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Mycoremediation potential and tolerance responses of *Oudemansiella radicata* in cadmium-pyrene co-contaminated soil

Rong Chen · Zhiren Zhou · Yuanxiu Liu · Juan Jiang · Qiao Li · Haihai Song · Donghui Pei · Heng Xu

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Abstract
Purpose In recent years, many previous studies have well documented that wild and cultivated edible mushrooms have the ability to bioaccumulate metal ions, but the process of mycoremediation and the detoxification strategy of mushrooms in co-contaminants (heavy metals and polycyclic aromatic hydrocarbons (PAHs)) are rarely reported. The study was to investigate the mycoremediation potential and tolerance responses of *Oudemansiella radicata* in cadmium and pyrene co-contaminated soil.
Materials and methods Soil samples collected and sieved from Sichuan province, China, which was spiked with cadmium (0, 5, 15, and 30 mg/kg) and pyrene (0, 200, and 400 mg/kg). After harvest, biomass, bioaccumulation of cadmium, residual pyrene, and antioxidant enzymes activities were measured.
Results and discussion Results showed that dry biomass was not apparently influenced by the co-contamination, even in highly polluted soils. In cadmium of 5 and 15 mg/kg, the bioaccumulation of cadmium enhanced when pyrene was added, the bioconcentration factor value even reached 1.09. The removal of pyrene (added at concentrations of 200 and 400 mg/kg) was significantly higher in *O. radicata*-planted soils than those in the unplanted soils and was inhibited in lower level of cadmium whereas promoted in higher level of cadmium, indicating that the highly adapted cadmium-resistant microbes could promote the dissipation of pyrene. Besides, antioxidant enzyme activities including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) represented significantly changes in co-contamination as compared with control.
Conclusions Using *O. radicata* for remediation of cadmium-pyrene-contaminated soils could be an interesting alternative, considering its short life time, metal tolerance, and bioaccumulation capacity.

Keywords Antioxidant enzymes · Cadmium · Co-contamination · *Oudemansiella radicata* · Pyrene

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1 Introduction

Mushrooms, as macro-fungi, possess a great tolerance to high levels of heavy metal pollution and are considered as an appealing approach to the remediation of heavy metal-polluted sites due to its low cost, high efficiency, easiness of operation, regeneration, and eco-friendly posttreatment (Chudzyński et al. 2011; Cocchi et al. 2006; Zhang et al. 2012b; Li et al. 2013b; Ji et al. 2009). But no work was done on the process of mycoremediation of co-contaminants (heavy metals and polycyclic aromatic hydrocarbons) and less information has been generated involving the tolerance and detoxification strategy of mushrooms. However, with the rapid urbanization and industrialization, the soil contaminated with both heavy metals and polycyclic aromatic hydrocarbons (PAHs) has become a serious environmental problem on a world scale, especially in China (Sun et al. 2011; Chigbo et al. 2012).

Cadmium (Cd) is a non-essential trace element. It represents severe threat to humans, animals, plants, and microorganisms due to its high mobility and toxicity, and the health risks

brought about by Cd-contaminated products through the food chain to humans are the cause of great concern (Liu et al. 2011; Nabulo et al. 2010). According to recent soil survey from 11 provinces in China, however, Cd contaminates more than 1.3× 105 km^2 of agricultural soils due to human activities, and 1.46×108 kg agricultural products are produced on the contaminated soils, posing risk to humans and other organisms in those areas (Wang 2002; Ye et al. 2014; Qian et al. 2010).

PAHs, a ubiquitous group of hazardous organic pollutants mainly produced by combustion processes, exist in more than 90 % of the surface soil (Wild and Jones 1995). Sixteen PAHs, listed as priority pollutants by the US Environmental Protection Agency (US-EPA) are considered to be a great environmental concern due to their strong carcinogenicity, teratogenicity, and toxicity (Bidaud and Tran Minh 1998; Lu et al. 2012). High concentrations of PAHs in soils and sediment could cause significant hazards to microorganisms and to higher organisms including human beings indirectly (Desalme et al. 2013; Sanchez et al. 2013). Therefore, remediation of heavy metal-PAHs co-contaminated soils has drawn much more attention recently (Lu et al. 2013; Li et al. 2013a).

Plants can directly absorb, accumulate, and degrade PAHs and indirectly accelerate the degradation by promoting the activity of rhizosphere microorganisms (Baek et al. 2006). Toxic metals, unlike organic pollutants, cannot be mineralized and degraded but can be removed mainly by phytoextraction (Lebeau et al. 2008; Vilas Boas et al. 2013). The soil with co-contamination might influence the process of phytoremediation through interacting among themselves and/or with plants and their rhizosphere biota (Li et al. 2013b). Previous research has shown that the effect of phytoremediation may be enhanced or inhibited in heavy metal-organic pollutant-combined system. Chigbo et al. (2012) showed that the ability of Cu phytoextraction was halved in 50 and 100 mg/kg Cu-contaminated soil relative to the absence of pyrene, and the addition of Cu to pyrene-contaminated soil inhibited the dissipation of pyrene. In contrast, in the presence of pyrene and Zn, the concentration of zinc in shoots of *Brassica juncea* significantly increased compared with soil contaminated with Zn lonely (Batty and Anslow 2008). However, many plants that are effective at removing metals from the soil are slow growing and small, thus leading to a long cycle. But mushrooms have a high yield and fast growth, which have been well demonstrated to accumulate high toxic heavy metal, such as mercury (Chudzyński et al. 2011), cadmium (Cao et al. 2012), lead (Garcia et al. 2009), and copper (Cen et al. 2012), showing great potential to extract heavy metals from contaminated soil (Jing et al. 2012). In addition, mushrooms, such as *Pleurotus eryngii* (Wang and Ng 2006b), *Lentinula edodes* (Nagai 2003), *Ganoderma lucidum* (Wang and Ng 2006a), *Clitocybe maxima* (Zhang et al. 2010), and *Pleurotus ostreatus* (Lettera et al. 2010) also show a significant superiority to degrade PAHs due to its ability to secrete extracellular enzymes (Mani and Kumar 2013), such as laccase, which could catalyze the oxidation of phenolic compounds and aromatic amines with molecular oxygen as the electron acceptor (Thurston 1994). However, to date, no studies have reported on the mycoremediation of mushrooms in heavy metal and PAH-polluted soil.

