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Phytoremediation of soil co-contaminated with Cd and BDE-209 using hyperaccumulator enhanced by AM fungi and surfactant

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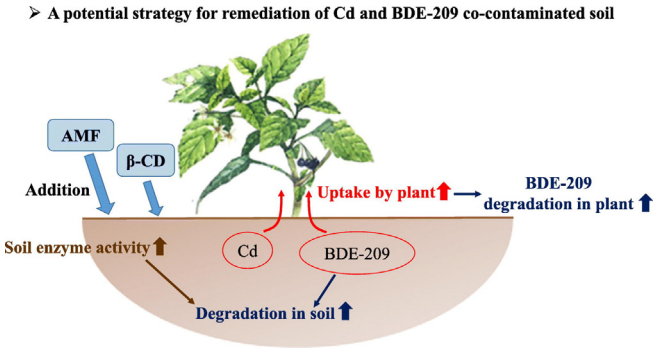
HIGHLIGHTS

- *S. nigrum* added with FM or β-CD significantly elevated Cd concentration in shoots.
- *S. nigrum* added with β-CD inoculated with or without AMF enhanced BDE-209 uptake.
- *S. nigrum* added with β-CD and FM had the highest dissipation efficiency of BDE-209.
- BDE-209 dissipation efficiency correlated positively to soil enzymatic activities.
- *S. nigrum*–β-CD with or without AMF was suitable to remediate co-contaminated soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Pot experiments were conducted to investigate the uptake and translocation of both Cd and decabromodiphenyl ether (BDE-209) in *Solanum nigrum*, under the treatments of two arbuscular mycorrhizal fungi [AMF, *Funneliformis mosseae* (FM) and *Rhizophagus intraradices* (RI)] and surfactant β-cyclodextrin (β-CD). Results showed that *S. nigrum* treated with either FM or β-CD significantly elevated shoot biomass and Cd concentrations and contents in shoots. The concentrations of BDE-209 in shoots and the dissipation and debromination efficiencies of BDE-209 in soil were significantly enhanced in *S. nigrum* treated with β-CD, inoculated with or without AMF. Moreover, significant positive correlations were found between the BDE-209 dissipation efficiency, the BDE-209 concentrations and contents in roots, and the soil enzymatic activities (polyphenol oxidase or dehydrogenase activities) and between the Cd and BDE-209 contents in shoots or roots. Higher concentrations of lower-brominated products and total PBDEs were detected in shoots than in roots suggesting that BDE-209 might be initially absorbed by roots, then translocated to shoots, and then degraded into lower brominated products in shoots. Considering the plant uptake of Cd and BDE-209 and the efficient removal of those chemicals in soils, the combination of *S. nigrum* and β-CD inoculated with or without AMF may be viable alternatives for phytoremediation of the co-contaminated soil.

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1. Introduction

Uncontrolled electronic-waste (e-waste) recycling has led to the release of hazardous contaminants during disposal activities, causing serious environmental problems (Wang et al., 2011c). Among all contaminants, polybrominated diphenyl ethers (PBDEs), used as brominated flame retardants in electronic circuit boards, have aroused extensive public health concerns over their persistence, bioaccumulation and potential adverse effects on humans and other organisms (Huang et al., 2011; Wang et al. 2015b). Due to the hydrophobic character of PBDEs, they are strongly bound to solid particles such as dust, soil, sediment and sewage sludge (Leung et al., 2011; Leung et al., 2007; Vrkoslavová et al., 2010). As the major component of commercial deca-BDE, decabromodiphenyl ether (BDE-209) is usually found at higher concentrations than other, lower-brominated PBDEs in sewage sludge (Li et al., 2015). In addition, the improper disposal of e-waste also results in the release of abundant heavy metals and PBDEs. Cadmium (Cd) is one of the most widely used toxic heavy metals in the electrical and electronics industry and is frequently detected in e-waste contaminated surroundings (Wu et al., 2014). The co-existence of Cd and BDE-209 has been shown to synergistically increase cytotoxicity and to cause greater inhibition of the diversity of the soil microbial community, compared with the effects of single exposure (Curcic et al., 2014; Zhang et al., 2012). Because soils from e-waste recycling sites are often subjected to simultaneous contamination with organic pollutants and heavy metals, there is an urgent need to develop suitable and efficient soil remediation technologies (Ye et al., 2015).

Phytoremediation is regarded as a cost-effective, efficient and environmentally friendly remediation strategy for the removal of heavy metals, radionuclides and organic pollutants from soil, with lower installation and maintenance costs than other remediation options (Ali et al., 2013). In the past few years, the plant uptake of either Cd (Khaokaew and Landrot, 2015; Lai, 2015; Liu et al., 2009) or PBDEs (Chow et al., 2015; Huang et al., 2011; Li et al., 2015; Wang et al., 2011a, 2011b; Zhao et al., 2012) from soil has been extensively investigated. However, to the best of our knowledge, only one study by Lu and Zhang (2014) has reported the simultaneous uptake of Cd, lead (Pb), zinc (Zn) and BDE-209 by *Sedum alfredii* co-planted with tall fescue in co-contaminated soil. *Solanum nigrum* is a Cd/Zn hyperaccumulator, with an exceptional ability to accumulate Cd and Zn in its aboveground parts, and consequently, the concentrations of these metals in the soil can be reduced (Gao et al., 2012; Marques et al., 2006; Sun et al., 2007). As for PBDEs, the uptake of PBDEs by *Nicotiana tabacum* and *S. nigrum* from sewage sludge contaminated with PBDEs was shown by Vrkoslavová et al. (2010). Therefore, it seems logical to consider employing *S. nigrum* to absorb Cd and BDE-209 from soil simultaneously.

