



Eco-toxicity and metal contamination of paddy soil in an e-wastes recycling area

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ABSTRACT

Paddy soil samples taken from different sites in an old primitive electronic-waste (e-waste) processing region were examined for eco-toxicity and metal contamination. Using the environmental quality standard for soils (China, Grade II) as reference, soil samples of two sites were weakly contaminated with trace metal, but site G was heavily contaminated with Cd (6.37 mg kg^{-1}), and weakly contaminated with Cu ($256.36 \text{ mg kg}^{-1}$) and Zn ($209.85 \text{ mg kg}^{-1}$). Zn appeared to be strongly bound in the residual fraction (72.24–77.86%), no matter the soil was metal contaminated or not. However, more than 9% Cd and 16% Cu was present in the non-residual fraction in the metal contaminated soils than in the uncontaminated soil, especially for site G and site F. Compared with that of the control soil, the micronucleus rates of site G and site F soil treatments increased by 2.7-fold and 1.7-fold, respectively. Low germination rates were observed in site C (50%) and site G (50%) soil extraction treated rice seeds. The shortest root length (0.2377 cm) was observed in site G soil treated groups, which is only 37.57% of that of the control soil treated groups. All of the micronucleus ratio of *Vicia faba* root cells, rice germination rate and root length after treatment of soil extraction indicate the eco-toxicity in site F and G soils although the three indexes are different in sensitivity to soil metal contamination.

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1. Introduction

Electronic-waste (e-waste) refers to the end-of-life electronic products including computers, printers, photocopy machines, television sets, mobile phones, and toys, which are made of sophisticated blends of plastics, metals, and other materials. Disposal of the e-wastes is an emerging global environmental issue, as these wastes have become one of the fastest growing waste types in the world. In China, More than 5.14 million home appliances and 4.48 million personal computers are becoming obsolete each year [1]. Moreover, being a developing country, over one million tons of e-waste from the U.S., Europe and other areas of the world are flooding into China every year, taking advantage of the lower labor costs and less stringent environmental regulations [2]. These discarded electronics are usually classified as hazardous waste as electronics contain heavy metals including lead, chromium, cadmium, mercury, and beryllium [3], and other hazardous materials. Furthermore, brominated flame retardants (BFR) can be a component of printed circuit boards, plastic covers and cables [4,5]. Also, plastics in electronics would create dioxins when burned [6]. Most

of these substances are considered hazardous and pose threats to human and environmental health. Therefore, if not disposed appropriately, they could become a source of toxic heavy metals or persistent toxic substances (PTS) [7].

Some studies have been focused on the environment pollution caused by e-wastes in China since the last decade. The accumulation of heavy metals, polybrominated diphenyl ethers (PBDEs) and PAHs in air [8], water [9], sediment [10,11] and soil [12–14] has been reported in some sites of e-waste recycling locations. However, since these chemical data do not take into account the possible combined effects of different contaminants, as well as their bioavailability, they provide only part of the knowledge necessary to evaluate the toxic potential for wildlives and human. Also it is difficult to make clearly hazard assessments and predictions of possible eco-toxicological effects of e-wastes based only on total concentrations. Therefore, a complementally bioassay strategy should be developed with a focus on the most important eco-toxicological effects. The biological endpoints are chosen according to their importance of known biological targets. Genotoxicity and/or the genome disruption are ones of the first targets concerned. Micronucleus (MN) assay in *Vicia faba* root cells was selected to evaluated the Genotoxicity and/or the genome disruption [15], which is highly sensitive and capable of detecting mutagens, clastogens and carcinogens from the environment, and showed excellent correlations with tests in the mammalian systems and human lymphocytes systems

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[16,17]. Seed (Rice seed) germination assay was selected to evaluate the phyto-toxicity of soil, which is a sensitive assay regulated by OECD to test the phyto-toxicity of chemical material [18].

Taizhou, situated on the winding east coast of middle China, is widely famed for its beautiful natural scenery. However, now it has become one of the biggest e-recycling areas in China during recent decades. It is pity that, except few reports of PBDE contamination in soil [13], the metal contamination and the available eco-toxicological data of this area are relatively limited. To understand the extent and nature of environmental pollution caused by these e-waste processing activity, this paper aimed to study the trace contamination of paddy soil in Taizhou. The objective of this study were (1) to establish a basic understanding of the level and extent of trace metal contamination in paddy soils around the e-wastes processing sites in Taizhou; (2) to examine mobility, solubility and potential bioavailability of heavy metals in the environment; and (3) to evaluate the eco-toxicity of paddy soils, and to find relationship between eco-toxicity and metal contaminations.

2. Materials and methods

2.1. 2.1. Sampling area

The research area is located in the southeast of Zhejiang province, China (Fig. 1), under the north sub-tropic monsoon climate. The mean annual rainfall is 1600–1700 mm, of which 60.2% occurs from May to September. Annual mean air temperature is 17 °C, with the highest of 40.8 °C and the lowest of –9.9 °C. The number of e-recyclers in Taizhou has grown exponentially and recycling bases have proliferated rapidly since early 1990s. Historically, local e-recyclers get e-wastes from two major sources: domestic pipelines and foreign imports mainly from U.S., Japan and Taiwan region of China. Different counties of Taizhou usually specialize in different stages of e-waste processing, from manual dismantling, circuit board cooking, acid bathing and stripping, to open burning and dumping. In fact, according to the Economic and Trade Commission of Taizhou city, as of January 2005, more than 40,000 peoples in Taizhou work in the e-recycling industry and around 2 million metric tons of e-waste annually are processed.



