Heavy Metal Accumulation and DNA Changes in Plants Around an Electronic Waste Dumpsite Suggested Environmental Management Plan

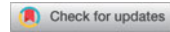Tawatchai Tanee, Arunrat Chaveerach, Runglawan Sudmoon, Jatuporn Teanma, Areerat Ragsasilp & Prapaparn Sirikhansaeng

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Heavy Metal Accumulation and DNA Changes in Plants Around an Electronic Waste Dumpsite Suggested Environmental Management Plan

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ABSTRACT

This research studied accumulation of heavy metals in soil and three plant species in E-waste dumpsite in Kalasin Province, Thailand. DNA changes in the plants were accessed by DNA fingerprinting and genomic template stability (GTS) analyses. Concentrations of the metals were in the order of Zn > Pb > Cd > Cr. The Bioconcentration Factor (BCF), Translocation Factor (TF), and Enrichment Factor (EF) values showed that *Typha angustifolia* was suitable for phytoremediation of Cd, Pb, and Zn. However, after the process of phytoremediation, appropriate abolishment of the heavy-metal containing plants should be taken to prevent the metals from passing along the food web. The GTS values ranged from 54.23 to 69.35%. These results suggest that heavy metals have affected the genotoxicity of plants grown in the electronic waste dumpsite.

Introduction

Electronic waste (E-waste) has caused a serious environmental problem, because it contains heavy metals, such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) (Fu et al. 2008; Leung et al. 2008; Olafisoye, Adefioye, and Osibote 2013). In E-waste dumpsites of developing countries, people still use outdated methods that tend to be more harmful for managing wastes, such as open burning of electronic equipment in order to melt plastics and to recover metals. This leads to disposal that causes a variety of environmental problems, such as atmospheric pollution and heavy metal contamination (e.g., Zn, Cu, Cd, and Pb) in water, soil, and organisms. Such contamination can be the result of immediate discharge, or long-term leachate subsurface discharge, or wind-blown erosion of deposited particles, or due to water surface runoff (Fu et al. 2008; Olafisoye, Adefioye, and Osibote 2013).

Some heavy metals are directly toxic, or form more toxic complexes like methyl mercury, which are harmful as much or more than through accumulation. However, some of them metals are non-biodegradable and thus persist for long periods of time in environmental ecosystems. Heavy metals that contaminate soil in E-waste dumpsites can transfer to and accumulate in the plants around the area. Plants can uptake the heavy metals through their roots and then translocate the metals to the stem and leaf. Heavy metal accumulation in plants not only affects plant growth, but can also be transferred to food chains of human and non-human animals such as endangered species which will be further serious issues. In addition, an excess of toxic heavy metals can damage the membranes, proteins, and DNA of a plant's cellular components (Manahan, 2003; Yang et al. 2004; Liu et al. 2009; Sun et al. 2009; Sudmoon et al. 2015; Tanee et al. 2016).

Recently, molecular technology was successfully applied to DNA analysis in the field of genotoxicology. Random Amplified Polymorphic DNA (RAPD) is a suitable technology that has been used to detect DNA damage and mutation in plants affected by heavy metals. Furthermore, RAPD banding patterns can be scored for genomic template stability (GTS) analysis to detect various types of DNA damage and mutations (point mutation, rearrangement, and small deletion or insertion of DNA). The RAPD technique has been applied successfully in a variety of plant species to the study of DNA damage and mutation from heavy metals (Liu et al. 2005, 2007, 2009; Duman, Altunkaynak, and Aras 2014; Sudmoon et al. 2015; Batir et al. 2015; Tanee et al. 2016).

