Pertanika J. Trop. Agric. Sc. 42 (1): 61 - 77 (2019)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

The Monitoring of Organophosphorus and Carbamate Insecticides and Heavy Metal Contents in Paddy Field Soils, Water and Rice (*Oryza sativa* L.)

Sirikul Thummajitsakul[1]*, Chanon Sangdee[1], Suteema Thaisa[1], Penpuck Ruengwiroon[1] and Kun Silprasit[2]

[1]*Division of Health Promotion, Faculty of Physical Therapy, Srinakharinwirot University, Ongkharak, Nakhon-Nayok, 26120, Thailand*
[2]*Faculty of Environmental Culture and Ecotourism, Srinakharinwirot University, Bangkok, 10110, Thailand*

ABSTRACT

The aim of this work was to monitor organophosphorus and carbamate insecticides in paddy field soils, water and rice plants by measuring anti-acetylcholinesterase activity, and heavy metal contents by an atomic absorption spectrometry. The results showed that the percentages of anti-acetylcholinesterase activities were found in the order of shoot > soil > root > grain > water. Concentrations of heavy metals in all samples were found in the following order: Fe>Mn>Ni>Pb>Zn, particularly Fe, Ni and Pb were found in concentrations exceeding their maximum permissible levels in all samples of water and rice plants. Interestingly, principal component analysis confirmed positive correlations (significantly: P <0.05) between the percentages of anti-acetylcholinesterase activities and heavy metal contents, between Zn and Mn contents, between Zn and Pb contents, between Pb and Mn contents, and between Pb and Ni contents. Additionally, the translocation factors (TF_{soil}, TF_{root}, and TF_{shoot}) and bioaccumulation factor (BAF) above 1 of the rice plant were observed in order of Zn>Fe>Mn>Ni or Pb, Zn or Ni> Pb or Mn >Fe, Pb>Zn or Mn or Fe>Ni, and Zn>Pb>Mn>Ni>Fe, respectively. These data support developing a useful biomarker to monitor organophosphorus and carbamate insecticides and heavy metals, and management programs in health promotion and toxic surveillance for insecticides and heavy metals in food and environments.

Keywords: Bioaccumulation, heavy metals, organophosphorus and carbamate insecticides, rice

ARTICLE INFO

Article history:
Received: 12 April 2018
Accepted: 08 October 2018
Published: 26 February 2019

E-mail addresses:
sirikul.thum@gmail.com (Sirikul Thummajitsakul)
chanon.hpswu@g.swu.ac.th (Chanon Sangdee)
IBGDRGN@hotmail.co.th (Suteema Thaisa)
viewillow@gmail.com (Penpuck Ruengwiroon)
kun.silprasit@gmail.com (Kun Silprasit)
* Corresponding author

ISSN: 1511-3701
e-ISSN: 2231-8542

INTRODUCTION

Nakhon Nayok is one of central provinces of Thailand where there are areas for rice growing of approximately 935.3 square km which provide 311,566 tons of rice products (National Statistical Office [NSO], 2014). Ongkharak is one of the most important districts of Nakhon Nayok province in rice cultivation. Nowadays, demand of agricultural products has continued to increase overtime because of Thailand's dramatically growing population. The use of pesticides is therefore essential and rapidly increasing. Previously, it has been reported that pesticides were imported to Thailand in 2016, which was higher than in the previous year by approximately 7.54% (Office of Agricultural Economics [OAE], 2017).

Generally, several chemicals used in paddy fields (i.e. inorganic fertilizers and pesticides) consist of heavy metals (Gimeno-García et al., 1996; Wuana & Okieimen, 2011). Therefore, the application of pesticides can lead to extensive distribution and long-term accumulation of heavy metals in soils (Jablonowski et al., 2012). Heavy metals from soils can enter water reservoirs, then accumulate in plants and animals, and transfer to humans *via* food webs (Gall et al., 2015). Heavy metals are toxic to human health as they directly effect biochemical and physiological functions in the human body (Jaishankar et al., 2014). Moreover, prevalence rates of toxic effect of pesticides have been reported at 14.47 cases per 100,000 people or 8,689 cases per year in 2016 (Bureau of Occupational and Environmental Diseases [BOED], 2016). Recently, insecticides of the organophosphorus and carbamate group are increasingly used to control pests, such as insects in agriculture regions. However, these compounds can impact the environment and human health.

The organophosphorus and carbamate insecticides (e.g., dichlorvos, parathion, fenamiphos, and N-methyl carbamates) are known as irreversible inhibitors of acetylcholinesterase activity and commonly used as biomarkers for environmental and human monitoring (Lionetto et al., 2013; Vale & Lotti, 2015). Corresponding with previous reports, the estimation of acetylcholinesterase inhibitory activities has been used to monitor or indicate the presence of organophosphorus and carbamate insecticides in vegetable and fruit juice (Korpraditskul et al., 2004). Similarly, the relationship between the use of the insecticides and the level of acetylcholinesterase in the blood has been significantly found in 236 farmers from Sam Chuk district, Suphan Buri province (Duangchinda et al., 2014).

Therefore, our major goals focused on monitoring organophosphorus and carbamate insecticides by anti-acetylcholinesterase activity, and measuring heavy metal content in soil, water, and rice from paddy fields in Ongkharak district, Nakhon Nayok province by an atomic absorption spectrometry. Bioaccumulation factor (BAF) and translocation factors (TFs) were also calculated for each rice sample and heavy metal. Our hypothesis was that percentages of anti-acetylcholinesterase activities correlated with each heavy metal content in rice plants. These basic data are useful for promoting awareness and surveillance of human health and environmental impacts. These benefits help to reduce the insecticide use of farmers in paddy fields, and to manage programs in sustainable prevention and treatment of human diseases from insecticides and heavy metals in food.

MATERIALS AND METHOD

Chemicals

Acetylthiocholine iodide was purchased from Sigma. Acetylcholinesterase enzyme, DTNB (5,5′-dithiobis, 2-nitrobenzoic acid) and nitric acid were obtained from Sigma-Aldrich. Absolute ethanol was obtained from Merk. Heavy metal standards (copper, lead, ferrous, nickel, zinc, manganese, and chromium) for an atomic absorption spectrometry were obtained from Sigma-Aldrich.

