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REGULAR ARTICLE

Main rhizosphere characteristics of the Cd hyperaccumulator *Rorippa globosa* (Turcz.) Thell

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Abstract
Aim This article was aimed to explore the main rhizospherial properties of the Cd hyperaccumulator *R. globosa* compared to those of the non hyperaccumulator *Rorippa palustris* (Leyss.) Bess. representing the same genus (*Rorippa*) of Cruciferae.
Method Pot culture experiments using soil spiked with Cd as $CdCl_2 \cdot 2.5H_2O$ and rhizobags were conducted to determine the differences in Cd accumulation *vs*. pH, dissolved organic carbon (DOC), Cd chemical fractionation, enzyme activities, and microorganism number in the rhizospheres of *R. globosa* and *R. palustris*, and in the bulk soils.
Results Experiments on Cd uptake by *R. globosa* and *R. palustris* from soil spiked with different doses of Cd ranging from 0 to 40 mg kg^{-1}, confirmed Cd-hyperaccumulating properties of *R. globosa* (Cd accumulation in the above-ground organs >100 mg kg^{-1}, enrichment factor EF> 1, translocation factor TF> 1, no significant biomass reduction at Cd doses >10 mg kg^{-1}) and the lack of such properties in *R. palustris*, which made these species suitable for comparative studies. The pH value was found to be a constant, specific property of the rhizosphere of *R. globosa* and *R. palustris*, and of the bulk soil, independent on the Cd dose, however the differences were rather small: by 0.2 unit lower in the rhizosphere of *R. globosa,* and only by 0.1 unit lower in the rhizosphere of R.. *palustris* compared to the bulk soil. Chemical fractionation of Cd, i.e. its affinity to pools of different binding strength, also appeared to be a specific feature of a rhizosphere and soil independent on the Cd dose. It exhibited a unique capability of the rhizosphere of the Cd-hyperaccumulator *R. globosa* to mobilize Cd, which enriched the most labile exchangeable fraction in 24.4 % and the immobile residual fraction in 42.3 %, compared to 19.3 % and 50.8 % in the bulk soil and in the rhizosphere of the non-hiperaccumulator *R.palustris* that did not show significant difference ($p<0.05$) from the bulk soil. In turn, DOC concentrations, enzymatic (urease and catalase) activity and microorganism (bacteria, fungi and actinomycetes) growth in rhizosphere soils were largely influenced by different Cd doses, although they were always considerably higher in the rhizosphere soils of *R globosa*, than in the rhizosphere of *R. palustris* and in the bulk soil, in particular at Cd doses ≥10 mg kg^{-1}.
Conclusion pH and DOC changes in the rhizosphere of the Cd-hyperaccumulator *R. globosa* were found to be of a minor importance. The alteration of Cd chemical fractionation consisting in substantial reduction of the immobile residual pool and Cd enrichment primarily in the most labile exchangeable fraction, along with over

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2-fold higher number of microorganisms was considered to be the driving force of Cd hyperaccumulation.

Keywords Cd hyperaccumulation · Cd hyperaccumulator *Rorippa globosa* (Turcz.) Thell · Non-hyperaccumulator *Rorippa palustris* (Leyss.) Bess · Specific rhizosphere properties

Introduction

Phytoremediation of soils polluted by trace elements with the use of hyperaccumulator plants having extraordinary ability to accumulate trace elements has been developing since the mid-1970s of the 20th century as an environmentally attractive and cost-effective alternative to traditional invasive and expensive techniques (e.g. topsoil exchange, washing, chemical leaching etc.). Phytoextraction is considered to be the most promising technique, in particular with respect to large areas of moderately polluted soils. Hyperaccumulation implies a higher trace element accumulation than in non-accumulator plants by active accumulation of element(s) via the roots in above-ground tissues at a level that is 2–3 orders of magnitude higher than in normal soils and at least one order of magnitude greater than in metalliferous soils (van der Ent et al. 2013). For practical operational purposes, the hyperaccumulation criteria can be summarized as follows: (1) element accumulation threshold, i.e. the minimum concentration in the shoots of a hyperaccumulator for different elements should be greater than 10,000 mg kg^{-1} for Mn, 3,000 mg kg^{-1} for Zn , 1,000 mg kg^{-1} for Ni, As, and Pb, 300 mg kg^{-1} for Cu, Co and Cr, and 100 mg kg^{-1} for Cd, Se and Tl (after Baker and Brooks 1989, modified by van der Ent et al. 2013); (2) translocation factor TF, i.e., shoot-to-root quotient of element concentrations should exceed 1 (TF> 1) (Chaney et al. 1997); (3) enrichment factor EF, i.e. shoot-to-soil (or other media) ratio of element concentrations should be higher than 1 (EF> 1) (Wei et al. 2005); and (4) tolerance criterion, i.e. high tolerance of a plant to trace elements. Besides, shoot biomass of hyperaccumulator plants should not decrease significantly when growing in contaminated media (Wei et al. 2012). Both high accumulation and high biomass are of particular importance in phytoextration, as the removal efficiency of a trace element by a hyperaccumulator plant is equal to the product of these values.

Cd is one of the most problematic trace elements. It is a non-essential element of a high ecotoxicity, showing adverse effects on humans, animals and plants. According to Kabata-Pendas (2010), its world average content in soil is estimated to be 0.41 mg kg^{-1}, but in natural soils it ranges from <0.01 up to 0.8 mg kg^{-1}, and in some regions up to 2.0–8.9 mg kg^{-1}. In anthropogenically contaminated soils in the world, the reported Cd concentrations range from 0.6 to 1,781 mg kg^{-1}, but most do not exceed 50–100 mg kg^{-1}. Although the average background concentrations of Cd in food plants, as well as in grasses and legumes are fairly low (0.001–0.4 mg kg^{-1}, d.m. and 0.07–0.46 mg kg^{-1}, d.m., respectively) in contaminated sites Cd contents in plant above-ground tissue may be as high as 0.5–22.8 mg kg^{-1}, d.m. (Kabata-Pendas 2010). The Cd concentration of the worldwide "standard reference plant" is 0.05 mg kg^{-1}, d.m. (van der Ent et al. 2013).