The electronic waste dumpsite in Kok Sa Ard Sub-District, Kongchai District, Kalasin Province, Thailand is an important site to study, as people continue to practice open burning of electronic equipment in order to melt plastics and recover metals. This activity has caused the release of heavy metals, possibly leading to the accumulation of heavy metals and effects on the DNA of plants in the area. This research aimed to study the accumulation of four heavy metals, including cadmium (Cd), chromium (Cr), zinc (Zn), and lead (Pb), in three plant species (*Typha angustifolia*, *Chromolaena odorata*, and *Pennisetum pedicellatum*), and to study the DNA changes of these plant species. These plants are invasive species; there is plenty of their population everywhere. Therefore, if they could be used for phytoremediation program, the program can be instantly started.

Materials and methods

Sample collection

The E-waste dumpsite area is in Kok Sa Ard Sub-District, Kongchai District, Kalasin Province, Thailand. The area was investigated and the dominant plant species were recorded. The site investigation was done in dry season, so the surface water was dried off. Three plant species, namely *Chromolaena odorata, Typha angustifolia*, and *Pennisetum pedicellatum* were selected and sites where the plants grown were marked. Finally, nine sites with 50 meter apart from each others were selected (Fig. 1). The plant samples including surrounded surface soil samples (depth of 0–30

Figure 1. Map of the nearest community, Nong Bua village and the studied E-waste dumpsite showing sample collection sites inside the dump area.

cm) were collected from the nine sampling sites, three sites for each plant species. For DNA analysis, leaves of each nine samples were kept at −20°C. Leaves of the control plant grown from seeds were also included. For heavy metal analysis, three collected soil samples for each plant species and the plant itself were mixed together.

Heavy metal analysis

The soil samples were dried in an oven (Binder, USA) at 105°C for 24 h. The three plant species were dissected into roots, stems and leaves, rinsed thoroughly with distilled water to remove any surface materials, and then dried in an oven (Binder, USA) at 105°C for 24 h. The completely dried samples were then ground with a mortar and pestle. One gram of each dried sample (soils and plant tissues) was added to 12 ml of an $HClO_4:HNO_3$ mixture (1:3) and boiled at 100°C (Sudmoon et al. 2015). The solutions were filtered and were brought up to a volume of 50 ml in a volumetric flask with deionized, distilled water. The digested samples were analyzed for Cd, Cr, Pb, and Zn using an AA 6200 Atomic Absorption Spectrophotometer (Shimadzu, Japan). All analyses were performed in triplicate.

DNA extraction

The total genomic DNA was extracted from plant leaves using a modified cetyltrimethylammonium bromide (CTAB) procedure (Porebski, Bailey, and Baum 1997). Briefly, 50 mg of each leaf sample was finely ground with a mortar and pestle in 600 μl of warm (65°C) extraction buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB). The homogenate was transferred to a

1.5 ml microcentrifuge tube; then, 5 µl of 10 mg/ml RNase A was added, and the samples were incubated at 65°C for 30 min, with the tube inverted every 10 min. An equal volume of chloroform-isoamyl alcohol (24:1 v/v) was then added. The sample tube was centrifuged at 8,000 × g for 10 min, and the aqueous phase was carefully transferred to a new tube. Finally, genomic DNA was precipitated with an equal volume of cold (−20°C) 2-propanol for 30 min and then centrifuged. The precipitate DNA was washed with 70% ethanol and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA was evaluated using 0.8% agarose gel electrophoresis and ethidium bromide staining.

RAPD procedures

RAPD amplification was carried out in 25 µl reaction mixtures consisting of GoTaq Green Master Mix (Promega, USA), 0.5 µM primers (Invitrogen, USA), and 20 ng of DNA template. Forty-four RAPD primers were screened, twelve primers successfully amplified clear bands. The reaction mixture was incubated at 94°C for 3 min, and amplification was performed using 35 cycles of the following: denaturation for 30 s at 94°C, annealing for 30 s at 40°C, and extension for 2 min at 72°C, followed by a 7 min final extension at 72°C. All amplification reactions were done using a Swift™ Maxi Thermal Cycler (Esco Micro Pte. Ltd., Singapore). The amplified products were detected using 1.2% agarose gel electrophoresis in TAE buffer and visualized using ethidium bromide staining. The resulting RAPD bands were used to analyze the percentage of genomic template stability (GTS).