As for the detoxification, reactive oxygen species (ROS) could cause wide ranging damage to nucleotides, proteins, carbohydrates, fatty acids, and lipids and eventually lead to cell death through cellular oxidative stress response. Previous research has reported that fungi display several antioxidant enzymes against ROS, including superoxide dismutases (SOD), catalases (CAT), and peroxidases (POD) in heavy metal-polluted soil (Cao et al. 2012; Zhang et al. 2012b; Lin et al. 2006). Liu et al. also reported that *Arabidopsis thaliana* can produce oxidative stress response to PAH-contaminated soil (Liu et al. 2009). However, oxidative stress of mushrooms in co-contaminants soil (heavy metal and PAHs) is poorly understood and relative investigation is needed.

This study chooses Cd and pyrene as the heavy metal and PAHs pollutant, respectively. *Oudemansiella radicata* is widely planted in Chengdu and has high yield due to mature cultivation techniques, which has been demonstrated to bioaccumulate metal ions (Zhang et al. 2012a; Cao et al. 2012). The aims of this study are to investigate (1) the effect of Cd-pyrene combined contamination on growth of *O. radicata*, (2) the influence of the co-contaminants on uptake and translocation of Cd in *O. radicata* and the pyrene removal from the soil by *O. radicata*, and (3) oxidative stress response of *O. radicata* (several antioxidant enzymes against ROS, including SOD, CAT, and POD) to co-contaminants.

2 Materials and methods

2.1 Chemicals

Pyrene was purchased from Chengdu Kelong Corp, China with a purity of >98 %. $CdCl_2 \times 21/_2H_2O$ (analytical grade) was obtained from Chengdu Kelong Corp, China. All other chemicals and organic solvents were reagent grade.

2.2 Soil preparation

Soil samples used for the study were collected from Sichuan province, China. Soil samples were air dried and sieved to pass through a 0.2 mm sieve for removing foreign substance. Soil was spiked with pyrene at concentration of 0, 200 and 400 mg/kg by dissolving in acetone, which were first homogeneous mixed into 25 % of the soil samples. All treatments contained the same amount of acetone. After acetone had evaporated off, the level of Cd (0, 5, 15, 30 mg/kg) was prepared, fully mixed

with the remaining 75 % of soil, and stored 60 days for use. The basic properties of the soil are listed in Table 1.

2.3 Pot experiment

Five kilograms of soil samples was placed in each plastic pot. Meanwhile, 0.5 kg cultivate bag of *O. radicata* was purchased from Huike, a mushroom production site in Chengdu. The uniform mycelia together with the compost (taken out from the bags) were transplanted into each pot and cultivated in a greenroom in order to remain uniform in growth at the same temperature (20±1 °C). Tap water was added daily to reach the field water-holding capacity. At the bottom of each pot, there was a plastic dish to collect any potential leachate. All experiments were arranged in a completely randomized design with three replicates.

2.4 Analysis for cadmium

After about 4 weeks, the fruit bodies of *O. radicata* began to grow out and the whole harvest process for all the treatments of experiment lasted for about 5 weeks. *O. radicata* was harvested once the fruit bodies unfolded and washed with deionized water three times. Every fruiting body was cut into two halves. One half of the samples were oven-dried to a constant weight at 55 °C to determine the dry weight and the contents of heavy metals. The other half of the samples was quick-frozen in liquid nitrogen to detect antioxidant enzymes. The concentrations of Cd in the samples (soil and *O. radicata*) were determined by flame atomic adsorption spectrometry (VARIAN, SpectrAA-220Fs) (Cen et al. 2012). The digestion of mushroom sample (0.2 g) was carried out in teflon containers, where they were mixed with HNO_3 (6 mL) and H_2O_2 (2 mL), incubated 30 min in a microwave digestion system at ≈180 °C at 30 bar pressure, and finally diluted to 25 mL with deionized water for analysis. The digestion of soil samples (0.2 g) was also performed by microwave digestion with mixture of HNO_3 (6 mL), HF (2 mL) and $HClO_4$ (1 mL), The digested soil was then diluted to 50 mL with deionized water.

Table 1 The physicochemical properties of the soil used in the experiment

Parameter	Soil value
pH	7.5±0.04
Water-holding capacity (%)	11.52±0.24
Cation exchange capacity (CEC; cmol kg^{-1})	10.55±0.21
Organic matter (OM; g kg^{-1})	18.18±0.48
Total N (g kg^{-1})	1.12±0.04
Total P (g kg^{-1})	1.78±0.03
Total K (g kg^{-1})	16.41±0.19

Data expressed as means±SD (n=3)

In order to estimate the accumulation of Cd by the fruiting body of *O. radicata*, Bioconcentration factors (BCF) were calculated according to the formula described from Malinowska et al. (Malinowska et al. 2004):

$$BCF = Me_{fungi}/Me_{soil} \quad (1)$$

Where Me_{fungi} is the mean concentration of a certain metal in the fruiting body of the mushroom and Me_{soil} is the mean concentration of the metal in the soil.

