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Effects of EDDS and plant-growth-promoting bacteria on plant uptake of trace metals and PCBs from e-waste–contaminated soil

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HIGHLIGHTS

- Application of EDDS increased soil DOC and facilitated the dissolution of soil PCBs.
- EDDS could solubilize metals and led to the enhanced metal uptake.
- PGPB improved plant growth and biomass resulting in a higher PCB accumulation.
- PGPB was superior to chelant EDDS in enhancing PCBs removal by plants.

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ABSTRACT

The present study investigated the effects of the biodegradable chelant *S,S*-ethylenediaminedisuccinic acid (EDDS) and the plant-growth-promoting bacterium DGS6 on pollutant uptake by corn from e-waste–contaminated soils. The highest concentration and total uptake of Cu and Zn in corn shoots were observed in the presence of EDDS and DGS6 + EDDS, respectively. The ΣPCB concentrations in shoots ranged from 0.53 to 0.72 ng g^{-1}, and the highest PCB concentration was observed in the presence of EDDS. This could be ascribed to the enhanced dissolved organic carbon, increased dissolution and efficient translocation of PCBs from roots to shoots, as well as potential root damage due to increased soluble metal levels in soil solution. In contrast, the highest total uptake of PCBs in shoots was observed in the presence of DGS6, likely due to enhanced shoot biomass and high levels of air deposition.

1. Introduction

In the past, uncontrolled e-waste recycling activities have resulted in serious environmental pollution in China. Abundant toxins, such as trace metals, polybrominated diphenyl ethers (PBDEs), and PCBs have been released into the environment due to use of primitive e-waste recycling processes [1–8]. High levels of persistent organic pollutants (POPs) and trace metals have been detected in the air, dust, soil, sediment, and biota samples around e-waste recycling sites, which may pose a threat to local residents and the ecosystem [9–12].

Soil pollution is an important reservoir of contaminants. First, pollutants in soils can be adsorbed by plant roots and translocated to the shoots, posing a serious risk to food safety and human health. Second, soil can become a secondary source of organic pollutants, which could be released into the atmosphere through evaporation, increasing air pollution. Extremely high levels of trace metals and POPs have been measured in vegetables and crops in the vicinity of e-waste recycling sites. Although more recent national and provincial laws have banned crude e-waste recycling methods, the decontamination of previously contaminated sites should be a priority [13,14].

Traditional chemically and physically based soil decontamination technologies, such as soil washing, are usually confined to small-scale and heavily polluted sites due to the high cost and potential damage to the soil property. Phytoremediation is

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an appealing alternative because it is environmentally friendly and cost effective, and is more applicable for the decontamination of large areas of light or medium pollution levels. Using chelants to reduce pollution levels has great potential in phytoremediation [15–19]. *S*,*S*-ethylenediaminedisuccinic acid (EDDS) is replacing the traditional metal-extracting chelant ethylenediamine tetraacetic acid (EDTA) due to its biodegradable characteristics, and its relatively negligible effect on the microbial communities [20]. Thus, it is considered to be a promising chelant for extraction of metals, such as Cu and Zn [17–19,21]. Besides metals, EDDS has also been used to evaluate the simultaneous removal of polycyclic aromatic hydrocarbons (PAHs) and metals from soils. EDDS enhanced the extraction of PAHs and increased Cu removal significantly [22,23]. Nevertheless, no study has explored the effects of EDDS on plant uptake of the more recalcitrant POPs, such as PCBs and PBDEs.

Rhizospheric remediation is an attractive approach to removal of organic pollutants from soils. This approach has many advantages over traditional bioremediation techniques for plant roots, and could supply organic carbon to the rhizosphere and sustain the growth of microorganisms, resulting in highly efficient and sustainable bioremediation [24,25]. In addition, this method is not lethal to the inoculated microorganisms, such as pollutant-degrading and plant-growth-promoting bacteria (PGPB), due to the support of plant roots and rich carbon sources. It was reported that PGPB improves plant growth in contaminated soils by hydrolyzing 1-aminocyclopropane-1-carboxylate (ACC) and enhancing the production of plant growth regulators and hormones [25–27]. Inoculation with PGPB could improve the accumulation of metals in plant tissues [28,29], allowing the organic pollutant to be removed through biodegradation or plant uptake [30,31].

