

Heavy Metal Contamination and Genetic Differentiation in Two Edible Aquatic Plants Near an Electronic Waste Dumpsite

Thitima Parkpoom¹, Sutee Chowrong¹, Somsak Intamat²,
Latsamy Soulivongsa³, and Lamyai Neeratanaphan^{1*}

¹ Division of Environmental Science, Faculty of Science, Khon Kaen University,
Khon Kaen 40002, Thailand

² Thatphanom Crown Prince Hospital, Nakornphanom 48110, Thailand

³ Faculty of Agriculture, National University of Laos, Vientiane 0604, Lao PDR

*Corresponding author: hlmya@kku.ac.th

Received: March 16, 2023; Revised: April 3, 2023; Accepted: April 9, 2023

Abstract

This study aimed to investigate As, Cd, Cr, Pb, Mn and Zn quantities in water, sediment and two edible aquatic plants (*Ottelia alismoides* and *Ipomoea aquatic*). The samples were collected from five sampling sites near an electronic waste (e-waste) dumpsite in Kalasin province of Thailand. Heavy metal quantity, bioaccumulation factors (BAFs) and inter-simple sequence repeats (ISSR) analysis were evaluated in two edible aquatic plants near an e-waste dumpsite and was compared with a reference area. The As and Pb quantities in the water samples from the e-waste dumpsite exceeded the surface water quality standard. In the sediment, heavy metal quantities were significantly higher than those in the reference area. The As, Cd, Cr, Pb and Zn quantities in *O. alismoides* near an e-waste dumpsite were higher than the food standards. The Pb quantity in *I. aquatic* near an e-waste dumpsite was greater than the food standards of the FAO/WHO. The highest BAFs of As (2.27 ± 0.12), Cd (1.40 ± 0.11) and Mn (22.73 ± 1.65) were found in *O. alismoides*. The genetic similarity values ranged from 0.43 - 0.99 in *O. alismoides* and from 0.42 - 0.98 in *I. aquatic*. These results indicated that heavy metals near e-waste accumulated in the two aquatic plants might be a factor in genetic differentiation.

Keywords: Aquatic plant; Heavy metal; E-waste; Genetic; Genotoxicity

1. Introduction

The manufacture of electronic and electrical equipment is one of the fastest-growing global activities. This situation results in a substantial increase in human-produced electronic waste (e-waste). E-waste is an obsolete product and can diffuse the toxicity of heavy metals to the surrounding environment (Kyere *et al.*, 2017). As, Cd, Cr, Pb, Mn and Zn has been detected in e-waste. In particular, As is detected in electric circuits, computers and mobile phones. Cd is released from batteries, cathode-ray tubes and printing press resistors. Cr is present monitors computers and cathode ray tube (CRT). Pb is leaked from the lead glass, battery paint, oil and CRT containing devices. Zn is found in the

battery, electrical circuits, cathode ray tube (CRT) and monitors (Jaishankar *et al.*, 2014). In addition, local workers improperly separate the valuable parts of e-waste. Residents have also released toxic substances from incorrectly disposed of e-waste that transported and burned at community dumpsites (Caravanos *et al.*, 2011). In addition, Pb, Ni and Mn were detected in the e-waste area (Saetang *et al.*, 2009). Consequently, the Agboghloshie of the Republic of Ghana found As, Cd, Cr, Pb, Mn, Ni and Zn in the e-waste area (Kyere *et al.*, 2017). In Thailand, the Khong Chai district in Kalasin province has the highest amount of e-waste stocks, which is reported to be above 72,000 tons per year. These e-waste dumpsites

release hazardous elements that affect the surrounding environment (Parkpoom et al., 2022). In addition, a previous study on e-waste found heavy metal quantities that exceeded standards in surface water, soil and aquatic plants surrounding this area. In particular, Pb (0.832 ± 0.957 mg/L), Cd (0.075 ± 0.079 mg/L) and Cr (0.31 ± 0.02 mg/L) in water were higher than the limits set by the government of Thailand (Thanomsangad et al., 2019). Saetang et al. (2009) detected that Ni (75 mg/kg), Mn (1,519 mg/kg) and Pb (79,520 mg/kg) in soil exceeded the soil quality standards. In addition, Neeratanaphan et al. (2017) reported Pb (1.06 ± 0.50 mg/L) in rice grains (*Oryza sativa*), which exceeded the standards set by FAO/WHO (0.2 mg/kg).

In this study, the e-waste dumpsite in Khong Chai district, Kalasin province has most of the edible aquatic plants, represented by duck lettuce (*Ottelia alismoides*; submerged plant) and water spinach (*Ipomoea aquatic*; floating plants). The effect of heavy metals in edible aquatic plants can result in a variety of adverse effects. The toxicities of heavy metals can inhibit DNA methylation, DNA repair, enzyme activities, gene expression and metabolism, and their mechanisms can lead to structural changes in chromosomes as well as genetic mutations. Furthermore, heavy metals can cause genetic differentiation by increasing DNA polymorphism (Hu et al., 2017), resulting in DNA changes in aquatic plants, chromosome aberrations in the roots of *Elodea canadensis* cells and genotoxicity and DNA damage in aquatic plant species such as water thyme (*Hydrilla verticillate*), hornwort (*Ceratophyllum demersum*), taro or elephant ear (*Colocasia esculenta*) and yellow velvetleaf (*Limnocharis flava*) (Gupta and Sarin, 2009). Therefore, aquatic plants could be used to study genotoxicity as a result of environmental pollution. Toxicity studies of heavy metals in contaminated areas are conducted using the genotoxicity method. The amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR) and randomly amplified polymorphic DNA (RAPD) methods are commonly used to indicate genetic relationships (Amom et al., 2017). ISSR markers are acceptable and capable of evaluating genetic differences

in aquatic plants because they are easy to apply, reliable, repeatable, inexpensive and informative. This marker can detect DNA polymorphisms in DNA sequences of aquatic plants contaminated with heavy metals (Wang et al., 2007). Thus, the purposes of this study were to detect heavy metal quantities in the environment and two edible aquatic plant species (*O. alismoides* and *I. aquatic*), including genetic differentiation by ISSR markers, in *O. alismoides* and *I. aquatic* near an e-waste dumpsite compared with the reference area.