Fig. 1. Maps of Taizhou and sampling sites. The map of Taizhou city was used as Ref. [19]. See Table 1 for a description of sampling sites.

2.2. Sample analyses

Based on the distribution of e-recyclers, seven sites (A to G) were selected for study (see location and description in Fig. 1 and Table 1). All the seven sites have e-waste recycling history, but only at site F and site G such operation were observed around the paddy soils during a personal interview. Site G is by far the biggest e-recycling village with 10 years of experience. All the soil samples were taken from the flooded paddy soils at the same time in December 2007. Soil samples were collected from the topsoil (0–20 cm soil layer) using a stainless steel shovel and stored in clean polyethylene bags to minimize sample contamination. The soil samples were firstly air dried at room temperature after transported to the laboratory, then sieved (<1 mm) for removing stones, roots and coarse materials, and finally stored in a desiccator prior to analysis. The pH, total organic carbon (TOC), total phosphorus, total nitrogen, and clay contents were determined according to the general methods [20]. Sequential chemical extractions were performed for the metal contaminated soil samples to fractionate the metal solid phases [21]. For total elemental concentrations, the dried and powdered soil samples were dissolved using a combination of concentrated nitric acid (HNO₃) and perchloric acid (HClO₄). Metal concentrations (Cd, Co, Cr, Cu, Fe, Mn, Pb, and Zn) in the digested solutions or extraction solutions were determined using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Iris Advantage 1000) by the Instrumental analysis center of the Shanghai Jiaotong University. Before using, the glassware and plasticware were soaked overnight in a 1:1 (v/v) concentrated nitric acid and concentrated hydrochloric acid, and then rinsed thoroughly with double distilled water. For quality control, reagent blanks and replicates were set at same time.

2.3. Soil extraction

Ten grams dried soil were used to make soil extraction with deionized water as extractant (liquid-to-solid ratio was 10:1). After an agitation extraction with a speed of 120 rpm for 8 h, the soil solution was centrifuged in 3500 rpm for 10 min, and then the supernatant was filtered through a 0.45- μ m membrane filter. The filtration was immediately prepared for MN assay in *Vicia faba* root cells and germination assay in rice seeds, respectively.

2.4. Micronucleus assay in *Vicia faba* root cells

Micronuclei are small masses of chromatin which appear beside the main nuclei when the plant is contacted with aneugenic agents (fusorial poisons) or clastogenic agents (inducing chromosome breaking). MN assay using *Vicia faba* or *Tradescantia* has been being one of the most frequently applied genotoxicity assays for detecting clastogenicity of contaminated soils [22], which has been recommended for using in mutation screening or monitoring by the Royal Swedish Academy of Sciences, Committee 17 of the Environmental Mutagen Society and the World Health Organization [23]. The micronucleus assay was performed as described by Ma et al. [23] with some minor modification. *Vicia faba* seeds bought from the local market were stored at 4 °C under dry conditions before using. Before soaking in distilled water for 24 h, the seeds were firstly surfacely disinfected with 5% calcium hypochlorite solution and thoroughly rinsed with distilled water. Then the seeds were allowed lying in two moist filter papers at 25 °C to germinate for 2 days. The seedlings with primary roots long to 2–3 cm were soaked for 4 h in darkness at 22 °C in soil extraction, distilled water (as negative control), and 50 mg L⁻¹ CuSO₄ (as positive control), respectively, followed by 22–24 h recovery before fixation. The roots tips were isolated and fixed at 4 °C for

Table 1
Description of the sampling sites.

Sampling site	Sample number	Description
A	3	No recycling operation was observed around the rice fields.
B	3	No recycling operation was observed around the rice fields.
C	4	No recycling operation was observed around the rice fields, but a few chipped circuit boards piled in the peasant's yard were observed.
D	4	No recycling operation was observed around the rice fields, also a few chipped circuit boards piled in the peasant's yard were observed.
E	5	No recycling operation was observed around the rice fields, but some un-dismantled computer was piled in the roadside.
F	5	Recycling operation was observed around the rice fields. We saw many peasants dismantle computer, washer, TV, etc., in their yard, or in the roadside.
G	5	This is a legal metal recycling centre surrounded by paddy fields. We saw many trucks transport in or out the center loaded with cables and others similar materials. We also saw many unsalvageable materials dumping in the river.
CK	3	Control, no e-recycling history and no serious environmental pollution were observed.

