of home and workplace furniture. Timber products are known to have ignition resistance and a low heat release rate. These characteristics delay burning and maintain the structural durability of a product, protecting both the occupants and their properties in a fire. Timber, however, experiences thermal degradation during combustion, yielding smoke, heat, toxic gases, and char when burned. To understand the fire conduct of timber, extensive knowledge in its process of decomposition is essential. This paper, therefore, reviewed the methods of flammability tests widely employed to investigate the reaction of timber and timber-based product to fire, namely cone calorimeter test, room-corner test, limiting oxygen index (LOI) test, furnace test, and single burning item test (SBI). In addition, an overview of the fire retardant treatments; impregnation and coatings was also presented. The potential effects of fire retardants on the durability, strength, hygroscopicity, corrosion, machinability, glueing characteristics, and paintability of the timber were also highlighted.

Keywords: Fire retardant, flammability test, natural, timber coatings, timber combustion, timber impregnation, wood combustion, wood impregnation

INTRODUCTION

Despite its numerous benefits, timber application as a constructional material has
been predominantly restricted by the stigma of burning (Delichatsios et al., 2003; Spinardi et al., 2017). To modify the behaviour of timber and timber-based products towards fire, it is vital to understand the fire conduct and the decomposition process. While it is well-known that timber is not easily burned, it still experiences thermal degradation during combustion, emitting smoke, heat, toxic gases, and char. With regards to the structure or architectural design of timber, it is imperative to perceive the distinction between light timber frame structures, where the fire resistance of wood may be protected by using lining materials such as gypsum and heavy timber structures which frequently depend on the anticipated charring rate (Bisby et al., 2013). Several comparative studies have been conducted on the smoke and heat evacuation from various materials in enclosure fires, extending from the manual counts based on empirical formulae over zone modelling to the utilization of computational fluid dynamics (Merci & Vandevelde, 2007). Nevertheless, limited studies have been conducted on timber and timber-based product.

To improve the ability of timber and timber-based product in fire incidents as to protect the occupants and properties, fire retardants solutions are used. Besides its function as “fire retardant”, much impacts experienced by timber and timber-based product on its natural ability; an area that almost overlooked in the study of the effects of fire retardants. Timber has its own natural ability in surviving the nature such as durability, strength and hygroscopicity. With the addition of fire retarding chemical, the timber and timber based product has also to disclose ability on resisting corrosion, machinability, glueing characteristics, and paintability, but there are not much studies on this especially on various species of tropical timbers. This paper, therefore, aims to provide an overview of the methods commonly employed to investigate the fire properties of timber and timber-based products including the potential fire-retardant treatments; impregnation and coatings. More importantly, this paper also reviewed works that investigated the effects of fire retardants on the natural ability of timber and timber-based products. This review may definitely serve as a starting point for more comprehensive research in the use of timber and timber-based product by providing fundamental information on this particular subject matter.

THE FUNDAMENTAL OF TIMBER BURNING
Generally, each part of a tree is an accumulation of cells which are arranged longitudinally in the stem. These cells are constituted by three main polymers, namely the cellulose (43%), the hemicellulose (28-35%), and the lignin (22-29%). The structural relations between these polymers are portrayed in Figure 1.

When timber is heated to 300°F (150°C), the cellulose, hemicellulose, and lignin are decomposed to unstable gases, tar (levoglucosan), and carbonaceous char (Lowden & Hull, 2013). The decomposition of timber follows a pattern which is considered as the mechanism of superposition of the individual components; starting with the decomposition.
Review of Timber Fire Testing and Retardants

of hemicellulose at 180-350°C, followed by the decomposition of cellulose at 275-350°C, and lastly the decomposition of lignin at 250-500°C (Kim et al., 2006). The chemical substance of timber is very similar to sugars as it largely consists of cellulose which is a polymer consolidated by the reoccurrence of glucose. The higher lignin content of timber, however, leads to a higher temperature rate of thermal degradation and its aromatic chemical structure provides a char yield as high as 35-38% at 1652°F (900°C) (Chung, 2010). At 500°C, the cross-linking response dehydrates the cellulose and the polymerized levoglucosan, producing aromatic structures before transforming to graphitic carbon structures. This process is known as pyrolysis, which is a complicated interconnection subject of chemistry, heat, and mass transfer (Spearpoint & Quintiere, 2001). There are two types of pyrolysis behaviour of solid materials; non-charring and charring. The overall char yield is mainly influenced by the decomposition of lignin (Browne, 1958). The aim of fire-retardants application over timber and timber-based products were to improve both the char production and combustion properties but sometimes only either one of the purpose can be achieved. Investigation on fire properties of Scots pine (Pinus sylvestris L.) treated with unmodified and modified silica dispersions, showed no improvement in char yields but the treatment with modified silica dispersions has enhanced the ignition properties including heat release rate (HRR), smoke production, and CO₂ development (Xiao et al., 2016). Conversely, thermal behaviour of oriental beech treated with 0.25%, 1% and 4.70% aqueous solutions of borax (BX), boric acid (BA), BA+BX(1:1), BA+BX(7:3) has decreased the maximum degradation temperature and increased residual char amount (Uner et al., 2016). The char properties of timber reduce the combustion rate and prevent the oxygen from easily entering the combustion zone (Pearce et al., 1981) which is important in fire safety design. The variability of charring rates and other fire properties within species and among different species largely depends on factors including density, moisture content, external heat flux, and oxygen concentration (Friquin, 2010). In timber and timber-based product advanced development, information on charring rate, HRR and the time to flashover are

Figure 1. (A) Wood cell wall; (B) Macrostructure of fibrils (Hoffmann & Jones, 1989)

the most valuable information as they probably support either to further investigations, explanations or pass or fail decisions.

During combustion, timber releases smoke which is a mixture of gases, most of which are harmful gases. In fact, it has been reported that about fifty percent of life loss cases in fires are caused by the smoke in the escape pathway and the high rate of toxicity (Drysdale, 2011). The factors influencing the composition of smoke are combustion condition, the pattern of decomposition, ventilation, temperature, and fuel chemical nature (Quintiere, 1982; Rasbash & Drysdale, 1982; Tewarson, 2002). Various compounds are present in the smoke gases from the wood combustion but most methods used to analyse the by-products of fire typically focus on the discharge of carbon monoxide (CO) and carbon dioxide (CO\(_2\)) and the decrease in oxygen concentration (Aseeva et al., 2014). Timber assimilates carbon dioxide from the atmosphere as it grows and transforms it into carbon molecules in its cellulose. Combustion of timber reverses this process, whereby about 1900g of carbon dioxide (CO\(_2\)) is discharged for every 1000g completely burnt timber.

**FIRE PERFORMANCE OF TIMBER AND TIMBER-BASED PRODUCT**

Fire performance refers to how an object reacts towards the fire. It is subject to the chemical composition and the atomic structure of a material. Identifying the fire performance of a material is vital as it defines the behaviour of fire in relation to a material in the fire incidents. The fire behaviour is a terminology used to depict the intensity, direction, and magnitude of how a fire spreads. The characters of timber in a fire are obtained for various reasons, such as to determine the fire performance of the untreated timber, to evaluate the performance of fire retardant treated (FRT) timber, and for research and development under the umbrella of fire safety engineering. Bench-scale fire test is used to investigate the fire characteristic of FRT and untreated timber by applying a small sample size. Meanwhile, the large-scale fire test is frequently used to test timber as a structural material to provide fire safety classifications for the end-use of it. There are several frequently investigated fire properties and those are important in determining the fire growth, spread, and impact to the occupants as well as the properties (Friedman et al., 2003; Hopkins & Quintiere, 1996; Lowden & Hull, 2013). For instance, (i) the ease of ignition, which defines how prompt the ignition is; (ii) the rate of flame spread, which defines how quick the fire spreads over a surface; (iii) the rate of heat release, which defines how much and how fast the heat is released, and; (iv) the ease of extinction, which defines how fast the flame extinguishes. On the other hand, the characters of smoke from the burning are evaluated in terms of the (i) smoke/toxic gas evolution and the outgrowth rate; and (ii) amount and composition of smoke released during the different phases of a fire.

The hazards of fire make timber an exceptionally attractive element for advanced investigation. The investigation of the fire properties of timber generally will be directed
to combustibility test followed by thermal analysis. Timber combustion results in transposition and transformation of the thermochemical and thermophysical aspect of the timber, involving mass loss, heat transfer, pyrolysis, and smouldering. This complex process produces the charring layer which is significant for the fire resistance of the timber and is closely related to the morphology and chemical structure of the timber species. The studies of the morphology and mineral content of the timber species are particularly crucial for the tropical timber species due to their rich and complex anatomy. The influence of these factors on the parameters that define the fire behaviour of timber has been another area of interest for the researchers in this field, whereby observations are often conducted using the scanning electron microscope (SEM).

**Combustibility Test**

Combustibility is an evaluation of how easily a material blasts into a fire, through flame or burning. Most of the combustibility tests for timber and timber-based products comprise heating a predefined quantity of the test sample for a set span and conducted to collect data on fire properties. The data are used for (i) fire modelling; (ii) prediction of real scale fire behaviour; and (iii) pass/fail tests (Lindholm et al., 2009). There are numbers of test have been developed since the early time in the effort to determine the fire properties of timber and timber-based product. However, the popular methods are cone calorimeter test (Figure 2), room corner test (Figure 3), limiting oxygen index (LOI) test (Figure 4), furnace test and single burning item test (SBI) (Figure 5). Even though the choice of combustibility test is customarily based on the purpose of research but the main concern is the cost involved. To simply understand the aforementioned fire tests, Table 1 detailed the functions and criteria of each test.

**Thermal Analysis**

Thermal analysis is one part of the materials science where the measurements of specified material properties are recorded as they alter from its natural condition due to the change of temperature. Generally, thermal analysis measures three group of physical parameters: (1) mass, (2) temperature or heat flow and (3) other parameters, such as dimension. The thermogravimetric analysis (TGA) is the simplest way of investigating the thermal property of degradation for wood as it provides quantitative information on the decomposition of polymeric substance (Beall & Eickner, 1970; Rowell & Dietenberger, 2012).

**Thermogravimetric Analysis.** TGA accommodates quantitative data on the decomposition of polymeric material and provides details regarding the degradation kinetics and the char formation (Crompton, 1989). It also analyses the changes in chemical and physical properties of the materials and measures them as a function of increasing temperature.
### Table 1

**Fire Test**

<table>
<thead>
<tr>
<th>Test</th>
<th>Function</th>
<th>Criteria</th>
</tr>
</thead>
</table>
| Cone calorimeter         | 1. For assessment of the quantifiable combustibility parameters, such as heat release rate (HRR), mass loss rate (MLR), smoke production, and other ignition properties  
                          2. Data obtained from this test is particularly used for the fire retardant study during which it evolves to:  
                          (i) Compare the fire reaction of substance to evaluate their fire resistance character for pyrolysis and fire models development;  
                          (ii) Procure the substance's specified parameters required as input in the mathematical models for the full-scale room or room/corner test investigation (the data correspond with the results derived from the full-scale room fires tests); and  
                          (iii) Categorize the substance according to a certain span table for regulatory purposes (Schartel & Hull, 2007)  
                          3. Primary results frequently observed from the cone calorimeter test for timber or timber composites are the curves for mass loss, HRR, and smoke production as a function of time | 1. Small-scale test  
                          2. Small sample size (maximum sample size: 100mm * 100mm *50mm)  
                          3. Officially accepted as the International Organization for Standardization (ISO 5660-1) standard to measure the HRR of a specimen in 1993 and the latest version (ISO 5660-2) has been revised in 2002.  
                          4. Test condition is only specific to a well-ventilated fire scenario with constant heat flux  
                          5. Evaluates one-dimensional fire propagation into a sample, considering no surface flame and denying the linking of the results for under-ventilated fires or post-flashover fires (Carpentor & Jansson, 2005)  
                          6. Test according to ISO 5660 could be performed to predict expected test results in EN 13823 – SBI.  
                          7. The cone calorimeter data appear to correspond with the results derived from the full-scale room fires tests (Chung, 2010; Delichatsios et al., 2003; Greca & Lulke, 2001; Lee et al., 2011; Spearspoint & Quintiere, 2001)  
                          8. Cost beneficial test |
| Room corner test         | 1. Measures most of important fire behaviours, including HRR, effective heat of combustion (EHC), MLR, ignitability, production rate of smoke and toxic gases and time to flashover  
                          - Flashover is the moment when the total HRR from the entire burning exceeds 1MW, during which the test would be terminated. In a case | 1. The test assesses the response of the timber and timber-based wall, surface lining and ceiling products including plywood, chipboards, panels, and wallboards to heat when being installed on the surface of a room and imposed directly to a predefined ignition source at the corner of the room with a single open doorway |
Table 1 (Continue)

<table>
<thead>
<tr>
<th>Test</th>
<th>Function</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>where flashover is not achieved, the test will be prolonged for a maximum of 20 minutes before being terminated 2. Among all the measured fire parameters in a room corner test, HRR and smoke production rate appear to be more dominant (Dillon, 1998), but the time to flashover may be regarded as the most important result of the test (Hansen &amp; Hoyde, 2002) 3. Determines the potential of fire growth in a space, whereby the flame spread over the furnishings and the interior finishing materials is considered 4. Considered as a reference scenario test for SBI 5. The test is accepted to be used as compartment fire test apparatus with some modification on its setting of the source of ignition (wooden cribs which are commonly used as the fuel load) and room furniture arrangement (Bartlett et al., 2017)</td>
<td>2. Considered as an ideal real fire model scenario as the material tested is exposed to fire as in orientation that represents the use of the material in actual situation under the well-ventilated conditions 3. The protocols to conduct a room-corner test are restricted to rules and regulations as listed by the internationally recognized bodies; American Society of Testing Materials (ASTM) and the International Standard Organization (ISO); ISO 9705 4. Involve high cost to conduct the test</td>
</tr>
<tr>
<td>Limiting oxygen index</td>
<td>1. Defines the minimum percentage of oxygen concentration required to sustain the burning of a material. 1. A mixture of oxygen and nitrogen is passed over a burning specimen positioned vertically in a glass chimney and the oxygen level is reduced until the flame is no longer supported at which the sample burned for a length of 5 minutes for a period of 3 min 2. The test protocols can be conducted according to ASTM D286 or the ISO 4589-2 standards</td>
<td></td>
</tr>
<tr>
<td>Furnace</td>
<td>1. Determine the charring properties of timber and timber-based products (Babg et al., 2013; Maraveas et al., 2015; Weikofn et al., 2011; Xu et al., 2015) 1. A furnace is an enclosed structure in which materials are heated up to a considerably high temperature 2. The test protocols are based on the ISO 834-1 (Hagi &amp; Weber, 2012)</td>
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</tbody>
</table>
Table 1 (Continue)

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<thead>
<tr>
<th>Test</th>
<th>Function</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single burning item</td>
<td>1. Timber and timber-based surface lining products are the commonly tested timber product in SBI test (Hagen et al., 2009)</td>
<td>1. The sample mounted on a trolley is placed in a frame (made of two vertical wings forming a right-angled corner) under an exhaust system. A triangular shaped propane diffusion gas burner at a HRR of 30 kW is used, representing a burning waste paper basket placed at the basis of the sample corner. The performance of the test sample is observed over a period of 20 minutes before the result is considered for the classification of building materials (Mierlo &amp; Sett, 2005)</td>
</tr>
<tr>
<td></td>
<td>2. The result of the test is used to categorize the building products (non-flooring products) according to its combustibility into Euro classes of A2, B, C, and D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. The HRR, total heat release (THR), and smoke production rate are measured, while the flame spread and burning droplets/particles are observed visually in this test</td>
<td>2. The test protocols are either the EN 13823 or EN ISO 17025</td>
</tr>
</tbody>
</table>

Figure 2. Schematic diagram of a cone (Lindholm et al., 2009)

Figure 3. Basic diagram of room corner test (Online Image), retrieved February 9, 2018 from https://www.sp.se/sv/units/resafe/safety/fire/PublishingImages/Material/ISO9705-col-txt.gif
with the constant heating rate. Thermogravimetric analysis frequently read as a function of temperature under the constant mass loss rate (Coats & Redfern, 1963). Eventually, when the sample (in milligram) is heated, it may lose weight due to its drying or diffusion as gases. In some cases, the substance may increase in weight through reaction towards the test atmosphere (Lowden & Hull, 2013). The mass loss in an inert atmosphere represents the production of fuel after ignition as the concentration of oxygen ($O_2$) under a flame is nearly 0% (Schartel & Hull, 2007).

**Scanning Electron Microscopy (SEM)**

It has been claimed by numerous researchers that the charring rate of timber is highly influenced by the anatomy of the timber (Aseeva et al., 2014; Babrauskas, 2005; Giraldo et al., 2016). This charring process is defined as the transformation in the surface structure and ultrastructure of timber during its responses to fire temperatures. The decomposition includes the thermophysical and thermochemical processes on a micro scale. To further investigate the characteristics of timber that influence its reaction to fire, SEM has been substantiated as a suitable instrument for the study of the micromorphology, surface topology, and surface ultrastructure of the biological materials (Echlin, 1968). SEM allows study of the materials in its natural or modified form by generating three-dimensional pictures (Borgin, 1970). Although the resolution of SEM is generally below that of the transmission electron microscope (TEM), this method is widely used to study the anatomical features of wood due to its great depth of field and the relatively easy requirement for sample preparation (Exley et al., 1974). Furthermore, SEM is a microscope that produces
a magnified image using the electrons instead of the light. In addition to the timber microstructure, the amount and nature of minerals present can also be observed by SEM (Giraldo et al., 2016).

FIRE RETARDANTS

Fire retardant is a substance used to delay or stop the spread of fire or reduce its intensity. Therefore, to produce a material with satisfactory fire resistance, the products have to be furnished with the most suitable fire retardant substance. A fire retardant material consists of a combination of certain chemicals, which can reduce the flammability of fuels or delay the burning when they are added to the potentially flammable materials, such as textiles, timber, plastics, and others. Fire retardant substances are obtainable mostly in the forms of powder, foam, gel, and liquid to suit the materials that vary in their physical nature and chemical composition. Most fire retardants act as the “synergists” to increase the fire protective benefits. As the elements in fire retardants applied on different materials react differently with fire, the selection of fire retardants must match appropriately to each type of material. In the case of Scots pine treated with phenol-formaldehyde (PF) and melamine- formaldehyde (MF) resins which is known as an efficient strategy to improve timber’s dimensional stability, mechanical strength, and durability, have actually caused different fire risk patterns: smoke issues for the PF-treated wood and heat hazard for the MF-treated wood (Xie et al., 2016). In most countries, a clear explanation of the practice of fire safety measures is documented in their national standard of building and fire codes. Generally, building codes incorporate height and area of a room, automatic sprinklers, fire stops, doors and other exits, fire detectors, and type of construction. Meanwhile, the fire codes may contain fire spread, flammability of materials, and fire endurance of a specific material. The common practice may differ with countries.

Timber has been utilized for most applications due to its poor thermal conductivity properties. Fire retardants have been developed to reduce the flammability of timber and encourage the char forming. The use of fire retardants for timber has started back when the Romans treated their ships with alum and vinegar for protection against fire, followed by Gay-Lussac using the ammonium phosphates and borax to treat the cellulosic textiles (Rowell & Dietenberger, 2012). In 1895, The U.S. Navy again enforced the utilization of fire retardants for their ship. This development was further applied in the building construction in 1899 when the City of New York required the use of fire retardants for a 12-storey building (Rowell & Dietenberger, 2012). In relation to the timber and timber-based products, the commonly applied methods to improve its fire retardant trait are impregnation and coatings (Davidson & Freas, 1987; Horrocks & Price, 2001). The selection of fire retarding methods largely depends on the suitability and cost involved.

The impregnation of fire retardants including polyphosphatic carbamate, and ceramic
coatings including alkoxy metal salt improved the fire performance of Japanese red pine (*Pinus densiflora* Siebold et Zuccarini) and Japanese linden (*Tilia japonica* Simonkai) (Harada et al., 2007). Table 2 illustrates the six categories of fire retardant treatment for timber (LeVan & Winandy, 2007).

Table 2

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Effect to timber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium dihydrogen orthophosphate</td>
<td>Promote the formation of increase char at a lower temperature</td>
</tr>
<tr>
<td>Bromine, chlorine</td>
<td>Free radical trap in flame</td>
</tr>
<tr>
<td>Sodium silicates (non-intumesce), polyol+phosphoricacid +dicyandiamide/melamine/urea/guanidine (intumesce)</td>
<td>Coating the timber surface</td>
</tr>
<tr>
<td>Metal alloy</td>
<td>Increase thermal conductivity of timber</td>
</tr>
<tr>
<td>Dicyandiamide and urea, borax</td>
<td>Dilute the combustible gases from the timber with non-combustible gases</td>
</tr>
<tr>
<td>Ammonium phosphate</td>
<td>Reduce the heat content of volatile gases</td>
</tr>
</tbody>
</table>

During the development process of an improved fire retardant, the tested FRT timber-based products undergo a credential bench-scale fire test which would then indicates the success or failure of the retardant improvement. The result from the bench-scale test can be used as input data to model the prediction of fire effects in different scale incident (Xu et al., 2017). The selected FRTs can be further tested in a large-scale fire test, which is costlier to accomplish the required rating. The ways to measure the performance of a fire retardant are presented in Table 3 (Rowell & Dietenberger, 2012).

Table 3

<table>
<thead>
<tr>
<th>Test</th>
<th>Expected Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermogravimetric analysis (TGA)</td>
<td>The weight of the sample recorded as a function of time at a constant temperature</td>
</tr>
<tr>
<td>Differential thermal analysis (DTA)</td>
<td>Amount of heat by measuring temperature differences between the sample and an inert reference</td>
</tr>
<tr>
<td>Differential scanning calorimeter (DSC)</td>
<td>Actual differential heat flow is measured when the sample and reference temperature are equal.</td>
</tr>
<tr>
<td>Cone calorimeter</td>
<td>Ignition time, mass loss rate (MLR), combustion products, heat release rate (HRR), and other parameters as a function of time</td>
</tr>
<tr>
<td>Tunnel flame-spread tests</td>
<td>Surface flame spread</td>
</tr>
<tr>
<td>Critical oxygen index test</td>
<td>The minimum concentration of oxygen in an oxygen-nitrogen mixture</td>
</tr>
</tbody>
</table>
Timber impregnation

The timber impregnation is conducted to stabilize the dimension, increase the strength and resistance to water, and reduce cracking of the timber. This process involves pressure-impregnation of chemical solutions using full-cell pressure processes (provided this is the most effective way to instil chemicals into the timber). The penetration of the chemicals highly depends on the species, microstructure, and moisture content of the timber. Three softwoods, Sugi (Cryptomeria japonica), Korean pine (Pinus koraiensis) and Hinoki (Chamaecyparis obtusa), vacuum–pressure impregnated with a fire retardant chemical consisting of ammonium phosphate polymer (APP), guanyl urea phosphate (GUP), phosphonic acid and minor amount of additives reduced the modulus of rupture (MOR) and static modulus of elasticity (MOE) compared with before treatment; conversely, the dynamic modulus of elasticity (DMOE) increased after treatment (Wen et al., 2014). There have been studies done to improve the ability of absorption or permeability of timber by the fire retardants, such as by having an additional step of microwave heating. This microwave pre-treatment is claimed to be able to increase the permeability of wood significantly for the substance such as metal alloy (Torgovnikov & Vinden, 2010; Vinden et al., 2011). For instance, the poplar samples impregnated with the ammonium polyphosphate (APP) fire retardant after being pre-treated with microwave heating demonstrated a significant improvement in its fire resistance (15.89% less at peak HRR, 5.69% less at total HRR, and 13.59% less at total smoke production, TSP) as compared to those without pre-treatment with microwave (He et al., 2015). The nano technology development has contributed not a small impact on the fire retardants research and development. The nano-silver solution as fire retarding formulation has significantly increased the fire retarding ability of four hardwood and one softwood species that is commercially used in the various industrial application in Iran (Taghiyari, 2012). Furthermore, the wood nanotechnology has allowed the Balsa wood (Ochroma pyramidale) to be delignified to form a hierarchically structured and nanoporous scaffold mainly composed of cellulose nanofibrils. These nanocomposites are impregnated with colloidal montmorillonite clay to form a nanostructured wood hybrid of high flame retardancy (Fu et al., 2017). This impregnation is almost similar to the chemical preservative treatments of timber except that there are higher retentions and absorption of chemicals acquired for the fire-retardant protection. The compounds which are highly reactive to the hydroxyl groups of cellulose, hemicellulose, and lignin components include epoxides, isocyanates, anhydrides, lactones, and diols (Mathias et al., 1991). It has been reported that the nitrogen-phosphorus impregnated Poplar and heat treated at 150°C decreased the moisture sorption by 57%, while the leach resistance values (LRV) increased by 70%, thus enhancing the fire retardant performance of the wood (Chu et al., 2017). Thermally treated Spruce demonstrated lower weight loss in fire resistance test (Čekovská et al., 2017). A significant change in the thermal properties of
treated sawdust reveals that the impregnation of hydrated-sodium metaborates into sawdust could create a significant amount of condensed phase char and provide a highly effective fire retardant protection with multiple modes of action (Nine et al., 2017). Apparently, combination of heat treatment and impregnation technique with improved formulation has further enhanced the timber’s reaction to fire and its working properties. Additionally, the Poplar wood treated with nitrogen and phosphorus (NP) based fire retardants and impregnated with polysodium silicate-aluminum dihydrogen phosphate (PSADP) also reduce the hygroscopicity of the wood, increase its LRV by 81% and reduce the moisture absorption rate by 40.3%, as compared to the wood which has been treated with NP but without PSADP (Zhang et al., 2016).

**Timber Coatings**

Another way to provide varying degrees of fire retardant to timber against fire is coatings. A coating is defined as an intelligible layer formed from a single or multiple application of a coating material to a substrate (DIN EN ISO 4618; 2.52). The fire performance of edge-jointed lumber of *Albizia* and *Gmelina* improved with the application of trimethylol melamine phosphoric acid coating (Subyakto et al., 2003). As indicated in the current standard (DIN EN ISO 4618; 2.53) a coating material is a material in fluid, paste or powder form which, when applied, forms a defensive and ornamental coating. Coating materials are complex chemical products including lacquers, paints, and other similar products. In most cases, coatings comprise of binders (also known as the film formers), pigments and extenders, solvents, and additives. Application of fire retardant coatings is to provide fire retardant properties, such as low flame spread, low smoke emission, and non-toxicity, without altering the performance of the substrate (U.S. Patent 6245842, 2001). In addition to its main function as a fire retardant, an ideal timber coating is also defined based on its effect on the hydrophobicity, toughness, oxidation protection, UV resistance durability (Liu et al., 2017). It has been proven that a hydrophobic coating based on synthetic resins and waxes exhibits a better durable performance against weathering and a higher hydrophobicity after 6 weeks of weathering, as compared to coating based on zirconium nanoparticles in butanol (Pánek et al., 2017). Coatings products are easy to apply using spray, brush or rollers. Generally, there are two types of coatings: (1) intumescent and (2) non-intumescent. Intumescent coatings will ‘intumesce’ to form an expanded low-density film on the surface of a material to protect the lower layer against the high temperatures and oxygen when exposed to fire. Prior reviews on the research and development of intumescent coatings have been reported by Vandersall (1971) and updated later by Kay et al. (1979). The intumescent coatings formulated through the combination of a dehydrating agent (e.g. polyammonium phosphate) a char former (e.g. starch, glucose, and dipentaerythritol) and a blowing agent (e.g. urea, melamine and chlorinate paraffin). Coatings that use additives, such as boric
acid, borax, chlorinated compounds, and antimony trioxide required an additional binding
substance. As for the wood plastic composites (WPC) which could be widely used in the
residential construction and the decking and furniture industry, the addition of 5 wt% of
zinc borate (ZB) or manganese dioxide (MnO₂), montmorillonite (MMT), and stannic oxide
(SnO₂) into intumescent flame retardants (IFR) created a fire retardant WPC with a V0
rating, exhibiting an excellent fire retardancy (Ren et al., 2015). As for the advancement in
binder-free coatings, hexagonal boron nitride (h-BN) nanosheets with anisotropic thermal
conductivity and low thermal diffusivity and effusivity properties made a high-performance
binder-free fire-resistant coating for wood up to 900 °C (Liu et al., 2017).

Formulation of Retardants

Chemicals used in the composition of fire retardants of timber range from the inexpensive
inorganic salts to the more complex and expensive chemicals. Fire retardants are generally
categorized by their corresponding chemical formation: (1) brominated, (2) phosphorus,
(3) nitrogen, (4) chlorinated, and (5) inorganic (Davidson & Freas, 1987). Inorganic salt
is the most used type of fire retardant for timber (LeVan & Winandy, 2007). It is frequently
used for interior timber products due to their good performance on mechanical and thermal
properties, optical behaviour, and higher bacterial resistance (Kartal et al., 2007). Examples
of the inorganic salts are monoammonium sulfate, diammonium phosphate, ammonium
sulfate, zinc chloride, sodium tetraborate, silicon dioxide, titanium dioxide, sodium
silicates, and boric acid. The inorganic salt, such as silicates and borates may involve a slow
impregnation up to 24 hours (US4612050A, 1986) and according to the fact that timber
is a highly hydrophilic material, this situation is undesirable in some cases. The solubility
of the inorganic salts in water highly depends on zinc chloride as it is the most soluble
salt (2 g/ml) while boric acid is the least soluble salt (0.056 g/ml) (Merck Index 1968).
Consequently, it appears that borax could possibly be used in the WPC as fire retardants
since the presence of boron after leaching has been shown with SEM-EDX and LOI tests
(Cavdar et al., 2015). Timber treated with sodium borate (agricultural borax) which is
environmentally friendly solution, performed better resistance to burning (Sogutlu et al.,
2011). In the process of looking for “green solvent”, a new formulation for impregnation
of Norway spruce (Picea abies) has proposed the application of the ionic fluid 1-ethyl-3-
methylimidazolium chloride as a carrier for calcium metasilicate, titanium dioxide, and
tungsten trioxide (Croitoru et al., 2015). The prevalence of ionic liquids as a method for
impregnation is the application of environmentally friendly solvents, but there is a need
for more findings on application to other species of timber.
Effects of Fire Retardants on the Main Characteristic of Wood

The goal of applying fire retardants to material whether by coating, impregnation, soaking or other possible ways, is to provide fire retardant ability to the material. The selection of formulation is always based on the natural level of fire retardant ability of the material which differs from one material to another. The fire retardants may increase the natural characteristic of the material or decrease it. In the case of a timber, which is one of the natural materials utilized in the interior and exterior products, the application of fire retardants has been shown to improvise and optimize its fire retarding performance (Ayrilmis et al., 2007; Bajaj, 1992; Camino et al., 1989; Goldstein & Oberley, 1966; Hao & Chow, 2003; Liu, 2000; Nine et al., 2017; Ostman & Tsantaridis, 1995). However, in the attempts to reduce the flammability rate of timber, the fire-retardants treatment may change its natural characteristics, including the durability, hygroscopicity, strength, corrosivity, machinability, surface appearance, gluability, and paintability (Ayrilmis et al., 2007; Bekhta et al., 2016; Chu et al., 2017; Hirata et al., 1991; Jiang et al., 2015; LeVan & Winandy, 2007; Wang, 2010). FRT timber regularly becomes moisture sensitive, discoloured or corrosive (Laranjeira et al., 2015). In order to investigate the pressure effects on the thermal degradation of FRT timber; the Sugar pine, Douglas-fir and California black oak treated with ammonium sulfate, boric acid, borax, diammonium phosphate zinc chloride were tested with differential scanning calorimetry (DSC) at 0, 10 and 30% moisture content and under 4 different pressure accordingly (Woo & Schniewind, 1987). The magnitude of the side effects, such as an increased moisture content, pressure effect, a reduced strength, and an increased potential to corrode metal connectors, relies on the fire-retardant chemicals utilized. The acceptance of the side effects depends on the intended use of the products.