2. Materials and Methods

2.1 Study areas

The study area was located in Kong Chai district, Kalasin province, northeastern Thailand. The geographic coordinates of this study site were latitude $16^{\circ}33'18''$ N and longitude $103^{\circ}39'31''$ E. The distance from five locations of the sample collection site to the e-waste dumpsite was approximately 50 meters. The surrounding environment of the e-waste dumpsite is a paddy field (Figure 1). The reference area was located in Muang district, Khon Kaen province, where there is no activity out of the contaminated heavy metals.

2.2 The analysis of heavy metals in the water, sediment, and aquatic plant samples

The water and sediment were collected from five sampling points surrounding an e-waste open dumpsite. Each sampling point collected the sample in three replicates. Water (20 mL) and 65% HNO₃ (1.25 mL) were added and incubated in a digestion block at 105 °C for 2 hr. Each sample (1.0 g) of the dried sediment was homogenized and mixed with HNO₃ (5 mL), HCl (15 mL) and H₂O₂ (10 mL). These samples were heated in a digestion block at 200 °C for 2 hr. The plant samples (0.5 g) were homogenized with HNO₃ (5 mL) and H₂O₂ (3 mL) and boiled on a hot plate at 120 °C until the solution evaporated to ambient aridity. Finally, all solution samples were adjusted to 25 mL for water and filtered through a membrane

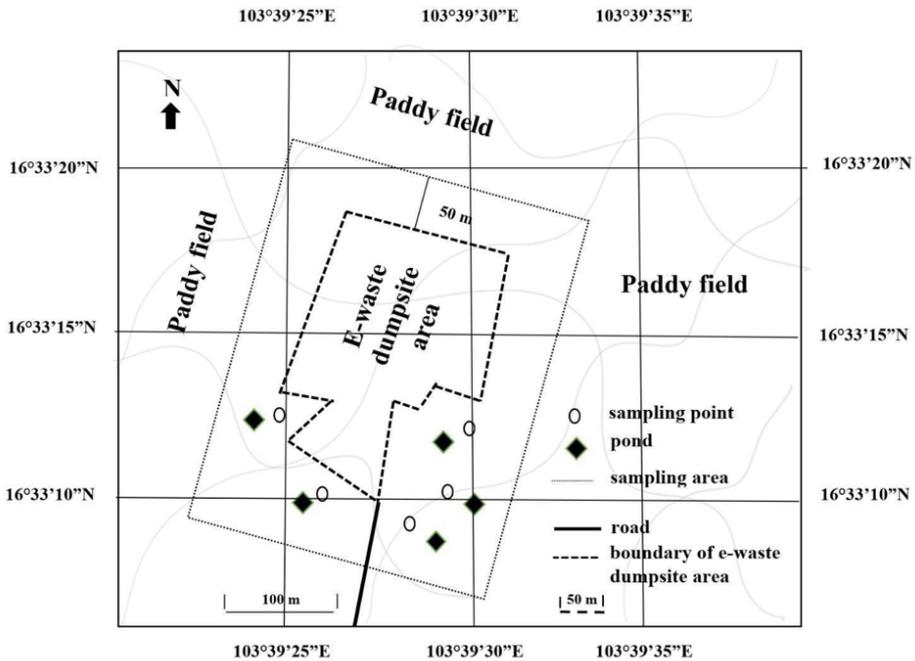


Figure 1. The e-waste dumpsite and the five locations of the sampling points

filter. Inductively coupled plasma-optical emission spectrometry (ICP-OES; model Optima 8300; USA) was used to analyze the concentrations of As, Cd, Cr, Pb, Mn and Zn in all samples. The limit of detection (LOD) for As, Cd, Cr, Pb, Mn and Zn was 0.006, 0.001, 0.001, 0.005, 0.002 and 0.001 mg/L, respectively. The wavelengths of As, Cd, Cr, Pb, Mn and Zn were 188.979, 226.502, 267.716, 220.353, 259.372 and 213.857 nm, respectively (Chowrong *et al.*, 2022). This method follows any standard method for the heavy metal analysis.

2.3 Bioaccumulation factor (BAF)

BAFs are defined as the potential of plants to collect specific metals with respect to their sediment concentrations. The BAF is calculated using the following equation (Thanomsangad *et al.*, 2019):

$$BAF = \frac{\text{Concentration of metal in plant tissue}}{\text{Concentration of metal in sediment}}$$

A BAF value >1 indicates that the edible aquatic plant's accumulation was greater than that of the sediment.

2.4 DNA extraction of the aquatic plant samples

Five random replicates of *O. alismoides* and *I. aquatic* samples were gathered from the paddy field near the e-waste dumpsite and reference area, and their tissues were preserved at -20 °C. The DNA of two edible aquatic plants was extracted from the genomic DNA extraction kit (RBC Bioscience, Taiwan). The DNA products were checked on a 0.8% agarose gel for the extracted DNA. All DNA samples were diluted to the final concentration (20 ng/μL) used as DNA templates in the polymerase chain reaction (PCR) (Tanee *et al.*, 2016; Ruchuwarak *et al.*, 2020).

2.5 Inter simple sequence repeat (ISSR) analysis

DNA fingerprinting of each edible aquatic plant sample (10 μL) was carried out using ISSR markers. Each reaction consisted of a combination of deionized water (3.8 μL), GoTaq Green Master Mix (5 μL), primers (0.2 μL) and extracted DNA (1 μL) for a final volume of 20 μL. The 40 ISSR primers that were screened are shown in Table 1.

The reaction mixture was incubated at 94 °C for 3 min 35 cycles consisting of 1) 30 secs at 94 °C for denaturation, 2) 30 sec at 50 °C for annealing, 3) 2 min at 72 °C for extension and 4) 7 min at 72 °C for final extension. The amplification products were detected using 1.2% agarose gel electrophoresis in TAE buffer and visualized under UV light. The DNA bands from all of the successful primers were reported as the following diallelic characters: present = 1 and absent = 0. The resulting ISSR bands were used for the dendrogram construction and the genetic similarity (S) values by the NTSYSpc 2.10 program (Neeratanaphan et al., 2014).