Plant–microbe symbioses are ubiquitous in the environment. As the most widespread type of mycorrhizae, arbuscular mycorrhizal fungi (AMF) can form symbiotic associations with the roots of most terrestrial species that grow under natural conditions, which can improve nutrition acquisition by the host plant and enhance plant growth and tolerance to soil pollutants (Liu et al., 2015; Marques et al., 2008b). Furthermore, AMF can stimulate soil microbial activity, improve the soil structure and contribute to the overall degradation of soil pollutants (Lu and Lu, 2015). The contribution of AMF to enhanced Cd and Zn uptake by *S. nigrum* in heavy metal-contaminated sites has been noted (Liu et al., 2015; Marques et al., 2008a; Marques et al., 2006). However, studies of the influence of AMF on the accumulation and degradation of BDE-209 in plants are very scarce. Only Wang et al. (2011a) have found that the AMF inoculation of *Funneliformis mosseae* increased the concentration of BDE-209 in ryegrass roots and enhanced its debromination in shoots. Therefore, it is of great interest to study the effects of AMF on the phytoremediation of soil co-contaminated with Cd and BDE-209.

Polybrominated diphenyl ethers are hydrophobic organic contaminants (HOCs) and tend to strongly adsorb onto organic matter in soil, thus becoming poorly available in the soil–water phase. The addition of solubility enhancing agents such as surfactants can improve the phytoremediation efficiency of HOCs (Chen et al., 2010). Cyclodextrins (CDs) have been proposed as non-toxic and biodegradable surfactants for the removal of hydrophobic compounds from contaminated soils because they can form inclusion complexes with a variety of organic compounds both in solution and in the solid state, consequently increasing their water solubility (Morillo et al., 2014; Romeh, 2015). Among CDs, β-cyclodextrin (β-CD) has been successfully applied to enhance the uptake of polychlorinated biphenyls (PCBs) by ryegrass (Chen et al., 2010). However, little attention has been paid to the use of a suitable plant species combined with β-CD for remediation of soils co-contaminated with heavy metals and HOCs, except for a study by Wang et al. (2015a) that developed a cysteine-β-CD–enhanced phytoremediation technology for the simultaneous removal of Pb and phenanthrene from contaminated soil. Therefore, it is speculated that β-CD may prove highly effective in the phytoremediation of soil co-contaminated with Cd and BDE-209.

Only a few studies on the phytoremediation of soil co-contaminated with heavy metals and organic pollutants are available. It was found that in soil co-contaminated with Cd and polycyclic aromatic hydrocarbons (PAHs), treatment with a combination of EDTA, cysteine, salicylic acid and Tween 80 not only increased the accumulation of Cd in *S. nigrum*, but also promoted the degradation of PAHs (Yang et al., 2011). Moreover, co-planting of *Sedum alfredii* with *Festuca arundinaceae* combined with the BDE-degrading bacterial strain JP12 significantly enhanced the dissipation of BDE-209 and phytoextraction of heavy metals (Cd, Pb and Zn) in co-contaminated soil (Lu and Zhang, 2014). However, no reports yet exist on the remediation of soil co-contaminated with heavy metals and organic pollutants using plants combined with microorganisms and biosurfactants. Herein, we attempted to develop a novel phytoremediation technology using a Cd-hyperaccumulator plant – *S. nigrum* – combined with AMF [*Rhizophagus intraradices* (RI) or *Funneliformis mosseae* (FM)] and a CD (β-CD) for the remediation of soil co-contaminated with Cd and BDE-209. The main objectives of the study were (i) to investigate the uptake and translocation of Cd and BDE-209 in *S. nigrum* and their removal in soil under different treatments; (ii) to characterise the environmental behaviour of BDE-209 in the soil–plant system by identifying the debrominated products in *S. nigrum* and soil; (iii) to determine the response of soil enzymatic activities to the dissipation of BDE-209; and (iv) to screen the most suitable combination for phytoremediation of soil co-contaminated with Cd and BDE-209.

2. Materials and methods

2.1. Chemicals

Standards of BDE-209 were obtained from Sigma-Aldrich (USA). A standard solution of PBDEs containing 27 native congeners (BDE-3, BDE-7, BDE-15, BDE-17, BDE-28, BDE-47, BDE-49, BDE-66, BDE-71, BDE-77, BDE-85, BDE-99, BDE-100, BDE-119, BDE-126, BDE-138, BDE-153, BDE-154, BDE-156, BDE-183, BDE-184, BDE-191, BDE-196, BDE-197, BDE-206, BDE-207 and BDE-209), MBDE-47 (^{13}C-BDE-47), MBDE-209 (^{13}C-BDE-209) and FBDE-4003S (6-fluoro-2,2′,4,4′-tetrabromodiphenyl ether) was purchased from Wellington Laboratories (Canada). The organic solvents (*n*-hexane, dichloromethane, methylbenzene and acetone) were of high-performance liquid chromatography grade and were supplied by Sigma-Aldrich (USA). Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) was purchased from Tianjin Kemiou Chemical Reagent Co. (China), and standard materials (GBW-08303 and GBW-07603) were obtained from the China Standard Materials Research Center. β-Cyclodextrin ($C_{42}H_{79}O_{35}$) was purchased from Sinopharm Chemical Reagent Co. (China). All other chemicals, including anhydrous sodium sulphate, alumina and silica gel (100 to-200 mesh), were supplied by Guangzhou Chemical Reagent Factory (China). Anhydrous sodium sulphate (Na_2SO_4), silica gel and alumina (100 to 200 mesh)

were used after heating overnight at 150 °C. Distilled water was used in all experiments.

2.2. Experimental design

Soils were collected from a depth of 0 to 20 cm in paddy experimental fields in Guangzhou, China. The soil contained 8.1% organic matter, 1.3 g kg^{-1} total N, 1.1 g kg^{-1} total P and 0.61 g kg^{-1} total K, with a pH of 5.9. The background concentration of Cd was 0.18 mg kg^{-1} without detectable BDE-209 (Li et al., 2016). After air-drying for 2 weeks, the soil samples were sieved through a 2-mm nylon sieve and autoclaved at 121 °C for 2 h to eliminate the indigenous microorganisms. The soil was then spiked with an aqueous solution of $CdCl_2$ as a source of Cd and incubated for 4 weeks. These sub-samples were then air-dried, fully homogenised, sieved again through a 2-mm sieve and then spiked with BDE-209 in dichloromethane, according to the method described by Lu and Zhang (2014). The final concentrations of Cd and BDE-209 were 14.8 mg kg^{-1} and 4.98 mg kg^{-1}, respectively, before plant cultivation.