Sampling

Samples from paddy soils (*N*= 30), rice (*N*=30) and water (*N*=10) were collected from 10 paddy fields located in Tambon Ongkharak (OK), Khlong Yai (KY), Sisa Krabue (SK), Bang Luk Suea (BLS), Sai Mun (SM), Bang Plakot (BP), Chumphon (CP), Bueng San (BS), Phra Achan (PA), and Bang Sombun (BSB) of Ongkharak district, Nakhon Nayok province between August 2017 and February 2018. Sampling locations were demonstrated with GPS (Global Positioning System) using Google Earth (version 7.1.2.2041) (Figure 1).

Water samples were collected by the grab sampling method at approximately 15 cm depth from the water surface, then kept in

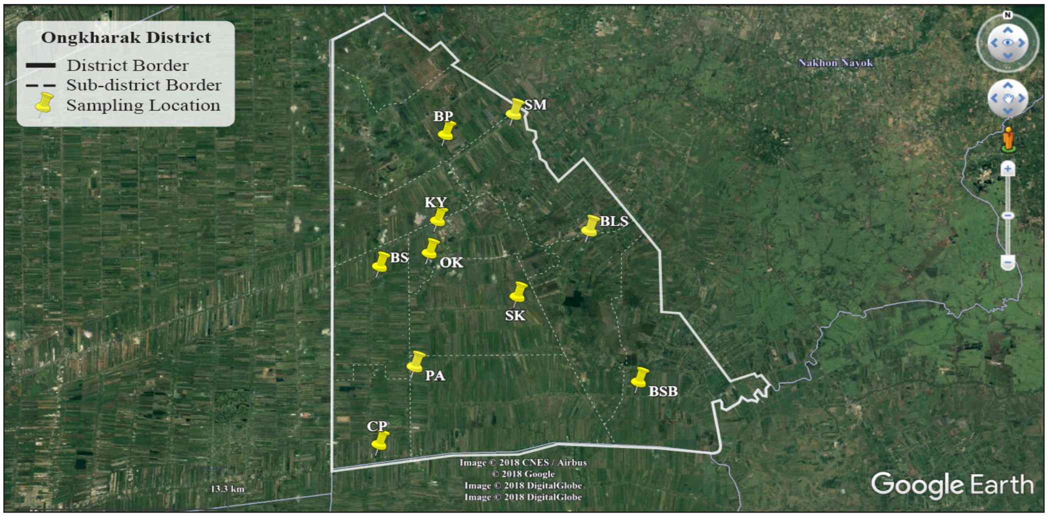


Figure 1. Sampling locations were revealed with GPS (Global Positioning System) using Google Earth (version 7.1.2.2041). Tambon Ongkharak = OK, Khlong Yai = KY, Sisa Krabue = SK, Bang Luk Suea = BLS, Sai Mun =SM, Bang Plakot = BP, Chumphon =CP, Bueng San =BS, Phra Achan =PA, and Bang Sombun =BSB.

50 ml polyethylene tubes at 4°C (Chowdhur et al., 2012; Thummajitsakul et al., 2015). Soil subsamples from 10 sites were collected at 15 depth from the surface by sampling tools, then combined into one sample, and each sample (approximately 500 grams) was used for drying at 50°C before preserving in plastic bags at a temperature of 4°C (Fery & Murphy, 2013; Thummajitsakul et al., 2015). For rice sampling, each subsample was collected from 10 sites per a paddy field, then combined into one sample, and divided into three parts (root, shoot and grain) according to the report of Kingsawat and Roachanakanan (2011). The different parts of the rice plant were cleaned with deionized water, then sectioned into small pieces, and dried at 50°C, followed by grinding with a homogenizer and preserving the powdered samples in plastic bag at 4° C.

Anti-acetylcholinesterase Activity

The anti-acetylcholinesterase activity was performed using Ellman colorimetric assay (Ellman et al., 1961) with some modifications. Each powdered sample (0.5 grams for rice samples and 2.5 grams for soil samples) was mixed with 5% ethanol solvent for 5 min, and left at room temperature for 15 min. Each soil solution was diluted 10 fold. Then, the extract or water sample (200 µl) was mixed with 15 mM acetylthiocholine iodide (200 µl), 3 mM DTNB (1 ml), and 0.3 U/ml of AChE enzyme (200 µl), and then incubated at 37 °C for 15 min. Sample extraction and reaction were performed in duplicate using the same method. An absorbance was determined at 410 nm by spectrophotometer (Model T60UV). Negative controls for quality control procedures were carried out using distilled water, and each sample extract without acetylcholinesterase enzymes. Methylcarbamate was used as positive control at concentrations 0, 0.2, 2, 20 and 200 µg/ml. Percentages of acetylcholinesterase inhibition were calculated following the below formula.

$$\text{\% Acetylcholinesterase inhibition} = \frac{[(A_{water} - A_{blank1}) - (A_{sample} - A_{blank2})]*100}{(A_{water} - A_{blank1})}$$

Note:

(a) A_{water} and A_{sample} were the absorbance of distilled water and sample extract with acetylcholinesterase enzyme, respectively.

(b) A_{blank1} and A_{blank2} were the absorbance of distilled water and sample extract without acetylcholinesterase enzyme, respectively.

Digestion and Sample Analysis

Sample digestion was performed according to Kingsawat and Roachanakanan (2011) and Thummajitsakul et al. (2018). The powdered samples of root, shoot, grain and soil (0.5 g), and water sample (50 ml) were digested with 10 ml of 70% HNO_3 (V/V) at 100°C on a hotplate until dried. After that, each digested sample was added to 1% HNO_3, then filtered through Whatman No. 1 paper and the volume adjusted to 50 ml with 1% HNO_3. The digestion process for each sample was performed in triplicate.

Heavy metals Cu, Pb, Fe, Cr, Ni, Mn and Zn were then detected by an atomic absorption spectrometry (Model 200 Series AA, Agilent Technologies). The 1% HNO_3 was used as a negative control. External standard solution at 1000 µg/ml concentration was diluted with 1% HNO_3, and used to generate linear standard calibration curves.

