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Assessment of Organophosphate and Carbamate Insecticides and Heavy Metal Contamination in Canal-Grown Water Morning Glory (*Ipomoea aquatica* Forssk) in Nakhon Nayok Province, Thailand

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Abstract

Excessive application of insecticides in agriculture has become an increasing trend in Thailand, where agriculture is common in the area. This problem leads to negative impacts on human health and environment. Therefore, this study focused on monitoring organophosphate and carbamate insecticides, and heavy metals (Cd, Cu, Pb, Ni, Hg, Zn and Mn) in *Ipomoea aquatica* Forssk, as well as water and soil from different canals in Ongkharak District, Nakhon Nayok Province, Thailand. The powder samples were prepared from roots, stems and leaves of *Ipomoea aquatica* Forssk, as well as soil. Then, anti-acetylcholinesterase activity of each sample was determined, and heavy metal contents were measured by atomic absorption spectrometry. The results showed that the highest anti-acetylcholinesterase activity was found in the soil samples, followed by those of leaves and stems, roots, and water, respectively. Additionally, the order of heavy metal concentration of stem and leaves, root, water and soil samples were Mn>Cu>Hg>Ni>Zn>Cd>Pb, Mn>Zn>Ni >Cu>Cd>Hg>Pb, Ni>Cu>Hg>Mn>Cd>Zn>Pb, and Mn>Cu>Ni>Zn>Pb>Cd>Hg, respectively. The concentration of Cu and Hg in stems and leaves were above the standard levels of Ministry of Public Health (2003), while Cu and Cd in root samples were above the standard levels. Additionally, the concentrations of Cd, Ni and Zn in all water samples were above the standard levels of Ministry of Natural Resources and Environment (1994), and the concentrations of Cu, Hg and Mn in certain water samples were above the standard levels. The translocation factors (TF_{root} and TF_{water}) and bioaccumulation factor (BAF) above 1 were observed in order of Cu>Ni>Cd>Zn>Mn, Mn>Zn>Ni >Cd, and Mn>Zn>Ni>Cd, respectively. The findings provide useful information to agricultural and public health authorities for a dialogue in policy development (i.e. the importation and use of pesticides) to minimize health risk of vulnerable population including farmers and nearby communities in the province, and thus strengthening equity in health.

Keywords: Organophosphate and carbamate insecticides; Anti-acetylcholinesterase activity; Heavy metals; Bioaccumulation; *Ipomoea aquatica* Forssk

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Introduction

Plant is a major source of several nutrients, such as vitamins and metals, and widely consumed by humans as part of a healthy diet [1]. However, several plants have found to be contaminated with chemical pesticide residues. In 2019, it has been reported that about 41.3% of fresh fruits and vegetables sold in supermarkets and fresh markets have pesticide residues over the maximum permissible levels [2]. In addition, previous studies have reported that soils, rice, and water from paddy fields in Nakhon Nayok province are contaminated with carbamate and organophosphate insecticides [3]. Moreover, above 60% of vegetable samples from Ongkharak market and family farms in the Sisa Krabue Community at Ongkharak District of Nakhon Nayok Province are contaminated with organophosphate and carbamate insecticides, and certain vegetable samples show insecticide contamination at harmful levels, and heavy metal contents (Fe, Cr and Pb) over permissible limits [4].

Although pesticide application in agricultural areas is a primary factor for increasing agricultural production, excessive use of pesticides can lead to environmental damage, especially in human health, such as the inhibition of acetylcholinesterase enzyme in the human body. Acetylcholinesterase (AChE) is a major enzyme involving the breakdown of neurotransmitter acetylcholine in the nervous system [5]. Determination of AChE inhibition has been used as an effective biomarker for monitoring carbamate and organophosphate insecticides involving occupational and environmental health [6]. Acetylcholinesterase inhibitory activities have been widely used to link exposure of carbamate and organophosphate insecticides in farmers [7], and the insecticide contamination is usually monitored from human bodily fluids, such as urine, blood and saliva [8]. However, it has been reported that organophosphorus and carbamate insecticides in paddy field soils, water, and rice plants can be monitored by determining acetylcholinesterase inhibition [9]. Moreover, many agricultural chemicals are composed of different heavy metals, such as Cd, Co, Cu, Zn, Pb, Fe, Mn, and Ni [10].

Heavy metal pollution is one of several serious global problems which effect environmental and human health [11-12]. The environment, and humans, can be exposed to heavy metals by agriculture, industries and medical use [13-15]. However, toxicities of heavy metals rely on dose, types, exposure methods, and personal factors that can lead to several disorders, such as oxidative stress, cancers, cardiovascular disease, and damaging biomolecules and organs in human body [16]. Heavy metals in chemicals and pesticides from several uses can become exposed to soils and water in the environment. Then, heavy metals in soils and water become exposed in the human body *via* the food chain: soil-plant-human system [17]. Additionally, several plants have properties in translocation and bioaccumulation of heavy metals that effect human health through the food chain [18].

Water morning glory (*Ipomoea aquatica* Forssk) is an edible leafy vegetable that is distributed throughout tropical Asia, in countries such as Thailand, and is popular for human consumption as a functional food [19]. It has been reported that *I. aquatica* Forssk can accumulate heavy metals, such as Pb, Cd and Hg [20]. Several studies of insecticide and heavy metal contamination in the environment and in plants have been carried out, but monitoring organo phosphate and carbamate insecticides in soil, water and water morning glory samples by determining anti-AChE activity, and evaluating concentration of heavy metals were novel in the targeted area. Thus, our major goals were to monitor organophosphate and carbamate insecticides in soil, water and *I. aquatica* Forssk from different canals, by determining AChE inhibition, and heavy metal contents were measured by using an atomic absorption spectrometry (AAS). These data are useful for surveillance of insecticide contamination in agricultural regions and to develop policy (i.e. the importation and use of pesticides in agriculture) for minimizing health risks of people in the communities, and helping to manage programs for human health promotion.

Method and materials

1) Chemicals

Absolute ethanol (Merck, USA), acetylthiocholine iodide (Sigma, Switzerland), DTNB (5,5′-dithiobis, 2-nitrobenzoic acid) (Sigma-Aldrich, Germany), and nitric acid (Sigma-Aldrich, USA) were obtained from Sac Sci-Eng Limited Partnership. Heavy metal standards (Cd, Cu, Pb, Ni, Hg, Zn and Mn) (Sigma-Aldrich, Switzerland), and acetylcho linesterase enzyme (Sigma-Aldrich, USA) were obtained from TTK Science Co., Ltd.