S. nigrum seeds were obtained from South China Botanical Garden, Guangzhou, China, sterilised with H_2O_2 for 15 s, washed thoroughly with deionised water and germinated on moist filter papers in the dark. Three days later, the seedlings were transplanted and cultured as stock in basins and supplied with 20% Hoagland–Arnon nutrient solution (Hoagland and Arnon, 1950). Seedlings of uniform height (5 cm) were then used for the pot trial.

The seven treatments combining AMF and/or β-CD are detailed in Table 1. Two species of AMF (FM and RI) were obtained from Mycagro Lab (France). For the mycorrhizal treatments, each pot contained five seedlings, 1.6 kg of soil and 30 g of inoculum. For the surfactant treatments, β-CD was supplied at a concentration of 5 g kg^{-1} on days 14 and 21. There were four replicates for each treatment. Hoagland–Arnon nutrient solution (20%) containing 10% KH_2PO_4 was added to each pot every week for 5 weeks. The soil was maintained at a 70% holding capacity on a daily basis, via the supply of distilled water.

2.3. Sample preparation

A quincunx sampling pattern was used to collect soil samples in each pot. The samples were sieved through a 100-mesh sieve and stored at 4 °C before analysis. The plants were harvested after 35 days of growth. They were gently removed from the substrate and separated into shoots and roots. Shoot and root samples were then rinsed thoroughly with distilled water, blotted with tissue papers and weighed. A part of the fresh root sub-sample from each treatment was used to determine AM colonization. The remainders of the samples were freeze-dried and stored at 4 °C until analysis.

2.4. Chemical extraction and analysis

Arbuscular mycorrhizal colonization was assessed following the same procedures as in our previous study (Li et al., 2011). Dried plant samples were weighed and ground. The powders were digested with an acid mixture of $HNO_3/HClO_4$ (4:1, v/v), and the Cd concentrations in the digestant were determined using an atomic absorption spectrophotometer (AA-770, Shimadzu).

The soil (1 g) or plant (0.5 g) samples were extracted according to the method provided in the Supplementary material. The sample extracts were analyzed by an Agilent 7890A gas chromatograph equipped with a single-quadrupole mass analyzer Agilent 5975C MSD, using negative chemical ionization (NCI) in the selected-ion monitoring (SIM) mode. A DB-XLB capillary column (15 m × 0.25 mm × 0.1 μm) was used to separate the PBDE congeners using helium as the carrier gas at a rate of 1.5 mL min^{-1}. The GC oven temperature was programmed as follows: from 80 °C (hold for 1 min) to 200 °C at a heating rate of 10 °C min^{-1}, then from 200 °C to 300 °C (hold for 15 min) at a heating rate of 20 °C min^{-1}.

Table 1
The treatments and labels.

Labels	Treatments	Labels	Treatments
CK	Unplanted control soil	Lβ	Planted soil added with β-CD
L	Soil planted with only *S. nigrum*	LβM	Planted soil inoculated with *F. mosseae* and added with β-CD
LM	Planted soil inoculated with *F. mosseae*	LβI	Planted soil inoculated with *R. intraradices* and added with β-CD
LI	Planted soil inoculated with *R. intraradices*		

2.5. Soil enzymatic activity assay

Soil polyphenol oxidase activity was determined by standard colorimetric methods and was expressed as mg purpurigallin kg^{-1} dry soil 2 h^{-1} (Dick et al., 1988). Soil dehydrogenase activity was measured by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) and was expressed as mg TPF kg^{-1} dry soil 24 h^{-1} (Chen et al., 2010; Shen et al., 2009).

2.6. Quality assurance and quality control

A procedure blank was included in each batch of extraction. The recoveries of PBDEs, which were determined by spiking a certain concentration of MBDE-47 and MBDE-209 into the samples, ranged from 84% to 102% and from 85% to 110% in soil and plants, respectively. The limit of detection (LOD) ranged from 0.01 to 0.2 ng g^{-1} dry weight for the four PBDE congeners. Standard reference materials for soil (GBW-08303) and plants (GBW-07603) were used to check the metal recovery rates. The recovery rates for Cd were within 90% ± 10%.

2.7. Data analysis

The bioconcentration factor (BF) and translocation factor (TF) of Cd and BDE-209 were determined as:

$$\text{BF} = \text{Cd or BDE-209 concentration in shoots}/\text{Cd or BDE-209 concentration in soil} \quad (1)$$

$$\text{TF} = \text{Cd or BDE-209 concentration in shoots}/\text{Cd or BDE-209 concentration in roots} \quad (2)$$

The plant uptake of Cd or BDE-209 was determined as:

$$\text{Plant uptake of Cd or BDE-209} = \text{Cd or BDE-209 content in shoots} + \text{Cd or BDE-209 content in roots} \quad (3)$$

The percentage removal of Cd by plants was determined as:

$$\text{Removal of Cd by plants (\%)} = (\text{plant uptake of Cd}/\text{initial Cd content in soil}) \times 100 \quad (4)$$

The removal of BDE-209 in soil was calculated as:

$$\text{Removal of BDE}-209 \text{ in soil} = \text{initial content of BDE}-209 \text{ in soil} - \text{content of BDE}-209 \text{ in soil after 35 days} \quad (5)$$

The dissipation efficiency of BDE-209 in soil was calculated as:

$$\text{BDE}-209\ \text{dissipation efficiency}\ (\%) = (\text{removal of BDE}-209\ \text{in soil}/\text{initial content of BDE}-209\ \text{in soil}) \times 100 \quad (6)$$

The data were subjected to statistical analysis with SPSS v16.0 software. All figures were drawn with the PC-based Origin program. The differences among the treatments were statistically evaluated in terms of standard deviations and by Tukey's test ($P < 0.05$). Correlations were determined by linear regression analysis.

3. Results

3.1. Root colonization rates

The root colonization rates of *S. nigrum* with the different treatments were determined in soil co-contaminated with Cd and BDE-209. As shown in Table 2, trace amounts (3.3% to 4.0%) of root colonization were found in the non-mycorrhizal treatments (L and Lβ). Arbuscular mycorrhizal fungi (FM and RI) significantly ($P < 0.05$) enhanced the root colonization rates (83.2% to 93.1%). However, no significant ($P < 0.05$) differences were found between the two AMF species (LM vs. LI, and LβM vs. LβI).