The present study investigated the effects of the biodegradable chelant EDDS and PGPB on metals and POPs in e-waste–contaminated soils. This study increases our understanding of phytoremediation in metal-POP co-contaminated soils, and could reveal the mechanism of metal and POP uptake by plants.

2. Materials and methods

2.1. Pot experiment

Contaminated soil (0–10 cm) was collected from an e-waste recycling site in Longtang Town of north Guangdong Province, China. Specific characteristics of the soil are provided in Table 1. After air drying, homogenization, and passage through a 2 mm sieve, soil was packed into 16 ceramic pots (700 g per pot) and the soil moisture was maintained at ~60% of the maximum water-holding capacity. Seeds of corn (*Zea mays* L. cv. Nongda 108) were grown in the pots after sterilization with 5% sodium hypochlorite solution for 5 min, washed three times with tap water, and rinsed with deionized water (DIW). The pot experiment was performed in a glass room at 22–28 °C, and DIW was added daily to maintain soil moisture of ~60% of the maximum water-holding capacity.

After growing for 1 week, plants were thinned to five per pot. Bacterial suspensions of PGPB which was identified as *Pseudomonas* sp. DGS6 isolated from the Cu-mining site at Jiuhuashan (N 32°04′40.16″, E 119°05′15.05″) [29] were sprayed onto the soil of half of the pots (8 pots), resulting in an inoculum density of $3.2\ 10^6$ CFU/g soil. For the remaining eight pots, the same dose of DIW was used for the spray irrigation. All plants were allowed to grow for a further 21 days. EDDS was applied to four pots with DGS6 and four pots with no DGS6 inoculation at 3 mmol kg^{-1} in the form of Na_3EDDS salt from Fluka Chemie GmbH, and the remaining pots were irrigated with an identical volume of DIW. Hence, four treatments with four replicates each (four pots per treatment) were included in the experiment; control (corn without any treatment), DGS6 strain inoculation, EDDS treatment, and DGS6 strain inoculation + EDDS treatment. A total of 10 days after EDDS application, the plants were harvested. Shoots and roots were separated, washed with tap water, rinsed with DIW, and freeze-dried for 48 h. Biomass was measured and the plant tissues ground to a fine powder for the following analysis.

2.2. Air sampling

A passive air sampler (PAS) was used to measure PCB deposition from the air using polyurethane foam (PUF, 14-cm diameter, 1.2-cm thickness, 0.035 g m^{-3}) disks. Two samplers were placed 1.5 m above the pots in the greenhouse, and another two samplers were placed 400 m from the greenhouse. The average sampling rate during PAS deployment was 3.5 m^3 days^{-1} measured by an active air sampler. At the end of cultivation, PUFs were collected and wrapped in solvent-rinsed aluminum foil envelopes, which were then placed in polyethylene zip bags and stored in the freezer before extraction.

2.3. Soil extraction

For the soil metal dissolution experiment, 5 g of soil (based on dry weight) was placed in a 50 mL polypropylene centrifuge tube. Approximately 3 mL of 5 mM EDDS was added to the soil sample, which corresponded to the total amount of chelant (3 mmol kg^{-1} soil) in the pot experiment. After 2 days, DIW was added to the soil (at a soil-to-water ratio of 1:5), and the suspension was shaken for 30 min. After centrifugation, the supernatant was filtered through 0.45-μm paper filter (Whatman Maidstone, UK). The total dissolved organic carbon (DOC) concentration was measured using a TOC analyzer (TOC-5000A, Shimadzu, Japan). The remaining supernatant was acidified with HNO_3 and analyzed in terms of the concentrations of various metals using ICP-AES [17].

2.4. Chemical analysis

2.4.1. Trace metal analysis

Both soil and plant samples were digested in a mixture of HNO_3 and $HClO_4$ (4:1 by volume), and the major and trace elements were assayed by ICP-AES. Certified standard reference materials (SRM 1515 for plants and SRM 2709 for soils) of the National Institute of Standards and Technology, USA, were used in the digestion and analysis as part of the QA/QC protocol. Reagent blank and analytical duplicates were also used where appropriate to ensure the accuracy and precision of the analysis. The recoveries of all metals in the reference materials were ~94 ± 5%.