2) Sampling

Eighteen samples of *I. aquatica* Forssk, soils and water were randomly collected from Ongkharak District, Nakhon Nayok Province, Thailand between 20 August 2018 and 3 February 2019. The collection positions were shown by GPS (Global Positioning System) using the Google Earth program (version 7.1.2.2041) (Figure 1); 3 sub-regions from Sisa Krabue Canal (SK, 14º.08'31.68"N 100º.97'62.48"E, 14º 09'75.61"N 100º 98'63.48"E and 14º08'87.38"N 100º 98'18.5"E), 2 sub-regions from TaPao Canal (TP, 14º 08'04.93"N 100º97'28.63"E and 14º 08'10.31"N 100º 97'21.98"E), 4 sub-regions from canal in Srinakharinwirot University (SU, 14º10'06.65"N 100º98'29.18"E, 14º10'15.35"N 100º98'43.93"E, 14º10'79.08"N 100º98'20.35"E and 14º10'92.55"N 100º98'69.45"E), 3 sub-regions from Bang Kajik Canal (BK, 14º07'47.83"N 101º03'14.23"E, 14º07'66.67"N 101º03'61.08"E and 14º07'83.09"N 101º04'04.3"E), 3 sub-regions from Satharana Prayoch Canal (SP, 14º10'14.63"N 100º99'85.2"E, 14º09'85.69"N 100º98'83.73"E and 14º10'10.99"N 100º99'32.42"E), and 3 sub- regions from Yeesib Ha Canal (YH, 14º.09'07.56"N 101º.02'45.43"E, 14º.08'58.66"N 100º.99'90.82"E and 14º.08'77.61"N 100º.98'53.24"E).

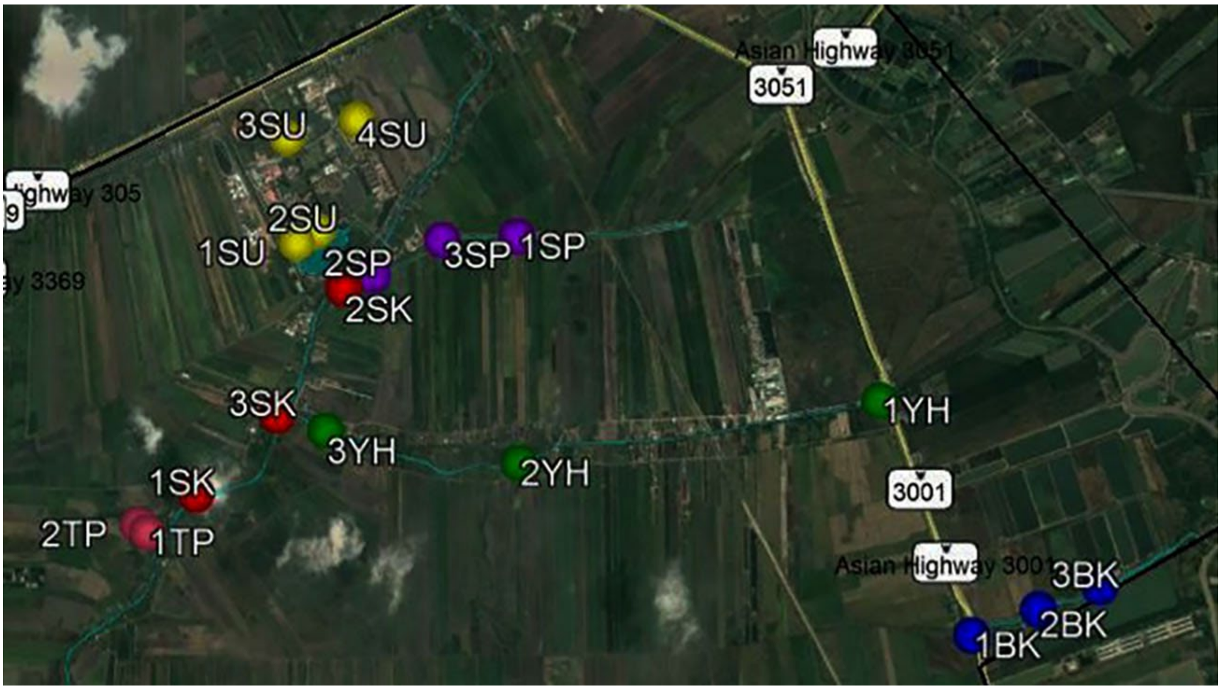

Figure 1 Sampling areas indicated by GPS and expressed on Google Earth (version 7.1.2.2041).

Each subsample of *I. aquatica* Forssk was separated into two parts (roots and parts of the stems and leaves). Every part of the samples were cleaned with deionized water, and cut into small pieces, then dried at 50 ºC, followed by homogenizing into powder, and being kept in plastic bags at room temperature for 10 min before use. Water samples were collected at depth of 15 cm based on grab sampling, and preserved in 50 mL polyethylene tubes at room temperature [3, 9, 21]. Each soil sample from the surface level to 15 cm depth from each location was collected, dried at 50 ºC, and kept in plastic bags at room temperature for 10 min before use [3, 9, 22].

3) Acetylcholinesterase inhibitory activity

Each 0.5 g sample of *I. aquatica* Forssk or soil for was extracted by mixing with 5% ethanol solvent for 5 min. After that, each extract or water sample (200 µL) was added with 15 mM acetyl thiocholine iodide (200 µL), 3 mM DTNB (1,000 µL), 0.3 U mL^{-1} of AChE enzyme (200 µL), and left at 37 ºC for 15 min. The absorbance was detected at a wavelength of 410 nm by a spectrophotometer (Model T60UV). Each reaction was performed in triplicate. For the positive control, methylcarbamate was used. Percentages of AChE inhibition were measured according to the formula of Thummajitsakul et al. (2019) [9] as shown in Eq. 1 where OD_{water} and OD_{sample} were the absorbance of each reaction with AChE enzyme of distilled water and sample, respectively. OD_{blank1} and OD_{blank2} were the absorbance of each reaction without acetylcholinesterase enzyme of distilled water and sample, respectively.

4) Sample preparation and digestion

The ground sample (0.5 g) and water sample (50 mL) were digested at 100 ºC with 65% HNO_3 (v/v) for 10 mL until dry. Each digested sample was mixed with 1% HNO_3, and filtered via Whatman No. 1 paper. The filtrate volume was adjusted to 50 mL with 1% HNO_3. Each digestion reaction was done in triplicate. Heavy metal contents (Cu, Pb, Cd, Ni, Mn, Zn and Hg) of each digested sample were measured by AAS (Model 200 Series AA, Agilent Technologies, USA). For quality assurance, at least two readings were performed in each experiment, including each standard solution prepared from dilution of 1,000 µg mL^{-1} stock concentration with 1% HNO_3. Concentration of each heavy metal was calculated by comparing with a linear standard calibration curve (y=0.00722x+0.00192 for Cu, y=0.10750x +0.00058 for Cd, y=0.02599x-0.00179 for Mn, y=0.01512x-0.00379 for Ni, y=0.01031x+0.00012 for Pb, y=0.30633x-0.00043 for Zn, y=0.00067x +0.00156 for Hg, R^2=1) [9].