3.2. Plant biomass

Compared with *S. nigrum* (L treatment), the addition of β-CD (Lβ treatment) or FM (LM treatment) resulted in a significant increase in shoot biomass (Fig. 1). The combination of β-CD and FM (LβM treatment) led to a markedly higher root biomass than the control (L treatment). Although the inoculation of FM increased the shoot biomass of *S. nigrum* significantly ($P > 0.05$), no significant differences in shoot and root biomass were found in *S. nigrum* inoculated with RI treatments.

3.3. Uptake and translocation of Cd in S. nigrum

As shown in Fig. 2a and b, the existence of β-CD (Lβ) or FM (LM) significantly increased the Cd concentrations and contents in the shoots of *S. nigrum*, compared with the non-mycorrhizal plant (L), and resulted in a significantly higher bioconcentration factor (Table 2) and Cd removal rate by the plant (Table 3). However, RI (LI treatment) reduced the Cd concentration and content of both shoots and roots (Fig. 2a and b). In all treatments, the accumulation of Cd in shoots was much greater than that in roots and accounted for a large proportion (70.6% to 83.6%) of the total Cd uptake (Fig. 2b). As presented in Table 2, single treatment with β-CD or AMF (FM or RI) resulted in a significantly higher TF. However, combined treatment with β-CD and AMF (i.e., LβM and LβI) resulted in a significantly lower TF compared with *S. nigrum*.

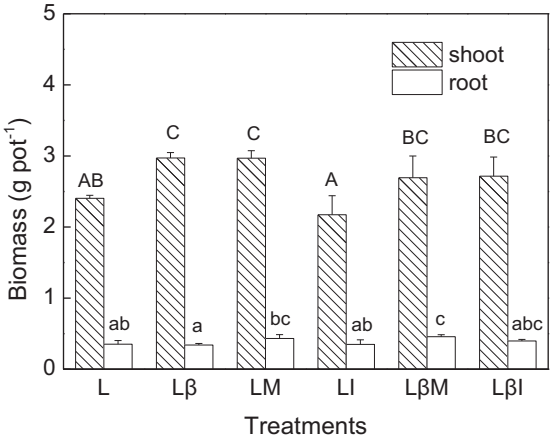

Fig. 1. Shoot and root biomass of *S. nigrum*. Bars (means ± SD, n = 4) with different uppercase/lowercase letters indicate significant differences ($P < 0.05$) of shoot/root biomass among different treatments.

3.4. Uptake, translocation and debromination of BDE-209 in S. nigrum

Compared with *S. nigrum*, the mycorrhizal treatments with or without β-CD significantly elevated the concentrations and contents of BDE-209 in both shoots and roots (Fig. 2c and d). No significant differences were found in the BDE-209 concentrations and contents of shoots between the L and Lβ treatments ($P > 0.05$), whilst these values in roots were significantly enhanced after the Lβ treatment. Furthermore, significant positive correlations were found between the Cd and BDE-209 contents both in roots and in shoots (Table 4, $P < 0.05$).

In general, all of the treatments significantly increased the total concentrations of PBDEs in both shoots and roots with the exception of the Lβ treatment (Fig. 3a and c), which is in line with the results of the BDE-209 concentrations in *S. nigrum* (Fig. 2c). Most PBDE congeners detected in shoots were di-, tetra- and hexa-BDEs, each of which accounted for approximately 20% of the total PBDEs (Fig. 3b). However, in roots, deca-BDEs accounted for the largest proportion regardless of the different treatments, ranging from 54.0% to 59.8% (Fig. 3d).

3.5. Dissipation and debromination of BDE-209 in soil

The contribution of plant uptake to the total BDE-209 dissipation in soil (P/S in Table 3) ranged from 0.05% to 0.43%. The concentrations of total residual PBDEs in soil did not vary significantly among all of the treatments (Fig. 3e), with deca-BDE accounting for the largest proportion (44.5% to 96.1%; Fig. 3f). Moreover, nona-BDE was the main debrominated products in soil, with proportions ranging from 2.8% to 40.1% (Fig. 3f), whilst the other congeners were present in relatively low concentrations and percentages. In soil planted with *S. nigrum* (L, Lβ, LM, LI, LβM and LβI), the residual deca-BDE concentrations were consistently lower than in the control (CK) (Fig. 3f). The total proportions of debrominated products (mono- through nona-) were in the

Table 2
Root colonization rates (means ± SD, n = 4), bioconcentration factors and translocation factors of Cd and BDE-209 in *S. nigrum*.

Treatments	Root colonization rate (%)	Cd		BDE-209	
		Bioconcentration factor (BF)	Translocation factor (TF)	Bioconcentration factor (BF)	Translocation factor (TF)
L	3.32 ± 0.86a	7.53c	0.46b	1.07a	0.58d
Lβ	4.04 ± 0.54a	8.57d	0.59d	1.60b	0.32a
LM	87.3 ± 6.47bc	8.34d	0.54c	1.54b	0.56d
LI	83.2 ± 5.85b	6.36b	0.52c	1.21a	0.48c
LβM	93.1 ± 6.61c	6.39b	0.41a	2.36c	0.34a
LβI	90.1 ± 7.50c	6.09a	0.41a	1.64b	0.38b

Values in each column with different letters indicate significant ($P < 0.05$) differences among different treatments.