Hyperaccumulating ability for Cd has been confirmed experimentally since 1994 jn several plant species, notably in *Thlaspi (Noccaea) caerulescens, Arabidopsis halleri, Viola baoshanensis, Solanum nigrum, Rorippa globosa, Sedum alfredii* (after van der Ent et al. 2013) or recently in *Amaranthus hybridus* (Zhang et al. 2010), These plants have great potential to be used for phytoremediation of Cd-contaminated soils, however, the mechanisms responsible for hyperaccumulation of Cd have not yet been fully understood (Su et al. 2013). Studies on hairy roots of the Cd hyperaccumulator *T. caerulescens* revealed storage of Cd in the root wall fraction and 7–10 days' delay in transmembrane uptake, which was presumed to be a defensive strategy, allowing time for activation of intracellular mechanisms for Cd detoxification (Nedelkoska and Doran 2001). The hydroponic experiments of Lombi et al. (2001) on Cd uptake and translocation in two contrasting ecotypes of *T. caerulescens* demonstrated existence of high-affinity Cd transporter(s) in the root cell plasma membranes of the hyperaccumulator ecotype, whereas no differences in sequestration of Cd in root cell vacuoles or in the efficiency of Cd translocation from roots to shoots has been observed. Apart from the need for studies of Cd uptake at a molecular level to characterize the Cd transporter(s), these experiments draw attention to rhizosphere processes that determine the ability of a plant to scavenge trace elements and, consequently, might screen for affinity of a plant to hyperaccumulate Cd. The role of the rhizosphere as the root-soil interface and the micro-ecosystem where roots access trace elements is difficult to underestimate.

Simultaneously, the most recent reviews of trace element phytoextraction underline a general lack of ecological understanding with respect to rhizosphere processes of hyperaccumulation (Wenzel 2009; Alford et al. 2010; Sessitsch et al. 2013).

So far, some rhizosphere mechanisms of trace element hyperaccumulation in plants have been explored, although the role of rhizosphere processes in the phytoextraction of trace elements is not yet satisfactorily elucidated and reviewed (Wenzel 2009; Alford et al. 2010; Sessitsch et al. 2013). Most of these studies were conducted on Ni, Zn, or As hyperaccumulators such as *T. goesingense, Pteris vittata, Sedum alfredii* and *T. caerulescens* (also regarded as a Cd hyperaccumulator) (e.g. McGrath et al. 1997; Whiting et al. 2001; Wenzel et al. 2003; Gonzaga et al. 2009; Wenzel 2009; Alford et al. 2010; Li et al. 2011a, b). Studies on the rhizosphere processes associated with Cd hyperaccumulation are much more limited, and need to be intensified, in particular that uptake and translocation of different trace elements may show marked differences not only between ecotypes, but within the same ecotype of a hyperaccumulator species, as was revealed for Zn and Cd uptake and translocation by *T. caerulescens* (Lombi et al. 2001). There are many processes in the rhizosphere that may influence trace element uptake, including root growth and scavenging properties, rhizosphere chemistry and activity of rhizosphere microorganisms. In a hydroponic experiment, Zhao et al. (2001) found that root secretion of *T. caerulescens* do not enhance Cd mobilization. However, Hammer and Keller (2002) reported that the roots of *T. caerulescens* mobilized Cd in rhizosphere soil. As for now, there is no univocal agreement regarding the unique capability of the root activity of hyperaccumulators to change rhizosphere chemistry and to mobilize trace elements in soil, and there are studies both confirming (e.g. Wenzel et al. 2003; Kidd et al. 2007; Gonzaga et al. 2009; Li et al. 2011a, b) or, in contrast, showing no indication of such property (these contradictions were discussed in the review by Alford et al. 2010).

Hyperaccumulation of Cd by *T. caerulescens* and its higher tolerance to this metal might lie in the strong anti-polarization role of its root membrane (Boominathan and Doran 2003). In a field experiment, Keller et al. (2003) suggested that the higher Cd accumulation capacity of *T. caerulescens* compared to several other plants might be related to a higher ratio of root density to shoot biomass. Qiu et al. (2008) found that the roots of the Cd hyperaccumulator *A. paniculata* had a strong antioxidative system, which might allow the plant to tolerate otherwise toxic levels of Cd. Rhizosphere chemistry is an important part of metal accumulation, in particular pH and dissolved organic carbon (DOC). DOC may have different functions in different hyperaccumulator plant species (Alford et al. 2010). Li et al. (2011a, b) reported decrease of pH and increase of DOC in the rhizosphere of the Zn and Cd hyperaccumulator *Sedum alfredii*, which could significantly reduce metal sorption and increase their mobility. The form of nitrogen was also found to have a noticeable effect on the rhizosphere dynamics and uptake of Cd and Zn by *T. caerulescens* (Xie et al. 2009). Other rhizosphere processes that enhance trace elements uptake by plants can be attributed to microbial activity (Sessitsch et al. 2013). Some rhizosphere bacteria might increase the accumulation of Cd in *T. caerulescens, S. alfredii* and other hyperaccumulator plants (Aboudrar et al. 2007; Xiong et al. 2008; He et al. 2009; Karimzadeh et al. 2012). Although there are a number of other studies on rhizosphere mechanisms of Cd hyperaccumulation, not many of them were focused on rhizosphere environmental characteristics (Alford et al. 2010; Dessureault-Rompré et al. 2010). Besides, the majority of studies so far were conducted on the Zn/Cd hyperaccumulator *T. caerulescens,* which represents a model metal hyperaccumulator species (Lombi et al. 2001). As can be derived from the available reports, mechanisms, associated processes and specifics of metal uptake and translocation may differ substantially in different hyperaccumulator species and even of different ecotypes of a hyperaccumulator plant (Lombi et al. 2001). So far, current information on rhizosphere conditions is based on a very limited subset (>10 %) of known hyperaccumulators (Alford et al. 2010). For these reasons, several new Cd hyperaccumulator plants require thorough studies to evaluate and find viable solutions to enhance their phytoremediation potential. This needs a sound understanding of the processes and interactions occurring in their rhizosphere. A promising Cd hyperaccumulator is *Rorippa globosa* (Turcz) Thell.

R. globosa is a newly found Cd hyperaccumulator identified from 13 weed species through pot culture, concentration gradient, and pollution field experiments (Wei et al. 2008). Some mechanisms of Cd hyperaccumulation by *R. globosa* have been explored in studies of organic acids and non-protein thiols in leaves, and root morphology of *R. globosa* compared

to those of the non-hyperaccumulator *Rorippa palustris* (Leyss.) Bess., which is in the same genus (*Rorippa*) of Cruciferae. (Sun et al. 2011; Wei et al. 2012). *R. palustris* is a weed species with similar leaf type, color, plant height and root morphology to *R. globosa*. It is very difficult to distinguish them at their seedling stages. *R. globosa* and *R. palustris* grow in the same environment, but the physiological similarities and differences of these two species are not known. The aim of this comparative study was to explore the main rhizosphere characteristics of the Cd hyperaccumulator *R. globosa* versus the same characteristics of the non-hyperaccumulator *R. palustris* as a step towards its field application for Cd phytoremediation under complex and heterogeneous environmental conditions.

Material and methods

Soil

Soil used in the study was meadow burozem collected from the field of the Shenyang Ecological Experimental Station, Chinese Academy of Sciences (41°31' N and 123°41′ E) from the surface (0–20 cm), air dried and sieved through a 4 mm stainless steel sieve. Basic characteristics of soil used in all experiments within this study are presented in Table 1.

