Ecotoxicology and Environmental Safety 111 (2015) 168–176

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Toxic assessment of the leachates of paddy soils and river sediments from e-waste dismantling sites to microalga, *Pseudokirchneriella subcapitata*

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ARTICLE INFO

Article history:
Received 13 June 2014
Received in revised form
23 September 2014
Accepted 1 October 2014

Keywords:
e-Waste dismantling activity
Pseudokirchneriella subcapitata
Leachates
Toxicity assessment
Chlorophyll *a* fluorescence
Anti-oxidative system

ABSTRACT

The potential adverse effects of e-waste recycling activity on environment are getting increasing concern. In this work, a model alga, *Pseudokirchneriella subcapitata*, was employed to assess the toxic effects of the leachates of paddy soils and river sediments collected from e-waste dismantling sites. Chemical analysis of the paddy soils and river sediments and their leachates were carried out and the growth rate, chlorophyll *a* fluorescence and anti-oxidative systems of the alga were measured. Results showed that two leachates decreased the amount of PSII active reaction centers and affected photosynthesis performance, interfered with chlorophyll synthesis and inhibited algal growth. Some chemical pollutants in the sediments and soils such as polybrominated diphenyl ethers (PBDEs) and metals derived from e-waste recycling activity may impose oxidative stress on algae and affect the activity of anti-oxidative enzymes such as GST, SOD, CAT and APX. The leachates of both river sediments and paddy soils are potentially toxic to the primary producers, *P. subcapitata* and the leachate from sediments was more deleterious than that from soils.

1. Introduction

Recently, the environmental impacts of pollutants derived from electronic waste (e-waste) dismantling activity have been of increasing concern (Leung et al., 2011; Ma et al., 2011), and the problem is progressively worse, especially in some developing countries like China. Large amounts of obsolete electronic products such as computers, TV, cell phones and breadboards are exported to developing countries for recycling due to local lower labor costs and less stringent environmental regulations (Wong et al., 2007). Guiyu, a small town in Shantou city, located in southeast Guangdong province, PR China, is one of the biggest e-waste dismantling places in the world. The dismantling and deposition activities are done with primitive methods such as mechanical crushing, smelting, burning and acid washing in family workshops scattering throughout the town. Because these small workshops lack technical instruction and knowledge of environmental protection, toxic compounds from the dismantling process have been directly discharged into the circumjacent environment without any further treatment.

Metals, polybrominated diphenyl ethers (PBDEs) and other persistent toxic substances were frequently detected in the air, waters, soils and biota within e-waste recycling sites (Leung et al., 2006; de Wit, 2002; An et al., 2011). Wang et al. (2005) reported that PBDEs ranged from 0.26 to 824 ng g^{-1} in sediments of brooks and soils in Guiyu town. Luo et al. (2007) found that PBDEs were very high (from 4434 to 16,088 ng g^{-1}) in sediments from the Nanyang river across Guiyu town. Ma et al. (2011) reported that workers in e-waste workshops in Taizhou, eastern China, had high concentrations of both PBDEs in their hair as compared with the people living in uncontaminated areas, as well as elevated levels of DNA damage (Wen et al., 2008). Huo et al. (2007) reported very high blood lead levels in children born between 2001 and 2007 in Guiyu. The low body weight ratio (6.5% vs 2.0%), premature births (8.9% vs 4.9%) and death rates (4.7% vs 0.52%) were also significantly higher in Guiyu town than those in the suburb in Xiamen city, away from Guiyu (Wu et al., 2012).

Although many researches on the environmental impacts of e-waste have been published, most of them focused on the chemical analysis of environmental samples. Very limited information is available about the assessment of the potential ecological effects

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http://dx.doi.org/10.1016/j.ecoenv.2014.10.012
0147-6513/

of e-waste cycling activities via the use of suitable biological models. Information based only on chemical monitoring does not address the total environmental problem. Documenting the response of biota may not only help identify the effects of pollutants, but also understand their mechanism of action. The Water Framework Directive (2000/60/EC) was the first report that defined both the chemical and ecological assays useful to determine the overall health of aquatic ecosystems (Wadhia and Thompson, 2007). Furthermore, the biota approach is cost-effective and an essential complement to chemical analysis. Moreover, it is practical to use the standard methods to assess the toxicity of environmental pollutants. Turner and Rice (2010) assessed the toxicity of tire wear particle leachate to algae and found different dilutions significantly reducing the photosynthesis efficiency of macroalgal. Wang et al (2009) utilized a multi-trophic approach to characterize the acute toxicity of river sediments collected from an e-waste site in Guiyu. These studies showed that the toxicity testing approach using extractive or leachate from solid wastes is valid and practicable in the environmental toxicity assessment. Most works mentioned above focused on either the chemical analysis or the toxic effects determination of samples from e-waste sites. However, the combination of chemical analysis with ecotoxicological assays to assess the toxic effects of leachate from the e-waste recycling activity is still not attempted.

The aim of the present study is to integrate both chemical analysis and biota responses to assess the potential ecological impacts of electronic products cycling activity. Algae as primary producers constitute the base of the food webs in aquatic ecosystems. Therefore, microalgae are often used as indicators for environmental pollution and water quality monitoring. The experiment was designed to determine and compare the response of a model alga, *Pseudokirchneriella subcapitata*, to the leachates of paddy soils and river sediments collected from Guiyu town. The growth rate, chlorophyll *a* fluorescence and anti-oxidative system of algae were investigated to assess the toxic effects of pollutants derived from e-waste dismantling activity.