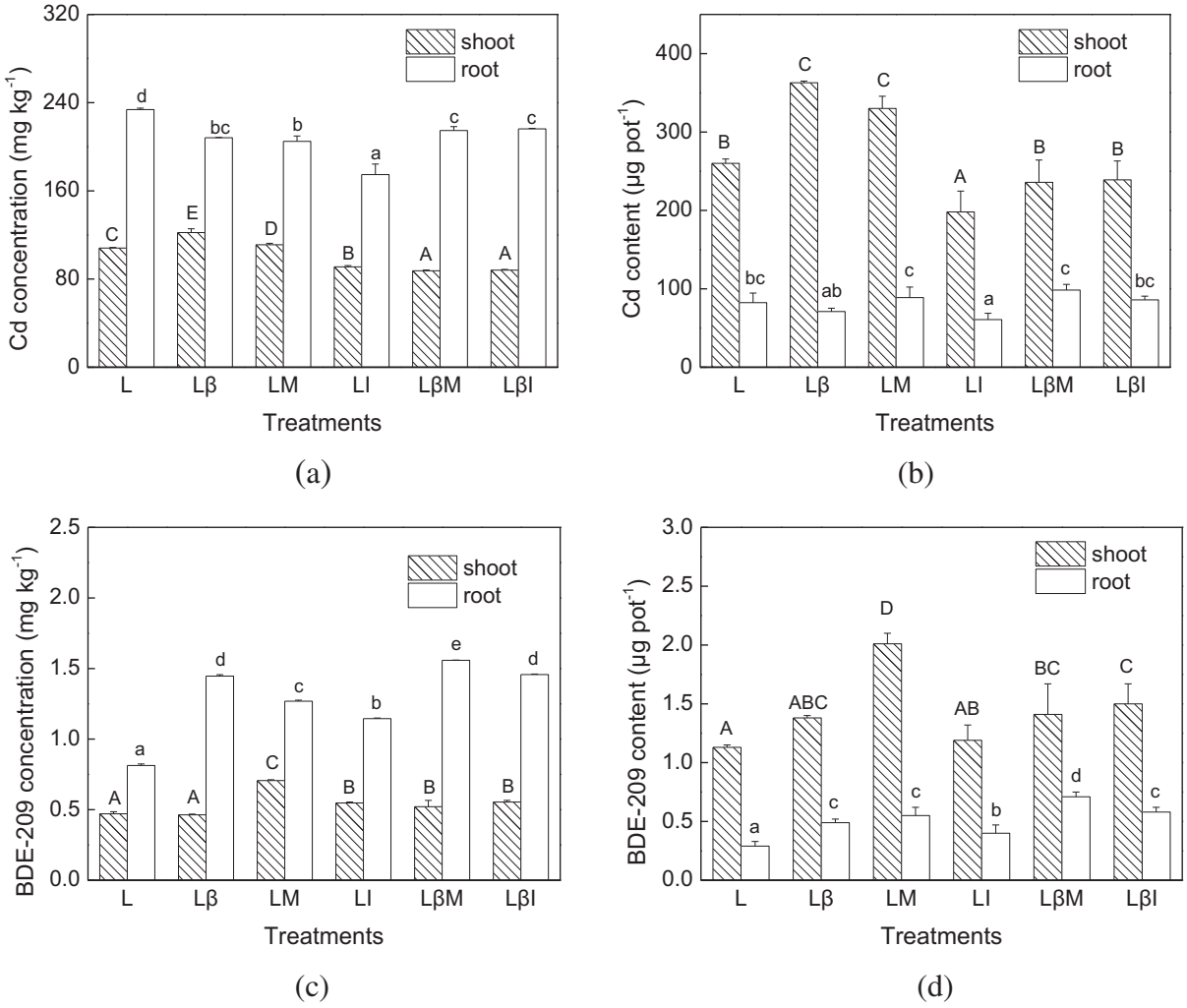

Fig. 2. Concentrations and contents of Cd and BDE-209 in shoots and roots of *S. nigrum*. Bars (means ± SD, n = 4) with different uppercase/lowercase letters indicate significant differences ($P < 0.05$) of Cd or BDE-209 concentration or content in shoots/roots among different treatments.

order of LβM > Lβ > LβI (Fig. 3f), which was the same order that had been found for the BDE-209 dissipation efficiencies in soil (Table 3). Furthermore, the proportions of the lower-brominated congeners (mono- through octa-) were higher in *S. nigrum* (Fig. 3b and d) than in soil (Fig. 3f).

3.6. Soil enzymatic activities

As shown in Fig. 4, the activities of polyphenol oxidase (Fig. 4a) and dehydrogenase (Fig. 4b) showed marked variations in response to different treatments, ranging from 184 to 363 mg purpurigallin kg^{-1} $2\ h^{-1}$ and 36.2 to 185 mg TPF kg^{-1} $24\ h^{-1}$, respectively. Furthermore, significant positive correlations ($P < 0.001$) were found between the BDE-209 dissipation efficiency in soil, the BDE-209 concentration and content in roots, and soil enzymatic activities (Table 4). The treatments of Lβ, LβM and LβI showed significantly greater polyphenol oxidase activities (Fig. 4a). Compared with *S. nigrum*, the addition of either AMF or β-CD markedly enhanced the dehydrogenase activities (Fig. 4b). Moreover, the mycorrhizal treatments with the addition of β-CD (LβM or LβI) possessed significantly higher polyphenol oxidase and dehydrogenase activities (Fig. 4b) than those without the addition of β-CD (LM and LI) or AMF (Lβ).

Table 3
Removal efficiencies of Cd and BDE-209 in soil (means ± SD, n = 4).

Treatments	Cd		BDE-209			
	Plant uptake (mg pot^{-1})	Removal by plant (%)	Plant uptake (P) (μg pot^{-1})	Removal in soil (S) (mg pot^{-1})	Dissipation efficiency (%)	P/S (%)
CK	–	0a	–	0.24 ± 0.05a	3.01a	–
L	0.35 ± 0.01b	1.48c	1.42 ± 0.03a	0.93 ± 0.06b	11.7b	0.15a
Lβ	0.43 ± 0.06c	1.82d	1.87 ± 0.02bc	3.34 ± 0.17d	41.9d	0.06a
LM	0.42 ± 0.01c	1.77d	2.65 ± 0.06c	0.63 ± 0.34b	7.91b	0.43b
LI	0.26 ± 0.03a	1.10b	1.59 ± 0.01ab	0.72 ± 0.23b	9.04b	0.22a
LβM	0.33 ± 0.03b	1.39c	2.12 ± 0.30b	4.42 ± 0.13e	55.5e	0.05a
LβI	0.33 ± 0.03b	1.39c	2.09 ± 0.21b	2.54 ± 0.05c	31.9c	0.08a

Values in each column with different letters indicate significant ($P < 0.05$) differences among different treatments.