Plant seedling culture

All seeds of *R. globosa* and *R. palustris* were collected from the field of the Shenyang Ecological Experimental Station, Chinese Academy of Sciences (41°31′ N and 123°41′ E) and sterilized by 0.1 % of NaClO. After washing with tap water, seeds of the same size were sown to a seedling pot filled with soil (Table 1). After the seeds germination in a glasshouse, seedlings 4 cm high were selected for soil pot experiments.

Table 1 Basic physico-chemical properties of soil used in the experiments

Item	Soil sample	Item	Soil sample
pH	6.7	Total-N (g kg^{-1})	0.89
CEC(cmol kg^{-1})	23.7	Available-P (mg kg^{-1})	12.46
Clay (%)	22.1	Available-K (mg kg^{-1})	81.02
Silt (%)	43.4	TOC (g kg^{-1})	15.2
Sand (%)	34.5	Total-Cd (mg kg^{-1})	0.15

Soil pot experiments

For pot culture experiments on *R. globosa* and *R. palustris*, a six –step concentration gradient of Cd in the soil (Table 1) was employed: control (Cd0) without Cd addition (with background Cd concentration 0.19 mg kg^{-1}), and treatments T_1-T_5, with 2.5, 5, 10, 20 and 40 mg Cd kg^{-1}soil (Cd2.5, Cd5, Cd10, Cd20, Cd40), respectively. The soil samples 2.5 kg (dry mass) each, spiked with $CdCl_2 \cdot 2.5H_2O$ solution according to the designed treatments, were, filled in plastic pots (φ= 20 cm, H=15 cm), and equilibrated for 2 months. In the pot experiments, a rhizobag technique was applied. The rhizobag was a cylindrical water-permeable nylon 500 mesh bag (φ=6 cm, H=15 cm) used to keep the rhisosphere soil separate from the non-rhisosphere. It also kept the roots intact and facilitated easy washing. Each rhizobag was filled with 500 g (dry weight) soil treated in accordance with the designed scheme. Outside the rhizobag, the same soil was placed in the pot to the same level. For each treatment, six seedlings were transplanted into a rhizobag of each of three replicate pots. All the pots were randomly relocated to a temperature-controlled glasshouse (20±5 °C). A 14-h photoperiod with a daily photosynthetic photon flux of 350 mmol m^{-2} s^{-1} was supplied by cool-white fluorescent lamps. Loss of water was replenished using tap water to sustain 80 % of soil water-holding capacity. The plants were planted in spring (April 2012) and harvested after 46 days of growth.

Sample analysis

Plant samples were separated into roots, stems and leaves, then rinsed with tap water and carefully washed with deionized water. Next, these samples were oven dried at 105 °C for 5 min, after that at 70 °C for about 2 days until becoming completely dry. About half of stems and leaves were mixed together to examine Cd concentration in shoots. The dried plant samples were ground to a powder and passed through a 0.3 mm sieve. For determination of total metal concentrations, plant samples were digested using mixed acids (87 % of HNO_3 and 13 % of $HClO_4$) and analyzed for Cd using an atomic absorption spectrophotometer (AAS, Hitachi 180–80 with a 1.3 nm spectral band width). For QA/QC, the measured values of Cd were

verified by using certified standard reference material (NIST SRM 1547, peach leaves). The enrichment factor (EF) was calculated as a ratio of Cd concentration in plant to that in soil, and the translocation factor (TF) was assessed as a ratio of Cd concentration in shoots to that in roots (Wei et al. 2012).

The soil in rhizobags adhering to roots, which was shaken off from the roots, was considered to be rhizosphere soil. The bulk soils were collected outside from the rhizobags at about 1 cm distance from their sides. Soil pH values at soil: water ratio=1:2.5 were measured with a pH meter (PHS-3B). For quantification of dissolved organic carbon (DOC) in soil, a method described in detail by Jones and Willett (2006) was used. In brief, it consisted in centrifugation of soil extracts (1:10 *w/v* soil to solution ratio) at 8,000×*g* for 10 min in Eppendorf 5804R centrifuge and analysis of DOC in supernatant using TOC-5050A analyzer (Shimadzu, Japan). Results were calculated as relative DOC concentrations per soil mass unit.

Chemical fractionation of Cd in soils was performed following the sequential extraction procedure by Tessier et al. (1979), which differentiates between exchangeable, carbonate, hydrous Fe-Mn oxide (reducible), organic matter (oxidizable) and residual fractions (Morera et al. 2001). Cd concentrations were determined using AAS (Hitachi 180–80). Phenol-NaClO colorimetric analysis method was used to determine soil urease activity (Liang et al. 2003). Catalase activity was measured by back-titrating residual H_2O_2 with $KMnO_4$ (Stepniewska et al. 2009). The number of cultivable microorganisms was estimated by viable count on serial spread plates (Liang et al. 2003).

Data processing

Data mean values and standard errors were calculated using Microsoft EXCEL. The results were analyzed using one-way ANOVA and Duncan's multiple range tests to separate means with the use of SPSS software. Differences were considered significant at the $p<0.05$ level (Wei et al. 2012).

Results

Cd -accumulating characteristics of *R. globosa vs R. palustris*

As shown in Table 2, at Cd treatment of soil ≥10 mg kg^{-1}, the Cd concentrations in stems, leaves and shoots of *R. globosa* were in all cases markedly above 100 mg kg^{-1} that is the hyperaccumulator threshold value for Cd. The TF and EF values for Cd in *R. globosa* all exceeded 1, confirming its properties as a Cd hyperaccumulator. Both above-ground and below-ground organs (roots) of *R. globosa* showed a progressive increase of Cd enrichment in parallel with the increase of Cd dose. Other experiments (Wei et al. 2012) showed no significant decrease ($p<0.05$) of *R. globosa* root and shoot biomasses at Cd treatments in a range from 0 to 20 mg kg^{-1}, which indicated its strong tolerance to Cd. Thus, *R. globosa* demonstrated typical concentrations, EF, TF values and tolerance properties of a Cd hyperaccumulator.

In contrast, although EF values for Cd in *R. palustris* were higher than 1, Cd concentrations in its stems, leaves and shoots were markedly lower than those in *R. globosa* and were either below or at the hyperaccumulator threshold 100 mg kg^{-1} for Cd treatments ≥20 mg kg^{-1}. This resulted in translocation factor TF values <1 for Cd treatments ≥10 mg kg^{-1}. Besides, another experiment showed significant decrease ($p<0.05$) of *R. palustris* root and shoot biomasses, indicating its weak tolerance to Cd (Wei et al. 2012). These properties qualify *R. palustris* as non-hyperaccumulator.

Thus, the Cd hyperaccumulator *R globosa* and the non-hyperaccumulator *R. palustris* as species of the same genus offer valuable opportunity for comparative studies aiming to identify unique properties and similarities of non-essential metal hyperaccumulators compared to non-accumulating plant species, and to make a step towards elucidation of their hyperaccumulation mechanisms and tolerance to Cd.