Data analysis

All experiments were replicated three times. The means and standard deviations (S.D.) of Cd in the soil and plant parts were calculated using Microsoft Office Excel 2010. The bioconcentration factor (BCF) was calculated as the ratio of the heavy metal concentration in the plant root to that in the soil; the translocation factor (TF) was calculated as the ratio of the heavy metal concentration in the plant shoot to that in the root; and the enrichment factor (EF) was calculated as the ratio of the heavy metal concentration in the plant shoot to that in the soil concentration (Lorestani, Cheraghi, and Yousefi 2011; Malik, Husain, and Nazir 2010). Hyperaccumulator plants were classified as such based on the following standards: (1) plants that have accumulated > 100 mgkg-1 of Cd, or > 1000 mgkg−1 of Cr or Pb, or > 10000 mgkg−1 of Zn; (2) plants having a BCF index greater than 1.0, sometimes reaching 50–100; and (3) plants having TF or EF indexes greater than 1.0 (Sun, Zhou, and Diao 2008; Sun et al. 2009; Lorestani, Cheraghi, and Yousefi 2011).

GTS (%) was calculated using the following equation: GTS (%) = (1 − a/n) × 100, where 'a' is the number of polymorphic bands detected in each sample and 'n' is the number of total bands in the control (Liu et al. 2007). Polymorphisms observed in the RAPD banding patterns included the disappearance of a normal band and the appearance of a new band when compared with the control RAPD profiles.

Results and discussion

Heavy metal concentration in soil and plants

The concentrations of Cd, Pb, Zn, and Cr in soil ranged from 3.24 to 25.44, 20.48 to 268.40, 67.55 to 932.33, and n.d. to 12.33 mg/kg, respectively, all concentrations lower than the standard level (Table 1). Heavy metal concentrations in the plants varied according to the plant species. Total Cd concentrations in roots, stems, and leaves ranged from 12.31 to 6.69, n.d. to 7.03, and 4.48 to 8.24 mg/kg, respectively, with *P. pedicellatum* roots and the stems and leaves of *C. odorata* reaching maximum levels. Total Pb concentrations in roots, stems, and leaves ranged from 20.48 to 268.40, n.d. to 67.56, and 27.56 to 69.65 mg/kg, respectively, with *P. pedicellatum* roots and the stems and leaves of *C. odorata* reaching maximum levels. Total Zn concentrations in roots, stems, and leaves ranged from 233.21 to 836.83, n.d. to 247.07, and 118.98 to 258.80 mg/kg, respectively, with *P. pedicellatum* roots and the stems and leaves of *C. odorata* reaching maximum levels. Cr concentrations were not found in any of the plant species (Table 1). The distribution of Cd, Pb, and Zn in each type of tissue was found in the diminishing order of roots > leaves > stems. Plants were able to take up the metals through the roots and then translocate them to the stems and leaves via physio-biochemical mechanisms (Lorestani, Cheraghi, and Yousefi 2011). However, *Typha* leaves may directly accumulated the metals from water not from the soil via translocation process as the plants were primarily grown in water. In addition, the direct deposition from contaminated air via waste burning process can be occurred. Therefore, air dispersion patterns that would connect the burning to potential deposition concentration gradients should be further studied. Furthermore, waste samples screening should be performed as it is a potential mechanism for more secure alternative forms of disposal of wastes, to avoid food web contamination risk.

Table 1. Heavy metal concentrations in soils and the different tissues of the three plant species.