Table 4
Correlation coefficients between Cd and BDE-209 contents in shoots and roots of *S. nigrum*, BDE-209 concentration in roots, BDE-209 dissipation efficiency in soil, and soil enzymatic activities.

	BDE-209 dissipation efficiency	BDE-209 concentration in roots	BDE-209 content in roots	BDE-209 content in shoots
Polyphenol oxidase activity	0.665**	0.536**	0.617**	NS
Dehydrogenase activity	0.675**	0.666**	0.725**	NS
Cd content in shoots	NS	NS	NS	0.241*
Cd content in roots	NS	NS	0.398**	NS

NS represents not significant.
* Re1presents significant at the level of $P < 0.05$.
** Represents significant at the level of $P < 0.01$.

4. Discussion

In this study, the mycorrhizal treatments (LM, LI, LβM and LβI) achieved high colonization rates even in soil co-contaminated with Cd and BDE-209. The root colonization rates of *S. nigrum* under FM treatment were observed to range from 67% to 72% in Cd-added soil (0 to 40 mg kg^{-1}) by Jiang et al. (2016). However, in this study, there were no significant ($P < 0.05$) differences between the two AMF species (LM vs. LI, LβM vs. LβI). Our results show that both β-cyclodextrin (Lβ treatment) and FM (LM treatment) had a positive effect on the growth of *S. nigrum* (Fig. 1). This effect of β-CD on plant biomass has previously been found in rice, alfalfa, ryegrass and tall fescue grown in PCB-contaminated soil treated with randomly methylated β-CD (Shen et al., 2009), which may be attributed to the increased permeability of the root cell membrane caused by the surfactants, resulting in more efficient uptake of nutrients and hence a larger plant biomass (Tribak et al., 2002). Moreover, Liu et al. (2015) confirmed that the biomass of shoots and roots in *S. nigrum* inoculated with *Glomus versiforme* had significantly increased Cd levels, ranging from 0 to 100 mg kg^{-1}. Acting as an intermediary between soil and plant, AMF species use their mycelia to obtain photosynthates from plants for use in their own metabolism and in turn absorb minerals and water from soil and transport them to the hosts (Liu et al., 2015). In this way, AMF can improve nutrient uptake and plant growth. Here, the inoculation of FM and RI exerted different effects on plant biomass (Fig. 1), which suggests a degree of functional diversity between different AMF symbioses (Li et al., 2011). Furthermore, the addition of β-CD exerted no toxic effects on the growth of mycorrhizal *S. nigrum*.

The existence of β-CD (Lβ) or FM (LM) significantly increased the Cd concentrations and contents in the shoots of *S. nigrum* (Fig. 2a and b), resulting in a notably higher bioconcentration factor (BF) (Table 2) and Cd removal rate by the plant (Table 3). Similarly, Wang et al. (2015a) reported that Pb concentrations in the shoots and roots of ryegrass were significantly elevated by cysteine-β-CD in soil co-contaminated with Pb and phenanthrene. In addition, Jiang et al. (2016) found that inoculation with FM improved the growth and total Cd uptake of *S. nigrum*, in agreement with our findings. The increased Cd concentrations and contents in the shoots of *S. nigrum* under FM treatment might be explained by the enhanced concentrations of phytoavailable Cd in soil following treatment (Liu et al., 2015). In contrast, the Cd contents both in shoots and roots of *S. nigrum* were notably decreased under RI treatment (Fig. 2b), as were the Cd concentrations in the shoots and roots (Fig. 2a). The contradictory effects of the FM and RI treatments on the shoot Cd concentrations and contents suggest that the selection of an appropriate AMF strain is crucial in phytoremediation because it may determine the plant's response to heavy metals (Hassan et al., 2013). The objectives of phytoremediation include the extraction of pollutants into bioavailable forms that can be easily taken up by plants and the enhancement of pollutant translocation from roots to shoots (Marques et al., 2008b). Therefore, in addition to evaluating the plant's ability to accumulate pollutants, the extent of pollutant translocation from roots to shoots is also an important consideration for the choice of a phytoremediation strategy. The translocation factor (TF), expressed as the pollutant concentration ratio of shoots to roots, is an indicator of pollutant transfer in plants (Wang et al., 2015a). The significantly higher TF in the treatments of Lβ, LM and LI (Table 2) suggested that addition of β-CD or AMF alone did not act as a Cd barrier in roots and indeed contributed to the translocation of Cd to shoots. Similar results were previously obtained for Zn uptake by *S. nigrum* inoculated with FM and RI (Marques et al., 2006). In contrast, the combination of β-CD and AMF (LβM and LβI treatments) led to a significantly lower TF than *S. nigrum* (Table 2), implying that the interaction between microorganisms (AMF) and a biosurfactant (β-CD) had complex effects that inhibited the translocation of Cd from roots to shoots. These results demonstrate that in soil co-contaminated with Cd and BDE-209, planting *S. nigrum* with the addition of either β-CD (Lβ) or FM (LM) was the most beneficial treatment for the uptake, translocation and removal of Cd.

In general, compared with *S. nigrum*, the mycorrhizal treatments with or without the addition of β-CD markedly increased the concentrations and contents of BDE-209 both in shoots and roots (Fig. 2c and d). Wang et al. (2011a) also observed that BDE-209 concentrations in the roots of ryegrass (*Lolium multiflorum* L.) were significantly increased after FM treatment. This enhancement may be attributed to the increased root access to soil that result from the formation of fine soil pores interwoven with AM hyphae and the invasion of fungi into the plant roots (Lu and Lu, 2015). No significant differences were found in the shoot BDE-209 concentrations and contents between the L and Lβ treatments ($P > 0.05$), whilst these values in roots were significantly enhanced after β-Cyclodextrin treatment (Fig. 2c and d). This contrast between shoots and roots may be due to the higher BDE-209 concentration in roots than in shoots and the fact that β-CD can form an inclusion complex with BDE-209, which possesses higher water solubility and thus is more available for root uptake (Romeh, 2015). Moreover, the significant positive correlations between the Cd and BDE-209 contents in roots and in shoots (Table 4, $P < 0.05$) indicated that the interactions between those two pollutants might promote their uptake by plants. The synergistic effects of Cd and BDE-209 in increasing cytotoxicity and suppressing the diversity of the soil microbial community were shown in previous studies (Curcic et al., 2014; Zhang et al., 2012). BDE-209 itself is generally considered to have low toxicity, but increasing evidence shows that it can break down in the environment into other, prohibited PBDEs with much greater toxicity (Wang et al., 2011a). Therefore, it is essential to investigate the uptake and subsequent degradation of PBDEs in plants. The majority of PBDE congeners detected in shoots were di-, tetra- and hexa-BDEs (Fig. 3b), indicating that BDE-209 was degraded to lower-brominated PBDEs in shoots. It is noteworthy that the total concentrations of the debrominated metabolites were higher in shoots than in roots (Fig. 3a and c), indicating that BDE-209 might be initially absorbed by roots, then translocated to shoots, and finally degraded there into lower-brominated products (Wang et al., 2011a). Moreover, as indicated in Table 2, all of the treatments led to a reduction of the TF of BDE-209, possibly because BDE-209 was degraded into lower-brominated products after being translocated to shoots.