2. Materials and methods

2.1. Sampling and materials preparation

2.1.1. Collection of samples

River sediments and paddy soils in the margins of the river were collected from three different sites within Guiyu town, where many small e-waste dismantling workshops and factories are scattered along the river (Fig. 1). Samples were collected by using an Ekman-Bridge grab sampler for surface sediments and a

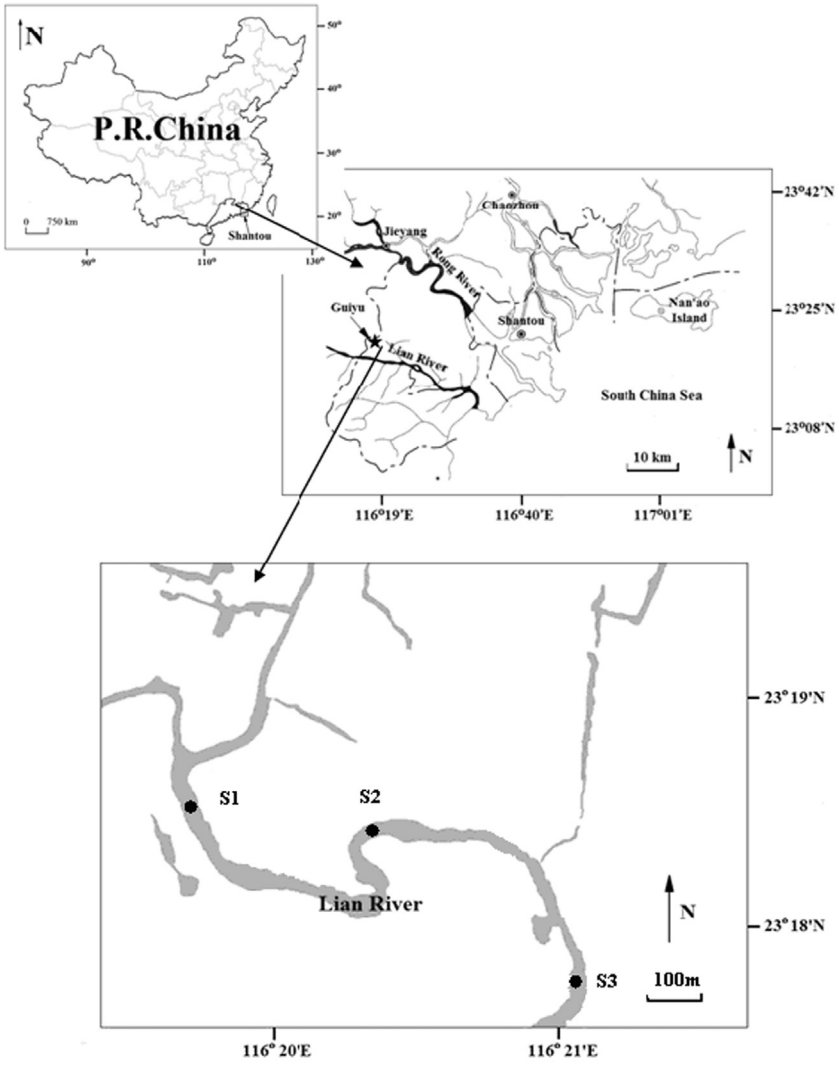

Fig. 1. The locations of sampling sites at Guiyu town in Guangdong Province, South China (S1, S2 and S3 indicated the sampling sites of river sediments and paddy soils).

shovel for paddy soils and kept at −5 °C in polypropylene containers until they were transported to the laboratory. Later, samples were freeze-dried and ground into a powder and sieved through a 100 μm mesh and then put into 500 mL brown bottles and stored at 4 °C for later use.

2.1.2. Extraction of leachates

Equal amounts of samples from three sites were taken and mixed homogenously. The 500 g of mixture was used for extraction. Three replicates were carried out. Samples were weighed and put into distilled water with a 1:1 ratio of sample:water (%, w:v), extracted on a horizontal oscillator with bubbling nitrogen for 6 h and then allowed to stand statically for 24 h. The leachates were first filtered (0.45 μm porosity filter paper) and then through a 0.22 μm membrane filter using a vacuum pump and then prepared for toxicity testing. The average pH of leachates was 6.98 ± 0.16 for river sediment and 7.22 ± 0.21 for paddy soil. The TOC was 1.86 ± 0.64% for sediment and 2.86 ± 1.26% for paddy soil.

2.2. Analysis of metals and PBDEs

2.2.1. Determination of metals

For soil, sediment and their corresponding leachate samples, Pb, Zn, Cu, Cd and Cr were analyzed by using inductively coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (OPTIMA 2000DV, Perkin-Elmer, USA).

2.2.2. Determination of PBDEs

PBDEs were analyzed following US EPA method 1614 (US EPA, 2007) with minor modifications. Briefly, the samples were spiked with 1 ng of a ^{13}C-PCB141 (Cambridge Isotope Laboratories Inc., Andover, MA, US), and mixed with anhydrous sodium sulfate and allowed to equilibrate for 2 h. The samples were extracted using 50:50 methylene chloride:hexane (v/v) in a sonication extractor; the extracts were purified and fractionated in silica gel and Florisil columns. The fraction extractions were concentrated to 2.0 mL under a gentle N_2 stream. Identification and quantification of individual PBDEs congeners were accomplished using a QP2010 GC–MS (Shimadzu, Japan). A gas chromatography column of DB-XLB ms fused-silica capillaries (30 m × 0.25 mm × 0.25 μm) (J&W Scientific) was used for PBDEs analysis. The injector and interface temperatures were 275 °C and 300 °C, respectively, and 2 μL samples were injected with the pulsed splitless mode (345 kPa). Recoveries of surrogate with spiked samples with ^{13}C-PCB141 (m/z =372, 374), PCB209 (m/z=498, 500) were in the range of 65–120%, RSD < 20%.