Heavy metal	Plant species	Concentration of heavy metals (mg/kg) ($\bar{x} \pm$ S.D.)			
		soil	root	Stem	leaf
Cd	*T. angustifolia*	3.24 ± 0.08	6.98 ± 0.34	nd.	4.48 ± 0.10
	C. odorata	5.74 ± 0.16	6.69 ± 0.28	7.03 ± 0.49	8.24 ± 0.58
	P. pedicellatum	25.44 ± 0.61	12.31 ± 1.74	5.78 ± 0.17	5.00 ± 0.10
	Standard level[a]	37	—	—	—
Pb	*T. angustifolia*	20.48 ± 7.32	145.06 ± 6.25	nd.	27.56 ± 5.73
	C. odorata	158.81 ± 29.13	145.48 ± 6.29	67.56 ± 3.31	69.65 ± 8.87
	P. pedicellatum	268.40 ± 5.20	208.81 ± 24.91	60.06 ± 5.73	40.06 ± 2.50
	Standard level[a]	400	—	—	—
Zn	*T. angustifolia*	67.55 ± 18.39	350.94 ± 115.06	nd.	118.98 ± 12.37
	C. odorata	286.02 ± 8.14	233.21 ± 34.25	247.07 ± 110.38	258.80 ± 84.77
	P. pedicellatum	932.33 ± 129.68	836.83 ± 215.05	94.51 ± 15.06	162.17 ± 137.67
	Standard level[a]	—	—	—	—

[a]Ministry of natural resources and environment (2004).
nd. = not detected.

Table 2. BCF, TF, and EF values of heavy metals in plant species.

	Cd			Pb			Zn		
Plant species	BCF	TF	EF	BCF	TF	EF	BCF	TF	EF
T. angustifolia	2.15	0.64	1.38	7.08	0.19	1.35	5.19	0.34	1.76
C. odorata	1.16	2.83	2.66	0.92	0.94	0.86	0.81	2.17	1.77
P. pedicellatum	0.48	0.88	0.42	0.78	0.05	0.04	0.17	0.31	0.28

Bioconcentration factor, translocation factor, and enrichment factor of heavy metals in plants

The BCF values of the Cd, Pb, and Zn in the three plants ranged from 0.48 to 2.15, 0.78 to 7.08, and 0.17 to 5.19, respectively. The TF values of Cd, Pb, and Zn in the three plants ranged from 0.64 to 2.83, 0.05 to 0.94, and 0.31 to 2.17, respectively. The EF values of the Cd, Pb, and Zn in the three plants ranged from 0.42 to 2.66, 0.04 to 1.35, and 0.28 to 1.77, respectively (Table 2). It is obvious that *T. angustifolia* can accumulate high amount of Cd, Pb, and Zn, consistent with reports by Chandra and Yadav (2011) and Chayapan et al. (2015) that it shows strong tolerance to heavy metal stress. This can be also the result that the plant was sitting in contaminated water as well as the soil. Therefore, the surface and ground water should be sampled for completion of the dataset. Accordingly, *C. odorata* can accumulate high amount of Cd, with BCF and EF values of that metal greater than 1.0. Whereas *P. pedicellatum* did not accumulate high amount of any studied metal, however, as the plant was able to grow in an area contaminated with heavy metals, the tolerance mechanisms of *P. pedicellatum* to such an area should be further studied.

For the phytoremediation process, BCF and TF values > 1 were used as parameters to evaluate the potential of plant species for phytoextraction, while a BCF value > 1 and a TF value < 1 were used as parameters to evaluate for phytostabilization (Lorestani, Cheraghi, and Yousefi 2011). *T. angustifolia* was suitable for phytostabilization of Cd, Pb, and Zn (BCF = 2.15, 7.08, and 5.19, and TF = 0.64, 0.19, and 0.34, respectively), whereas *C. odorata* was suitable for phytoextraction of Cd (BCF = 1.16 and TF = 2.83). These results suggest that *T. angustifolia* is suitable for phytoremediation of heavy metals in an area with contaminated soil, an outcome consistent with previous research that used *T. angustifolia* for phytoremediation of soil and water contaminated with heavy metals (Panich-Pat et al. 2004; Bareen and Khilji, 2008; Chandra and Yadav, 2011; Wu et al. 2014; Chayapan et al. 2015). It should be considered that after the phytoremediation, the plant accumulated the metals for a period of time, and then the metals can be passed along the food web or released from the plants. Therefore, the heavy-metal containing plants should be destroyed by security landfill closure or other appropriated method.