5) Data analysis

Descriptive statistics (e.g. percentages, mean and SD) were used for acetylcholinesterase inhibitory activities and heavy metal contents. The relationships between % AChE inhibition and heavy metal contents of samples were performed by Pearson correlation. PSPP program version 0.10.5 [23], and Paleontological statistic program version 3.16 [24] were used for all analyses. Additionally, bioaccumulation factor (BAF) and translocation factors (TF_{root} and TF_{water}) were determined by the following equations.

$$\% \text{ AChE inhibition} = [(OD_{water}-OD_{blank1})-(OD_{sample}-OD_{blank2})] \times 100 / (OD_{water}-OD_{blank1}) \quad \text{(Eq.1)}$$

$$BAF = C_{leaves\ and\ stem} / C_{water}$$

$$TF_{root} = C_{leaves\ and\ stem} / C_{root}$$

$$TF_{water} = C_{roots} / C_{water}$$

where $C_{leaves\ and\ stem}$, C_{roots} and C_{water} were metal concentration in stem and leaves, roots, and water (mg kg^{-1} for solid and mg L^{-1} for water), respectively. Both TFs and BAF of *I. aquatica* Forssk over 1 indicated that it was a heavy metal accumulator [25-26].

Results and discussion

Excessive application of organophosphate and carbamate insecticides in agriculture has become an increasing trend in Thailand. This problem is causing negative impacts on human health and the environment. Organophosphorus and carbamate insecticides are toxic to human health by their action of inhibiting the AChE enzyme, leading to excessive accumulation of acetylcholine at neuronal synapses and neuro muscular junctions, which causes symptoms of muscarinic and nicotinic poisoning (i.e. nausea, vomiting, diarrhoea, bradycardia, and flaccid paralysis) [27-28].

1) AChE inhibition

In our study, the result showed that organo phosphorus and carbamate insecticides were found in all samples by determining anti-AChE activities. Difference of % acetylcholinesterase inhibition among sample groups (root, stem and leaves part, water, and soil) was insignificantly found at P-value > 0.05. However, the result represented that the highest AChE inhibition was observed in soil samples (ranged from 58.3 to 90.4%), followed by stem and leaves of *I. aquatica* Forssk (ranging from 65.7 to 91.4%), water samples (ranging from 49.6 to 82.4%), and roots of *I. aquatica* Forssk (ranging from 49.1 to 80.1%), as shown in Table 1.

Previously, Thummajitsakul et al. (2019) reports that organophosphorus and carbamate insecticides are found in paddy field soils, water and rice plants by determining AChE inhibition, and heavy metals (Fe, Mn, Ni, Pb, and Zn) are also observed in all shoots, soils, roots, grains and water samples, while Fe, Ni and Pb in all water and rice plants are shown to be over their maximum permissible levels [9].

Table 1 The percentages of AChE inhibition of root samples, stem and leaves samples of *I. aquatica* Forssk, water samples, and soil samples from each sampling location

Sampling locations*	% AChE inhibition (Mean±SD)			
	Root	**Stem and leaves**	**Water**	**Soil**
SK	63.8±26.2	75.4±21.1	49.6±11.1	87.7±7.8
TP	49.1±10.5	68.2±12.1	82.4±17.6	66.3±23.1
SU	80.1±11.6	70.7±19.5	79.0±30.5	77.7±15.4
BK	75.4±19.7	65.7±25.1	77.8±20.6	76.3±26.9
SP	74.4±36.2	91.4±9.6	77.8±16.2	58.3±30.6
YH	64.1±11.4	75.3±5.6	72.1±15.5	90.4±7.3
Total	69.5±20.6	74.6±17.0	72.9±20.7	76.8±20.2
P-value	**0.930***			

Remark: *Difference of % AChE inhibition among sample groups (root, stem and leaves, water, and soil) was significant at P-value < 0.05.

2) Heavy metal concentration in *I. aquatica* Forssk, water and soils

Our result showed that heavy metals, namely Mn, Cu, Hg, Ni, Zn, and Cd, were found in all samples of stem and leaves, roots, water and soils, while soil samples were contaminated with Mn, Cu, Pb, Ni, Zn, and Cd. Consequently stem and leaf, root, water and soil samples showed the mean concentration of each heavy metal in the order of Mn>Cu>Hg>Ni>Zn>Cd>Pb (do the same in other point), Mn>Zn>Ni> Cu>Cd>Hg>Pb, Ni>Cu>Hg>Mn>Cd>Zn>Pb, and Mn>Cu>Ni>Zn>Pb>Cd>Hg, respectively. Among all heavy metals, Cu, Cd, Ni, Zn, and Mn showed higher concentrations in root than stem and leaves, significantly (P-value < 0.05) (Table 2). Moreover, difference among sample groups (root, stem and leaves part, water, and soil) of each heavy metal, except for Cu, was found significantly at P-value < 0.05.

The mean values of Mn ranged from 97.5 to 974.3 mg kg^{-1} in stem and leaves, 1,700.4 to 33,089.7 mg kg^{-1} in roots, ND to 4.6 mg L^{-1} in water, and 89.4 to 964.4 mg kg^{-1} in soil. Order of Mn content in the samples was roots>leaves and stem>soil>water. This result was compared with the maximum permissible level of each sample shown in Table 2. The result showed that most of the water samples (66.7%) exceeded the maximum permissible level, while all soil samples were below its maximum permissible level (Table 3). Although, there was no criterion value of Mn for food and vegetables, *I. aquatica* Forssk showed high Mn levels, especially in the roots. High amounts of Mn in vegetables can be toxic and negatively affect human health. It has been reported that human who are exposed to high level of Mn for several years can suffer from disorders of several bodily systems, such as the central nervous system, reproductive system, and immune system [29].

The mean concentrations of Cd were in the range of 1.9 to 2.5 mg kg^{-1} for stem and leaves, 2.9 to 12.5 mg kg^{-1} for roots, 2.1 to 2.3 mg L^{-1} for water, and 3.0 to 3.3 mg kg^{-1} for soil. The order of Cd content in the samples was roots> soils>stem and leaves>water (Table 2). In comparison with the maximum permissible level of each sample, the result showed that most of water (100%) and root samples (83.3 %) showed above the maximum permissible levels, while all stem and leaf, and soil samples were below the maximum permissible limits (Table 3). It has been reported that enhancement of soil Cd concentration increases Cd accumulation in all parts of *I. aquatica* [30]. Although contamination of Cd in stem and leaves of *I. aquatica* Forssk did not exceed its permissible level, consumption of the exposed vegetables for long-term may cause toxicity in humans. Humans can obtain Cd *via* contaminated food (i.e. vegetables, water and food) leading to health impacts in the long-term. Cd can impact several human organ systems, such as the cardiovascular system, immune system, and cause blood-testis barrier damage, and the disturbing of insulin receptors [31].

