

# Evaluation of enhanced soil washing process with tea saponin in a peanut oil–water solvent system for the extraction of PBDEs/PCBs/PAHs and heavy metals from an electronic waste site followed by vetiver grass phytoremediation

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## Abstract

**BACKGROUND:** Problems associated with PBDEs/PCBs/PAHs and heavy metals mixed contaminated sites have received wide attention. To address the associated environmental concerns, innovative remediation techniques are urgently needed.

**RESULTS:** 5.0 mL L<sup>-1</sup> peanut oil and 5.0 g L<sup>-1</sup> tea saponin were found to be effective in extracting 94.6%, 97.1%, 95.1%, 83.5%, and 87.1% of PBDEs, PCBs, PAHs, Pb, and Ni, respectively, after two successive washing cycles. Cultivation of vetiver grass and addition of nutrients for 4 months further degraded 45.1%, 36.2%, and 40.2% of the residual PBDEs, PCBs and PAHs, respectively. Pb and Ni removal efficiencies by phytoextraction were 4.1% and 2.0%, respectively. The combined treatment partially restored the microbiological functions of washed soil, as indicated by a significant increase in the number, biomass C, N, and functioning diversity of soil microorganisms ( $P < 0.05$ ). After treatment, the residual organic pollutants and heavy metals mainly existed as very slowly desorbing fractions and residual fractions, as evaluated by Tenax extraction combined with a first-three-compartment model and sequential extraction. The secondary environmental risk of residual mixed pollutants in the remediated soil was limited.

**CONCLUSION:** The proposed combined cleanup strategy proved to be effective and environmentally friendly.

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Supporting information may be found in the online version of this article.

**Keywords:** mixed-contaminated site; soil washing; tea saponin; peanut oil; vetiver grass cultivation; Tenax extraction

## INTRODUCTION

Electronic waste (E-waste) is an emerging global concern as illegal dismantling activities have been found to release many persistent toxic substances into our environment.<sup>1</sup> Serious environmental problems throughout the world are caused by excessive inputs from end-of-life electronic products, such as computers, printers, photocopy machines, television sets, mobile phones, and toys that are made up of sophisticated blends of plastics, metals, and other materials.<sup>2</sup> Some pieces of electronic equipment contain high concentrations of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals (such as Pb, Cd, Cr, Zn, Cu and Ni)<sup>3</sup>. These contaminants could persist in the environment for long periods and are biomagnified through the food chain – through microorganisms, plants, animals, and humans.<sup>4</sup> With the signing of the Stockholm Convention and the development of global monitoring programs, many E-waste sites are identified in major cities in China and other industrialized countries worldwide.<sup>5</sup> Soils from these sites are often subjected to organic–inorganic mixed

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contamination caused by the extensive and primitive disposal or recycling of E-waste.<sup>6</sup> Currently, most of these contaminated sites require urgent land-use conversion and pose a likely threat to the health of residents and wildlife.<sup>7</sup> Therefore, developing new soil remediation techniques that are suitable and effective is necessary.

Proper remediation that can simultaneously remove organic and inorganic pollutants from mixed-contaminant sites is essential.<sup>7</sup> *Ex situ* soil washing with environmentally friendly additives may be a promising technology for achieving this goal. However, great changes in soil structure during soil washing processes result in significant disturbance of soil microcosms.<sup>8</sup> Thus, restoring the microbiological functions of washed soil through phytoremediation may be needed as a follow-up procedure after soil washing.

Vegetable oil and saponins are nonhazardous, environmentally friendly, and effective solvents used to extract organic and inorganic pollutants from contaminated soils for remediation purposes.<sup>7,9–11</sup> Gong *et al.*<sup>12</sup> investigated the effectiveness of soybean oil in removing PAHs from a heavily polluted site. They found that approximately 80% of PAHs could be extracted from soil. Gusiatin and Klimiuk<sup>11</sup> demonstrated the feasibility of using 3% w/w saponin to remove 44%–61% of Cu, 60%–76% of Cd and 68%–84% of Zn from loamy sand soil. Song *et al.*<sup>13</sup> observed that saponin at a concentration of 3750 mg L<sup>-1</sup> saponin has a removal rate of 88% and 76% of Cd and phenanthrene, respectively, from mixed-contaminant soil. However, research on using vegetable oil and saponins to simultaneously extract PBDEs, PCBs, PAHs, and heavy metals from contaminated sites is limited. In particular, studies that consider the subsequent restoration of residual soil with plant cultivation are few. Furthermore, the mobility and bioaccessibility of these mixed pollutants in washed soil are often neglected. Little is also known about the subsequent environmental risk of washed soil.

To understand the uncertainty of this process, an empirical model commonly used to quantify the triphasic nature of organic pollutants desorption has recently been established.<sup>14</sup> This model assumes that organic pollutant desorption occurs from three compartments in soil defined by fast, slow, and very slow rates, each following first-order kinetics and could be predicted by consecutive desorption for 100 h with Tenax extraction.

In the present study, the feasibility of using peanut oil and tea saponin (TS) solution to wash PBDEs, PCBs, PAHs, and heavy metals from an E-waste site was evaluated. Peanut oil is relatively inexpensive and readily available because of its high production in China. Between 2001 and 2010, the annual production of peanut oil was more than  $2.0 \times 10^6$  tons in China.<sup>12</sup> In addition, peanut oil is considered a natural resource that can be rapidly biodegraded in soil under the optimal microbial conditions.<sup>9,12</sup> TS is a type of natural surfactant derived from tea seeds. TS is a colorless crystal consisting of hydrophilic and hydrophobic groups, particularly glycosides with their aglycones related to either sterols or triterpenes.<sup>15</sup> The hydrophilic groups contain hydroxy and ester groups. TS has low toxicity to bacterial cells and can complex heavy metals such as Cd, Cu, Ni, and Zn in the presence of various organic contaminants.<sup>11</sup> The combined effects of peanut oil and TS concentration, as well as cycles of successive washing, on removal efficiency were examined. The subsequent microbiological functions of residual soil were restored by vetiver grass cultivation [*Vetiveria zizanioides* (L.) Nash] and nutrient addition. *V. zizanioides* (L.) Nash is a tall (1 m to 2 m), fast-growing, perennial tussock grass.<sup>16</sup> It is used to rehabilitate contaminated soils because of its unique morphological, physiological, and ecological characteristics,<sup>17</sup> as

well as its tolerance to different adverse climatic and edaphic conditions, such as elevated levels of persistent organic pollutants and heavy metals.<sup>18</sup> The effectiveness of the combined cleanup strategy was assessed using biological tests. The secondary environmental risk of residual PBDEs, PCBs, and PAHs in washed soil was evaluated by Tenax extraction and a first-three-compartment model. The redistribution of different fractions of heavy metals in soil before and after decontamination was also determined through a sequential extraction procedure. The results of this study can be used as a reference for the remediation and environmental management of sites contaminated with organic and inorganic pollutants.