Changes of pH in the rhizosphere and the bulk soil

In general, rhisosphere pH values of both species, as well as that of the bulk soil, were fairly stable and did not show any significant differences ($p>0.05$) between different Cd treatments. No significant differences between pH values (similarly to other measured values in the experiment) were also recorded for all bulk soil samples, therefore data for the bulk soil from *R. globosa* and *R. palustris* pots were interpreted as the same bulk soil analyzed in six replicates (Fig. 1). The pH values of *R. globosa* were 6.44–6.47 and the rhizosphere pH of *R. palustris* was 6.53–6.59. The rhizosphere pH of *R. globosa* was thus by 0.1 unit lower than that of *R. palustris*. The pH of bulk soil ranged from 6.62 to 6.68. Compared to the bulk soil,

Table 2 Cd accumulative characteristics of the rhizosphere of *R. globosa* and *R. palustris* and of the bulk soil (mg kg^{-1})

Plant	Treatment	Root	Stem	Leave	Shoot	EF	TF
R. globosa	Cd0	0.7±0.02	0.9±0.03	1.1±0.04	1.0±0.05		
	Cd2.5	36.3±1.64	33.9±1.46	50.9±2.17	45.6±2.13	18.2	1.3
	Cd5	57.5±2.86	36.1±2.75	88.8±3.94	68.3±3.06	13.7	1.2
	Cd10	101.8±6.02	114.4±4.79	130.5±5.38	116.8±5.48	11.7	1.1
	Cd20	134.6±6.89	137.0±5.72	152.0±5.79	141.6±6.46	7.1	1.1
	Cd40	189.7±8.51	189.1±6.08	223.0±6.21	196.3±7.83	4.9	1.0
R. palustris	Cd0	0.6±0.03	0.8±0.04	1.1±0.06	0.9±0.03		
	Cd2.5	23.6±1.12	30.2±1.38	34.7±1.27	32.5±1.28	13.0	1.4
	Cd5	46.6±2.37	58.5±2.07	63.6±3.18	60.4±3.26	12.1	1.3
	Cd10	101.3±6.53	87.2±3.28	96.8±6.84	90.3±5.17	8.7	0.9
	Cd20	126.3±7.21	91.7±4.01	100.8±4.37	93.9±4.09	4.7	0.7
	Cd40	179.9±8.06	97.9±4.75	105.3±4.86	100.8±5.21	2.5	0.6

(Cd treatments Cd0, Cd2.5, Cd5, Cd10, Cd20 and Cd40 mean spiked doses 0, 2.5, 5, 10, 20 and 40 mg kg^{-1}, respectively)

the mean pH value in the rhizosphere soil of *R. globosa* was roughly 0.2 unit lower, and that of *R. palustris* was 0.1 unit lower (Fig. 1).

Effect of the rhizosphere on DOC

In the bulk soils of *R. globosa* and *R palustris,* DOC concentrations differed insignificantly ($p>0.05$). Thus the bulk soil of both plants was interpreted as one sample with six replicates. (Due to insignificant differences, the same interpretation was applied with respect to all other parameters measured in bulk soil). Moreover, DOC concentrations in bulk soils were stable throughout the experiment and did not exhibit any significant differences in the entire range of Cd treatments (from 0 to 40 mg kg^{-1}), while DOC concentrations in the rhizosphere soils of two plants showed clear dependence upon the treatment and aligned in order: rhizosphere of R. globosa > rhizosphere of R. palustris ≥ bulk soil (Fig. 2). DOC concentrations in rhizosphere soils of *R. globosa* were the highest and insignificantly different ($p>0.05$) for the Cd treatment range from 0 to 5 mg kg^{-1}, At higher Cd doses, DOC concentrations in rhizosphere soils of *R. globosa* progressively decreased to the significant level ($p<0.05$) at Cd dose ≥10 mg kg^{-1}, up to 33 % at 40 mg Cd kg^{-1} compared to control. (Cd0).

In turn, DOC concentrations in the rhizosphere soil of *R. palustris* followed similar pattern as in the rhizosphere of *R. globosa*, retaining at the same level at

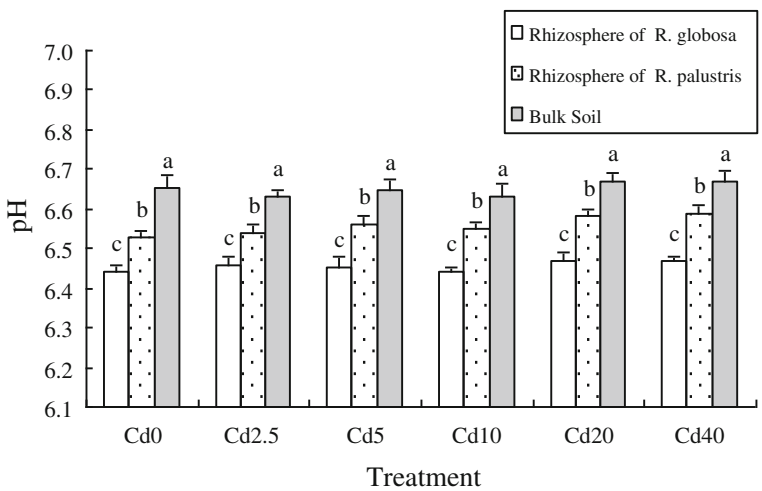

Fig. 1 pH values in the rhizosphere of *R. globosa* and *R. palustris*, and in the bulk soil. (The data for the bulk soil was the mean for the bulk soil in the pots with *R. globosa* and *R. palustris* ($p<0.05$). In the same treatment, data followed by same letter over column bar are not significantly different ($p<0.05$))

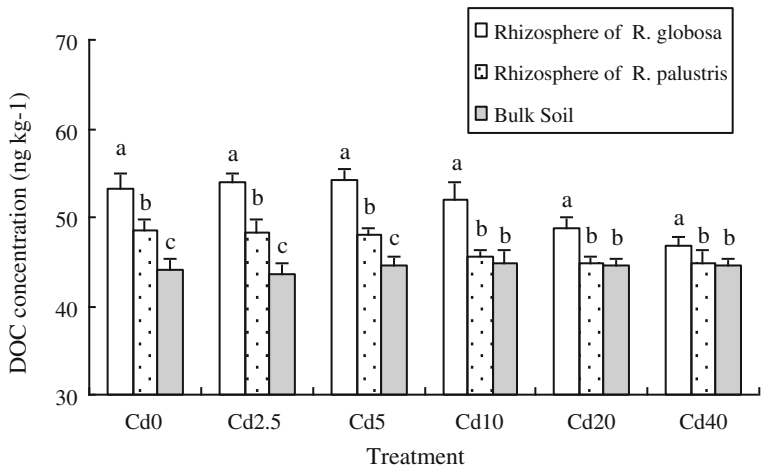

Fig. 2 DOC concentrations in the rhizosphere of *R. globosa* and *R. palustris*, and in the bulk soil. (The data for the bulk soil was the mean for the bulk soil in the pots with *R. globosa* and *R. palustris* ($p<0.05$). In the same treatment, data followed by same letter over column bar are not significantly different ($p<0.05$))

Cd treatments from 0 to 5 mg kg^{-1}, and significantly decreasing ($p<0.05$)compared to control at Cd treatments ≥10 mg kg^{-1}. At these Cd doses, DOC values in the rhizosphere of *R. palustris* reached the level insignificantly different ($p<0.05$) from DOC in the bulk soil.

