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Rhizosphere characteristics of phytostabilizer *Athyrium wardii* (Hook.) involved in Cd and Pb accumulation

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ABSTRACT

Soil contamination with Cd and Pb shows interactive effects on rhizosphere microenvironment and further uptake of Cd and Pb by plants. Thus, the rhizosphere characteristics of the mining ecotype (ME) and non-mining ecotype (NME) of *Athyrium wardii* in response to Cd and Pb was investigated through a pot experiment in this study. Compared with Cd or Pb alone, the contamination of Cd and Pb inhibited the growth of the two ecotypes of *A. wardii*, and ME presented lower decrease of 56.22% and 55.07% for above-ground part biomass than NME. The combination of Cd and Pb promoted Cd accumulation both in above-ground and under-ground parts of ME, as well as Pb accumulation in under-ground parts of ME, with the increase of 92.68%, 102.77% and 35.87%, respectively. As a result, increased bioaccumulation coefficients (BCF) for Cd and Pb and decreased translocation factors (TF) for Pb of ME were observed when exposed Cd and Pb. ME presented much greater BCF values for Cd and Pb, and much lower TF values for Cd and Pb than NME. The rhizosphere soil pH of ME with the exposure of Cd and Pb reduced by 0.12–0.13 units compared with single Cd or Pb. The dissolved organic carbon (DOC) concentrations in rhizosphere soils of ME exposed to Cd and Pb increased in comparison with single Cd or Pb, and ME showed greater increase (53.31–59.00%) than NME. These suggested that pH reduction and exudation of greater amounts of DOC may have contributed to the promotion of Cd and Pb accumulation in ME. In addition, the combination of Cd and Pb inhibited soil microbial biomass carbon (MBC) and soil respiration (SR) of *A. wardii* compared with Cd or Pb alone. However, greater MBC and SR, and lower metabolic quotients were found in rhizosphere soils of ME when exposed to Cd and Pb. ME showed better soil biophysical conditions in rhizosphere soils when exposed to Cd and Pb. These improvements presented great benefit for ME to phytostabilize soils co-contaminated with Cd and Pb.

1. Introduction

Soil contamination with Cd and Pb resulted from anthropogenic activity, especially mining activities, is a serious and widespread problem (Li et al., 2014; Kim and Hyun, 2015; Shen et al., 2017). Cd and Pb, two persistent environmental hazards, are often present in soils concomitantly (Huang et al., 2016; Jia et al., 2016). High contents of Cd and Pb in mining areas easily induce environmental pollution that affects soil, plant and potential human health (Li et al., 2014; Kim and Hyun, 2015; Zhu et al., 2016; Shen et al., 2017). Phytostabilization, involving the use of metal tolerant plant species, shows great practice in remediation of soils contaminated with heavy metals in mining areas (Mendez and Maier, 2008; Bolan et al., 2011; Pérez-Esteban et al., 2014). It has been reported that some plant species, such as *Vetiveria zizanioides* and *Lupinus luteus*, showed potential for the phytostabilization of soils contaminated with Cd and Pb (Aibibu et al., 2010; Dary et al., 2010; Meeinkuirt et al., 2013). These phytostabilizers immobilize metals mainly through accumulation into roots and precipitation within the rhizosphere (Mendez and Maier, 2008; Bolan et al., 2011), thus the root-soil system is considered to be the key process for phytostabilization. Although some progress has been made towards an understanding of metal accumulation in plants for phytostabilization, the rhizosphere process associated with metal accumulation has not yet been fully elucidated among plant species for phytostabilization.

The rhizosphere is a dynamic microenvironment where plant root-soil-microbe interactions take place. These interactions alter soil components of the dynamic rhizosphere system, including heavy metals, pH, Eh, CEC, organic compounds and microorganisms, which in turn influence heavy metal availability and further uptake by plants (Gonzaga et al., 2009; Dessureault-Rompré et al., 2010; Zhang et al., 2016; Antoniadis et al., 2017). In especial, the single most important factor affecting heavy metal availability is soil pH. Lower rhizosphere soil pH was observed in the hyperaccumulating ecotype (HE) of *Sedum alfredii* grown in soils contaminated with Zn, as well as *Thlaspi*

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Table 1
Characteristics of the experimental soils after homogenization.