2.3. Algal exposure

Pseudokirchneriella subcapitata (Korshikov) Hindak, (Formerly *Selenastrum capricornutum*), commonly used as a freshwater model alga in standard toxicity tests, was provided by the Freshwater Algae Culture Collection Center of the Institute of Hydrobiology (FACHB-Collection), Wuhan City, China.

Exposure experiments were undertaken following the guidelines of the Organization for Economic Cooperation and Development (OECD) for testing chemicals no. 201 with minor modifications (OECD, 1984). Aliquots of leachates prepared at diluted ratio of 25%, 50% and 100% of the original leachate mentioned above were dispensed into a series of 250 mL culture flasks and distilled water was served as a control. The volume was brought up to 200 mL with the autoclaved algal medium. Then 20 mL of algae culture in the exponential growth phase were inoculated into treated flasks with initial cell densities of 1×10^5 cell mL^{-1}. The flasks were covered with perforated polyethylene film to avoid contamination. All flasks were placed into a plant incubator (FPG-3, Ningbo, China) at 24 ± 1 °C with a light:dark period of 12:12 h at 60 μmol m^{-2} s^{-1} illumination. Flasks were shaken by hand three times daily to minimize cell sedimentation. Three replicates were conducted for each treatment. Each replicate was sampled at fixed intervals for cell counts, chlorophyll *a* content, fluorescence parameters; and the biochemical analysis were performed only at 96 h exposure.

2.4. Bioassay tests

2.4.1. Growth rate inhibition determination

The growth rate was measured by directly counting the number of cells in a Goryaev hemocytometer at 24, 48, 72 and 96 h after inoculation. The growth rates were calculated according to the OECD guideline (OECD, 1984).

2.4.2. Chlorophyll a content analysis and chlorophyll a fluorescence measurement

Samples of the algal culture (50 mL) were collected at 24, 48, 72 and 96 h exposure and centrifuged at 10,000*g* for 10 min. The chlorophyll *a* contents were measured by a UV/vis spectrophotometer (Shimadzu Ltd., Japan).

One mL of algal solution under different treatments was taken at 96 h and diluted 5 times with the sterilized medium. Samples then underwent the step of dark adaptation for at least 30 min before measurement. Chlorophyll *a* fluorescence was measured by a Plant Efficiency Analyzer (PEA, Hansatech Ltd., England) with an excitation light intensity of 3000 μmol m^{-2} s^{-1}.

The fluorescence transient was analyzed according to Strasser and Strasser (1995) and Appenroth et al. (2001) by using the fluorescence intensities at 300 μs (K-step), 2 ms (J-step) and 30 ms (I-step). Selected JIP-test parameters quantifying PSII behavior were derived from the fluorescence induction kinetics according to Strasser et al. (2000) as following: Fo: the fluorescence intensity at 0 s; F_M: the maximum fluorescence intensity; PIabs: performance index (on an absorption basis); φPo: maximum yield of primary photochemistry; φEo: quantum yield of electron transport; Ψo: efficiency with which a trapped excitation can move an electron into the electron transport chain further than Q_A; φDo: Quantum yield of dissipation; ABS/RC: absorption flux per reaction center; TRo/RC: trapping flux per reaction center (at t=0); ETo/RC: electron transport flux per reaction center (at t=0); DIo/RC: Dissipated energy flux per reaction center (at t=0); RC/CSo: the amount of active PSII reaction centers per excited cross section (at t=0).

2.4.3. Measurement of enzyme activity

The antioxidant enzymes extraction methods were modified from Knörzer et al. (1996). Algae cells from 100 mL culture media after 96 h exposure were re-suspended into 4 mL ice-cold extraction buffer containing 50 mM Hepes–KOH (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, 20% (v/v) glycerol and 2% (w/v) PVPP. The suspension was kept in an ice bath and ultra-sonicated (UH-950B, Tianjing, China) for 15 min with 5 second intervals per minute in a 4 °C ice-bath, and then centrifuged at 12,000 rpm for 10 min at 2 °C. The supernatant was immediately used for the measurement of the antioxidant enzymes. Soluble protein content was measured according to Bradford (1976).

Ascorbate peroxidase (APX) activity was measured by monitoring the decrease of absorbance at 290 nm (extinction coefficient of 2.8 mM^{-1} cm^{-1}) for 1 min (20 s lag period) using the method described in Nakano and Asada (1981).

Superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) according to the protocol of Yu and Rengel (1999) with minor modifications.

Catalase (CAT) activity was measured by monitoring the decrease the absorbance at 240 nm (extinction coefficient of 36 M^{-1} cm^{-1}) for 3 min (1 min lag period), according to the method described in Aebi (1984).

Glutathione-S-transferase (GST) activity was measured by utilizing the tissue GPX/GST activity quantitative assay kit (Jiancheng Bioengineering Institute, Nanjing, China) with the enzyme extract prepared according to the method described above.

2.4.4. Determination of lipid peroxidation and total GSH

Lipid peroxidation in the algal cells was determined by measuring the concentration of malondialdehyde (MDA) (Buege and Aust, 1972). GSH was measured using the tissue GSH quantitative assay kit (Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Statistical analyses

The experimental data were reported as mean values of three measurements ± standard deviation (SD). The differences in the means of the various treatments were tested with a one-way ANOVA. Significance is accepted at $P < 0.05$.