Effect of the rhizophere on Cd fractionation

Chemical fractionation of Cd spiked to soil in doses ranging from 2.5 to 40 mg kg^{-1} showed that the pattern of Cd distribution between fractions of different binding strength, both in the rhizosphere of studied plants and in the bulk soil, was fairly similar for all treatments and aligned in order: R (Residual)>EXC (Exchangeable) > CARB (Carbonate) > Fe/Mn_{ox} (Fe/Mn-oxides > OM. (Organic matter) (Fig. 3). Of these fractions, Cd was the most enriched in the residual fraction (R) of the highest binding strength, and in the most labile exchangeable fraction (EXC), while fractions of Cd bonds of decreasing mobility (CARB > Fe/Mn_{ox} > OM) subsequently decreased (Fig. 3). Moreover, Cd fractionation appeared to be a specific property of the given rhizosphere or the bulk soil, independent of the Cd concentration (for all the studied treatments within the Cd range from 2.5 to 40 mg kg^{-1}, Cd fractionation in the rhizospheres of each species and in the bulk soil was constant and showed insignificant differences at $p<0.05$ level). In the bulk soil, Cd distribution in fractions (mean values, in % of total Cd extracted, d.m.) was as follows: 50.8 % (R)>19.3 % (EXC)>13.5 % (CARB)>9.5 % (Fe/Mn_{ox})>6.9 % (OM). In the rhizosphere of the Cd hyperaccumulator *R. globosa*, significant changes in Cd mobility occurred compared to the bulk soil. Cd enrichment in the strongly bound residual fraction (R) decreased by 19 %, while the fraction of Cd of the highest lability (EXC) increased by 20.9 %. Relative Cd enrichment of other fractions in the rhizosphere soil of *R. globosa* compared to that of the bulk soil also increased, by 4.9 % for the carbonate fraction (CARB), by 8.6 % for Fe/Mn oxides ($Fe-Mn_{ox}$), and by 16.9 % for organic matter (OM), with enrichment of $Fe-Mn_{ox}$ and OM fractions having significant relative changes ($p<0.05$). Of these, the highest absolute changes occurred in the residual and exchangeable fractions, for which Cd enrichment was 42.3 % (R) and 24.4 % (EXC)

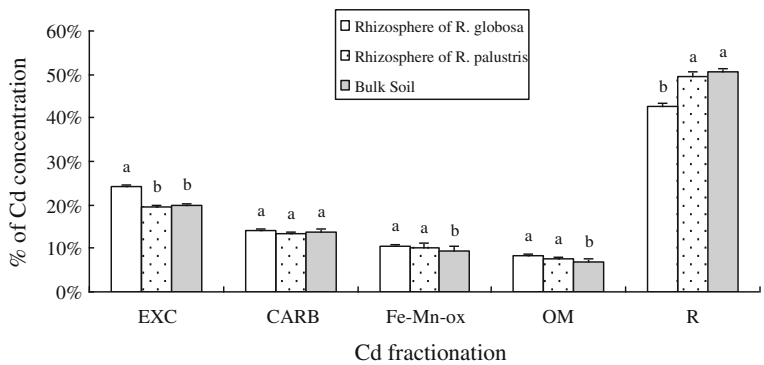

Fig. 3 Chemical fractionation of Cd in the rhizosphere of *R. globosa* and *R. palustris*, and in the bulk soil. (The data for each fraction was the mean for treatments Cd2.5, Cd5, Cd10, Cd20, and Cd40 ($p<0.05$). In the same fraction, data followed by the same letter over column bar are not significantly different ($p<0.05$)

of total Cd load in the rhizosphere of *R. globosa*, versus 50.8 % (R) and 19.3 % (EXC) in the bulk soil.

In contrast, although slight differences in Cd binding strength, generally in the same direction, also occurred, neither relative nor absolute significant ($p<0.05$) changes in Cd enrichment in the fractions of different mobility in the rhizosphere of the non-accumulator *R. palustris* compared to the bulk soil was observed, with exception of a slight increase of the relative Cd enrichment by 10.4 % and 5.9 % in OM and Fe/Mn_{ox} fractions, respectively. These fractions though played a rather marginal role in the chemical fractionation of Cd in soil, thus absolute changes in Cd enrichment in these fractions were insignificant.

Due to insignificant ($p<0.05$) differerences between Cd enrichment in the major R and EXC fractions in the rhizosphere of the non-hyperaccumiulator *R. palustris* and in the bulk soil, the relative and absolute differences between Cd enrichment in these fractions in the rhizosphere of the Cd hyperaccumulator *R globosa* and non-hyperaccumulator *R. palustris* were at the similar level as between each of these plants and the bulk soil.

It should be added that the total loads of Cd extracted sequentially from the rhizosphere soil of both plants and the bulk soil was at the level of 89.2–97.0 % of nominal doses spiked to soil and did not differ significantly ($p<0.05$) for any treatment.

Effect of the rhizosphere on soil enzyme activities

As shown in Fig. 4, urease and catalase activities in the rhizosphere soils of both plants were significantly higher than those in bulk soils ($p<0.05$), urease activity both in the bulk soil and in the rhizosphere being more strongly affected by Cd doses than catalase activity. In both cases, enzyme activities at all Cd treatments were the highest in the rhizosphere soil of *R. globosa*, markedly lower in the rhizosphere soil of *R. palustris* and were the lowest in the bulk soil. In the rhizosphere soils of *R. globosa*, urease activities were practically at the same level at Cd treatments within the range 0–5 mg kg^{-1}, but showed a distinct descending trend and reached a level of significant difference from control at Cd ≥10 mg kg^{-1}.

Urease activities in the rhizosphere soil of *R. palustris* appeared to be more sensitive to Cd doses those of catalase and started to decline significantly at Cd ≥5 mg kg^{-1}. Contrary to this, urease activity in the bulk soil, although substantially lower than in the rhizosphere of either plant, was fairly stable within a Cd range 0–10 mg kg^{-1} and significantly declined only at Cd ≥20 mg kg^{-1}.

The highest reduction of urease activities in rhizosphere of both plants and the bulk soil was recorded at Cd treatments ≥20 mg kg^{-1}. Compared to their controls, at Cd dose 40 mg kg^{-1}, urease activities in the rhizospheres of *R. globosa*, *R. palustris* and in the bulk soil decreased by 45.9 %, 66.7 % and 55.5 %, respectively.