16 h in freshly prepared 3:1 ethanol/glacial acetic acid mixture. Then the root tips were immersed in distilled water for 5 min and hydrolyzed in 5 M HCl at 22 °C for 1 h. After subsequent staining in Schiff reagent for 24 h, the root tips were finally prepared for enumerating micronucleated cells. Micronucleated cells were scored on at least 1000 cells/seedling under a microscope (Olympus CX41, Japan). MN frequency (MN%) was calculated as follows:

$$\text{MN \%} = \left(\frac{\text{Number of cells containing micronucleus}}{\text{Total number of cells counted}} \right) \times 1000 \quad (1)$$

For each treatment, six seedlings were observed, and for each soil extraction, three replicates were performed.

2.5. Germination assay

In the current study the influence of soil extraction on the germination of rice seeds was also studied by measuring the root length and counting the number of germinated seeds. After surface sterilized with 5% calcium hypochlorite solution, the rice seeds with germination rates higher than 80% were rinsed with distill water for three times, then were laid between two filter papers for germination at 25 °C in the dark. The filter papers were pre-moistened with 10 mL of different soil extraction and placed in a 9-cm glass Petri dish. For each dish, 5 seeds were put in one direction, and in total, 50 seeds were used for one treatment. Distilled water was used as the negative control and copper sulphate solution (50 mg L⁻¹ CuSO₄) as the positive. After germination for 2 days, the seedlings were taken out and washed with de-ionized water, germinated seeds were counted and root length was measured to the nearest mm.

2.6. Statistical analysis

Statistical analysis was performed by one-way ANOVA and multiple stepwise regressions, using the statistical package for social sciences 11.5 for windows.

3. Results and discussion

3.1. Soil characteristics and metal concentrations

All the soil samples were paddy soils derived from the quaternary red earth with longtime irrigation. The basic characteristics are the follows: pH 4.43–5.89, organic carbon 9.3–42.29 mg kg⁻¹, total N 783–2351 mg kg⁻¹, total P 251–557 mg kg⁻¹ and clay 13–21%. The heavy metal concentrations measured in soil samples are shown in Table 2 together with the environmental quality standard for soils (China, Grade II) [24]. The standard of Grade II is the limits for protecting normal agriculture and human health. The result showed that Fe, Zn, Cu and Cd were the most abundant metals among the soil samples (Table 2). The concentrations of Cd at site E, F and G were higher than 0.3 mg kg⁻¹, which is beyond the limit of Grade II

soil of Chinese environmental quality standard [24]. And the concentration of Cd in site G soil is even higher than in one early report in Guiyu, another famous e-waste recycling site [7], but lower than another report of soil metal contamination in Guiyu [12]. The difference in metal concentrations may attribute to the different of sampling sites. The Cu concentrations at these three sites exceeded 50 mg kg⁻¹, which also exceeds the limit of Grade II soil of Chinese environmental quality standard [24]. The Zn concentrations of soils at site F and G were 281.38 mg kg⁻¹ and 209.85 mg kg⁻¹, respectively; both are slightly higher than the value of Grade II soil of Chinese environmental quality standard [24]. The concentrations of other metals are lower than the values of Grade II soil of Chinese environmental quality standard. But the Fe concentration at site E, F and G are approximately one time higher than that of the control soil. Therefore, according to the limit values for Cd, Cu and Zn in the regulation of environmental quality standard for soils (China, Grade II), site E, F and G soils could be declared as polluted. However, metal concentrations in the control soil were generally below to the limits for Grade II agriculture soil as designed by the Chinese soil quality standard.

Although the e-waste is not deposited in the paddy soil, some recyclers are settled down in the vicinity of paddy soil. Thus the soil will be contaminated through the native e-waste processing operations such as the dumping, dismantling and burning of waste materials. The receiving of wet or dry depositions accounts for the first and most important reason for metal pollution of paddy soil. Secondly, with relative high vapor pressure [25], Cd and Zn is easier than the other metals to volatilize from the open burning of plastics for reducing waste volume and copper wires for recovering valuable metals, e.g., copper. Moreover, the long time flooding of paddy soil will also increase the metal concentration through the irrigation with native stream, which receives the discharging of waste solution. And the dumping of large amount of unsalvaged e-waste may also be one important reason for metal concentration in paddy soil. The low content of Pb and Cr might be a reflection of the effects of different types of e-wastes processing activities and their resultant discharged.

3.2. Chemical speciation of trace metals in the soils

When considering heavy metals in soil, the bioavailability is a very important factor in the ecotoxicological evaluation of the soil. The metal bioavailability in soil or sediment depends on both soil and sediment properties and environmental factors (pH, redox potential. . .) [26]. While sequential soil extractions could give a rough estimate of metal associations, as well as the mobility and bioavailability of toxic metals in soils [27,28] and sediments [11]. Generally, the metal in exchangeable (dissolved) fraction and carbonate fraction is of high mobility. The changes in major cationic composition may cause an ion release due to ion exchange. Metal associated with Fe–Mn oxides has a medium mobility. The changes

Table 2
Total metal concentrations of soil samples taken from Taizhou (mg kg⁻¹ dried soil).