The mean concentrations of Ni were in range of 21.6 to 23.5 mg kg^{-1} for stems and leaves, 24.6 to 119.2 mg kg^{-1} for roots, 12.0 to 12.9 mg L^{-1} for water, and 30.3 to 40.4 mg kg^{-1} for soils. The order of Ni content in the samples was roots>soils>stem and leaves>water (Table 2). In comparison with the maximum permissible level of each sample, the result showed that 100% of water samples showed above their maximum permissible level, while soil samples were below their maximum permission levels. Although, there was no criterion value of Ni for food and vegetables, long-term or short-term exposure of Ni can effect human health via drinking water and food, such as contact dermatitis, cardiovascular diseases, kidney diseases, and cancers [32].

Table 2 Concentrations of heavy metals found in root samples, stem and leaves samples of *I. aquatica* Forssk, water samples, and soil samples from each sampling location

Sampling locations	Samples	Concentration of heavy metals (mg kg^{-1} for solid or mg L^{-1} for liquid)						
		Cu	**Cd**	**Pb**	**Ni**	**Hg**	**Zn**	**Mn**
SK	Stem and leaves	124.9±11.4	2.0±0.6	ND*	21.6±0.5	39.6±15.8	19.5±2.6	167.7±96.0
	Root	26.4±16.1	8.4±9.6	ND*	67.0±73.5	ND*	56.6±17.3	1700.4±382.3
	Soil	95.7±46.6	3.3±0.3	7.7± 0.5	33.8±4.5	ND*	57.3±58.5	248.8±221.4
	water	ND	2.1±0.2	ND	12.0±0.6	ND	1.3±0.4	1.1±0.8
TP	Stem and leaves	1.3±1.7	2.3±0.8	ND*	22.9±1.2	20.9±21.1	21.2±11.3	301.2±214.2
	Root	41.3±56.8	4.1±0.6	ND*	32.7±15.8	ND*	42.3±3.6	2616.4±3241.1
	Soil	52.7±7.7	3.1±0.3	6.8± 8.6	38.9±10.2	ND*	25.7±2.7	964.4±819.1
	water	ND	2.1±0.2	ND*	12.7±0.0	ND*	1.7±0.2	1.3±0.8
SU	Stem and leaves	21.9±14.3	2.3±0.2	ND*	22.5±1.5	43.9±19.8	16.6±3.2	97.5±137.3
	Root	114.5±66.0	2.9±0.6	ND*	25.6±3.3	ND*	30.0±5.0	8630.8±15089.6
	Soil	77.6±19.6	3.2±0.3	5.1± 3.7	30.6±1.4	ND*	15.9±3.6	110.8± 67.3
	water	ND	2.1±0.2	ND*	12.3±0.4	6.7±5.3	1.4±0.4	ND*
BK	Stem and leaves	73.8±83.5	1.9±0.2	ND*	23.1±1.2	20.9±21.1	14.4±2.1	547.3± 589.7
	Root	69.9±71.9	12.5±16.6	ND*	119.2±161.0	ND*	322.7±462.8	4904.3± 7116.3
	Soil	70.5±32.6	3.3±0.4	5.6± 1.4	32.0±1.1	20.9±0.0	24.8±7.5	192.2± 117.3
	water	ND	2.3±0.3	ND*	12.6±0.9	ND*	1.7±0.1	ND*
SP	Stem and leaves	33.5±20.0	2.0±0.3	ND	23.2±1.0	40.8±15.5	15.7±3.5	974.3±389.1
	Root	24.9±15.1	3.1±0.2	ND*	24.6±1.6	ND*	82.8±19.3	33089.7±8132.8
	Soil	49.4±5.4	3.3±0.3	4.2± 3.9	40.4±13.2	ND*	24.0±8.5	538.3±777.4
	water	ND	2.2±0.1	ND*	12.9±0.3	3.0±0.0	1.2±0.2	4.0±3.6
YH	Stem and leaves	46.6±46.8	2.5±0.2	ND*	23.5±1.3	53.2±21.3	17.2±2.3	936.1±579.4
	Root	1.8±0.0	4.7±0.7	ND*	39.2±2.8	10.0±0.0	62.6±26.1	13475.2±18736.8
	Soil	24.5±8.24	3.0±0.3	4.5± 5.3	30.3±1.3	ND*	19.6±3.6	89.4±20.5
	water	8.9±0.0	2.2±0.3	ND*	12.5±0.8	ND*	1.6±0.2	4.6±1.0

Table 2 Concentrations of heavy metals found in root samples, stem and leaves samples of *I. aquatica* Forssk, water samples, and soil samples from each sampling location (*continued*)

Total (mean±SD)	Stem and leaves	50.3±43.8	2.2±0.2	ND*	22.8±0.7	36.5±13.0	17.4±2.5	504.0±381.9
	Root	46.5±40.2	5.9±3.8	ND*	51.4±36.7	10.0±0.0	99.5±110.8	10736.1±11774.6
	Soil	61.7±24.9	3.2±0.1	5.7±2.9	34.4±4.3	ND*	27.9±14.9	357.3±338.5
	water	8.9±0.0	2.2±0.1	ND*	12.5±0.3	4.9±2.6	1.5±0.2	2.7±1.8
MPL**	Food and Vegetables	20[a]	3[a]	1[a]	NV***	0.5[a]	100[a]	NV***
	Soil	250[d]	37[b]	400[b]	1,600[b]	23[b]	500[d]	1,800[b]
	water	0.1[c]	0.005[c] or 0.05[c]	0.05[c]	0.1[c]	0.002[c]	1.0[c]	1.0[c]
P-value[1]		0.053	0.009	-	0.010	0.000	0.031	0.012
P-value[2]	Stem and leaves	0.000						
	Root	0.002						
	Soil	0.001						
	water	0.000						

* ND = non detected, ** MPL = Maximum permission level (mg kg^{-1} for solid or mg L^{-1} for liquid), *** NV = no criterion value,

[a] Ministry of Public Health (2003) [48], [b] Ministry of Natural Resources and Environment, Thailand (2004) [49], [c] Ministry of Natural Resources and Environment, Thailand (1994) [50], [d] U.S. Environmental Protection Agency (1999) [51].

[1] difference of each heavy metal among sample groups (root, stem and leaves part, water, and soil) was significant at P-value < 0.05.

[2] difference among heavy metals in each sample group (root, stem and leaves part, water, and soil) was significant at P-value < 0.05.