Statistical Analysis

Descriptive statistics (i.e. percentages, mean and SD, etc.) were used for anti-acetylcholinesterase activities and heavy metal contents. Principle component analysis (PCA) and Pearson' correlation were performed to analyze the relationships between % acetylcholinesterase inhibition and heavy metal concentrations of paddy soils, water, and different parts of the rice plant. All statistical analysis was done using the PSPP program version 0.10.5 (Pfaff et al., 2013), and Paleontological statistic program version 3.16 (Hammer et al., 2001). Moreover, bioaccumulation factor (BAF) and translocation factors (TFs) were calculated for each rice sample and heavy metal by formulas below:

$$BAF = C_{grain}/C_{soil}$$
$$TF_{soil} = C_{root}/C_{soil}$$
$$TF_{root} = C_{shoot}/C_{root}$$
$$TF_{shoot} = C_{grain}/C_{shoot}$$

where C_{soil}, C_{grain}, C_{shoot} and C_{root} were concentrations of heavy metals in soil, rice grain, rice shoot and rice root (mg/kg), respectively. The TF_{soil}, TF_{root}, and TF_{shoot} above 1 indicate effectively metal translocation from soil to root, root to shoot, or shoot to grain, respectively. Similarly, if BAF exceeds 1, rice is recognized as hyperaccumulator which absorbed and accumulated heavy metals from the soils (Satpathy et al., 2014; Neeratanaphan et al., 2017).

RESULTS AND DISCUSSION

This work focused on monitoring organophosphorus and carbamate insecticides by determining anti-acetylcholinesterase activities, and heavy metals in paddy field soils, water and rice samples by an atomic absorption spectrometry. The results showed that the percentages of anti-acetylcholinesterase activities were observed in the following order: shoot (72.74 -93.25%) > soil (66.88 -93.10%) > root (61.42 - 93.82%)> grain (26.51-89.66%)> water (5.39 - 76.01%) (Table 1).

Previously, it has been reported that pesticides are not found only in the plant outside, but they can be absorbed, redistributed and accumulated into the tissue of rice plants (Ge et al., 2017). For example, insecticides in organochlorine, organophosphate and synthetic pyrethroid groups can be transferred and accumulated into tomato, pineapple and mango fruits (Agyekum et al., 2015). However, the level of acetylcholinesterase inhibition of samples may be affected by other factors, such as natural acetylcholinesterase inhibitors from plants (i.e. alkaloid, terpenoids, flavonoids and phenolic agents) (Murray et al., 2013), and certain heavy metals Cd, Cu, Zn and Hg *in vitro* (Frasco et al., 2005).

Table 1
Percentages of acetylcholinesterase inhibition of water, soil, rice shoot, rice grain, and rice root samples in agricultural regions of Ongkharak district, Nakhon Nayok Province

Sampling location	% Acetylcholinesterase inhibition (mean±SD)				
	Water	Soil	Shoot	Grain	Root
KY	31.61±7.90	74.93±10.88	72.74±16.39	70.55±21.48	61.42±32.91
SK	56.03±12.35	82.40±7.94	76.01±15.49	80.60±17.63	66.52±14.90
BLS	42.67±3.72	66.88±21.29	76.58±15.59	87.28±9.76	85.06±11.39
SM	5.39±1.74	73.49±19.61	89.94±5.67	84.55±9.48	71.21±15.90
BP	17.39±5.23	75.14±18.67	73.36±25.48	74.50±8.66	75.29±12.39
CP	32.54±3.73	89.44±5.02	80.60±16.41	26.51±4.60	87.36±1.18
BS	76.01±5.51	78.30±13.85	75.65±11.16	64.08±21.22	84.20±8.71
OK	38.36±6.17	89.66±6.31	89.73±9.98	75.14±12.39	93.82±1.61
PA	27.01±11.80	77.30±19.59	93.25±1.00	89.66±5.81	85.78±7.35
BSB	25.29±6.00	93.10±3.27	89.37±12.09	39.80±12.86	82.04±18.77
Total	**36.39±20.00**	80.06±15.35	81.11±15.06**	69.09±25.26**	77.60±20.37

In this study, all heavy metals were found in all samples, except for Cr that was not detected in any samples and Cu that was detected in only one rice sample. The heavy metals were found in the paddy field soils, water, shoot, grain and root of rice plants in order of Fe>Mn>Ni>Pb>Zn. The amount of each heavy metal was mainly higher in root, shoot and grain of rice plants than that of soil and water samples (Table 2). Although, concentrations of heavy metals in soil samples did not exceed the maximum permissible level of each heavy metal, concentrations of Fe, Ni and Pb in all water and rice samples did exceed maximum permissible levels (Table 3).

Table 2
Concentration of each heavy metal found in water, soil, shoot, grain and root from 10 sampling locations of paddy fields

Heavy metals	Concentration of each heavy metal (mean±SD) (mg/kg for rice and soil, mg/l for water)				
	Water	Soil	Shoot	Grain	Root
Mn					
Min	0.32±0.22	70.00±7.78	67.78±3.14	24.44±12.02	40.28±33.78
Max	1.40±0.15	127.41±37.39	1,109.00±279.76	349.26±59.23	154.44±40.18
Mean	1.03±0.26	100.19±18.37	219.19±50.47	102.80±25.60	105.18±22.28
Zn					
Min	0.04±0.02	14.65 ± 7.72	17.00±11.75	12.53±6.09	18.08±8.47
Max	0.16±0.11	29.38±5.43	175.74±151.23	116.91±5.72	49.16±5.96
Mean	0.10±0.06	20.24±8.27	42.59±24.78	39.86±8.36	26.50±7.17

Table 2 *(continue)*

Heavy metals	Concentration of each heavy metal (mean±SD) (mg/kg for rice and soil, mg/l for water)				
	Water	Soil	Shoot	Grain	Root
Ni					
Min	0.34±0.25	66.58±41.67	30.76±25.27	49.61±31.97	28.53±27.35
Max	1.08±0.70	117.48±73.09	578.15±54.19	106.17±45.96	113.37±17.28
Mean	0.77±0.51	89.15±53.22	131.31±44.37	75.40±54.34	77.70±36.75
Fe					
Min	4.67±3.25	176.19±33.67	673.33±321.46	534.29±132.92	10,952.38±5,569.60
Max	111.35±59.98	40,476.19±30,799.38	5,904.76±2147.26	1,562.86±852.25	29,309.52±4,592.79
Mean	19.82±9.95	14,968.87±7549.43	1,515.59±592.75	876.15±477.94	17,216.55± 6951.86
Pb					
Min	0.31±0.14	26.80±21.17	22.8±11.31	12.80±8.48	19.80±13.61
Max	0.74 ±0. 6 3	77.20±38.74	78.00±17.98	94.80±39.60	82.80±50.79
Mean	0.46±0.29	51.85±35.62	54.97±25.52	65.24±39.32	51.29±34.00
Cu					
Min	ND*	ND*	ND*	ND*	ND*
Max	ND*	ND*	ND*	ND*	ND*
Mean	ND*	ND*	ND*	ND*	ND*
Cr					
Min	ND*	ND*	ND*	ND*	ND*
Max	ND*	ND*	ND*	ND*	ND*
Mean	ND*	ND*	ND*	ND*	ND*

*ND is not detected.