If the BCF value of a metal is higher than 1.0, it means the fungi can accumulate the metal from the soil effectively.

2.5 Analysis for pyrene

Residual pyrene in soils was analyzed by the procedure described from Gao and Zhu (Gao and Zhu 2004) with some modifications. The soils from planted or non-planted pots were carefully collected, homogenized and finely sieved to pass through a 0.2 mm sieve. 2 g of soil sample was spiked with anhydrous Na_2SO_4 to remove moisture and submited to ultrasonication in 10 mL of dichloromethane for 1 h, followed by centrifugation at 4,000 rpm below 40 °C. Then 3 mL of supernatant was filtered through 2 g of silica gel column with 11 mL1:1 (v/v) elution of hexane and dichloromethane. The solvent fractions were then concentrated by evaporation of the dichloromethane under a stream of nitrogen, and the residue was dissolved in methanol with a final volume of 2 mL. After the mixture was filtered through a 0.22-mm filter, the treated soil and plant tissue extracts were analyzed by HPLC fitted with a 4.6×250 mm reverse phase C18 column using methanol–water (85:15) as the mobile phase at a flow rate of 1 mL/min. Chromatography was performed at 30 °C. Pyrene was detected at 238 nm.

2.6 Activities of antioxidant enzymes in *O. radicata*

The fresh samples (0.5 g) were quickly frozen in liquid nitrogen and grinded by a precooled mortar and pestle, and then extracted in 5 mL of 200 mM potassium phosphate buffer (pH 7.8) at 4 °C. The homogenate was centrifuged at 5500 rpm for 20 min, and the resulting supernatant was further centrifuged at 12,000 rpm for 30 min at 4 °C. Finally, the obtained supernatant was used for measuring the activities of antioxidant enzymes. The activity of SOD, POD, and CAT was determined by the method of Beauchamp and Fridovich (1971), Chance and Maehly (1955), and Montavon et al. (2007) with some modifications, respectively.

2.7 Posttreatment process

In this study, the posttreatment process were carried out in biogas digester. The fruit bodies of *O. radicata* were ground into powder as the material of biogas fermentation and were put into biogas digester. After fermentation completely, the method of chemical precipitation was applied to remove heavy metals in biogas slurry, then the biogas residue and precipitation containing heavy metals were concentrated landfill.

2.8 Statistical analysis

All treatments were replicated three times in the study. Results represented the mean with standard deviation (S.D.) using Microsoft Office Excel 2007. Statistical assays were carried out by one-way ANOVA using the Tukey HSD test to evaluate whether the means were significantly different, taking $p \leq 0.05$ as significant. These analyzes were performed with SPSS software.

3 Results and discussion

3.1 Growth response

The yield (means ± SD) and total Cd metal of the fruiting bodies from every potting group are listed in Table 2, and Cd content in fruiting bodies is shown in Fig. 1. One-way analysis of variance indicated that there is no apparent change in dry biomass among the treated groups. Many similar findings were reported for a broad range of species (Wang et al. 2012; Xu et al. 2011a). These results, however, were different to some previous reports. Chigbo et al. reported that the biomass of *B. juncea* tended to decrease under joint stress of Cu and pyrene and the effects were statistically significant (Chigbo et al. 2012). Besides, Zhang et al. reported that an addition of PAHs significantly increased the total biomass of *Juncus subsecundus* in Cd-polluted treatments compared with the Cd treatments only (Zhang et al. 2012c). Therefore, the growth response to co-contamination might be related to the plant species and the characteristics of pollutants. It seems that the addition of Cd and pyrene did not influence the growth of *O. radicata*, even in highly polluted treatments, indicating *O. radicata* can tolerate higher level of Cd and pyrene.

Table 2 Mushroom yield (dry weight), total Cd accumulation, and the calculated BCFs in the pot experiment

Cd added (mg/kg)	Pyrene added (mg/kg)	Yield (g/pot)	Total Cd accumulation (μg)	BCF
0	0	25.50±1.61	Not detected	Not detected
0	200	24.57±2.25	Not detected	Not detected
0	400	24.67±3.67	Not detected	Not detected
5	0	26.93±0.78	69.75±2.02a	0.52±0.03b
5	200	26.43±2.65	88.80±8.90a	0.67±0.07 cd
5	400	25.53±1.60	139.40±8.74b	1.09±0.12f
15	0	21.87±1.17	242.76±12.98c	0.74±0.04de
15	200	24.81±2.81	289.53±32.79d	0.79±0.10e
15	400	23.97±2.42	297.23±30.010d	0.82±0.03e
30	0	23.67±1.98	437.90±36.63e	0.62±0.06bc
30	200	25.30±1.55	404.04±24.75e	0.53±0.01b
30	400	22.40±1.59	242.37±17.20c	0.41±0.07a

Results are expressed as means±SD (n=3). Data within columns with different letters indicate a significant difference (Tukey HSD $p \leq 0.05$)

ND not detected

3.2 Cd bioaccumulation

The concentration of Cd in the fruiting bodies of *O. radicata* significantly influenced by the concentration of Cd, PAHs, and their interactions. In the absence of pyrene, the results of the BCF values (Fig. 1) first significantly increased in 15 mg/kg Cd, then decreased in 30 mg/kg Cd, about 142.31 % and 119.23 % higher than low Cd (5 mg/kg), respectively. When soil co-contaminated with Cd and pyrene, however, pyrene significantly influenced Cd concentration and accumulation, which depends on the various levels of Cd treatment. For example, in 5 mg/kg Cd in soil, BCF values increased following the pyrene concentration gradient of soil and even reached 1.09 (higher than 1.0 in treated groups) when 400 mg/kg of pyrene was added. While in 15 mg Cd/kg soil, values of BCF showed similar trend as the initial concentration of 5 mg/kg Cd mixed both levels of pyrene, whereas no significant difference was observed. But in high dose of Cd (30 mg/kg), the BCF values tended to decrease with an increase pyrene concentration, about 14.1 and 33.9 % reduction in soils co-contaminated with 200 and 400 mg/kg of pyrene compared with soils contaminated with 30 mg/kg Cd only. That is to say, in lower Cd-polluted soil, pyrene would increase the extracting efficiency of Cd, and in highly Cd-polluted soil, pyrene would decrease the extracting efficiency of Cd due to their complex interactions.