Durability. The durability of timber refers to the ability to resist elemental and natural forces of decay. There have been studies in the USA and the UK which investigated the adverse effects of fire retardants on wood (Östman et al., 2001). The general practice used to evaluate the durability level of FRT timber is the comparison of fire performance prior and after weathering. This weathering phase may by natural exposure or accelerated. Application of FRT timber in the exterior requires durability in humid conditions when exposed to rain, good LRV, and resistance to weather including the UV radiation. In many circumstances, FRT timber appears to be moisture sensitive, discoloured, corrosive, and mechanically weak, making it not durable in the exterior applications. The degradation may result from the fungus that could be caused by the cycles of rain, moisture, termites, putrefaction, and destructive insects. Most of the fire retardant formulations increase the durability in some characteristics but decline others. Decay resistance tests revealed that solid wood specimens treated with quaternary ammonia compounds didecyl dimethyl ammonium chloride (DDAC) and didecyl dimethyl ammonium tetrafluoroborate (DBF)
showed resistance against the fungi tested, however, monoammonium phosphate (MAP), diammonium phosphate (DAP) and ammonium sulphate (AS) did not provide complete protection but inversely the DDAC and DBF resulted higher HRR compared to MAP, DAP and AS (Terzi et al., 2011). It has been reported that the graphite (G), aluminium trihydrate (ATH), and titanium oxide (TiO₂) prevent the discolouration of the wood-polypropylene composites under the accelerated weathering of a xenon-arc lamp source during 1000h but reduce the tensile properties (Turku & Kärki, 2016). Furthermore, the stabilized WPC with aluminium hydroxide as fire retardant has shown a lower fading degree but a reduced outdoor durability (García et al., 2009). The expandable graphite (EG) and ammonium polyphosphate (APP) has been demonstrated to improve the flame retardancy of acrylonitrile–butadiene–styrene-based WPCs but worsen the mechanical properties (Zheng et al., 2014). Overall, the timber treated with inorganic salt is suitable for application where the humidity never exceeds eighty percent, which corresponds to the water-soluble properties of the fire retardant and hygroscopic properties of the timber. As for exterior application, higher leach resistance is desirable to increase the durability of the timber.

**Strength.** Fire-retardant treatment may incur a reduction in the strength properties of the timber (Croitoru et al., 2015; Davidson & Freas, 1987; Kadir et al., 2015; LeVan & Winandy, 2007; Mathias et al., 1991). In some cases, physically observed conditions have demonstrated that in the length of three to eight years of service, timber would turn brash, brittle, and fragmented. The main factors that decrease the strength of FRT timber are the thermal degradation, chemicals reaction, and effect of elevated temperature (LeVan & Winandy, 2007). FRT timber roof and truss members which are imposed for thermal degradation have shown a loss in bending (and possibly tensile) strength (Kasal, 1999). Most of the fire-retardant substances did not increase the strength properties of timber. Furthermore, the addition of ammonium polyphosphate (APP) and silica as fire retardants could cause a reduction in the mechanical properties of the composites wood-fibre/polypropylene (PP), except for the tensile strength of the small amount of silica-filled wood-fibre/PP composite (Zhang et al., 2012). The use of wood flour enhances the mechanical properties of thermoplastics but additionally increases the burning rate of WPC (Arao et al., 2014). The combination of elevated temperature conditions and the acidic nature of the fire retardants may have accumulated the rate of acid hydrolysis of the timber and reasoned the loss of strength and embrittlement of FRT timber (LeVan & Winandy, 2007). This finding explains the long-term impact of elevated temperatures and acids on the composition of the FRT timber. To effectively measure the strength properties of FRT timber, mechanical test could be conducted or strength losses for any combination of exposure conditions and times may be calculated by assuming that the total strength loss
due to fire retardant treatment and thermal exposure is the sum of an intrinsic loss due to physical changes, and a loss due to chemical changes which is measured by the fractional conversion (Berndt & Schniewind, 1990). The requirement for the strength of FRT timber has been stated in some building codes including the International Building Code (IBC), the ANSI/TPI 1-2014 National Design Standard for Metal Plate Connected Timber Truss Construction, and the ANSI/AWC NDS-2015 National Design Specification (NDS) for Timber Construction. Due to the strength issue, most of the building codes require some reductions in the design values and the capacity of connectors used in conjunction with the treated timber.

**Hygroscopicity.** Hygroscopic is an ability of a substance exposed in the room temperature to absorb and adsorb water from the surrounding atmosphere. It may modify the original features and properties of the exposed substance, such as an increase in volume, boiling point or viscosity as the water molecules become trapped in between the molecules. Physically, a hygroscopic substance tends to become damp and waterlogged when exposed to the surroundings containing salt and sugar. Based on the existing studies, timber treated with inorganic fire-retardant salts are more hygroscopic than the untreated timber (Ayrilmis et al., 2007; Winandy et al., 2002), particularly at a high relative humidity. One of the initial problems associated with hygroscopicity of the FRT timber is the corrosion of metal fasteners and fittings such as metal truss plates. An alternative solution to decrease the hygroscopic effect using the silicon compounds and nano-wollastonite-based substance may be considered (Cai et al., 2016; Fufa et al., 2010; Soltani et al., 2016). The combination of sodium silicate, boric acid, ammonium borate, and di-ammonium phosphate has been shown to improve the leaching resistance, while the formulation of sodium silicate boric acid and di-ammonium phosphate increases the hygroscopic property and the metal corrosive efficacy of Japanese pine (*Pinus densiflora*) (Won et al., 2014). There have been limited studies on the hygroscopicity effect of FRT timber, instead, more research works have been done to investigate the hygroscopicity level after heat treatment, elevated temperature, against humidity, and other properties (Kumar & Shakher, 2016; Martin et al., 2013; Time, 2002).

**Corrosions.** Metal fasteners have a vital function in the development of timber construction. Nevertheless, these fasteners are subject to corrosion that could decrease the capacity of the structural joint. The inorganic salt fire-retardant compounds for timber are corrosive to metals in the humid or moist environments. Furthermore, the hygroscopicity characteristic of the FRT timber may impose aggressive corrosion even at a low relative humidity. The degree of corrosions by FRT timber varies according to the formulation of the chemical involved. Based on a 3-year corrosion test of eleven wood fastener materials in the wood
treated with copper-containing waterborne salt additives, the fastener materials that are cathodic with respect to copper should be selected when a long service life is needed under wet conditions (Baker, 1980). The application of FRT timber in the exterior has been a case of study in many countries with some reports made accessible. In relation to the historic buildings in the Czech Republic, the original roof timber was seriously corroded by the ammonium phosphate and sulfate-based fire-retardant coatings, whereby repetition of coating led to an accumulation of salts in the timber and caused an undesirable side effect of structural elements thinning (Kucerova et al., 2007). Some formulations including the corrosion inhibitors, such as sodium dichromate and ammonium thiocyanate have been reported (Tuomi, 1980). Nonetheless, the reports on the resistance to corrosion with the non-leachable type of fire retardant for timber remain limited. One aspect that has gained little attention in the exterior usage of timber is the fungal attack. Therefore, the formulation of borate-based fire retardants such as zinc borate does not only benefit the timber in biostatic, tannin stain resistance, flame retardant properties, corrosion prevention but also renders the use of a separate dry film fungicide unnecessary (Schoeman & Lloyd, 1999). As the usage of FRT timber expands globally, the building codes have evolved accordingly. Since 2009 to 2015, there has been a serial revision for the International Building Code (IBC) under the section 2304.9.5; “Fasteners in preservative-treated and fire-retardant-treated wood”. The revisions include many new subsections (2304.9.5.1 through 2304.9.5.4) dealing with timber treatments in a different condition of environmental applications. Specifically, the subsection 2304.9.5.3 as shown in Figure 9, states that fasteners (including nuts and washers) utilized with FRT timber in exterior or in other wet or damp condition, must be hot-dipped zinc-coated galvanized steel, stainless steel, silicon bronze or copper. This segment permits other types of fasteners (barring nails, wood screws, timber rivets, and lag screws) to be mechanically galvanized as per ASTM B 695, Class 55 at the minimum.

Figure 6. Section 2304.9.5.3 of the 2012 IBC (Online image). 2015. Retrieved February 9, 2018 from http://seblog.strongtie.com/wp-content/uploads/2015/04/figure1.jpg
**Machinability.** Machinability of wood means the ability of wood to undergo typical machining process such as sawing, milling or drilling. There is no clear clarification on the indicators that can directly relate to the machinability of timber. The performance of timber and timber-based product in machinability frequently measured by relative indicators which used to have high connection on the physical process and the results generally referred under identification of cutting resistance of the timber. Torque and axial force have been selected as relative indictors for machinability of innovative wood based materials with addition of styrene-butadiene rubber (SBR) gum granulate in the effort of determination of its cutting resistance by Wilkowski et al., 2014. The machinability of drilling process of hardboard and novel wood-fiber material with lignins as binder has been investigated with three relative indicators of machinability taken into account: cutting power, the specific cutting coefficients associated to torque and the specific cutting coefficients associated to the thrust force for variable cutting parameters (three cutting speeds, five feeds rate) (Wilkowski et al., 2011).

There are only a few studies of the machinability of FRT timber available and mainly on the practice of the machining process (Davidson & Freas, 1987). The regular practice of preparing FRT timber for trimming and mouldings is to cut the timber approximately to the finished size prior to the treatment to minimize the machining (Davidson & Freas, 1987). This is because of the appearance of salt crystal in FRT timber which has harsh effects on the cutting tools. The use of regular high-speed steel tools may only allow cutting of not more than a few hundred feet of the FRT timber. An increased tool life is obtainable by using the cutting and shaping tools tilted with tungsten carbide or similar abrasion-resistant alloys (Davidson & Freas, 1987; Kultermann & Spence, 2016).

**Gluability.** Gluability of timber is often recognized as evaluation of shear strength of timber at the glued joint. Since the early years, the intended end-use of the timber and timber-based product determines the formulation of the glue. Classified as the thermosetting polymers, phenol resorcinol-formaldehyde (PRF) provides excellent weather, water, and humidity resistance when cured for interior or exterior uses but has a poor effect on the FRT timber. The deterioration rate of wood glue joints of the epoxy resin is the highest, followed by the aquapolymer-isocyanate resin, and lastly the resorcinol resin (Wang et al., 1993). Timber of the Southern pine species treated with ammonium salt fire retardant, glued with resorcinol-resin adhesives under conventional gluing conditions (hot-press phenolic adhesives) resulted in a lower joint quality as compared to the similar untreated specimens in block-shear evaluation (Schaeffer, 1966). To enhance the gluing characteristic of FRT
timber, a combination of resorcinol-resin glue with a liquid formalin hardener have been proven to produce better joints and structural bonding than the glues with paraformaldehyde hardener when cured at 65°C or higher temperature (Schaeffer, 1966).

In recent years, with the increasing awareness towards safer environment, apparently there are advancement in the fire retardants formulation. Plywood prepared from Poplar veneers glued using urea formaldehyde resin and treated with seven different fire retardant compositions passed the glue shear strength test according to Indian Standard (IS) (Samani & Khali, 2016). Additionally, the sol-gel technique enables timber to be permanently silified to a specific degree by coating or impregnating it with chemically or physically modified silica sols, which has considerably improved the fire retardancy and perform better gluing properties (Mahltig et al., 2008).

**Paintability.** Generally, the fire-retardant treatment of timber has no conflict with the decorative paint coatings, except for the treated timber which has accumulated moisture content. With regards to the timber treated with hygroscopic inorganic salts, the moisture content must be reduced to twelve percent or less when applying the coating. In the exposure to a relatively high humidity, an element of crystallization can be seen over the surface of the paint coatings applied on the timber treated with hygroscopic salt. The natural finishes used for the fire-retardant treatments may vanish as the treatment and subsequent drying may cause darkening and irregular staining on the painted surface. To overcome this situation, manufacturers usually prepare a decorative fire-retardant timber by gluing a thin and untreated decorative veneer facing the treated core. The effects of fire retardants on the paint applied on FRT timber have not been extensively studied, with only limited research reporting the effects of paint on the combustion resistance of untreated timber and the development of paint formulation as fire retardants (El-Wahab et al., 2010; Staggs et al., 2003).

**CONCLUSION**

Timber is one of the most sustainable, appealing, versatile, durable, eco-friendly, and renewable raw material available. The past years have witnessed growing interests in the use of timber and timber-based products in building construction and furnishings with present practices seeing its vast potentials to be further developed for various uses. However, in some circumstances, the utilization of timber and timber-based products is unfeasible due to its combustible properties, which may cause unwanted fires that could cause fatalities to the occupants and damage to properties. Therefore, the usage of timber is limited by certain safety requirements and regulations. In order to enhance its reaction to fire, timber and timber-based products are treated with fire retardants and surely safety
requirements to fulfill such as performance requirements lined by the American Wood Preservers’ Association and may differs according to region and country. Fire retardants generally decrease the effective heat of combustion, the initial peak heat release, and the average heat release rate. On the contrary, the ignition times are usually increased and have minimal impact on charring rates and the fire endurance of structural members. The excessive levels of fire retardants can increase the production of smoke. FRT timber and timber-based product also results in reduction in their mechanical properties and become more brittle compared to the untreated.

From this review, it can be concluded the issues of thermal degradation and fire performance of solid timber, FRT timber and timber products in either elevated temperature or direct fire exposure circumstances are most likely overlooked. Although considerable studies have been done on some species of timber and timber-based product, the finding is not feasible for generalisation even for the same category of timber due to complexity of the processes involved. This situation leaves many gaps in attaining full understanding of thermal degradation and fire behaviour of solid timber, FRT timber and timber-based products especially for the tropical species. Additionally, there are not many in-depth studies focusing on the effects of fire retardants on the ability of strength, durability, hygroscopicity, corrosion, machinability, glueing and paintability of different species of commercially traded timber and timber-based products across the world.

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REFERENCES


The Influence of Change Management and E-Learning in Malaysian Private Higher Education Institutions

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ABSTRACT
The purpose of this research is to analyse the influence of change management and e-learning in Malaysian private higher education institutions focusing on educators’ perspectives. The conceptual framework was modified in combination of various theories from Systemic Change Models and E-learning Cycle Models. A self-administered questionnaire adapted from Siebel 4.0-2 Survey Questionnaires (SSQ) by Hambling, 2010 was the data collection instrument. The sample consisted of educators from private higher education institutions with visions or missions based on e-learning implementation in Malaysia. As per findings, through review of the visions and missions, the selected private higher education institutions integrated teaching and learning with Stepwise Multiple regression analysis, has a significant relationship on independent variables that contribute to e-learning implementation.

Keywords: Change management, e-learning implementation, mission, private higher education, vision

INTRODUCTION
Information and Communication Technology (ICT) is key to value the education in higher education institutions globally (National Information Technology Council, 2008). In 6th Malaysia Plan, ICT was broadly emphasised as an enabler in Malaysia’s education system. In 7th Malaysia Plan, the National Institute for Trial Advocacy (NITA) aimed of developing education as profitable industry in Malaysia (National Information Technology Council, 2008). Ravet and Layte (2008) concluded that educators in higher education institutions were managing educational transformation to monitor the efficacy of the learning resources. They also stated that there were many courses conducted through e-learning, like Problem Based Learning (PBL), Self-Directed Learning (SDL), Process Oriented
Guided Inquiry Learning (POGIL), and Distance Learning. The Centre of Educational Technology (2005) stated that students preferred usage of e-learning as the core approach to learning. “Three decades from the present moment only the large university campuses would be left, universities would not endure” (Clayton, 2000).

Change management is an approach taken to make a smoother transition of individuals, teams, and organizations to the desired future state (Bresnahan et al., 2002). It is a structured approach to ensure that the achievement would benefit the education industry (Brusilovsky, & Millan, 2007). Prime Minister of Malaysia, Tun Dr. Mahathir emphasised that people were our ultimate resource (Mohamad, 2007). Malaysia needs to contribute to the improvement of human resources programmes to support the changes needed to achieve vision 2020, especially in core areas such as education, training and managerial skills (Mohamad, 2007).

**Problem Statement**

Supyan (2011) stated that the main problem with education today was that people did not understand, nor come to a consensus on why changes were needed and how to proceed with the change. This causes many difficulties and failures in the change management process (Jeremi et al., 2012). Most studies in the field of change management have only focused on who plays the major role in change management. Therefore, this research analyses and provides a clear picture of how change management needs to be done, and who is going to face the implications. These aspects exposed a gap in the practice on implementing and sustaining e-learning in the Malaysian education system (Embi, 2011).

Noraini and Nor (2010) found that effectiveness of change management in the implementation of e-learning in universities within Malaysia. Adding to that, Embi (2011) and Alhabshi (2006) finding showed that there were gaps in practicing e-learning implementation. Based on a real life example, a private higher education institution in Malaysia that had a vision and mission on the implementation of e-learning, found the adoption part of e-learning was not a smooth and pleasant process because majority of educators in this institution were unable to adapt to the new e-learning environment, which led to several resignations.

There has been no comprehensive study involving educators group on the implementation of e-learning within private higher education institutions in Malaysia (Jowati, 2011). Therefore, a comprehensive study is to focus on the educators’ perspective on change management and bridge the identified gaps in the concepts, theories, methodology and practices. This research will focus on the educators in institutions that have vision or mission towards e-learning implementation.
Influence of Change Management

Research Question
The research question is to find out which change management variables that is the most influential in e-learning implementation in private higher education institutions within Malaysia from the perspective of educators.

Research Objective
The objective of this research is to identify which change management variables most influence e-learning implementation in private higher education institutions within Malaysia from the perspective of educators.

Research Hypotheses
H0: All change management variables have the same degree of influence in e-learning implementation in private higher education institutions within Malaysia from the perspective of educators.

METHODS
The research instruments were modified according to the conceptual framework based on theoretical framework. In this research the survey method, cross sectional study and exploratory method were used as the research strategy to conduct this research in Malaysia’s private higher education institutions. This survey method was used because the data obtained was standardized, facilitated comparison and analyzed using quantitative means. A self-administered questionnaire was chosen as the data collection instrument for the quantitative method and interviews were conducted as the qualitative method. The samples were educators from private higher education institutions with visions or missions based on e-learning implementation in Malaysia. The questionnaire was adapted from the Siebel 4.0-2 Survey Questionnaire (SSQ) by Hambling (2010).

The rationale for adapting the ideas of the SSQ was because the research that Hambling conducted used the Systemic Change Model in implementation of the Siebel 4.0-2, an e-learning platform from the perspective of the ‘people’ who were the users. SSQ was cited by 14 research articles that used Systemic Change Model from the year 2010-2012 (MS Academia, 2013). Besides that, this research questionnaire used the ideas of SSQ with modified variables to suit the conceptual framework and answer the research questions. For this study, the researcher selected participants from institutions with university status situated in Kuala Lumpur that incorporate e-learning implementation in their vision and mission. Private higher education institutions in Kuala Lumpur were selected as the sample because the majority of the university status private higher education institutions with visions and missions on e-learning were situated in Kuala Lumpur. In line with government
ambition to make Malaysia a regional hub for education, higher education in Kuala Lumpur aims at attracting top world institutions with innovative teaching and learning (Ministry of Education, 2013). Besides that higher education institutions in Kuala Lumpur are envisioned as leading in teaching and learning facilities with a major contribution to the education sector of the country. There are 37 private universities in Malaysia with total 282928 students studying in Malaysia (Ministry of Education, 2013). Universities in Malaysia have been shaped with conducive monitoring from the Universities and University Colleges Act 1971 (Ministry of Higher Education Report, 2012). The number of students and educators was determined by the ratio of 1:13 which means that in total there were 21763 of educators in the year of 2014 (Ministry of Education, 2014). There were nine private higher education institutions with university status that were qualified to participate in this research after analyzing the vision and mission of the institutions. However, only private higher education institutions in Kuala Lumpur with university status that included e-learning implementation in their institute’s vision and mission statement were selected. Educators in this research consist of teachers, tutors, instructors and lecturers in the selected private higher education institutions based on their qualifications. Besides that, Krejcie and Morgan’s (1970) model was used because it was an appropriate model to get the sample size and this model had been cited in 394 studies.

RESULTS AND DISCUSSIONS

Content Validity

Content validity was determined through using scales which were adopted from established empirical studies (Narver et al., 1993; Jaworski & Coupland, 2014). The questionnaire validity and reliability was ascertained by conducting Cronbach alpha. Even though validity and reliability of SSQ were mentioned, the researcher were still conduct a pilot test to determine the actual validity in the context of this research. Convenience sampling consisted of 50 educators from private universities that had vision and mission on implementation of e-learning in their institutions were included in pilot study. The test was not used for statistical purposes and responses from the pilot test were not included in the research findings.

In fact, only a preliminary reliability evaluation was carried out with Cronbach’s Coefficient Alpha Reliability Analysis. The researcher did not carry out the factor analysis due to small sample size. Tabanchnick and Fidell (2007) reviewed this subject with the purpose of reassuring to encompass the smallest amount of 300 cases for factor analysis (Saunders & Thornhill, 2003). Therefore, factor analysis was not needed in the pilot test (Saunders & Thornhill, 2003). While recommended by Nunnally (1979) in the initial point of the research reliability in the range of 0.8 to 0.9 was adequate. The summary results of Cronbach’s Alpha stated in Table 1.
Additionally, participants were encouraged make suggestions for improvement. Comments were solicited on the clarity of the questions and the editing was done in order to simplify the questions. The pilot test results identified ambiguities in the questionnaire items. Problems concerning instructions given for completing the questionnaire were also solved. A final version of the questionnaire was prepared for use in the actual research.

**Reliability Assessment**

In order to ensure that the developed scales and factors measured consistently intended to measure, the Cronbach’s Alpha Coefficient (Nunnally, 1967) was employed to test their reliability. A post test of the reliability of the survey instrument used in this study was measured by internal consistency approach (Churchill, 1979). The Cronbach’s Alpha was computed on each of the Likert scale items that were factor loaded into the nine factors mentioned earlier. The internal consistency reliability scores ranged from 0.641 to 0.854 as in Table 2 after removing some items with low corrected item-total correlations value.

**Table 1**

**Summary results of Cronbach’s Alpha**

<table>
<thead>
<tr>
<th>Construct</th>
<th>No. of items</th>
<th>Means</th>
<th>Std-deviation</th>
<th>Cronbach’s Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stakeholders involvement</td>
<td>2</td>
<td>75.39</td>
<td>14.76</td>
<td>0.8002</td>
</tr>
<tr>
<td>Systems view</td>
<td>8</td>
<td>45.89</td>
<td>4.67</td>
<td>0.9001</td>
</tr>
<tr>
<td>Evolving mindset</td>
<td>4</td>
<td>59.56</td>
<td>7.87</td>
<td>0.8395</td>
</tr>
<tr>
<td>Understanding transition</td>
<td>3</td>
<td>64.56</td>
<td>9.87</td>
<td>0.8279</td>
</tr>
<tr>
<td>System design</td>
<td>8</td>
<td>80.70</td>
<td>14.87</td>
<td>0.8007</td>
</tr>
<tr>
<td>System evaluation</td>
<td>2</td>
<td>80.66</td>
<td>14.89</td>
<td>0.8021</td>
</tr>
<tr>
<td>Academic transform</td>
<td>6</td>
<td>53.86</td>
<td>11.54</td>
<td>0.7910</td>
</tr>
<tr>
<td>Service and satisfaction</td>
<td>10</td>
<td>64.63</td>
<td>9.32</td>
<td>0.8153</td>
</tr>
<tr>
<td>Ownership control</td>
<td>10</td>
<td>62.83</td>
<td>11.28</td>
<td>0.8522</td>
</tr>
</tbody>
</table>

**Table 2**

**Reliability for each variable**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Final no. of items</th>
<th>Final internal reliability (Cronbach’s Alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evolving mindset</td>
<td>4</td>
<td>0.641</td>
</tr>
<tr>
<td>Academic transform</td>
<td>6</td>
<td>0.742</td>
</tr>
<tr>
<td>Understanding transition</td>
<td>3</td>
<td>0.652</td>
</tr>
<tr>
<td>System evaluation</td>
<td>2</td>
<td>0.704</td>
</tr>
<tr>
<td>Service and satisfaction</td>
<td>10</td>
<td>0.732</td>
</tr>
<tr>
<td>System view</td>
<td>8</td>
<td>0.712</td>
</tr>
<tr>
<td>Stakeholders involvement</td>
<td>2</td>
<td>0.668</td>
</tr>
<tr>
<td>Ownership control</td>
<td>10</td>
<td>0.790</td>
</tr>
<tr>
<td>System design</td>
<td>8</td>
<td>0.854</td>
</tr>
</tbody>
</table>
Number of Questionnaires Distributed, Returned and Usable

In order to capture the targeted sample size of 381 respondents, 550 survey questionnaires were distributed to private higher education institutions in Kuala Lumpur that have visions and missions on e-learning implementation. A total 493 were returned, representing a response rate of 89.6%. Out of the 493 returned, 487 were found to be usable (98.8%) and 6 questionnaires were rejected due to incomplete responses (1.21%). From this feedback, it was concluded that respondents were willing to give their cooperation in answering the survey questions at their convenience. This provides evidence that if a survey is monitored and administered properly, much information can be gathered from the respondents.

Means and Standard Deviation of Study Variables

All variables were measured on five points Likert type scale. The mean scores for all the variables range between 20.23 and 67.54. This indicates that change management variables and e-learning variables are in moderate level. The standard deviation scores range from 6.03 to 22.67 (Table 3).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stakeholders involvement</td>
<td>36.67</td>
<td>20.54</td>
</tr>
<tr>
<td>Systems view</td>
<td>46.22</td>
<td>19.53</td>
</tr>
<tr>
<td>Evolving mindset</td>
<td>33.47</td>
<td>8.24</td>
</tr>
<tr>
<td>Understanding transition</td>
<td>26.98</td>
<td>17.24</td>
</tr>
<tr>
<td>System design</td>
<td>59.81</td>
<td>11.18</td>
</tr>
<tr>
<td>System evaluation</td>
<td>42.13</td>
<td>22.67</td>
</tr>
<tr>
<td>Academic transform</td>
<td>20.23</td>
<td>6.03</td>
</tr>
<tr>
<td>Service and satisfaction</td>
<td>52.19</td>
<td>16.28</td>
</tr>
<tr>
<td>Ownership control</td>
<td>67.54</td>
<td>22.11</td>
</tr>
</tbody>
</table>

Skewness and Kurtosis of Study Variables

All variables were measured on a five points Likert type scale. The mean scores for all the variables range between 20.23 and 67.54. This indicates that change management variables and e-learning variables are at a moderate level. The standard deviation scores range from 6.03 to 22.67. The normality distribution of the data, the skewness and kurtosis of each variable were examined. The critical value for both measures of normality has drawn the distribution. The skewness and kurtosis for the nine main variables of this study were examined. By applying the above criteria to the skewness values for each of the study variables, it is shown that none of the variables fall outside the more and less 2.58 range.
of skewness. Thus, the data for this study is normal with regards to skewness. Univariate skewness and univariate kurtosis values range from -0.501 to 0.062 and -0.402 to 0.564 respectively.

The relatively large value of Mardia’s normalized multivariate estimate kurtosis (23.623) shows evidence that the data are slightly not multivariate normal. In order to address the issue of multivariate non-normality, bootstrapping is conducted to assess the stability of parameter estimates and report them more accurately. Within the context of the Structural Equation Model, bootstrapping provides a mechanism for addressing situations where the statistical assumptions of large samples and multivariate normality may not hold (Boon, 2003). In this study the Bollen-Stein bootstrap procedure (Bollen & Scott, 1993) was employed. It is a modified bootstrap method for the $\chi^2$ goodness of fit statistic which provides means to test if the specified model is correct. In particular, it can be used to correct for the standard error and fit statistic bias that occurs due to non-normal data. It tests the adequacy of the hypothesized model based on the transformation of the sample data such that the model is made to fit the data perfectly.

In this study, 1000 bootstrap samples were drawn with replacement from this transformed sample. The Bollen-Stein bootstrap p-value is 0.356 (> .05) indicating that there is sufficient evidence to reject the hypothesized model. Considering the feasibility and statistical significance of all parameter estimates, the substantially good fit of the final model and the lack of any substantial evidence of model misfit, the author concludes that the nine dimensions (ownership control, academic transform, service and satisfaction, stakeholders involvement, system view, evolving mindset, understanding transition, system design and system evaluation) can represent an adequate description of educators’ perspectives of change management due to e-learning implementation in private higher education institutions. The Cronbach’s alpha was computed on each of the Likert scale items that were factor loaded into the nine factors. The internal consistency reliability scores ranged from .641 to .854 after removing some items with low corrected item-total correlations value. Reliability is also an indicator of convergent validity (Hair et al., 2006). According to Hair et al., (2006) coefficient alpha is generally an internal measure of reliability as in most practical cases it is only the lower bound on reliability.

**Correlation Analysis**

The present researcher would like to clarify that the main objective of exploratory factor analysis is data reduction and exploration of the factors loaded in the present study. As mentioned earlier Exploratory Factor Analysis was carried out, using the Varimax Orthogonal. The results in forms of rotation were almost identical. However, the orthogonal rotation has the strong likelihood that correlated factors and theoretically justified. Thus, Orthogonal Varimax Factor Analyses are used for further analysis.
To identify the underlying dimensions of independent variables (ownership control, academic transform, and service and satisfaction) the principal component factor analysis with orthogonal rotation was conducted. A total of nine constructs (60 items) namely stakeholders’ involvement, system view, evolving mindset, understanding transition, system design, system evaluation, ownership control, academic transform, service and satisfaction were the factors that analysed to identify the number of dimensions derived. As suggested by Aaker (1971), factors with eigenvalues greater than 1.00 were retained.

Besides that, an exploratory factor analysis (EFA) was performed to reduce the large number of variables (items) to a smaller set of underlying factors that summarize the essential information contained in the variables. The detailed explanation of the analysis and its interpretation are presented in the following section. To determine the underlying factors, principal axis analysis was employed as an indicative test to determine if the 53 items were tapping onto the same construct. The nine factors have eigenvalues greater than 1.0 as referred to appendix P. To ensure that only very significant loadings are considered the variables for a factor are selected only when the absolute size of their factor loadings is 0.5 or more (Hair et al., 2006). The Bartlett’s test of sphericity was significant (Approx. $x^2 = 21265.198$, d.f = 2145, p=0.00), Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.896, far greater than 0.6 which is acceptable and conformed to the multivariate normality of the data as refer to appendix O. An inspection of the anti-image correlation matrix revealed that all the measures of sampling adequacy were well above the acceptable level of 0.5.