2.6 Statistical analyses and data calculation

The statistical comparisons of heavy metal quantities in water, sediment and two edible aquatic plants from the e-waste dumpsite and reference area were statistically analyzed using the Mann–Whitney U test. The genetic differentiation of two edible aquatic plants was evaluated by the genetic similarity (S) values. All

statistical analyses were carried out using SPSS software version 24.0 at the 95% confidence level.

3. Results and Discussion

3.1 Heavy metal quantities in water and sediment

Table 2 shows the heavy metal quantities in water and sediment samples from the reference area and e-waste dumpsite. The Pb quantity from the e-waste dumpsite (0.096 ± 0.060 mg/L) exceeded the surface water quality standard (0.05 mg/L) (TPCD, 1994). The Pb and Zn quantities of the water samples from the e-waste dumpsite were higher than those from the reference area ($p < 0.05$). The heavy metals in sediments from both areas were lower than the allowable level established using the soil quality standard (TPCD, 2021). In the sediment samples; As, Cd, Cr, Pb, Mn and Zn from the reference area and e-waste were significantly different ($p < 0.05$).

Table 1. The base sequences of 40 ISSR primers

Primer	Base sequence of primer	Primer	Base sequence of primer
A1	CTCTCTCTCTCTCTTG	P5	CACACACACACAGT
A4	AGAGAGAGAGAGAGAA	P6	CACACACACACAAG
A5	AGAGAGAGAGAGAGAGC	P7	CACACACACACAGG
A6	AGAGAGAGAGAGAGAGT	P8	GAGAGAGAGAGAGG
A7	CACACACACACACACACC	P9	GTGTGTGTGTGTGG
A8	CACACACACACACACAAA	P10	GAGAGAGAGAGACC
A9	CACACACACACACACAT	P11	GTGTGTGTGTGTCC
A10	CACACACACACACACAG	P12	CACCACCACGC
A11	AGAGAGAGAGAGAGAAA	P13	GAGGAGGAGGCC
A12	AGAGAGAGAGAGAGAAC	P14	CTCCTCCTCGC
A13	AGAGAGAGAGAGAGAAG	P15	GTGGTGGTGGC
A14	AGAGAGAGAGAGAGAAT	P16	ACTGACTGACTGATCG
I1	CCTACCACACACACACACA	P17	GACAGACAGACAGACA
I2	AGAGAGAGAGAGAGCTGCT	P18	GTGTGTGTGTGTGTGTC
I3	CACACACACACACACACA	P19	ACACACACACACACACG
I4	AGAGAGAGAGAGAGAGAG	P20	ACACACACACACACACCG
P1	AGAGAGAGAGAGAGAGG	P21	CCCTCCCTCCCTCCCT
P2	CTCTCTCTCTCTCTTAC	P22	CCCCGTGTGTGTGTGT
P3	CTCTCTCTCTCTCTTGC	P23	AGAGAGAGAGAGAGAG
P4	CACACACACACAAC	P24	GAGAGAGAGA

Table 2. Heavy metal quantity in water and sediment from the reference area and e-waste dumpsite

Sample/Area	Quantity of heavy metals					
	As	Cd	Cr	Pb	Mn	Zn
Water (mg/L)						
Reference area (n = 5)	ND	0.001 ± 0.002	0.001 ± 0.001	0.018 ± 0.008 ^a	ND	0.179 ± 0.037 ^a
E-waste dumpsite (n = 5)	0.013 ± 0.005	0.002 ± 0.003	0.002 ± 0.003	0.096 ± 0.060 ^b	0.692 ± 0.176	0.374 ± 0.093 ^b
Standard*	0.01	0.05	0.05	0.05	1.00	1.00
Sediment (mg/kg)						
Reference area (n = 5)	0.665 ± 0.162 ^a	0.331 ± 0.122 ^a	2.376 ± 0.573 ^a	15.85 ± 4.227 ^a	93.797 ± 8.572 ^a	109.336 ± 10.1
E-waste dumpsite (n = 5)	2.576 ± 0.150 ^b	2.408 ± 0.154 ^b	11.971 ± 1.753 ^b	66.163 ± 8.451 ^b	158.475 ± 5.438 ^b	425.782 ± 54.1
Standard**	≤ 6.00	≤ 67.00	≤ 17.5	≤ 400.00	≤ 1710.00	-

Note: *Surface water quality standard of Thailand (TPCD, 1994)

**Soil quality standards for habitat and agriculture (TPCD, 2021)

ND = Not detected

^{a,b} Different characters in the same column demonstrate significant differences ($p < 0.05$)

Heavy metals from the e-waste dumpsite are ultimately discharged to water, soil and sediment in the surrounding environment. These data indicated that the e-waste dumpsite was the point source of heavy metal pollution distributed to the surrounding area. In this study, the Pb quantity was greater than the As, Cd and Cr quantities because the e-waste dumpsite contains a large quantity computers and televisions, of which Pb is the major component (Thanomsangad *et al.*, 2019). In sediment samples from the e-waste dumpsite and reference area, the heavy metals in water samples were more than the criteria because the suspension of sediments absorbs the heavy metals from the water as an aqueous formula and precipitation (Weber *et al.*, 2013). Heavy metals contaminate water and soil and accumulate in aquatic plants. Heavy metal contamination showed an effect on the soil biota, even though the low concentration of heavy metals might interrupt the physiological metabolism of plants. Plants can take up heavy metals and accumulate via the food chain, exposing them to animals and posing a risk to human health (Singh and Kalamdhad, 2011). In addition, the amount of organic matter and clay were critical in the increased level of heavy metals and can translocate to accumulate in the aquatic plants. Moreover, phytoextraction is the process of decreasing heavy metals from the soil by a plant accumulating in a significant quantity of heavy metals in its shoots (Ruchuwarak *et al.*, 2018).

3.2 Heavy metal quantities in *O. alismoides* and *I. aquatic*

Table 3 shows the heavy metal quantity in *O. alismoides* from the reference area and

e-waste dumpsite. The As (5.86 ± 0.30 mg/kg), Cd (3.37 ± 0.27 mg/kg), Cr (7.07 ± 0.84 mg/kg), Pb (6.50 ± 0.73 mg/kg) and Zn (222.19 ± 16.07 mg/kg) quantities in *O. alismoides* from the e-waste dumpsite exceeded set standards (2, 0.2, 2, 1, 100, respectively). The statistical analysis demonstrated that As, Cd, Cr, Pb, Mn and Zn quantities in *O. alismoides* between the e-waste dumpsite and reference area were significantly different ($p < 0.05$). The Zn quantity in *O. alismoides* from the reference area and e-waste dumpsite exceeded set standards. Table 4 shows the Pb quantity (2.28 ± 0.50 mg/kg) in *I. aquatic* from the e-waste dumpsite more than the criterion (1 mg/kg). Statistical tests showed that the As, Cr, Pb and Mn quantities in *I. aquatic* between the reference area and the e-waste dumpsite were significantly different ($p < 0.05$).