Cd, unlike pyrene, cannot be degraded and could be removed mainly by extraction. In co-contaminated soil, heavy metal accumulation and remediation depend on the concentrations and properties of heavy metal and PAHs, plant species, and soil conditions. For example, Lin et al. represented that Cu concentrations in maize increased with increasing Cu level in soil, but the ability of Cu phytoextraction would be inhibited under co-contamination of high level of pyrene in highly Cu-polluted soil (Lin et al. 2008). Zhang et al. reported that the soil combined with low Cd and low PAH lessened Cd toxicity to emergent wetland species, resulting in improving plant

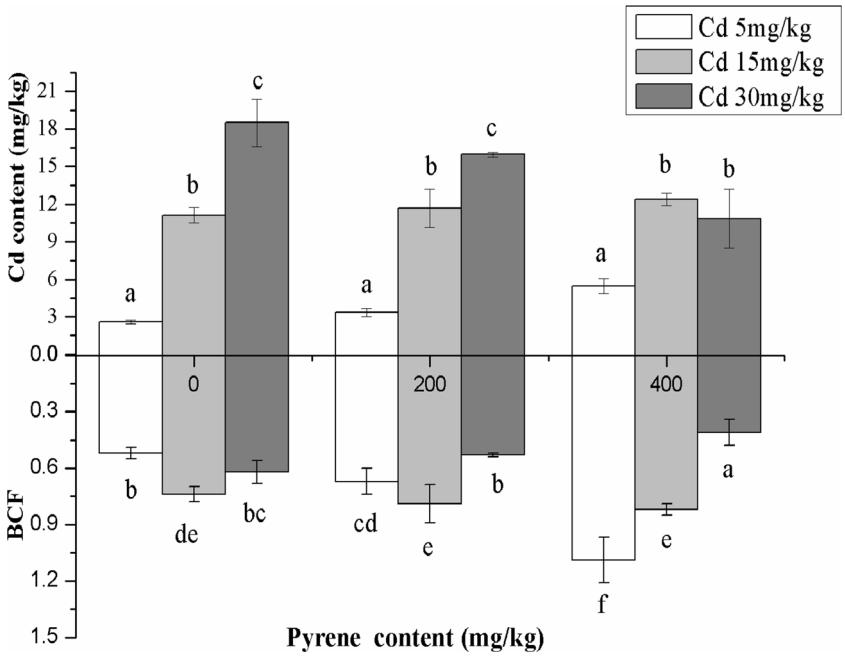


Fig. 1 Cd(II) accumulation and BCF in the fruiting body of *O. radicata* with different treatment. Average±standard deviation from three samples. *Different letters in the same column* indicate statistical difference (Tukey HSD $p \leq 0.05$)

growth and increasing Cd accumulation in plant tissues (Zhang et al. 2011). Almeida et al. reported that the accumulation of metal in salt marsh plants was significantly higher when 104 μg l^{-1} of Cu was mixed with PAHs (Almeida et al. 2008). However, Wang et al. showed that the presence of PAHs decreased the effects of Cd on plant biomass (*Sedum alfredii*) and Cd concentration, thus decreased Cd phytoextraction efficiency (Wang et al. 2012). Results presented in this experiment suggested that the fruiting bodies of *O. radicata* uptake of Cd enhanced with an increasing Cd concentration in the absence of pyrene. Meanwhile, pyrene, to some extent, could promote the accumulation of Cd in lower level of Cd and could inhibit the process in high level of Cd.

In this experiment, BCF values reached 1.09 (higher than 1.0) in 5 mg/kg Cd mixed with 400 mg /kg pyrene in soil and the levels of Cd in mushrooms showed significantly positive correlation with the Cd concentrations in soil, which indicated that *O. radicata* has well mycoremediation potential to Cd-polluted soil.

3.3 Dissipation of pyrene in soil

The concentrations of pyrene in soil after about 9 weeks are shown in Fig. 2. The residual concentrations of pyrene in the *O. radicata* planted soil were significantly lower than those in the unplanted soil. In 200 mg/kg pyrene and 0, 5, 15, and 30 mg/kg Cd-spiked soils, the removal ratios of pyrene was 87.2, 75, 64.9, and 86.7 % in *O. radicata*-planted soils, whereas in unplanted soil it was 64.8, 46.5, 53.8, and 64.7 %. In 400 mg/kg pyrene and 0, 5, 15, and 30 mg/kg Cd-spiked soils, the removal ratios of pyrene was 82.2, 72.1, 75.1, and 78.3 % in *O. radicata*-planted soils, whereas in unplanted soil, it was 57.8, 61.8, 62.6, and 64.1 %. The results indicated that the removal of pyrene was clearly aided or enhanced by planting the mushroom. The result was well in agreement with previous reports (Zhang et al. 2012b; Lu et al. 2010).