Genotoxicology in the three plant species

Heavy metal accumulation in plants can affect plant growth, and an excess of toxic heavy metals can damage membranes, proteins, and the DNA of a plant's cellular components (Yang et al. 2004; Liu et al. 2009; Sun et al. 2009; Sudmoon et al. 2015;
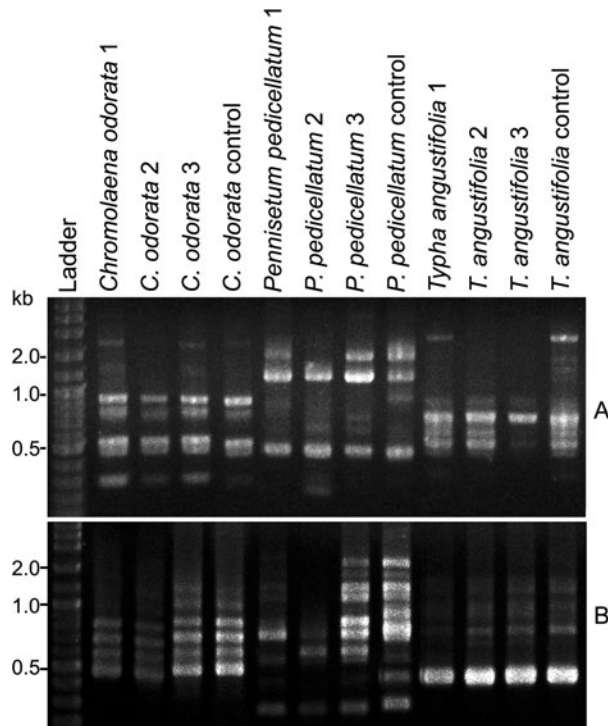
Figure 2. Examples of RAPD banding patterns from primers CAGGCCCTTC (A) and CAGCACCCAC (B) for the three plant species from the E-waste dumpsite and for the control plants.

Tanee et al. 2016). In the field of genotoxicology, DNA fingerprinting analysis, especially RAPD profiling, has been applied to detect altered DNA. An RAPD assay can be used to successfully detect in the plants genomic DNA changes induced by DNA-damaging heavy metals (Liu et al. 2007, 2009; Duman, Altunkaynak, and Aras 2014; Sudmoon et al. 2015; Tanee et al. 2016). The RAPD banding patterns show variation, principally with the loss of normal bands and appearance of new bands in the plants exposed to heavy metals when compared with the control plants that have not been exposed to heavy metals. In this study, the twelve successful primers produced 672 bands ranging in size from 100–3000 bp. RAPD profile examples are shown in Fig. 2. The RAPD banding patterns showed substantial differences between the plants exposed to heavy metals and the control plants (the plants grown from seeds, with no heavy metal exposed) with apparent changes (disappearance and/or appearance) in the number of DNA bands produced by each primer. The DNA changes in the plants exposed to heavy metals were expressed as decreases in percentages of GTS, reflecting a measure of the qualitative changes in the RAPD banding patterns. The lowest GTS values represent the highest DNA changes. In this study, the GTS values of *T. angustifolia, C. odorata*, and *P. pedicellatum* were from 0 to 100%, −100 to 100%, and 33.33 to 100%, respectively (Table 3). The results showed *T. angustifolia* as having the most dramatic changes in DNA, with 54.23% of its GTS value, while the lowest DNA changes were observed in *P. pedicellatum*, with 69.35% of its GTS value. A decrease in GTS values was observed to correlate with an increase in BCF values (Fig. 3). The results indicate that heavy metal exposure has led to DNA

Table 3. GTS value (%) of the three plant species for all primers.