2.4.2. PCB analysis

Approximately 5 g of soil samples or PUF disks, spiked with relevant recovery standards (2,4,5,6-tetrachloro-*m*-xylene (TCMX), PCB 30, PCB 198, and PCB 209), were extracted for 48 h with dichloromethane (DCM). Approximately 5 g of plant samples were homogenized with 5 g of anhydrous sodium sulphate, spiked with surrogate standards, and extracted with 100-mL hexane/acetone (3:1, v/v) for 72 h. The fractionated soil extracts, PUF, or plants were concentrated to ~0.5 mL after solvent-exchange to hexane. The extracts of soil or PUF were directly cleaned-up by passing through a multi-layer column, which contained anhydrous Na_2SO_4, 50% (w/w) sulfuric acid-silica gel, neutral silica gel (3% deactivated), and neutral alumina (3% deactivated) from top to bottom with an eluent of 20 mL hexane/DCM (1:1, v/v). The plant extracts were washed with sulfuric acid and purified on the multi-layer column. After being evaporated to ~50 μL, ^{13}C-PCB 141 was added as an internal standard before analysis.

Table 1
Soil physical–chemical properties.

Texture (%)			pH	OM	Metals (mg kg^{-1})		PCBs (ng g^{-1})
Sand	Silt	Clay			Cu	Zn	
56 ± 3.4	31 ± 1.5	13 ± 1.0	6.8 ± 0.3	1.8 ± 0.1	840 ± 9	235 ± 7	49 ± 1.2

Samples were analyzed on a GC-EI-MS (Agilent GC7890) coupled with a 5975C mass-spectrometer detector using a Varian capillary column (50 m × 0.25-mm id × 0.25-μm film thickness), and PCB congener analytical details were as described previously [7].

A procedural blank, a spiked blank consisting of all chemicals, and a duplicated sample were run with each batch of 10 samples to assess potential sample contamination and the repeatability of the analysis. No target compounds were detected in laboratory blanks. The surrogate recoveries for TCMX, PCB 30, PCB 198, and PCB 209 in all samples were 64 ± 11%, 59 ± 19%, 80 ± 14%, and 78 ± 14%, respectively. The results of this study were corrected based on the surrogate recovery rates.

2.5. Statistical analysis

All statistical calculations, such as correlations and significant differences, were performed using SPSS ver. 17.0. The statistical significance of differences and variance ($p < 0.05$) of pollutant accumulation in plants among treatments were determined by one-way ANOVA (LSD).

3. Results

3.1. Soil characteristics

The physical–chemical properties of soil are shown in Table 1. The concentrations of Cu and Zn were 840 and 235 mg kg^{-1}, respectively, and the concentration of PCBs reached 49 ng g^{-1}. The effects of EDDS on soluble metals and DOC in soils are shown in Table 2. The extractable Cu and Zn levels in EDDS-treated soils reached 107 and 24 mg kg^{-1}, respectively, which were 59- and 27-fold higher than the control (soil with no EDDS application). The concentration of DOC in the EDDS-treated soil reached 125 mg L^{-1}, which was 5.6-fold higher than the control soil.

3.2. Plant growth

The biomass of plant roots and shoots is shown in Fig. 1. The highest biomass was observed in DGS6 inoculated plants, at 13.6 and 4.2 g pot^{-1} in shoots and roots, respectively. In comparison with the control, the biomass increased by 98% in shoots and 22% in roots. EDDS did not affect shoot biomass. However, it reduced root biomass significantly, by 43% compared with control samples. Regarding the DGS6 + EDDS treatment, inoculation with the DGS6 strain significantly improved plant growth compared with the EDDS treatment alone; the increase in the shoots, but not the roots, was significant (Fig. 1).

Table 2
Effects of EDDS on soluble metals and dissolved organic carbon.

	Extractable metals (mg kg^{-1})		DOC (mg L^{-1})
	Cu	Zn	
Control	1.8 ± 0.2 a	0.9 ± 0.1 a	22.3 ± 1.7 a
EDDS	107 ± 9 b	24 ± 2 b	125 ± 11 b

3.3. Plant uptake of trace metals

The concentrations and total uptake of Cu and Zn are shown in Fig. 2. The addition of EDDS resulted in the highest Cu concentration in shoots, which was 4.9-, 7.5-, and 1.4-fold higher than in the control, DGS6 inoculation, and DGS6 + EDDS treatment groups, respectively. The total uptake of Cu in shoots showed a trend similar to the Cu concentration, with the EDDS treatment being highest, followed by the DGS6 + EDDS treatment. The total uptake of Cu by roots was similar; the highest values were observed in the DGS6 + EDDS treatment, followed by EDDS application and DGS6 inoculation.