Figure 2 also shows the effect of Cd on the pyrene dissipation in the *O. radicata*-planted soil. Compared with the control, the residual pyrene increased when the soil was polluted with Cd, indicating that cadmium, in some extent, inhibited the degradation of pyrene. But in soils that were polluted by different concentrations of Cd, the residual pyrene tended to increase at the lower treatment concentrations of Cd (0, 5, 15 mg/kg) and decrease at high dose of Cd (30 mg/kg) as a whole. In 200 mg/kg pyrene-contaminated soil, the addition of 5 and 15 mg/kg of Cd significantly decreased the removal ratio from 87.2 to 64.9 % whereas increased to 86.7 % in 30 mg/kg Cd-polluted soil. In 400 mg/kg pyrene, with the increment of Cd level, the removal ratio first decreased to 72.1 %, then increased to 75.1 and 78.3 %, respectively. The result represented that higher-level dose of Cd, to some extent, could promote the dissipation of pyrene in co-contaminated soil and lower dose of Cd inhibited the dissipation of pyrene. What is more, both in 200 and 400 mg/kg pyrene-spiked soils, the removal ratio in unplanted soils also show the similar trend, implying that at high level of Cd, highly adapted Cd-resistant microbes might have promoted dissipation of pyrene.

In order to demonstrate whether highly adapted Cd-resistant microbes have the ability of promoting the dissipation of pyrene in planted soils. The study of dissipation of pyrene by microbes in polluted soils was conducted. The soil in each pot contaminated by Cd (0, 5, 15, and 30 mg/kg) and pyrene (200 mg/kg) was randomly and evenly sampled (5 g) of triplicates, followed by the addition of 100 mL of sterile

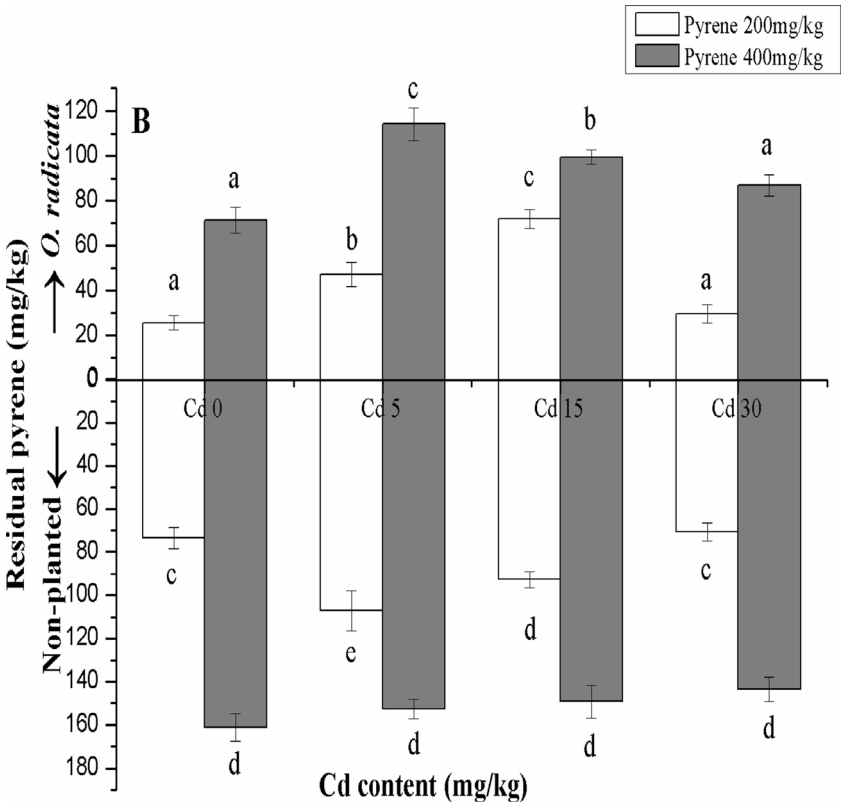


Fig. 2 Residual of pyrene in planted and non-planted soils after 9 weeks. Average±standard deviation from three samples. *Different letters in the same column* indicate statistical difference (Tukey HSD $p \leq 0.05$)

water in 250 mL Erlenmeyer flask, which was well mixed under aseptic conditions, then was shaken at 160 rpm and 37 °C for 24 h in the dark, finally was let stand for 10 min to obtain supernatant. The supernatants (2 mL) were then added into 150 mL MSM medium (K_2HPO_4 2 g, $(NH_4)_2SO_4$ 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.1 g, $FeCl_3$ 0.5 g, $CaCl_2$ 0.5 g) amended with pyrene of 200 mg/kg under aseptic conditions and cultured in 250 mL Erlenmeyer flasks, then were incubated at 37 °C on a rotary shaker at 160 rpm in the dark and the degradation rate was measured after 5 and 10 days, respectively. At the same time, the soil in each pot contaminated by Cd (0, 5, 15 and 30 mg/kg) and pyrene (400 mg/kg) was also randomly and evenly sampled (5 g) of triplicates, then the above steps were repeated, in addition to the MSM medium amended with pyrene of 400 mg/kg.

As Fig. 3a, b shows, in the concentration of 200 mg/kg pyrene, there is almost no obvious regularity of the difference on the microbial degradation after 5 days. But after 10 days, the removal of pyrene by soil microorganisms obtained in Cd-(0, 5, 15, and 30 mg/kg) and pyrene-polluted (200 mg/kg) soils increased with the increment of Cd concentration, and removal ratios were 84.8, 88, 93.2, and 93.8 %, respectively. In pyrene of 400 mg/kg, the removal of pyrene by soil microorganisms significantly increased when the soils spiked with Cd compared with control after 5 days. However, the trend of the removal ratios first decreased in 5 mg/kg Cd from 88.7 to 83.9 %, then increased to 88.2 and 91.4 % in 15 and 30 mg/kg Cd after 10 days. In general, both in 200 and 400 mg/kg pyrene, microbes in higher level of Cd could remove pyrene better than in lower level of Cd, implying that the highly adapted Cd-resistant microbes, to some extent, could promote the dissipation of pyrene.