The communalities of the items range from 0.327 to 0.753 and these were acceptable. A communality represents the variance in that variable accounted for all the factors and was calculated by summing the squared of all factors loadings for a variable. Low communality indicates that the factor model was not effective and the variable should be omitted from the model. On the other hand, low communalities across the set of variables indicated that the variables were weakly related to each other. Usually, a communality of 0.75 was considered high and a communality of 0.25 was considered low. However, it was vital that communalities were construed with the interpretability of the factors. A communality value greater than one signals cause spurious solution due to insufficient sample size or the number of factors was either big or small. The principal components analysis extracted nine factors having eigenvalues greater than 1.0. The nine factors accounted for 54.4% of total variance. Factor 1 was loaded with four items from evolving mindset scales (EM_26_1, EM_26_2, EM_26_3, EM_26_4), explaining 14.3% of the variance. Factor 2 comprised six loaded items from academic transform scales (AT_26_5, AT_26_6, AT_27_4, AT_27_5, AT_27_6, AT_27_7) explaining 9.3% of the variance. Factor 3 comprised three loaded items from understanding transition (UT_27_1, UT_27_2, UT_27_3) explaining 5.6% of variance. Factor 4 from system evaluation (SE_28_1 and
SE_28_2) explaining 2.9% and factor 8 comprised 2 items from stakeholders’ involvement (SI_30_1 and RECODE_SI_30_3) and 4.3% of total variance. RECODE_SI_30_3 was reversed scored for further analysis to increase the alpha value in the range of 0.508 to 0.802. Factor 5 and 7 comprised 10 items explaining 4.4% and 5.6% of total variance. Factor 5 was from service and satisfaction scale (SS_28_2, SS_28_4, SS_31_2, SS_31_5, SS_31_6, SS_31_7, SS_31_12, SS_31_13, SS_31_14) and factor 7 was from ownership and control scales (OC_29_4, OC_29_5, OC_29_6, OC_29_7, OC_29_10, OC_29_11, OC_29_13, OC_29_16, OC_30_2, OC_30_4). Factor 6 and 9 were comprised 8 items explaining 4.6% and 2.9% of total variance respectively. Factor 6 comprised of system view (SV_29_1, SV_29_2, SV_29_3, SV_29_8, SV_29_9, SV_29_12, SV_29_14, SV_29_15) and factor 9 comprise of system design (SD_31_1, SD_31_3, SD_31_4, SD_31_8, SD_31_11, SD_31_4, SD_31_15). The results show that educators need to focus on these nine dimensions to influence change management due to e-learning implementation. For further analysis, only these nine dimensions were used on the reliability and the number of items loaded.

Change Management Variables Influencing E-Learning Implementation

This section reports on the findings that address on independent variables (ownership control, academic transform, and service and satisfaction) most influences the dependent variables of stakeholders’ involvement, system view, evolving mindset, understanding transition, system design, and system evaluation. For the purpose of that stepwise multiple regressions were performed. In order to determine which variables contributed to independent variables were regressed against dependent variables. Table 4 displays the summary of Stepwise Multiple Regression analysis for independent variables that were predicted to contribute to the dependent variables.

According to the analysis, there are change management variables that influence e-learning implementations. For stakeholders involvement, with all variables entered into the equation, OC yield an adjusted $R^2$ of 0.074 (F (1, 486) = 31.54, $p<0.005$). AT produced an adjusted $R^2$ of 0.096 (F (2, 486) = 18.78, $p<0.005$). No other variables entered the equation. For stakeholders’ involvement, OC was the primary predictor accounting 7.4% of the variance. Other independent variable did not achieve significance. For system view, with all variables entered into the equation, OC yield an adjusted $R^2$ of 0.058 (F (1, 486) = 15.24, $p<0.005$). AT produced an adjusted $R^2$ of 0.063 (F (2, 486) = 9.48, $p<0.005$). No other variables entered the equation. For system view, OC was the primary predictor accounting 5.8% of the variance. Other independent variable did not achieve significance.

For evolving mindset, with all variables entered into the equation, AT yield an adjusted $R^2$ of .217 (F (1, 486) = 9.23, $p<0.005$). SS produced an adjusted $R^2$ of 0.184 (F (2, 486) = 12.53, $p<0.005$). No other variables entered the equation. For evolving mindset, AT was
the primary predictor accounting 2.17% of the variance. Other independent variable did not achieve significance. For understanding transition, with all variables entered into the equation, AT yield an adjusted $R^2$ of 0.013 ($F$ (1, 486) = 10.24, $p<0.005$). SS produced an adjusted $R^2$ of 0.146 ($F$ (2, 486) = 9.21, $p<0.005$). No other variables entered the equation. For system view, AT was the primary predictor accounting 1.3% of the variance. Other independent variable did not achieve significance.

For system design, with all variables entered into the equation, SS yield an adjusted $R^2$ of 0.321 ($F$ (1, 486) = 9.64, $p<0.005$). AT produced an adjusted $R^2$ of 0.205 ($F$ (2, 486) = 16.45, $p<0.005$). No other variables entered the equation. For system design, SS was the primary predictor accounting 32.1% of the variance. Other independent variable did not achieve significance. For system evaluation, with all variables entered into the equation, SS yield an adjusted $R^2$ of 0.182 ($F$ (1, 486) = 12.63, $p<0.005$). AT produced an adjusted $R^2$ of 0.224 ($F$ (2, 486) = 9.32, $p<0.005$). No other variables entered the equation. For system evaluation, SS was the primary predictor accounting 18.2% of the variance. Other independent variable did not achieve significance. Thus, there are some findings in response to the research question on identifying the independent variables that significantly

\begin{table}[h]
\centering
\caption{Summary of Stepwise Multiple regression analysis for variables predicting dependent variables}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
IV & Model Summary & ANOVA & Coefficients \\
& & $R^2$ & Adjusted $R^2$ & F-value & p & Beta & t & p \\
\hline
\hline
(Stakeholders’ involvement) & \hline
OC & 0.078 & 0.074 & 31.54 & 0.000* & 0.257 & 5.02 & 0.000* \\
OC+AT & 0.102 & 0.096 & 18.78 & 0.000* & 0.127 & 2.47 & 0.002* \\
\hline
(System view) & \hline
OC & 0.064 & 0.058 & 15.24 & 0.000* & 0.632 & 3.63 & 0.000* \\
OC+AT & 0.074 & 0.063 & 9.48 & 0.000* & 0.452 & 2.52 & 0.003* \\
\hline
(Evolving mindset) & \hline
AT & 0.234 & 0.217 & 9.23 & 0.000* & 0.342 & 3.63 & 0.001* \\
AT+SS & 0.192 & 0.184 & 12.53 & 0.000* & 0.213 & 3.51 & 0.000* \\
\hline
(understanding transition) & \hline
AT & 0.022 & 0.013 & 10.24 & 0.000* & 0.234 & 3.52 & 0.003* \\
AT+SS & 0.159 & 0.146 & 9.21 & 0.000* & 0.523 & 4.12 & 0.000* \\
\hline
(System design) & \hline
SS & 0.324 & 0.321 & 9.64 & 0.000* & 0.654 & 2.34 & 0.000* \\
SS+AT & 0.214 & 0.205 & 16.45 & 0.000* & 0.353 & 2.64 & 0.001* \\
\hline
(System evaluation) & \hline
SS & 0.188 & 0.182 & 12.63 & 0.000* & 0.742 & 3.46 & 0.000* \\
SS+AT & 0.236 & 0.224 & 9.32 & 0.000* & 0.453 & 2.32 & 0.000* \\
\hline
\end{tabular}
\end{table}

*significant mean effect
contributed to e-learning implementation. Therefore, the null hypothesis is rejected. In general, it could be concluded that the contribution of independent variables decreases from OC and SS between 31.54% to 9.23%.

**CONCLUSION**

The findings revealed that there were change management variables that influenced the e-learning implementation. It was revealed that in general, it could be concluded that the ownership control most influenced stakeholders’ involvement. The finding also showed that ownership control was also a predictor for the system view. The findings were consistent with related studies done by Ding and Wermers (2012), that system view had a significant effect on the system of governance and ownership control. With regards to the factor of change such as stakeholders involvement reacts as crucial factors for ownership control (Garrison, 2011). Furthermore, this finding also disclosed that the e-learning variables that most influenced evolving mindset and understanding transition, were only academic transform, service and satisfaction. Academic transform was the primary predictors for both evolving mindset, and the system view. A related study conducted by Suktrisul (2004) examined the phenomenon of resistance to change. They argued that changes were dependent on peer groups, their values, and patterns of behavior typical of people’s attitude towards academic transforms service and satisfaction. A similar study done by Sirinaruemir (2004) who discussed service and satisfaction was needed to understand transition especially in a group of people who work in same job scope. It was to comprehend that progress in change management is measured by the time taken by small teams led by a process facilitator (Caine & Jenlink, 1997). Therefore, the researcher concluded that in order to have high relevance of evolving mindset and understanding transition for e-learning implementation, academic transforms, service and satisfaction were important factors. The key efforts of the process teams are to cultivate an in-depth understanding of the change management, develop individual thinking and support the private higher education community to progress through dialogue, design, and active participation to help implement an ideal educational system.

This study showed that e-learning variables that most influenced system design and system evaluation, with all variables entered into the equation were only service and satisfaction and academic transform. Service and satisfaction was the primary predictor for system design, and system evaluation. These findings were consistent with related studies done by Wang (2011). The study found that system design and system evaluation proposed in developing a Web-Based assessment system, the Peer-Driven Assessment Module as a way to compromise on service and satisfaction to evaluate the system. Some scholars also highlighted the use and usability of educational design patterns for designing and evaluating the system, and enhance service and satisfaction as part of an e-learning framework approach (Derntl & Calvo, 2011).
As a concluding remark, the researcher would like to state that change management due to e-learning from the perspective of educators needed concerted efforts and support from stakeholders, educators and the management team of the institution itself. The fullest cooperation and support from the university administration, all concerned departments, and continuous training to update lecturers on e-learning skills can help in improving the change management adaptation and assist in enhancing the e-learning utilisation among educators in private higher education institutions that have vision and mission on e-learning implementation.

REFERENCES


The Execution of the Green Building Project in Klang Valley, Malaysia: A Pilot Study

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ABSTRACT

Execution of the green building project specifically among the housing developers in Malaysia is still at its infancy. As of August 2016, there are only 16 housing projects certified by the Green Building Index (GBI) under the category of Residential New Construction (RNC) since the assessment systems established in 2010. The authors address this issue in the context of the factors affecting the execution of the green building project among the housing developers in Klang Valley. A pilot study by using questionnaire survey was conducted among 30 housing developers from 10 different zones divided by the Ministry of Housing and Local Government, Malaysia. This paper aimed to report the results of the reliability test of the designed questionnaire. The results based on the Cronbach’s Alpha analysis showed that the items used in the questionnaire were reliable and obtained an acceptable level of internal consistency, which were relevant for the main study. The pilot study revealed that only 9 out of 30 respondents had the completed or ongoing green building projects. The findings exposed the level of knowledge, emotion, value, attitude, behavior and the potential factors affecting the execution of the green building projects among the housing developers in Klang Valley.

Keywords: Execution, green building, housing developers, Malaysia

INTRODUCTION

Green building is a building that exhibits energy efficiency, resource depletion and protection of health and the environment. Green building has proven in contributing toward sustainability as it can reduce 30% to
80% of carbon dioxide (CO₂) emission due to energy efficient consumption (Lutzkendorf & Lorenz, 2006). Rapid urban development in Malaysia has contributed to the increasing of CO₂ emission. The future trend in residential energy consumption and carbon emission in 2020 will be 4397 kilotons of oil equivalent (ktoe) with the CO₂ emission of 11,689,308 tonnes (Ministry of Energy, Green Technology and Water [MEGTW], 2011). Housing is one of the fastest growth sectors in Malaysia due to the rapid increase in population and increasing demand of houses. Most housing developers in Malaysia had their main offices located in Klang Valley, in which a main metropolitan area of Malaysia (Isa et al., 2018). The green housing development is the solution to fulfill the housing demand, while, protecting the environment. The fact is, currently, the green housing projects are not a popular choice among the housing developers of the country. As of August 2016, there are only 16 housing projects certified by the Green Building Index (GBI) under the category of Residential New Construction (RNC) since the assessment systems established in 2010. This fact showed that the number of the green housing projects was at a low level compared to a total of 818 housing projects delivered in Klang Valley since 2010 (Ministry of Housing and Local Government [MHLG], 2016). Even though Klang Valley had the highest number of registered green projects (GBI, 2017; Isa et al., 2018), however more than 50% of them had less than five years work experience on the green housing projects (Isa et al., 2018).

This study dedicated for testing the internal consistency of the proposed items and reliability of the design instrument, was a set of questionnaire for the main study. The authors aim to contribute to the theory and practice in the development of the green housing project through identifying the factors affecting the execution of the green building project among the housing developers. The authors provide an extended review of the literature on the factors affecting the green housing project execution based on the pro-environmental behavior (PEB) theories and provide analytical framework that views research from the perspective of the housing developers in order to provide basis for further research and practice on housing developers’ capabilities.

**Pro-Environmental Behaviour Theories**

A number of studies revealed that the PEB theories as a tool to influence change and behavior to a more sustainable manner. PEB is defined to minimize the negative impact of one’s action on the nature. Several internal and external factors were highlighted as significant antecedents of PEB as follows:

**Internal Factors.** The variables of internal attribution of PEB are knowledge, emotion, value and attitude (Kollmuss & Agyeman, 2002). What people know about the environment, how they feel about it, and what action they take that may help or harm the environment are critical for establishing a sustainable community. The old model of PEB emphasizes
that a linear progression of environmental knowledge leading to environmental awareness and attitudes which lead to pro-environmental behavior. The knowledge of green building and the benefit gained from the development of the project are crucial for the developers as a starting point to change their attitude, value and behavior towards executing the green project. The industry begins to realize that green buildings benefit on the functionality, flexibility, accessibility, as well as economic, environmental and productivity performances (Isa et al., 2014a). By having more knowledge concerning the green building and environmental protection, it will increase the implementation of the green building in the industry.

Emotion is another internal factor of the green building implementation among developers. Emotion is often intertwined with mood, temperature, personality, disposition, and motivations. It is also linked to behavioral tendency and driving the force behind motivation, positive or negatives. Ekman (1992) stated, there were six basic emotion measurements, which were anger, disgust, fear, happiness, sadness and surprise. Emotional involvement is very important in shaping human beliefs, values, and attitudes towards the environment (Kollmuss & Agyeman, 2002). In the meantime, values are responsible for shaping a human intrinsic motivation. The environmental values are based on one’s life experience that has shaped the beliefs and value of active environmentalists. Schwartz (2012) stated that values had a positive influence on environmental behavior in openness to change and universalism. Meanwhile, attitude is the enduring positive or negative feeling about some person, object or issue. Attitude can directly influence the developers’ behavior together with their beliefs and values concerning the benefits of the green building practices. Attitude is the predictors of behavior and behavioral intentions (Ajzen, 1991). The knowledge of green building may affect the developers’ emotional, value, and attitude towards the implementation of the project. It may also encourage developers to value the importance of the implementation of the green building project.

**External Factors.** The external factors of PEB consist of institutional or political, economic, social and cultural aspects. The enforcement of the green building policies and regulation will improve the housing developers’ involvement in the green building projects. Promoting of the green building product is a part of support from the government in creating a capable and viable local construction sector. There are many efforts by the government and the professional bodies to encourage the development of the green building among developers in Malaysia (Isa et al., 2014a). The government has provided tax exemption equivalent to 100% of the additional capital expenditure to get the GBI certificate and Green Technology Financing Scheme, which are worth of RM1.5 billion as a part of soft loan to use green technology and materials for the developers and owners (Elias & Lin, 2015).
Social and culture play a very important role in shaping a person’s behavior. The provision of proper education concerning the economically viable of a green building development for the developers and buyers will improve the interest of execution and demand of the green project. The government and private sectors and public engagement are important in leading the green building market. Education and public awareness of the green building are essential to drive clients demand, tenants’ satisfaction, and reduce the environmental problems by generating a sense of social responsibility (Albahori et al., 2017; Safee et al., 2015). Economical factors have a strong influence on a person’s decision and behavior (Kollmuss & Agyeman, 2002). Developers and buyers are affected by the economic incentives to behave environmentally. They intent to choose green building if the payback time for the energy saved is reasonable (Albahori et al., 2017).

Based on the literature review discussed above, the internal and external factors of the green building execution were identified. A questionnaire was designed according to the objectives of identifying the factors affecting the execution of the green building development among housing developers. Then, this paper identified the reliability of the questionnaire used during the pilot study to confirm the usefulness of the research tool for the main study.

**METHODS**

The sampling method used for this study was non-probability convenience sampling. This method was selected because it was convenient and often used during preliminary research efforts to get a gross estimate of the results, without incurring cost or time required to select a random sample. This method was significant for a pilot study as it was highly recommended by many scholars and researchers when the time was short and where the information was needed fast (Sekaran, 2000). Sample size calculations are not required for some pilot studies as long as they are enough to provide useful information about the aspects that are being assessed for feasibility (Thabane et al. 2010). For this research, the pilot study was done involving a total of 30 respondents. The respondents were randomly picked based on the list of developers obtained from the MHLG (MHLG, 2016). The developers were located in 10 different zones of housing projects in Klang Valley. Three (3) committed respondents were selected to involve in the survey representing the developers of each zone.

One set of structured questionnaire survey questions was designed for the piloting purposes. The questionnaire was divided into seven sections. Section A involves 11 questions that cover gender, working position, level of education, working experience and their involvement in green building project. Based on the Likert scale, the respondents were required to choose within the scale of agreement (i.e. 1= strongly disagree, 2= disagree, 3= neither agree nor disagree, 4= agree, and 5= strongly agree, for question 1 (section B),...
question 4 (section C), question 6 (section D), question 8 and 9 (section E)). Meanwhile, question 2 (section B) is based on the scale of emotion (i.e. 1=angry, 2=fear, 3=tender, 4=happy, and 5=excited). Question 3 (section C) is based on a scale of importance (i.e. 1=not important at all, 2=not important, 3=neutral, 4=important, 5=very important). The other two questions are about the implementation of the green building principles based on knowledge, emotion, value, and attitude, political, economic, social and cultural factors. The final part of this questionnaire asks the respondents to give their opinion and suggestion on four open-ended questions. The first and second column of Table 1 shows the distribution of the items according to the variables.

Analysis of the data was undertaken using Statistical Package for the Social Sciences (SPSS). Cronbach’s Alpha analysis was used to measure the internal consistency of the items used for the questionnaire and indicates how well the items in the set were correlated to one another. For the purpose of describing and indicating the weighting value of each item, the data was analyzed using descriptive statistic and Relative Importance Index (RII) methods. The mean score was divided into three levels: high (0.00-0.249), moderate (2.50-3.49) and low (3.50-5.00) as suggested by Chua (2012). In addition, by using the mean values, the resulted RII value was transformed into three important levels: high (0.8≤RII≤1), medium (0.5≤RII≤0.8) and low (0≤RII≤0.5) (Tam et al, 2007). The RII ranges are from zero to one and the factors will be ranked based on the biggest value (Isa et al., 2014b).

RESULTS AND DISCUSSION

The following subsections discuss the analysis results on the factors affecting the execution of the green building project among the housing developers. The respondents were dominated by males, which is (73.3%) than females (26.7%). The disproportionate of genders showed that males tend to work in the construction industry of the country. Majority of the respondents (46.7%) were from the engineering background, 30% from the architecture and the rest was from the building surveying or other background. About 77% of the respondents had less than five years’ experience in green building projects, 20% had six to ten years of experience in green building projects, while the rest of the respondents (3%) had experience of eleven to fifteen years in green building projects. Minority of the respondents (9 out of 30) had several ongoing green building projects. Table 1 summarizes the results with specific factors categorized into three aspects, internal and external factors and the barriers, their reliability test, mean score and weight within and across the categories.

Reliability Test

The results indicate that the items of the internal factors and external factors of the green building execution were reliable and consistent with the Cronbach’s Alpha value of,
0.951 and 0.966 respectively (refer Table 1). In details, the Alpha reliability scale of the internal factors was 0.938 for the developers’ knowledge of the green building principles, 0.674 for their emotion towards execution of the green principles, 0.925 for their value and 0.927 for the developers’ attitude on the execution of the green principles for their projects. Meanwhile, the values of Cronbach’s Alpha for the external factors are 0.940 for the political factors on affecting the developers’ decision of executing green building projects, 0.926 for the social and cultural factors, and 0.913 for the economic factors of the green building execution among them. The overall coefficient values of Cronbach’s Alpha are above 0.9, which is 0.962. This indicates the variables had excellent internal consistency and achieved high reliability values. There was only one variables achieved $0.6 \leq \alpha < 0.7$, however it is still acceptable as the unacceptable value of Cronbach’s Alpha is $<0.5$ (George & Mallery, 2003). Thus, the findings of the pilot study showed that the items used for the questionnaire were reliable and obtained an acceptable level of internal consistency. The authors conclude that the instrument was reliable and understandable. Each of the items would remain in the existing set of dimensions based on the literature review.

### Table 1

<table>
<thead>
<tr>
<th>Factors</th>
<th>No. of items</th>
<th>Cronbach’s Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Developers knowledge of the green building principles</td>
<td>24</td>
<td>0.938</td>
</tr>
<tr>
<td>2. Developers emotion on implementing the green building principles</td>
<td>18</td>
<td>0.674</td>
</tr>
<tr>
<td>3. Developers value on green building principles</td>
<td>18</td>
<td>0.925</td>
</tr>
<tr>
<td>4. Developers attitude on executing green building principles</td>
<td>18</td>
<td>0.927</td>
</tr>
<tr>
<td>External Factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Political factors affecting the green building implementation</td>
<td>17</td>
<td>0.940</td>
</tr>
<tr>
<td>6. Social and cultural factors affecting the green building implementation</td>
<td>19</td>
<td>0.926</td>
</tr>
<tr>
<td>7. Economic factors affecting the green building implementation</td>
<td>12</td>
<td>0.913</td>
</tr>
<tr>
<td>OVERALL</td>
<td>126</td>
<td>0.962</td>
</tr>
</tbody>
</table>

### Descriptive Analysis and Establishing the Weight for the Factors

The results as illustrated in Table 2 revealed that most of the respondents agreed with 24 items of the principles of a green building as listed in the questionnaire with the mean score of 3.81. Other than acceptance of the items, the results also showed that the level of the developers’ knowledge concerning the green building project was at a high level. Meanwhile, the level of the developers’ positive emotion, value and attitude concerning the execution of the green building project were also high with the mean values of 3.68, 3.90 and 3.96 respectively.
The Execution of the Green Building Project in Klang Valley, Malaysia

Table 2
Descriptive and relative importance analysis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Factors affecting the execution of the green building development among housing developers (The items)</th>
<th>No. of items</th>
<th>Mean Score</th>
<th>Weight (RII)</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal factors</td>
<td>Developers knowledge of green building principles</td>
<td>24</td>
<td>3.81</td>
<td>0.762</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Developers emotion on implementing the green building principles</td>
<td>18</td>
<td>3.68</td>
<td>0.736</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Developers value on green building principles</td>
<td>18</td>
<td>3.90</td>
<td>0.780</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Developers attitude on executing green building principles</td>
<td>18</td>
<td>3.96</td>
<td>0.792</td>
<td>1</td>
</tr>
<tr>
<td>External Factors</td>
<td>Political factors affecting the green building implementation</td>
<td>17</td>
<td>3.82</td>
<td>0.764</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Social and cultural factors affecting the green building implementation</td>
<td>19</td>
<td>3.83</td>
<td>0.766</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Economic factors affecting the green building implementation</td>
<td>12</td>
<td>3.75</td>
<td>0.750</td>
<td>6</td>
</tr>
</tbody>
</table>

Mean Score: 0.00-2.49 = low; 2.50-3.49 = moderate; 3.50-5.00 = high

It shows that most of the developers were happy to incorporate the green building principles for their future projects. They also valued the 24 listed items as important for delivering a green building and they were willing to execute the green principles into their future building projects. The findings of this study initially contradicted the old linear progression model of PEB, which claimed that environmental knowledge, awareness and concern lead to the pro-environmental behavior. The fact was, only 9 out of 30 respondents had the completed or ongoing green building projects. In term of the external factors, the results showed that most of the respondents highly agreed with all those 48 items of the factors affecting execution of the green building project among the housing developers in Malaysia. The factors grouped into the aspects of political, social and economic as listed in the questionnaire with the mean score of 3.82, 3.83 and 3.75 respectively.

The RII values of the internal factors group are within the range of 0.792 to 0.736 and the external factors are within the range of 0.766 and 0.750, which are above 0.8 representing a high level of importance.

CONCLUSION

This paper has revealed the results of a pilot study for testing the consistency of the items and reliability of the instrument. The results showed that the instrument was reliable for the main study. This paper also identifies the potential factors affecting the developers’ decision on choosing the green building development for their housing projects. The results showed that the level of the developers’ knowledge, positive emotion, value and attitude concerning the execution of the green building project were high and they were happy to incorporate...
the green building principles for their future projects. Surprisingly, the execution of the green building project among the housing developers were still limited. To understand the situation, the main study of this research should be answering an important question; What are the exact factors affecting the green building project execution among the developers? In conclusion, the green building gives enormous impacts towards the sustainability of building, environment and human well-being. Developers should play an important role for the sustainability through the housing project execution.

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REFERENCES


Quality of Life among Patients with Brain Pathology in a Malaysian Hospital

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ABSTRACT

The objective of this study was to determine the prevalence of symptoms and problems in Malaysian brain pathology patients. A total of 100 respondents in Kuala Lumpur Hospital were included in this cross-sectional study. The study utilized European Organization for Research and Treatment of Cancer Quality Of Life questionnaire (EORTC QLQ-C30). Patient with a “symptom/problem” having the minimum response of “a little”. A response of “quite a bit” was defined as having a “severe symptom/problem”. The two most prevalent “symptoms/problems” among the neurological disorder patients were fatigue (65%; severe: 28%) and reduced cognitive functioning (64%; severe: 25%). The mean number of “symptoms/problems” ranged from 4.63 (meningioma) to 6.80 (cerebellar edema) while the mean number of “severe symptoms/problems” ranged from 1.39 (astrocytic glioma) to 2.8 (cerebellar edema). Therefore special attention should be given to these patients in order to improve the overall quality of life of the patients.

Keywords: Brain pathology, neurological disorder, quality of life, severity, symptomatology
INTRODUCTION

The cancer incidence is 3.3 per 100,000 populations among brain and other nervous system disorders. This consists of male 3.6 and female 3.1 per 100,000 population in peninsular Malaysia (Omar et al., 2006). There are various forms of brain cancer which include malignant gliomas (34.6%), followed by medulloblastoma 11.3% and meningothelial tumours (3.1%) of all nervous system tumours in the year 2003 till 2005 in Peninsular Malaysia (Lim et al., 2008).

The main aim of brain tumour management was on tumour removal while the physician role was to develop state free of this disease. The quality of life of patients with brain disorder is often neglected and it leads to the deterioration of physical, emotional, and social functioning (Kim et al., 2016). The cancer patients appear to have poor quality of life even prior to their high dose chemotherapy treatment (Larsen et al., 2003). Study also reported that the cancer patients with various types of diagnoses showed variation in ‘symptoms/problems’ which impacted on their quality of life (Johnsen et al., 2009a; Johnsen et al., 2009b). Moreover the patients with metastasis stage found to have weak physical, psychosocial and poor quality of life (Yen et al., 2006). Therefore special attention should be given to the patient with poor quality of life to improve their overall curability of the disease.

A great deal of literature has been published from western data on other cancer patients (Brown et al., 2009; Costanzo et al., 2006; Distefano et al., 2008; Dodd et al., 2010; Mehnert et al., 2009; Mystakidou et al., 2005; Okamura et al., 2002; Oozeer et al., 2006; Reid-Arndt et al., 2009; Reimer et al., 2006; Sampogna et al., 2009; Zenger et al., 2009). According to what we know so far, there are limited published articles concerning the quality of life specifically for Malaysians brain pathology patients. Thus, the main aim of this study was to evaluate the quality of life in the patients with brain pathology in a referral centre in Malaysia.

MATERIALS AND METHODS

The study was done at Hospital Kuala Lumpur (HKL), a referral centre for neurological disorder cases. All the patients who visits neurosurgery clinic between (April 2016 to December 2016) with neurological disorder during the sampling period and fulfilled the inclusion criteria were included in the study.

The study respondents were selected following these criteria. First, the respondent must be with brain pathology. Second, the age of participant must be at least 18 years. Second, the participants should be able to well-versed in Malay, English, Mandarin or Tamil. Finally, the participant must be conscious and able to respond to our questionnaire.

The EORTC-QOL-30 questionnaire has been pre-tested and validated. This disease-specific questionnaire is used to evaluate the quality of life of cancer patients. The
questionnaire comprised of four languages including English, Malay, Mandarin and Tamil, was used in the study (Aaronson et al., 1993; Mustapa and Yian, 2007). The Malay version had been validated among Malaysian cancer patients and it has internal consistencies for Global Health Status (0.91), Functional domains (0.50-0.89) and Symptoms domains (0.75-0.99) and the sensitivity of the scale found in all the domains (Na et al., 2014).

The participants were excluded from the study if the patient wanted to withdraw from the study, mentally disabled, not able to be interviewed or required immediate treatment.

This study used European Organization for Research and Treatment of Cancer Quality Of Life (EORTC QLQ-C30) likert scale format questionnaire, version 3.0. The consent was obtained from the patient before the questionnaires were distributed. Information such as socio-demographic profiles and clinical status were assessed from the patients and medical records.

All the scales ranged from 1 to 4 except for the global health status scale points with the scale from 1 to 7 (Aaronson et al., 1993). The questionnaire was divided into functioning scales, symptoms scales and global health status scale. The functioning scale comprised physical, role, cognitive, emotional and social domains. The symptoms scale consisted of fatigue, pain, nausea/vomiting, dyspnea, insomnia, appetite loss, constipation, diarrhea and financial difficulties. The linear transformation was done referring to the EORTC scoring manual and all scores ranged from 0 to 100. A higher score indicates better functioning and good global health statuses. Conversely higher score for the symptom scale indicates more symptoms among the patients (Fayers et al., 2001).

The questionnaire did not have any cut-off points or defined thresholds to interpret functioning and symptom score as a case. Thus the percentage of frequencies of “symptoms/problems” and the frequencies of “severe symptoms/problems” were computed by referring to previous study methodology. Patient with a symptom/problem having the minimum response of “a little” and had function scale scores ≤ 67 and symptoms scale scores ≥33. While a response of “quite a bit” was defined as having a “severe symptom/problem” had function scale scores ≤34 and symptoms scale scores ≥ 66. The explanation of this method is shown in Figure 1 (Johnsen et al., 2009b). The number of “symptom/problem” and “severe symptom/problem” answers were determined for each person ranging from 0 to 14. The global health status scale was removed from this method, thus leaving 14 scales in total.