## EXPERIMENTAL

### Materials and methods

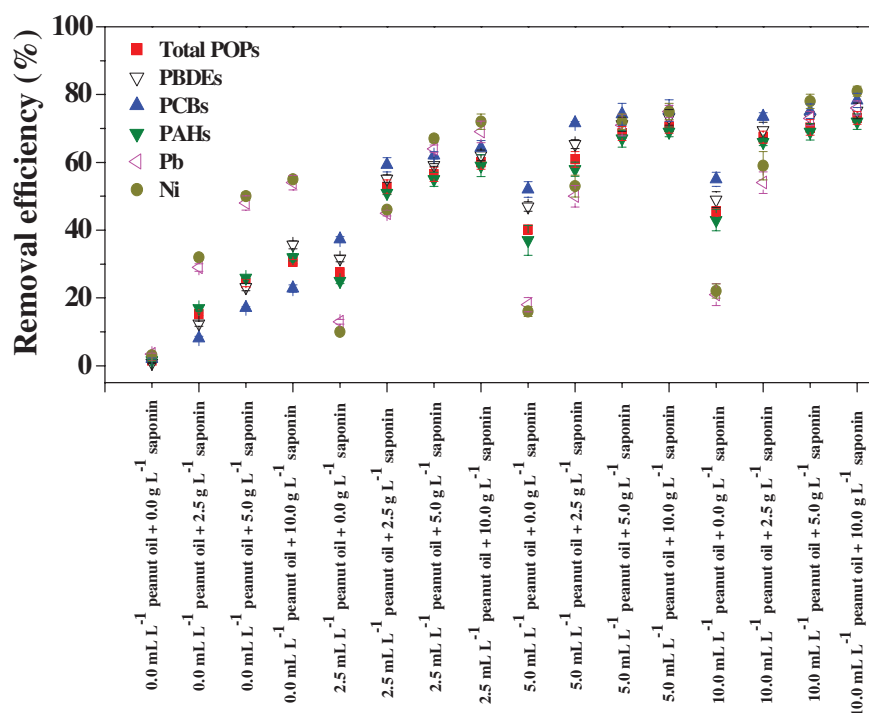
Standard samples of PBDEs, PCBs, and PAHs (purity > 99.5%) and aqueous metal standard solutions of Pb and Ni (1000 mg L<sup>-1</sup>) were obtained from Shanghai Chemicals Technology Co, Ltd. Peanut oil used in this study was purchased from a local supermarket (Jiangsu, China). It had a relative density of 0.91 g cm<sup>-3</sup>, an oleic acid content of 35% to 67%, and a linoleic acid content of 13% to 43%. TS (60% pure) was purchased from Nanjing Plant Technology Co, Ltd (Jiangsu, China). It has a molecular weight of 1222, critical micelle concentration of 0.5%, and surface tension of 32.9 mN m<sup>-1</sup>. Before use, the Tenax beads (60 to 80 mesh) were rinsed with acetone and hexane before drying overnight at 75 °C.

### Site description and soil preparation

All soil samples were collected from an abandoned E-waste disposal plant in the old district of Jiangnin County, Nanjing City, Jiang Province, China (31°94'40" N, 118°85'32" E). This plant dismantled various kinds of electronic products between 2000 and 2013. The soil samples were collected from the surface to a depth of 20 cm. All soil samples were air dried for 7 days, homogenized, ground to pass through a 2 mm sieve, and then stored in glass bottles at 4 °C prior to analysis. The samples were pH 6.1 (in water) and composed of 16.2% sand, 68.7% silt, 15.1% clay, 1.9% organic matter, 0.4 g kg<sup>-1</sup> total N, 37.7 mg kg<sup>-1</sup> hydrolysable N, 0.1 g kg<sup>-1</sup> total P, and 22.6 mg kg<sup>-1</sup> Olsen P on dry weight basis. The concentrations of total organic pollutants (OPs), PBDEs, PCBs, and PAHs in the samples were  $198.3 \pm 18.1$ ,  $12.9 \pm 0.6$ ,  $33.3 \pm 1.9$ , and  $152.1 \pm 15.5$  mg kg<sup>-1</sup> dry soil, respectively (Supplementary material 1). Specifically, the soil samples contained  $350.6 \pm 14.2$  mg kg<sup>-1</sup> of Pb, and  $220.3 \pm 12.3$  mg kg<sup>-1</sup> of Ni.

### Experimental design and soil washing

In an 8 L stainless steel tank with a stirring apparatus, 1000 g of contaminated soil and 5 L of different washing solvents were added. A complete randomized block design (four concentrations of peanut oil  $\times$  four concentrations of TS) in triplicate was used to investigate the combined effect of peanut oil and TS on the removal efficiency of OPs and heavy metals. The operating concentrations of the two-phase partitioning system (the volume fraction of peanut oil divided by the total volume fraction of distilled water plus peanut oil) ranged from 0.0 mL L<sup>-1</sup> to 10.0 mL L<sup>-1</sup>. The operating concentrations of the TS solution (w/v) ranged from 0.0 g L<sup>-1</sup> to 10.0 g L<sup>-1</sup>. The tank was stirred at  $30 \pm 2$  °C at 50 rpm for 60 min. Then, soil was separated from the supernatant by centrifugation at 2000 rpm for 30 min for another washing cycle. Three successive washing cycles were performed to evaluate the maximum removal



**Figure 1.** Removal efficiency of POPs and heavy metals from Jiangnjin soil with the combination of 0.0, 2.5, 5.0, 10.0 mL L<sup>-1</sup> peanut oil and 0.0, 2.5, 5.0, 10 g L<sup>-1</sup> tea saponin. Values are means  $\pm$  standard deviation of triplicate measurements.

efficiency. After successive washing, the soil samples were combined and air dried for subsequent microcosm studies.