3. Results

3.1. Chemical analysis of leachates from paddy soils and river sediments

According to the previous studies (Wong et al. 2007; Luo et al., 2007), PBDEs and metals are very typical pollutants in environmental matrices from e-waste recycling activity. Therefore, they were chosen as priority monitoring targets of analysis in present investigation. PBDEs and metals analysis of paddy soils, river sediments and their corresponding leachates were shown in Table 1. The average of total PBDEs in the paddy soils was 433.85 ± 284.4 ng g^{-1} (dw, the same afterwards), much lower than the content in sediments (7349.32 ± 1103.8 ng g^{-1}). Major congeners of PBDEs in paddy soil were BDE209, 207, 206 and BDE208. Among them, BDE 209 represented 90% of the total PBDEs. As for sediments samples, BDE209, 183, 207, 206, 99 and BDE47 constituted most of the PBDEs, and BDE 209 contributed to 72% relatively to the total PBDEs. The low brominated BDEs such as BDE47 and 99 in sediments were much higher than in paddy soils. In terms of leachates, similar patterns were found for PBDEs, but the ratio of low-brominated BDEs to total PBDEs increased. BDE183, accounted for about 5.6% of total PBDEs in sediments, but decreased greatly in leachates.

Copper in sediment averaged 2977.43 μg g^{-1} (dw), about 115 times that in paddy soil (Table 1). Pb, Zn and Cd content in sediments were also higher but that not significant as copper. No significant difference was found in leachates between river sediments and paddy soils for Pb, Zn and Cd. But copper was 6.5 times higher in sediment than that in paddy soil.

Table 1
Concentrations of PBDEs and heavy metals in sediments and soils and their counterpart leachates.

	Paddy fields soils	Leachates of paddy soils	River sediments	Leachates of river sediments
PBDEs congeners (ng g^{-1}, dw or ng L^{-1})				
BDE28	0.243 ± 0.31	0.093 ± 0.08	21.965 ± 18.86	0.281 ± 0.09
BDE47	1.577 ± 1.61	0.057 ± 0.05	155.828 ± 125.53	0.542 ± 0.21
BDE66	0.333 ± 0.26	0.007 ± 0.01	51.333 ± 45.03	0.019 ± 0.02
BDE99	1.863 ± 1.81	0.134 ± 0.12	228.265 ± 182.32	0.951 ± 0.13
BDE100	0.250 ± 0.09	0.007 ± 0.01	25.518 ± 18.24	0.017 ± 0.01
BDE153	0.420 ± 0.14	nd	85.232 ± 61.44	0.012 ± 0.01
BDE154	0.191 ± 0.04	nd	17.595 ± 9.97	0.014 ± 0.01
BDE138	0.316 ± 0.06	0.004 ± 0.01	11.199 ± 4.78	0.022 ± 0.01
BDE183	2.384 ± 1.51	0.007 ± 0.01	478.426 ± 231.43	0.091 ± 0.05
BDE206	10.748 ± 7.79	0.952 ± 0.58	309.123 ± 74.33	0.851 ± 0.05
BDE207	18.436 ± 13.75	0.264 ± 0.21	482.997 ± 63.89	3.627 ± 2.12
BDE208	6.374 ± 4.34	0.032 ± 0.06	147.635 ± 26.27	1.049 ± 1.08
BDE209	390.674 ± 159.11	0.589 ± 0.38	5319.057 ± 1276.47	17.064 ± 6.46
∑PBDE	433.859 ± 284.48	2.146 ± 1.33	7349.320 ± 1103.86	24.080 ± 8.21
Heavy metal (mg kg^{-1}, dw or mg L^{-1})				
Pb	71.631 ± 15.72	nd	385.150 ± 43.12	0.153 ± 0.06
Zn	99.322 ± 19.54	0.112 ± 0.03	744.691 ± 36.54	0.181 ± 0.05
Cu	25.831 ± 9.83	0.241 ± 0.03	2977.432 ± 358.63	1.551 ± 0.68
Cd	1.313 ± 0.56	nd	14.081 ± 1.22	0.022 ± 0.01
Cr	31.991 ± 5.82	0.021 ± 0.02	40.931 ± 7.93	0.033 ± 0.02

Note: nd means no detection.

Table 2
Growth rate inhibition (ρ_{ti}, as a percentage of control) of *Pseudokirchneriella. subcapitata* exposed to the various dilution ratio leachate solutions of paddy fields soils and river sediments collected from e-waste sites over exposure time.

Concentration (percentage %)	Growth rate inhibition (ρ_{ti}, %)			
	24 h	48 h	72 h	96 h
Soils leachate				
25	19.02 ± 1.57	17.27 ± 1.23	13.27 ± 1.68	13.21 ± 1.26
50	21.12 ± 1.09	21.67 ± 1.53	17.02 ± 2.88	16.17 ± 0.81
100	28.75 ± 1.76*	31.79 ± 3.25*	24.66 ± 2.49*	23.46 ± 1.24
Sediments leachate				
25	43.56 ± 2.09*	41.96 ± 5.87*	40.78 ± 5.76*	41.61 ± 6.94*
50	45.49 ± 1.53*	47.87 ± 6.29*	48.26 ± 7.57*	50.11 ± 11.70*
100	55.01 ± 1.94*	61.33 ± 6.84*	56.82 ± 6.24*	58.92 ± 9.38*

[a] Values are mean ± standard deviation. [b] ρ_{ti} = Inhibition ratio.
* $P < 0.05$.