Statistical Package for Social Sciences (SPSS) program version 22.0 was used to analyze the data in this study. Descriptive statistics including mean, percentages and ranges were used to describe the sample characteristics.

Ethical clearance approval was sought from Human Research Ethics Committee, Universiti Sains Malaysia (USM/JEPeM/16050178) and Medical Research & Ethics Committee (MREC) at the Ministry of Health (MOH) (NMRR-16-1134-29874 (IIR)).
RESULTS

The study had a response rate of 93.5%. The mean age of the respondents was 45.3 years (95% CI= 42.6, 47.9). Tables 1 and 2 shows the socio demographic profiles and clinical characteristics of the respondents respectively. Patients with brain pathology with majority of brain tumour patients were included in the study. The neurological disorders patients in the Table 2 defined as patients who was shown with brain tumour in the Magnetic resonance imaging (MRI) report but had not been confirmed in the biopsy results. The majority of the patients underwent surgery.

Table 1
Socio-demographic characteristics of neurological disorder respondents in HKL (n=100)

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<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>Percentage %</th>
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<tbody>
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<tr>
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Figure 1. Illustration of the definition of ‘symptom/problem’ and ‘severe symptom/problem’
Source: Johnsen et al., 2009b
### Table 1 (continue)

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<thead>
<tr>
<th>Characteristics</th>
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<th>Percentage %</th>
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<td>Buddhist</td>
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<tr>
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<tr>
<td>Government</td>
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<td>21.0</td>
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<tr>
<td>Non government</td>
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<tr>
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<tr>
<td>Not working</td>
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<td><strong>Total monthly income household (RM )</strong></td>
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<td>3001-6000</td>
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</tr>
<tr>
<td>6001-9000</td>
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<td>8.0</td>
</tr>
<tr>
<td>&gt;9001</td>
<td>4</td>
<td>4.0</td>
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<tr>
<td>others</td>
<td>22</td>
<td>22.0</td>
</tr>
</tbody>
</table>
Table 2
Clinical characteristic of brain disorder respondents in HKL

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>Percentage (%)</th>
</tr>
</thead>
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<tr>
<td>Year of diagnosis (n=92)</td>
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<tr>
<td>2015-2016</td>
<td>34</td>
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<tr>
<td>2013-2014</td>
<td>17</td>
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</tr>
<tr>
<td>2011-2012</td>
<td>11</td>
<td>11.0</td>
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<tr>
<td>2009-2010</td>
<td>6</td>
<td>6.0</td>
</tr>
<tr>
<td>2007-2008</td>
<td>7</td>
<td>7.0</td>
</tr>
<tr>
<td>2005-2006</td>
<td>3</td>
<td>3.0</td>
</tr>
<tr>
<td>&lt;2005</td>
<td>14</td>
<td>14.0</td>
</tr>
<tr>
<td>Neurological disorders (n=100)</td>
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<td></td>
</tr>
<tr>
<td>Astrocytic glioma</td>
<td>13</td>
<td>13.0</td>
</tr>
<tr>
<td>Meningioma</td>
<td>19</td>
<td>19.0</td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>15</td>
<td>15.0</td>
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<tr>
<td>Carvenoma</td>
<td>7</td>
<td>7.0</td>
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<tr>
<td>Schwanoma</td>
<td>5</td>
<td>5.0</td>
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<tr>
<td>Craniopharyngioma</td>
<td>3</td>
<td>3.0</td>
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<tr>
<td>Ethmoid</td>
<td>1</td>
<td>1.0</td>
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<tr>
<td>Frontal lobe tumour</td>
<td>1</td>
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<tr>
<td>Fibrosarcoma</td>
<td>1</td>
<td>1.0</td>
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<tr>
<td>Cerebellar edema</td>
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<tr>
<td>Germinoma</td>
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<tr>
<td>Hemorragic brain</td>
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<tr>
<td>Brain lesion</td>
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<td>Mucopyocele</td>
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<td>Radiotherapy group</td>
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</tbody>
</table>

Table 3 shows the mean score of five multi-item function scales. The most severe impairment in functioning was the cognitive functioning (mean score=61). The cut-off value, indicates that 63% of brain pathology patients had impairment in their cognitive functioning (severity=25%). Another leading symptom and problem among the patients was fatigue (severity=28%) which affected 65% of the patients.

Thirty-six percent of the patients had impairment in their physical functioning (severity=5%), followed 44% of the patients with impaired role functioning (severity=2%). Social functioning was impaired in 26% of patients (severity=9%). In the symptoms
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>PF Mean score</th>
<th>RF Mean score</th>
<th>EF Mean score</th>
<th>SF Mean score</th>
<th>CF Mean score</th>
<th>QL Mean score</th>
<th>FA Mean score</th>
<th>NV Mean score</th>
<th>PA Mean score</th>
<th>DY Mean score</th>
<th>SL Mean score</th>
<th>AP Mean score</th>
<th>CO Mean score</th>
<th>DI Mean score</th>
<th>FI Mean score</th>
<th>Mean No of %symptom</th>
<th>No of %severe symptom</th>
<th>No of %severe symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>74.2</td>
<td>78.33</td>
<td>67.08</td>
<td>61.16</td>
<td>82.33</td>
<td>59.92</td>
<td>44.89</td>
<td>11.67</td>
<td>29.33</td>
<td>10.00</td>
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<td>12.67</td>
<td>4.67</td>
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<td>(30.71)</td>
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<td>(22.02)</td>
<td>(31.10)</td>
<td>(23.98)</td>
<td>(30.16)</td>
<td>(21.97)</td>
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<td>(32.31)</td>
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<td>88.46</td>
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<td>(32.03)</td>
<td>(32.03)</td>
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<tr>
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<td>74.56</td>
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<td>63.16</td>
<td>39.77</td>
<td>6.14</td>
<td>31.58</td>
<td>8.77</td>
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<td>(12.49)</td>
<td>(36.99)</td>
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<td>78.89</td>
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<td>(39.79)</td>
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<td>(36.85)</td>
<td>(9.13)</td>
<td>(44.72)</td>
<td>(14.91)</td>
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<td>(44.72)</td>
<td>(29.81)</td>
<td>(0.00)</td>
<td>(43.36)</td>
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<td>(16.67)</td>
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<td>(16.67)</td>
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<td>(26.28)</td>
<td>(37.40)</td>
<td>(26.70)</td>
<td>(35.20)</td>
<td>(33.75)</td>
<td>(36.12)</td>
<td>(28.04)</td>
<td>(41.96)</td>
<td>(30.05)</td>
<td>(24.98)</td>
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<td>(44.52)</td>
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<td>15.8</td>
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<td>42.1</td>
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</tbody>
</table>

PF, Physical Functioning; RF, Role Functioning; EF, Emotional Functioning; CF, Cognitive Functioning; SF, Social Functioning; FA, Fatigue; NV, Nausea And Vomiting; PA, Pain; DY, Dysphoria; SL, Insomnia; AP, Appetite loss; CO, Constipation; DI, Diarrhoea; FI, Financial difficulties.

% symptoms, proportion of patients scoring 67 at the most for function scales or at least 33 for symptoms

% severe symptoms, proportion of patients scoring 34 at the most for function scales or at least 66 for symptoms.

No. of "symptoms", mean number of symptoms using the same cut-off point as in % "symptom" (quality of life excluded)

No. of "severe symptoms", mean number of symptoms using the same cut-off point as in % "severe symptom" (quality of life excluded)
counterparts, the most severe symptom was fatigue (mean score = 44.89), followed by financial difficulties (mean score = 34), insomnia (mean score = 29.67), pain (mean score = 29.33), appetite loss (mean score = 17), constipation (mean score = 12.67), nausea and vomiting (mean score = 11.67), dyspnea (mean score = 10) and diarrhea (mean score = 4.67). Using the cut-off values, 65% of the patients had fatigue (severity 28%), 51% had financial difficulties (severity = 30%), 47% had pain (severity = 20%), 43% had insomnia (severity = 29%), 26% had appetite loss (severity = 16%), 24% had constipation (severity = 10%), 15% had nausea and vomiting (severity = 7%), 20% had dyspnea (severity = 9%) and 12% had diarrhea (severity = 1%).

In the study, the mean number of “symptoms/problems” was 5.22 while the mean number of “severe symptoms/problems” was 2.10. Overall, the cerebellar edema patients had the most symptoms and problems with a mean number of 6.80. The mean number of “symptoms/problems” ranged from 4.63 (meningioma) to 6.80 (cerebellar edema); the mean number of “severe symptoms/problems” ranged from 1.39 (astrocytic glioma) to 2.8 (cerebellar edema). Most shockingly, the study found 90% of the patients had at least one “symptom/problem”.

DISCUSSION

The present study was aimed to determine the symptoms and problems affecting the quality of life of brain pathology patients. The study showed that the symptoms and problems were frequent among the patients. The cut-off value, indicates that 63% of the patients had impairment in cognitive functioning (severity = 25%). This finding is in agreement with previous case control studies, which found the patients with brain tumour had more cognitive impairment compared to healthy patients. The study not only demonstrated that the patients suffered more in cognitive impairment compared to healthy adults but the cognitive functioning worsened after the surgery (Zarghi, 2014). In the previous study among Malaysians with primary intracranial tumor, a small number of patients (n=38) was included in the analyses. The patients were identified with reduced cognitive functioning, emotional functioning and global health status before the surgery compared to after the surgery. The patients also tended to have more fatigue and pain symptoms before the surgery (Ooi & Mazlina, 2013).

In the current study also found the most severe symptom was fatigue which had the highest mean score followed by financial difficulties, insomnia and pain. This defined 65% of the patients had fatigue (severity = 28%), financial difficulties (51%; severity = 30%), pain (47; severity = 20) and insomnia (43%; severity = 29%). The current study found 90% of all brain pathology patients having at least one “symptoms/problems”. Similarly in a
study done by Johnsen et al (2009a) also reported that the cancer patients had higher mean scores on the symptom scale and experienced fatigue followed by pain and insomnia. Comparing the cancer patients in each domain, fatigue (55%), reduced role functioning (49%), insomnia (46%), pain (37%) and dyspnoea (36%) were identified in the study. The study found 82% of all cancer patients fulfilled the criteria of at least one ‘symptoms/problems’ in their quality of life. The functional scale of role and physical functioning were found to be severely impaired in the patients (Johnsen et al., 2009b). The patients with reduced global quality of life found to have poor physical, cognitive, social and role functioning and more appetite loss and financial difficulties (Kim et al., 2016). In another study it was shown the patients had elevated symptoms of fatigue, pain, sleep disturbance and depression and reported poor functional status and low quality of life (Dodd et al., 2010). The patients who were appeared to be more fearful of their disease relapse had much more reduced quality of life, specifically in their fatigue, financial difficulties, emotional functioning, and appetite domains (Franssen et al., 2009).

The cancer patients treatment modalities also lead to increased level of fatigue, poor appetite and physical functioning and reduced quality of life (Johnsen et al., 2009b). At the pre and post operative stage, the patients are mostly effected in their emotional functioning and dyspnea symptoms. The patients also having more symptoms of fatigue before the surgery (Kim et al., 2016). Therefore it is becoming increasingly difficult to ignore the patient’s poor quality of life and more attention needs to be given together with supportive care in order to improve the overall curability of the patients (Ernstmann et al., 2009).

CONCLUSION
The study proved that the patients with brain pathology in referral centre in Malaysia caused significant impairment in the quality of life. The fatigue and reduced cognitive functioning were two leading symptoms and problems among the patients. There is a significant improvement in cancer management, however the overall treatment will remain poor if medical personnel does not apply a balanced pharmacotherapy and psychotherapy management in order to improve the patients’ quality of life.

ACKNOWLEDGEMENT
This project was funded by University Sains Malaysia (USM) under the short term grant, 304/PPSP/6315007 and Priscilla Das is a holder of MyBrain15-MyPhd scholarship. We also would like to thank the staff of the Department of Neuroscience and the patients who participated in the study.
REFERENCES


Quality of Life among Patients with Brain Pathology


Development and Validation of a Stability Indicating UV-Spectrophotometric Assay Method for the Determination of Naratriptan Hydrochloride

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³College of Pharmacy, Prince Sattam bin abdul aziz University, Kharg, KSA
⁴IKGT university, Jalandhar, Punjab, India

ABSTRACT

The research in this paper discusses about the development and validation of a novel, cost effective, simple, reproducible and accurate UV-spectrophotometric method to estimate naratriptan hydrochloride in bulk as well as pharmaceutical formulations. Naratriptan hydrochloride was analyzed at 223 nm in simulated saliva (pH 6.8) and phosphate buffer (pH 7.4). Linearity was present in the concentration range of 1–40 μg/ml for both media. The method was validated as per different parameters according to International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines of Q2 (R1). The suggested method was successfully used for the analysis of naratriptan hydrochloride in-house oral film formulation as linearity values for the same were found to be 1–40μg/mL. The results revealed the suitability of the technique for the estimation of naratriptan hydrochloride in the oral films for its accuracy, precision, and reproducibility.

Keywords: ICH, Naratriptan hydrochloride, phosphate buffer, Q2 (R1), simulated saliva, UV-spectrophotometric
INTRODUCTION

2-[3-(1-Methyl-piperidin-4-yl)-1H-indol-5-yl]-ethanesulphonic acid methylamide hydrochloride or also known as Naratriptan hydrochloride is a serotonin 5-HT_{1B/1D} receptor agonist. The chemical structure of naratriptan hydrochloride is as per shown in Figure 1.

![Chemical structure of naratriptan hydrochloride](image)

It is a serotonin 5-HT_{1B/1D} receptor agonist and used for the treatment of acute migraine. Activation of 5-HT_{1B} receptor, which is present on the vascular smooth muscles, causes vasoconstriction of smooth muscles (Longmore, et al., 1997). 5-HT_{1D} receptors are located on the sensory trigeminal terminals. Activation of these receptors inhibits the release of sensory and vasoactive neuropeptides as well as vasodilator transmitters (Reuter et al., 2002; Saxena et al., 2018).

Acute migraine is a disabling disorder characterized by moderate to severe throbbing and pulsating pain often associated with phonophobia, photophobia, nausea, and vomiting. It worsens with day to day activity of patient (Goadsby, 2003; Vecchia & Pietrobon, 2012). An untreated migraine attack can last for 4 to 72 hours (Tepper & Spears, 2009; Skaer, 2018). Migraine can also cause sexual dimorphism i.e. prevalence ratio among men and women is 3:7 (Estemalik & Tepper, 2013) (Dhillon et al., 2011).

The suggested analytical method will be beneficial since few have been reported in the literature for the proposed drug. This method analyzes the drug sample by using UV spectrophotometer. The observation of analysis and the method validation data confirms the reproducibility of the method. The novelty of this work lies on the broad linearity and lower standard deviation values as compared to previous work done (Sreelakshmi et al., 2013; Borse & Shirkhedkar, 2012; Shelke, 2015).

MATERIALS AND METHODS

Materials and Reagents

Naratriptan hydrochloride was obtained as a gift sample from Apotex Pharmachem Pvt. Ltd., India. The oral film formulations contain 5 mg of proposed drug per film and was...
formulated in-house. The polymers used were hydroxyl propyl methyl cellulose (HPMC) E5 and propylene glycol in the formulation along with other excipients obtained from SD-Fine Chemicals, India. All other chemicals such as dibasic sodium phosphate, disodium hydrogen phosphate, monobasic sodium phosphate, sodium chloride and HCl used in the method were of Analytical Grade (AR) obtained from LOBA Chemie, India.

**Instruments**

A double beam UV-visible spectrophotometer (U-2900/U-2910 Shimadzu Co. Ltd., Japan) provided with UV-Probe software was used. For intermediate precision studies, another UV-visible spectrophotometer (SL 159 Elico Ltd., India) was used. Automatic wavelength accuracy check was previously performed on both instruments, which was found to be 0.1 nm and provided with matched quartz cells of 1.0 cm cell path length.

**Method Development**

Analysis of the proposed drug formulations was performed in various media. The criterion for the selection of specific media was the solubility of the drug, cost of solvents, sensitivity of the method, applicability and method robustness (Verma et al., 2014).

**Preparation of Simulated Saliva (pH 6.8).** Weighed amounts of dibasic sodium phosphate (0.19g), disodium hydrogen phosphate (2.38g), and sodium chloride (8g) were put in a 1000-ml capacity volumetric flask and dissolved in a small amount of distilled water. The volume was made up to 1000 mL with distilled water. The pH was adjusted to 6.8 by using NaOH and HCl.

**Preparation of Phosphate Buffer (pH 7.4).** Weighed amounts of monobasic sodium phosphate (2.62g) and dibasic sodium phosphate (11.50g) were taken and dissolved in distilled water. The final volume was made up to 1000 ml with distilled water. The pH was adjusted to 6.8 by using NaOH and HCl (India et al., 1985).

**Procedure for Calibration Curve.** Naratriptan hydrochloride (10mg) was dissolved in 100 ml of phosphate buffer (pH 7.4) and simulated saliva (pH 6.8) to prepare a stock solution of 100μg/ml concentration. Standard volumetric flasks were used to form dilutions of six different concentrations in the range of 2-12 μg/ml in phosphate buffer (pH 7.4) and 1-10 μg/ml in simulated saliva (pH 6.8) for a calibration curve. Samples were analyzed for both solutions at 223 nm.

**Sample Preparation.** The film formulation of the proposed drug was dissolved in each medium separately. Suitable dilutions were done to get final concentration of 5μg/ml and filtered through nylon filters.
Validation of Developed Analytical Method (Guideline, 2005)

Specificity. Naratriptan hydrochloride solution (5μg/ml) in both media and placebo solutions were scanned in the range of 400 to 200 nm to analyze the absorbance shift at the respective wavelength. The standard deviations were determined after making out comparison of above results.

Accuracy. For determination of accuracy, three levels of API concentrations i.e. higher concentration (HC), intermediate concentration (IC), and lower concentration (LC) were prepared from stock solution and analyzed \( (n = 3) \). The mean, standard deviation (SD), percentage relative standard deviation (RSD) were calculated at each level. The overall SD, overall percentage RSD, and compiled percentage recovery were also calculated from the data.

Precision. Different drug concentrations were analyzed for repeatability studies. Determinations of Inter-day, inter instrument variation and intra-day were carried out to find the intermediate precision. Intra-day and inter-day variation were determined to analyze the samples at three different times periods per day and three different days respectively. Same procedure was followed for inter-instrument variations and samples were re-analyzed using a U-2900/U-2910 Shimadzu Co. Ltd., Japan. \( (n = 3) \).

Linearity. For determining linearity, six concentrations ranging 1–10μg/mL of the proposed drug were prepared from the stock solution and least square regression analysis was employed.

Robustness. Proposed method was analyzed for robustness by: (a) changing wavelength maxima by ±5 nm i.e. 228 nm and 218 nm and (b) changing pH of the respective media by ±0.4unit.

RESULTS AND DISCUSSION

Method Development

Various media like water, 0.1N HCl, acetate buffers (pH4.5), simulated saliva (pH 6.8) and phosphate buffer (7.0-10.2) were investigated for optimization of the medium. On the basis of outcomes simulated saliva (pH 6.8) and phosphate buffer (pH 7.4) were finally selected as the suitable media. The spectra of naratriptan hydrochloride in both media were determined and \( \lambda_{\text{max}} \) was found to be 223 nm (Figure 2).
UV-Spectrophotometric Assay Method for Naratriptan Hydrochloride

Figure 2. UV spectra of naratriptan hydrochloride in simulated saliva pH 6.8 (A) and phosphate buffer pH 7.4 (B)

Calibration Curve

The linear regression equations were obtained at 223nm, \( (0.0991 \times \text{concentration in } \mu\text{g/ml}) + 0.0534 \), with a regression coefficient of 0.9982 and \( (0.0882 \times \text{concentration in } \mu\text{g/ml}) - 0.0006 \), with a regression coefficient of 0.9962 for simulated saliva (pH 6.8) and phosphate buffer (pH 7.4) respectively (Figure 3).

Figure 3. Calibration curves of naratriptan hydrochloride in simulated saliva pH 6.8 (A) and phosphate buffer pH 7.4 (B)
Validation of Developed Analytical Method

Specificity. Since the UV-spectrum of the proposed drug was similar for film in both media, (Figure 2) it was found that no major difference was observed between the mean absorbance. Also, no interference peaks were present in blank and placebo solutions on 223nm. Hence the proposed analytical method features specificity for naratriptan hydrochloride.

Accuracy. The percentage mean recovery values observed were close to 100%. The standard deviation values for overall recovery data were less than 1.41 for both medias. The observed standard deviation values for overall accuracy were less than 1.73 and 1.35 in simulated saliva pH 6.8 and phosphate buffer pH 7.4, respectively representing the higher accuracy of the proposed analytical method. In simulated saliva pH 6.8, the %RSD values for percentage mean recovery for higher, intermediate and lower drug concentrations levels were found to be 97.69, 101.02 and 98.61, respectively. In phosphate buffer pH 7.4, these values were found to be 96.98, 99.52 and 98.99, respectively (Table 1 and Table 2).

Table 1
Data for overall recovery in simulated saliva and phosphate buffer

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
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<tr>
<td>Overall mean</td>
<td>98.90</td>
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<tr>
<td>Overall S.D.</td>
<td>1.41</td>
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<tr>
<td>Overall %RSD</td>
<td>1.43</td>
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Table 2
Accuracy data in simulated saliva and phosphate buffer

<table>
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<td>Simulated saliva pH 6.8</td>
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<tr>
<td>Accuracy Level 1</td>
<td>Mean 97.69</td>
</tr>
<tr>
<td></td>
<td>S.D. 0.42</td>
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<td>%RSD 0.53</td>
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<tr>
<td>Accuracy Level 2</td>
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<td>S.D. 1.05</td>
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<tr>
<td></td>
<td>%RSD 1.06</td>
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<tr>
<td>Accuracy Level 3</td>
<td>Mean 97.69</td>
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<td></td>
<td>S.D. 0.52</td>
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<td></td>
<td>%RSD 0.43</td>
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<td>Overall data for accuracy</td>
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<td></td>
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<td>Accuracy Level 1</td>
<td>Mean 96.98</td>
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<td></td>
<td>%RSD 0.43</td>
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<tr>
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<td></td>
<td>S.D. 0.34</td>
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<td></td>
<td>%RSD 0.34</td>
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<tr>
<td>Accuracy Level 3</td>
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<td>S.D. 0.43</td>
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<td>%RSD 0.49</td>
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<td>Overall data for accuracy</td>
<td>Mean 98.50</td>
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<td></td>
<td>S.D. 1.33</td>
</tr>
<tr>
<td></td>
<td>%RSD 1.35</td>
</tr>
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</table>
**Precision.** Intermediate precision and repeatability were performed for sample concentration in simulated saliva pH 6.8 and phosphate buffer pH 7.4. The percent RSD were found to be less than 1 for both media (Table 3).

Table 3  
*Data for repeatability and intermediate precision in simulated saliva pH 6.8 and phosphate buffer pH 7.4*

<table>
<thead>
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<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Repeatability*</td>
<td>0.54 (abs.)</td>
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<tr>
<td>Inter. precision*</td>
<td>99.41</td>
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<tr>
<td>Repeatability**</td>
<td>0.44(abs.)</td>
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<tr>
<td>Inter. precision**</td>
<td>99.72</td>
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</table>

* In simulated saliva pH 6.8; **in phosphate buffer pH 7.4

**Linearity.** The linearity of the analysis for the proposed drug was found to be 1–40μg/mL ($r^2 = 0.9864$) and 1–40μg/mL ($r^2 = 0.9901$) in simulated saliva pH 6.8 and phosphate buffer pH 7.4, respectively. High precision of the proposed methods was confirmed from lower values of standard error (S.E.) and the mean slope and intercept values are within the 95% confidence interval (Table 4).

Table 4  
*Linear regression data for the linearity curve in simulated saliva (pH 6.8) (n = 9) (I) and in phosphate buffer (pH 7.4) (n = 9) (II)*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I</th>
<th>II</th>
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</thead>
<tbody>
<tr>
<td>Correlation coefficient(r)</td>
<td>0.9978</td>
<td>0.9976</td>
</tr>
<tr>
<td>&quot;r&quot; squared</td>
<td>0.9958</td>
<td>0.9953</td>
</tr>
<tr>
<td>Slope</td>
<td>0.1015</td>
<td>0.0926</td>
</tr>
<tr>
<td>S.E. slope</td>
<td>0.0025</td>
<td>0.0024</td>
</tr>
<tr>
<td>95% confidence limit of slope</td>
<td>0.0955–0.1074</td>
<td>0.0870–0.0983</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0304</td>
<td>0.0312</td>
</tr>
<tr>
<td>S.E. Intercept</td>
<td>0.0092</td>
<td>0.0088</td>
</tr>
<tr>
<td>95% confidence limit of intercept</td>
<td>0.0087–0.0521</td>
<td>0.0105–0.0520</td>
</tr>
</tbody>
</table>

**Robustness.** It was found that small variation in the pH of the selected media i.e. ±0.4 did not have significant effect on the absorbance of the naratriptan hydrochloride. The percent mean recoveries (±S.D.) were found to be 98.70 (±1.75) and 98.99 (±0.53) in simulated saliva pH 6.8 and phosphate buffer pH 7.4.
Analysis of Oral Film Formulations

In simulated saliva pH 6.8, the measure estimations of drug for various formulations were in the range of 98.60% to 99.41% along with a standard deviation not more prominent than 0.42. In phosphate buffer pH 7.4 medium, these values were in the range of 97.64% to 99.22% along with a standard deviation not more than 0.81. The assay results of formulation were not having significant differences. This demonstrates that there is no significant effect of excipients for the estimation of naratriptan hydrochloride in the oral films by using the proposed method.

The above proposed analytical method is accurate, rapid, simple, inexpensive, and precise. Therefore, it can be used for the estimation of naratriptan hydrochloride in different dosage forms. The recoveries of naratriptan hydrochloride from formulation were found to be in good agreement with their respective claims for this API, which tells about there is no interference of formulation excipients which are present in the oral film. Moreover, this method is also very fast with respect to analysis time as compared to HPLC.

The %RSD values of all the parameters for the proposed analytical method for the analysis of naratriptan hydrochloride are lying between 0.34 – 1.06 as these are lesser as compared to previous work.

CONCLUSION

The above proposed analytical method is accurate, rapid, simple, accurate, inexpensive, and precise. Therefore, it can be used for the estimation of naratriptan hydrochloride in different dosage forms.

REFERENCES


Post-Weaning Exposure to Bisphenol A Induces Histological Changes in the Liver

Siti Sarah Mohamad Zaid1*, Siti Nur Hajar Rohim1, Goh Yong Meng2 and Noordin Mohamed Mustapha3

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ABSTRACT

Bisphenol A (BPA) is an endocrine disrupting chemical (EDC) widely used in industry as a plasticizer for the production of polycarbonate plastics and epoxy resins. The liver is highly sensitive to BPA, even at low doses. The objective of the study is to investigate the effect of BPA on histo-architecture of the liver in post-weaning rats. Post-weaning female rats were exposed to BPA by oral gavage over a six weeks period. The results showed that even at low environmental doses, BPA exposure had adverse effects on the liver histo-architecture, thereby disrupting the functions of cellular. The administration of BPA resulted in severe hepatocytes necrosis, dilated sinusoid, and depicting features of conspicuous Kupffer cells. The results may be due to the generation of reactive oxygen species (ROS) by BPA. In conclusion, post-weaning exposure of BPA resulted in significant histological alterations due to ROS generation.

Keywords: Bisphenol A, histology, liver, oxidative stress, plastic

INTRODUCTION

In the last few decades, exposure to environmental toxicants has become a serious health concern (Monisha et al., 2014). Bisphenol A (BPA) is a type of xenobiotic or endocrine disrupting chemical (EDC) widely used in industry as...
a plasticizer (Lim et al., 2017; Von et al., 2010). EDCs are substances in the environment which may disrupt the normal function and development of body system (Miao et al., 2011).

The liver is one of the major organs for the detoxification of xenobiotics and metabolism, and is thus highly sensitive to effects of BPA even at low levels of exposure (Moon et al., 2012). EDCs bind to a nuclear receptor (NR) and interfere with a hormone action by altering the hormone responsive tissues (Diamanti-Kandarakis et al., 2009). According to the Center for Disease Control and Prevention (CDC), children are highly exposed and more susceptible to BPA than the adults (Diamanti-Kandarakis et al., 2009). In children, BPA exposure may cause liver abnormalities, diabetes, hormone and brain disruption (Brouard et al., 2016; Frederica & Julie, 2011). A study has shown that BPA can induce liver inflammation through the formation of reactive oxygen species (ROS) (Eid et al., 2015). ROS plays a key role in pathological conditions and can cause tissue damage, inactivation of many enzymes, and the alteration of receptor proteins involved with cell signaling (Prahalathan et al., 2004).

In this study, we investigated the effects of BPA on liver histo-architecture in pre-pubertal rats. The rats were exposed to BPA by oral gavage over a six-week period. Using an image analyzer, histological changes in the liver were measured to assess the degree of abnormalities at the cellular level. This study provides scientific information regarding the degree of disruptive effects of BPA on the liver, a major metabolizing and excretory organ for toxicants, particularly at the cellular level. In addition, this study is intended to create awareness among students, scholars, academics, and NGOs working with environmental issues about the effects of BPA on human health.

MATERIALS AND METHODS

Animals

The study was performed with 24 of prepubertal female Sprague Dawley (SD) rats at the age of 28 days (P28) with a range of 100-120 g of body weight, obtained from the Animal Resources Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia. SD rats were chosen because they are relatively easy to handle and are widely used in toxicological experiments worldwide. At the Animal House, Comparative Medicine and Technology Unit (COMeT), Institute of Biological Science, the rats were maintained under standard laboratory conditions (temperature 25±2°C, 50±15% relative humidity and normal photoperiod of 12 h dark and 12 h light). They had ad libitum access to rat chow (Gold Coin Feedmills Pte. Ltd, Malaysia) and water. To minimize exposure to EDC, drinking water was provided in glass bottles with rubber stoppers surrounded by a steel ring.
Experimental Design

The experimental design and procedures were conducted under protocols in compliance with EU Directive 2010/63/EU that was approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (Approval no: UPM/IACUC/AUP-U004/2017). After one week of acclimatization, the rats were randomly divided into three groups (n=8 in each group). Group I (NC) served as control and received a treatment of palm oil alone at 0.2 ml. Group II (BPA LD-low dose) and group III (BPA HD- high dose) were treated with 10 and 100 mg/kg body weight of BPA, respectively. The dose selection of BPA at 10 mg/kg body weight was based on a previous study in which BPA was shown to induce histological changes on the liver at this dose (Richter et al., 2007). Additionally, the dose selection of 100 mg/kg body weight was intended to investigate the disruptive changes of BPA at a 10× higher dose. Administration was performed every day (between 09:00 am to 10:00 AM) by oral gavage (to mimic the most likely route of human exposure) for six consecutive weeks. After the last dose of treatment, all rats were sacrificed at the onset of the diestrous phase.