In this study, the removal of pyrene was clearly aided or enhanced by planting *O. radicata*, which were consistent with much previous study. Lu et al. reported that the residual percentage for pyrene in planted soil with *Bidens maximowicziana* was 5–8 % and 17–24 % in unplanted soil (Lu et al. 2010). The results of Zhang et al. also reported that pyrene removal by *Scirpus triqueter* was higher in the rhizospheric soils (49.8–60.8 %) than that in the unplanted soils (17.5–41.6 %) (Zhang et al. 2012b).

The fates of PAHs in spiked soils include volatilization, leaching, photo-degradation (contaminated at the surface), plant uptake, biodegradation, and other abiotic losses (Sheng et al. 2009). The effect of heavy metal on dissipation of PAHs may be either positive or negative. The soil contaminated with Cd and pyrene represented that lower level of Cd inhibited the removal of pyrene and the higher level of Cd promoted the process in Cd-pyrene-polluted soil, which may be closely related to the highly adapted Cd-resistant microbes could promote the dissipation of pyrene.

Many similar results were also reported (Chigbo et al. 2012; Khan et al. 2009). However, Lin et al. shows that

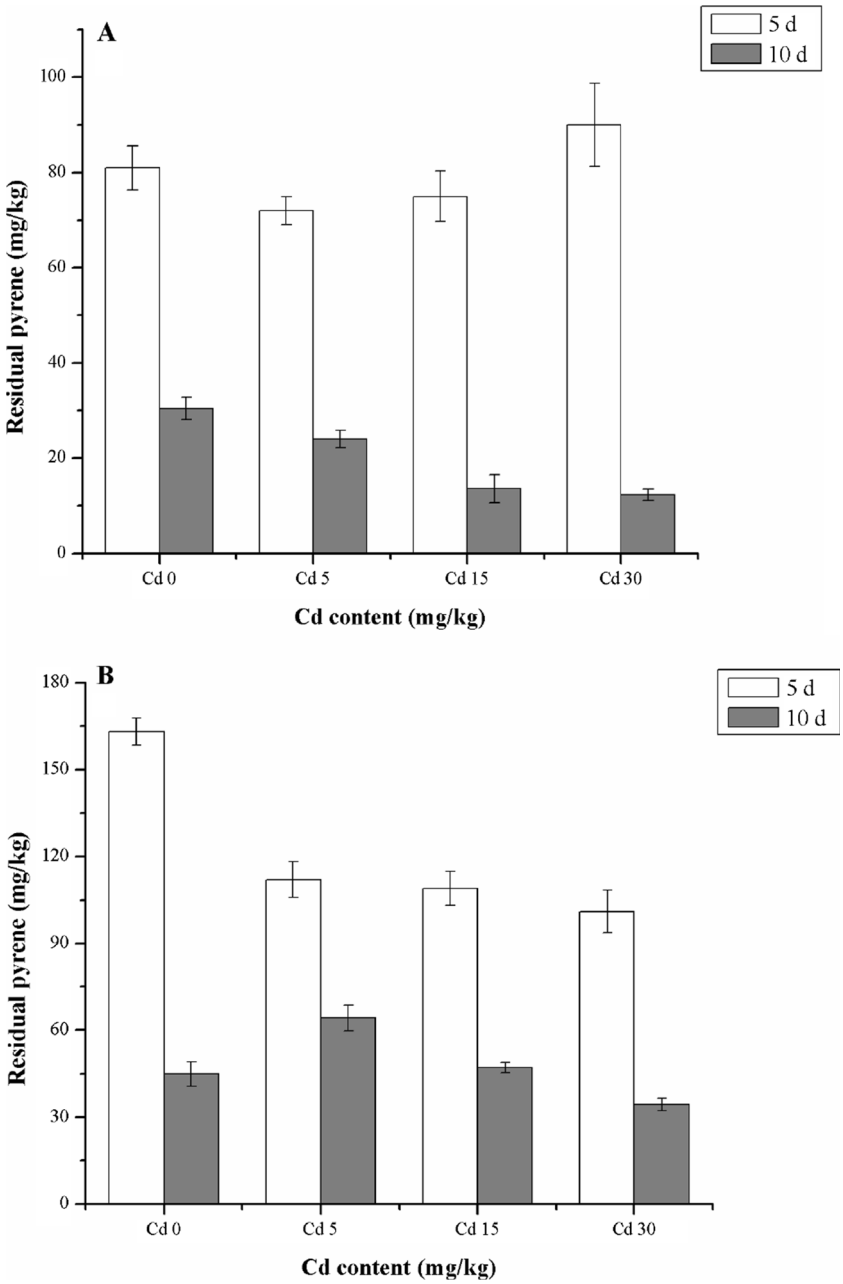


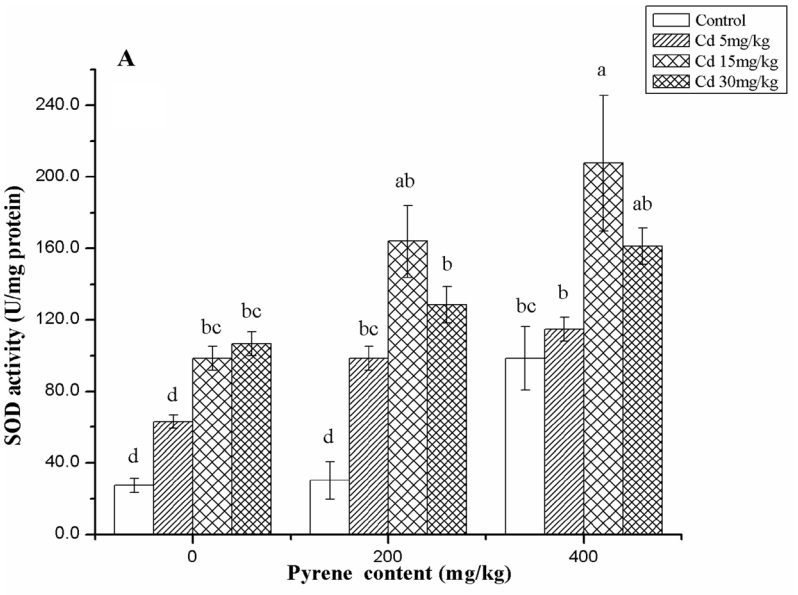
Fig. 3 Effect of Cd-resistant microbes on the removal of pyrene for short term (5 days) and long term (10 days) at initial pyrene concentration of 200 (**a**) and 400 mg/kg (**b**), respectively. Results are expressed as means± SD