In the sediment of e-waste dumpsites, aquatic plants take up large quantities of heavy metals. They can absorb heavy metals through roots by the absorption of heavy metals that are transported to the xylem. The characteristics of the stems or leaves affect the accumulation and absorption of heavy metals in the tissue of plants (Stankovic *et al.*, 2014). In e-waste dumpsites, the heavy metals (As, Cd, Cr, Pb and Zn) in *O. alismoides* and Pb in *I. aquatic* were higher than the standards. Stems or leaves are the factors that absorbed heavy metal contamination because leaves, stems, and other parts of plants were the major pathway of absorption (Talbot *et al.*, 2013). The highest As, Cd, Cr, Pb, Mn and Zn were detected in *O. alismoides*, which is done through submerged fibrous roots, tiny stems and leaves that grow completely underwater. In contrast, *I. aquatic* are floating plants with long stems and leaves that form large clusters

that float on the water surface (Sheoran et al., 2016). Therefore, the submerged plants absorbed heavy metals from water and sediment. The two edible aquatic plants have diverse morphological, physiological and genetic differences. In addition, the effectiveness of various aquatic plant species in absorbing metals is evaluated by either plant uptake or sediment by plant carry factors associated with the metals. Heavy metals accumulate and are stored mainly in stems and roots and are distributed in a harmless form (Sharma et al., 2021). Active and passive absorption are the mechanisms

by which plants take up and translocate ions through aquatic plants, affecting the potential absorption of different heavy metals in each species. Dhir (2014) detected that the habitats of floating and submerged plants were associated with concentrations of heavy metals. Thus, *O. alismoides* has the highest potential to stock up heavy metals. In conclusion, if residents consume these aquatic plant species regularly, they will affect human health. This toxicity in humans can result in damage to the liver, kidney, digestive system and brain and cause mutagenesis (Manova and Gruszka, 2015).

Table 3. Heavy metal quantity in *O. alismoides* from the reference area and e-waste dumpsite

	Quantity of heavy metals (mg/kg)					
	As	Cd	Cr	Pb	Mn	Zn
Reference area						
Individual 1	0.41	0.19	0.74	0.79	690.94	115.76
Individual 2	0.30	0.12	0.63	0.68	440.46	128.43
Individual 3	0.20	0.11	0.61	0.76	692.67	126.52
Individual 4	0.43	0.17	0.67	0.88	289.78	142.17
Individual 5	0.36	0.09	0.59	0.60	455.08	128.90
Mean ± SD	0.34 ± 0.09 ^a	0.14 ± 0.04 ^a	0.65 ± 0.60 ^a	0.74 ± 0.11 ^a	513.79 ± 174.92 ^a	128.52 ± 9.26 ^a
E-waste dumpsite						
Individual 1	6.23	3.62	7.84	7.10	3968.70	240.04
Individual 2	5.80	3.46	6.43	6.14	3550.01	214.37
Individual 3	5.76	3.57	7.87	6.03	3742.19	235.05
Individual 4	6.07	3.22	7.23	5.78	3456.67	200.12
Individual 5	5.45	2.98	5.98	7.45	3290.45	221.34
Mean ± SD	5.86 ± 0.30 ^b	3.37 ± 0.27 ^b	7.07 ± 0.84 ^b	6.50 ± 0.73 ^b	3,601.60 ± 262.21 ^b	222.19 ± 16.07 ^b
Standard	2 [*]	0.2 ^{**}	2 [*]	1 [*]	-	100 [*]

Note: ^{*}Joint FAO/WHO food standards program (Codex Alimentarius Commission, 2011).

^{**} FAO: Food and Agriculture Organization of the United Nations (FAO, 2001).

^{a, b} Different characters in the same column demonstrate significant differences ($p < 0.05$)

Table 4. Heavy metal quantity in *I. aquatic* from the reference area and e-waste dumpsite

	Quantity of heavy metals (mg/kg)					
	As	Cd	Cr	Pb	Mn	Zn
Reference area						
Individual 1	0.22	0.01	0.42	0.67	81.03	28.19
Individual 2	0.18	0.02	0.32	0.78	72.53	18.36
Individual 3	0.21	0.01	0.48	0.65	65.91	47.28
Individual 4	0.18	0.02	0.61	0.77	66.23	35.45
Individual 5	0.22	0.13	0.37	0.66	60.61	23.67
Mean ± SD	0.20 ± 0.02 ^a	0.04 ± 0.05	0.44 ± 0.11 ^a	0.71 ± 0.60 ^a	69.26 ± 7.82 ^a	30.59 ± 11.24
E-waste dumpsite						
Individual 1	0.74	0.09	1.16	2.13	271.56	43.66
Individual 2	0.64	0.14	1.15	2.11	253.40	45.33
Individual 3	0.46	ND	2.47	3.14	441.54	50.89
Individual 4	0.65	0.06	1.23	2.12	256.34	55.34
Individual 5	0.51	0.22	2.32	1.89	232.23	43.44
Mean ± SD	0.60 ± 0.12 ^b	0.10 ± 0.08	1.67 ± 0.67 ^b	2.28 ± 0.50 ^b	291.01 ± 85.30 ^b	47.73 ± 5.21
Standard	2 [*]	0.2 ^{**}	2 [*]	1 [*]	-	100 [*]

Note: ^{*}Joint FAO/WHO food standards program (Codex Alimentarius Commission, 2011).

^{**} FAO: Food and Agriculture Organization of the United Nations (FAO, 2001).