### Soil microcosms

Soilmicrocosms were set up using nine pots (upper diameter, 35 cm; lower diameter, 25 cm; height, 20 cm), each containing 4000 g of air-dried soil. Each pot contained washed soil after two sequential washing  $\times$  four times. The following three treatments were designed, each treatment was replicated three times: (1) initial polluted soil sample; (2) second washed soil: obtained by two sequential washing employing 5.0 mL L<sup>-1</sup> peanut oil and 5.0 g L<sup>-1</sup> tea saponin; and (3) second washed soil for 4 months of vetiver grass cultivation and nutrient solution addition (NH<sub>4</sub>NO<sub>3</sub> as the N source and K<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub> as the P source). The nutrient solution contained 47.5 g N L<sup>-1</sup> and 2.8 g P L<sup>-1</sup>. Seeds of vetiver grass were germinated, and 50 seedlings of uniform size were selected and then transplanted to the designated pots. The microcosms were incubated in a greenhouse at a daily average temperature of 28  $\pm$  2 °C. Every 3 weeks, approximately 20 g of soil was collected by taking four random soil samples up to a depth of 10 cm from each pot. The samples were combined to give one composite sample per pot (80 g). At the end of the experiment, all plants that survived were harvested. All soil and plant samples were stored at 4 °C for subsequent analysis.

### Desorption determination by consecutive Tenax extraction

Tenax extraction was used according to the references of.<sup>14,19</sup> The consecutive desorption data were fitted into a triphasic kinetic model to obtain the kinetics parameters:

$$S_t/S_0 = F_r \exp(-k_r t) + F_{sl} \exp(-k_{sl} t) + F_{vl} \exp(-k_{vl} t) \quad (1)$$

where  $S_0$  and  $S_t$  are the amounts of OPs in soil at the start (0) and at time  $t$  (h) of the desorption experiments, respectively;  $S_t/S_0$  is the

residual fraction of OPs in soil at time  $t$  (h); and  $F_r$ ,  $F_{sl}$ , and  $F_{vl}$  are the rapidly, slowly, and very slowly desorbing fractions, respectively.

### Heavy metals fraction in soil before and after decontamination

Sequential extractions proposed by the European Communities Bureau of Reference were performed on 5 g portions of the soil samples to assess the redistribution of heavy metals among operationally defined pools.<sup>20</sup>

### Microbial activity

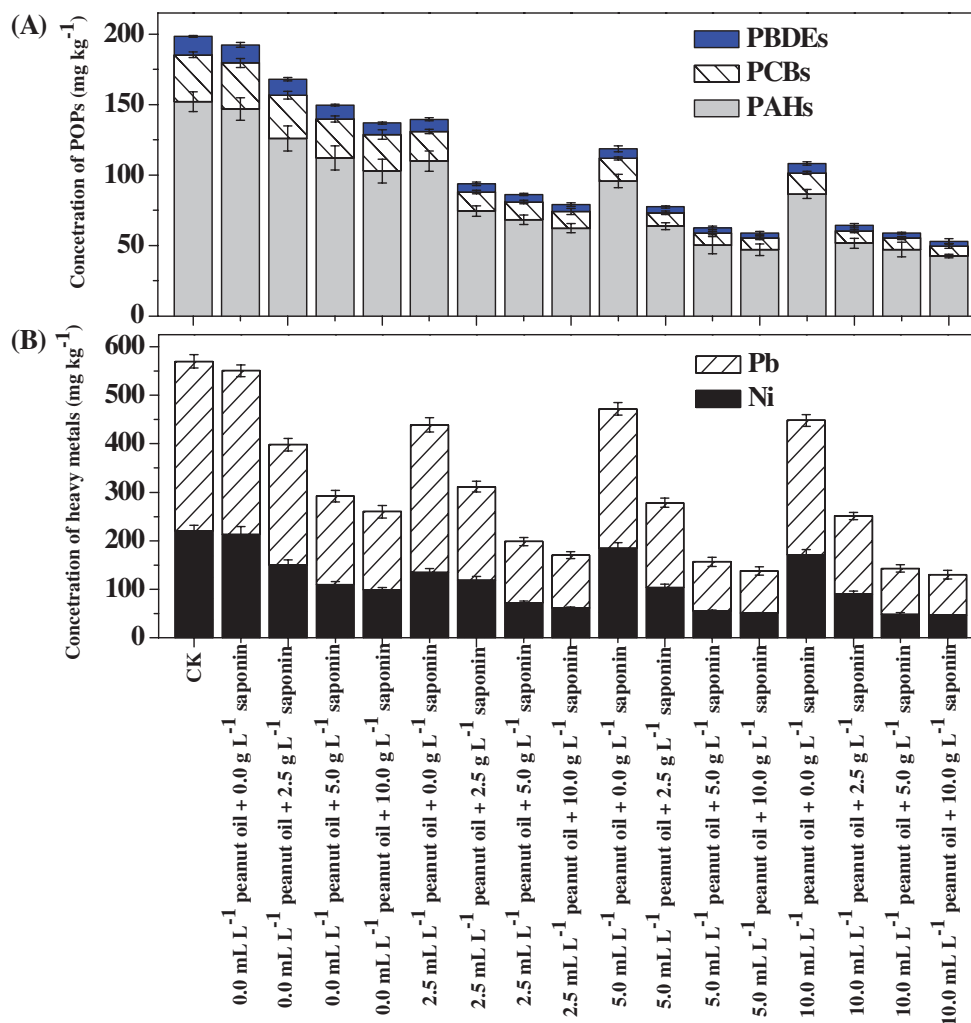
Fast-growing cultivable microorganisms in soil were counted by the plating method.<sup>5</sup> Changes in soil microbial biomass C and N were determined by the fumigation-extraction method.<sup>5</sup> The physiological profiles of the soil microbial community were obtained using Gram-negative microplates.<sup>8</sup>