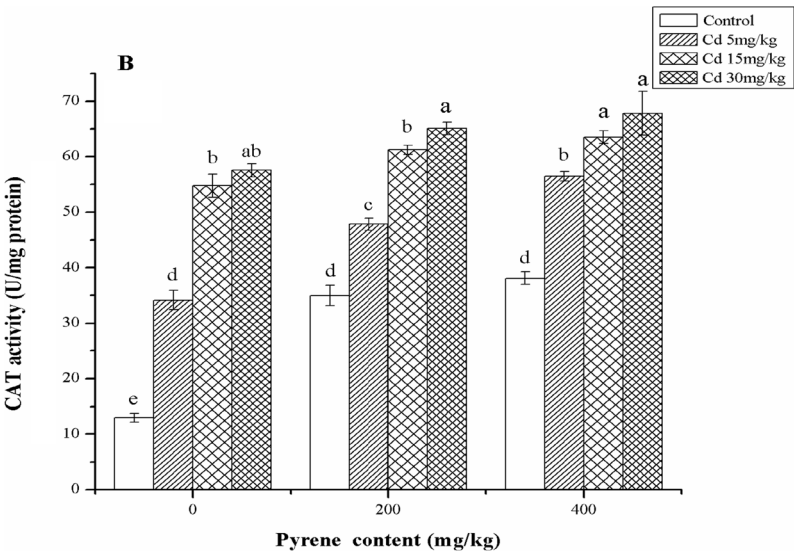
the soil with *Zea mays*, residual pyrene in the planted soil tended to increase with the increment of Cu level, was probably inhibited the dissipation of pyrene (Lin et al. 2008). Zhang et al. (2012c) presented that the dissipation of PAHs from soils with *Juncus subsecundus* was not significantly affected by Cd additions (Zhang et al. 2012c). This suggested that the microbial composition and microbial activity or the modified root physiology were changed under joint contaminated resulting in the difference on the pyrene removal. Since the mechanisms of the dissipation of pyrene mixed with Cd were complex, further studies need to be done.

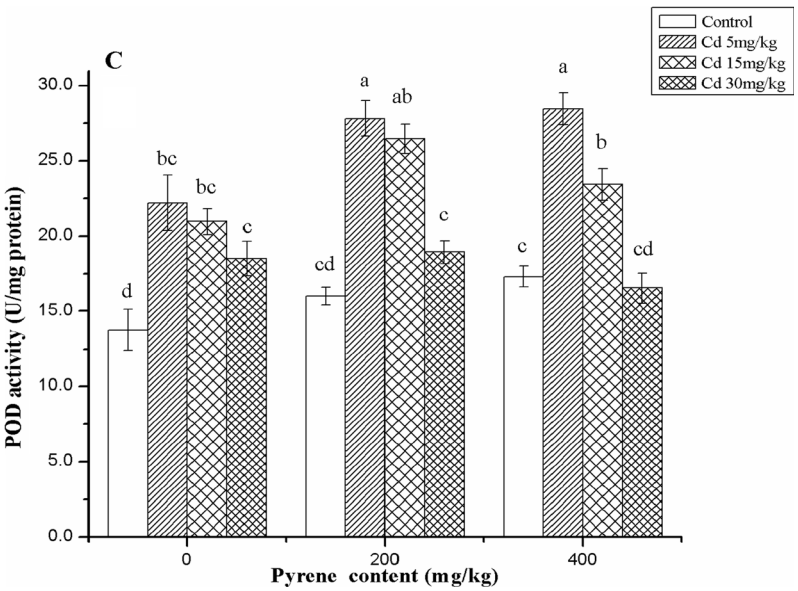
3.4 Antioxidant enzymes activities

As Fig. 4 shows, the joint contamination with Cd and pyrene had induced a strong antioxidative response in the fruiting body of *O. radicata*. SOD, an efficient scavenger of ROS, destroys the free superoxide by converting it to peroxide and oxygen, and then the peroxide can be degraded by CAT and POD (Bai et al. 2003).

SOD activity (Fig. 4a) represented significant increase under joint stress of Cd and pyrene in comparison with control and reached maximum when 15 mg/kg of Cd was mixed with 400 mg/kg of pyrene, about 598.54 % higher than control.