Table 3
Maximum permissible levels for each heavy metal and % samples from 10 sampling locations that showed concentrations above the maximum permissible levels

	Mn	Zn	Ni	Fe	Pb
*MPL_{soil}	1,800[a]	7,500[b]	1,600 [a]	20,000-550,000[b]	400[a]
*MPL_{water}	1.0[c]	1.0[c]	0.1[c]	0.5[d]	0.05[c]
*MPL_{rice}	NV[g]	100[e]	1[f]	20[e]	0.5[e]
% samples > *MPL_{soil}	0	0	0	0	0
% samples > *MPL_{water}	60	0	100	100	100
% samples > *MPL_{rice}	-	10	100	100	100

* MPL = Maximum permissible levels for heavy metal (mg/kg for soil and rice samples, mg/l for water samples)
[a] Ministry of Natural Resources and Environment [MNRE], Thailand (2004)
[b] Data from Chawpaknum et al. (2012)
[c] Ministry of Natural Resources and Environment [MNRE], Thailand (1994)
[d] Department of Groundwater Resources [DGR], Thailand (2008)
[e] Ministry of Public Health [MOPH], Thailand (1986)
[f] United States Department of Agriculture - Foreign Agricultural Service [USDA – FAS] (2014)
[g] NV is the no criterion value

Moreover, correlations between heavy metal contents and % acetylcholinesterase inhibition of all investigated samples were analyzed. Interestingly, the strongly and moderately positive correlations between % anti-acetylcholinesterase activities and concentrations of Pb and Zn were respectively found, significantly. Additionally, the % anti-acetylcholinesterase activities showed weakly positive correlation with Mn, Ni, and Fe contents, significantly. Therefore, this study implied that an increase of insecticide levels in water, soil and rice samples was associated with higher concentration of heavy metals. Moreover, very strongly positive correlation was observed between Zn and Mn, while Zn showed moderately positive correlation with Pb, significantly. The Pb content also showed weakly positive correlation with concentrations of Mn and Ni contents, significantly (Table 4). It implied that an increase of Zn concentration was associated with higher concentrations of Mn and Pb in the samples. Similarly, an increase of Pb concentration correlated with the presence of higher concentrations of Mn and Ni in the samples.

The organophosphorus and carbamate chemicals are insecticides that inhibit acetycholinesterase enzymes (Vale & Lotti, 2015). Therefore, determination of anti-acetylcholinesterase activities can be used to link the level of organophosphorus and carbamate insecticides in targeted samples (Korpraditskul et al., 2004). In this study, it showed that the presence of the Pb, Zn, Mn, Ni, and Fe heavy metals might help either to stabilize the insecticides used in paddy fields, or the insecticides composed of the heavy metals. Similarly, Zn contributed to the Mn and Pb stabilization, and Pb facilitated the Mn and Ni stabilization. Similarly, previous report revealed a positive correlation between acetamiprid insecticides and Cu, and indicated that Cu facilitated the stabilization of acetamiprid insecticides (Tariq et al., 2016).

Table 4
Pearson's correlation matrix between the percentages of acetylcholinesterase inhibition and each heavy metal, and between heavy metal pair

	% AChE inhibition**	**Mn**	**Ni**	**Pb**	**Zn**	**Fe**
% AChE inhibition	1.00					
Mn	0.361*	1.00				
Ni	0.330*	0.043	1.00			
Pb	0.680*	0.370*	0.372*	1.00		
Zn	0.423*	0.870*	0.116	0.527*	1.00	
Fe	0.337*	0.067	0.120	0.171	0.018	1.00

*Correlation was significantly found at $P < 0.05$
**% AChE inhibition is the percentages of acetylcholinesterase inhibition

Furthermore, bioaccumulation factor (BAF) and translocation factors (TFs) were analyzed. The results showed that the order of BAF values of heavy metals was Zn>Pb>Mn>Ni>Fe. The BAF > 1 of grain samples indicated that the rice plant was able to accumulate the heavy metals, especially Pb and Zn (Table 5). The transfer factors (TFs) of heavy metals from soil to root (TF_{Soil}), root to shoot (TF_{Root}), and shoot to grain (TF_{Shoot}) were also analyzed. The averages of TF_{soil}, TF_{root}, and TF_{shoot} were demonstrated in the following order: Fe>Zn>Mn>Pb>Ni, Mn>Ni>Zn>Pb>Fe, and Pb>Zn>Ni>Fe>Mn, respectively (Table 5). It indicated that translocation of Fe, Mn and Pb from soil to root, root to shoot, shoot to grain was greater than other heavy metals, respectively. The concentrations of Fe, Mn and Pb revealed the highest values in root, shoot and grain, respectively. Pb was highly accumulated in grain corresponding to the BAF above 1. The sample percentages, which there were TF_{soil}, TF_{root}, TF_{shoot} and BAF values above 1, were shown in the order of Zn>Fe>Mn>Ni or Pb, Zn or Ni> Pb or Mn >Fe, Pb>Zn or Mn or Fe>Ni, and Zn>Pb>Mn>Ni>Fe, respectively (Table 6). However, all heavy metals could be transferred from soil and accumulated in the grain of rice plants. The pathway of heavy metal translocation in plants is a major component in heavy metal redistribution through different parts of the plants (Page & Feller, 2015).

Similarly, it has been reported that Zn, Cd and Cu are mostly found in paddy field soils, water, and different parts of rice plant, namely root, shoot, grain and husk (Kingsawat & Roachanakanan, 2011). Corresponding to the previous report, heavy metals (Cd, Cr, Pb, Zn, As, Mn, and Hg) were higher in root than straw, and grain of rice plants (Singh et al., 2011). Additionally, it has been reported that Cd, Pb, Ni, and Zn in rice crop (*Oryza sativa* L.) were highly accumulated in rice stem and grain (Rahimi et al., 2017).