Dissipation of BDE-209 into soil can result from degradation, plant uptake or abiotic dissipation (including leaching and volatilization), but the abiotic loss of BDE-209 is limited due to its fairly low vapour pressure ($\log K_{OW} \approx 10$) (Lu and Zhang, 2014). In this study, plant

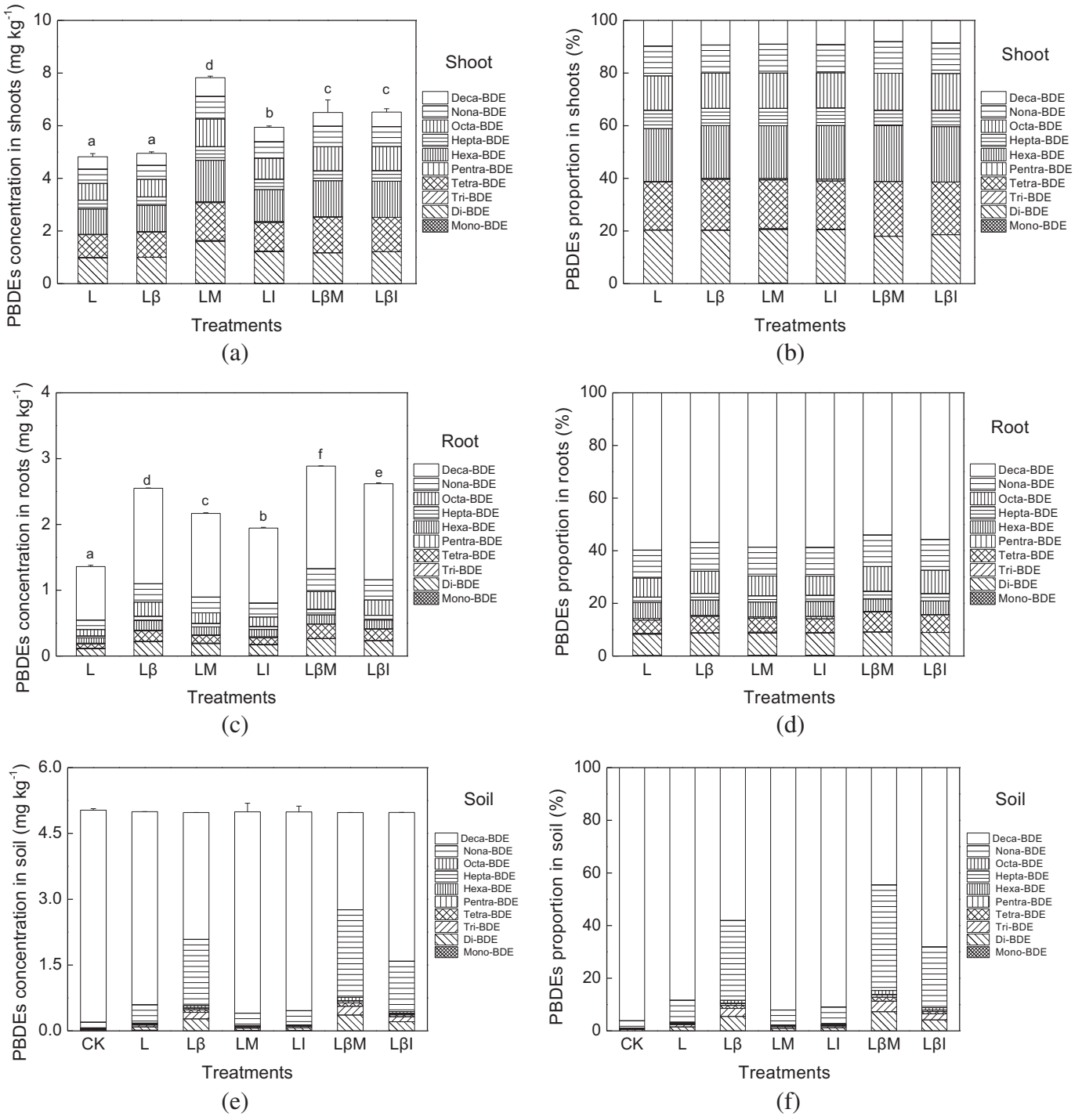

Fig. 3. Concentrations and proportions of PBDEs in shoots and roots of *S. nigrum* and soil. Bars (means ± SD, n = 4) with different letters indicate significant differences ($P < 0.05$) of PBDEs concentration in shoots/roots among different treatments.

uptake made an extremely low contribution to the total BDE-209 dissipation in soil (P/S in Table 3), whereas the degradation of BDE-209 in soil was the major contributor to its dissipation. Nona-BDE was the main debrominated products in soil, whilst the other congeners were present in relatively low concentrations and percentages (Fig. 3f), which suggests that BDE-209 most commonly loses a single bromine atom to form nona-BDEs (Wang et al., 2011a). In soil planted with *S. nigrum*, the residual deca-BDE concentrations were consistently lower than in the control (CK), indicating that *S. nigrum* enhanced the debromination of deca-BDE in soil. Furthermore, the proportions of the lower-brominated congeners (mono- through octa-) were higher in *S. nigrum* (Fig. 3b and d) than in soil (Fig. 3f), suggesting that either further debromination of PBDEs occurred inside the plants or that lower-brominated congeners were more liable to be taken up by the plants (Huang et al., 2010). Moreover, it may be possible for the lower-brominated products in soil to be further mineralized by prolonging the remediation time, thus inducing the toxicity to be further reduced. Compared with the other treatments in this study, the Lβ, LβM and LβI treatments achieved significantly higher BDE-209 uptake in *S. nigrum* (Fig. 2c), as well as significantly higher dissipation