The highest Zn concentrations in both shoots and roots were observed in the DGS6 + EDDS treatment, being 1.5- and 1.7-fold higher than the control, respectively, followed by the EDDS treatment. The lowest values were observed after DGS6 inoculation, which can be attributed to the larger biomass caused by the inoculation of DGS6 (Figs. 1 and 2). The highest total Zn uptake was observed in the DGS6 + EDDS treatment for both shoots and roots, being 1.8- and 1.7-fold higher than the control, respectively, followed by the DGS6 inoculation.

3.4. PCB accumulation in plants

PCB accumulation in corn is shown in Table 3. The homologues of PCBs analyzed included tri-PCBs (PCB 28 and 37), tetra-PCBs (PCB 44, 49, 52, 60, 66, 70, 74, 77), penta-PCBs (PCB 82, 87, 99, 101, 105, 114, 118, 126), hexa-PCBs (PCB 128, 138, 153, 156, 158, 166, 169), and hepta-PCBs (PCB 170, 179, 183, 180, 187, 189). As shown in Table 3, EDDS application resulted in the most significant increase in PCB concentration in shoots, followed by DGS6 + EDDS treatment. In the roots, the highest concentration was observed for DGS6 + EDDS treatment, followed by EDDS treatment. Regarding the total uptake of PCBs, DGS6 inoculation resulted in the highest

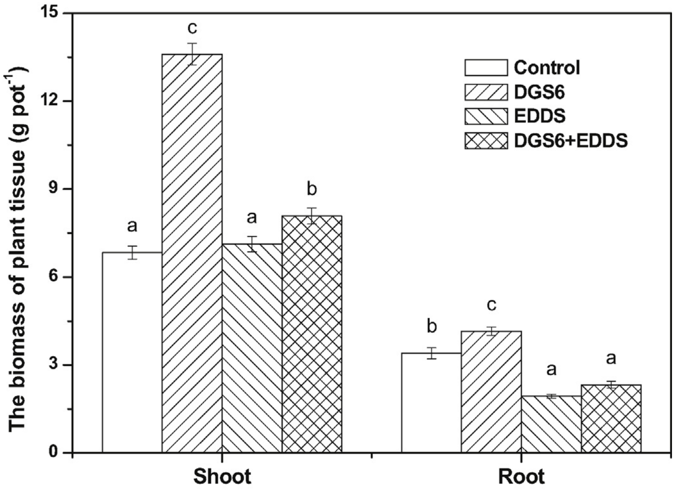

Fig. 1. The biomass of plant shoots and roots of different treatments (g pot^{-1}). Control, corn without any treatment; DGS6, inoculation with DGS6; EDDS, treatment with EDDS; DGS6 + EDDS, inoculation with DGS6, and followed by EDDS application. The small letters (a–c) stand for statistical significance at the 0.05 level with the LSD test.