Soil characteristics	Treatment			
	CK	Cd25	Pb800	Cd25 + Pb800
TCd (mg kg^{-1})	nd	24.77 ± 0.13	nd	24.74 ± 1.62
TPb (mg kg^{-1})	9.74 ± 0.87	14.28 ± 1.38	811.74 ± 16.76	823.07 ± 7.09
ECd (mg kg^{-1})	nd	9.90 ± 0.32	nd	9.87 ± 0.14
EPb (mg kg^{-1})	11.87 ± 0.50	9.53 ± 0.32	382.17 ± 10.06	391.63 ± 8.74
pH	6.52 ± 0.03	6.55 ± 0.02	6.50 ± 0.02	6.57 ± 0.02
OM (g kg^{-1})	14.58 ± 0.98	15.50 ± 0.81	15.14 ± 0.56	15.71 ± 0.26
TN (g kg^{-1})	0.74 ± 0.07	0.72 ± 0.07	0.81 ± 0.08	0.80 ± 0.02
AN (mg kg^{-1})	68.91 ± 2.38	67.65 ± 3.54	75.30 ± 2.66	73.06 ± 4.04
AP (mg kg^{-1})	24.14 ± 3.01	21.22 ± 1.13	21.22 ± 1.13	24.94 ± 3.38
AK (mg kg^{-1})	47.08 ± 2.12	43.58 ± 2.83	48.08 ± 3.54	45.58 ± 4.24
DOC (mg kg^{-1})	82.65 ± 3.39	82.50 ± 0.99	83.18 ± 1.17	83.30 ± 3.04
MBC (mg MBC kg^{-1} dry soil h^{-1})	53.21 ± 1.43	50.49 ± 2.12	51.42 ± 1.30	53.41 ± 0.94
SR (mg CO_2-C g^{-1} dry soil h^{-1})	23.41 ± 1.35	22.05 ± 1.23	21.52 ± 2.11	23.30 ± 2.23

TCd, total Cd content. TPb, total Pb content. ECd, available Cd. EPb, available Pb. pH, soil/water, 1:2.5. OM, organic matter. TN, total nitrogen. AN, available nitrogen. AP, available phosphorus. AK, available potassium. DOC, dissolved organic carbon. MBC, microbial biomass carbon. SR, soil respiration. nd, not detected. Data are means ± standard deviation of four replicates.

caerulescens exposed to Cd and Zn, resulting in higher mobility of Cd and Zn and thus greater plant uptake (Wang et al., 2006; Li et al., 2011b; Antoniadis et al., 2017). The pH reduction in rhizosphere soils may be due to the secretion of root exudates, which is one of the main sources of soil dissolved organic carbon (DOC) (Gonzaga et al., 2009; Li et al., 2013b). DOC presented in root exudates is involved in metal activation by reducing soil pH and forming soluble chelate complexes with heavy metals (Gonzaga et al., 2009; Dessureault-Rompré et al., 2010; Montiel-Rozas et al., 2016). Increased DOC concentrations in soils were observed after the cultivation of plants, such as *T. caerulescens*, *S. alfredii* and *Thlaspi goesingense* (Knight et al., 1997; Wenzel et al., 2003; Li et al., 2011a). Increased DOC concentrations were also observed in rhizosphere soils of *S. alfredii* and *T. goesingense* exposed to Zn and Ni, respectively (Wenzel et al., 2003; Li et al., 2011a). These researches highlight that DOC in rhizosphere of hyperaccumulators plays an important role in heavy metal mobilization.