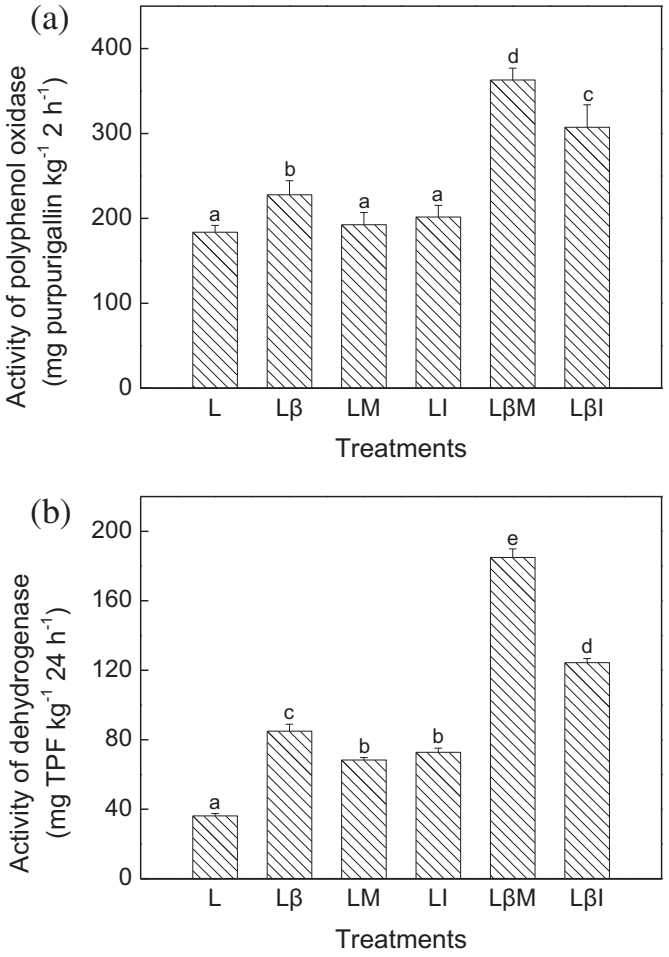

Fig. 4. Activities of (a) polyphenol oxidase and (b) dehydrogenase in soil. Bars (means ± SD, n = 4) with different letters indicate significant differences ($P < 0.05$) of soil enzymatic activity among different treatments.

and debromination efficiencies of BDE-209 in soil (Table 3). Satisfactory removal of Cd was also observed in the Lβ and LM treatments (Table 3). Therefore, Lβ, LβM and LβI show promise as treatments for the phytoremediation of soil co-contaminated with Cd and BDE-209.

Soil enzymes are the mediators and catalysts of most soil transformation processes. The activities of soil enzymes are sensitive indicators, and a reduction in enzymatic activities implies that soil is exposed to toxic stress (Zhang et al., 2012). Soil polyphenol oxidase is an oxygen-transferring enzyme acting on specific recalcitrant pollutants to induce their precipitation or transformation to other products, and thus polyphenol oxidase activity is closely related to BDE-209 degradation in soil (Liu et al., 2008). The treatments of Lβ, LβM or LβI possessed significantly higher polyphenol oxidase activities (Fig. 4a) and tended to show higher dissipation efficiencies of BDE-209 in soil (Table 3). Since dehydrogenase exists in all microorganisms, it can serve as an indicator of total viable microbial cells (Shen et al., 2009). Moreover, the groups treated with mycorrhizal fungi and β-CD (LβM and LβI) possessed significantly higher BDE-209 dissipation efficiency (Table 3) and dehydrogenase activities (Fig. 4b) than those without β-CD (LM and LI). This significant increase in dehydrogenase activity (i.e., soil biological activity) suggests that β-CD can be used directly by microbes as carbon sources and therefore stimulate microbial activities (Shen et al., 2009). In addition, root exudates from *S. nigrum* may increase the bioavailability of contaminants, provide more substrates for co-metabolic degradation and modify the soil environment to make it more suitable for microbial transformation (Shen et al., 2009). The polyphenol oxidase and dehydrogenase activities were significantly higher in the LβM and LβI treatments than in the Lβ, LM and LI treatments (Fig. 4), suggesting that β-CD and AMF acted synergistically to enhance the soil enzymatic activities. Generally, the soil enzymatic activities in the LβM, LβI and Lβ treatments were significantly higher than in the other treatments, verifying that these methods efficiently mitigated the toxic effects of co-contamination with Cd and BDE-209.

5. Conclusions

This study developed a novel phytoremediation technology employing *S. nigrum* combined with AMF (FM or RI) and β-CD, for the simultaneous removal of Cd and BDE-209 from soil. The interactions between Cd and BDE-209 promoted their uptake by plants. Moreover, our study demonstrates that *S. nigrum* added with β-CD under either mycorrhizal or non-mycorrhizal treatments could achieve satisfactory Cd and BDE-209 uptake by plant as well as higher BDE-209 dissipation efficiencies, and these treatments may therefore be suitable alternatives for the remediation of soil co-contaminated with Cd and BDE-209. The groups receiving these treatments had higher activities of polyphenol oxidase and dehydrogenase in soil, and possessed significantly higher BDE-209 concentrations in the plants and dissipation and debromination efficiencies of BDE-209 in soil. Furthermore, BDE-209 can be degraded into lower-brominated products in plants and soil, which may be further mineralized through prolonging the remediation time. These findings provide new insights into the development of bioremediation strategies for sites co-contaminated with heavy metals and PBDEs, and suggest that the interactions among different types of pollutants, plants, chemicals and microorganisms should be taken into consideration when selecting a soil phytoremediation technology.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.09.066.

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