### Extraction and analysis of OPs and heavy metals

All extraction procedures for soil and plant OPs were performed with an accelerated solvent extractor system.<sup>1</sup> An Agilent 6890 GC coupled with a model 5973 N MS detector in selected ion mode was used for compound analysis.<sup>2,5,21</sup> The mean recovery of OPs in spiked soil samples was 91.7%  $\pm$  3.2% for PBDEs, 94.5%  $\pm$  1.7% for PCBs, and 93.8%  $\pm$  4.6% for PAHs. The concentrations of Cd and Pb were quantified with a Thermo Flame Atomic Absorption Spectrophotometer.<sup>8</sup>

### Statistical analysis

Statistical analysis was performed using SPSS 14.0. Equation (1) was calculated using SigmaPlot 10.0 software. Mean values were compared by least significant difference at probability level  $\leq$  5%. The removal rates of pollutants were subsequently calculated.<sup>21</sup>



**Figure 2.** Residual concentration of PBDEs, PCBs, PAHs, Pb and Ni in Jiangnin soil by a complete randomized block design for washing solvents (0.0, 2.5, 5.0, 10.0 mL L<sup>-1</sup> peanut oil × 0.0, 2.5, 5.0, 10 g L<sup>-1</sup> tea saponin). CK: control. Values are means ± standard deviation of triplicate measurements.

## RESULTS AND DISCUSSION

### Removal of OPs and heavy metals

All single peanut oil and TS solvents showed significant ( $P < 0.05$ ) removal efficiency for OPs. The removal efficiency of the treatments containing only peanut oil and TS for total OPs ranged from 27.5% to 45.5% and 15.2% to 30.7%, respectively, whereas that of the control was only 1.5% (Fig. 1). A similar trend was observed for PBDEs, PCBs, and PAHs. The removal efficiency for OPs increased with increased peanut oil concentration from 0.0 mL L<sup>-1</sup> to 5.0 mL L<sup>-1</sup>, after which the increase in removal efficiency slowed down (Fig. 1). Furthermore, peanut oil mixed with distilled water exhibited a two-phase partitioning system, in which the water-immiscible washing solvent (peanut oil) constituted the non-aqueous-phase liquid.<sup>22</sup> This strategy had three distinct advantages. First, despite being a less organic extraction solvent, peanut oil could constantly be in contact with the contaminated soil because the soil particles evenly dispersed in the *ex situ* soil washing system. Second, the adsorption of peanut oil in soil particles could be reduced because water exerted a barrier effect on the sorption.<sup>23</sup> Third, any peanut oil residue in soil could be rapidly biodegraded if optimal microbial conditions were maintained.<sup>12</sup> Similarly, the removal of OPs increased with increased concentration of TS to 10 g L<sup>-1</sup> (Fig. 1). These data were consistent with

previous findings that TS can facilitate the solubilization and desorption of nonpolar solutes in water, thereby increasing OP removal.<sup>11,13</sup>

Peanut oil and TS solution can also significantly remove heavy metals ( $P < 0.05$ ). The removal efficiencies of the treatments containing only peanut oil and TS for Pb ranged from 13.1% to 21.3% and 29.2% to 54.6%, respectively, whereas that of the control was only 3.5% (Fig. 1). The removal efficiencies of the treatments containing only peanut oil and TS for Ni ranged from 10.3% to 22.7% and 32.6% to 55.6%, respectively, whereas that of the control was only 3.1% (Fig. 1). These results agreed well with previous findings that peanut oil could complex heavy metals because of its high contents of oleic acid and linoleic acid.<sup>9,12</sup> Meanwhile, TS can bind cationic metals by complexation interactions between the metal ions and the acidic functional groups (e.g. carboxyl) associated with the external surfaces of structure, thereby further improving the possibility of metal chelation and increasing removal efficiency.<sup>13,15</sup>

The effect of peanut oil on the removal of OPs was greater than that of TS, whereas the effect of peanut oil on the removal of heavy metals was lower than that of TS. Obtaining high removal efficiency for simultaneously removing OPs and heavy metals from the soil is difficult when peanut oil or TS is used alone. Therefore,



a complete randomized block design was employed to investigate the combined effect of the two solvents on the removal efficiency of mixed pollutants. The combined treatments with peanut oil and TS resulted in removal efficiency of 52.7% to 73.1% for total OPs, 55.2% to 75.2% for PBDEs, 59.3% to 78.3% for PCBs, 51.6% to 72.2% for PAHs, 45.4% to 76.1% for Pb, and 46.5% to 81.4% for Ni (Fig. 1). Furthermore, two-way ANOVA analysis (Supplementary Material 2) demonstrated that the interaction between peanut oil and TS was significant ( $P < 0.05$ ). The surfactant TS increases the solubility of OPs and heavy metals, consequently promoting the desorption of these pollutants from soil particles into the aqueous phase. The organic and inorganic pollutants then move from the aqueous phase into the more hydrophobic peanut oil, which serves as a sink for the solubilized contaminants.<sup>7</sup> This sink sets up a concentration gradient which results in the continuous release of more pollutants from the soil.<sup>22</sup> The concentrations of 5.0 and 10.0 mL L<sup>-1</sup> peanut oil with 5.0 and 10.0 g L<sup>-1</sup> TS showed the highest removal efficacy for mixed-contaminants. However, from the perspective of minimal solvent usage, the combination of 5.0 mL L<sup>-1</sup> peanut oil and 5.0 g L<sup>-1</sup> TS were used in the development of a clean-up procedure.

### Successive soil washing

Preliminary tests indicated that a single washing cycle was not sufficient to extract most of the mixed pollutants from soil with 5.0 mL L<sup>-1</sup> peanut oil and 5.0 g L<sup>-1</sup> TS. The residual total OPs, PBDEs, PCBs, PAHs, Pb and Ni in first washed soil were still  $3.72 \pm 1.2$ ,  $8.6 \pm 1.1$ ,  $50.2 \pm 6.1$ ,  $101.5 \pm 9.4$  and  $55.2 \pm 2.1$  mg kg<sup>-1</sup>, respectively (Fig. 2). Therefore, three successive washing cycles were utilized, and the cumulative removal of OPs and heavy metals increased with increased washing cycles (Table 1). After three successive washing cycles, the removal efficiencies for total OPs, PBDEs, PCBs, PAHs, Pb, and Ni were 98.6%, 99.2%, 98.3%, 98.8%, 91.4%, and 93.1%, respectively. But the total OPs, PBDEs, PCBs, PAHs, Pb, and Ni desorbed fast in the first two washing cycles, whereas washing beyond two cycles exhibited limited contribution to contaminant desorption. The results obtained were consistent with the hypothesis that *ex situ* soil washing leads to a balanced redistribution of contaminants between the solid and liquid phases, and that a new distribution equilibrium of contaminants is established once a steady state for the transfer of contaminants is achieved in each washing cycle.<sup>7</sup> Therefore, two washing cycles were considered optimum for the soil clean-up strategy.