ND = Not detected

^{a, b} Different characters in the same column demonstrate significant differences ($p < 0.05$)

3.3 Bioaccumulation factors of heavy metals in *O. alismoides* and *I. aquatic*

Table 5 shows the BAFs of heavy metals in *O. alismoides* and *I. aquatic* from the e-waste dumpsite. The BAFs of As, Cd, Cr, Pb, Mn and Zn in *O. alismoides* as an effect of absorption from sediment followed the sequence of Mn > As > Cd > Cr > Zn > Pb. The BAF values in *O. alismoides* of As, Cd and Mn were higher than 1. The highest BAF values were found for Mn in *O. alismoides*. In addition, the BAFs of As, Cd, Cr, Pb, Mn and Zn in *I. aquatic* as a yield of sediment uptake followed the sequence of Mn > As > Cr > Zn > Cd > Pb. The BAF value of Mn in *I. aquatic* was more than 1. The highest BAF value of Mn (22.73 ± 1.65) was found in *O. alismoides*. The BAF value of Zn was more than 1 in the two species.

BAF demonstrated the potential of edible aquatic plants to accumulate heavy metals or hyperaccumulator plants (Ruchuwararak et al., 2020). The BAFs of As, Cd, Cr, Pb, Mn and Zn in the two edible aquatic plants were used to evaluate the level of risks and steady state conditions of accumulated metals in plants. In addition, many factors indicated that the abiotic environmental factors affecting BAF values were oxidation–reduction, hydrogen ions, salinity and alkalinity (Manova and Gruszka, 2015). BAFs of heavy metals greater than one demonstrated the potential of edible aquatic plants for phytoextraction and phytostabilization of metals (Neeratanaphan et al., 2016). In addition, phytotransformations are the breakdown of contaminants of metals taken up by plants through metabolism pathways (Li et al., 2016). The BAFs of As, Cd and Mn in *O. alismoides* and the BAF of Mn in *I. aquatic* exceeded one, which demonstrated the high potential absorption.

Plant characteristics, microorganism-plant interactions, translocation and tolerance mechanisms are factors in the potential of aquatic plant species to absorb toxic substances. This species of plant can absorb and hyperaccumulate the contaminants in roots and shoots of tissue (Van der Ent et al., 2013) due to heavy metals being transferred from roots to shoots. In addition, *O. alismoides* has larger leaves and shorter stems than *I. aquatic* (Manova and Gruszka, 2015), while *I. aquatic* has long stems. Therefore, if the local people consumed two aquatic plants near the e-waste dumpsite, these aquatic plants would be affected to human health.

3.4 ISSR profiles and similarity index of *O. alismoides* and *I. aquatic*

From the 40 screened primers, 17 successful primers are A5, A7, A8, A9, A11, A14, P1, P2, P3, P12, P14, P15, P16, P17, P19, P20 and P22 in *O. alismoides*, 11 successful primers are A4, A6, A11, A14, P1, P4, P6, P9, P16, P20 and P22 in *I. aquatic*. The 17 primers of *O. alismoides* indicated 927 total DNA bands, and the 11 primers of *I. aquatic* detected 530 total DNA fragments ranging from 100 to 2,500 bp. The ISSR profiles of primers A8, P17 and P19 of *O. alismoides* are shown in Figure 2. The ISSR profiles of primers A11, A6 and P22 of *I. aquatic* are shown in Figure 3. These DNA bands were used for the dendrogram and similarity index. The dendrogram analyzes the *O. alismoides* samples into 2 main groups based on the study sites. The first group comprises individual *O. alismoides* samples (1.1-1.5) from the reference area, and the second group comprises individual *O. alismoides* samples (2.1-2.5) from the e-waste dumpsite shown in Figure 4.

Table 5. The BAF values of heavy metals in *O. alismoides* and *I. aquatic* from the e-waste dumpsite

Heavy metals	BAF values	
	<i>O. alismoides</i>	<i>I. aquatic</i>
As	2.27 ± 0.12	0.23 ± 0.04
Cd	1.40 ± 0.11	0.04 ± 0.03
Cr	0.59 ± 0.07	0.14 ± 0.06
Pb	0.10 ± 0.01	0.03 ± 0.01
Mn	22.73 ± 1.65	1.84 ± 0.54
Zn	0.52 ± 0.04	0.11 ± 0.01

In addition, the dendrogram demonstrated that *I. aquatic* samples were divided into 2 main groups based on the study sites. The first group was made up of individual *I. aquatic* samples (1.1 - 1.5) from the reference area, and the second group was made up of individual

I. aquatic samples (2.1 - 2.5) from the e-waste dumpsite shown in Figure 5. The similarity index demonstrated the genetic relationships among the 2 study sites. In this study, the range similarity values of *O. alismoides* and *I. aquatic* were 0.43 - 0.99 and 0.42 - 0.98, respectively.

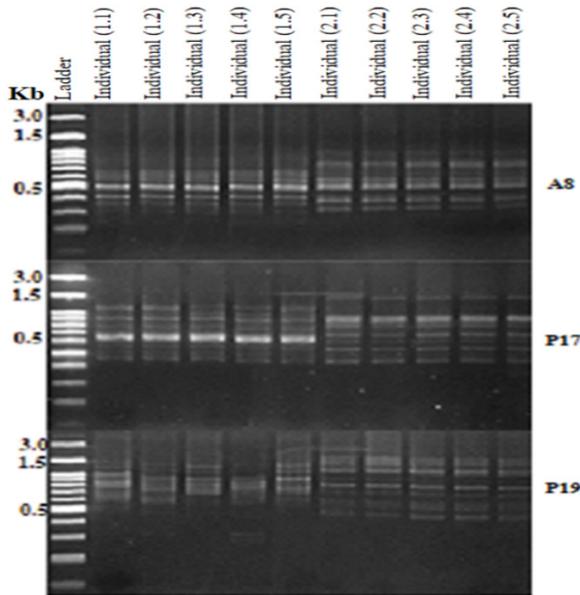


Figure 2. ISSR profiles for *O. alismoides* from the reference area (1.1 - 1.5) and the e-waste dumpsite (2.1 - 2.5) by primers A8, P17 and P19