In Thailand, it has been reported that concentrations of heavy metals (Cd, Cr, Pb, Cu, Ni and Zn) in paddy field soils for organic rice cultivation are much below maximum permissible levels of the heavy metals (Chinoim & Sinbuathong, 2010). The uptake and accumulation of certain heavy metals (Pb, Cd, Fe, Cr, Ni, and Zn) into Thai jasmine rice in paddy field soil from Mea Sod in Thailand have been reported that Pb, Cd and Fe are mostly accumulated in roots, and Cr, Ni and Zn are mostly accumulated in stems and grains (Thongsri et al., 2010). Concentrations of soil heavy metals (Zn, Cd and Pb) influence contents in different parts of rice according heavy metal concentrations in soil, and accumulation of heavy metals is found higher in root than grain and straw (Roongtanakiat & Sanoh, 2015).

In this study, the principle component analysis was used to demonstrate correlations among % acetylcholinesterase inhibition and heavy metal contents by principle components 1 and 2 that showed 46.4% and 21.8% of total variance, respectively. The Mn, Pb and Zn contents and % anti-acetylcholinesterase activities

Table 5
*Translocation factors (TF_{Soil}, TF_{Root}, and TF_{Shoot}) and bioaccumulation factor (BAF) of the rice plant (*Oryza sativa *L.)*

Heavy metals / Sampling locations	KY	SK	BLS	SM	BP	CP	BS	OK	PA	BSB	Mean±SD
Mn											
TF_{soil}	1.29	0.93	1.01	1.02	0.33	2.00	0.82	1.45	1.13	0.82	1.08±0.44
TF_{Root}	2.22	1.57	1.04	9.33	3.45	0.65	0.87	0.59	0.85	1.17	2.17±2.66
TF_{Shoot}	0.24	1.16	0.21	0.31	0.28	0.60	0.34	1.26	2.02	0.46	0.69±0.60
BAF	0.68	1.69	0.22	3.01	0.33	0.78	0.24	1.09	1.96	0.45	1.04±0.91
Zn											
TF_{soil}	1.32	1.11	0.62	1.73	3.36	1.57	0.84	1.00	1.47	1.14	1.42±0.76
TF_{Root}	1.21	2.01	0.94	5.19	0.40	1.12	1.27	1.54	0.73	1.44	1.58±1.34
TF_{Shoot}	0.71	1.54	3.39	0.67	1.11	0.85	0.47	0.99	0.97	0.75	1.14±0.84
BAF	1.14	3.44	1.96	5.97	1.48	1.49	0.50	1.52	1.04	1.23	1.98±1.60
Ni											
TF_{soil}	0.84	0.43	0.34	0.58	1.11	1.27	1.14	1.09	1.32	0.79	0.89±0.35
TF_{Root}	0.35	2.73	3.49	1.02	0.61	0.79	5.84	1.04	1.07	1.40	1.83±1.40
TF_{Shoot}	2.22	0.64	0.59	0.79	0.89	1.24	0.18	0.87	0.92	0.82	0.91±0.82
BAF	0.65	0.75	0.71	0.47	0.60	1.25	1.18	0.98	1.30	0.90	0.88±0.90
Fe											
TF_{soil}	0.46	1.41	0.93	1.64	1.35	64.68	1.68	1.74	1.15	0.65	7.57±20.07
TF_{Root}	0.05	0.04	0.09	0.29	0.05	0.09	0.04	0.05	0.09	0.11	0.09±0.08
TF_{Shoot}	0.75	1.20	1.04	0.14	0.69	0.98	0.78	0.55	1.24	0.60	0.80±0.33
BAF	0.02	0.07	0.08	0.07	0.05	5.50	0.05	0.04	0.12	0.04	0.60±1.72
Pb											
TF_{soil}	1.49	1.27	0.79	2.33	0.34	0.64	1.13	0.71	0.82	1.37	1.09±0.57
TF_{Root}	0.57	1.40	1.05	0.94	3.85	1.26	1.19	1.12	0.66	0.73	1.28±0.94
TF_{Shoot}	2.27	1.30	1.78	1.03	0.98	0.38	0.80	0.76	2.02	1.33	1.27±0.60
BAF	1.93	2.32	1.48	2.27	1.29	0.30	1.08	0.61	1.10	1.33	1.37±0.66

Table 6
Percentages of rice samples that there were TF and BAF values above 1 for each heavy metal

	% of rice samples				
	Mn	Zn	Ni	Fe	Pb
$TF_{soil} > 1$	60	80	50	70	50
$TF_{Root} > 1$	60	70	70	0	60
$TF_{Shoot} > 1$	30	30	20	30	60
BAF > 1	40	90	30	10	80

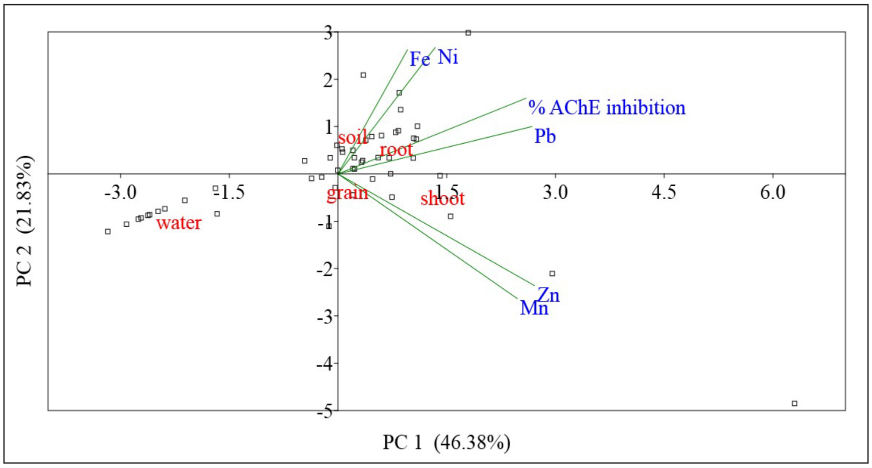
were clustered in PC1, and Ni and Fe in PC2 on positive view of the PCA biplot. The result showed that the PCA biplot confirmed positive correlation of Fe, Ni, Pb, Zn, Mn accumulation and % anti-acetylcholinesterase activities in shoot, grain and root of the rice plant (Figure 2A and 2B).