### Assessment of soil microcosms

Apart from removing pollutants, restoring the ecological function of washed soil is also important. The residual OPs and heavy metals in the second washed soil can still negatively affect soil microbial functional diversity, thereby influencing soil fertility and plant growth.<sup>22</sup> Therefore, cultivation of vetiver grass and addition of nutrients for 4 months were conducted to rehabilitate the ecological functions of washed soil. After 4 months cultivation, vetiver grass survived and grew well in the second washed soil, and the average biomass of vetiver grass was  $337.6 \pm 36.8$  g kg<sup>-1</sup> dry soil (Table 2). Although the removal efficiencies of total OPs, PBDEs, PCBs and PAHs by vetiver cultivation were approximately 38.2%, 45.1%, 36.2%, and 40.2%, respectively, the percentages of total OPs, PCBs, and PAHs phytoextracted by vetiver grass were only about 0.6%, 0.3% and 0.6%, respectively (Table 2). No significant concentration of PBDEs was detected in vetiver biomass

**Table 1.** Removal efficiency of pollutants after successive washing cycles with 5.0 mL L<sup>-1</sup> peanut oil and 5 g L<sup>-1</sup> tea saponin

	Washing cycle		
	1st Removal (%)	2nd Removal (%)	3rd Removal (%)
Total OPs	68.5 ± 2.1 b	95.3 ± 4.3 a	98.6 ± 4.5 a
PBDEs	71.2 ± 2.8 b	96.4 ± 2.1 a	99.2 ± 5.1 a
PCBs	74.2 ± 3.2 b	97.1 ± 3.6 a	98.3 ± 4.0 a
PAHs	67.1 ± 2.5 b	95.1 ± 4.2 a	98.8 ± 5.3 a
Pb	71.7 ± 3.2 b	83.5 ± 4.7 a	91.4 ± 5.1 a
Ni	74.7 ± 2.3 b	87.1 ± 3.9 a	93.1 ± 2.4 a

Values are means ± SD of triplicate measurements, mean values with the same letter are not significantly different among treatments by LSD at the 5% level.

( $P > 0.05$ ). These results were consistent with previous findings. Sun *et al.*<sup>5</sup> observed that PAH uptake in alfalfa and tall fescue accounts for a small portion of PAH biodegradation from soil. Although phytoextraction has a limited function in organic pollutant biodegradation, phytoremediation can still facilitate organic pollutant biodegradation by enhancing the activity of rhizosphere microorganisms.<sup>22</sup> The microbial consortia in the rhizosphere effectively enhance the breakdown of organic pollutants. In the present study, Pb and Ni removal efficiencies by vetiver cultivation were approximately 4.3% and 2.1%, respectively, and the concentrations of Pb and Ni detected in vetiver biomass were  $2.6 \pm 0.3$  and  $0.6 \pm 0.1$  mg kg<sup>-1</sup>, respectively. This finding was consistent with previous reports. Meanwhile, during vetiver grass cultivation, a fair amount of organic substances (such as polysaccharide, protein, lipid, and enzyme) was excreted.<sup>16,17</sup> These substances facilitated the phytoextraction of heavy metals, but the amount of heavy metals that accumulated in vetiver tissue was lower than in previous findings. Chen *et al.*<sup>18</sup> reported that up to 2280 mg kg<sup>-1</sup> of Pb can accumulate in vetiver tissue. A possible reason for the discrepancy was the low level of bioaccessibility of residual heavy metals or the low residual concentration of heavy metals in the second washed soil that led to limited phytoextraction.<sup>7</sup> In China, the standard for total PCBs, total PAHs, Pb and Ni is 0.015 mg kg<sup>-1</sup>, 0.148 mg kg<sup>-1</sup>, 80 mg kg<sup>-1</sup> and 90 mg kg<sup>-1</sup> according to the Environmental quality standards for soil (GB15618-2008).<sup>24</sup> But there is no standard for PBDEs. In this study, although residual concentrations of total PCBs and PAHs were still higher than the standard, acquired data demonstrated that it was extremely difficult for the residual organic pollutants to redesorb from soil particles, suggesting a significant reduction of transfer risk. Meanwhile, residual concentration of Pb and Ni has met the requirement of guideline.

In addition, soil microorganisms are sensitive to shifts in ecosystem functions because their activity is often easily altered by perturbation.<sup>8</sup> Microbiological parameters such as culturable microorganism count, microbial biomass, and functional diversity may serve as indices of the impact of pollution on soil health. Previous studies have proven that successive enhanced soil washing can result in great disturbance of soil microcosms.<sup>25</sup> The numbers of bacteria, actinomycetes, and fungi, as well as the values of microbial biomass C and microbial biomass N after successive soil washing, were significantly lower than those of the control (CK) ( $P < 0.05$ , Table 3). However, these parameters significantly increased after 4 months of vetiver grass cultivation and nutrient

**Table 2.** Percentage mass removal and phytoremediation of pollutants by 4 month vetiver cultivation and nutrient addition

	Pollutants content in initial soil (mg kg <sup>-1</sup> )	Pollutants content in the 2nd washed soil (mg kg <sup>-1</sup> )	Pollutants content in final soil (mg kg <sup>-1</sup> )	Pollutants removal efficiency by vetiver cultivation (%) <sup>a</sup>	Pollutants content in vetiver biomass (mg kg <sup>-1</sup> ) <sup>b</sup>	Percentage of pollutants phytoextracted (%) <sup>c</sup>
Total OPs	198.3 ± 18.1	9.3 ± 0.4	5.7 ± 0.4	38.2 ± 2.4	(1.9 ± 0.1) × 10 <sup>-2</sup>	0.6 ± 0.2
PBDEs	12.9 ± 0.6	0.5 ± 0.1	0.3 ± 0.1	45.1 ± 1.5	None <sup>d</sup>	None <sup>d</sup>
PCBs	33.3 ± 1.9	0.9 ± 0.1	0.6 ± 0.2	36.2 ± 3.1	(0.1 ± 0.1) × 10 <sup>-2</sup>	0.3 ± 0.1
PAHs	152.1 ± 15.5	7.5 ± 0.3	4.7 ± 0.1	40.2 ± 1.6	(1.8 ± 0.1) × 10 <sup>-2</sup>	0.6 ± 0.1
Pb	350.7 ± 7.3	57.9 ± 2.7	52.4 ± 1.3	4.3 ± 0.2	2.6 ± 0.3	4.1 ± 0.1
Ni	221.4 ± 4.2	28.6 ± 1.1	25.4 ± 0.6	2.1 ± 0.3	0.6 ± 0.1	2.0 ± 0.2

Values are means ± standard deviation of triplicate measurements.