Histopathological Analysis of Liver

The formalin-fixed tissue (liver) was processed by dehydration in a graded series of ethanol, cleared by xylene, and infiltrated in paraffin using an automatic tissue processor (Leica 1020, Germany). Subsequently, these processed tissues were embedded in paraffin blocks at the embedding centre (Leica EG1150H & Leica EG1159C, Germany). Sections of about 5 μm thickness using microtome (Jung Multicut 2045, Germany) were deparaffinized in xylene, dehydrated in a graded series of ethanol, cleared in xylene and stained with Harris hematoxylin and eosin (H&E) for histological study. Histological analysis was conducted under a light microscope attached to an image analyser.

Statistical Analysis

All values have been expressed as median (IR) for eight animals (n = 8) in each group. Significant differences between the groups were determined using Statistical Package for Social Sciences (SPSS Inc. Chicago, IL, USA, version 22.0) by performing Kruskal-Wallis test for multiple comparisons, followed by Mann-Whitney U-test to compare differences between two groups. A difference was considered significant at p < 0.05.

RESULTS AND DISCUSSION

Liver Weight

To determine whether BPA exposure induces toxicity effects, the liver weights of all rats were taken at the end of experiment (Table 1). Figure 1 shows the relative weight of the liver in all experimental groups. The significant decline in relative weight of liver (1.03%)
was observed in the HD group compared to the NC group after six weeks of BPA exposure. This is because some studies have shown that high doses of BPA exposure may alter liver weight in rats (Moon et al., 2012).

Table 1
Weight of liver in all experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver wet weight (g)</th>
<th>Liver relative weight (wet weight (mg)/body weight (kg))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>9.80 (±0.95)</td>
<td>52.36 (±7.89)</td>
</tr>
<tr>
<td>LD</td>
<td>10.54 (±1.13)</td>
<td>53.02 (±7.54)</td>
</tr>
<tr>
<td>HD</td>
<td>9.12 (±2.40)</td>
<td>49.99 (±8.46)</td>
</tr>
</tbody>
</table>

All values are expressed as the median (IR). There is no significant difference between all the groups. NC, control group; LD, low dose group (10 mg/kg); HD, high dose group (100 mg/kg).

Figure 1. Liver relative weight in all experimental groups NC, control group; LD, low dose group (10 mg/kg); HD, high dose group (100 mg/kg)

Histopathological Findings of Liver

Histopathological analysis of the representative sections of liver from all experimental groups are shown in Figures 2, 3 and 4. In Figure 2, a photomicrograph of a rat liver in control group shows normal limits feature where there is an absence of massive necrosis, congestion or widened sinusoids. The liver in the low dose of BPA 10 mg/kg (LD BPA group) shows an abnormally extended sinusoidal cavity, a feature of conspicuous Kupffer cell (KC), in addition to necrotic hepatocytes, granularly degenerated hepatocytes and dilated sinusoid (Figure 3). In the high dose of BPA 100 mg/kg (Figure 4), a photomicrograph of the liver shows massive loose spaces due to cytoplasmic vacuolation arising from hepatocytic degeneration, massive hepatocytes with either cytoplasmic vacuolation or granulation, and conspicuous Kupffer cells.
**Bisphenol A Induces Histological Changes in the Liver**

*Figure 2.* (a) Normal limit features were observed in control group (H&E, ×10). (b) Normal limit features except for the slightly more congested central vein (H&E, ×40) (NC group)

*Figure 3.* (a) Abnormally extended sinusoidal cavity (H&E, ×10). (b) Features of conspicuous Kupffer cell in addition to necrotic hepatocytes (arrow), granularly degenerated hepatocytes and dilated sinusoid (H&E, ×40) (LD group)

*Figure 4.* (a) Massive loose spaces due cytoplasmic vacuolation arising from hepatocytic degeneration (H&E, ×10). (b) Massive hepatocytes with either cytoplasmic vacuolation (blue arrow) or granulation (green arrow) and conspicuous Kupffer cells (arrow) (H&E, ×40) (HD group)
Bisphenol A (BPA) is used in the production of polycarbonate plastics and epoxy resins. Thus, BPA is present all around us in the environment. BPA is an endocrine disruptor that can imitate the body’s hormones, and it may disrupt the secretion, transport, action, production, function, and elimination of hormones (Von et al., 2010). Young children are highly sensitive to the BPA (Diamanti-Kandarakis et al., 2009). The liver is one of the major organs for the detoxification of xenobiotics and metabolism (Knaak & Sullivan, 1996). The liver is highly sensitive to BPA, even at low doses. For that reason, the present study has investigated the disruptive effects of BPA on the post-weaning of female rat (as an animal model) that occur in the liver.

According to previous studies, in vivo and in vitro animal data revealed that BPA is able to increase the ROS generation and disrupt the enzyme activity in rat livers, as well as induce cytotoxicity effect at high doses on rat hepatocytes (Diel et al., 2000; Hanioka et al., 1998). In toxicological studies, analysis of weight of selected organs is a very sensitive indicator for adverse effects of toxicants, as reflected in histological findings. The present study shows that the organ weight in the BPA-exposed rats was higher compared to the normal group. The result is in agreement with a previous study that found that fat starts to accumulate in the liver of BPA-exposed rats by disrupting the metabolic functions (Adams et al., 2009). In addition, another study has shown that high dose of BPA exposure can alter liver weight and decrease the viability of hepatocytes (Moon et al., 2012).

Some studies have revealed that BPA exposure in perinatally or juvenile-exposed animals can affect liver homeostasis, causing altered gene expression and accumulation of fat (Marmugi et al., 2012; Ronn et al., 2013). In this study, the administration of BPA resulted in severe hepatocytes necrosis, dilated sinusoid, and depicting features of conspicuous Kupffer cells. These results were similar to the acute and chronic effects of BPA as documented in previous studies (Marmugi et al., 2012; Ronn et al., 2013). Since the liver is the primary site for getting rid of xenobiotics, the observed increase in its weight in the present study may represent a homeostatic mechanism to deliver more BPA into the liver for detoxification. Liver enlargement may be due to a combination of hepatocyte hypertrophy and smooth endoplasmic reticulum proliferation, which is presumed to stimulate a hepatic physiological adaptation to an increased workload demand.

From the histological findings, morphological changes in the liver of BPA-exposed rats are associated with oxidative stress. BPA is a phenolic compound which may cause abnormalities, DNA damage, and genotoxicity in the liver of rats and mice (Iso et al., 2006). It has been evidently reported by previous studies (Marmugi et al., 2012; Ronn et al., 2013). A previous study stated that abnormal liver function and altered insulin homeostasis was found to associate with BPA (Abdel-Wahab, 2014). The hepatocytes are approximately 80% of the total liver (Kmieć, 2001). One of the functions of hepatocytes is the production of
proteins, including albumin (25% of hepatic protein production), lipoproteins, globulins, clotting factors, and certain hormones (Talwar & Srivastava, 2002). When DNA is broken at late stages of necrosis, it is easier to distinguish from apoptotic cells on morphological grounds when morphological alterations are unambiguous. These may be explained by BPA-induced apoptosis in the liver cell. The greater extent of hepatocytes apoptosis seen in the liver sections of BPA-exposed rats is in agreement with previous studies.

In this study, histological findings revealed that even at low dose, the liver cells were affected. It has been observed that BPA can cause degenerative changes in the hepatic cells as observed by vacuolated hepatocytes, congested blood vessels, dilated sinusoids, Kupffer cell, and necrosis. The findings are in agreement with a previous study which found that BPA exposure led to membrane damage and cell rupture, necrosis, cell infiltration of erythrocytes, vacuolated hepatocytes (Hanioka et al., 1998).

CONCLUSION
Liver weight in the group that had been exposed to BPA was higher compared to the normal group because high doses of BPA exposure could alter liver weight in rats. Histological analysis revealed vacuolated hepatocytes, congested blood vessels, dilated sinusoids, Kupffer cell, and necrosis. It can be concluded that even at low dose exposure of environmentally relevant concentrations, BPA can cause significant histological alterations in the liver that consequently may cause deleterious hazardous effects on human health. Moreover, exposure to high dose of BPA could have severe effects on the liver. Awareness should be built among stakeholders including students, scholars, academics, and NGOs working with environmental issues about the effects of BPA on human health. Furthermore, the results of the present study suggest that in a population of high use of plastics where there is a chance of exposure to BPA, the population may suffer adverse health effects. Thus, the use of BPA in other industries and plasticizers should be limited, and incorrect handling of plastic containers must be avoided, to reduce the health risks resulting from exposure to endocrine disruptors such as BPA.

ACKNOWLEDGEMENTS
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Bisphenol A Induces Histological Changes in the Liver


Review Article

Chemical Constituents and Biological Activities of South East Asia Marine Sponges: A Review

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ABSTRACT

The ocean has an exceptional resource with various groups of natural products that are potentially useful for biomedical and other applications. Marine sponges have prominent characteristic natural products with high diversity. They produce many vital therapeutic metabolites with prominent biological activities. Marine invertebrates and microbial communities are the primary producers of such metabolites. Among the richest sources of these metabolites, class Demospongiae and the order Haplosclerida and genus Xestopongiae from family Petrosiidae are of interest. This review summarizes the research that has been conducted on two classes, eight orders, twelve families and fourteen genera of marine sponges available in the South East Asia region, covering the literature of the last 20 years. Ninety-five metabolites including alkaloids, sterols, terpenoids, quinones isolated from marine sponges collected in South East Asia along with their bioactivities especially cytotoxicity and antibacterial activities were reported in this review. Chemistry and biology are highly involved in studying marine sponges. Thus, tight collaboration is needed for understanding their taxonomy aspects. This
review will outline chemistry and biological aspects, challenge, limitation, new idea and a clear future perspective on the discovery of new drugs from South East Asia’s marine sponges.

Keywords: Bioactivities, chemistry, marine sponges, South East Asia

INTRODUCTION

The world’s biodiversity is a wellspring for new discoveries. As a result of life’s having developed multiple solutions to recurring challenges, organisms have produced billions of diverse compounds to enhance their chance of survival, and many of these compounds are potentially useful to mankind (Bhakuni & Rawat, 2005). In addition to its huge inventory of flora, fauna and other forms of life, the world’s biodiversity also contains all of the interactions, energy pathways, symbioses and other elements that contribute to the lives of these species. The oceans, which comprise 95% of the Earth’s hydrosphere, are the greatest centers of biodiversity (Jaksha, 2010). Within the realm of these oceans lies a treasure trove of undiscovered metabolites with novel chemical structures. These molecules demonstrate diverse biological properties of potential medical interest, such as anticancer, anti-inflammatory, anti-HIV, anti-infectives and treatments for a myriad of other diseases (El-Amraoui et al., 2013; Youssef et al., 2013; Blunt et al., 2007; Keyzers & Davies-Coleman, 2005; Simmons et al., 2005; Phuwapraisirisan et al., 2003).

Marine organisms are amazing living chemical factories. They produce primary metabolites (include sugars, fats, nucleic acids and proteins). These organisms are needed for life-sustaining and simple physiological functions, such as growth, metabolism and respiration. They are also important in energy storage, transfer, and genetic information management. In addition, molecular structural diversity of marine organism was higher than their terrestrial counterparts due to their longer evolutionary history. Similar to the terrestrial plants and animals, marine organisms also contain secondary metabolites that are essential contributors to their fitness and survival. Certain invertebrates that contain specific natural product compounds in their tissues may have less chance to be attacked by grazers and predators compared to similar organisms, which do not have such compounds. For example, the predators may be able to overcome the built-in defenses of their food source by sequestering the defense metabolites in their own tissues for their own protection.

Marine plants and animals have been drawn attention worldwide biomining effort to isolate useful natural product compounds from the marine environment in the past 30-40 years. A slight number of marine plants, microbes, and animals was reported to provide more than 13000 novel natural product molecules (Blunt et al., 2011). Since then, hundreds of new compounds have been yearly discovered. The interest in marine natural
products is further fueled by the fact that marine natural products tend to be more potent than those isolated from terrestrial organisms. This is probably as an essential arsenal in their chemical defense systems, marine organisms have to produce toxins that are potent at very low concentrations to overcome the enormous dilution factors that result from the dispersal of chemicals into the sea. Thus, the most potent cytotoxins known, such as apratoxin A and theopederins A to E, have been found in marine organisms (Huang et al., 2016; Fusetani et al., 1992).

In reality, the number of marine natural products that has made it to commercial application is still small compared to the terrestrial organism-derived bioproducts (Newman & Cragg, 2016). However, this is more likely to have been due to the relative infancy of marine bioprospecting activities than to a lack of potential for discovery. However, in the last two decades biodiscovery programs on marine natural products have indeed resulted in significant enrichment of marine natural product chemical libraries as exemplified by the two most notable marine databases, The Dictionary of Marine Natural Products (DMNP) and MarinLit. The biodiversity in marine species is extremely rich on coral reefs, where there are approximately 1000 species per m$^2$ especially in the Indo-Pacific Ocean where the marine diversity is maximal (Brahmachari, 2012; National Research Council, 2002). In fact, the Indo-Pacific oceans in particular, in which Malaysia is so opportunely situated, house the world’s greatest tropical marine biodiversity. Despite this great promise, however, many vast ocean regions of the world, especially in the tropics, remain almost entirely unexplored. There is now a growing concern that many of the oceans’ unknown resources are under threat from both climatic and human-effected changes on the earth’s ecosystem. These bioresources may forever be lost, even before they could be fully understood let alone harnessed for the benefit of mankind. This calls for a more concerted, coordinated and sustainable effort to explore this valuable bioresources.

**MARINE SPONGES AS A SOURCE OF MARINE NATURAL COMPOUNDS**

Marine sponges (Porifera) have found for more than 700 million years and are claimed to be the most primitive of the multicellular animals. Taxonomically, there are four classes of Porifera: the Demospongidae, Hexactinellida, Calcarea and Sclerospongia. Globally, there are more than 10000 species of marine sponges and most are classified as Demospongia (Koziol et al., 1997; Kruse et al., 1998). Marine sponges exclusively inhabit the marine environment and are widely found in the intertidal zones to areas of thousands of meters deep. They have a very strong pumping ability to filter large volumes of water via their tissues for the consumption of food and oxygen. Marine scientists believed that they have well-developed and efficient defenses against foreign organisms that may attack them. It is hence not unexpected that sponges are able to survive in a nutrient-poor environment and have evolved special chemical resistance strategy to defend against possible predators.
Marine sponges contain novel compounds on the basis that they yield the biggest quantity of structurally diversified natural products (Blunt et al., 2009; Rifai et al., 2005). They are indeed the top source of marine bioactive compounds, which serve as an important feedstock for the pharmaceutical industry. The bioactive compounds isolated from marine sponge species (*Neopetrosia* sp.) can interact with multiple key aspects of the cell cycle, enzymes and other targets, contributing to antidiabetic, antimicrobial, antifungal and cytotoxic activities (Ramanjooloo et al., 2015; Qaralleh et al., 2010). These interactions provide insights and potential inroads into the development of new therapeutics that have significant biological activities (Qaralleh et al., 2016; Ramanjooloo et al., 2015; Guzmán et al., 2011; Qaralleh et al., 2010; Takei et al., 2010; Coello et al., 2009; Rao et al., 2006; Lucas et al., 2003). These studies have deeply impacted the progress of drug discovery in the field of pharmacology, where new applications and potentials of certain compounds from the marine sponge can be more thoroughly characterized through studies of the interactions between the drugs and human systems (Dembitsky et al., 2005; Kim & Park, 2002).

**OCCURRENCE OF MARINE SPONGES IN SOUTH EAST ASIA**

Studies on the diversity of South East Asia sponges have been conducted by several groups, who have reported on the discovery and taxonomic identification of many species of sponges in the Indo-Pacific region. Among these were 168 species identified around the Derawan Islands, Indonesia (De Voogd et al., 2009), 118 species from Jakarta Bay, Indonesia (De Voogd & Cleary, 2008), 45 species from the Mo Ko Thale Tai National Park in the Gulf of Thailand (Putchakarn, 2007), 33 species from Cebu, Philippines (Longakit et al., 2007), 128 species from the Mariana Islands (Kelly et al., 2003), 126 species from the Eastern Gulf of Thailand (Kritsanapuntu et al., 2001), 151 species from Southwest of Sulawesi (de Voogd et al., 1999) and 3 species from Malaysia (Qaralleh et al., 2011). Table 1 shows the classification of the marine sponges.

Table 1

<table>
<thead>
<tr>
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<th>Family</th>
<th>Genus</th>
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</tr>
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<tbody>
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<td>Demospongiae</td>
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<td>Suberitidae</td>
<td><em>Aaptos</em></td>
<td>Malaysia</td>
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<td></td>
<td>Haplosclerida</td>
<td>Petrosiidae</td>
<td><em>Xestospongia</em></td>
<td>Indonesia, Philippines, Thailand and Vietnam</td>
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<td>Calafibropsongiidae</td>
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In this review, the metabolites isolated from 12 families and 13 genera together with their bioactivities are summarized. The current review is intended to present an overview of the findings done by other researchers on South East Asia marine sponges, which reflect the rich structural diversity of the sponges in the South East Asia region as well as their great potential to yield lead compounds for future drug discovery. The information summarized herein will be useful in planning future research and development activities on the marine bio-resources of the South East Asia region.

Metabolites from the Genus *Aaptos*

Sponges of the genus *Aaptos* (family; Suberitidae, order; Hadromerida, and class; Demospongiae) are greyish-yellow or dark reddish-brown in color, with irregular and hispid surfaces but without prominent papillae (Boxshall et al., 2016). With firm and hard character traits, they are commonly 3 to 5 cm in diameter, occasionally larger to fist-sized and are described as lumpy and bluntly lobate. Sponges of this genus are typically found in deep waters, but they are also sometimes found in shallow sublittoral areas. The class of compounds reported from several *Aaptos* sp. includes alkaloids and sterols. Table 2
summarizes the compounds isolated from this genus and their bioactivities. The structures of the compounds are presented in Figure 1.

Aaptamine (1) and two other aaptaminoids, 3-(phenethylamino)demethyl(oxy) aaptamine (2) and 3-(isopentylamino) demethyl(oxy)aaptamine (3) were isolated from an *Aaptos sp.* collected from the coastal waters of Terengganu, Malaysia (Shaari et al., 2008). These compounds were isolated from the chloroform fraction through bioassay-guided isolation in which the preliminary screening showed significant cytotoxic activities towards a range of cancer cell-lines, including human promyelocytic leukemia cells (HL-60), human T4-lymphoblastoid cells (CEM-SS), human breast cancer cells (MCF-7), human cervical cancer cells (HeLa), human colon cancer cells (HT-29) and mouse fibroblast cells (L929) with CD50 values, ranging from 3.2 to 24.1 µg/mL. Aaptamine was also isolated from another *Aaptos sp.* collected from Pulau Bidong, Terengganu (Mohamad et al., 2009).

The sterol cholestanol known as cholestan-3β-ol (4) was isolated from the non-polar fraction whereas aaptamine was isolated as greenish-yellow crystal from a semi-polar fraction during the screening of methanol extracts that showed strong antibacterial activity towards *Bacillus subtilis*, *Streptococcus fecalis* and *Streptococcus agalata* and weak antibacterial activity against *Bacillus proteus* and *Escherichia coli* as well as strong free-radical scavenging activity at 78.8% with IC50 value of 0.12 mg/mL (Mohamad et al., 2009).

Table 2
*The bioactivity and isolated compounds from Genus Aaptos*

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound</th>
<th>Bioactivity</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>3-(phenethylamino) demethyl(oxy)aaptamine</td>
<td>cytotoxic activities towards human promyelocytic leukaemia cells (HL-60), human T4-lymphoblastoid cells (CEM-SS), human breast cancer cells (MCF-7), human cervical cancer cells (HeLa), human colon cancer cells (HT-29) and mouse fibroblast cells (L929)</td>
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</tr>
<tr>
<td></td>
<td>3-(isopentylamino) demethyl(oxy)aaptamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholestanol</td>
<td>cholestan-3β-ol</td>
<td>antibacterial activity against <em>Bacillus subtilis</em>, <em>Streptococcus fecalis</em> and <em>Streptococcus agalata</em> and weak antibacterial activity against <em>Bacillus proteus</em> and <em>Escherichia coli</em></td>
<td>Mohamad et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>strong free radical scavenging activity</td>
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Aaptos species collected from Pulau Kapas, Pulau Perhentian and Pulau Redang were found to exhibit anti-amoebic capacity against the morbific Acanthamoeba castellanii (IMR isolate) (Nakisah et al., 2012). The study concentrated on cell growth inhibition, membrane penetrability and morphological features determined using scanning electron microscopy (SEM). The sponge extracts, whose anti-amoebic IC\textsubscript{50} values ranged from 0.615 to 0.876 mg/mL, induced extensive cell blebbing, surface morphology changes, cell size reduction, cystic appearance, and reduction of the acanthapodia as well as the food cup. From the SEM analysis, it was found that the extracts not only affect the viability of A. castellanii but also induced apoptotic cell death. The sponge extracts were also shown to be genotoxic, inducing significant DNA damage in A. castellanii. The metabolites that were isolated from this species showed cytotoxicity, antibacterial properties and strong free radical-scavenging activity, and the extracts also exhibited potential anti-amoebic activity and genotoxicity.

Figure 1. Chemical structures of isolated compounds from South East Asia marine sponges from genus Aaptos for (1)-(4), from genus Haliclona for (5)-(16), from genus Petrosiidae or Xestospongia for (17)-(23)
Figure 1. Chemical structures of isolated compounds from South East Asia marine sponges from genus Petrosiidae or Xestospongia for (24)-(40), from genus Axinyssa for (41)-(42), from genus Penares for (43)-(57)
Figure 1. Chemical structures of isolated compounds from South East Asia marine sponges from genus *Penares* for (58), from genus *Mycale* for (59)-(61), from genus *Isodictya* for (62)-(63), from genus *Lendenfeldia* for (64)-(68), from family Aplysinellidae for (69)-(76)
Figure 1. Chemical structures of isolated compounds from South East Asia marine sponges from family Aplysinellidae for (77)-(80), from genus Ianthella for (81)-(83), from genus Leucetta for (84)-(89) and from genus Spongia for (90)-(95)
Metabolites from the Genus *Haliclona*

*Haliclona* sponges (family; Chalinidae, order; Haplosclerida, and class; Demospongiae) are typically clumps of hollow cylinders that appear smooth and delicate on the surface (Beedessee et al., 2012). With firm and corky characteristic traits, they are commonly found under the stones with flat crusts and on rocks from the intertidal zone downwards to 30 m, where they serve as homes to baby starfish or echinoderms. The genus *Haliclona* has been chemically studied quite extensively since it has been discovered in many areas of the world. It elaborates a diverse array of compounds comprising alkaloids, hemiketals, polyacetylenes, quinones, terpenoids and sterols. Many of the *Haliclona* metabolites have been displayed to have significant biological activities, such as cytotoxicity, antibacterial, antifungal, anti-tumor and enzyme inhibition (Damodaran et al., 2013; Williams et al., 2012; Lakshmi et al., 2010; Casapullo et al., 2009; Jang et al., 2009; Abdo et al., 2007). *Haliclona* species are also common to the South East Asia region, and studies on them have yielded a myriad of compounds with interesting biological activities (Table 3).

A tetracyclic alkylpiperidine alkaloid, 22-hydroxyhaliclonacyclamine B (5) and haliclonacyclamines A (6) and B (7) were isolated from *Haliclona* sp. obtained from Flores Island, Indonesia (Arai et al., 2009). The alkaloids revealed strong anti-mycobacterial activity under aerobic and hypoxic (oxygen-depleted) conditions towards *Mycobacterium smegmatis* and *M. bovis* with MIC values of 2.5 µg/mL and 1.0 µg/mL, respectively, for both conditions. In contrast, the positive control (isoniazid) gave much weaker MIC values for the two strains (25 and >100 µg/mL, respectively) under hypoxic condition whereas it demonstrated similar or more potent MIC values (2.5 and 0.03 µg/mL, respectively) under aerobic conditions. Furthermore, 22-hydroxyhaliclonacyclamine B exhibited a bactericidal effect only on *M. bovis* under both aerobic and hypoxic states using colony-forming-unit (CFU) assay.

Papuamine (8) was isolated from a *Haliclona* sp. that was collected from Indonesia. The crude extract of the marine sponge displayed strong cytotoxicity and stimulation of apoptosis against human solid tumor cells (Kanno et al., 2013). The cytotoxicity effects of papuamine on human breast tumor cells (MCF-7) were found to be time- and concentration-dependent. Further studies showed that papuamine’s mechanism of action was related to autophagy and is time-dependent. In this process, the mitochondrial membrane prospective showed a concentration- and a time-dependent reduction due to exposure to papuamine, which eventually caused dysfunction of the mitochondria. Furthermore, exposure to papuamine increased the phosphorylation or activation of c-Jun N-terminal protein kinase (JNK) through the release of cytochrome C that was also caused by the decrease in the membrane potential of mitochondria, which contributed to the reduction of cell survival and the activation of apoptotic cell death.
Papuamine (8) and haliclonadiamine (9) were also isolated from another *Haliclona* sp. obtained from Indonesia waters. In the preliminary screening, the ethanol extract of the marine sponge was cytotoxic to MCF-7 (MIC value 1.40 µg/mL), LNCap (MIC value 1.80 µg/mL), Caco-2 (MIC value 2.39 µg/mL) and HCT-15 (MIC value 2.25 µg/mL) cells (Yamazaki et al., 2013). The purified compounds were further found to be cytotoxic to six human cancer cell lines, i.e., breast cancer MCF-7, liver cancer Huh-7, prostate cancer PC-3, colorectal adenocarcinoma HCT-15, histiocytic lymphoma U937 and human Jurkat leukemia cells. The compounds were notably very potent against U937, with IC$_{50}$ values of 0.93 µg/mL and 1.00 µg/mL, respectively. Further investigation on the mechanism of action revealed that the compounds trapped U937 cells at the sub-G1 phase, which contributed to condensation of chromatin and fragmentation of the nuclei (apoptosis of U937 cells).

Maleimide-5-oxime (10), 3,4-dihydroxybenzoic acid (11) and tetillapyrone (12) were isolated from *Haliclona baeri* collected in the coastal area of Chonburi Province, Thailand (Wattanadilok et al., 2007). Maleimide-5-oxime was isolated from the ethyl acetate extract as a viscous yellow mass. The isolated compounds showed potential antifungal activities tested on 7 yeasts, including *Candida albicans*, *C. krusei*, *C. glabata*, *C. parapsilosis* *C. dubliniensis*, *C. tropicalis*, and *Cryptococcus neoformans*. Moreover, 3 non-dermatophyte filamentous fungi known as *Aspergillus fumigatus*, *A. flavus* and *A. niger* were also inhibited as well as 5 dermatophyte fungi, including *Microsporum gypseum*, *M. canis*, *Trichophyton mantagrophytes*, *T. rubrum*, and *Epidermophyton floccosum*. Furthermore, the in vitro growth inhibition was examined against 3 cancer cell lines, the human breast adenocarcinomas (estrogen-dependent ER(+) MCF-7 and estrogen-independent ER(-) MDA-MB-231) and a non-small cell lung cancer cells (NCI-H460). However, maleimide-5-oxime showed very weak activities with MIC > 250 µg/mL for the antifungal assay and GI$_{50}$ > 200 µM for the growth inhibition assay.

The marine sponge *Haliclona cymaeformis* collected in Mahatao, Batanes, Philippines yielded p-sulfooxyphenyl-pyruvic acid (13) as a mixture with p-hydroxyphenylpyruvic acid (14) (Bugni et al., 2002). Attempts at purification via HPLC were not successful and the compounds were therefore evaluated as the mixture. However, the mixture showed a very weak bioactivity for tyrosine kinase inhibition in the $^3$H-thymidine incorporation assay.

Halicloic acid A (15) and halicloic acid B (16) were two merohexaprenoids isolated from the Philippines marine sponge *Haliclona* sp. collected in Culasian Point, Leyte (Williams et al., 2012). Both acids inhibited indoleamine 2,3-dioxygenase (IDO), which plays a central role in tumor cell evasion of T-cell-mediated immune rejection, with IC$_{50}$ values of 10 µM and 11 µM, respectively, in an in vitro assay of the inhibition of purified recombinant human IDO. Overall, the secondary metabolites isolated from *Haliclona sp.* show great potential for antibacterial, cytotoxicity and antifungal activities in which the results showed that each compound is selective.
Metabolites from the Genus *Xestospongia*

The genus *Xestospongia* (family; Petrosiidae, order; Haplosclerida, and class; Demospongiae) consists of tubular sponges of oscules or large openings at the apex with a rough surface (Beedessee et al., 2012). Appearing as a massive vase or volcano-like shape, some of which are encrusted and bulbous, these stony and brittle sponges are commonly found on the wall of sea cliffs or caves, in the reef and at the sea bed (Qaralleh et al., 2011). These species are also known to contain more siliceous spicules than spongin. There have been many chemical investigations on *Xestospongia* collected from many parts of the world, which revealed it to elaborate a diverse array of compounds which includes alkaloids, macrolides, polyacetylenes, quinones, terpenoids and sterols (Lorente et al., 2015; Mejia et al., 2013; Laurent et al., 2006). Some of the reported bioactivities and compounds isolated from *Xestospongia* collected in the South East Asia region are summarized in Table 4.

Table 3

The bioactivity and isolated compounds from genus *Heliclona*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids haliclonaclamines A and B</td>
<td>strong anti-mycobacterial activity under aerobic and hypoxic conditions</td>
<td>Arai et al. (2009)</td>
</tr>
<tr>
<td>22-hydroxyhaliclonaclammine B</td>
<td>bactericidal effect against <em>M. bovis</em> Bacille de Calmette ed Guerin (BCG) under aerobic and hypoxic conditions</td>
<td>Arai et al. (2009)</td>
</tr>
<tr>
<td>papuamine</td>
<td>potent cytotoxicity, induce apoptosis against human solid cancer cells</td>
<td>Kanno et al. (2013)</td>
</tr>
<tr>
<td>papuamine and haliconadiamine</td>
<td>cytotoxicity against MCF-7, hepatoma Huh-7, prostate fencer PC-3, HCT 15, histolytic lymphoma U937 &amp; Jurkat cells</td>
<td>Yamazaki et al. (2013)</td>
</tr>
<tr>
<td>haliscosamine</td>
<td>antifungal</td>
<td>El-Amraoui et al. (2013)</td>
</tr>
<tr>
<td>Malamides maleimide-5-oxime</td>
<td>antifungal</td>
<td>Erickson et al. (1995); Wattanadilok et al. (2007)</td>
</tr>
<tr>
<td>Terpenoids p-sulfooxyphenylpyruvic acid</td>
<td>Indoleamine 2,3-dioxygenase (IDO) inhibition</td>
<td>Bugni et al. (2002)</td>
</tr>
<tr>
<td>halicloic acid A and B</td>
<td></td>
<td>Williams et al. (2012)</td>
</tr>
</tbody>
</table>
Aaptaminoids were isolated from an Indonesian *Xestospongia* collected off the Jakarta coast (Calcul et al., 2003). The methanolic extract of the sponge revealed a potent antimicrobial activity against bacteria of Gram-negative (*Escherichia coli* and *Vibrio anguillarum*) and Gram-positive (*Staphylococcus aureus*). It was also tested against fungi (*Candida tropicalis*) and showed potential activity. Thus, it was subjected to bioassay-guided isolation to yield the alkaloids 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine and four new compounds renieramycins T and U cytotoxic to human colon carcinoma (HCT 116), human lung carcinoma (QG 56), human pancreatic adenocarcinoma (AsPC1) and human ductal breast epithelial tumor (T47D) renieramycins W, X and Y cytotoxic to human colon carcinoma (HCT 116), human lung carcinoma (QG 56), human pancreatic adenocarcinoma (AsPC1) and human ductal breast epithelial tumor (T47D) and four new compounds renieramycins T and U cytotoxic to human colon carcinoma (HCT 116), human lung carcinoma (QG 56), human pancreatic adenocarcinoma (AsPC1) and human ductal breast epithelial tumor (T47D).

### Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine and four new compounds renieramycins T and U</td>
<td>cytotoxic</td>
</tr>
<tr>
<td>Quinones</td>
<td>xestosaprol D and E</td>
<td>Xestosaprol D weakly inhibits the aspartic protease BACE-1</td>
</tr>
<tr>
<td>Sterols</td>
<td>ibisterol sulfates B and C and 4β,5β-epoxy-2β,3α,12β,22S-tetrahydroxy-14α-methylcholesta-7,9(11)-dien-6,24-dione</td>
<td>Inhibitors of HIV-1 integrase</td>
</tr>
<tr>
<td></td>
<td>aragusterol I, aragusterol B, xestokerol A, 21-O-octadecanoyl-xestokerol A, 7α-hydroxytergesterol, 7β-hydroxytergesterol, 7-oxopetrosterol and petrosterol</td>
<td>Antifouling</td>
</tr>
</tbody>
</table>

Aaptaminoids were isolated from an Indonesian *Xestospongia* collected off the Jakarta coast (Calcul et al., 2003). The methanolic extract of the sponge revealed a potent antimicrobial activity against bacteria of Gram-negative (*Escherichia coli* and *Vibrio anguillarum*) and Gram-positive (*Staphylococcus aureus*). It was also tested against fungi (*Candida tropicalis*) and showed potential activity. Thus, it was subjected to bioassay-guided isolation to yield the alkaloids 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine (17), 2-ethyl-3-methyl-10-methoxy-3H-1,3a,6-triazapyrene (18), 11-methoxy-1H-[1,6]naphthyridino[6,5,4-def]quinoxalin-2(3H)-one (19), 2-isopropyl-10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine (20) and 10-methoxybenzimidazo[6,7,1-def][1,6]naphth...
def][1,6]naphthyridine (21). Various compounds from the aaptamine class were isolated together with the previously found aaptamine, isoaaptamine and demethyl(oxy)aaptamine from Indonesian marine sponge Xestospongia sp. obtained off Jakarta. All the isolated compounds were examined for antimicrobial against Gram-negative bacteria of Escherichia coli and Vibrio anguillarum as well as Gram-positive bacteria of Staphylococcus aureus. The Candida tropicalis fungi was also tested. In addition, the compounds were cytotoxic against human buccal carcinoma KB cells. Aaptamine, isoaaptamine, demethyl(oxy) aaptamine and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine displayed moderate antibacterial activity with MIC values ranging from 6 to 100 μg/mL. In particular, the compounds were more active against Vibrio anguillarum with MIC values of 12 μg/mL for both aaptamine and isoaaptamine, and 100 μg/mL for both demethyl(oxy)aaptamine and 10-methoxybenzimidazo[6,7,1-def][1,6]naphthyridine. With respect to the antifungal activity, only aaptamine and isoaaptamine showed activity against Candida tropicalis with MIC values of 25 μg/mL and 12 μg/mL, respectively. As for cytotoxicity, 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine as well as aaptamine, isoaaptamine and dimethyl(oxy) aaptamine showed cytotoxic activities with ID_{50} values of 3.5 μg/mL, 3.7 μg/mL, 0.5 μg/mL and 1.8 μg/mL, respectively.

Two bistetrahydroisoquinoline marine natural products, renieramycins T (22) and U (23) were isolated together with renieramycins M (24) and S (25) from a Thai Xestospongia sp. collected in the vicinity of Sichang Island (Daikuhara et al., 2009). These compounds were isolated from the methanolic extract pretreated with KCN through partitioning and column chromatography. Both renieramycins T and U were isolated as amorphous powders. All of the isolated compounds were tested for cytotoxicity against human colon carcinoma (HCT 116), human lung carcinoma (QG 56), human pancreatic adenocarcinoma (AsPC1) and human ductal breast epithelial tumor (T47D) cells. Renieramycin T, showed strong cytotoxicity with IC_{50} values ranging from 4.7 to 98 nM while renieramycin M showed the strongest cytotoxicity with IC_{50} values ranging from 0.84 to 20 nM.

Three bistetrahydroisoquinoline marine natural products, including renieramycins W (26), X (27) and Y (28) and two renieramycins M (24) and T (22) were identified from a Philippine Xestospongia sp. collected in the vicinity of Puerto Galera at Oriental Mindoro of Mindoro Island (Tatsukawa et al., 2012). These compounds were isolated from the ethyl acetate extract pretreated with KCN via column chromatography. Renieramycin W was isolated as pale-yellow amorphous powder, renieramycin X as a pale yellow amorphous solid, and renieramycin Y as a pale-yellow solid. These renieramycins W and X were examples of tiglic acid esters derivatives at the C-1 side chain, while renieramycin Y is the example, having pentasubstituted phenol in the A-ring.

Xestosaprol D (29) and E (30), two xestosaprol derivatives, and a series of pentacyclic compounds, including adociaquinones A and B, xestosaprol A,
13,14,15,16-tetrahydroxestoquinol and 3,13-dideoxo-1,2,13,15-tetrahydro-3,13-dihydroxyhalenaquinone were isolated from *Xestospongia* sp. obtained from Turtle Bay, Sangalaki, Indonesia (Millán-Aguinaga et al., 2010). Xestosaprol D was isolated as an optically active yellow solid, while xestosaprol E was identified as an optically dynamic solid through bioassay-guided fractionation. In a preliminary screening, an ethyl acetate extract showed potent antimicrobial activity against multi-drug resistant *Staphylococcus aureus* (ATCC 43300). However, pure xestosaprol D only poorly inhibited the aspartic protease BACE-1, a key enzyme in the etiology of Alzheimer’s disease, with an IC$_{50}$ value of 30 μg/mL. In addition, neither xestosaprol D nor E displayed any appreciable antimicrobial activity against Vancomycin-Resistant Enterococci, *Escherichia coli*, Methicillin-resistant *Staphylococcus aureus* or cytotoxicity against SKOV-3 cells (IC$_{50} >$ 50 μg/mL), which is a human ovarian tumor cell line.

Two sulfated sterols, ibisterol sulfates B (31) and C (32) and an extraordinary non-sulfated sterol, 4β,5β-epoxy-2β,3α,12β,22S-tetrahydroxy-14α-methylcholest-7,9(11)-dien-6,24-dione (33) together with halistanol sulfate and ibisterol sulfate were elucidated in a Philippines *Xestospongia* sp. obtained off Boracay Island (Lerch & Faulkner, 2001). These compounds were isolated through bioassay-guided fractionation and column chromatography since the methanolic extract showed some selectivity in initial cytotoxicity screening against a 25-cell-line panel. All compounds were obtained as white powders in which the sulfated sterols ibisterol sulfates B and C were the major metabolites while the non-sulfated 4β,5β-epoxy-2β,3α,12β,22S-tetrahydroxy-14α-methylcholest-7,9(11)-dien-6,24-dione was a minor metabolite. All isolated compounds were tested in an HIV-integrase inhibition assay in which they exhibited mild inhibition with IC$_{50}$ values of 0.4 μg/mL, 2.3 μg/mL, 1.8 μg/mL and 26 μg/mL for halistanol sulfate, ibisterol sulfate B, ibisterol sulfate C and 4β,5β-epoxy-2β,3α,12β,22S-tetrahydroxy-14α-methylcholest-7,9(11)-dien-6,24-dione, respectively. This species yields compounds with strong cytotoxicity properties regardless of geographical area of origin, which may be further targets for study for drug development.

Three new sterols, aragusterol I (34), 21-O-octadecanoyl-xestokerol A (37) and 7β-hydroxyxypetrosterol (38b) and five known compounds, aragusterol B (35), xestokerol A (36), 7α-hydroxyxypetrosterol (38a), 7-oxopetrosterol (39) and petrosterol (40) were isolated from *Xestopongia testudinaria* collected in Truong Sa archipelago, Khanh Hoa, Vietnam (Nguyen et al., 2013). Aragusterol B and 21-O-octadecanoyl-xestokerol A showed the most active antifouling activity with EC$_{50}$ value (60 and 10 μM, respectively) where EC$_{50}$ value of 21-O-octadecanoyl-xestokerol A is comparable to tributyltin oxide (EC$_{50} = 12$ μM), a current marine anti-biofouling agent while not exhibiting toxicity up to 200 μM in growth inhibition and viability assay on *Polaribacter* sp., TC5.
Metabolites from the Genus *Axinyssa*

The genus *Axinyssa* (family; Halilchondriidae, order; Halichondrida, and class; Demospongiae) are digitate cushion-shaped sponges with hispid and conulose surfaces (Boxshall et al., 2016). This genus is commonly found in soft sediment in deeper water ranging from 40 m to 80 m. *Axinyssa* is known for having sesquiterpenes that contain unusual nitrogenous functional groups and that usually possess anthelmintic, antimalarial and antifouling properties. The compounds isolated, and bioactivities reported from genus *Axinyssa* are presented in Table 5.

A Thai *Axinyssa* sp. collected from the Andaman Sea yielded two new nitrogenous germacrane sesquiterpenes identified as (1Z,4Z)-7αH-11-aminogermacr-1(10),4-diene (41) and N,N-11-bis-[(1Z,4Z)-7αH-germacr-1(10),4-dienyl]urea (42) from the ethyl acetate extract (Satitpatipan & Suwanborirux, 2004). (1Z,4Z)-7αH-11-aminogermacra-1(10),4-diene showed strong antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zones 23 mm and 22 mm, respectively. The compound also exhibited potent antifungal activity against *Candida albicans* with an inhibition zone of 27 mm. However, N,N-11-bis-[(1Z,4Z)-7αH-germacr-1(10),4-dienyl]urea did not show any antimicrobial activity at the same test concentration.

Table 5

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids</td>
<td>(1Z,4Z)-7αH-11-aminogermacr-1(10),4-diene (1) and N,N-11-bis-[(1Z,4Z)-7αH-germacr-1(10),4-dienyl]urea</td>
<td>antimicrobial against <em>Staphylococcus aureus</em> and <em>Bacillus subtilis</em> and anti fungal against <em>Candida albicans</em></td>
</tr>
</tbody>
</table>

Metabolites from the Genus *Penares*

Sponges of the genus *Penares* (family; Plakinadaceae, order; Homosclerophorida, and class; Demospongiae) usually consist of several rounded parts joined at their bases. Sometimes these sponges are also described as shapeless (Lyakhova et al., 2012). They may be structurally hard or slightly compressible and whitish-beige or greyish in color. This genus is well known to elaborate triterpenoids, indole and azetidine alkaloids, sphingolipids, macrolides and fatty acids. Some of the reported bioactivities and compounds isolated from genus *Penares* are presented in Table 6.

Two unusual bromine-containing alkaloids, 7-bromo-1-(6-bromo-1H-indol-3-yl)-9H-carbazole (43) and 3,11-dibromo-13H-indolo[3,2-k]phenanthridine (44) were elucidated...
from a South China Sea *Penares* sp. obtained from the Vietnamese waters (Lyakhova et al., 2012). 7-bromo-1-(6-bromo-1*H*-indol-3-yl)-9*H*-carbazole was observed as pale yellow amorphous powder while 3,11-dibromo-13*H*-indolo[3,2-κ]phenanthridine was obtained as light brown solid. Compound (43) showed moderate inhibition of the human cancer cell lines, human promyelocytic leukemia HL-60 (IC\(_{50}\) of 16.1 µM) and human cervical carcinoma HeLa (IC\(_{50}\) of 33.2 µM). However, compound (44) was inactive.

Six triterpenoids (45 - 50) together with penasterone, acetylpenasterol and ergosta-4,24(28)-dien-3-one were identified from the non-polar fractions of a *Penares* sp. collected from Vietnamese waters (Kolesnikova et al., 2013). Cytotoxic activity for all of these triterpenoids compounds were tested against numerous human cancer cell lines, including human promyelocytic leukemia (HL-60), Ehrlich ascites carcinoma, and human cervical carcinoma (HeLa). It was also examined on normal murine epithelial cells (JB6 C141). The results showed that compound 50 was cytotoxic against human HL-60 cells with an IC\(_{50}\) value of 9.7 µM.

Eight triterpenes, derivatives of oxidized lanostane and nor-lanostane (51 - 58) were isolated from the non-polar extract of another *Penares* sp. collected from the Vietnam waters (Lyakhova et al., 2015). The compounds were identified as 29-nor-24(R),25-dihydroxy-penasterone (51), 29-nor-25-hydroperoxy-3-oxo-7β,8β-epoxy-5α-lanost-23(E)-en-30,9α-olide (52), 24(R),25-dihydroxy-penasterone (53), 24(S)-25-dihydroxy-penasterone (54), 24(R)-hydroxy-3-oxo-7β,8β-epoxy-5α-lanost-25-en-30,9α-olide (55), 24(S)-hydroxy-3-oxo-7β,8β-epoxy-5α-lanost-25-en-30,9α-olide (56), 29-nor-24ξ-hydroxy-25-chloro-3-oxo-7β,8β-epoxy-5α-lanost-30,9α-olide (57) and 3β,24-dihydroxy-7β,8β-epoxy-5α-lanost-24-en-30,9α-olide (58).

Table 6
*The bioactivity and isolated compounds from genus Penares*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>7-bromo-1-(6-bromo-1<em>H</em>-indol-3-yl)-9<em>H</em>-carbazole</td>
<td>cytotoxic against human tumour cell lines, human promyelocytic leukaemia HL-60 and human cervical carcinoma HeLa</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Six new triterpenoids, penasterone, acetylpenasterol and ergosta-4,24(28)-dien-3-one</td>
<td>cytotoxic against human promyelocytic leukaemia HL-60</td>
</tr>
<tr>
<td></td>
<td>Eight oxidised lanostane and nor-lanostane derivatives, penasterol and 24-ethylcholesta-4,24(28)-dien-3-one</td>
<td></td>
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</tbody>
</table>
Metabolites from the Genus *Mycale*

The genus *Mycale* (family; Mycalidae, order; Poecilosclerida, and class; Demospongiae) comprises yellow or yellowish sponges that look like cushions or sheets, which are found either under the littoral boulders or under rocks and shells in the sublittoral areas (Boxshall et al., 2016). This genus has a soft to firm consistency, occasionally compressible, with a fibrous interior. It has few but large oscules at the summit of the lobes. This genus is known for producing secondary metabolites that exhibit antiviral and antitumor activities (Habener et al., 2016). Some of the compounds and their bioactivities are presented in Table 7.

A cyclic noristerterpenoid peroxide, mycaperoxide H (59), as well as a known noristerterpenoid peroxide, mycaperoxide B, were isolated from a Thai *Mycale* sp. collected from Sichang Island (Phuwapraisirisan et al., 2003). These peroxides were isolated from the polar fractions of the sponge via bioassay-guided isolation. Mycaperoxide H exhibited cytotoxicity against HeLa cells with an IC$_{50}$ value of 0.8 μg/mL while mycaperoxide B is a known compound with cytotoxic properties.

5-Pentadecyl-1H-pyrrole-2-carbaldehyde (60) and (6’E)-5-(6’pentadecenyl)-1H-pyrrole-2-carbaldehyde (61) were isolated as a mixture from ethyl acetate fraction through column chromatography and DAD-HPLC. It was based on the results from bioassay guided fractionation of *in vitro* growth inhibition screening on mouse lymphoma cell line (L5178Y) (Hertiani et al., 2009).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaldehydes</td>
<td>5-pentadecyl-1H-pyrrole-2-carbaldehyde and (6’E)-5-(6’pentadecenyl)-1H-pyrrole-2-carbaldehyde</td>
<td>Growth inhibition of mouse lymphoma cell line (L5178Y)</td>
</tr>
<tr>
<td>Norsesterpenes peroxide</td>
<td>mycaperoxide H and B</td>
<td>cytotoxic against HeLa cells</td>
</tr>
</tbody>
</table>

Metabolites Isolated from the Genus *Isodictya*

Sponges from genus *Isodictya* (family; Isodictyidae, order; Poecilosclerida, and class; Demospongiae) are being described as colourful (bright yellow, brownish, maroon, greenish and black) daisy-like, large, and hard but chalky sponge. Typically found in narrow opening of rocks in shallow waters to 40 m deep waters, these sponges have many small, branch-like fistules on an exhalant-liked perforated large subhemisperical body (Lim et al., 2008; Fattorusso et al., 2006). Compounds isolated, and their bioactivities are summarized in Table 8.
Table 8
The bioactivity and isolated compounds from genus *Isodictya*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ent-isocopalane diterpenes</td>
<td>coelodiol and coeloic acid</td>
<td>Fattorusso et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>cytotoxic against human gastric adenocarcinoma</td>
<td></td>
</tr>
</tbody>
</table>

Coelodiol (62) and coeloic acid (63), both are rare *ent*-isocopalane diterpenes were isolated from the ethyl acetate fractions of the *Isodictya* genus (Fattorusso et al., 2006). For cytotoxicity screening, both isolated compounds showed inhibition in *in vitro* growth of human gastric adenocarcinoma cell line (MKN-45) with 20 µg/mL for coelodiol and 40 µg/mL for coeloic acid.

**Metabolites Isolated from the Genus *Lendenfeldia***

Sponges from genus *Lendenfeldia* (family; Spongiidae, order; Dictyoceratida, and class; Demospongiae) are less described with details and it is mostly found near Australian waters with ample of cytotoxic compounds isolated from it. However, few of this genus were reported in South East Asia and the isolated compounds and their bioactivities for *Lendenfeldia* sp. found in South East Asia are summarized in Table 9.

A new naphthalene dimer, (S)-2,2′-dimethoxy-1,1′-binaphthyl-5,5′,6,6′-tetraol (64), a new furanolipid, 3-[(3E,7E)-4,8-dimethylpentadeca-3,7-dienyl]-furan (65) and three known homoscalarane sestertepenes, 16β,22-dihydroxy-24-methyl-24-oxoscalaran-25,12β-olactone (66), 24-methyl-12,24,25-trioxoscalar-16-en-22-oic acid (67), and 12,16-dihydroxy-24-methylscalaran-25,24-olide (68) were isolated from marine sponge, *Lendenfeldia* sp. collected from Indonesia (Dai et al., 2007). The bioassay-guided isolation was performed using column chromatography to yield the new colourless gum, (S)-2,2′-dimethoxy-1,1′-binaphthyl-5,5′,6,6′-tetraol, and oil, 3-[(3E,7E)-4,8-dimethylpentadeca-3,7-dienyl]-furan besides obtaining the three known homoscalarane sestertepenes. All isolated compounds showed inhibition in hypoxia-induced HIF-1 activation and iron chelator (1, 10-phenanthroline)-induced HIF-1 activation in T47D breast tumour cells except 3-[(3E,7E)-4,8-dimethylpentadeca-3,7-dienyl]-furan with IC$_{50}$ values ranges from 0.64 to 6.90 µM with 24-methyl-12,24,25-trioxoscalar-16-en-22-oic acid, which was the most potent compound. Besides, the naphthalene dimer and homoscalarane sestertepenes showed similar results on both human breast tumour cell lines (T47D and MDA-MB-231) under normoxic conditions (containing 21% oxygen).
Metabolites Isolated from the Family Aplysinellidae

Sponges originated from the family Aplysinellidae of the Order Verongiidae have a dendritic fibrous skeleton (Bergquist & de Cook, 2002). The skeleton of sponges from this family is typically made up of fibres with bark and pith elements of different proportion of fibre-mass to soft tissues volume from one species to another. However, sponges from this family are well-known for its alkaloids containing bromotyrosine residues (Dai et al., 2016). The compounds isolated and their bioactivities reported are presented in Table 10.

Seven new bromotyrosine-derived metabolites and six known compounds were isolated from the family Aplysinellidae collected from Manta Point, Sangalaki, Indonesia (Dai et al., 2016). The new metabolites isolated were purpuramine M (69), purpuramine N (70), araplysillin VII (71), araplysillin VIII (72), araplysillin IX (73), araplysillin X (74) and araplysillin XI (75). In the same study, there was also six known compounds, namely hexadellin A (8), araplysillin II (76), araplysillin IV (77), purpurealidin I (78), aplysamine 4 (79), and purpuramine G (80). They are isolated together with the new compounds from the methanol extract through silica flash column chromatography and reversed-phase HPLC. The isolated compounds were tested for inhibition of aspartic protease, BACE-1 (memapsin-2) and differential cell viability in five cancer cell lines, ovarian cancer A2780S and cisplatin-resistant variant A2780CP (SCP5), non-small cell lung cancer A549, human breast cancer MCF-7, and glioma U251MG cells and a control cell line of normal mouse fibroblasts of NIH3T3. Araplysillin X and purpurealidin I showed the most significant activity while purpuramine M had inhibition of growth in ovarian cancer cell lines (A2780S...
and A2780CP (SCP5)) and glioma cancer cell line U251MG with IC$_{50}$ values 20, 40 and 50 µM respectively.

Table 10

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Bioactivity</th>
<th>Reference</th>
</tr>
</thead>
</table>

Metabolites Isolated from the Genus *Ianthella*

Normally being found in shallow waters attached to dead or alive coral head, sponges from genus *Ianthella* (family; Lanthellidae, order; Verongiidae, and class; Demospongiae) are known to be bright-coloured and tube-liked (Brunt and Davies, 1994). Compounds isolated from sponges of this genus and their bioactivities are summarized in Table 11.

Petrosterol-3,6-dione (81) and 5α,6α-epoxy-petrosterol (82) are two new cyclopropane ring sterols isolated together with a known compound, petrosterol (40) from a Vietnamese sponge, *Ianthella* sp. collected from Namyet Island in Khanh Hoa province in Vietnam (Tung et al., 2009a). The isolated compounds exhibited cytotoxicity activities against multiple cancer cell lines (A549, HL-60, MCF-7, SK-OV-3 and U937) with IC$_{50}$ ranges from 8.4 to 22.6 µM especially in HL-60 cells. Apoptosis events (chromatin condensation and increase of the amount of sub-G1 hypodiploid cells) were observed in treated cells, suggesting the possibility of potential leukemia treatment.

In addition, aragusteroketal B (83) and aragusterol B (35) were isolated from ethyl acetate soluble fractions through silica gel and resins column chromatography from *Ianthella* sp. obtained from Vietnam waters (Tung et al., 2009b). Cytotoxicity activities against three human tumour cell lines (MCF-7, SK-Hep-1 and HeLa) were conducted on isolated compounds presenting moderate activities with aragusteroketal B giving lower IC$_{50}$ against HeLa (24.6 µM). Aragusterol B had lower IC$_{50}$ against MCF-7 (12.8 µM) and SK-Hep-1 (18.5 µM).
Table 11

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterols</td>
<td>petrosterol-3,6-dione, 5α,6α-epoxy-petrosterol and petrosterol</td>
<td>cytotoxic against A549, HL-60, MCF-7, SK-OV-3 and U937</td>
</tr>
<tr>
<td>aragusteroketal B and aragusterol B</td>
<td>cytotoxic against MCF-7, SK-Hep-1 and HeLa</td>
<td>Tung et al. (2009b)</td>
</tr>
</tbody>
</table>

Metabolites Isolated from the Genus *Leucetta*

With massive, subspherical or pear-shaped outline, sponges from genus *Leucetta* (family; Leucettidae, order; Clathrinida, and class; Calcarea) usually consists of large oscules surrounded by elevated margin with visible subcortical cavities (Hooper, 2014). Mostly found on the hard substrate on the outer reef slope, the sponges of this genus are often being described in bright coloured and with compact or firm structure but chalky or friable. Due to presence of imidazol alkaloids with interesting bioactivities profile, this genus is frequently being studied. Table 12 summarizes the compounds isolated from genus *Leucetta* with their bioactivities.

New imidazole alkaloids, naamine F (85), naamine G (86), kealiinine A (87), kealiinine B (88) and kealiinine C (89) were isolated together with known compound, naamine A (84) from sponges of *Leucetta chagosensis* collected near the coast of Kapoposang Island, Indonesia (Hassan et al., 2004). These alkaloids were isolated from the ethyl acetate and butanol fractions of methanol crude extract using reversed-phase silica and Sephadex LH20. Isolated compounds were tested on antimicrobial assays and antiproliferative assay against human cervix carcinoma cell line (HeLa), mouse lymphoma cells L5178Y and rat brain tumour cell line PC12. Naamine G exhibited strong antifungal activity against *Cladosporium Herbarum* and weak cytotoxicity against L5178Y and HeLa cell lines and inactive against the PC12 cell lines. However, for brine shrimp assay, kealiinine A showed the most active result, contributing to a mortality rate of 50 % at 20 µg/mL.

Table 12

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>Reference</th>
</tr>
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</table>
Metabolites Isolated from the Genus *Spongia*

With firm, rubbery and compressible texture, sponges of genus *Spongia* (family; Spongiiidae, order; Dictyoceratida, and class; Demospongiae) are identified as dark cream or brownish coloured sponge of subspherical, fig-shaped and stalk tapered with wide base found in lagoons on top of rocks or pinnacles (Hall, 2012). Meroterpenoids from this genus showed a variety of interesting bioactivities. Table 13 presents the bioactivity and isolated compounds from genus *Spongia*.

Three new sesquiterpene phenols (langconol A (90), langconol B (91), and langconol C (92)) and a new sesquiterpene hydroxyquinone (langcoquinone C (93)) were isolated from marine sponge *Spongia* sp. obtained from Vietnam waters. In addition, two others known meroterpenoids, polyfibrospongol A (94) and smenospongorine (95) were identified from this sponge (Nguyen et al., 2017). Langcoquinone C and smenospongorine exhibited strong antibacterial activity against Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus* (MIC values ranges from 6.25 to 12.50 µM) which is comparable with positive control (ampicillin, MIC: 6.25 µM). Langconol A and langconol C showed moderate antibacterial activity against *Bacillus subtilis* (MIC value of 12.50 and 25.00 µM, respectively). For cytotoxicity testing against three cancer cell lines, lung cancer (A549), breast cancer (MCF-7) and cervix cancer (HeLa) and one human normal cell line, fibroblast (WI-38), langconol C, langcoquinone C and smenospongorine had strong to moderate cytotoxicity against all tested cell lines including both cancer cell lines and normal cell line. However, langconol A, langconol B and polyfibrospongol A exhibited weak cytotoxicity (IC$_{50}$ > 50 µM) for all cell lines.

Table 13

*The bioactivity and isolated compounds from genus Spongia*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meroterpenoids langconol A, langconol B, and langconol C</td>
<td>antibacterial and cytotoxicity</td>
<td>Nguyen et al. (2017)</td>
</tr>
<tr>
<td>langcoquinone C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>polyfibrospongol A and smenospongorine</td>
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</table>
CONCLUSION

In summary, the marine environment of South East Asia has great potential to yield new insights on novel bioactive sponge metabolites because of the wide array of compounds of diverse structures isolated from only a small number of sponge species collected from this region. Although marine organisms are potential sources of many bioactive compounds and are expected to provide more that could combat various chronic diseases, research in this area is still scarce. The discovery of compounds with potential bioactivities from marine organisms is valuable to pharmaceutical research and industry. Many of these compounds show interesting and potent biological activities that suggesting that they may have potential uses as therapeutic agents. Certainly, exploration of the wealth of these marine organisms using cutting-edge technologies needs to be pursued with greater coordination and vigor to fully tap their real value for the betterment of health and the regional economy. Many marine organisms are found in various locations, and some of them show great potentiality, regardless of their geographical origin. In view of the high significance of these marine organisms for pharmaceutical research, further exploration and identification of precious metabolites that may serve as the important basis for the future of drug development require more efforts.

ACKNOWLEGDEMENTS

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Chemical Constituents and Biological Activities of South East Asia Marine Sponges: A Review


LCMS/MS Metabolite Profiling and Analysis of Acute Toxicity Effect of the Ethanolic Extract of *Centella asiatica* on Zebrafish Model

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\(^4\) School of Pharmaceutical Science, Universiti Sains Malaysia, 11800 USM, Minden, Pulau Pinang, Malaysia

**ABSTRACT**

*Centella asiatica* or known as ‘pegaga’ in Malaysia, is a popular medicinal herb, which is being used as main ingredient or incorporated into various herbal products. Apart from efficacy, the chemical profile and potential toxic effect of the plant are two important aspects of concern towards ensuring product satisfaction and safety of consumers. This paper reports the qualitative and quantitative chemical analysis of the leaf ethanolic extract of *C. asiatica* using LCMS/MS. The acute toxicity effect of the extract and selected marker chemical constituents were further analysed using a zebrafish model. Twenty constituents, were identified and the main chemical marker constituents of the plant viz asiaticoside, asiatic acid, and madecassic acid were further quantified. Asiaticoside was found to be present in higher concentration than the other marker constituents. Meanwhile in the acute toxicity test, the LD\(_{50}\) of the extract on the zebrafish model was determined to be 1250 mg/L while 100% mortality was observed at the highest test concentration of 2500 mg/L. However, acute toxicity...
evaluation on four marker triterpenoids of the herb, i.e asiatic acid, madecassic acid, asiaticoside and madecassoside, indicated them to be quite safe on the zebrafish model, with no mortality shown for test concentrations between 10 to 500 mg/kg BW.

**Keywords:** Acute toxicity, *Centella asiatica*, HPLC-DAD-LCMS/MS, metabolite profile, zebrafish model

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**INTRODUCTION**

Plant secondary metabolites have numerous properties with potential applications as pharmaceuticals, agrochemicals, industrial chemicals, flavours and fragrances. Profiling these secondary metabolites, especially in cases where the metabolites are used in their original metabolome such as, extracts, resin or exudates, is an important element in ensuring productivity, efficacy and quality of the botanical product. There is already a growing commercial interest that is fostering the application of metabolite profiling technologies and development of metabolite profiles of various botanical products (Verpoorte et al., 2007). Meanwhile, drug discovery research is very intensive in terms of both costs and the time from discovery to development (Nguyen et al., 2014). Among the reasons is the fact that preclinical researches have been using mainly rodents as the translational animal model which have several limitations such as high costs, low throughput and time-consuming, among others (Stewart et al., 2014). A complementary model to the rodent model would certainly be a beneficial addition towards obtaining a better insight into the pathology of diseases.