There are, however, still other factors (i.e. plant species, bioactive agents and metal category) that can effect on the BAF and TF values of plants (Balabanova et al., 2015; Page & Feller, 2015). Moreover, all samples in this study were collected from 10 sampling locations in Ongkharak district of Nakhon Nayok province. Among these sampling locations, the public landfill is located in Tambon Sai Mun, which accepts enormous quantities of waste from other areas in Nakhon Nayok province, such as Srinakharinwirot University, Ongkharak (Yunak et al., 2016). A landfill area is an important factor of heavy metal contamination in soils and plants (Chuangcham et al., 2008; Kasam et al., 2018), especially if disposing of hazardous materials. The large amount of spent household batteries discarded in landfills cause infiltration of higher heavy metals, such as Mn and Zn, into surroundings (Karnchanawong & Limpiteeprakan, 2009).

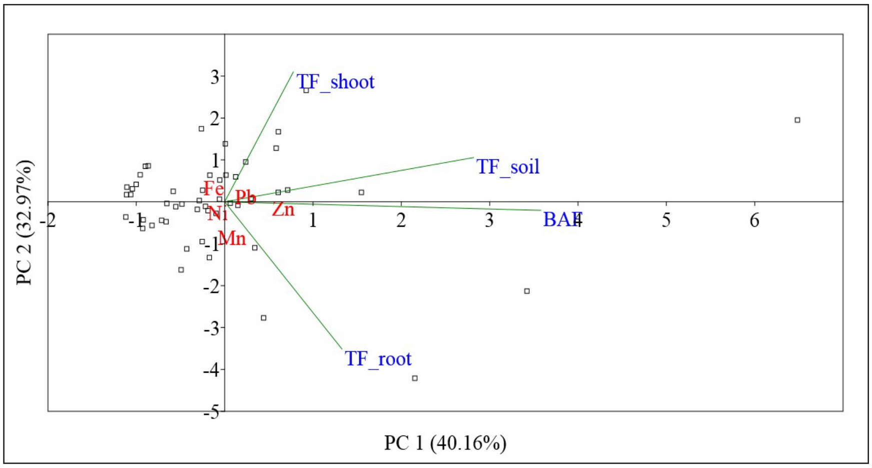
Heavy metals can, therefore, accumulate from natural sources or biogeochemical cycles in geological structures of the earth, or from anthropogenic sources (Garrett, 2000; Satpathy et al., 2014). They can accumulate in water resources *via* natural methods, such as rainfall, flowing and infiltrating of water into canals and soils. Generally, heavy metals are deposited on the upper part of soils, and accordingly the top soils are frequently used to monitor heavy metals occurring from human activities. Additionally, heavy metals from soils can affect the ecosystem and human health by entering into groundwater, or transferring to plants (Bhagure & Mirgane, 2011).

Several previous studies have reported that the application of fertilizers and pesticides in paddy fields can release certain toxic heavy metals (Zn, Mn, Cd, Cu, Cr and Pb) into soil, which can be transferred into different parts of rice plant (*Oryza sativa* L.), especially grains (Satpathy et al., 2014). Moreover, insecticides, fertilizers, herbicides and fungicides are major sources of heavy metals, such as Cu, Cd, Pb, Co, Ni, Zn, Fe and Mn (Chiroma et al., 2007; Gimeno-García et al., 1996; Tariq et al., 2016).

Thus, the uncontrolled application of pesticides in paddy fields can cause several environmental problems, such as heavy metal contamination in plants, soil and water (Kingsawat & Roachanakanan, 2011). Moreover, the long-term and extensive use of pesticides can also effect human health *via* the food web, and working or living near farms and industries (Al-Saleh & Abduljabbar, 2017; Jaga & Dharmani, 2003; Mahmood & Malik, 2014). Heavy metals cause poisoning to kidney, lung, liver and brain of humans by activating oxidative stress and inhibiting enzymes in metabolic processes which can cause mutations and cancers (Jadoon & Malik, 2017; Morales et al., 2016; Sharma et al., 2014).



(A)



(B)

Figure 2. Principle component analysis for heavy metal concentrations and the percentages of acetylcholinesterase inhibition of water, soil, rice shoot, rice grain, and rice samples from different sampling sites (A), and for bioaccumulation factor (BAF), translocation factors (TFs), and heavy metals of the samples (B)

CONCLUSION

In the current studies, organophosphorus and carbamate insecticides in water, soils and different parts of the rice plant (shoot, grain and root) were monitored by determining acetylcholinesterase inhibitory activities. The order of % anti-acetylcholinesterase activities was shoot > soil > root > grain > water, and the order of heavy metal contents was Fe>Mn>Ni>Pb>Zn. The Fe, Mn, Ni, Pb and Zn contents of soil samples were below maximum permissible levels, but the Fe, Ni and Pb contents of all water and rice samples were above maximum permissible levels. Moreover, the percentages of anti-acetylcholinesterase activities correlated positively with concentrations of Pb, Zn, Mn, Ni, and Fe, significantly. Positive correlations were also significantly found between Zn and Mn, between Zn and Pb, between Pb and Mn, and between Pb and Ni. The order of TF_{soil}, TF_{root}, TF_{shoot} and BAF values which exceeded 1 was Zn>Fe>Mn>Ni or Pb, Zn or Ni> Pb or Mn >Fe, Pb>Zn or Mn or Fe>Ni, and Zn>Pb>Mn>Ni>Fe, respectively. These useful data may be applied to develop a potential biomarker for monitoring organophosphorus and carbamate insecticides and heavy metals in food and environments, and to manage programs for human health promotion to avoid toxicity from the insecticides and heavy metals.

ACKNOWLEDGEMENTS

This research was supported by a grant from Faculty of Physical Therapy, Srinakharinwirot University (Grant number: 263/2560). The authors would like to thank the laboratory of Faculty of Environmental Culture and Ecotourism of Srinakharinwirot University. The authors declared that there was no conflict of interest.

REFERENCES

Agyekum, A. A., Ayernor, G. S., Saalia, F. K., & Bediako-Amoa, B. (2015). Translocation of pesticide residues in tomato, mango and pineapple fruits. *Journal of Food Science and Engineering*, *5*(2015), 142-149.

Al-Saleh, I., & Abduljabbar, M. (2017). Heavy metals (lead, cadmium, methylmercury, arsenic) in commonly imported rice grains (*Oryza sativa*) sold in Saudi Arabia and their potential health risk. *International Journal of Hygiene and Environmental Health Health*, *220*(7), 1168-1178. doi: 10.1016/j.ijheh.2017.07.007.

Balabanova, B., Stafilov, T., & Bačeva, K. (2015). Bioavailability and bioaccumulation characterization of essential and heavy metals contents in *R. acetosa*, *S. oleracea* and *U. dioica* from copper polluted and referent areas. *Journal of Environmental Health Science and Engineering*, *13*(2), 1-13.