<sup>a</sup> Ratio of pollutants removal content by vetiver cultivation to pollutants content in the second washed soil.

<sup>b</sup> The average biomass of vetiver grass are 417.5 ± 24.5 and 337.6 ± 36.8 g kg<sup>-1</sup> dry soil in the uncontaminated soil and second washed soil, respectively.

<sup>c</sup> Ratio of pollutants content in vetiver biomass to pollutants removal content by vetiver cultivation.

<sup>d</sup> No significant data have been detected or calculated ( $P < 0.05$ ).

**Table 3.** Enumeration of bacteria, actinomycetes, fungi and changes of microbiomass carbon (MBC), microbiomass nitrogen (MBN), Shannon–Weaver Index, Simpson Index in different treatments

	Initial soil	2nd washed soil	Vetiver grass cultivation*
Bacteria (log CFU g <sup>-1</sup> dry soil)	6.1 ± 0.2 b	3.4 ± 0.2 c	8.2 ± 0.7 a
Actinomycetes (log CFU g <sup>-1</sup> dry soil)	3.5 ± 0.4 b	1.6 ± 0.1 c	5.7 ± 0.5 a
Fungi (log CFU g <sup>-1</sup> dry soil)	4.6 ± 0.3 b	2.2 ± 0.2 c	6.8 ± 0.3 a
MBC (µg C g <sup>-1</sup> dry soil)	296.2 ± 24.3 b	114.3 ± 8.6 c	375.7 ± 21.1 a
MBN (µg N g <sup>-1</sup> dry soil)	55.2 ± 3.6 b	23.4 ± 2.1 c	87.4 ± 6.3 a
Shannon–Weaver Index	4.6 ± 0.5 b	3.1 ± 0.3 c	6.5 ± 0.2 a
Simpson Index	3.2 ± 0.2 b	1.2 ± 0.3 c	5.4 ± 0.3 a

Values are means ± standard deviation of triplicate measurements. Mean values with different letters are significantly different among treatments by LSD at the 5% level.

\*The second washed soil with 4 months cultivation of *Vetiveria zizanioides* (L.) Nash and nutrient addition.

addition ( $P < 0.05$ , Table 3). These results indicated that the cultivation of vetiver grass provided a more suitable microcosm for the survival and colonization of soil microorganisms.

The cultivation of vetiver grass increased the Shannon–Weaver and Simpson indices (Table 3). The Shannon–Weaver index provides information on the distribution of C source utilization by microbial communities and on the potential metabolic diversity of communities;<sup>7</sup> the Simpson index provides information on the number of species and on the relative abundance of each species.<sup>22</sup> Therefore, 4 months of vetiver grass cultivation and nutrient addition resulted in the partial restoration of the microbial and biological functions of washed soil.

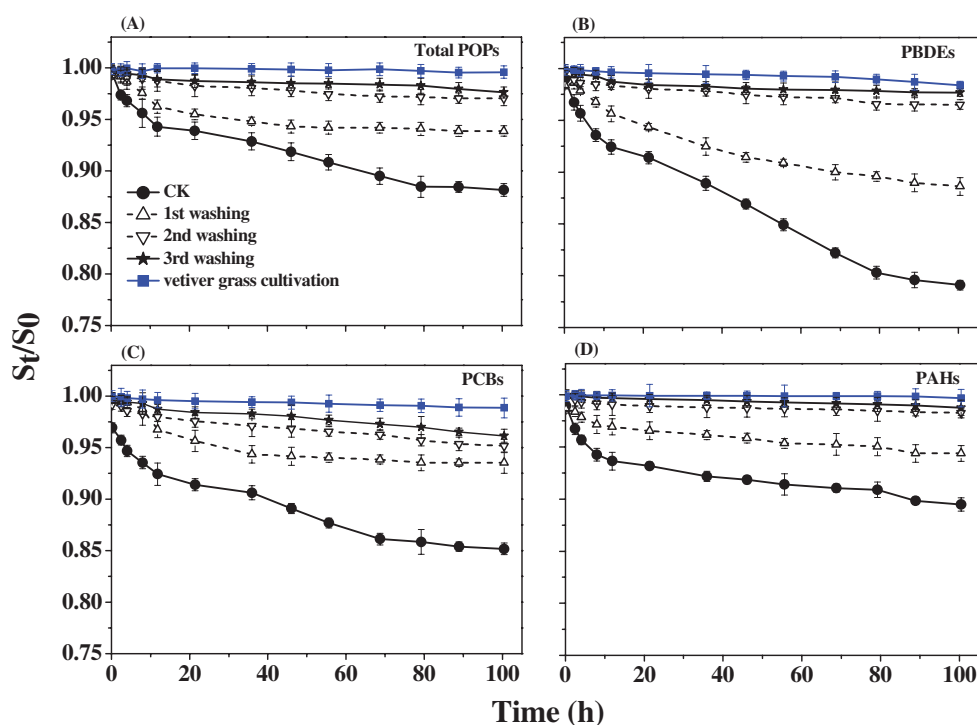
### Tenax extraction of OPs before and after treatments

Previous studies have mainly focused on pollutant removal efficiency when *ex situ* soil washing technology is used.<sup>9</sup> To date, little is known about the secondary environmental risk of residual OPs in washed soils. In the present study, OP desorption kinetics before and after different treatments were investigated by 100 h Tenax extraction (Fig. 3 and Table 4). The solid and dotted lines were obtained through curve fitting using Equation (1).