The zebrafish is a new promising model in drug discovery research (Howe et al., 2013). This is due to several positive points about its use, including high physiological homology to human (70% of human gene have at least one obvious zebrafish orthologue), high throughput value, genetic tractability, low cost, sensitive to various drugs and pharmacological agents (such as ethanol) and have a short reproductive cycle (Stewart et al., 2013). The pennywort (*Centella asiatica* Linn), is a small, perennial, herbaceous creeper from the plant family Apiaceae. The genus *Centella* itself consists of 50 species which is well-distributed in tropical and subtropical regions of the world (Azerad, 2016). The species is native to tropical countries such as India, Sri Lanka, China, Indonesia, Malaysia, South Africa and Madagascar (Orhan, 2012). The plant is also known by several synonyms such as *C. coriacea* Nannfd., *Hydrocotyle asiatica* L., *H. lunata* Lam., and *Trisanthus cochinchinensis* Lour. Meanwhile, the vernacular names of the plant are also many, such as Indian pennywort, brahma-manduki, brahmi-buti (India), tungchian, luei gong gen (China), gotu kola (Western), pegaga (Malaysia), *Hydrocotyle asiatica* (French), asiatischer wassernabci (German), idrocotile (Itali), bavilacqua (Mauritius) and blasteostimulina (Spain) (Gray et al., 2018). The evergreen herb have been reported to
grow well, mostly in moist, sandy or clay soils (Roy et al., 2009). The small, fan-shaped or round leaf, of about 1.4 to 1.7 cm in size, is the main vegetative part, used as vegetable and traditional medicine in various communities of the world. Based on the available literature, *C. asiatica* is a popular medicinal plant with reported uses ranging from treatment of skin problems (Azis et al., 2017), asthma and digestive disorders (Choudhury et al., 2015), hepatitis and syphilis (Xing et al., 2009), stomach ulcers, mental fatigue, diarrhoea, epilepsy (Kumar & Gupta, 2002), anxiety (Wijeweera et al., 2006) and for memory enhancement (Subathra et al., 2005; Soumyanath et al., 2012). Its dermatological use is the basis for its wide acceptance as an active ingredient in various commercially available cosmetic products (Schaneberg et al., 2003). In Malaysia, an ethnomedicinal survey of plants used by Orang Asli communities in the state of Perak, reported that the leaves were boiled to prepare an infusion used for postpartum treatment (Samuel et al., 2010). The plant is also a popular ‘ulam’ or raw vegetable side dish consumed as part of a healthy diet among the locals. *Centella asiatica* is also widely used in the treatment for epilepsy, for healing wounds and promoted as a healthy tonic drink in ancient times (Brinkhaus et al., 2000). The plant has also been prescribed in other traditional medicine systems such as Ayurveda (Kumar & Gupta, 2002), Traditional Chinese Medicine, Kampo Medicine and African traditional medicine for various related uses (Long et al., 2012). In Traditional Chinese Medicine, the plant is prescribed for dermal problems, heatstroke, diarrhoea, leprosy, hepatitis, jaundice, acute glomerulonephritis, diabetes and cerebrospinal meningitis (Luo et al., 2015; Hsu et al., 2015; Xia et al., 2015). In fact, *C. asiatica* is listed in the Indian Herbal Pharmacopoeia, and the Pharmacopoeia of the People’s Republic of China, as well as in the European Pharmacopoeia (Schaneberg et al., 2003). The main chemical class of compounds found in *C. asiatica* is the pentacyclic triterpenoids present either in their free or glycosidic forms, asiatic acid, madecassic acid, asiaticoside and madecassoside (Figure 1), being a few examples (Shao et al., 2014). Apart from triterpenoids, flavonoids, phenolic acids and sterols (Orhan, 2012) as well as essential oils (Francis & Thomas, 2016) have also been reported.

Botanical products containing *C. asiatica*, either on its own or in combination with other herbs or medicinal plants, are now commonly found in the herbal and nutraceutical products market. Product quality, efficacy and toxicity are relevant issues that require regular and close monitoring so as to ensure the safety of consumers. This paper reports the LCMS/MS metabolite profile and the acute toxicity effect of the alcoholic extract of a locally grown *C. asiatica*, which are vital ground work for the ensuing research on standardization and pharmacological evaluation of the plant extract.
MATERIALS AND METHOD

General Instrumentation
High performance liquid chromatography (HPLC) was performed on Agilent HPLC (1200 series, USA) system equipped with binary gradient pump and a UV detector. The system was controlled by a personal computer using Agilent Chemstation software. Liquid chromatography mass spectrometry (LCMS/MS) analysis was obtained using an ion-trap mass spectrometer equipped with an electrospray ionization (ESI) source, coupled to an Agilent HPLC equipped with a UV detector, binary gradient pumps, C-18 (4.0 x 250 mm I.D, 1.8 µm particle size, Agilent Technology), column oven, sample auto-injector, and controlled with Chemstation software.

Chemicals and Reagents. Analytical grade solvents (methanol and ethanol) were purchased from Fischer Scientific, Malaysia and HPLC grade acetonitrile was supplied by Merck, Germany. Ultrapure water with a resistivity greater than 18m, obtained from a certified Milli-Q system (Millipore, Bedford, MA, USA) was used for HPLC and LCMS/MS analysis. Asiaticoside, asiatic acid and madecassic acid (purity: 98%) were purchased from Sigma Aldrich (St. Louis, USA).

Plant material. The ethanolic leaf extract of C. asiatica was provided by Atta-ur-Rahman Institute for Natural Product Discovery (AuRINS), Universiti Teknologi MARA (UiTM) Puncak Alam, Selangor. A reference voucher specimen (CA-K017X) has been deposited at the Herbarium of AuRINS. The extract was prepared by maceration of powdered, air-dried leaves of C. asiatica in 70% aqueous ethanolic solution, filtered (Whatman filter paper No 1), dried in vacuo and further lyophilized. The final extract was kept at -20°C prior to use.

Zebrafish. Wild type, shortfin adult zebrafishes (Danio rerio), weighing 0.5±0.02 g, were used for all fish experiments. Zebrafish supply was obtained from 3B Aquatics (B.B.
Bangi, Selangor, Malaysia). Each time, upon delivery, the zebrafishes (mixed sex) were maintained at 26 ± 2°C, in holding water (aerated, dechlorinated tap water, pH 7.0 ± 0.5, total ammonia < 0.01 mg/l), on a 14:10 hours (light/dark) cycle. The zebrafishes were acclimatized for at least 7 days before utilized in the toxicity test. All zebrafishes used in the experiment were experimentally naive. They were fed with Tetramin Tropical fish flakes, daily (twice a day) except 24 hours prior to and until the end of experiment time. All fish experimental protocols were approved by Institutional Animal Care and Use Committee (IACUC) Universiti Putra Malaysia (Approval no: AUP-R013/2015). All experiments were conducted in a specially designated room for fish experiment where unwanted disturbances were kept to the minimum.

**Qualitative and Quantitative HPLC Analysis**

**Chromatographic Conditions.** Chromatographic separation was performed on an Agilent column (RP-18, 4.0 mm x 250 mm, 1.8 μm) by gradient elution with mobile phase system made up of water (solvent A) and HPLC grade acetonitrile (solvent B). The mobile phase composition (A:B) was held at 5:95 for the first 5 mins, gradually increased to 15:85 over the next 15 min, was again held constant at 15:85 for the ensuing 5 mins, and then changed to 85:5 in the final 5 mins. The injection volume and temperature were 20 µL and ambient temperature, respectively. The flow rate was kept at 1 mL/min throughout the analysis which was carried out at ambient temperature. The wavelength for peak detection was set to 210 nm. The gradient system was used for both qualitative and quantitative analysis.

**Preparation of Standard and Sample Solutions.** Standard stock solutions of asiaticoside, asiatic acid, and madecassic acid were prepared by accurately weighing and dissolving 2 mg of each standard in 1 mL HPLC grade methanol to give a concentration of 1 mg/mL (1000 ppm). Serial dilutions of 500 ppm, 250 ppm, 125 ppm and 62.5 ppm were then made using HPLC grade methanol solutions. A 1 mg/mL solution of the sample was also prepared in HPLC grade methanol. The prepared solutions were filtered through 0.22 μm filter membrane and the resultant filtrates submitted to HPLC analysis.

**HPLC Method Development and Calibration.** For each of the prepared standard solutions, triplicate HPLC runs were performed and the calibration curves for each was constructed by plotting mean area under curve versus concentration of the standards (µg/µl). The relative standard deviation of the content of each standard was obtained from repeated injections (n=3) of the sample solution. The recovery rate and accuracy of the method was determined by adding 62.5 ppm of each standard to 2000 ppm of the extract. The quantitative determination was carried out twice. Before the calibration curve was determined, the noise level of the system was first determined by running a blank sample
dissolved in methanol. The HPLC method was then validated for its specificity, linearity, accuracy, and sensitivity according to guidelines published by the International Conference on Harmonisation (ICH), 2009.

For assessment of linearity of the developed method, six concentrations of the standard solutions were prepared i.e 2000 ppm, 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 62.5 ppm. Three individually prepared replicate of each concentration was analyzed. The linearity of the method was evaluated by visual inspection of the plot of peak areas as a function of anayte concentration i.e via determination of the regression equation, \( y = mx + c \) (where \( y \) = peak area, \( x \) = concentration of standard, \( c \) = y intercept) and coefficients \((R^2)\) corresponding to the standard compounds. The mean, standard deviation and relative standard deviation (RSD) values for each test concentration was calculated.

Limit of detection (LOD) was measured by conducting serial dilutions of the sample. The lowest concentration of the standard solution was determined by sequentially diluting the sample. The chromatogram was observed and the lowest detectable concentration and RSD was recorded. Six replicates for the lowest detectable concentration was prepared. From this replicates, the standard deviation of the signal obtained was calculated as below equation. Meanwhile the limit of quantitation (LOQ) is the concentration that gives a signal-to-noise ration of 10:1 (a peak with height at least 10 times as high as baseline noise level). The LOD and LOQ values were calculated based on the standard deviation of the response and the slope obtained from the linearity plot of each standard compound, as represented by the following equations:

\[
LOD = \frac{3.3 \delta}{S} \quad \text{LOQ} = \frac{10\delta}{S}
\]

where \( S \) is slope of calibration curve (x axis) and \( \delta \) is standard deviation (y intercept from regression line) and \( S \) is slope of calibration curve.

Accuracy of the method was assessed from sample recoveries which was evaluated by spiking a known concentration of the mixed standards into the extract. Basically, to a prepared the sample solution, 62.5 ppm of each standards were added to 2000 ppm of the extract. The mixture was then subjected to HPLC analysis and the percent recovery calculated according to the following equation:

\[
\text{Recovery} = \frac{\text{detected amount}}{\text{added amount}} = \frac{(A-B)}{C} \times 100\%
\]

where \( A \) is the amount of sample solution with spiked standards, \( B \) is the amount of sample solution, and \( C \) is the added amount of the standards.

**Conditions for LCMS/MS Analysis**

The LC column used for LC-MS/MS analysis was an Agilent C18 reverse phase column with dimensions 4.0 mm (id) x 250 mm (length) and 1.8 µm particle size. Column
temperature was maintained at 50°C. Sample elution was performed in a gradient manner using mobile phase comprising of water containing 0.1% acetic acid (solvent A) and HPLC grade acetonitrile containing 0.1% acetic acid (solvent B). The mobile phase composition (A:B) was gradually increased from 5:95 to 15:85 over 25 min and returned to initial condition (95%) for 5 min for solvent A, and 5% to 85% for 25 min and then decreased to initial condition (5:95) over the next 5 min, for a total LC run time of 30 min. The injection volume was 20 µL and elution was with a constant flow rate of 1.00 mL/min.

For the mass analysis, the source conditions were: nebulizer pressure was 40 psi, drying gas flow was set at 12 L/min, and drying gas temperature was 350°C. Data acquisition was performed by Agilent MassHunter workstation Data Acquisition, while data processing was carried out with MassHunter Qualitative Analysis software. The MS acquisitions were performed in the positive and negative electrospray ionization mode, for the mass range of 50 to 1000 m/z. Additionally, MS/MS experiments were carried out in the automatic and multiple reaction monitoring (MRM) mode. Automatic MS/MS low-energy collision dissociation (CID) was performed at 5-8 eV collision energy. Peak identification was carried out based on comparison with literature values.

**Zebrafish Acute Toxicity Study**

Acute toxicity was evaluated in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines for the Testing of Chemicals (Rufli, 2014).

**Preparation of Test Solutions.** Test solutions were prepared fresh each time. Stock solution of the extract was prepared by dissolving 10 g in 4 L distilled water to form a 2500 mg/L solution. From the stock solution of the extract, serial dilutions were made to prepare 1250, 625, 312.5, and 156.5 mg/L test concentrations. Since pure standard compounds were insoluble in water, test solutions of the compounds were prepared in phosphate-buffered saline (PBS) solution, with vortexing to homogenize the solution. In addition, based on the previous acute toxicity study by Duggina and co-workers (2015) who reported an LD_{50} value of 1000 mg/kg in rats for *C. asiatica* saponin fraction, the test concentrations for the pure compounds in the acute toxicity test were set to 10, 50, 100, and 500 mg/kg.

**Acute Toxicity Test Procedure.** Fifty adult zebrafishes were divided into five groups of ten fishes (n=10), to represent four test concentration groups and one control (distilled water) group. Plastic fish tanks of dimensions 20 cm (length) x 12.5 cm (width) x 11 cm (height) with a 2L volume capacity were used for the test. The tanks were filled to the brim with the respective test solutions. Each group of adult zebrafishes were then released into the respective tanks. Fish mortality was recorded after 2, 6, 24, 48, 72 and 96 hours. The LC_{50} (test concentration that kills 50% of the fishes) value was then determined from
a plot of number of mortality versus concentration. The test was a static test i.e no water or solution change after throughout the experiment.

**Acute Toxicity Test Procedure for Pure Compounds.** One hundred and seventy adult zebrafishes were divided into seventeen groups, with ten fishes per group (n=10), to represent four test concentration groups for each of the four selected pure compounds, and one control (distilled water) group. Test solutions of the pure compounds were intraperitoneally injected to the zebrafishes, with an injection volume of 10 µl as all the fishes have similar body weight (0.5 ±0.02 g). After injection, the fishes were released into test chambers or tanks containing distilled water and their mortality recorded as before. Again, the test was a static test, thus the pH and temperature of the water in the test chambers were monitored daily.

**RESULTS AND DISCUSSIONS**

**LCMS/MS Metabolite Profile of *Centella asiatica* Extract**

The LCMS full scan and total ion chromatograms in the positive and negative modes are shown in Figure 2A to 2C. Careful analysis of the base peaks and MS/MS fragments obtained from the LCMS/MS analysis allowed the tentative assignment of twenty chemical constituents comprising several of the marker triterpenoids of *C. asiatica*, several phenolic acids and flavonoids. Tentative identification of major constituents existed in *C. asiatica* ethanolic extract were listed in Table 1. In the LC chromatogram, the peak at retention time (R<sub>T</sub>) 2.45 min could be assigned to the presence of any of the three isomers chlorogenic acid, crypto-chlorogenic acid or neo-chlorogenic acid based on the [M-H]⁻ at m/z 353.09 and fragment ion at m/z 191.06 for the loss of the caffeoyl moiety ([M-C₉H₇O₃]) in its MS/MS spectrum (Supplementary information). These isomeric phenolic acids, also known as caffeoylquinic acids (CQAs) have been reported previously as major constituents in *C. asiatica* (Abas et al., 2014; Alqahtani et al., 2015; Long et al., 2012). Meanwhile, dicaffeoylquinic acid (DCQA) was also detected at R<sub>T</sub> 4.76 min, based on its [M-H]⁻ ion at m/z 515.12 and fragment ion at m/z 191.06 (Supplementary information). Similarly, the peak was assigned to any of the possible isomers of DCQAs, which also have been reported previously as constituents of *C. asiatica* (Roy et al., 2009).

Apart from phenolic acids, flavonoids are another group of compounds reported in *C. asiatica* (Gray et al. 2018; Krishnaiah et al. 2009). Both positive and negative ion modes were utilized to ionize the flavonoid glycosides and their aglycones, but the negative mode ESI was more sensitive for detection of the flavonoid glycosides in the extract. Quercetin and kaempferol were amongst the major flavonoids detected in the extract. The peak at R<sub>T</sub> 6.33 min showed [M-H]⁻ ion at m/z 301.04 (Supplementary information), which suggested it to be that of quercetin. This was supported by the [1,3-B-H]⁻ ion at m/z 151.00 due to loss
of a \( \text{C}_8\text{H}_6\text{O}_2 \) radical (ring B). Similarly, the peak at \( R_T \) 7.39 min with [M-H]⁻ at 285.04 could be assigned to kaempferol based on the \([^{1/2}\text{B-H}]^{-}\) and \([^{0/4}\text{A-H}]^{-}\) ions at m/z 133.03 and 108.02, respectively, due to losses of \( \text{C}_8\text{H}_6\text{O}_2 \) (ring B) and \( \text{C}_4\text{H}_4\text{O}_2 \) (ring A) radicals. Meanwhile, the flavonoid glycoside, rutin was detected by the peak at \( R_T \) 3.8 min with [M-H]⁻ ion of m/z 609.15, with fragment ions at m/z 301.06 due to the [M-H-Glc-Rha]⁻ ion. Other flavonoids detected were myricetin (\( R_T \) 0.55 min), castiliferol (\( R_T \) 3.06 min), patuletin (\( R_T \) 7.77 min) and naringin (\( R_T \) 13.69 min), tentatively identified based on their [M-H]⁻ and MS/MS fragmentations. All these flavonoids have been reported previously to occur in \textit{C. asiatica}.

It has been reported that the triterpenoid saponins (asiaticoside and madecassoside) and their aglycones, (asiatic acid and madecassic acid) were the most abundant constituents of \textit{C. asiatica} (Gray et al., 2018). In the present study, these pentacyclic triterpenoid compounds were also detected. Asiatic acid was assigned to the peak at \( R_T \) 10.52 min, which showed an [M-H]⁻ ion at ion of m/z 487.34 (Supplementary information). Loss of two molecules of \( \text{H}_2\text{O} \) gave the fragment ion at m/z 451 while a low abundance fragment ion at m/z 441.34 was assigned as a [M-H-HCOOH]⁻ ion, in accordance with that reported by Xia et al. (2015). However, MS/MS experiment failed to produce any significant fragment ions. The peaks for asiaticoside and madecassic acid were detected at \( R_T \) 6.48 and 9.36 min, respectively. Asiaticoside exhibited an [M-H]⁻ at m/z 487, at fragment ions at m/z 453.34, 407.33, which were due to the ions [M-H-2Glc-Rha]⁻ and [M-H-2Glc-Rha-HCOOH]⁻, respectively (Supplementary information). As was the case with asiatic acid, MS/MS experiment on the deprotonated ion of asiaticoside failed to produce any significant fragment ions for asiaticoside.

Madecassic acid, exhibited a pseudomolecular ion [M+2Na-H]⁻ at m/z 549.34 (Supplementary information). Fragment ion at m/z 487 was attributable to [M-2Na-H-HCOOH]⁻. MS/MS experiment on the [M-H]⁻ ion of madecassic acid also failed to produce any significant fragment ions. The peak at \( R_T \) 5.91 min in the LCMS spectrum was tentatively identified as madecassoside based on the [M-H]⁻ ion at m/z 973.50 (Supplementary information). MS/MS fragment ion at m/z 487.34 was due to [M-H-3Glc]⁻ while the fragment ions at m/z 469.33 and 451.32 suggested a consecutive losses of 2 molecules of \( \text{H}_2\text{O} \) to give a [M-H-3Glc-H\text{2O}]⁻ and [M-H-3Glc-2H\text{2O}]⁻ ions, respectively. In addition to these chemical markers for \textit{C. asiatica}, two other triterpenoid saponins were detected, tentatively identified as brahminoside B (\( R_T \) 3.54 min) and quadranoside IV (\( R_T \) 5.90 min), based on their [M-H]⁻ and MS/MS fragmentations.
Quantitative HPLC Analysis of Marker Triterpenoids of *Centella asiatica*

An HPLC method was developed for the quantification of several triterpenoid saponins in the leaf ethanolic extract of *C. asiatica*. To ensure the methodology used for HPLC quantification was reproducible, method validation was carried out using external calibration. The regression and coefficients ($R^2$) corresponding to the triterpenoids are given in Table 2. The calibration curves (Supplementary information) were linear over the selected concentration range (62.5 to 1000 ppm) with $R^2$ equal to or higher than 0.997. According to Shabir 2004, a method may be classified as precise, sensitive and accurate for quantification of compounds, if the precision is around 3% RSD and below, and when the LOD and LOQ is less than 15.

The contents of three *C. asiatica* marker triterpenoids (Table 3) were quantified using the validated HPLC method. The analytical HPLC run was completed in 40 mins. Under the optimized chromatographic conditions, sufficient separation was achieved for the compounds in the *C. asiatica* extract, as shown in Figure 3A. The three marker triterpenoids i.e asiaticoside, madecassic acid, and asiatic acid were detected at $R_T$ 16.01, 20.46 and 22.72 min, respectively. Based on the chromatogram the triterpenoids were not the major constituents of the extract. The chromatograms of the reference standards for confirmatory identification of asiaticoside, asiatic acid, and madecassic acid are shown in Figures 3B to 3D.

The contents of three *C. asiatica* marker triterpenoids (Table 3) were quantified using the validated HPLC method. The analytical HPLC run was completed in 40 mins. Under the optimized chromatographic conditions, sufficient separation was achieved for the compounds in the *C. asiatica* extract, as shown in Figure 3A. The three marker triterpenoids i.e asiaticoside, madecassic acid, and asiatic acid were detected at $R_T$ 16.01,
Table 1

*Tentative identification of major chemical constituents in leaf ethanolic extract of Centella asiatica.*

<table>
<thead>
<tr>
<th>No.</th>
<th>Tentative assignment</th>
<th>R&lt;sub&gt;t&lt;/sub&gt; (min)</th>
<th>Molecular Weight</th>
<th>[M+H]&lt;sup&gt;+&lt;/sup&gt; (m/z)</th>
<th>[M-H]&lt;sup&gt;-&lt;/sup&gt; (m/z)</th>
<th>Molecular formula</th>
<th>MS/MS fragment ions</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Myricetin</td>
<td>0.55</td>
<td>318.24</td>
<td>317.05</td>
<td>317.05</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;</td>
<td>290.05, 215.11</td>
<td>METLIN, ID 3448</td>
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<tr>
<td>2</td>
<td>Bornyl acetate</td>
<td>0.57</td>
<td>196.29</td>
<td>195.05</td>
<td>195.05</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>160.84, 119.80</td>
<td>NIST, No 413981</td>
</tr>
<tr>
<td>3</td>
<td>Catechin</td>
<td>0.71</td>
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<td>289.02</td>
<td>289.02</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>133.01</td>
<td>Massbank : BS003015</td>
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<tr>
<td>4</td>
<td>CQA isomer</td>
<td>2.45</td>
<td>354.31</td>
<td>353.09</td>
<td>353.09</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O&lt;sub&gt;9&lt;/sub&gt;</td>
<td>191.06</td>
<td>Massbank : FIO00626</td>
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<td>5</td>
<td>Castilliferol</td>
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<td>431.19</td>
<td>431.19</td>
<td>C&lt;sub&gt;24&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>123.95, 103.92, 87.92</td>
<td>METLIN, ID 50408</td>
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<tr>
<td>6</td>
<td>Rutin</td>
<td>3.80</td>
<td>610.52</td>
<td>609.15</td>
<td>609.15</td>
<td>C&lt;sub&gt;27&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O&lt;sub&gt;16&lt;/sub&gt;</td>
<td>301.06</td>
<td>Massbank : FIO00596</td>
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<td>7</td>
<td>DCQA isomer</td>
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<td>515.12</td>
<td>515.12</td>
<td>C&lt;sub&gt;25&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O&lt;sub&gt;12&lt;/sub&gt;</td>
<td>191.06, 179.03, 173.04</td>
<td>METLIN, ID 87146</td>
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<tr>
<td>8</td>
<td>Ursolic acid</td>
<td>5.22</td>
<td>456.71</td>
<td>455.19</td>
<td>455.19</td>
<td>C&lt;sub&gt;30&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O&lt;sub&gt;13&lt;/sub&gt;</td>
<td>453.34, 407.33</td>
<td>METLIN, ID 53791</td>
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<td>9</td>
<td>Quadranside IV</td>
<td>5.90</td>
<td>650.85</td>
<td>651.41</td>
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<td>C&lt;sub&gt;36&lt;/sub&gt;H&lt;sub&gt;58&lt;/sub&gt;O&lt;sub&gt;10&lt;/sub&gt;</td>
<td>325.08, 183.99</td>
<td>Pubchem (CID 10372074)</td>
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<tr>
<td>10</td>
<td>Madecassosside</td>
<td>5.91</td>
<td>975.13</td>
<td>973.50</td>
<td>973.50</td>
<td>C&lt;sub&gt;48&lt;/sub&gt;H&lt;sub&gt;57&lt;/sub&gt;O&lt;sub&gt;30&lt;/sub&gt;</td>
<td>191.06</td>
<td>METLIN, ID 94663</td>
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<tr>
<td>11</td>
<td>Quercetin</td>
<td>6.33</td>
<td>302.24</td>
<td>-</td>
<td>301.04</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;</td>
<td>151.00, 121.03, 107.01</td>
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<td>12</td>
<td>Asiaticoside</td>
<td>6.48</td>
<td>959.13</td>
<td>959.52</td>
<td>959.52</td>
<td>C&lt;sub&gt;48&lt;/sub&gt;H&lt;sub&gt;57&lt;/sub&gt;O&lt;sub&gt;19&lt;/sub&gt;</td>
<td>453.34, 407.33</td>
<td>Massbank : BS003708</td>
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<td>13</td>
<td>Asiaticoside B</td>
<td>7.20</td>
<td>975.13</td>
<td>974.50</td>
<td>974.50</td>
<td>C&lt;sub&gt;48&lt;/sub&gt;H&lt;sub&gt;57&lt;/sub&gt;O&lt;sub&gt;20&lt;/sub&gt;</td>
<td>577.14, 361.19</td>
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<tr>
<td>14</td>
<td>Kaempferol</td>
<td>7.39</td>
<td>286.23</td>
<td>285.04</td>
<td>285.04</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>229.28, 150.04, 143.05</td>
<td>Massbank : BML81511</td>
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<td>15</td>
<td>Campesterol</td>
<td>7.39</td>
<td>400.69</td>
<td>-</td>
<td>399.03</td>
<td>C&lt;sub&gt;28&lt;/sub&gt;H&lt;sub&gt;40&lt;/sub&gt;O</td>
<td>229.28, 183.04, 159.04</td>
<td>METLIN, ID 167</td>
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<td>No.</td>
<td>Tentative assignment</td>
<td>Rt (min)</td>
<td>Molecular weight</td>
<td>[M+H]+ (m/z)</td>
<td>[M-H]- (m/z)</td>
<td>Molecular formula</td>
<td>MS/MS fragment ions</td>
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<td>16</td>
<td>Patuletin</td>
<td>7.77</td>
<td>332.26</td>
<td>332.16</td>
<td>-</td>
<td>C_{16}H_{22}O_{3}</td>
<td>51298</td>
<td>METLIN, ID :</td>
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<tr>
<td>17</td>
<td>Madecassic acid</td>
<td>9.36</td>
<td>504.71</td>
<td>503.34</td>
<td>-</td>
<td>C_{30}H_{48}O_{6}</td>
<td>800387</td>
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<td>18</td>
<td>Asiatic acid</td>
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<td>487.34</td>
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<td>810786</td>
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<td>19</td>
<td>Naringin</td>
<td>13.69</td>
<td>580.54</td>
<td>579.28</td>
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<td>C_{27}H_{32}O_{14}</td>
<td>22501.1649.99</td>
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### Table 2
Calibration parameters, linear ranges, limit of detection (LOD) and limit of quantification (LOQ) values for HPLC method for quantitative analysis of marker triterpenoid compounds in leaf ethanolic extract of *Centella asiatica*.

<table>
<thead>
<tr>
<th>Reference standards</th>
<th>Rt (min)</th>
<th>Regression line equation</th>
<th>Linear range (mg/mL)</th>
<th>R²</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asiaticoside</td>
<td>16.01</td>
<td>y=0.874x+10.715</td>
<td>0.0625-1.00</td>
<td>0.997</td>
<td>2.8842</td>
<td>8.7400</td>
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<td>Madecassic acid</td>
<td>20.46</td>
<td>y=0.4045x+1.295</td>
<td>0.189-1.27</td>
<td>0.999</td>
<td>1.3348</td>
<td>4.0450</td>
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<tr>
<td>Asiatic acid</td>
<td>22.72</td>
<td>y=2.7694x+73.41</td>
<td>0.0625-1.00</td>
<td>0.997</td>
<td>1.1915</td>
<td>3.6100</td>
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</table>
Figure 3. HPLC chromatogram of (A) leaf ethanolic extract of Centella asiatica (500 µg/L) (B) asiaticoside (C) madecassic acid (D) asiatic acid, analyzed at 256 nm.

Table 3
Concentration of three marker triterpenoids in the leaf ethanolic extract of Centella asiatica (n=3)

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Asiaticoside (mg/g)</th>
<th>RSD (%)</th>
<th>Asiatic acid (mg/g)</th>
<th>RSD (%)</th>
<th>Madecassic acid (mg/g)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.61±0.01</td>
<td>1.60</td>
<td>0.39±0.002</td>
<td>0.51</td>
<td>0.46±0.01</td>
<td>2.17</td>
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<tr>
<td>2</td>
<td>0.63±0.003</td>
<td>0.47</td>
<td>0.40±0.002</td>
<td>0.5</td>
<td>0.49±0.002</td>
<td>0.41</td>
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<tr>
<td>3</td>
<td>0.59±0.001</td>
<td>0.17</td>
<td>0.39±0.01</td>
<td>2.50</td>
<td>0.47±0.001</td>
<td>0.21</td>
</tr>
</tbody>
</table>
Generally, asiaticoside and madecassoside are the most abundant triterpenoids found in *C. asiatica*, and the concentrations of these compounds may vary depending on the origin, environment, and time of harvest, as well as the processing methods used. In the present study, the range of concentrations for asiatic acid, asiaticoside and madecassic acid were between 0.39-0.40, 0.59-0.63, and 0.46-0.49 mg/g of extract, respectively. The amount of asiaticoside in was slightly higher compared to the content reported for the plant from India with 0.31 mg/g (Gupta, et al., 2014), and from Germany with 0.18-0.52 mg/g (Günther & Wagner, 1996). However, another recent study reported higher contents of the triterpenes in *C. asiatica* from Malaysia, with 7.9 and 11.5 mg/g of asiaticoside and 9.7 and 16.5 mg/g of madecassoside (Azerad, 2016). Due to unavailability of the reference standard, madecassoside was not quantified in the present study. However, the contents of the triterpenoid glycosides are usually reflective of the triterpenoid aglycones (Günther & Wagner, 1996). In the present study, the content of madecassic acid was found to be slightly higher than asiatic acid. Thus, in the same way, the content of madecassoside in the present extract was also expected to be higher than that of asiaticoside.

**Acute Toxicity Effect of *Centella asiatica* Extract on Zebrafish Model**

The acute toxicity effect of the leaf ethanolic *C. asiatica* extract (RECA) on adult wild-type zebrafish was further assessed. Exposing the zebrafishes to the extract at test concentrations of 156.5 and 312.5 mg/L for up to 96 hours did not produce any mortality in the zebrafishes. However, at higher test concentrations, the mortality of the zebrafish model was affected. Half of the zebrafishes died at a test concentration of 1250 mg/L, while at the highest test concentration of 2500 mg/L, 100% death was recorded. From the dose-response curve shown in Figure 4, the LD_{50} for the extract was determined to be 1250 mg/L.