Bhagure, G. R., & Mirgane, S. R. (2011). Heavy metal concentrations in groundwaters and soils of Thane Region of Maharashtra, India. *Environmental Monitoring and Assessment*, *173*(1–4), 643–652. doi: 10.1007/s10661-010-1412-9.

Bureau of Occupational and Environmental Diseases. (2016). *Situation report: Occupational and environmental diseases and health for 2016*. Retrieved December 5, 2017, from file:///C:/Users/Administrator/Downloads/01_envocc_situation_59.pdf

Chawpaknum, C., Boon-ngam, J., & Palajaroen, S. (2012). *Heavy metal in the Bangpakong River*.

Retrieved December 28, 2017, from http://www.inlandfisheries.go.th/research/details.php?id=5

Chinoim, N., & Sinbuathong, N. (2010). Heavy metal contamination of soils from organic paddy fields in Thailand. *The Proceeding of The 19th World Congress of Soil Science, Soil Solutions for a Changing World*, Brisbane, Australia: The Australian Society of Soil Science. Retrieved January 4, 2018, from http://iuss.org/19th%20WCSS/Symposium/pdf/0176.pdf

Chiroma, T. M., Abdulkarim, B. I., & Kefas, H. M. (2007). The impact of pesticide application on heavy metal (Cd, Pb and Cu) levels in spinach. *Leonardo Electronic Journal of Practices and Technologies*, *11*, 117-122.

Chowdhury, A. Z., Banik, S., Uddin, B., Moniruzzaman, M., Karim, N., & Gan, H. S. (2012). Organophosphorus and carbamate pesticide residues detected in water samples collected from paddy and vegetable fields of the Savar and Dhamrai Upazilar in Bangladesh. *International Journal of Environmental Research and Public Health*, *9*(9), 3318-3329. doi: 10.3390/ijerph9093318

Chuangcham, U., Wirojanagud, W., Charusiri, P., Home, W. M., & Lertsirivorakul, R. (2008). Assessment of heavy metals from landfill leachate contaminated to soil: A case study of Kham Bon landfill, Khon Kaen Province, NE Thailand. *Journal of Applied Sciences*, *8*(8), 1383-1394.

Department of Groundwater Resources, Thailand. (2008). *Notification of the Ministry of Natural Resources and Environment*. Retrieved January 4, 2018, from http://www.dgr.go.th/water/DGRlaw/law2551-01.pdf

Duangchinda, A., Anurugsa, B., & Hungspreug, N. (2014). The use of organophosphate and carbamate pesticides on paddy fields and cholinesterase levels of farmers in Sam Chuk District, Suphan Buri Province, Thailand. *Thammasat International Journal of Science and Technology*, *19*(1), 39-51.

Ellman, G. L., Courtney, K. D., Andres, V. Jr., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88-95.

Fery, M., & Murphy, E. (2013). *A guide to collecting soil samples for farms and gardens*. Retrieved December 1, 2017, from https://catalog.extension.oregonstate.edu/ec628

Frasco, M. F., Fournier, D., Carvalho, F., & Guilhermino, L. (2005). Do metals inhibit acetylcholinesterase (AchE)? Implementation of assay conditions for the use of AchE activity as a biomarker of metal toxicity. *Biomarkers*, *10*(5), 360–375.

Gall, J. E., Boyd, R. S., & Rajakaruna, N. (2015). Transfer of heavy metals through terrestrial food webs: A review. *Environmental Monitoring and Assessment*, *187*(4), 1-21. doi: 10.1007/s10661-015-4436-3

Garrett, R. G. (2000). Natural sources of metals to the environment. *Human and Ecological Risk Assessment: An International Journal*, *6*(6), 945-963. https://doi.org/10.1080/10807030091124383

Ge, J., Cui, K., Yan, H., Li, Y., Chai, Y., Liu, X., … Yu, X. (2017). Uptake and translocation of imidacloprid, thiamethoxam and difenoconazole in rice plants. *Environmental Pollution*, *226*(2017), 479-485. doi: 10.1016/j.envpol.2017.04.043

Gimeno-García, E., Andreu, V., & Boluda, R. (1996). Heavy metals incidence in the application of inorganic fertilizers and pesticides to rice farming soils. *Environmental Pollution*, *92*(1), 19-25.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). *PAST: Paleontological statistics software* *package for education and data analysis*. Retrieved January 23, 2018, from http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Jablonowski, N. D., Linden, A., Köppchen, S., Thiele, B., Hofmann, D., Mittelstaedt, W., … Burauel, P. (2012). Long-term persistence of various 14C-labeled pesticides in soils. *Environmental Pollution*, *168*(2012), 29-36. doi: 10.1016/j.envpol.2012.04.022

Jadoon, S., & Malik, A. (2017). DNA damage by heavy metals in animals and human beings: An overview. *Biochemical Pharmacology*, *6*(3), 1-8.

Jaga, K., & Dharmani, C. (2003). Sources of exposure to and public health implications of organophosphate pesticides. *Revista Panamericana de Salud Pública 14*(3), 171-185.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, *7*(2), 60–72. doi: 10.2478/intox-2014-0009

Karnchanawong, S., & Limpiteeprakan, P. (2009). Evaluation of heavy metal leaching from spent household batteries disposed in municipal solid waste. *Waste Management*, *29*(2), 550-558.

Kasam, Rahmawati, S., Iresha, F. M., Wacano, D., Fauziah, I. F., & Amrullah, M. A. (2018). *Evaluation of heavy metal exposure to soil and paddy plant around the closed municipal solid waste landfill: Case study at Gunung Tugel landfill, Banyumas-Central Java*. Retrieved January 23, 2018, from https://iopscience.iop.org/article/10.1088/1757-899X/299/1/012012/pdf

Kingsawat, R., & Roachanakanan, R. (2011). Accumulation and distribution of some heavy metals in water, soil and rice fields along the Pradu and Phi Lok canals, Samut Songkhram province, Thailand. *Environment and Natural Resources Journal*, *9*(1), 38-48.

Korpraditskul, R., Rattanakreetakul, C., & Aussawasujindarat, S. (2004). The inhibition of enzyme acetylcholinesterase by organophosphorus and carbamate pesticide in vegetable and fruit juice. *Proceedings of The 42nd Kasetsart University Annual Conference* (pp. 376-383). Bangkok, Thailand: Thai National AGRIS Centre.