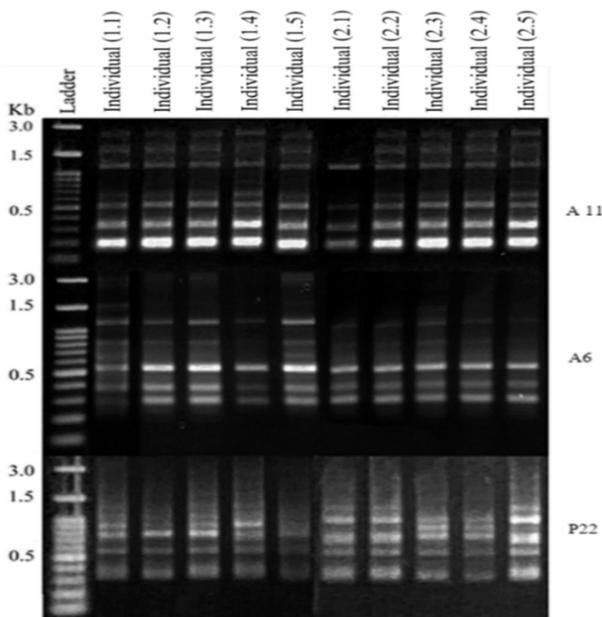


Figure 3. ISSR profiles for *I. aquatic* from the reference area (1.1 - 1.5) and the e-waste dumpsite (2.1 - 2.5) by primers A11, A6 and P22

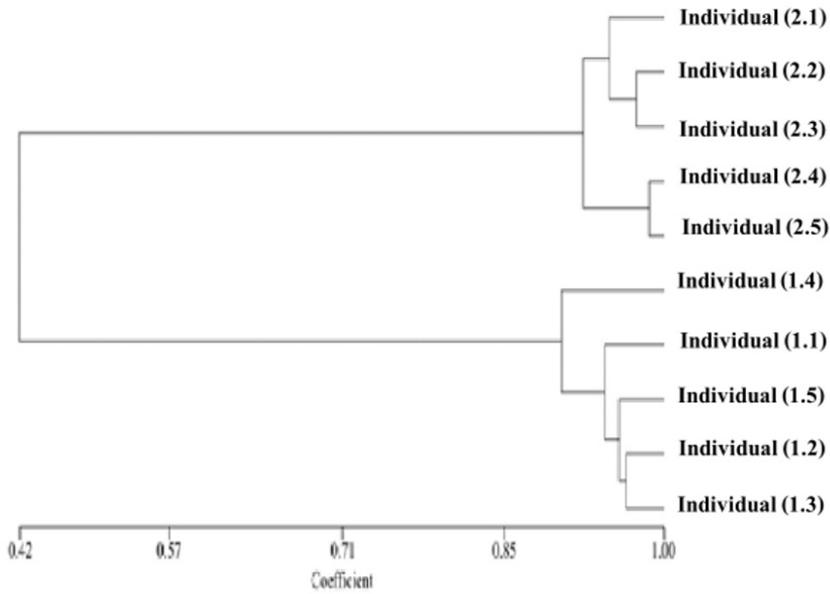


Figure 4. The dendrograms of *O. alismoides* from the reference area (1.1 - 1.5) and the e-waste dumpsite (2.1 - 2.5) by 17 primers

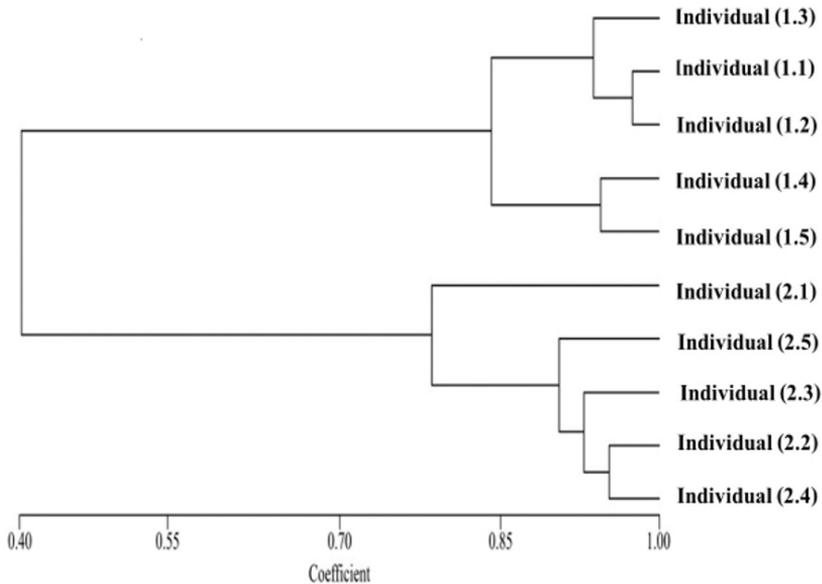


Figure 5. The dendrograms of *I. aquatic* from the reference area (1.1 - 1.5) and the e-waste dumpsite (2.1 - 2.5) by 11 primers

The dendrogram of *O. alismoides* and *I. aquatic* separated the sample results into two main groups. These results correlated with the heavy metal quantity in the two edible aquatic plant samples. The ISSR profile of *O. alismoides* and *I. aquatic* results in this study could analyze changes in ISSR patterns presenting changes in the absence of DNA bands and the appearance of new DNA bands.

These results induced the difference in the similarity index between each aquatic plant from the reference area and the e-waste dumpsite. The accumulation of heavy metals in each group of two aquatic plants is related to genetic differentiation. Genetic differentiation demonstrated that the plant can endure various environments, including that with heavy metal contamination. High heavy

metal concentrations induce increased genetic differentiation or decreased genetic similarity (Boonmee et al., 2015). In addition, the similarity index demonstrates the relationships between the reference and e-waste dumpsite. The similarity index within species should be in the range of 0.85 - 1.00 (Neeratanaphan et al., 2016)