Sampling sites	Cd	Co	Cu	Cr	Fe	Mn	Pb	Zn
A	0.09 ± 0.20	3.71 ± 1.12	41.39 ± 18.81	9.78 ± 7.97	13175.1 ± 10772.69	1775.8 ± 153.48	28.13 ± 19.29	74.88 ± 62.77
B	0.17 ± 0.02	18.18 ± 2.58	46.93 ± 17.89	10.40 ± 8.71	20649.93 ± 2588.90	461.02 ± 5.33	49.63 ± 20.55	94.64 ± 20.89
C	0.09 ± 0.01	16.45 ± 2.67	39.94 ± 15.42	10.64 ± 8.32	18749.93 ± 1988.81	461.02 ± 10.67	49.63 ± 25.64	114.46 ± 33.58
D	0.11 ± 0.13	14.42 ± 2.84	46.63 ± 1.20	21.66 ± 6.02	23675.61 ± 3153.70	435.25 ± 0.99	65.16 ± 2.40	138.0 ± 7.51
E	0.33 ± 0.08	47.26 ± 48.49	51.19 ± 10.64	33.49 ± 28.74	30144.69 ± 11541.92	508.52 ± 93.47	67.04 ± 19.90	145.45 ± 29.35
F	0.62 ± 0.92	61.12 ± 9.85	97.90 ± 50.34	20.95 ± 2.63	31571.88 ± 4053.07	430.11 ± 79.76	44.29 ± 16.33	281.38 ± 127.99
G	6.37 ± 5.91	65.92 ± 18.87	256.38 ± 99.70	26.75 ± 6.09	33000.00 ± 3528.96	366.56 ± 57.75	46.84 ± 12.18	209.85 ± 32.89
CK	0.15 ± 0.01	8.72 ± 2.43	32.08 ± 2.11	6.33 ± 1.03	15598.68 ± 1046.56	324.81 ± 24.66	33.45 ± 1.33	111.99 ± 37.77
Environmental quality standard for soils (China, Grade II)								
pH < 6.5	0.3	-	50	250	-	-	250	200
pH 6.5–7.5	0.3	-	100	300	-	-	300	250
pH > 7.5	0.6	-	100	350	-	-	350	300

in redox conditions may cause a release, but some metal precipitates are insoluble if they present in sulfide mineral. Metals associated with organic matter may have medium or high mobility, depending on the occurring of decomposition/oxidation of organic matter [29]. As metals in the residual fraction are not easily released into the mobile and bioavailable phases, this fraction remain relatively stable and inert [21].

The sequential chemical distribution of Cu, Cd and Zn in site E, F, G and CK is presented in Fig. 2. The distinctive speciation patterns in soil E, F, G and CK indicate differences in the mobility, solubility and potential bioavailability of heavy metals in different soils. Generally, soil E, F and G have more metals present in the exchangeable fraction than the CK. As an index of the plant-available fraction [30], the high fraction of exchangeable metal may migrate from the contaminated soils into ground water, and pose a health risk to human *via* food chain contamination. Most of the Zn in the tested soils was slightly available, being mostly bound to the residual fraction, ranging from 72.24% to 77.86%. However, it was observed that site E, F and G have more Zn present in the exchangeable fraction than the control ($P < 0.05$) and slightly few Zn being present in the Fe–Mn oxidizing fraction and organic fraction than the control. However, no consistent variance in the carbonate

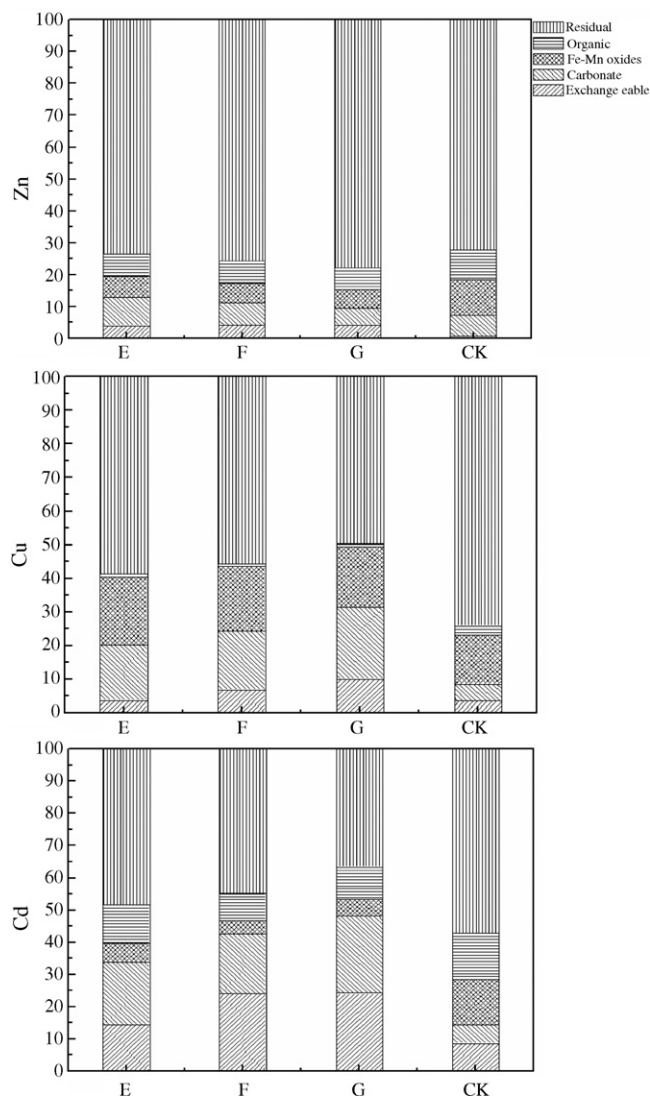


Fig. 2. Sequential chemical distribution of Zn, Cu and Cd in selected paddy soils.

fraction and residual fraction between the metal contaminated soils and the control soil was observed.