Table 3 The percentages of root samples, stem and leaves samples of *I. aquatica* Forssk, water samples, and soil samples showing concentration of each heavy metal above the maximum permission level

	Cu	Cd	Pb	Ni	Hg	Zn	Mn
$MPL_{stem\ and\ leaves}$	83.3	0	0	0	100.0	0	NV***
MPL_{root}	83.3	83.3	0	0	16.7	0	NV***
MPL_{soil}	0	0	0	0	0	0	0
MPL_{water}	16.7	100.0	0	100.0	33.3	100.0	66.7

The mean concentrations of Zn were in the range of 14.4 to 21.2 mg kg^{-1} for stem and leaves, 30.0 to 322.7 mg kg^{-1} for roots, 1.2 to 1.7 mg L^{-1} for water, and 15.9 to 57.3 mg kg^{-1} for soil. The order of Zn concentration in the samples was root>soil>stem and leaves>water (Table 2). In comparison with its maximum permissible level of each sample, the result showed that 100% of water samples showed above their maximum permissible level, while other samples below their maximum permissible levels. However, toxicity can occur if consumers obtain excessive amount of Zn, with effects such as nausea, vomiting, diarrhea, abdominal pain, lethargy, anemia, and dizziness [33].

The mean concentrations of Cu were in the range of 1.3 to 124.9 mg kg^{-1} for stem and leaves, 1.8 to 114.5 mg kg^{-1} for roots, 24.48 to 95.7 mg kg^{-1} for soil, ND to 8.86 mg L^{-1} for water. The order of Cu concentration was soil>stem and leaves>root>water (Table 2). In comparison with the maximum permissible level of each sample, the results showed that stem and leaves (83.3%), root samples (83.3%), and water samples (16.7%) showed levels above the maximum permissible, while all soil samples were below the maximum permissible limit. Cu toxicity can lead to damage chromosomal and DNA by a free-radical-mediated process, and effect the liver and gastrointestinal system [34].

The mean concentrations of Hg were in the range of 20.9 to 53.2 mg kg^{-1} for stem and leaves, ND to 10.0 mg kg^{-1} for roots, ND to 6.7 mg L^{-1} for water, ND to 20.9 mg kg^{-1} for soil. The order of Hg concentration in the samples was stem and leaves>soil>root>water (Table 2). In comparison with the maximum permissible level of each sample, the results showed that stem and leaves (100%), water samples (33.3%), and root samples (16.7%) were above the maximum permissible levels, while all soil samples were below the maximum permissible limit. Hg toxicity can affect the functions of several bodily systems and organs, such as peripheral nerves, kidneys, and the immune, endocrine, and muscle systems [35].

The mean concentrations of Pb were only observed in soil samples ranging from 4.2 to 7.7 mg kg^{-1}, which were below its maximum permissible limit (Table 2). However, Pb is one of the major toxic heavy metals found in our environment, and can cause serious effects on renal, reproductive, and nervous systems in the human body [36].

3) Heavy metal accumulation and translocation of *I. aquatica* Forssk

Consequently, BAF and TFs of stem and leaves of *I. aquatica* Forssk were also calculated. The result revealed that the order of BAF were Mn>Zn>Ni>Cd. The BAF value exceeding 1 of stem and leaves samples indicated that *I. aquatica* Forssk has high potential to accumulate the heavy metals, especially Mn (Table 4). The transfer factors (TFs) of each heavy metal from water to roots (TF_{water}), and roots to stem and leaves (TF_{root}) were also evaluated. The mean of TF_{water} and TF_{root} were shown in the order of Mn>Zn>Ni>Cd, and Cu>Ni>Cd>Zn>Mn, respectively (Table 4).

The result revealed that TF_{root} of Cu was above 1 indicating Cu moved freely from root to stem and leaves of the plant sample, and TF_{water} of Cd, Zn, Ni and Mn were above 1 indicating Cd, Zn, Ni and Mn moved freely from water to the roots of *I. aquatica* Forssk. To confirm our finding, our current study was compared with other previous studies, shown in Table 5.

Generally, the mechanism of heavy metal translocation is an important factor for the distribution of heavy metals in plants [37]. In previous studies heavy metals, namely Pb, Cd and Hg, are found in *I. aquatica* from Bangkok province in Thailand, and in particular Hg found in leaves was higher than in stem [20]. Additionally, *I. aquatica* is a good accumulator for Mn and Cd, which can be transferred from roots to shoots, and in fact accumulated in larger quantities at roots than shoots [38]. Heavy metals, namely Cd, Cu, Ni, Pb, Sb, and Zn, have been also found in *I. aquatica* and sediments near wastewater inlets [39].

Previously reports have been made about different vegetables from local markets and family farms in Ongkharak District, Nakhon Nayok Province. Some vegetables, which contained organophosphate and carbamate insecticides at harmful levels, are also contaminated with heavy metals, namely Pb, Fe, Cr, Zn and Cu, and carry potential health risks in human [4].

Heavy metals may be in environments *via* natural processes or via human activities (i.e. agriculture and industry), and accumulate in water via overland flow or infiltration of rain water into ponds or the top surface soils, which are a major constituent of heavy metal accumulation [40-41]. Natural processes and human activities can impact on the qualities of surface and groundwater in rural and urban regions, such as contamination of heavy metals, and pesticides [42]. Heavy metals from the soil affect human health and the environment by travelling from soil to plants or by infiltration of water into soil and groundwater [43].

Heavy metals can move through the food chain (i.e. soil to water, water to plants, and plants to animals or humans), then enter the human body via consumption of contaminated plants. This process has an effect on human health, for which there are several determinants (i.e. behaviour, physiology, and diet), and overall contributes to heavy metal accumulation in humans [17, 44].

Noticeably, the target area of this current study has been reported about pesticide application. For example, the use of organophosphate and carbamate pesticides (i.e. chlorpyrifos, profenofos, metamidofos, fenobucarb and carbosulfan) of famers in Sisa Krabue Sub-district, Ongkharak District, Nakhon Nayok Province has been reported, especially mixing of several hazardous pesticides before use [58]. Moreover, effects of organophosphate insecticides on farmer and non-farmer health in the agricultural regions have been reported that the farmers have higher health risk than non-farmers who live nearby or around the agricultural area [58]. Pesticides can also be leached through the soil structure to groundwater, by flow or the infiltration process of rain water, but this depends on many factors (i.e. dose, method, application, and properties of the pesticide) [45].