Primer	Nucleotide sequence	GTS value (%)		
		T. angustifolia	*C. odorata*	*P. pedicellatum*
1	CAG GCC CTT C	25.00	80.00	71.43
2	TGC CGA GCT G	75.00	83.33	100.00
3	AGT CAG CCA C	0.00	100.00	83.33
4	GGG TAA CGC C	66.67	75.00	33.33
5	CAG CAC CCA C	88.89	100.00	75.00
6	TCT GTG CTG G	83.33	100.00	100.00
7	CAA ACG TCG G	100.00	100.00	50.00
8	GGA CTG GAG T	28.57	90.91	33.33
9	TGC GCC CTT C	25.00	100.00	100.00
10	CTG AGA CGG A	50.00	− 100.00	60.00
11	GGT GGT CAA G	25.00	− 100.00	85.71
12	CTG CGC TGG A	83.33	75.00	40.00
	Average	54.23	58.69	69.35
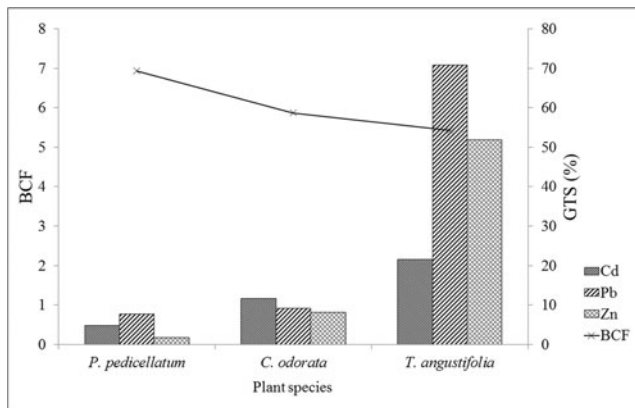
Figure 3. Correlation between genomic template stability (GTS) of the three plant species and Bio-concentration Factor (BCF) values calculated from concentration of the heavy metals in the soil and in the plants.

changes of the plants in the contaminated area. According to the GTS data, while the plants can grow properly in the contaminated area, DNA changes result in the plants. These results are consistent with those from previous studies which have suggested that heavy metals can induce DNA changes in plant DNA (Manahan 2003; Liu et al. 2007, 2009; Sudmoon et al. 2015; Tanee et al. 2016). Furthermore, bioaccumalations or effects of the metals on DNA changes should be further studied in native plant species, especially the edible species which are important in food web.

Conclusion

The three plant species that grow in the E-waste dumpsite, *T. angustifolia, C. odorata*, and *P. pedicellatum*, have the potential to uptake heavy metals (Cd, Pb, Zn) from the contaminated soil, leading to accumulation of the metals in their roots, stems and leaves. Population of these plants are plenty everywhere, therefore phytoremediation program can be instantly started, however with the appropriate

downstream abolishment of the plants. It is clear that *T. angustifolia* can accumulate Cd, Pb, and Zn in a high amount, so it is suitable for remediation of Cd, Pb, and Zn with BCF values > 1 and TF values < 1. While *C. odorata* can accumulate Cd, thus it is suitable for Cd remediation, with BCF and TF values > 1. Moreover, in this study, it was shown that the heavy metals induced DNA changes in the three studied plants evidenced by the RAPD analysis results. These results can be used as a basis for environmental monitoring, especially of the heavy metals accumulated in vegetable plants in the E-waste dumpsite and around that area. Serious enactment of education and management concerning E-wastes in electronic waste villages should be taken to prevent the effects of waste disposal problems. Moreover, appropriate waste dumpsites farther away from residential buildings and agricultural fields should be chosen to avoid the harmful effects of indiscriminate disposal of the wastes.

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