The majority of Cu in all the tested soils was associated with the residual fraction (49.76–74.14%) and slightly with the Fe–Mn oxides fraction (14.49–20.31%), carbonate fraction (5.02–21.26%) and exchangeable fraction (3.57–10.05%). Although Cu had high affinity with organic matter [31], the organic fraction in flooded paddy soil was very low (0.76–2.79%). In comparison, site F and G have more Cu being bounded in exchangeable fraction ($P < 0.05$), carbonate fraction, and Fe–Mn fraction than the control soil, and fewer Cu in the organic fraction and residual fraction than the control soil. But the fractionation pattern of Cu in soil E is similar to that of the control.

Different from Zn and Cu, the fraction of residual Cd is relatively low, only ranging from 36.60% to 57.14%. This indicates the bioavailability of Cd in the paddy soil is higher than that of Zn and Cu. Compared with the control soil, the metal contaminated soils have more Cd being present in the exchangeable fraction ($P < 0.05$), carbonate fractions and few Cd in the Fe–Mn oxides fraction, organic fraction, the residual fraction. The high percentages of exchangeable and carbonate fractions of Cd in the metal contaminated soils might be the co-effect of metal characteristics and different geochemical conditions of the tested soils. Cadmium is the most mobile and potentially bioavailable metal and primarily scavenged by non-detrital carbonate minerals, organic matter, and iron-manganese oxides minerals [32]. On the other hand, strong acid leaching of printed circuit boards for recovering precious metals, from which the waste acids were discharged into nearby streams, will lead the soil acidification with the long time soil flooding.

3.3. Micronucleus assay in *Vicia faba* root cells

The results of MN assay are present in Fig. 3. MN% of the soil extraction-exposed groups were 10.33–37.28, all were higher than that of the negative group (3.07 ± 0.8). The lowest MN% value was observed in the control soil extraction-exposed groups, with the mean value of only 10.33. But high MN% values were appeared in the soil G and soil F extraction-exposed groups, which increased 2.6 folders and 1.7 folders, respectively, than that of the control soil exposed group. The MN% values are significantly positive correlated with the content of Cu ($P < 0.01$), Cd ($P < 0.05$), Co ($P < 0.05$) and Fe ($P < 0.05$) in soil. The result of stepwise regression indicates

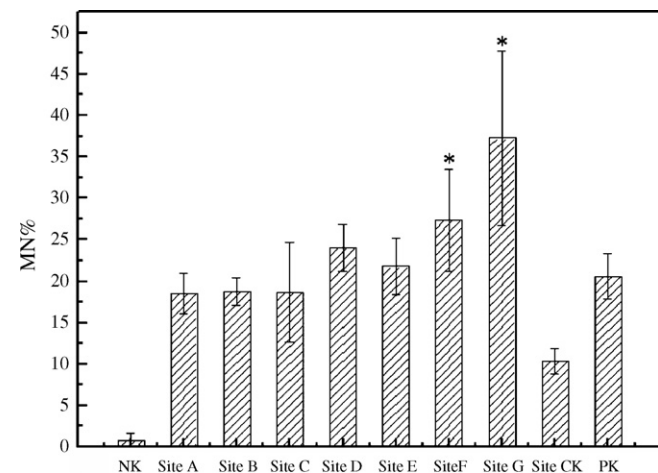


Fig. 3. Effect of soil extraction on micronucleus rate of *Vicia faba* root tip cells. * $P < 0.05$, significant difference is observed between the control soil extraction-exposed groups and the selected soil extraction-exposed groups by Tukey's test.