Table 4 Values of TF and BAF of stem and leaves of *I. aquatica* Forssk

	Cu	Cd	Ni	Hg	Zn	Mn	HI
TF_{root}	5.4±9.9	0.5±0.2	0.6±0.3	-	0.3±0.2	0.1±0.0	-
BAF	-	1.0±0.1	1.8±0.0	-	11.9±2.2	199.1±46.6	-
TF_{water}	-	2.7±1.7	4.1±2.9	-	64.5±63.6	4,234.5±3,534.5	-

Table 5 Heavy metal contamination of *I. aquatica* Forssk, water and soil from previous studies

Heavy metals	Distribution of heavy metals	Heavy metals exceeding MPL	References
- Mn>Cu>Hg>Ni>Zn>Cd>Pb (for stem and leaves) - Mn>Zn>Ni>Cu>Cd>Pb or Hg (for roots) - Ni>Cu>Hg>Mn>Cd>Zn>Pb (for water) - Mn>Cu>Ni>Zn>Pb>Cd>Hg (for soils)	- found in all parts of *I. aquatica* Forssk in canals in Ongkharak District, Nakhon Nayok Province, Thailand - root>stem and leaves (for Mn, Ni, Zn, and Cd) - water samples - soil samples	- Cu and Hg for stem and leaves - Cu and Cd for roots - Cu, Hg, Cd, Ni and Zn for water samples	Our current study
- Cu>Pb>Cr>Zn>Ni>Cd for *I. aquatica* Forssk -Cr, Pb>Ni, Cd for water samples - Pb>Zn, Cu>Cr>Ni>Cd for soil samples	- root>stem>leaves - water samples - soil samples	- Cd, Cr, Pb, and Zn in the stem and roots - Cr and Pb in groundwater - Cd and Cu in landfill soils and its vicinity	[52]
Zn>Pb>Cu	roots>shoots	Pb in shoots	[53]
As>Ni>Pb>Hg	- found in all parts of *I. aquatica* Forssk in Tha Chin River, Thailand - roots>stems>branches > leaves>shoots - water in Tha Chin River, Thailand	No heavy metals	[54]
As>Cd>Cr>Ni>Fe>Cu>Zn>Pb	- found in all parts of *I. aquatica* Forssk in industrial areas around Dhaka City, Bangladesh - leaves>roots - soil and water samples	- As, Cd, Cr, Ni, Fe, Cu, and Zn in leaves and roots - As and Cd in water sample - As and Cr in soil sample	[55]
Zn>Cu>Pb>Cd	- found in all parts of *I. aquatica* Forssk in Tha Chin River, Samut Sakhon, Thailand - root>leaves>stems - water samples	- Cu and Zn in water samples	[56]
Pb>Cd	- found in all parts of *I. aquatica* Forssk in Laguna de Bay - root>leaves	No heavy metals	[57]
Hg>Pb>Cd	- found in all parts of *I. aquatica* Forssk in Bangkok, Thailand - leaves>stems (for Hg)	Hg in leaves	[20]

Interestingly, it has been reported that several inorganic fertilizers and pesticides are important sources of heavy metals, such as Cd, Co, Cu, Zn, Pb, Fe, Mn, and Ni [10]. Similarly, heavy metals namely As, Cr, Co, Pb and Ni have been identified in 22 pesticides. Importantly, a combination of pesticides and heavy metals can provide a synergistic toxic interaction in humans and animals [46]. Therefore, the present research implies that the heavy metals occur from human activities through the application of pesticides.

Previously, positive correlations between percentages of anti-acetylcholinesterase activities and heavy metal concentrations (Fe, Mn, Ni, Pb, and Zn), including translocation and accumulation, have been observed in rice grain [9]. In the current study, correlations between the percentages of anti-AChE activities and all heavy metal contents in root, stem and leaves, water, and soil were performed by using principle component analysis. The first principal component (PC1) showed 36.39% of total variability, and the second principal component (PC2) had 20.28% of the variance. The Cd, Ni and Zn contents were grouped in the positive region of PC1 axis, while Cu, Pb, Hg, Mn and % AChE inhibition were clustered in PC2 (Figure 2). The result showed that correlation between the percentages of anti-AChE activities and concentration of all heavy metals in *I. aquatica* Forssk, soil and water samples was found, insignificantly (P-value $>$ 0.05). However, strong negative and positive correlation between the percentage of anti-AChE activity and Ni content ($r = -0.836$), and between Ni and Mn contents ($r = 0.863$) in soil sample was significantly found (P-value $<$ 0.05). Strong positive correlation between Cd and Ni ($r=0.993$), between Cd and Zn ($r=0.845$), and between Ni and Zn ($r=0.897$) in root sample was also found, significantly (P-value $<$ 0.05). Furthermore, the result confirmed that Cu, Cd, Ni, Zn, and Mn had higher concentrations in root than stem and leaves, significantly (P-value $<$ 0.05). Hence, the PCA result implies that mechanism of heavy metal uptake by this plant is complicate. It may involve several factors affecting translocation of each heavy metal, such as soluble of each heavy metal, interaction between heavy metals, and properties of plant tissue.

Moreover, our study indicated that *I. aquatica* Forssk was a hyperaccumulator of some heavy metals. This data is useful for monitoring heavy metals or cleaning contaminated water sources. Moreover, consumption of *I. aquatica* Forssk affects on bioaccessibility of other contaminated food [47].

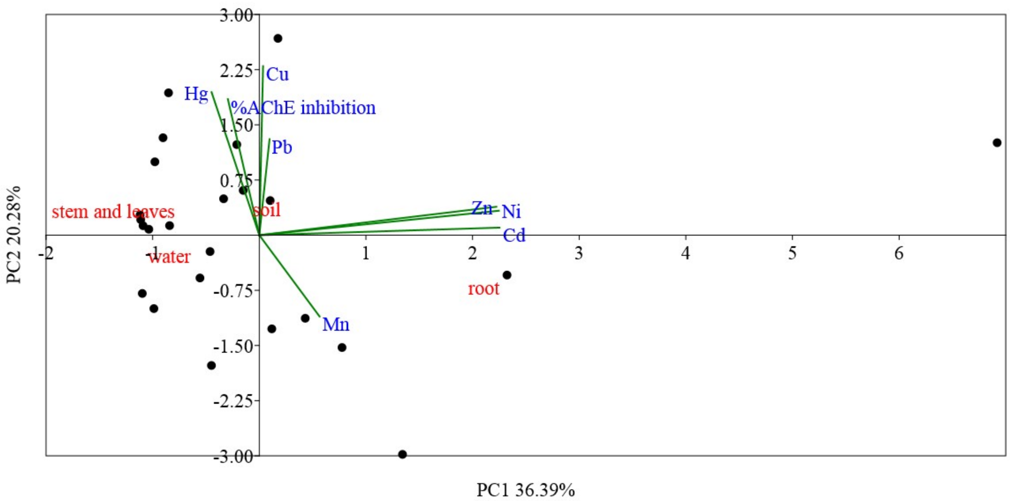

Figure 2 Biplot (PC1 x PC2) for principle component analysis of heavy metal contents and the percentages of acetylcholinesterase inhibition of root, and stem and leaves of *I. aquatica* Forssk, including water, and soil.