3.2. Growth inhibition effects of leachates on P. subcapitata

The growth of *P. subcapitata* under different dilutions of the leachates was shown in Table 2. No significant inhibition was found in the 25% dilution of paddy soils ($P > 0.05$), but really did in 100% of leachates from paddy soils with the 28.7% of growth inhibition. However, with longer exposure time, the cell density of algal gradually increased in the paddy soil leachates and the growth inhibition was lowered. The inhibition of growth was more obvious in leachates of sediments than that at paddy soils. In the 25% dilution of sediments, the growth was inhibited by 43.5% after 24 h exposure. Furthermore the growth inhibition increased with the increasing concentration of leachates. Algae growth was increasingly inhibited in the 50% and 100% leachates along the exposure time. At the 96 h of exposure, 100% sediment leachates inhibited 58.9% of growth, being noticed the morphological changes of the cells such as swelling and fragmentation.

3.3. Effects of leachates on the chlorophyll a content of P. subcapitata

A similar change was found for the content of chlorophyll *a* under exposure to various ratios of leachates. No significant reduction was observed in chlorophyll *a* in 25% of paddy soil leachate. But the amount of chlorophyll *a* gradually decreased under higher concentration leachates with prolonged exposure time (Fig. 2). After 96 h, the chlorophyll *a* content was about one-third of the control in the 100% exposure groups and characterized with a yellow-green color.

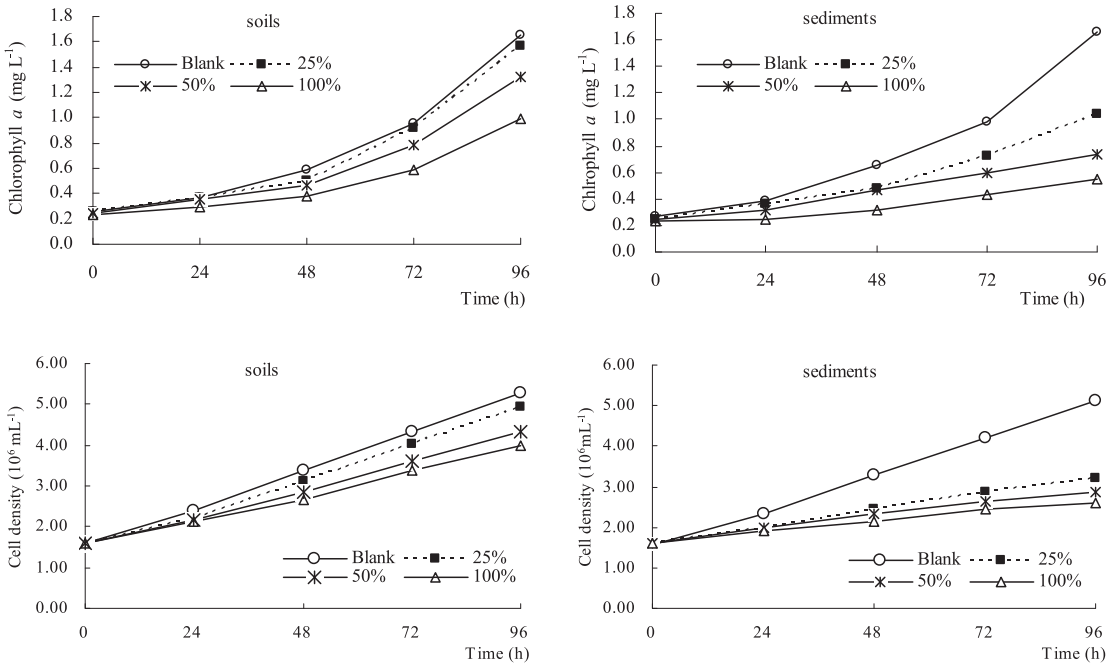

Fig. 2. Effects of leachates on the growth of *Pseudokirchneriella subcapitata*.

3.4. Toxicity of leachates on the chlorophyll a fluorescence parameters of P. subcapitata

Fig. 3 showed that all leachates can induce a significant decrease of RC/CSo and PIabs but increase of ABS/RC and DIO/RC. And φEo and ψo, also decreased slightly. However, φPo and ETO/RC did not show significant variation statistically compared to the control. Generally, similar response patterns of chlorophyll *a* fluorescence parameters were observed for both leachates from paddy soil and sediments, but variation levels of algae chlorophyll *a* fluorescence performances were different.

RC/CSo significantly decreased by 37% and 31% in the leachates of the river sediments and paddy soils compared to the control, respectively, while ABS/RC increased by 9% and 12%, respectively. It suggested that the exposure to leachates mainly inhibited the density of reaction centers and increased the dissipated energy. A large quantity of the superfluous light energy can be released off in form of only heat dissipation, so the DIo/RC was significantly elevated. Finally, PIabs, the performance index of PSII, was significantly reduced.

As mentioned above, PIabs were reduced under leachate treatments, no matter from paddy soil or sediments. When the concentration of leachates was 100%, PIabs of *P. subcapitata* was 75% of the control value for sediment and 65% of the control value for paddy soil, respectively.

However, the primary photochemistry reactions like φPo and ETO/RC were affected by leachate from neither sediment nor paddy soil. RC/ABS and ψo, two main components constituted of PIabs, could be slightly decreased under leachates treatments. This suggested that the concentration of active reaction centers was highly sensitive to leachate treatment. Generally, the effects of leachate treatments from paddy soils on PS II of *P. subcapitata* were much smaller than that from river sediment.