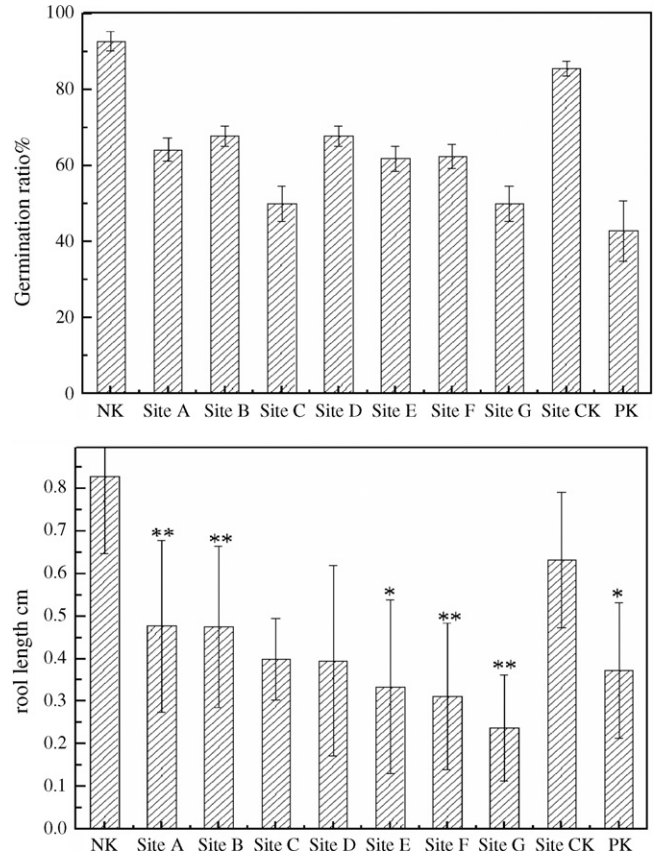


Fig. 4. Effect of soil extraction on the germination rate (a) and root length (b) of rice. * $P < 0.05$, significant difference is observed between the control soil extraction-exposed groups and the selected soil extraction-exposed groups by Tukey's test; ** $P < 0.01$, very significant difference is observed between the control soil extraction-exposed groups and the selected soil extraction-exposed groups by Tukey's test.

that Cu gives large contribution to the value of MN%, the equation is

$$Y = 15.036 - 0.092Cu \quad (r^2 = 0.776, P = 0.004, F = 20.728) \quad (2)$$

These imply that the MN assay is sensitive to the accumulation of Cu, Cd, Cu, and Fe in soil, especially the accumulation of Cu.

The high MN% values in soil F and G treated *Vicia faba* indicating clastogenicity. The clastogenicity can be explained by the co-effect of high metal content and metal mobility. The high MN% values in soil F and G treated groups also show that the MN assay in *Tradescantia* or *Vicia faba* is sensitive to the metal contamination of soil. These are consistent to other reports. The induction of micronuclei in *Tradescantia* is significantly related to the soil concentration of several metals (e.g., Sb, Cu, Cr, As, Pb, Cd, Ni, Zn) [31], and the frequency of micronuclei (MN) will increase with the increasing metal concentration in soils from identical locations [33]. *Vicia faba* micronuclei frequencies of the chromium contaminated soil samples show linear dose responses to chromium contents in the soil [34]. The *Vicia faba* MN frequencies in the paddy soil polluted with wastewater irrigation were 2.2–48.4 times higher than the control [35]. All these research prove that the MN assay is a suitable assay to be used for contaminated soil evaluation.

3.4. Seed germination assay

The results of seed germination assay are present in Fig. 4. Low germinations rates of 50–85% were observed after seeding at 25 °C for 2 days germination (Fig. 4a), but the highest one was in the

control soil extraction treated seeds, which is close to the distilled water treated group. The lowest germination rate of 50% appeared in the soil C and soil G treated seeds, both were close to the positive control (42%). The germination rates of other soil extraction treated groups ranged from 61.90% to 67.86%. None of the metal concentration of the soil was linearly correlated with the germination rates although the low germination rates were observed in metal contaminated soil treated seeds. This pointed out the insensitive of germination to soil contaminants concentrations, which is similar to previous results [35–38]. The low germination of site C may suggest the existing of other contaminants, and may be the effect of co-exposure of metal with other contaminants. Although the concentration of individual contaminant was very low, the co-exposure may cause toxic effect [39]. The root length of the soil treated seeds is ranging from 0.2377 cm to 0.6327 cm (Fig. 4b), all being shorter than that of the distilled water treated seeds. The longest one of 0.6327 cm appeared in the control soil treated seeds; meanwhile, the shortest of 0.2377 cm did in the site G soil treated seeds, which is even shorter than the positive control. The root length is negatively significant correlated with the soil concentration of Fe ($P < 0.01$), Co ($P < 0.05$) and Cr ($P < 0.05$). When analyzed with stepwise regression, the equation is

$$Y = 0.731 - 1 \times 10^5 Fe \quad (r^2 = 0.737, P = 0.006, F = 16.791). \quad (3)$$

These demonstrate the root length is sensitive to the accumulation of Fe in soil.

Many contaminants can be detrimental to seed germination via direct toxicity or indirect changing of soil micro-environment. The lowest germination rate in site C and G imply the degeneration of soil quality. Compared with germination rate, the root length is obviously affected by the concentration of soil contaminants [40].

4. Conclusions

The inappropriately disposal of e-waste make them become a source of heavy metal contaminant. The presented results of the chemical analyses of seven soil samples (A–G) indicate that the tested paddy soils have been moderately contaminated by Cd, Cu and Zn referencing the environmental quality standard for soils (China, Grade II) [24]. But the soil at site G was heavily contaminated, especially by Cd. The metal polluted soils tend to have more Cd and Cu associated with the non-residual fraction, especially for site F and site G. Zn is mainly concentrated in the residual fraction, no matter the soil is metal contaminated or not. Combined with chemical analysis, the *Vicia faba* MN test and germination assay validate the eco-toxicity of some polluted paddy soil. Water extraction of soil F and G show a trend toward increasing numbers of micronuclei formation with the *Vicia faba* MN test. Although the germination assay is not sensitive to metal contamination of soil, a trend toward decreasing germination rate and shortening root length is also observed in water extraction of soil G. Therefore, our work proved the hazardous implications of e-waste recycling on the agriculture environment. However, our data are still very limited and much more works are needed to be done about the extent and long-term effects of these particular e-wastes on environments and human health.