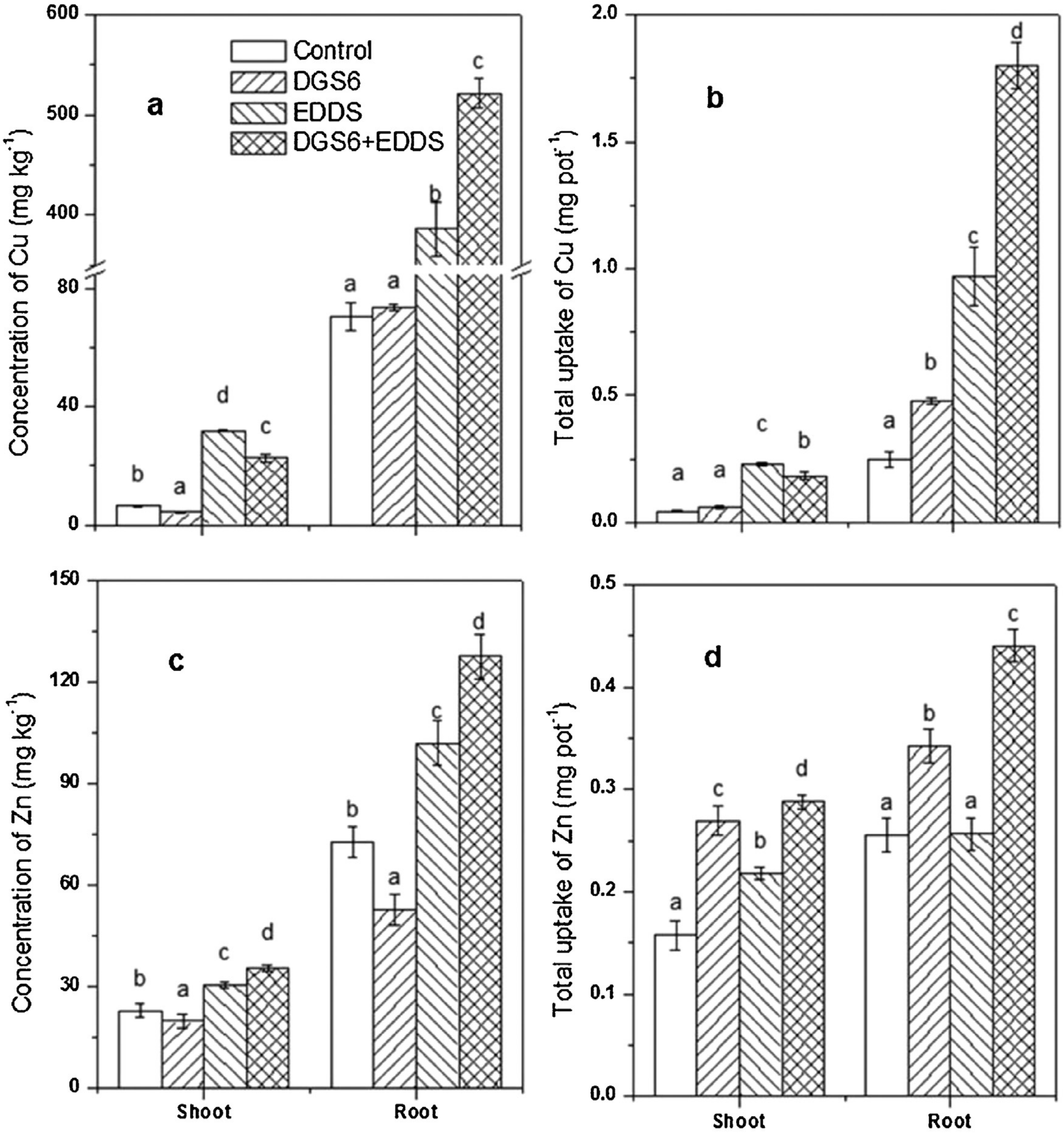

Fig. 2. The concentration (mg kg^{-1}) of Cu (a) and Zn (c), and total uptake (mg pot^{1}) of Cu (b) and Zn (d) in corn. The small letters (a–d) stand for statistical significance at the 0.05 level with the LSD test.

values in both shoots and roots, being 2.2-, 1.6-, and 1.5-fold, and 1.2-, 1.6-, and 1.3-fold higher than the control, EDDS addition, and DGS6 + EDDS treatment in shoots and roots, respectively.

The PCB compositions of soil, air, and corn tissue are plotted in Fig. 3. Tetra- and penta-PCBs were the dominant congeners in soil samples, accounting for 32% and 30%, respectively, of the total PCBs. In the air sample, tetra-PCBs were the dominant congeners, accounting for more than 58% of the total PCBs. The PCB composition in the roots was similar to that in soil, with penta- and tetra-PCBs accounting for 30% of the total. In the shoots, PCB compositions in plants inoculated with DGS6 were similar to that in the air. In contrast, PCB compositions

Table 3
Concentrations and total uptake of PCBs in plant tissues.

	Shoot		Root	
	Concentration (ng g^{-1} DW)	Total uptake (ng pot^{-1})	Concentration (ng g^{-1} DW)	Total uptake (ng pot^{-1})
Control	0.53 ± 0.01 a	3.65 ± 0.02 a	33 ± 1 a	111 ± 12 b
DGS6	0.60 ± 0.05 b	8.16 ± 0.38 d	33 ± 3 a	135 ± 9 c
EDDS	0.72 ± 0.04 d	5.14 ± 0.19 b	43 ± 4 b	83 ± 5 a
DGS6 + EDDS	0.66 ± 0.07 c	5.36 ± 0.37 c	44 ± 2 c	103 ± 10 b

The values are means ± SD (n = 3). The small letters (a–d) stand for statistical significance at the 0.05 level with the LSD test.