Conclusion

This study explored the environmental pollution of the water, soil, and edible plants in the canals of Thailand. Organophosphate and carbamate insecticides, determined by anti-AChE activity, were found in all samples of soils, stem and leaves, water, and roots, respectively. Moreover, stem and leaves, roots, and water samples were contaminated with Mn, Cu, Hg, Ni, Zn, and Cd, while soil samples were contaminated with Mn, Cu, Pb, Ni, Zn, and Cd. Most stem and leaves showed Cu and Hg above the maximum permissible levels, while most root samples showed Cu and Cd above maximum permissible levels. Additionally, all water samples had concentrations of Cd, Ni and Zn above maximum permissible levels, and certain samples showed Cu, Hg and Mn above their maximum permissible levels. Moreover, our study showed that *I. aquatica* Forssk was a hyperaccumulator of some heavy metals, and therefore, it may be used as a bioindicator to monitor contamination of heavy metals or to develop a new method for cleaning water that was contaminated with the heavy metals. However, consumption of *I. aquatica* Forssk from ponds nearby agricultural areas should be a concern for human health. There should be frequently monitoring of water, soil and this plant in the canals, and education about organic agriculture and chemical waste management should be provided for people in the area.

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References

[1] Slavin, J.L., Lloyd, B. Health benefits of fruits and vegetables. Advances in nutrition, 2012, 3(4), 506-516.

[2] Thai Pesticide Alert Network. The result of testing on chemical pesticide residues in fruits and vegetables for 2019. Thai Pesticide Alert Network's website, 2019. [Online] Available from: https://www.thaipan.org/wp-content/uploads/2019/pesticide_doc58.pdf?fbclid=IwAReGpd-mJ6 SirHCYX1 KsRQTRnYsfgELV358 Ug9liLNYhrriASK5 kLeVPk [Accessed 23 April 2019]

[3] Thummajitsakul, S., Praditpol, H., Poolaoi, J., Silprasit, K. Carbamate and organophos phate contamination in soil, rice, and water samples from rice paddy fields in Nakhon Nayok Province. Applied Environmental Research, 2015, 37(3), 103-109.

[4] Thummajitsakul, S., Subsinsungnern, R., Treerassapanich, N., Kunsanprasit, N., Puttirat, L., Kroeksakul, P., Silprasit, K. 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, 2018, 41(3), 987-1001.

[5] Soreq, H., Seidman, S. Acetylcholinesterase - New roles for an old actor. Nature Reviews Neuroscience, 2001, 2, 294-302.

[6] Lionetto, M.G., Caricato, R., Calisi, A., Giordano, M.E., Schettino, T. Acetylcho linesterase as a biomarker in environmental and occupational medicine: New insights and future perspectives. BioMed Research International, 2013, 1-8.

[7] Rendón von Osten, J., Epomex, C., Tinoco-Ojanguren, R., Soares, A.M., Guilhermino, L.

Effect of pesticide exposure on acetylcho linesterase activity in subsistence farmers from Campeche, Mexico. Archives of Environmental Health, 2004, 59(8), 418-25.

[8] Kapka-Skrzypczak, L., Cyranka, M., Skrzypczak, M., Kruszewski, M. Biomo nitoring and biomarkers of organophosphate pesticides exposure - State of the art. Annals of Agricultural and Environmental Medicine, 2011, 18(2) 294-303.

[9] Thummajitsakul, S., Sangdee, C., Thaisa, S., Ruengwiroon, P., Silprasit, K. The monitoring of organophosphorus and carbamate insecticides and heavy metal contents in paddy field soils, water and rice (Oryza sativa L.). Pertanika Journal of Tropical Agricultural Science, 2019, 42(1), 61-77.

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

[11] Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N. Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology, 2004, 7(2), 60-72.

[12] Mansour, S.A. Heavy Metal contamination as a global problem and the need for prevention/reduction measurements. Wiley Online Library's website, 2014. [Online] Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/9781118474563.ch 14 [Accessed 23 April 2019]

[13] Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J. Heavy metals toxicity and the environment. Molecular, Clinical and Environmental Toxicology, 2012, 101, 133-164.

[14] He, Z.L., Yang, X.E., Stoffella, P.J. Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology: Organ of the Society for Minerals and Trace Elements, 2005, 19(2-3), 125-40.

[15] Rehman, K., Fatima, F., Waheed, I., Akash, M.S.H. Prevalence of exposure of heavy metals and their impact on health consequences. Wiley Online Library's website, 2017. [Online] Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jcb.26234[Accessed 23 April 2019]

[16] Alissa, E.M., Ferns, G.A. Heavy metal poisoning and cardiovascular disease. Journal of Toxicology, 2011, 1-21.

[17] Gall, J.E., Boyd, R.S., Rajakaruna, N. Transfer of heavy metals through terrestrial food webs: A review. Environmental Moni toring and Assessment, 2015, 187(4), 201.

[18] Khan, A., Khan, S., Khan, M.A., Qamar, Z., Waqas, M. The uptake and bioaccumu lation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: A review. Environmental Science and Pollution research, 2015, 22, 13772-13799.

[19] Prasad, K.N., Shivamurthy, G.R., Aradhya, S.M. Ipomoea aquatica, an underutilized green leafy vegetable: a review. International Journal of Botany, 2008, 4(1), 123-129.

[20] Göthberg, A., Greger, M., Bengtsson, B.E. Accumulation of heavy metals in water spinach (Ipomoea aquatica) cultivated in the Bangkok region, Thailand. Environmental Toxicology and Chemistry, 2002, 21(9), 1934-1939.

[21] Chowdhury, A.Z., Banik, S., Uddin, B., Moniruzzaman, M., Karim, N., Gan, H.S. 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, 2012, 9(9), 3318-3329.

[22] Fery, M., Murphy, E. (2013). A guide to collecting soil samples for farms and gardens. The Oregon State University Extension Service's website, 2013. [Online] Available from: https://catalog.extension.oregonstate.edu/ec628 [Accessed 23 April 2019]

[23] Pfaff, B., Darrington, J., Stover, J., Satman, M.H., Beckmann, F., Williams, J., Kiefte, M., Kobly, P., van Son, R. GNU PSPP version's website, 2013. [Online] Available from: https://www.gnu.org/software/pspp/ [Accessed 23 April 2019]

[24] Hammer, Ø., Harper, D.A.T., Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. The Palaeontological Association's website, 2001. [Online] Available from: http://palaeo-electronica.org/2001_1past/issue1_01.htm [Accessed 23 April 2019]

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

[26] Neeratanaphan, L., Khamma, S., Bencha wattananon, R., Ruchuwararak, P., Appa maraka, S., Intamat, S. 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, 2017, 23(5), 1086-1098.

[27] Vale, A., Lotti, M. Organophosphorus and carbamate insecticide poisoning. Handbook of Clinical Neurology, 2015, 131, 149-68.

[28] Adeyinka, A., Kondamudi, N.P. Cholinergic crisis. National Center for Biotechnology Information's website, 2019. [Online] Available from: https://www.ncbi.nlm.nih.gov/books/NBK482433/ [Accessed 23 April 2019]

[29] Keen, C.L., Zidenberg-Cherr, S. Manganese. ScienceDirect's website, 2003. [Online] Available from: https://doi.org/10.1016/B0-12-227055-X/00732-X [Accessed 23 April 2019]

[30] Huang, B., Xin, J., Dai, H., Liu, A., Zhou, W., Liao, K. Translocation analysis and safety assessment in two water spinach cultivars with distinctive shoot Cd and Pb concentrations. Environmental Science and Pollution Research International, 2014, 21, 11565-11571.