Catalase activities, in general, were weakly affected by the increase of Cd doses (Fig. 4). The significant decrease ($p<0.05$) of catalase activity in the rhizosphere soil of *R. globosa and R. palustris* and in the bulk soil occurred at the Cd treatment 40 mg kg^{-1}.

Effect of rhizophere on soil microorganism number

Table 3 shows numbers of bacteria, fungi and actinomycetes in the rhizosphere of *R. globosa* and *R. palustris,* and in the bulk soil. Microorganism numbers were the highest in the rhizosphere soil of *R. globosa,* lower in that of *R. palustris* and the lowest in the bulk soil. Despite progressive decrease in parallel with increasing Cd doses, the number of every studied microorganism community (bacteria, actinomycete, fungi) in the rhizosphere of the Cd hyperaccumulator *R. globosa* was substantially higher than in the bulk soil. However, bacteria numbers in the rhizosphere of *R. globosa* appeared to be sensitive to Cd even at the lowest treatments and regularly declined at every Cd dose, while bacteria number in the rhizosphere of *R. palustris* and in the bulk soil displayed a significantly declining trend at Cd ≥10 mg kg^{-1}.

Fungi were the least numerous (roughly two orders of magnitude less than that of bacteria), but followed the same order: rhizosphere of *R. globosa*>rhizosphere of *R. palustris*>bulk soil. Significant decrease of fungi number occurred at Cd ≥20 mg kg^{-1} for *R. globosa* and the bulk soil, and Cd ≥10 mg kg^{-1} for *R. palustris.*

Actinomycete numbers were one order of magnitude lesser than bacteria, and showed similar trends as bacteria, i.e. regular declining trend in the rhizosphere of *R. globosa* up to a Cd dose 40 mg kg^{-1}, when a sharp drop by 48.5 % occurred, and significant decrease in the rhizosphere of *R. palustris* and in the bulk soil at Cd dose ≥10 mg kg^{-1}.

To summarize observed trends, the relation between microorganism numbers in the rhizosphere of the Cd-hyperaccumulator *R. globosa* and the non-hyperaccumulator *R. palustris* was not univocal. In

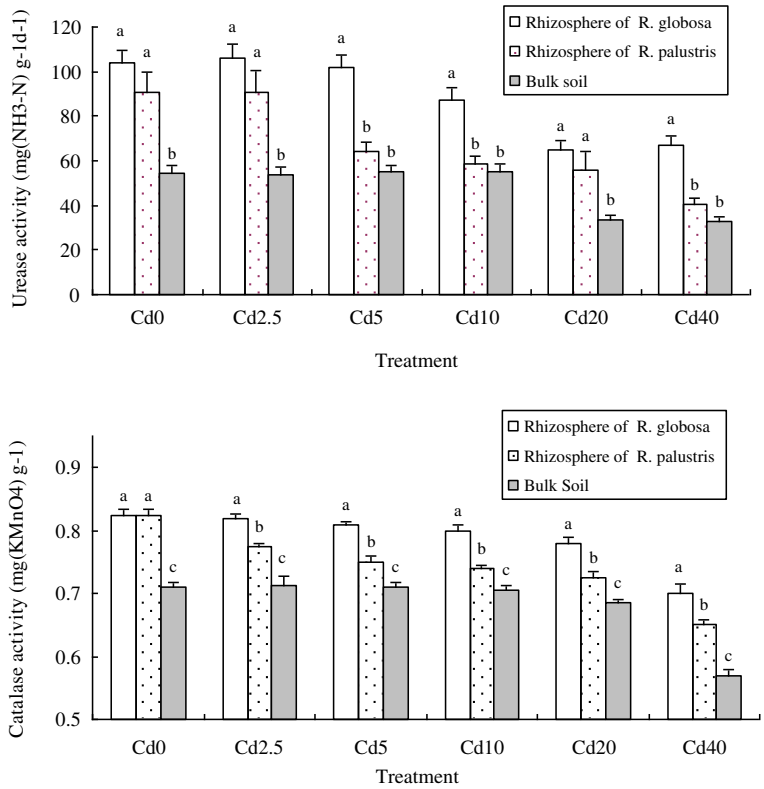

Fig. 4 Enzyme activities in the rhizosphere of *R. globosa* and *R. palustris*, and in the bulk soil. (The data for the bulk soil was the mean for the bulk soil in the pots with *R. globosa* and *R. palustris* ($p<0.05$). In the same treatment, data followed by same letter over column bar are not significantly different ($p<0.05$))

the untreated soil, bacteria, fungi and actinomycete numbers in the rhizosphere of *R. globosa* were 50 %, 14 % and 6 % higher than in the rhizosphere of *R. palustris*, and 2.2-, 2.4- and 1.4-fold higher than in the bulk soil, respectively. The lowest spiked dose of 2.5 mg Cd kg^{-1} resulted in a sharp (by 23 %) substantial decrease of bacteria, and a very slight decrease of fungi and actinomycete number in the rhizosphere of *R. globosa*, while in the rhizosphere of *R. palustris* all three kinds of microorganisms did not show significant reduction of their number. The number of microorganisms in the rhizosphere of both plants and in the bulk soil decreased in parallel with growing Cd doses, while the highest alterations of the microorganism

Table 3 Microorganism numbers in the rhizosphere of *R. globosa* and *R. palustris,* and in the bulk soil

Microorganism	Cd0	Cd2.5	Cd5	Cd10	Cd20	Cd40
Bacteria number ($\times10^6$) in rhizosphere of *R. globosa*	65.2±5.1a	50.0±7.3b	47.4±6.7b	39.5±3.8c	36.4±2.5d	28.5±1.9e
Bacteria number ($\times10^6$) in rhizosphere of *R. palustris*	43.1±4.1a	42.4±3.9a	43.8±2.6a	37.4±2.1b	25.7±2.8c	17.1±1.8d
Bacteria number($\times10^6$) in bulk soil	30.2±3.9a	27.1±4.1b	26.8±3.1b	21.4±2.9c	14.8±1.8d	11.2±1.1
Fungi number ($\times10^4$) in rhizosphere of *R. globosa*	45.3±4.6a	42.3±4.3a	40.5±4.4a	40.8±3.1a	28.8±3.0b	25.4±3.5c
Fungi number ($\times10^4$) in rhizosphere of *R. palustris*	39.7±4.2a	39.1±4.0a	35.7±3.8b	29.4±2.6c	21.5±2.2d	12.4±1.5e
Fungi number ($\times10^4$) in bulk soil	19.1±2.7a	19.4±2.4a	22.4±2.9a	19.4±2.4a	13.0±1.8b	9.6±0.7c
Actinomycete number ($\times10^5$) in rhizosphere of *R. globosa*	99.6±9.8a	98.3±9.5a	90.2±9.9b	88.1±9.2b	86.6±9.7b	44.6±5.3c
Actinomycete number ($\times10^5$) in rhizosphere of *R. palustris*	93.5±9.8a	83.3±8.7b	83.4±8.5b	44.5±4.6c	42.3±5.1c	17.3±2.1d
Actinomycete number ($\times10^5$) in bulk soil	72.6±8.2a	61.5±7.5b	63.7±8.2b	39.8±4.5c	37.2±4.1c	23.8±2.8d