Approximately 11.9% of the total OPs desorbed within 100 h of Tenax extraction in CK (Fig. 3(A)). With increased number of washing cycles from 1 to 2, the desorption of total OPs decreased from 6.2% to 2.9%. However, no significant reduction occurred between the second and third washings ( $P > 0.05$ ). Similar phenomena were

observed for PBDEs, PCBs, and PAHs ((Fig. 3(B)–3(D)). Therefore, the secondary environmental risk of residual OPs in the washed soil decreased with increased number of washing cycles. In addition, the  $F_r$  of total OPs in Jiangnin soil was only 2% after the second washing (Table 4).  $F_r$  can be deemed as the dissolved plus surface-sorbed compound, which can be easily extracted by soil-washing solvents.<sup>14</sup> Similarly, the  $F_{s,i}$  of total OPs decreased to approximately 1% compared with the 4% in CK. Although  $F_{s,i}$  may be more strongly bound with soil particles, it is still reversible and diffusionally retarded from release from internal soil locations.<sup>19</sup> In contrast, the  $F_{v,i}$  of total OPs increased to more than 97%. The  $k_r$ ,  $k_{s,i}$ , and  $k_{v,i}$  of total OPs significantly decreased with increased washing times ( $P < 0.05$ ; Table 4). After the second washing, the  $k_{s,i}$  and  $k_{v,i}$  of total OPs were only  $5.5 \times 10^{-6} \text{ h}^{-1}$  and  $4.6 \times 10^{-6} \text{ h}^{-1}$ , respectively. These results suggested that the residual OPs in the soil after the second washing mainly existed in the form of  $F_{v,i}$ , which probably included the well-aged, well-sequestered OPs, whose releases were thermodynamically and kinetically constrained.<sup>7</sup>

For vetiver grass cultivation treatment, 100 h of Tenax extraction was also performed to evaluate the environmental risk of residual OPs in soil. The  $F_r$  of total OPs decreased to approximately 0%, whereas the  $F_{v,i}$  of total OPs increased to approximately 99%. The  $k_{s,i}$  and  $k_{v,i}$  of total OPs decreased to  $2.8 \times 10^{-6} \text{ h}^{-1}$  and  $2.2 \times 10^{-6} \text{ h}^{-1}$ , respectively. Such changes in desorption fractions and rates can be attributed to the root exudates from vetiver grass



**Figure 3.** Desorption kinetics for total POPs (A), PBDEs (B), PCBs (C) and PAHs (D) after different treatments.  $S_t/S_0$  was the residual fraction of POPs in soil at time  $t$  (h). Vetiver cultivation: the soil after the second washing with 4 months cultivation of *Vetiveria zizanioides* (L.) Nash and nutrient addition. Values are means  $\pm$  standard deviation of triplicate measurements.

and other byproducts during indigenous microbial metabolite processes.<sup>16,18</sup> Furthermore, these data indicated that the desorption of residual OPs from the micropore structures of soil particles was difficult without strong physicochemical or biological disturbance, resulting in low transfer risk for residual OPs in remediated soil.<sup>26</sup>

### Heavy-metal fractions in soil before and after decontamination

The chemical forms of heavy metals in soils can greatly influence their fates in terms of leaching and subsequent environmental transport risk.<sup>27</sup> The sequential extraction procedure is used to evaluate the redistribution of heavy metals in soils. Pb in soils before successive washing cycles was primarily found in the residual and Fe–Mn oxide fractions, followed by carbonate, exchangeable, and organic matter fractions, which were 31.2%, 28.5%, 24.6%, 9.5%, and 6.2%, respectively (Fig. 4(A)). However, the redistribution of Pb dramatically changed after successive washing cycles. The organic matter and exchangeable fractions of Pb were almost removed from soils after the second washing; therefore, the great extractability of Pb by 5.0 mL L<sup>-1</sup> peanut oil and 5.0 g L<sup>-1</sup> TS can be explained by the presence of large, weakly bound fractions. In addition, Pb mainly accumulated in the residual, Fe–Mn oxide, and carbonate fractions. This result indicated that the transport risk of Pb in the washed soil significantly decreased with increased number of washing cycles ( $P < 0.05$ ).

A similar phenomenon was observed for Ni (Fig. 4(B)). The residual, Fe–Mn oxide, and carbonate fractions accounted for 59.1%, 20.5% and 14.1% of the total amount in the second washed soil. These results were expected because these fractions were usually determined by extraction with different strong acids.<sup>20</sup> Hence, successive washing cycles can reduce the mobility of heavy

metals by removing the labile fractions while further removing some strongly bound fractions. The remaining fractions of heavy metals were predominantly present in the (chemically and biologically) least accessible residual soil fractions.<sup>11</sup>

For vetiver grass cultivation treatment, the contributions of different fractions for Pb and Ni had no significant changes ( $P > 0.05$ , Fig. 4(A) and 4(B)) compared with those in the second washed soil. Thus, the residual heavy metals after two cycles of washing were probably tightly bound with the organic and inorganic components of the soil, resulting in extremely low levels of bioaccessibility.<sup>13</sup> In spite of vetiver grass cultivation for 4 months, the redistribution of heavy metals in the second washed soil through phytoremediation was still difficult. Referring to a previous study, if the grass was established longer (i.e. 6 months to 2 years), fair amounts of organic acids could be excreted, which facilitated the increase in contributions of exchangeable heavy metals.<sup>17,18</sup> This phenomenon could apparently enhance the environmental risk of residual heavy metals. However, increased contributions of exchangeable heavy metals could further advance the removal of residual heavy metal by phytoextraction and accumulation in the vetiver grass reversely. Therefore, we conservatively concluded that the environmental transfer risk of Pb and Ni in the second washed soil after 4 months of vetiver cultivation was limited.