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References

- [1] A. Terazona, S. Murakami, N. Abe, B. Inanc, Y. Moriguchi, S.I. Sakai, M. Kojima, A. Yoshida, J.H. Li, J.X. Yang, M.H. Wong, A. Jain, I.S. Kim, G.L. Peralta, C.C. Lin, T. Mungcharoen, E. Williams, Current status and research on E-waste issues in Asia, *J. Mater. Cycles Waste Manag.* 8 (2006) 1–12.
- [2] J.H. Li, B.G. Tian, T.Z. Liu, H. Liu, X.F. Wen, S.I. Honda, Status quo of e-waste management in mainland China, *J. Mater. Cycles Waste Manag.* 8 (2006) 13–20.
- [3] Y.C. Jang, T.C. Townsend, Leaching of lead from computer printed circuit boards and cathode ray tubes by municipal solid waste landfill leachates, *Environ. Sci. Technol.* 37 (2003) 4778–4784.
- [4] K.S. Betts, PBDEs, PCBs in computers, cars, and homes, *Environ. Sci. Technol.* 40 (2006) 7452–7454.
- [5] S. Heart, Environmental impacts and use of brominated flame retardants in electrical and electronic equipment, *Environmentalist*, doi:10.1007/s10669-007-9144-2.
- [6] S.A. Cormier, S. Lomnicki, W. Backes, B. Dellinger, Origin and health impacts of emissions of toxic by-products and fine particles from combustion and thermal treatment of hazardous wastes and materials, *Environ. Health Perspect.* 114 (2006) 810–817.
- [7] A. Leung, Z.W. Cai, M.H. Wong, Environmental contamination from electronic waste recycling at Guiyu, southeast China, *J. Mater. Cycles Waste Manag.* 8 (2006) 21–33.
- [8] W.J. Deng, P.K.K. Louie, W.K. Liu, X.H. Bi, J.M. Fu, M.H. Wong, Atmospheric levels and cytotoxicity of PAHs and heavy metals in TSP and PM_{2.5} at an electronic waste recycling site in southeast China, *Atmos. Environ.* 40 (2006) 6945–6955.
- [9] C.S.C. Wong, N.S. Duzgoren-aydin, A. Adnan, M.H. Wong, Evidence of excessive releases of metals from primitive e-waste processing in Guiyu, China, *Environ. Pollut.* 148 (2007) 62–72.
- [10] D.L. Wang, Z.W. Cai, G.B. Jiang, A. Leung, M.H. Wong, W.K. Wong, Determination of polybrominated diphenyl ethers in soil and sediment from an electronic waste recycling facility, *Chemosphere* 60 (2005) 810–816.
- [11] C.S.C. Wong, S.C. Wu, N.S. Duzgoren, A. Aydin, M.H. Wong, Trace metal contamination of sediments in an e-waste processing village in China, *Environ. Pollut.* 145 (2007) 434–442.
- [12] M.H. Wong, S.C. Wu, W.J. Deng, X.Y. Yu, A.O.W. Leung, C.S.C. Wong, W.J. Luksemburg, A.S. Wong, Export of toxic chemicals—a review of the case of uncontrolled electronic-waste recycling, *Environ. Pollut.* 149 (2007) 137–140.
- [13] Z.W. Cai, G.B. Jiang, Determination of polybrominated diphenyl ethers in soil from e-waste recycling site, *Talanta* 70 (2006) 88–90.
- [14] X.Z. Yu, Y. Gao, S.C. Wu, H.B. Zhang, K.C. Cheung, M.H. Wong, Distribution of polycyclic aromatic hydrocarbons in soils at Guiyu area of China, affected by recycling of electronic waste using primitive technologies, *Chemosphere* 65 (2006) 1500–1509.
- [15] K.W. Schramm, A. Hofmaier, O. Klobasa, A. Kaune, A. Kettrup, Biological in vitro emission control, *J. Anal. Appl. Pyrol.* 49 (1999) 199–210.
- [16] W.F. Grant, The present status of higher plant bioassays for the detection of environmental mutagens, *Mutat. Res.* 310 (1994) 175–185.
- [17] T.H. Ma, The international program on plant bioassays and the report of the follow-up study after the hands-on workshop in China, *Mutat. Res.* 426 (1999) 103–106.
- [18] Organization of Economic Cooperation Development (OECD), Proposal for updating guideline 208: terrestrial (non-target) plant test 208A—Seedling emergence and seedling growth test OECD Guideline for Testing of Chemicals, vol. 208, OECD, European Committee, Paris, France, 2000, pp. 208–209.
- [19] Surveying and Mapping Team of Zhejiang Province, The Map of Taizhou City, Hunan Map Press, Changsha, China, 2001.
- [20] Institute of Nanjing Pedology Chinese Academy of Sciences, Physicochemical Analysis of Soil, Third ed., Science Press, Beijing, 1981.
- [21] A. Tessier, P.G.C. Campbell, M. Bisson, Sequential extraction procedure for the speciation of particulate trace metals, *Anal. Chem.* 51 (1979) 844–850.
- [22] P.A. White, L.D. Claxton, Mutagens in contaminated soil: a review, *Mutat. Res.* 567 (2004) 227–345.
- [23] T.H. Ma, Z.D. Xu, C. Xu, H. McConnell, E.V. Rabago, G.A. Arreola, H. Zhang, The improved Allium/Vicia root tip micronucleus assay for clastogenicity of environmental pollutants, *Mutat. Res. Environ. Mutagen. Rel. Subjects* 334 (1995) 185–195.
- [24] GB 15618–1995, Environmental quality standard for soils, China.
- [25] E. Kurek, J.M. Bollag, Microbial immobilization of cadmium released from CdO in the soil, *Biogeochemistry* 69 (2004) 227–239.
- [26] E.D. Van Hullebusch, P.N.L. Lens, H.H. Tabak, Developments in bioremediation of soils and sediments polluted with metals and radionuclides. 3. Influence of chemical speciation and bioavailability on contaminants immobilization/mobilization bio-processes, *Rev. Environ. Sci. Bio. Technol.* 4 (2005) 185–212.
- [27] G. Renella, P. Adamo, M.R. Bianco, L. Landi, P. Violante, P. Nannipieri, Availability and speciation of cadmium added to a calcareous soil under various managements, *Eur. J. Soil Sci.* 55 (2004) 123–133.
- [28] F. Garrido, V. Illera, C.G. Campbell, M.T. García-González, Regulating the mobility of Cd, Cu and Pb in an acid soil with amendments of phosphogypsum, sugar foam, and phosphoric rock, *Eur. J. Soil Sci.* 57 (2006) 95–105.