A
Control
Cd 5mg/kg
Cd 15mg/kg
Cd 30mg/kg
SOD activity (U/mg protein)
240.0
200.0
160.0
120.0
80.0
40.0
0.0
d
d
bc
bc
d
bc
ab
b
bc
b
a
ab
0
200
400
Pyrene content (mg/kg)


B
Control
Cd 5mg/kg
Cd 15mg/kg
Cd 30mg/kg
CAT activity (U/mg protein)
70
60
50
40
30
20
10
0
e
d
b
ab
d
c
b
a
d
b
a
a
0
200
400
Pyrene content (mg/kg)


C
Control
Cd 5mg/kg
Cd 15mg/kg
Cd 30mg/kg
POD activity (U/mg protein)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
d
bc
bc
c
cd
a
ab
c
c
a
b
cd
0
200
400
Pyrene content (mg/kg)

With the initial concentration of 0, 5, 15, and 30 mg/kg Cd in soil, the activity of SOD tended to increase with the increasing level of pyrene from 0 to 400 mg/kg in soil. On the contrary, both in pyrene of 200 and 400 mg/kg, SOD activities first increased in 0, 5, and 15 mg/kg, and then decreased at 30 mg/kg Cd, decreasing 21.6 and 22.3 % compared with 15 mg/kg of Cd, respectively.

CAT activity (Fig. 4b) kept increasing while the concentration of pyrene and Cd increased and the significant induction was observed at all concentrations in comparison with the control. The maximum activity of CAT was observed when 30 mg/kg of Cd was mixed with 400 mg/kg of pyrene, which was 523.38 % higher than control.

POD activity was more complex than SOD activity and CAT activity (Fig. 4c) and reached maximum at 5 mg/kg Cd and 400 mg/kg pyrene, about 206.88 % higher than control. As the figure shows, the activity of POD tended to increase with the increasing level of pyrene in comparison with the soil spiked with Cd of 0, 5, and 15 mg/kg alone, especially in pyrene of 200 mg/kg. With the same concentration of pyrene, however, POD activity first increased significantly at 5 mg/kg Cd, and then showed downward trend.

Heavy metal and PAHs are known to induce oxidative stress through over-production of reactive oxygen species (ROS) (Xu et al. 2011b; Liu et al. 2009; Fontes et al. 2013), and the increased activity of antioxidant enzymes can partly reduce oxidative stress (Fontes et al. 2013). In *O. radicata*, the main enzymes involved in these defense mechanisms are the ROS-eliminating enzymes, such as SOD, CAT, and POD (Cao et al. 2012), whose increase or decrease depended on contamination, concentration, and the tested species (Tian et al. 2011; Vega et al. 2013).

The results presented in this study showed that antioxidant enzymes (SOD, POD, and CAT) had various responses to Cd and pyrene. In general, SOD and POD increased at lower Cd concentrations and both concentration of pyrene, then declined with higher Cd concentrations, CAT increased with increase in concentration of all concentrations. As shown in Fig. 4, in the Cd co-contaminated soil, with the initial pyrene concentration of 0, 200, and 400 mg/kg, SOD activity, converting superoxide into peroxide, significantly increased with the increment of soil Cd level (0, 5, and 15 mg/kg) but decreased in the concentration of 30 mg/kg Cd. POD activity showed the similar trend as the SOD activities but decreased in 15 mg/kg Cd in soil. However, CAT activity continues to increase with the increment of Cd and pyrene. The increase in SOD activity owed to the increase in superoxide radical concentration, which might be the result of genes induction of SOD by superoxide-mediated signal transduction during biosynthesis of SOD. The reason for the decline may be inactivation of the enzyme by H_2O_2 or binding metal to the active centers of the enzyme (Liu et al. 2011; Liu et al. 2009). The increase of the CAT and POD activities might be due to the increase of the substrate produced by SOD or directly formed by biochemical pathways (Zhang et al. 2012a). The decrease of POD at high might be due to the toxic ROS load, which may have exceeded the capacity of the *O. radicata* antioxidant systems. Thus, CAT activity was stimulated under all conditions. SOD and POD activities could be activated under rather mild stress, while under acute stress, the activity decreased.

These results showed that increases in antioxidative enzyme activity induced in *O. radicata* was a defense against Cd-pyrene stress, indicating that *O. radicata* has the ability to cope with heavy metal and PAH stress, which is related to its ability to incite an efficient defense against oxidative stress to a certain extent. These results may contribute to a better understanding of the response mechanisms of macrofungi to heavy metal and PAH stress and to gain insights into heavy metal-PAH-fungi interactions in natural environments. What is more, in the experiment, significant decrease in SOD and POD activities were both observed in high level of Cd, showing that Cd might exert more effects compared with pyrene on cell damage.

◀ **Fig. 4** Effect of Cd and pyrene on SOD activity (**a**), CAT activity (**b**), and POD activity (**c**) in the fruiting body of *O. radicata*. Average± standard deviation from three samples. *Different letters in the same column* indicate statistical difference (Tukey HSD $p \leq 0.05$)

4 Conclusions

The results obtained in this study proved that *O. radicata* could remediate Cd and pyrene co-contaminated soils. In this study, there are no significant differences in the growth response of *O. radicata*, but the dissipation of pyrene from soil and accumulation of Cd in *O. radicata* might be affected by the co-contamination due to their interaction. It was observed that pyrene could promote the accumulation of Cd in the soil with lower concentration of Cd, and the BCF value even reached 1.09, but the value of BCF significantly decreased under co-contamination of 30 mg/kg Cd. The dissipation of pyrene from the planted soil was significantly enhanced in comparison with the unplanted soil. It was noticed that low level of Cd inhibited the removal of pyrene whereas higher level of Cd promoted the dissipation of pyrene in co-contaminated soil, indicating that highly adapted Cd-resistant microbes could facilitate the dissipation of pyrene. Generally, the activities of antioxidant enzymes in the fruiting body of *O. radicata* showed significant changes to against oxidative stress with the increment concentration of pollutants, especially the Cd added. *O. radicata* represented well a potential to serve as the bioaccumulator in the joint polluted soil for their short lifetime, metal tolerance, and bioaccumulation capacity.

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