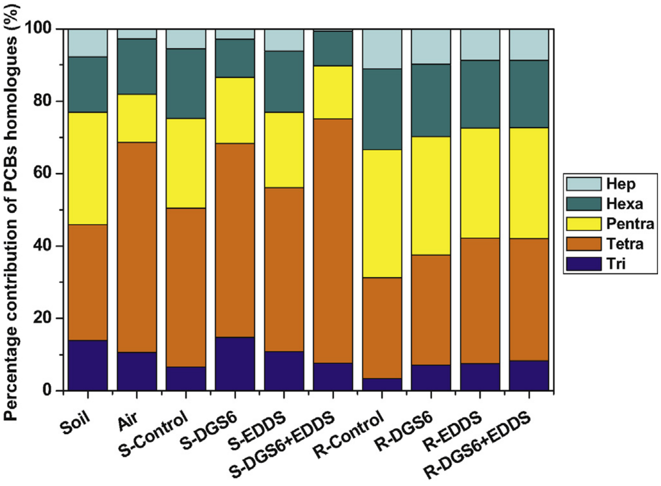

Fig. 3. Composition of PCB homologue groups. (S-Control: shoot of control; S-DGS6: shoot of treatment with DGS6; S-EDDS: shoot of treatment with EDDS; S-DGS6 + EDDS: shoot of treatment with EDDS and DGS6; R-Control: root of control; R-DGS6: root of treatment with DGS6; R-EDDS: root of treatment with EDDS; R-DGS6 + EDDS: root of treatment with EDDS and DGS6).

of control and EDDS-treated plants were similar to that of the soil.

4. Discussion

4.1. Effects of DGS6 and EDDS on corn growth

It has been found the DGS6 strain could enhance the availability and uptake of plant nutrients; produce substances promoting plant growth, such as indole-3-acetic acid (IAA); and improve plant stress tolerance by hydrolyzing ACC [29]. The survival of DGS6 can be seen from the obvious enhancement of biomass and nutrients in the inoculated plants (with 40% and 35% increase in the Fe and P contents of the shoots compared with the control, data not shown). This plant-growth-promoting ability was confirmed in the present study, as evidenced by the significantly enhanced biomass in DGS6 inoculated corn (Fig. 1). The application of EDDS did not affect shoot biomass. However, it significantly reduced the biomass of roots, which was consistent with the observation that roots were more sensitive to the toxicity of soil suspensions [32]. EDDS was slightly toxic to plant roots [33] and could solubilize soil metals, but its effects on the soil microbial growth and activity can be ignored [20]. As shown in Table 2, means of 59- and 27-fold increases in extractable Cu and Zn were achieved after the application of EDDS, and the solubilized metals exerted toxic effects on plant tissue [17,19,34,35].

4.2. Effects of DGS6 and EDDS on trace metal uptake

After solubilization of metals by EDDS, corn roots would likely undergo damage, which could lead to a breakdown of the root exclusion mechanisms and indiscriminate uptake of solutes by plants [17]. Therefore, the translocation of metals from roots to shoots was enhanced, as evidenced by identification of the highest translocation factor (shoot concentration/root concentration) in the EDDS treatment (about 0.08 for Cu and 0.29 for Zn). A higher phytoextraction efficiency was achieved when more metals were translocated to the shoots. This result is consistent with a previous report that EDDS was most effective in terms of solubilizing soil Cu for root uptake and translocation into aboveground biomass [17,19]. The high root concentration of metals (Cu or Zn) in the DGS6 + EDDS treatment group could be the result of: (1) the high biomass and the presence of dissolved metals in the soil solution, which would increase the root volume and allow roots to adsorb more metals; and (2) siderophores produced by the inoculant DGS6, which would solubilize metals and facilitate metal uptake by plant roots [29,36]. In the present study, the plant tissues were washed with only DIW before freeze drying instead of being soaked in solution (such as $CaCl_2$) to dissolve metals bound to the cell walls, which would contribute to the root uptake of metals. The fact that lower Cu concentration in shoots in the presence of DGS6 + EDDS compared with EDDS alone was in agreement with the improved plant growth (greater biomass) by the inoculation of DGS6 (see Fig. 1).