- [29] W. Salomons, Environmental impact of metals derived from mining activities: processes, predictions, prevention, *J. Geochem. Explor.* 52 (1995) 5–23.
- [30] J.L. Everhart, J.D. McNear, E. Peltier, L.D. Van, R.L. Chaney, D.L. Sparks, Assessing nickel bioavailability in smelter-contaminated soils, *Sci. Total Environ.* 367 (2006) 732–744.
- [31] A.M. Imperato, P. Adamo, D. Naimo, M. Arienzo, D. Stanzione, P. Violante, Spatial distribution of heavy metals in urban soils of Naples city (Italy), *Environ. Pollut.* 124 (2003) 247–325.
- [32] B.G. Prusty, K.C. Sahu, G. Godgul, Metal contamination due to mining and milling activities at the Zawar zinc mine, Rajasthan, India. 1. Contamination of stream sediments, *Chem. Geol.* 112 (1994) 275–292.
- [33] B.J. Majer, D. Tschërko, A. Paschke, R. Wennrich, M. Kundi, E. Kandeler, S. Knasmüller, Effects of heavy metal contamination of soils on micronucleus induction in *Tradescantia* and on microbial enzyme activities: a comparative investigation, *Mutat. Res.* 515 (2002) 111–124.
- [34] H.Q. Wang, Clastogenicity of chromium contaminated soil samples evaluated by *Vicia* root-micronucleus assay, *Mutat. Res. Fund. Mol. Mech.* 426 (1999) 147–149.
- [35] Y.F. Song, B.M. Wilke, X.Y. Song, P. Gong, Q.X. Zhou, G.F. Yang, Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and heavy metals (HMs) as well as their genotoxicity in soil after long-term wastewater irrigation, *Chemosphere* 65 (2006) 1859–1868.
- [36] Y.J. An, Soil ecotoxicity assessment using cadmium sensitive plants, *Environ. Pollut.* 127 (2004) 21–26.
- [37] H.C. Cao, J.N. Wang, X.L. Zhang, Ecotoxicity of Cadmium to maize and soybean seedling in black soil, *Chin. Geograph. Sci.* 17 (2007) 270–274.
- [38] P. Alvarenga, P. Palma, A.P. Gonçalves, R.M. Fernandes, A. de Varennes, G. Vallini, E. Duarte, A.C. Cunha-Queada, Evaluation of tests to assess the quality of mine-contaminated soils, *Environ. Geochem. Health* 30 (2008) 95–99.
- [39] J.G. Hengstler, U. Bolm-Audorff, A. Faldum, K. Janssen, M. Reifenrath, W. Götte, D. Jung, O. Mayer-popkken, J. Fuchs, S. Gebhard, H.G. Bienfait, K. Schlink, C. Dietrich, D. Fause, B. EPe, F. Oesch, Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected, *Carcinogenesis* 24 (2003) 63–73.
- [40] R. Cuevas, M.E. Gallegos-Martínez, F. Cruz-Sosa, M. Gutiérrez-Rojas, In vitro evaluation of germination and growth of five plant species on medium supplemented with hydrocarbons associated with contaminated soils, *Bioresource Technol.* 99 (2008) 6379–6385.