In this study, the ranges of the similarity index in *O. alismoides* and *I. aquatic* were 0.43 - 0.99 and 0.42 - 0.98, respectively. The similarity index in *O. alismoides* and *I. aquatic* presented high genetic differentiation. The difference in the similarity index between the reference area and the e-waste dumpsite could be affected by heavy metals, especially As and Pb quantities in water and sediment as well as the high quantity in sediment from the e-waste dumpsite. In addition, the heavy metal quantities in *O. alismoides* and *I. aquatic* from the e-waste dumpsite exceeded those of the reference area. The contamination of heavy metals in plants can induce genotoxicity through several mechanisms of action, including binding to sulfhydryl groups within proteins and enzymes. Heavy metals stimulate the inhibition of DNA repair to induce damage to DNA and oxidative stress-related reactive oxygen species (ROS), which induce DNA damage (Dutta et al., 2018). For example, Cr, Pb and As induce many stress responses and damage components, such as DNA, proteins and membranes, at the cellular level in various plants (Baker et al., 2016). Exposure revealed induction of oxidative DNA damage, changes slowly in DNA methylation schemes, gene amplification, inhibition of DNA repair and affected chromatin formation (Moore et al., 2013). Pb can induce oxidative damage, chromosome aberration, mutation, DNA breakage and DNA synthesis inhibition (Silbergeld, 2003). Cd causes cell apoptosis, ploidy changes, DNA damage and deletions (Zhou et al., 2013) Cr can cause DNA damage, stable DNA-chromium complexes, Cr-DNA adducts and protein-Cr-DNA crosslinks (Neeratanaphan et al., 2020). Wahyudi et al. (2020) reported DNA of *Phaseolus vulgaris* damage induced by heavy metals (Pb, Cu, Cd and Mn) that were reflected in different RAPD profiles; the absence of bands and the presence of new bands occurred in the generated

profiles. In addition, As induced genotoxicity in *Colocasia esculenta* (Boonmee et al., 2015).

The e-waste dumpsite has heavy metal amounts that exceed those of the reference area, which might be due to the main factor associated with genetic differentiation (Olafisoye et al., 2013). In addition, *O. alismoides* can tolerate and adapt to environmental conditions more readily than *I. aquatic* because the quantity of heavy metals in *O. alismoides* was higher than that in *I. aquatic*. The range of the similarity index in *O. alismoides* was similar to that in *I. aquatic*. These results demonstrate the ability of *O. alismoides* to tolerate and adapt to the condition of the e-waste dumpsite, which was contaminated with high heavy metals, by repairing DNA damage (Manova and Gruszka, 2015). Similarly, Correia et al. (2014) reported that DNA polymorphisms detected by ISSR markers were more unstable in *Plantago almogravensis* than in *Plantago lagopus*. However, *P. lagopus* species were exposed to Al longer than *P. almogravensis*. The DNA damage of *P. lagopus* may be assumed to be repaired by the mechanisms itself. DNA replication was not completely inhibited, as reflected by the high level of genomic stability. Therefore, the communities near the e-waste dumpsite should increase awareness and seek improvements in the waste disposal system. Consumption of polluted aquatic plants should be avoided.

4. Conclusion

Heavy metal quantities in water and sediment, including edible aquatic plant species around an e-waste dumpsite, were found in this study. These edible aquatic plants may be a risk to the local population. Heavy metal contamination from the e-waste dumpsite induced genotoxicity in edible aquatic plants. The range of the similarity index in *O. alismoides* was similar to that in *I. aquatic*, indicating that *O. alismoides* had a greater ability than *I. aquatic* to tolerate and adapt to poisonous environmental conditions and survive. The local administration should correctly manage the contamination associated with this e-waste dumpsite. The public should

be informed of these reports in order to protect residents from consuming aquatic plants growing in these polluted areas.

Acknowledgement

The authors would like to thank the Research Project on Ecotoxicology, Natural Resources and Environment, Khon Kaen University, Khon Kaen, Thailand.

Conflict of interest

The authors declare no conflicts of interest.

References

- Amom T, Nongdam P. The use of molecular marker methods in plants: a review. *International Journal of Current Research and Review* 2017; 9(17): 1-7.
- Baker EJ, Miles EA, Burdge GC, Yaqoob P, Calder, PC. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Progress in Lipid Research* 2016; 64: 30-56.
- Boonmee S, Neeratanaphan L, Tanee T, Khamon P. The genetic differentiation of *Colocasia esculenta* growing in gold mining areas with arsenic contamination. *Environmental Monitoring and Assessment* 2015; 187(227): 1-8.
- Caravanos J, Clark E, Richard FR, Lambertson C. Assessing worker and environmental chemical exposure risks at an e-waste recycling and disposal site in Accra Ghana. *Journal of Health and Pollution* 2011; 1: 16-25.
- Chowrong S, Suemram L, Tengjaroenkul B, Sriuttha M, Patawang I, Neeratanaphan L. Chromosomal aberration and genetic differentiation of *Oreochromis niloticus* affected by heavy metals from an iron ore mine area. *International Journal of Environmental Studies* 2022; 1-17.
- Codex Alimentarius Commission. Working document for information and use in discussions related to contaminants and toxins in the GSCTFF. Joint FAO/WHO food standards program. Codex Committee on Contaminants in Foods. 5th edition. The Hague, The Netherlands. 2011; 21-25.
- Correia S, Matos M, Ferreira V, Martins N, Gonçalves S, Romano A, Pinto-Carnide O. Molecular instability induced by aluminum stress in *Plantago* species. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 2014; 770: 105-111.
- Dhir B. Potential of biological materials for removing heavy metals from wastewater. *Environmental Science and Pollution Research* 2014; 21: 1614-1627.
- Dutta S, Mitra M, Agarwal P, Mahapatra K, De S, Sett U, Roy S. Oxidative and genotoxic damages in plants in response to heavy metal stress and maintenance of genome stability. *Plant Signaling and Behavior* 2018; 13: e1460048.
- Food and Agriculture Organization (FAO). Codex alimentarius commission food additives and contaminants, FAO/WHO, Rome, Italy, ALINORM 01/12A. 2001; 1-289.
- Gupta M, Sarin NB. Heavy metal induced DNA changes in aquatic macrophytes: Random amplified polymorphic DNA analysis and identification of sequence characterized amplified region marker. *Journal of Environmental Sciences* 2009; 21: 686-690.
- Hu S, Li G, Yang J, Hou H. Aquatic plant genomics: advances, applications and prospects. *International Journal of Genomics* 2017; 2017: 1-9.
- Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metal. *Interdisciplinary Toxicology* 2014; 7: 60-72.
- Kyere VN, Greve K, Atiemo SM, Ephraim J. Spatial assessment of potential ecological risk of heavy metal in soils from informal e-waste recycling in Ghana. *Environmental Health and Toxicology* 2017; 32:1-7.
- Li Y, Zhang J, Zhu, G, Liu, Y, Wu B, Ng, WJ, Tan SK. Phytoextraction, phytotransformation and rhizodegradation of ibuprofen associated with *Typha angustifolia* in a horizontal subsurface flow constructed wetland. *Water Research* 2016; 102: 294-304.
- Manova V, Gruszka, D. DNA damage and repair in plants—from models to crops. *Frontiers in Plant Science* 2015; 6: 885.