![Figure 4. Dose-response curve for acute toxicity effect of leaf ethanolic extract of *Centella asiatica* (RECA)](image-url)
A previous experiment on rodents showed that up to 1000 mg/kg BW of *C. asiatica* water extract showed no mortality on the animals (Chivapat et al., 2011). An LD$_{50}$ value of 1000 mg/kg on the rodents was reported for the triterpenoid saponin-rich fraction of the extract. Deshpande et al. (2015) also reported no toxicity on rodents when they were exposed for up to 14 days to a standardized water extract of *C. asiatica* at a test concentration of 2000 mg/kg BW. In another acute and sub-acute toxicity study of the acetone leaf extract of *C. asiatica*, it was found that up to 1000 mg/kg BW of oral administration did not cause any significant effect on behaviour, sensory nervous system, breathing and sensory nervous system response in mice (Chauhan & Singh, 2012).

The acute toxicity results in the present study showed that as a test organism, zebrafish is more robust and sensitive compared to rodents. Since it is a smaller animal compared to rodents, a zebrafish’s ability to detect toxicity of a substance is better because the range of toxicity can be better observed. Another advantage is that the experiment is easier to perform because the fishes need to be only immersed in the test solution, whereas in rodents, the test materials have to be administered orally or via injection. Nevertheless, a major drawback in using zebrafish as model for toxicity studies compared to rodents is that some pathological protocol may be quite difficult to perform due to the small size of the zebrafish’s organs.

The acute toxicity effect of four marker triterpenoids of the extract, i.e asiatic acid, madecassic acid, asiaticoside and madecassoside, were also assessed. A ninety-six hours exposure to each of the compounds at test concentrations of 10 to 500 mg/kg did not cause any mortality in the adult, wild-type zebrafishes.

**CONCLUSION**

Toxicity evaluation is an important aspect of pharmacological research and the quality control of plant-based health products. Due its special characteristics, the zebrafish have become an emerging vertebrate model organism in toxicological and pharmacological studies because it offers a viable, quick and inexpensive alternative to test hypothesis and generate strategies for complementary research using rodent models and human subjects (Altemus et al., 2014; Greenleaf et al., 2014). In the present study, undertaken to determine the lethal concentration prior to its use in further experimental pharmacological studies, the standardized leaf ethanolic extract of *C. asiatica* affected the adult zebrafish survivability at concentrations above 1000 mg/kg BW for exposure up to 96 hours. This was in contrast to previous reports on the medicinal herb’s non-toxic effect on rodents at similar range of test concentrations. However, the toxicity was not due to asiatic acid, madecassic acid, asiaticoside and madecassoside, the marker constituents which were qualitatively and quantitatively shown to be present in the ethanolic extract, based on the zero mortality
shown up to 500 mg.kg BW. It is possible that other constituents may be contributing to the toxicity of the extract.

ACKNOWLEDGEMENTS
The authors thank the Ministry of Agriculture (MoA) for financial support through FRGS grant (No 538006), and Prof Ilham Adenan (AURINS) for providing *C. asiatica* extract. Fauziahanim Zakaria thanks Universiti Sains Malaysia for providing scholarship (ASTS scheme).

REFERENCES


The Mineralogy and Chemical Properties of Sedimentary Waste Rocks with Carbon Sequestration Potential at the Selinsing Gold Mine, Pahang

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ABSTRACT

Waste rocks are a non-economical by-product of mining operations, which can lock up carbon dioxide into a carbonate form and thereby help reduce greenhouse gases emissions. The aims of this research are to determine the mineral and chemical composition of the sedimentary waste rocks of gold mines and to classify the potential of silicate minerals to be a feedstock for carbonation mineralization. The sampling was undertaken at the Selinsing gold mine, where waste rocks were collected from the waste dump, stockpiles, the borrow pit, and the main pit. The mineralogical and chemical component of the sedimentary waste rocks were explored using X-ray diffraction and energy dispersive X-ray spectroscopy. The findings indicated that the presence of divalent cations, of 55.12% for CaO, 9.09% for MgO, and 16.24% for Fe₂O₃ from gold mine waste, capable of sequestering carbon dioxide into calcium, magnesium and iron carbonates, respectively, through carbonation of mineral. The domination of silicate minerals such as quartz, muscovite, kaolinite, chlorite, albite, and carbonate minerals such as calcite, have been found to be widespread in sedimentary waste rocks. However, the natural silicates (chlorite, muscovite) and carbonates (calcite) are potential minerals which can be consumed as feedstock for carbonation processes because they contain the magnesium, iron, and calcium elements which can form stable carbonates in the...
presence of carbon dioxide. The mineralogy and chemical composition of sedimentary waste rocks from the Selinsing gold mine provides a better understanding of the future carbonation reaction to sequester more carbon dioxide in response to climate change.

**Keywords:** Carbonation reaction, carbon sequestration, chemical composition, mineralogy, sedimentary rock, Selinsing gold mine

**INTRODUCTION**

Carbon dioxide (CO$_2$) emissions are at a current level of 257.69 million tonnes (Mt), as of 2014, and are expected to increase to 12.1 tonnes of CO$_2$ emission per capita by 2020 (Zaid et al., 2015). To reduce the release of CO$_2$ into the environment, carbon capture and storage (CCS) is one reliable technique which can help achieve 20% CO$_2$ emission reductions (Benson & Cole, 2008), made possible by storing CO$_2$ permanently in a stable carbonate form (Arce et al., 2017; Lackner et al., 1995; Renforth, 2011). The formation of carbonate requires potential divalent cations, such as magnesium, iron, and calcium (Mg-Fe-Ca), which are more reactive with CO$_2$ in terms of forming carbonate minerals (Lechat et al., 2016; Wilson et al., 2009). The availability of divalent cations in mine waste rocks from ultramafic-hosted ore residue, are usually favorable for mineral carbonation (Hitch et al., 2010). Therefore, the waste of the mine can be used as potential feedstock for carbon sequestration.

Rocks and minerals play an important part, as reactive agents, in decreasing CO$_2$ discharge during carbon sequestration (Li & Hitch, 2015; Renforth et al., 2011; Wilson et al., 2009). For instance, igneous rocks such as basalts contain silicate minerals, including feldspars, plagioclase, pyroxenes, and others, which can react with dissolved CO$_2$ to form carbonates (Jorat et al., 2017). Additionally, sedimentary rock such as shales have the potential to store significant quantities of CO$_2$ dissolved in liquid formation, trapped by mineral carbonation or absorbed into organic mixtures or the surfaces of minerals (Busch et al., 2008).

In this study, the potential of obtaining sedimentary rock from gold mining waste has been explored as a potential feedstock for carbon sequestration through the mineral carbonation process. This study’s objective are to determine the mineral and chemical compositions of waste rock samples from various types of gold mine waste, and to classify the potential of silicate minerals as a feedstock for carbonation mineralization in the waste rock samples of gold mine waste.

**MATERIALS AND METHODS**

**The Sampling Area of the Selinsing Gold Mine**

Field sampling was undertaken at an active gold mining area in Selinsing, Pahang with coordinate of N 4°15'0'', E 101°47'10'', which itself is in a prominent gold mining region
in Peninsular Malaysia (Makoundi et al., 2014; Yeap, 1993). Waste rock samples had been collected at seven sampling points which consisted of stockpiles of high grade (HG), lower grade (LG), super lower grade (SLG), waste dump, borrow pit, and the main pit which consisted of open pit 1 and open pit 2 (Figure 1). The host rock at the Selinsing gold mine consists of sedimentary rocks, including siltstone, argillite, phyllite, carbonaceous shale, grey-black limestone, sandstone, and tuffaceous conglomerate (Makoundi et al., 2014; Pour & Hashim, 2015). Minerals such as quartz (SiO$_2$), dolomite [CaMg(CO$_3$)$_2$], and pyrite (FeS$_2$) are all widely distributed in gold mines (Makoundi et al., 2014).

**Mineralogical Analysis**

Prior to analysis, waste rocks were first crushed manually, grounded into fine particles, and then sieved to a one mm size component using grain sieves. Then the sieved samples were grounded again into a very fine powder form, using an octagonal agate mortar and pestle made of natural quartz. About 1 ± 0.5 g of the fine powder samples were placed in a 2.5 cm diameter circular specimen holder of polymethylmethacrylate (PMMA), before being attached to an X-ray machine. The fine powder of waste rocks was analysed using an X-ray diffractometer (XRD) instrument (model Philips X’Pert Pro Pranalytical-PW3440/60, Netherlands) at a 1°/min rate (0.02° step size), counting for 0.2 s per step over the scattering angle range between 5-50°. The detection limit range was between 1 to 2%. The integrated peak areas intensity from the single peak function was determined using Diffrac AT EVA software version 9.0, and the d-spacing was identified using the OriginPro 8 software (Originlab Corporation, Northampton, UK).
Chemical Analysis

A chemical composition analysis was conducted using an energy dispersive X-ray (EDX) to measure in a percentage the elemental composition of waste rock. The whole waste rocks from the Selinsing gold mine were analysed in a fine powder form (< 2 mm size fraction). Samples were made to be as homogenous as possible, so to minimize error while performing EDX. About 1 g of homogenous fine powder samples were introduced to the EDX machine, for the purpose of phase determination. The values obtained for Ca, Mg, Fe, Si, Al, and K were then converted in terms of oxides correspondence, with 0.1% detection limits.

RESULTS AND DISCUSSION

Mineralogy and Chemical Composition of Waste Rock

Seven types of crystalline phases were classified in the waste rock samples drawn from the various kinds of gold mining wastes at Selinsing, Pahang (Figure 2). The mineralogy of the gold mining waste consists of silicate minerals, such as quartz (SiO$_2$), muscovite [K(Mg,Fe)$_3$(AlSi$_3$O$_{10}$)(OH)$_2$], chlorite [(Mg,Fe,Al)$_6$(Si,Al)$_4$O$_{10}$(OH)$_8$], kaolinite [Al$_2$Si$_2$O$_5$(OH)$_4$], albite (NaAlSi$_3$O$_8$); and carbonate minerals such as calcite (CaCO$_3$). The highest peak of quartz, signified a major mineral found to be widespread in the waste rock of gold mining wastes. This is because quartz is known to be a primary mineral that is resistant to weathering and commonly discovered in every type of rock (Kusin et al., 2018; Shamshuddin, 2011).

Figure 2. The XRD diffractograms of the waste rock from: (a) the waste dump (WD), the borrow pit (BP), stockpile (HG, LG, SLG); and from (b) the main pit, included limestone (LS), open pit 1 (OP1), and open pit 2 (OP2). The inserted symbols indicate the peaks for all type of mining wastes, with the orange circle being quartz, the green square being graphite, the purple triangle being muscovite, the red star being calcite, the blue diamond being chlorite, and the black hexagon being kaolinite.
Results have shown a domination of silicate minerals, explained by high percentage of SiO₂ and Al₂O₃, which were widely discovered at the stockpile SLG and at the waste dump at 71.06% and 24.35% respectively (Table 1). A high percentage of SiO₂ and Al₂O₃ shows the existence of muscovite, kaolinite, chlorite, and albite in gold mining wastes. This is because some of these minerals are formed during the chemical weathering of alumino-silicates (Kusin et al., 2017; Shamshuddin, 2011), which are found in different types of rock including conglomerate, phyllite, carbonate, shale, and tuffs (Makoundi et al., 2014; Pour & Hashim, 2015). Since the host rock at the Selinsing gold mine comes from sedimentary rocks, those minerals are expected to be found in each type of mining waste.

**Table 1**  
The chemical composition of waste rock, extracted from gold mining waste at the Selinsing, Pahang

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sampling Location</th>
<th>Waste Dump</th>
<th>Borrow Pit</th>
<th>Stockpile HG</th>
<th>Stockpile LG</th>
<th>Stockpile SLG</th>
<th>Limestone</th>
<th>Open Pit 1</th>
<th>Open Pit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgO*</td>
<td>-</td>
<td>5.74*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.14*</td>
<td>2.21*</td>
<td>-</td>
</tr>
<tr>
<td>SiO₂</td>
<td>54.81</td>
<td>62.91</td>
<td>70.17</td>
<td>61.35</td>
<td>71.06</td>
<td>22.92</td>
<td>61.53</td>
<td>68.34</td>
<td>-</td>
</tr>
<tr>
<td>CaO*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>55.12*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe₂O₃*</td>
<td>5.71*</td>
<td>4.85*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.89*</td>
<td>1.79*</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>24.35</td>
<td>22.27</td>
<td>24.17</td>
<td>21.62</td>
<td>14.55</td>
<td>2.60</td>
<td>18.64</td>
<td>18.23</td>
<td>-</td>
</tr>
<tr>
<td>K₂O</td>
<td>3.94</td>
<td>6.51</td>
<td>5.10</td>
<td>8.91</td>
<td>4.85</td>
<td>-</td>
<td>5.09</td>
<td>6.82</td>
<td>-</td>
</tr>
<tr>
<td>SO₃</td>
<td>-</td>
<td>0.57</td>
<td>3.31</td>
<td>-</td>
<td>0.26</td>
<td>-</td>
<td>1.11</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Note:* *indicate the divalent cation which can influence the mineral carbonation process

**The Potential of Silicate Minerals for Mineral Carbonation**

This studies’ findings have indicated that the total 9.09% of MgO can be explained through the presence of chlorite in the waste rocks of the waste dump, limestone, and open pit 1, while the 55.12% of CaO apparently come from calcite in limestone (Table 1). Both MgO and CaO are potential divalent cations required for the mineral carbonation process to occur (Lechat et al., 2016; Wilson et al., 2009). The carbonation reaction of MgO and CaO in the existence of CO₂, producing carbonate minerals such as magnesium carbonate (MgCO₃) (reaction 1) and calcium carbonate (CaCO₃) (reaction 2), respectively (Lackner et al., 1995; Lechat et al., 2016; Power et al., 2013; Renforth, 2011; Wilson et al., 2009):

\[
\text{MgO} + \text{CO}_2 \rightarrow \text{MgCO}_3 \quad (\text{Delta Hr} = 118 \text{ Kj/mol}) \quad (1)
\]

\[
\text{CaO} + \text{CO}_2 \rightarrow \text{CaCO}_3 \quad (\text{Delta Hr} = 179 \text{ Kj/mol}) \quad (2)
\]
In the context of carbon sequestration, CO$_2$ reacts with the divalent cation (Ca-Mg-Fe) to produce permanent carbonates (Lechat et al., 2016; Wilson et al., 2009). The results suggest that chlorite is the most promising mineral for the carbonation process, due to the presence of the divalent cation of Mg in chlorite, which makes it capable of reacting with CO$_2$ to form a carbonate mineral, as proven in the reaction below (Hitch et al., 2010; Power et al., 2013; Renforth et al., 2011):

$$(\text{Mg,Fe,Al})_6(\text{Si,Al})_4\text{O}_{10}(\text{OH})_8 + 6\text{CO}_2 \rightarrow 6(\text{Mg,Fe,Al})\text{CO}_3 + 4(\text{Si,Al})\text{O}_2 + 4\text{H}_2\text{O}$$

The finding is in line with recent research, which has indicated that chlorite is the potential silicate mineral containing the divalent Mg, which is promising for the carbonation of mineral (Hasan et al., 2018). Furthermore, carbon sequestration is very effective in the Mg and Ca-rich minerals included in mining waste, in term of sequestering more CO$_2$ (Assima et al., 2014; Wilson et al., 2009). The occurrence of silicate minerals of Mg in the waste dump, and in open pit 1, indicates that CO$_2$ can be sequestered to form a magnesium carbonate such as magnesite (MgCO$_3$) (Jacobs, 2014). Therefore, the availability of chlorite in gold mining waste, is a potential silicate minerals which can be consumed as feedstock for the mineral carbonation process.

Carbonate minerals such as calcite are present in the limestone extracted through gold mining, because it is favorable in all types of rock, including in the sedimentary rocks of the Selinsing gold mine (Makoundi et al., 2014). In carbon sequestration, calcite may act as a reservoir for carbon storage, because it is already in a stable form. Accordingly, CO$_2$ is naturally stored in limestone rock (Mani et al., 2008).

The highest percentage of ferric oxide (Fe$_2$O$_3$) has been found in the waste dump (5.71%) (Table 1), known as a potential divalent cation which can be sequestered into iron carbonate (FeCO$_3$) (Vogeli et al., 2011). The total 16.24% of Fe$_2$O$_3$ is explained through the presence of muscovite at all sampling points, and of chlorite at the main pit and the waste dump (Figure 2). Therefore, the Fe-silicate minerals in gold mining wastes, such as muscovite and chlorite, can be utilized as feedstocks for enhancing the process of mineral carbonation.

CONCLUSION

This study describes the mineralogy and chemical composition of sedimentary waste rocks from gold mining waste, in term of their potential for carbon sequestration. The waste of the Selinsing gold mine in Pahang has the potential for permanent CO$_2$ storage, due to the presence of divalent cations, including MgO at 9.09%, CaO at 55.12%, and Fe$_2$O$_3$ at 16.24%, which can promote the formation of magnesium, calcium, and iron carbonate, respectively, through the carbonation process. The presence of natural silicate minerals like chlorite and muscovite, and of carbonate minerals like calcite in gold mine waste, can be
Carbon Sequestration Potential at Gold Mine, Pahang, Malaysia

potential feedstock for carbon sequestration. This is because the minerals are rich in the Mg, Fe, and Ca divalent cations, which are effective in the mineral carbonation process. Therefore, the potential of mineral and chemical components of gold mining waste for supporting the mineral carbonation process, can help sequester long term CO$_2$ storage.

ACKNOWLEDGEMENTS

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REFERENCES


Fabrication of Scaffold in Tissue Engineering using Selective Laser Sintering Process

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²Pillai College of Engineering, New Panvel, Navi Mumbai, Maharashtra 410206, India

ABSTRACT

Bone defects are serious complications that are caused by extensive trauma or tumor. The traditional therapies fail to repair these defects. Tissue engineering scaffold can be used to regenerate the damaged tissue. This paper describes an experimental investigation of bone scaffold to measure the porosity using gas porosimeter and suggest an alternative to bone scaffold. The prototype was made using additive manufacturing of selective laser sintering technique. The material chosen for the study was synthetic polymer PA12. The behaviour of actual bone and prototype had been observed under compressive load of fixed interval loading condition. Mechanical properties of polymer had been evolved and compared with actual bone.

Keywords: Additive Manufacturing (AM), bone scaffold, polymer (PA12), Selective Laser Sintering (SLS), Young’s Modulus (E)

INTRODUCTION

Every day a number of surgical procedures is performed to replace or repair tissue that has been damaged through disease or trauma, combining cells from the body with highly porous scaffold biomaterials. Tissue engineering is a science in which the damaged tissues can be replaced with the porous biomaterials (O’brien, 2011; Atala, 2004; Bonassar & Vacanti, 1998). Literature review shows that researchers are facing problem to replace or regenerate the tissue in the human body.

Surgical treatment which typically focuses on transplanting tissue from one site to another in the same patient is called as autograft and from one individual to another called as a transplant or allograft (O’brien, 2011). Problems faced in these techniques are harvesting autografts. An
autograft which is expensive, painful, constrained by anatomical limitations. Autograft is associated with donor-site morbidity due to infection and hematoma (O’Brien, 2011). Similarly, allografts or transplants also have serious constraints due to problems with accessing enough tissue for all of the patients who require them. Also there are risks of rejection by the patient’s immune system and the possibility of introducing infection or disease from the donor to the patient. 

Alternatively, tissue engineering helps to regenerate damaged tissues, instead of replacing them, by developing biological substitutes that restore, maintain or improve tissue function (O’Brien, 2011; Atala, 2004; Bonassar & Vacanti, 1998). The basic requirement of scaffold is that it must be biocompatible and its strength should be in such a way that it should withstand body weight. An attempt has been made to suggest an alternate material which will fulfill basic requirement of scaffold. In this paper an experimental investigation of bone scaffold is carried out to suggest an alternative material (PA12). Mechanical properties of PA12 are compared with actual bone scaffold. The material chosen for the study is PA12, which is a synthetic polymer (Lu et al., 2000; Oh et al., 2003; Rowlands et al., 2007).

As discussed in previous section bone scaffold must be porous. Porosity is the ratio of void volume to the total volume. Porosity is essential in any bone scaffold because it gives strength to the replaced part when the cell grows. Also it goes into the porous structure and provides strength to the scaffold. As porosity increases strength of the bone decreases. Literature shows various techniques to measure porosity of bone (Amziane & Collet, 2017). These techniques can be employed based on the size of the pores. Experimental techniques to measure porosity are as follows.

**Mercury Intrusion Porosimetry (MIP)**

In MIP, the volume of liquid that penetrates a solid is measured as a function of applied pressure. The analysis is based on the capillary law governing liquid penetration into small pores. Since mercury is a non-wetting liquid for most materials, an externally imposed pressure is required to force it into the pores of a porous solid. The smaller the pore size, the greater the pressure required to force the mercury, into the pore. This technique is effective when pore size is larger than about 3.5 nm (Lawrence et al., 2007).

**Gas Expansion Method**

This is one of the techniques used to measure porosity of bone sample as well as polymer. A sample is placed into an airtight holder and pressure is applied to a reservoir of known volume. After the pressure has stabilized, valve is opened, which permits the gas within the reservoir to expand into the holder. After equilibrium is reached, the new pressure of the system is measured and recorded. It works on the principle of Boyle’s law.
After reviewing extensive literature (Lawrence et al., 2007; OFITE, 2009; Wong & Hernandez, 2012; Slotwinski & Garboczi, 2015; Mueller, 2012; Bidanda & Bártolo, 2007), it is observed that prototype of scaffold can be prepared by AM techniques. Widely used AM techniques are Stereo Lithography (SLA), Fuse Deposition Modelling (FDM), 3D Printing (3DP) and Selective Laser Sintering (SLS) (Salmoria et al., 2008). Using these techniques solid object can be created. Out of these techniques one of the applicable techniques is SLS technique. This technique uses layer by layer through the heating and fusion of powder material using an infrared laser beam. The main advantage of this technique is that porous prototype can be fabricated. This will help get the required strength and surface finish. The other advantage of SLS technique is that no post curing required.

MATERIALS AND METHODS

Sample Preparation

Scaffold bone specimen and its prototype (PA12) specimen were chosen for the study. Procedure to prepare samples for the analysis is as follows:

Procedure for Scaffold Bone. A fresh, part of femur bone was chosen as a specimen for the study. The size of bone chosen for the study was 2:1 ratio (length is two times its diameter). Figure 1 shows actual bone sample specimen. The size of actual femur goat bone is having outside diameter of 13.47 mm, thickness of 2.04 mm and length of 26.97 mm. Considering limitation of experimental setup and availability of actual bone sample the above size had been chosen for the study.

The specimen dipped into a hydrogen peroxide solution for 12 hours to clear the soft tissue or flesh. Hydrogen peroxide is a natural antiseptic chemical which bleaches the bone without damaging it and also soften the tissue. The mixture of hydrogen peroxide and water was used for cleaning the bone and also less concentric mixture was used, which helped to sterilize the bone and also bone would not dissolve the tissue. Specimen was rinsed well in cold water to remove white powder residue on the bone surface.

Figure 1. Actual bone scaffold of goat under investigation
Procedure for Scaffold Bone Prototype. The criterion for the selection of material in order to prepare a prototype of bone scaffold is its biocompatibility and strength. A synthetic polymer PA12 material was used to prepare prototype of actual bone. AM technology (SLS) was used with CO₂ laser which had a wave length of 10.6 mm and laser beam diameter of 250 mm. The process parameter used to prepare prototype had a laser scan speed of 45 mm/s and chamber temperature 120°C. The property of PA12 material and bio-characteristics are shown in Table 1 and Table 2.

Table 1
Properties of PA12 material

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Properties</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tensile modulus</td>
<td>1500 – 1800 MPa</td>
</tr>
<tr>
<td>2</td>
<td>Ultimate strength</td>
<td>40 – 45 MPa</td>
</tr>
<tr>
<td>3</td>
<td>Elongation at break</td>
<td>10 – 20 %</td>
</tr>
</tbody>
</table>

Table 2
Bio-characteristic of PA12 material

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Bio-characteristic</th>
<th>Human Body Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Biocompatibility</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Biofunctional</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Bioactive</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Bioinert</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Sterilizable</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Figure 2. Schematic of Gas Porosimeter for measurement of grain volume of bone scaffold
The procedure to measure the porosity is as follows:

Boyle’s law used to find the grain volume \( V_g \) of the scaffold (Figure 2). Bulk volume \( V_b \) can be obtained by using hand held scanner and difference of bulk volume and grain volume to the bulk volume gives the porosity.

Porosity is calculated by using following formula

\[
\text{% Porosity} = \left[ \frac{V_b - V_g}{V_b} \right] \times 100
\]

**Experimental Setup**

An experimental set up shown in Figure 3 had been developed to measure the porosity of bone scaffold and its prototype. The test was carried out at the constant temperature. Nitrogen gas was used to carry out test on goat sample with a pressure of up to 1.37895 N/mm\(^2\) (200 PSI). A reference cell was having a known volume, \( V_1 \) and pressure, \( P_1 \). The sample cell was for testing core samples of approximately 4 cm in diameter by 5 cm long with volume \( V_2 \) and pressure \( P_2 \). A digital pressure gauge with 0.01 N/mm\(^2\) accuracy was attached to find the pressure difference between sample cell and reference cell. The vacuum pump was used for evacuation of nitrogen gas.

![Figure 3. Gas porosimeter setup to measure the grain volume of bone scaffold](image)

Two chambers with known volumes were connected by a non return valve. The tested sample was placed in the sample cell whose volume was \( V_2 \). The pressure in this chamber was \( P_2 \). The second reference cell (volume \( V_1 \)), initially at pressure \( P_1 \), was connected to the first by opening the valve between them, thus permitting the gas to expand isothermally. \( P_3 \) was the equilibrium Pressure in the system with Sample. Volume \( V_3 \) was obtained by Boyle’s law \( P_1 V_1 = P_3 V_3 \) Grain volume \( V_g \) was obtained by difference of volume of the system \( V_2 \) without sample and the volume of system was with sample \( V_3 \). By scanning
the bulk volume \(V_b\) was obtained as 2.13 cm\(^3\). The procedure to obtain bulk volume \(V_b\) is shown in sec 2.1.2. This bulk volume \(V_b\) was required to compute percentage porosity of bone scaffold. For the prototype of bone scaffold the percentage of porosity was obtained as 16.94%. Whereas the percentage of scaffold bone was 14.04%. To evolve mechanical properties the percentage porosity plays a key role which is discussed in next section.

**RESULTS**

**Structural Analysis of Bone Scaffold and Polymer**

Table 3 shows test result for bone sample and PA12 polymer prototype. Test was conducted on universal testing machine (UTM) under uniaxial compressive load. Compressive load was applied in the interval of 100 N. Behaviour of polymer specimen had been compared with bone scaffold. Evolution of material properties like young’s modulus (E), stiffness are discussed in the following paragraph.

Figure 4 shows the load versus deflection for the actual bone and PA12 polymer. The behaviour of actual bone and PA12 material was observed at an interval of 100 N. It was observed that initially up to 600 N deflections were directly proportional to load. Only 7.34 % variation was observed in actual bone and polymer. After 600 N, 16.31 % variation in deflection was observed. Maximum load i.e. at (2280N) the deflection of 4.112 mm was for actual bone sample and for polymer prototype 4.511 mm was observed.

The behaviour of both samples under specific interval loading condition was observed by plotting stress versus strain graph. Figure 5 shows the stress versus strain plot for the bone and PA12 polymer. Initially stress is directly proportional to strain for both the samples, this shows elasticity of material. From the above Figure 4, it is observed that
Table 3
Test results for PA12 specimen and bone scaffold

<table>
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<th>Sr. No</th>
<th>Load (N)</th>
<th>Deflection (mm)</th>
<th>Strain</th>
<th>Stress (MPa)</th>
<th>Youngs Modulus (MPa)</th>
<th>Stiffness (N/mm)</th>
<th>Deflection (mm)</th>
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polymer specimen shows 9 % deviation with the bone sample. This difference may be because of porosity difference in the samples. Maximum ultimate stress for bone sample and prototype is 31.148 MPa. As discussed in above section, approximately 20% porosity difference is observed in prototype, which affects strength of the polymer. Hence this study can be further extended to improve strength of the polymer material by using composite material, which should be biocompatible and should give adequate strength, to withstand load.

Figure 5 shows the stress-strain curve for polymer prototype and bone sample.

Figure 6 shows the deflection versus Young’s modulus for the polymer (PA12) and bone. At each point young’s modulus of PA12 is observed. It is also seen that PA12 material does not have adequate strength as compared to bone material. It is observed that at maximum deflection of bone is at 4.511 mm, the young’ modulus is a 186.22 MPa and the deflection for PA12 is 4.112 mm and the Young’s modulus is 209.04 MPa. The difference between PA12 is 10.44 % difference with the bone sample. Further study had been extended to observe the characteristic property of material i.e. stiffness. The effect of various stiffness ratios on change in deflection had been studied.

Figure 7 shows effect of various stiffness ratios ($K_{poly}/K_{bone}$) on percentage change in deflection. From the Figure 7, it is observed that polymer material shows 30.23 % difference with the bone sample. From the graph it is observed that with increase in stiffness ratio percentage, difference of polymer with the bone material is less than 9 %. As brought out in above section. This study could be useful to get exact value of stiffness of the PA12 material. It is seen that the % deflection in case of bone is 30% more compared to that of bone scaffold.
DISCUSSIONS

Recently biomechanical science is an emerging area to regenerate damaged tissue. Hence study has been performed to suggest an alternate for human scaffold using PA12 biocompatible material. An important property of PA12 polymer is porosity, which gives strength to the replaced part. An experimental investigation has been performed to measure porosity of bone scaffold and its prototype. Behaviour of bone scaffold and its prototype has been analyzed. It is observed that strength of the polymer can be enhanced by using composite biomaterials.

CONCLUSION

Biocompatible polymers are synthetic or natural polymers used to replace part of a living system or to function in intimate contact with living tissue. It intended to interface with biological system to evaluate, treat or replace any tissue, organ or function of the body. A
polymer will be considered biocompatible, if it allows the body to function without any complications such as allergic reactions or other side effects. Considering this issue polymer PA12 had been selected which was compatible with human body.

The study reveals that to replace actual bone, synthetic polymer can be used. The material should be biocompatible and should have sufficient mechanical strength to withstand load. The material chosen for the study is PA12 synthetic polymer material. The prototype of scaffold bone has been manufactured using selective laser sintering, which is an additive manufacturing technique. An experimental set-up has been developed to measure porosity of actual bone and polymer scaffold.

The structural analysis of bone and polymer has been performed. Material property of polymer like young’s modulus (E) shows 1.03% difference with the bone sample. The prototype shows more deformation before ultimate load. It is seen that polymer material does not have adequate strength as compare to bone material. The study can be further extended to get required strength in the polymer. Therefore there is need to have composite biomaterials for the human scaffold like chitin, chitosan, nanocrystalline cellulose. This can enhance the mechanical properties of bone.

REFERENCES


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