The enhanced mobility of heavy metals induced by DOC leads to increasing heavy metal availability in soils, which may affect soil microbial growth and activity. Numerous studies have shown that heavy metal stress had an inhibitive effect on soil microbial biomass and soil respiration (Shentu et al., 2014; Huang et al., 2016; Zhou et al., 2017). Significant increase of metabolic quotient (qCO_2) in plant rhizosphere soils with increasing heavy metal concentrations was also reported (Lu et al., 2013; Bian et al., 2015). However, this inhibitive effect may be mitigated by the increased organic compounds presented in root exudates in plant rhizosphere with the exposure of heavy metals (Wei and Twardowska, 2013; Huang et al., 2016). These organic compounds provided energy source for microorganisms for proliferation and enzyme synthesis. Thus, the microbial biomass and respiration improved significantly with plant cultivation, and was greater in the rhizosphere than the bulk soils (Huang et al., 2016, 2017; Zhou et al., 2017).

The mining ecotype (ME) of *Athyrium wardii* (Hook.), originally growing in an old Pb-Zn mine in Yingjing, Ya'an, Sichuan Province, PR China, was found to show larger root system, greater accumulation of Cd or Pb in underground parts and lower translocation factors than the non-mining ecotype (NME), thus being a promising candidate for phytostabilization of soils contaminated with Cd or Pb (Zou et al., 2011; Zhang et al., 2012). Although previous works have investigated the rhizosphere process associated with Cd or Pb accumulation in *A. wardii* exposed to single Cd or Pb (Zou et al., 2011; Zhang et al., 2014; Zhao et al., 2016), information about rhizosphere process in relation to Cd and Pb accumulation in *A. wardii* exposed to Cd in combination with Pb has not been reported. Thus, the changes of pH, DOC, microbial growth and activity in rhizosphere soils of *A. wardii* involved in Cd and Pb accumulation, with the contamination of Cd and Pb, was investigated in present work.

2. Materials and methods

2.1. Plant and soil preparation

For the two ecotypes of *A. wardii*, ME was collected from a Pb-Zn mine area in Yingjing, Ya'an, Sichuan Province, PR China (102°31′E, 29°47′N), and NME was obtained from Yucheng, Ya'an, Sichuan Province, PR China (102°51′ − 103°12′E, 29°40′ − 30°14′N) (Zhao et al., 2016). The ferns were separated into similar size, cultivated in vermiculite media and transplanted into pots as previously described by Zhao et al. (2016).

Soils consisted of calcareous alluvial soils and humic substances. Calcareous alluvial soils were obtained from a farmland uncontaminated with Cd and Pb in Dujiangyan, Sichuan Province, PR China, air-dried and sieved through 2-mm mesh screen before further use. Humic substances derived from mature plant litter were obtained from the Lingyan Mountain in Dujiangyan, Sichuan Province, PR China, and then mixed thoroughly with calcareous alluvial soils and prepared for the pot experiment (Zhan et al., 2016).

2.2. Pot experiment

The treatments composed of one control (CK), 25 mg kg^{-1} Cd (Cd25), 800 mg kg^{-1} Pb (Pb800) and a combination of 25 mg kg^{-1} Cd + 800 mg kg^{-1} Pb (Cd25 + Pb800), with four replicates for each treatment. Sufficient amount of NH_4NO_3 were used as a supplement to balance the supply of nitrogen. Each pot (5 L) was filled with 5 kg of soils prepared before. Pot soils were then mixed thoroughly with Cd and/or Pb solutions, and homogenized for 30 days (Zhang et al., 2012; Zhao et al., 2016). The characteristics of the homogenized soils were presented in Table 1. After homogenization, healthy and uniform plants were transplanted into pots with two plants per pot, resulting in 32 plants for each ecotype in total. The pot experiment was performed in an experimental station of Sichuan Agricultural University in Dujiangyan, Sichuan Province, PR China, with natural light. A total of 32 pots were arranged randomizedly and interchanged positions regularly to get uniform illumination. During the experiment, all plants were watered with deionized water to maintain soils with approximate 70% of field capacity.

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