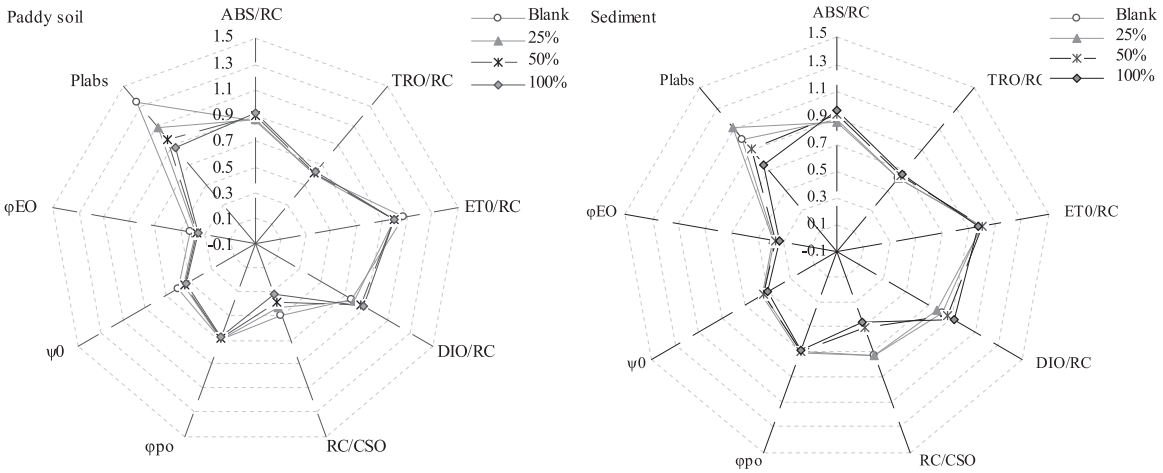

Fig. 3. Effects of leachates on the chlorophyll *a* fluorescence parameters of *Pseudokirchneriella subcapitata*.

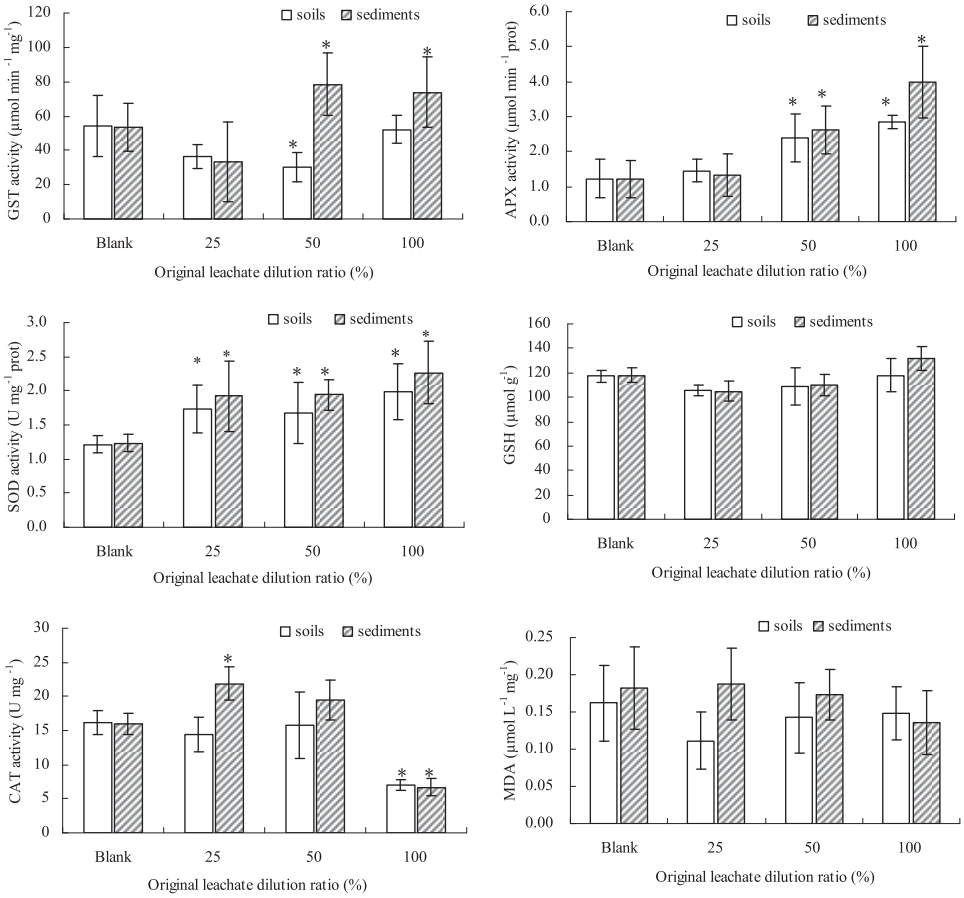

Fig. 4. Effects of leachates on the antioxidative system of *Pseudokirchneriella subcapitata*. Significance of differences to control is indicated by * ($p < 0.05$).

3.5. Toxicity of leachates on the antioxidative system of P. subcapitata

As shown in Fig. 4, the 25% and 50% of leachates from paddy soils and 25% of leachate from sediments caused obvious inhibition of GST activity, but 100% of leachate from paddy soil and 50% and 100% of leachates from river sediments stimulated GST activity. SOD exhibited significant induction effects in all treatments of both paddy soil and river sediment leachates ($P < 0.05$). In 100% of sediment leachate exposure, SOD activity was 1.8 folds higher than the control.

Soil leachates caused no obvious changes in CAT at low and middle concentrations (25% and 50% dilutions), but CAT was significantly inhibited by 43.6% compared with the control at high concentrations (100%). On the other hand, CAT displayed weak induction at low and middle concentrations and strong inhibition at high concentrations (100%) for the leachates of river sediments.