Lionetto, M. G., Caricato, R., Calisi, A., Giordano, M. R., & Schettino, T. (2013). Acetylcholinesterase as a biomarker in environmental and occupational medicine: New insights and future perspectives. *BioMed Research International*, *2013*(2013), 1-8.

Mahmood, A., & Malik, R. N. (2014). Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry*, *7*(1), 91-99. doi.org/10.1016/j.arabjc.2013.07.002

Ministry of Natural Resources and Environment, Thailand. (1994). *Announcement of national environment committee no. 8 standard level on water surface quality*. Retrieved January 18, 2018, from http://www.pcd.go.th/count/lawdl.cfm?FileName=3_14_water.pdf.

Ministry of Natural Resources and Environment, Thailand. (2004). *Announcement of national environment committee no. 25 standard level on soil quality*. Retrieved January 18, 2018, from http://www.pcd.go.th/count/lawdl.cfm?FileName=9_1_soil.pdf.

Ministry of Public Health, Thailand. (1986). *Announcement of ministry of public health no. 98. Standard level on contaminants in foods*. Retrieved January 27, 2018, from http://food.fda.moph.go.th/data/Admin/food_law.pdf.

Morales, M. E., Derbes, R. S., Ade, C. M., Ortego, J. C., Stark, J., Deininger, P. L., & Roy-Engel, A. M. (2016). Heavy metal exposure influences double strand break DNA repair outcomes.

PLoS One, *11*(3), 1-21. doi.org/10.1371/journal.pone.0151367

Murray, A. P., Faraoni, M. B., Castro, M. J., Alza, N. P., & Cavallaro, V. (2013). Natural AChE inhibitors from plants and their contribution to Alzheimer's disease therapy. *Current Neuropharmacology*, *11*(4), 388–413. doi: 10.2174/1570159X11311040004

National Statistical Office. (2014). *Statistics of rice cultivation, whole kingdom: Crops year 2005–*2014. Retrieved January 27, 2018, from http://service.nso.go.th/nso/web/statseries/statseries14.html

Neeratanaphan, L., Khamma, S., Benchawattananon, R., Ruchuwararak, P., Appamaraka, S., & Intamat, S. (2017). Heavy metal accumulation in rice (*Oryza sativa*) near electronic waste dumps and related human health risk assessment. *Human and Ecological Risk Assessment: An International Journal*, *23*(5), 1086-1098. doi.org/10.1080/10807039.2017.1300856

Office of Agricultural Economics. (2017). *Quantity and value of pesticide imports in Thailand*. Retrieved January 27, 2018, from http://www.oae.go.th/ewt_news.php?nid=146.

Page, V., & Feller, U. (2015). Heavy metals in crop plants: Transport and redistribution processes on the whole plant level. *Agronomy*, *5*(3), 447-463.

Pfaff, B., Darrington, J., Stover, J., Satman, M. H., Beckmann, F., Williams, J., … van Son, R. (2013). *GNU PSPP version*. Retrieved November 20, 2017, from thttps://www.gnu.org/software/pspp/

Rahimi, G., Kolahchi, Z., & Charkhabi, A. (2017). Uptake and translocation of some heavy metals by rice crop (*Oryza sativa*) in paddy soils. *Journal of National Agricultural and Food Centre*, *63*(4), 163−175. doi.org/10.1515/agri-2017-0016

Roongtanakiat, N., & Sanoh, S. (2015). Comparative growth and distribution of Zn, Cd and Pb in rice, vetiver and sunflower grown in contaminated soils. *Kasetsart Journal (Natural Science)*, *49*(5), 663 – 675.

Satpathy, D., Reddy, M.V., & Dhal, S.P. (2014). Risk assessment of heavy metals contamination in paddy soil, plants, and grains (*Oryza sativa* L.) at the East Coast of India. *BioMed Research International*, *2014*, 1-11.

Sharma, B., Singh, S., & Siddiqi, N. J. (2014). Biomedical implications of heavy metals induced imbalances in redox systems. *BioMed Research International*, *2014*, 1-25. doi: 10.1155/2014/640754

Singh, J., Upadhyay, S. K., Pathak, R. K., & Gupta, V. (2011). Accumulation of heavy metals in soil and paddy crop (*Oryza sativa*), irrigated with water of Ramgarh Lake, Gorakhpur, UP, India. *Journal Toxicological & Environmental Chemistry*, *93*(3), 462-473. doi.org/10.1080/02772248.2010.546559

Tariq, S. R., Shafiq, M., & Chotana, G. A. (2016). Distribution of heavy metals in the soils associated with the commonly used pesticides in cotton fields. *Scientifica*, *2016*, 1-11.

Thongsri, T., Visoottiviseth, P., & Kosiyachinda, P. (2010). Accumulation and distribution of heavy metals into Thai jasmine rice in Mae Sod paddy field, Thailand. *Proceeding of the 15th International Conference on Heavy Metals in the Environment* (pp. 323-325). Gdańsk, Poland: Gdańsk University of Technology.

Thummajitsakul, S., Praditpol, H., Poolaoi, J., & Silprasit, K. (2015). Carbamate and organophosphate contamination in soil, rice, and water samples from rice paddy fields in Nakhon Nayok province. *Applied Environmental Research*, *37*(3), 103-109.

Thummajitsakul, S., Subsinsungnern, R., Treerassapanich, N., Kunsanprasit, N., Puttirat, L., Kroeksakul, P., & Silprasit, K. (2018). Pesticide and heavy metal contamination: potential health risks of some vegetables and fruits from a local market and family farm in Ongkharak district of Nakhon Nayok province, Thailand. *Pertanika Journal of Tropical Agricultural Science*, *41*(3), 1005-1019.

United States Department of Agriculture - Foreign Agricultural Service. (2014). *China's maximum levels of contaminants in foods*. Retrieved January 27, 2018, from https://gain.fas.usda.gov

Vale, A., & Lotti, M. (2015). Organophosphorus and carbamate insecticide poisoning. *Handbook of Clinical Neurology*, *131*, 149-168. https://doi.org/10.1016/B978-0-444-62627-1.00010-X

Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Network ISRN Ecology*, *2011*, 1-20.

Yunak, W., Wannawoharn, A., & Sasuk, U. (2016). *The planning of solid waste management at Srinakharinwirot University, Ongkharak* (Bachelor thesis, Srinakharinwirot University, Thailand). Retrieved January 27, 2018, from http://ir.swu.ac.th/xmlui/handle/123456789/5059