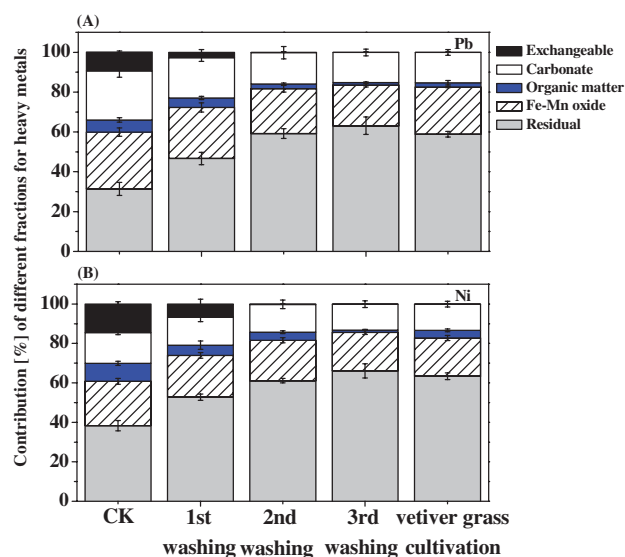
### CONCLUSIONS

Two successive washings with 5.0 mL L<sup>-1</sup> peanut oil and 5.0 g L<sup>-1</sup> TS were effective in extracting OPs and heavy metals from E-waste contaminated soil. Subsequent cultivation of vetiver grass and addition of nutrients for 4 months further degraded residual OPs, phytoextracted residual Pb, and Ni, and also restored the microbiological functions of soil. The secondary environmental

**Table 4.** Fractions and rate constants for the rapid, slow and very slow desorption of OPs from Jiangnin soil after different treatments predicted by consecutive Tenax extraction

		CK	1st washing	2nd washing	3rd washing	Vetiver cultivation*
Total OPs	$F_r$	0.08	0.05	0.02	0.01	0.00
	$F_{sl}$	0.04	0.02	0.01	0.01	0.01
	$F_{vl}$	0.88	0.93	0.97	0.98	0.99
	$k_r$ ( $h^{-1}$ )	0.4	0.2	$3.6 \times 10^{-2}$	$4.1 \times 10^{-2}$	$1.4 \times 10^{-3}$
	$k_{sl}$ ( $h^{-1}$ )	$3.7 \times 10^{-2}$	$3.1 \times 10^{-4}$	$5.5 \times 10^{-6}$	$2.1 \times 10^{-6}$	$2.8 \times 10^{-6}$
	$k_{vl}$ ( $h^{-1}$ )	$4.6 \times 10^{-4}$	$2.2 \times 10^{-5}$	$4.6 \times 10^{-6}$	$1.5 \times 10^{-6}$	$2.2 \times 10^{-6}$
PBDEs	$F_r$	0.15	0.09	0.02	0.01	0.00
	$F_{sl}$	0.06	0.03	0.02	0.02	0.01
	$F_{vl}$	0.79	0.88	0.96	0.97	0.99
	$k_r$ ( $h^{-1}$ )	0.5	0.3	$4.2 \times 10^{-2}$	$3.2 \times 10^{-2}$	$2.5 \times 10^{-3}$
	$k_{sl}$ ( $h^{-1}$ )	$4.3 \times 10^{-2}$	$3.7 \times 10^{-4}$	$2.5 \times 10^{-6}$	$1.7 \times 10^{-6}$	$1.4 \times 10^{-6}$
	$k_{vl}$ ( $h^{-1}$ )	$5.7 \times 10^{-4}$	$2.7 \times 10^{-5}$	$3.9 \times 10^{-6}$	$2.2 \times 10^{-6}$	$2.8 \times 10^{-6}$
PCBs	$F_r$	0.10	0.04	0.02	0.01	0.00
	$F_{sl}$	0.05	0.03	0.03	0.02	0.01
	$F_{vl}$	0.85	0.93	0.95	0.97	0.99
	$k_r$ ( $h^{-1}$ )	0.4	0.2	$5.5 \times 10^{-2}$	$3.6 \times 10^{-2}$	$4.2 \times 10^{-3}$
	$k_{sl}$ ( $h^{-1}$ )	$5.2 \times 10^{-2}$	$2.8 \times 10^{-4}$	$4.3 \times 10^{-6}$	$3.3 \times 10^{-6}$	$2.7 \times 10^{-6}$
	$k_{vl}$ ( $h^{-1}$ )	$3.8 \times 10^{-4}$	$3.5 \times 10^{-5}$	$4.7 \times 10^{-6}$	$2.7 \times 10^{-6}$	$2.3 \times 10^{-6}$
PAHs	$F_r$	0.07	0.04	0.01	0.01	0.00
	$F_{sl}$	0.04	0.03	0.01	0.01	0.01
	$F_{vl}$	0.89	0.97	0.98	0.98	0.99
	$k_r$ ( $h^{-1}$ )	0.3	$6.8 \times 10^{-2}$	$3.5 \times 10^{-2}$	$2.4 \times 10^{-2}$	$7.8 \times 10^{-3}$
	$k_{sl}$ ( $h^{-1}$ )	$1.7 \times 10^{-2}$	$4.5 \times 10^{-4}$	$5.8 \times 10^{-6}$	$3.4 \times 10^{-6}$	$3.1 \times 10^{-6}$
	$k_{vl}$ ( $h^{-1}$ )	$4.8 \times 10^{-5}$	$2.6 \times 10^{-5}$	$4.2 \times 10^{-6}$	$2.7 \times 10^{-6}$	$3.3 \times 10^{-6}$

$F_r$ ,  $F_{sl}$  and  $F_{vl}$  are the rapid, slow and very slow fractions, respectively.  $k_r$ ,  $k_{sl}$  and  $k_{vl}$  ( $h^{-1}$ ) are the first-order rate constants for rapid, slow and very slow desorption, respectively. \*The second washed soil with 4 months cultivation of *Vetiveria zizanioides* (L.) Nash and nutrient addition.



**Figure 4.** Contribution [%] of different fractions for Pb (A) and Ni (B) after successive washing cycles with  $5.0 \text{ mL L}^{-1}$  peanut oil and  $2.5 \text{ g L}^{-1}$  tea saponin. Values are means  $\pm$  standard deviation of triplicate measurements.

risk of mixed contaminants in the remediated soil was limited, as evaluated by Tenax extraction and sequential extraction. The results of this study demonstrated that the combined cleanup strategy was an environmentally friendly technology important

for risk assessment and management in mixed-contaminant sites. Meanwhile, the reuse of peanut oil and TS is very important to reduce the remediation cost, and the work of selecting proper sorbing substance to recover surfactants and vegetable oil is under way.

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## Supporting Information

Supporting information may be found in the online version of this article.

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