A significant increase of APX activity was observed at the middle and high concentrations for the two types of leachates, being 2.3 folds of the control for soils and 3.2 folds for sediments, but not at low concentrations groups (25%).

GSH was not affected by both leachates types. For all paddy soil leachates treatments, MDA contents were lower than the control, but no significant differences ($P > 0.05$) were observed. Similarly, for the sediments leachates treatments, no significant changes were observed in MDA content.

4. Discussion

4.1. Toxic comparison of two leachates on the growth of P. subcapitata

The sediments and soils are undoubtedly sinks of many lipophilic or hydrophobic compounds like PBDEs (Zhang et al., 2009). High concentrations of PBDEs and heavy metals in river sediments and paddy soils samples indicated that local environment has been strongly contaminated by the e-waste dismantling activities.

However, in sharp contrast, very low PBDEs and metals were detected in two types of leachates, which may be related to the intrinsic hydrophobic characteristics of pollutants and other factors such as humic acid content, pH and TOC etc. (Chen et al., 2011). Breitholtz and Wollenberger (2003) reported that only minor fractions (0.4–2.34%) were found in solutions and instead major fractions were remained in the particulate material smaller than 0.3 mm. This is in agreement with many other studies in which high levels of PBDEs could be detected in sediments but immeasurable levels of PBDEs in water (Law et al., 2006; Allchin et al., 1999).

In our experiment, although the PBDEs and metals in leachates were lower than that in their parent samples, the growth inhibition aggravated significantly with the increasing concentrations of two leachates, indicating that leachates of river sediments and paddy soils are toxic to algal growth. Also the toxicity was higher

in river sediments than in paddy soils, which is parallel to higher content of pollutants like PBDEs and metals in the leachates. We supposed that the difference in algal responses to two leachates might be primarily attributed to heavy metals especially copper rather than PBDEs. It is well known that copper is a strong inhibitor of photosystem II (PSII) electron transport activity associated with photolysis of water. Copper may alter the energy storage capacity in algae during photosynthesis and decrease chlorophyll *a* content (Mallick and Mohn, 2003; Sabatini et al., 2009). Bossuyt and Janssen (2004) reported that the exposure of 100 μg L^{-1} of copper led to an obvious decrease of algal biomass as well as in the growth rate. Our study also showed a great decrease in chlorophyll *a* content and growth rate with the increase of leachates concentration. This trend is much stronger in leachate from river sediments than that from paddy soils.

Remarkably, a great difference was observed in copper ion concentration between two leachates, 1.55 mg L^{-1} in leachates from river sediments and 0.24 mg L^{-1} from paddy soils. Although there was also higher concentration of total PBDEs in sediments (24.1 ng L^{-1}) than in soils (2.15 ng L^{-1}), the majority of PBDEs belongs to high-brominated substituted diphenyl ethers like BDE209, 207 and 208, it was reported that these high-brominated substituted diphenyl ethers are less toxic to organisms (de Wit, 2002). Of course, other compounds like PAHs etc in leachates may also contribute to the performance of tested organisms. Kumerová et al. (2008) reported that PAHs caused the inhibition of the photosystem II and led to the decrease of cell growth. But in present study PAHs were not analyzed. Therefore further investigation should be required.

4.2. Toxic effects of the leachates to the chlorophyll a fluorescence of P. subcapitata

Medium and high concentration of leachates (50% and 100%) both from river sediments and paddy soils could cause inhibition of algal growth and pigment synthesis. The adverse effects were also displayed in the photosynthetic activities. The chlorophyll *a* fluorescence parameters reflect different aspects of photosynthetic performance. So the fluorescence transients test is a useful method to detect the impacts of pollutants on the energy fluxes and the transportation of electron in PSII (Strasser et al., 2000).

Leachates, neither from sediment or soil, decreased the quantity of active reaction centers, which was embodied as the decrease of RC/CSo and the increase of the ABS/RC. These changes may be attributed to many pathways, such as the reversible inactivation of reaction centers and conversion of PS II units into heat sink units (Ivanov et al., 2008). Diminished amount of active PSII center may provoke the activation of light energy dissipation via non-photochemical pathway (Müller et al., 2001). It was reported that non-photochemical energy dissipation became an important part of the regulatory system in energy transfer when the photosynthetic electron transport system was inhibited by chemicals (Singh and Jajoo, 2013; Eullaffroy et al., 2009). In present study, it was also demonstrated indirectly through the analysis of chemicals like Copper and PBDEs in leachates. It was reported that Cu prevent the conversion of light energy absorbed by chlorophyll antenna complex into photosystem II electron transport and increases non-photochemical quenching (Oukarroum et al., 2012). It means that excess excitation energy has been converted into thermal dissipation in order to keep the energy balance between absorption and utilization, and minimize the potential of photooxidative damage. Otherwise, the excess excited chlorophyll molecules can induce the formation of reactive oxygen species (ROS) if the excess excitation energy could not be converted timely (Müller et al., 2001; Foyer and Noctor, 2009). Therefore, the lack of PS II reaction centers should also be regarded as a protection mechanisms with which algae avoid the damage of photosynthetic structure via the reversible inactivation of PS II reaction centers and decrease the utilization efficiency of light energy under stress conditions (Sing and Jajoo, 2013). More energy was dissipated to maintain the cellular homeostasis, which may be used to explain the decrease of cell growth under various environment stresses (Eullaffroy et al., 2009; Ivanov et al., 2008). This may be the possible mechanism of leachates function on the reaction centers of *P. subcapitata*.