Cd treatments Cd0, Cd2.5, Cd5, Cd10, Cd20 and Cd40 mean spiked doses 0, 2.5, 5, 10, 20 and 40 mg Cd kg^{-1}, respectively. Data for the bulk soil was the mean for the bulk soil in pots with *R. globosa* and *R. palustris* ($p<0.05$). Data in the same lines followed by the same letter were not significantly different at $p<0.05$ level)

number occurred in the range between Cd10 and Cd40 treatments. Particularly severe reduction of the microorganism growth was observed at Cd40. When compared to untreated soil Cd0, the number of bacteria, fungi and actinomycetes in the rhizosphere of *R. globosa* decreased 2.3-, 1.8- and 2.2-fold, in the rhizosphere of *R .palustris* 2.5, 3.2 and 5.4-fold and in the bulk soil 2.7-, 2- and 3-fold, respectively. These data exhibit the lowest decrease of microorganism growth in the rhizosphere of *R. globosa*, which resulted in their highest number, 2- to 2.6 fold higher than .in the bulk soil.

Discussion

The experiment confirmed all Cd hyperaccumulator properties of *R. globosa* within Cd concentrations in soil in the entire studied range, up to 40 mg kg^{-1}, and its efficiency as a Cd scavenger (Tab.2). It is obvious that rhizosphere processes are decisive for enhanced metal uptake by a plant, which is one of the major prerequisites of hyperaccumulation. Assessment of alteration of basic parameters of soil chemistry (pH, DOC, Cd chemical fractionation) and enzyme activity and microbiology by a root system of the Cd hyperaccumulator *R. globosa* compared to the non-hyperaccumulator *R. palustris* and the bulk soil exhibited the role of these parameters in the uptake of this non-essential metal. An important observation is the small effect of the rhizosphere on pH values (pH reduction by 0.1 unit only in the rhizosphere of the non-accumulator *R. palustris* compared to the bulk soil, and by 0.1 unit in the rhizosphere of the Cd hyperaccumulator *R. globosa* compared to the non-accumulator *R. palustris*). Therefore, it is unlikely that Cd hyperaccumulation by *R. globosa* can be explained by the reduction of rhizosphere pH. The pH is considered to be one of the most important chemical factors influencing bioavailability of trace elements, and some authors reported increase of element uptake by hyperaccumulators due to the significant reduction of rhizosphere pH (e.g. Fitz et al. 2003; Gonzaga et al. 2009; Li et al. 2011a, b). However, in recent reviews Wenzel (2009) concluded that "hyperaccumulation could not be related to changes in rhizosphere pH which were found to be typically small" that was indicated also by Alford et al. (2010). This conclusion thoroughly concurs with own observations regarding the Cd hyperaccumulator *R. globosa*. Slightly reduced pH, as found in this study, is a specific property of the rhizosphere of both the Cd hyperaccumulator *R. globosa* and non-hyperaccumulator *R. palustris*. It remained constant throughout the experiment over the entire range of Cd treatments. A similar acidification property has been demonstrated for the rhizospheres of many plants, both non-accumulators and hyperaccumulators. In this particular case, the small extent of rhizosphere acidification can be considered a supporting, but not a driving force for the hyperaccumulation. Stability of the pH values independent of Cd doses suggests that it is attributable to the species root activity.

Simultaneously, in comparison with Cd fractionation of the rhizosphere of the non-accumulator *R. palustris* (that did not show significant influence on rhizosphere chemistry compared to the bulk soil), the rhizosphere of the Cd hyperaccumulator *R. globosa* appears to exhibit a considerably reduced fraction of Cd strongly bound in the residual pool, and increased the most labile binding of Cd in the exchangeable fraction, and to a lesser extent onto organic matter, Fe/Mn oxides and carbonates of high or moderate mobility. Moreover, this pattern of considerably weaker Cd bonds and therefore higher mobility in the rhizosphere of the Cd hyperaccumulator *R. globosa* relative to that for *R. palustris* and the bulk soil was found to be independent of the Cd concentration in soil within the studied range from 2.5 to 40 mg Cd kg^{-1} (Fig. 3). High stability of the Cd fractionation indicates that for the observed changes in rhizosphere chemistry and markedly higher Cd mobility in the rhizosphere of *R. globosa* is responsible mostly its root activity, most likely root exudates. This comparative study indirectly proved that the root system of the Cd hyperaccumulator *R. globosa* displays specific mechanisms for Cd mobilization in soil, which enables its uptake and further transport into the above-ground organs. There are still controversies concerning capability of the root activity of hyperaccumulators to change rhizosphere chemistry. There is evidence both indicating and not confirming this capability (Alford et al. 2010). Apart from the lack of agreement concerning the mechanisms of different trace element hyperaccumulation in diverse hyperaccumulator species, this study provided clear evidence of Cd mobilization in the rhizosphere of the Cd hyperaccumulator *R. globosa* due to its root activity as a unique property of this species. A question

arises concerning the specific role and importance of root exudates and microbial activity in mobilizing Cd in the rhizosphere, as both activities may be key factors in these processes. It should be added that Cd fractionation in natural soils, in particular of geogenic origin, might differ from the studied soils amended only with Cd. In particular, greater residual fraction and stronger Cd bonds in the natural soils may occur. Therefore, the potential of *R. globosa* to change rhizosphere chemistry has to be investigated also with the use of natural highly Cd-contaminated soils in further studies. However, since Cd-hyperaccumulating properties of *R.globosa* were proved in contaminated field experiments (Wei et al. 2008), all mechanisms of Cd uptake by this species are expected to be the same, while the type of soil and origin of Cd pollution may cause certain alterations of Cd fractionation in soil, and consequently also in the rhizosphere of *R. globosa*.

DOC, enzyme activities and microbial growth in the rhizosphere of the Cd hyperaccumulator *R. globosa* were found to be always higher compared to those of the non-hyperaccumulator *R. palustris* and the bulk soil. All these parameters were the highest in the rhizosphere. They displayed some stability at a relatively low Cd concentration range (from 0 to 5 mg kg^{-1}), and next showed declining trend, being though always significantly higher than the relevant parameters in the rhizosphere of the non-hyperaccumulator *R. palustris* and the bulk soil.