[31] Bernhoft, R.A. Cadmium toxicity and treatment. The Scientific World Journal, 2013, 1-7.

[32] Duda-Chodak, A., Blaszczyk, U. The impact of nickel on human health. Journal of Elementology, 2008, 13(4), 685-696.

[33] Plum, L.M., Rink, L, Haase, H. The essential toxin: impact of zinc on human health. International Journal of Environmental Research and Public Health, 2010, 7(4), 1342-1365.

[34] National Research Council. Copper in drinking water. National Academy of Sciences's website, 2000. [Online] Available from: https://doi.org/10.17226/9782 [Accessed 23 April 2019]

[35] Bernhoft, R.A. Mercury toxicity and treatment: A review of the literature. Journal of Environmental and Public Health, 2012, 1-10.

[36] Wani, A.L., Ara, A., Usmani, J.A. Lead toxicity: A review. Interdisciplinary Toxicology, 2015, 8(2), 55-64.

[37] Page, V., Feller, U. Heavy metals in crop plants: Transport and redistribution processes on the whole plant level. Agronomy, 2015, 5, 447-463.

[38] Guan, B.T.H., Mohamat-Yusuff, F., Halimoon, N., Yong, C.S.Y. Uptake of Mn and Cd by wild water spinach and their bioaccu mulation and translocation factors. Environment Asia, 2017, 10(1), 44-51.

[39] Marcussen, H., Dalsgaard, A., Holm, P.E. Element concentrations in water spinach (Ipomoea aquatica Forssk.), fish and sediment from a wetland production system that receives wastewater from Phnom Penh, Cambodia. Journal of Environmental Science and Health, Part A, 2009, 44(1), 67-77.

[40] Pachana, K., Wattanakornsiri, A., Nanuam, J. Heavy Metal transport and fate in the environmental compartments. NU Science Journal, 2010, 7(1), 1-11.

[41] Sun, R., Chen, L. (2016). Assessment of heavy metal pollution in topsoil around Beijing Metropolis. PLoS One, 2016, 11(5), e0155350.

[42] Khatri, N., Tyagi, S. Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas. Journal Frontiers in Life Science, 2015, 8(1), 23-39.

[43] Bhagure, G.R., Mirgane, S.R. Heavy metal concentrations in groundwaters and soils of Thane region of Maharashtra, India. Environmental Monitoring and Assessment, 2011, 173(1-4), 643-652.

[44] Hapke, H.J. Heavy metal transfer in the food chain to humans. Springer's website, 1996. [Online] Available from: https://doi.org/10.1007/978-94-009-1586-2_73 [Accessed 24 June 2019]

[45] Pérez-Lucas, G., Vela, N., Aatik, A.E., Navarro, S. Environmental risk of groundwater pollution by pesticide leaching through the soil profile. Intechopen's website, 2018. [Online] Available from: doi: 10.5772/intechopen.82418 [Accessed 23 April 2019]

[46] Singh, N., Gupta, V.K., Kumar, A., Sharma, B. Synergistic effects of heavy metals and pesticides in living systems. Frontiers in Chemistry, 2017, 5, 70.

[47] Yang, U., Yoon, S.R., Chung, J.H., Kim, Y. J., Park, K.H., Park, T.S., Shim, S.M. Water spinach (Ipomoea aquatic Forsk.) reduced the absorption of heavy metals in an in vitro bio-mimicking model system. Food and Chemical Toxicology, 2012, 50(10), 3862-3866.

[48] Ministry of Public Health. Standard of foods contained contaminants. Bureau of Food. Ministry of Public Health's website, 2003. [Online] Available from: http://food.fda.moph.go.th/law/announ_moph1-150.php [Accessed 23 April 2019]

[49] Ministry of Natural Resources and Environment, Thailand. Announcement of National Environment Committee No. 25 Standard level on soil quality. Ministry of Natural Resources and Environment's website, 2004. [Online] Available from: http://www.pcd.go.th/info_serv/reg_std_soil01.html [Accessed 23 April 2019]

[50] Ministry of Natural Resources and Environment, Thailand. Announcement of National Environment Committee No. 8 Standard level on water surface quality. Ministry of Natural Resources and Environment's website, 1994. Online] Available from: http://www.pcd.go.th/info_serv/reg_std_water05.html [Accessed 23 April 2019]

[51] U.S. Environmental Protection Agency (USEPA). Background report on fertilizer use, contaminants and regulations. U.S. Environmental Protection Agency's website, 1999. [Online] Available from: https://nepis.epa.gov/Exe/ZyNET.EXE?ZyActionL=Register&User=anonymous&Password=anonymous&Client=EPA&Init=1 [Accessed 23 April 2019]

[52] Vongdala, N., Tran, H.D., Xuan, T.D., Teschke, R., Khanh, T.D. Heavy metal accumulation in water, soil, and plants of municipal solid waste landfill in Vientiane, Laos. International Journal of Environmental Research and Public Health, 2019, 16(1), 1-13.

[53] Ng, C.C., Rahman, M.M., Boyce, A.N., Abas, M.R. (2016). Heavy metals phyto-assessment in commonly grown vegetables: water spinach (*I. aquatica*) and okra (*A. esculentus*). SpringerPlus, 2016, 5, 1-9.

[54] Rattanapaiboon, W., Dampin, N., Chankao, K. The quantity of heavy metal accumulated in water spinach (Ipomoea aquatica Forsk) cultured in Tha Chin River. Naresuan University Journal: Science and Technology, 2015, 23(1), 82-93.

[55] Nahar, K.K., Alam, S.S. (2015). Physical and chemical parameters and trace metal flux in water, soil and plant samples collected from industrial effluent affected areas in and around Dhaka city. Bangladesh Journal of Botany, 2015, 44(2), 237-244.

[56] Veschasit, O., Meksumpun, S., Meksumpun, C. Heavy metals contamination in water and aquatic plants in the Tha Chin River, Thailand. Kasetsart Journal (Natural Science), 2012, 46, 931-943.

[57] Baysa, M.C., Anuncio, R.R.S., Chiombon M.L.G., Cruz, J.P.R.D., Ramelb, J.R.O. Lead and cadmium contents in Ipomoea aquatica Forsk. grown in Laguna de Bay. Philippine Journal of Science, 2006, 135 (2), 139-143.

[58] Wilaiwan, W., Siriwong, W. Assessment of health effects related to organophosphate pesticides exposure using blood cholinesterase activity as a biomarker in agricultural area at Nakhon Nayok Province, Thailand. Journal of Health Research, 2014, 28(1), 23-30.