Compared with Cu, the increase in the concentration and uptake of Zn in the presence of EDDS was much lower (Fig. 3). This may be attributed to the lower concentration of Zn than Cu in the soil (Tables 1 and 2). Furthermore, the chemical affinity of EDDS for Cu ($\log Ks = 18.4$) was greater than that for Zn ($\log Ks = 13.4$), and EDDS was the most efficient solubilizer of Cu among several chelants, such as trans-1,2-cyclohexanediaminetetraacetic acid, ethylene bis tetraacetic acid, and EDTA [8,17,19].

4.3. Effects of DGS6 and EDDS on PCB accumulation by plants

Two primary pathways for POPs entering vegetation have been proposed: the first is uptake via roots, followed by translocation to shoots along with transpiration; the second is through air deposition onto leaves, which is dependent on the hydrophobicity, lipophilicity, and volatilization of pollutants [37,38]. In most cases, both pathways contribute when vegetation is grown [39]. EDDS is an organic acid, and its application to the soil significantly increases the concentration of dissolved organic carbon in soil solution (see Table 2), which may enhance the desorption of PCBs and increase the amount of PCBs dissolved into soil solution. However, with more metals such as Cu and Zn solubilized by EDDS and the impaired roots, roots might non-selectively absorb a greater quantity of PCBs from soil solution by transpiration, which would subsequently be translocated from roots to shoots. The significant correlation between the PCBs and Cu concentrations may support this hypothesis ($R = 0.80$, $P < 0.05$).

For the DGS6 + EDDS treatment, the roots were robust and likely secreted root exudates composed of various organic acids [40]. The exudates may enhance PCB desorption in the presence of EDDS, leading to high concentrations of PCBs in roots. However, the root damage due to the high levels of dissolved metals associated with EDDS application was counteracted to some extent by the DGS6 strain, and the translocation was not as great that following EDDS treatment alone. Hence, the shoot PCB concentration was lower than that in plants that underwent EDDS treatment (Table 3). The highest total uptake by DGS6 inoculation could be ascribed to the high biomass of the shoots and roots. In the present study, the air PCB concentration in the greenhouse was 64 pg m^{-3}, which was similar to that 400 m from the greenhouse, suggesting minimal volatilization of PCBs from soil to air during cultivation. The large surface area of leaves associated with the high shoot biomass would increase air deposition, which could contribute to shoot uptake of PCBs in addition those translocated from the roots. The high total PCB uptake in roots was thought to be the result of the large volume and biomass of roots, and active root secretion of various organic compounds.

4.4. PCB compositions of plants

In the absence of the DGS6 strain (control and EDDS treatments), PCB compositions in the shoots tended to be similar to that in soil, which suggests that translocation of PCBs from roots to shoots was active. The inoculation of the DGS6 strain (DGS6 and DGS6 + EDDS treatments) resulted in PCB compositions that were similar to that of air. This indicated that the large biomass of shoots associated with DGS6 strain inoculation could increase leaf adsorption of PCBs from the air. The translocation of PCBs from roots to shoots may also play a role in shoot PCB accumulation after these two treatments (for at least DGS6 + EDDS) based on the decreased root biomass and increased shoot PCB concentrations. These results suggest that the proportion of PCBs in shoots due to air deposition was higher than that originating from root uptake after DGS6 + EDDS treatment.

5. Conclusion

Uncontrolled e-waste recycling activity results in metal-POP co-contamination in the surrounding sites. Effective management is important for the safety of local residents and ecosystems. The present study demonstrated that inoculating PGPB to plant roots was superior to the application of the biodegradable chelant EDDS in terms of enhancing PCB removal and plant growth, increasing the biomass and leading to considerable PCB absorption by the roots and deposition from the air onto the shoots. The application of EDDS solubilizes metals and impairs plant roots, resulting in higher metal concentrations in plant tissues. The DOC of soil increased after these treatments, which may have facilitated the dissolution of PCBs into the soil solution, and so increased PCB uptake by roots and translocation upward to the shoots. Future studies should examine the impact of PGPB inoculation on the accumulation of other POPs present in e-waste soils, such as PBDEs, and field experiments should be conducted to evaluate the potential of PGPB in phytoremediation of e-waste soils.

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