In addition, due to the lack of PS II reaction centers, the main process of the primary photochemistry could be inhibited, expressing in the form of the decrease of parameters φEo, ψo and Plabs. Finally, the decrease of photosynthesis performance caused by leachates exposure also reflected in the growth of algae and chlorophyll *a* content as mentioned above.

4.3. Toxic effects of the leachates to antioxidative system of P. subcapitata

The antioxidative defense systems play a very important role in the detoxification process of xenobiotics. Therefore the use of antioxidative enzymes has gained recognition and related parameters are well-documented as biomarkers in pollution biomonitoring and risk assessment.

In present study, there was no significant difference for GST, APX and CAT (just for soil leachates) at low concentration of leachates compared to the control, but with the increasing of the concentrations, significant induction of GST, SOD and APX were observed at high concentration leachates of river sediments and paddy soils, suggesting that oxidative stress occurred within algae. Many pollutants can disturb the electron transfer flow of the photosynthesis electronic transfer chain in chloroplasts (Lemaire and Livingstone, 1997) and consequently lead to the generation of ROS. It was reported that copper exposure may lead to the alteration of PSII activity and caused the formation of superoxide and hydroxyl radicals in wheat (Navari-Izzo et al., 1998) and also induce the activities of CAT, APX and GST in *Scenedesmus obliquus* (Dewez et al., 2005). Similar performance was also found at high concentration leachates. As mentioned previously, the antioxidative systems may be ignited with increasing of ROS, but once the defense system can no long cope with the excess ROS, oxidative stress will occur and subsequently the physiological process will subsequently be disrupted (Winston and Giulio, 1991).

It is well known that GST participates in the elimination of pollutants from cells (Torres et al., 2008; Mittler, 2002). GST directly detoxifies pollutants with electrophilic groups by conjugating with GSH. It was reported that PBDEs and its metabolites like OH-PBDEs could cause the increase of ROS after short time exposure in organism (Ji et al., 2011). The increase of GST activity was observed in high concentration leachates exposure to *P. subcapitata* may indicate the detoxification of PBDEs or metals.

SOD is an essential component as the first line of antioxidative defense system in plants to scavenge superoxide, which serves as a precursor to some highly reactive species (Foyer and Noctor, 2009). Accumulation of superoxidate both derived from the normal metabolism process such as photosynthesis and environment pollutant exposure (Liu et al., 2014) will initiate the SOD response. Enhanced SOD activity resulted in the increase of H_2O_2, Thus the induction of SOD coincided with an increase in the activity of enzymes removing H_2O_2. CAT activity may be induced by oxidative stress and remove most of H_2O_2 in cells (Willekens et al., 1997). But many reports about CAT responses to pollutants exposure were contradictory. Both enzyme activation and inhibition have been described in the exposure of different pollutants (Contreras et al., 2009). In this study, the decrease in CAT activity was observed in *P. subcapitata* under high concentrations exposure of

leachates. It is generally recognized that ROS accumulate when the subtle balance between SOD and CAT is disrupted.

However, there are other regulation pathways for H_2O_2 besides CAT. GSH and AsA are well known as reductant in the ROS scavenging pathway in plant. The H_2O_2 can be also reduced into H_2O and O_2 by APX and subsequently generates monodehydroascorbate, which is (non-enzymatically) converted to dehydroascorbate and then reduced to ascorbate by dehydroascorbate reductase with consumption of GSH or from monodehydroascorbate to ascorbate by monodehydroascorbate reductase without consumption of GSH (Torres et al., 2008). In present study, no obvious change of GSH was observed in all leachates exposures. However, interestingly, significantly induction of APX was found in middle and high concentration leachates groups. APX was also in accordance with increased SOD activity in *P. subcapitata*, thus the substantial decline in CAT activity at high concentration leachates may likely be compensated by the APX activity induction. Therefore, we suppose that APX play an important role in the reduction process of H_2O_2. This is a reasonable explanation for why GSH content stays relatively constant.

Although it was reported that MDA in macro phytoplankton tissues was a useful index to evaluate pollution levels and could be used to assess the toxic effects of pollutants such as metals and other pollutants (Liao et al., 2005), in this study, MDA levels did not show significant change at various dilution ratio leachates. As previously mentioned, the responses of some parameters like GSH and MDA were not completely in agreement with the literatures (Sabatini et al., 2009). The regulation of the antioxidative system with SOD, CAT, APX, and GST activities and non-enzyme antioxidants appeared to be complex involving in the fine balance between production and quenching of ROS in algae and need further investigations.

5. Conclusion

The leachates of river sediments and paddy soils collected from e-waste dismantling sites are potentially toxic to the primary producers, *P. subcapitata*. The chemical pollutants in leachates may decrease the amount of PSII active reaction centers of algae, interfere with the electron transfer process and subsequently affect the photosynthesis performance of algae, meanwhile they may also lead to the generation of ROS, disturb GST, SOD and APX etc antioxidant enzyme activity and furthermore cause the inhibition of algal growth. The sediment leachate is capable of higher deleterious effects on the algae than that of paddy soils. Copper and PBDEs as well as its metabolites in the leachates of both river sediments and paddy soil might be partially responsible for the adverse effects on *P. subcapitata*. However, further investigations and analysis are required to understand exactly the toxic mechanisms of leachates on algae.

Acknowledgment

This work was partly supported by the National Science Foundation Committee of China (U1133005) and the National Science and Technology Support Program (2012BAD18B01) and Grant of JNU Science Research and Innovation Cultivation (21611609). The authors also thank Larry B. Liddle from Long Island University, USA for his kindly help in improving the language of the manuscript.

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