- Moore LD, Le T, Fan G. DNA methylation and its basic function. *Neuropsychopharmacology* 2013; 38: 23-38.
- Neeratanaphan L, Sudmoon R, Chaveerach A. Assessment of genotoxicity through ISSR marker in *Pistia stratiotes* induced by lead. *EnvironmentAsia* 2014; 7: 99-107.
- Neeratanaphan L, Boonmee S, Srisamoot N, Tanomtong A, Tengjaroenkul B. Analysis of genetic similarity of *Limnocharis flava* individuals growing around a gold mining area with arsenic contamination. *Applied Ecology and Environmental Research* 2016; 14: 105-114.
- Neeratanaphan L, Khamma S, Benchawattananon R, Ruchuwarak P, Appamaraka S, Intamat S. Heavy metal accumulation in rice (*Oryza sativa*) near electronic waste dumps and related human health risk assessment. *Human Ecological Risk Assessment* 2017; 23: 1086-1098.
- Neeratanaphan L, Kamoller C, Suwannathada P, Suwannathada P, Tengjaroenkul B. Genotoxicity and oxidative stress in experimental hybrid catfish exposed to heavy metals in a municipal landfill reservoir. *International Journal of Environmental Research and Public Health* 2020; 17: 1980.
- Olafisoye OB, Adefioye T, Osibote OA. Heavy metals contamination of water, soil and plants around an electronic waste dumpsite. *Polish Journal of Environmental Studies* 2013; 22: 1431-1439.
- Parkpoom T, Intamat S, Phoonaploy U, Neeratanaphan L. Ecological and human health risk assessment of heavy metals by consuming the aquatic plant species near an electronic waste open dumpsite in Thailand. *Applied Environmental Research* 2022; 44: 86-98.
- Ruchuwarak P, Intamat S, Tengjaroenkul B, Neeratanaphan L. Bioaccumulation of heavy metals in local edible plants near a municipal landfill and the related human health risk assessment. *Human and Ecological Risk Assessment* 2018; 24: 1-13.
- Ruchuwarak P, Intamat S, Neeratanaphan L. Genetic differentiation and bioaccumulation factor after heavy metal exposure in edible aquatic plants near a municipal landfill. *EnvironmentAsia* 2020; 13: 36-49.
- Saetang P, Rojanapraiwoong S, Muksuwan WA. Preliminary survey of impact and solution towards participatory waste management: a case of Khok Sa Ard subdistrict, Khong Chai district, Kalasin province. Preliminary Report Submitted to Asia Foundation, Thailand. 2009.
- Sharma P, Tripathi S, Sirohi R, Kim SH, Ngo HH, Pandey A. Uptake and mobilization of heavy metals through phytoremediation process from native plants species growing on complex pollutants: Antioxidant enzymes and photosynthetic pigments response. *Environmental Technology and Innovation* 2021; 23: 101629.
- Sheoran V, Sheoran AS, Poonia P. Factors affecting phytoextraction: a review. *Pedosphere* 2016; 26(2): 148-166.
- Silbergeld EK. Facilitative mechanisms of lead as a carcinogen. *Mutation Research /Fundamental and Molecular Mechanisms of Mutagenesis* 2003; 533: 121-133.
- Singh J, Kalamdhad AS. Effects of heavy metals on soil, plants, human health and aquatic life. *International Journal of Research in Chemistry and Environment* 2011; 1: 15-21.
- Stankovic S, Kalaba P, Stankovic AR. Biota as toxic metal indicators. *Environmental Chemistry Letters* 2014; 12: 63-84.
- Talbot MJ, White RG. Methanol fixation of plant tissue for scanning electron microscopy improves preservation of tissue morphology and dimensions. *Plant Methods* 2013; 9: 1-7.
- Tanee T, Sudmoon R, Thamsenanupap P, Chaveerach A. Effect of cadmium on DNA changes in *Ipomoea aquatic* Forssk. *Polish Journal of Environmental Studies* 2016; 25: 311.
- Thailand Pollution Control Department (TPCD). Surface water quality standards. Notification of the National Environmental Board, No. 8, Ministry of Natural Resources and Environment, Bangkok. 1994.
- Thailand Pollution Control Department (TPCD). Soil quality standards for habitat and agriculture. Notification of the National Environmental Board; in the Royal Gazette on 11 March 2021, Bangkok. 2021.

- Thanomsangad P, Tengjaroenkul B, Sriuttha M, Neeratanaphan L. Heavy metal accumulation in frogs surrounding an e-waste dump site and human health risk assessment. *Human and Ecological Risk Assessment* 2019; 26: 1313-1328.
- Van der Ent A, Baker AJ, Reeves RD, Pollard AJ, Schat H. Hyper-accumulators of metal and metalloids trace elements: facts and fiction. *Plant and Soil* 2013; 362: 319-334.
- Wahyudi D, Hapsari L, Sundari, S. RAPD analysis for genetic variability detection of mutant soybean (*Glycine max* (L.) Merr). *Journal of Tropical Biodiversity and Biotechnology* 2020; 5(1): 68-77.
- Wang P, Zhang Y, Zhao L, Mo B, Luo T. Effect of gamma rays on *Sophora davidii* and detection of DNA polymorphism through ISSR marker. *BioMed Research International* 2017; 2017: 1-6.
- Weber P, Behr ER, Knorr CDL, Vendruscolo DS, Flores EM, Dressler VL, Baldissotto B. Metals in the water, sediment, and tissues of two fish species from different trophic levels in a subtropical Brazilian river. *Microchemical Journal* 2013; 106: 61-66.
- Zhou Z, Wang C, Liu H, Huang Q, Wang M, Lei Y. Cadmium induced cell apoptosis, DNA damage, decreased DNA repair capacity, and genomic instability during malignant transformation of human bronchial epithelial cells. *International Journal of Medical Sciences* 2013; 10: 1485-1496.