DOC consists of several types of low molecular weight organic carbon compounds that are known to be produced by roots and may create ligand-induced mobilization of trace elements and thus increase their solubility (Christensen and Christensen 1999; Alford et al. 2010). While DOC in bulk soil remained practically at the same level at all treatments, an excess of DOC in the rhizosphere of both studied plants with respect to soil may be attributed to the release of root exudates. Substantial reduction of DOC in the rhizosphere of *R. globosa* at 40 mg Cd kg^{-1}, close to the low level occurring in bulk soil suggests that, similarly to pH, in this particular case, DOC is rather a supportive, but not a key factor inducing observed deep changes in Cd mobility, but may somewhat increase Cd uptake by the hyperaccumulator from specific pools/fractions (Fig. 2).

The relatively high enzymatic activity and microorganism number in the rhizosphere of both plants and in the bulk soil confirmed their potentially important role in Cd hyperaccumulation. Processes involved in microbial mobilization/immobilization of elements in contaminated soil are discussed in many recent publications (e.g. Liang et al. 2003; Kuffner et al. 2008; Stepniewska et al. 2009; Epelde et al. 2010; Wangeline et al. 2011) and in reviews where a current state of knowledge is summarized (Wenzel 2009; Sessitsch et al. 2013). In brief, plant-microbe interaction is a reciprocal process influenced by root activities. Changes in enzyme and microbial activities impact the growth of the root, which might directly or indirectly affect the accumulation of trace elements in plants. Microorganisms can enhance element solubility and change its fractionation through decomposition of soil organic matter and metal chelation by exudation of siderophores and organic acids, or increase element uptake by the plant root through stimulation of root growth and induction of genes involved in metal uptake. This study showed that enzyme activity, in particular that of urease in the Cd hyperaccumulator *R. globosa* was relatively high, roughly two fold higher than in the bulk soil at all Cd treatments, although it decreased at Cd doses ≥10 mg kg^{-1} (Fig. 4). Similar trends in microbial growth (Table 3) indicate that rhizosphere microorganisms may play an important role in Cd mobilization and its uptake by *R. globosa*. Comparison of data on microorganism number in the rhizosphere of Cd-hyperaccumulator *R. globosa* with that of the non-accumulator *R. palustris* and the bulk soil at increased Cd contents in soil showed, that despite invariably the highest mobility of Cd in the rhizosphere soil of *R. globosa,* which potentially might have a toxic effect on microbial growth and activity, the decrease of enzyme activity and microorganism growth was there the lowest. At Cd40 treatment, bacteria, fungi and microorganism numbers were1.7-, 2- and 2.6-fold greater than in the rhizosphere of the non-accumulator *R. palustris* and 2.5-, 2.6-, and 1.9-fold greater than in bulk soil, respectively. Chemical fractionation of Cd in the rhizosphere of *R. globosa* showed high Cd enrichment in the most mobile pools. This, along with the highest, though decreasing enzyme activity and microorganism number suggests that the root activity of this Cd-hyperaccumulator actually has a unique capability to mobilize Cd, and simultaneously supports growth of rhizosphere microorganisms reciprocally promoting root growth and metal uptake. Both root exudates and rhizosphere microorganisms seem to play a key role in Cd mobilization and enhancing uptake, although root activity seem to be a major driving force of these processes. Further comparative studies on *R. globosa* root growth, exudates function and composition, and rhizosphere

microbial activity might provide more insight into the Cd hyperaccumulation mechanism. This would allow finding ways of optimal rhizosphere manipulation to enhance the efficiency of phytoremediation. .

Conclusions

Throughout the experiment with different Cd treatments, the rhizosphere soil of the Cd hyperaccumulator *R. globosa* exhibited lower pH, and higher DOC contents, Cd enrichment in mobile fractions, enzyme (urease and catalase) activities and microorganism numbers compared to those of the non-accumulator *R. palustris* and bulk soil. Analysis of these factors has shown that they might be of different importance for Cd mobilization and uptake by *R. globosa*. While pH and DOC changes in the hyperaccumulator rhizosphere were found to provide a minor effect, permanent alteration of the Cd chemical fractionation, consisting in substantial reduction of the immobile residual pool and Cd enrichment primarily in the most labile exchangeable fraction, along with over 2-fold higher microorganism number and enzyme activity, was considered to be the principal driving force of Cd hyperaccumulation. These unique features of the rhizosphere of the Cd-hyperaccumulator *R. globosa* support reports on the hyperaccumulator-specific capability of the root activity to mobilize trace elements and to enhance microbial growth reciprocally improving root development, element solubilization and uptake.

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References

Aboudrar W, Schwartz C, Benizri E, Morel JL, Boularbah A (2007) Soil microbial diversity as affected by the rhizosphere of the hyperaccumulator *Thlaspi caerulescens* under natural conditions. Intern J Phytoremediat 9:41–52

Alford ÉA, Pilon-Smits EAH, Pashke MW (2010) Metallophytes – a view from the rhizosphere. Plant Soil 337:33–50

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126

Boominathan R, Doran PM (2003) Organic acid complexation, heavy metal distribution and the effect of ATPase inhibition in hairy roots of hyperaccumulator plant species. J Biotechnol 101:131–146

Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8:279–284

Christensen JB, Christensen TH (1999) Complexation of Cd, Ni, and Zn by DOC in polluted groundwater: a comparison of approaches using resin exchange, aquifer material sorption, and computer speciation models (WHAM and MINTEQA2). Environ Sci Technol 33:3857–3863

Dessureault-Rompré J, Luster J, Schulin R, Tercier-Waeber ML, Nowack B (2010) Decrease of labile Zn and Cd in the rhizosphere of hyperaccumulating *Thlaspi caerulescens* with time. Environ Pollut 158:1955–1962

Epelde L, Becerril JM, Barrutia O, Gonza´lez-Oreja JA, Garbisu C (2010) Interactions between plant and rhizosphere microbial communities in a metalliferous soil. Environ Pollut 158:1576–1583

Fitz WJ, Wenzel WW, Zhang H, Nurmi J, Stipek K, Fischerova Z, Schweiger P, Kollensperger G, Ma LQ, Stingeder G (2003) Rhizosphere characteristics of the arsenic hyperaccumulator *Pteris vittata* L. and monitoring of phytoremoval efficiency. Environ Sci Technol 37:5008–5014

Gonzaga MIS, Ma LQ, Santos JAG, Matias MIS (2009) Rhizosphere characteristics of two arsenic hyperaccumulating Pteris ferns. Sci Total Environ 407:4711–4716

Hammer D, Keller C (2002) Changes in the rhizosphere of metal-accumulating plants evidenced by chemical extractants. J Environ Qual 31:1561–1569

He L-Y, Chen Z-J, Ren G-D, Zhang Y-F, Qian M, Sheng X-F (2009) Increased cadmium and lead uptake of a cadmium hypraccumulator tomato by cadmium-resistant bacteria. Ecotox Environ Safe 72:1343–1348

Jones DL, Willett VB (2006) Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol Biochem 38:991–999

Kabata-Pendas A (2010) Trace elements in soil and plants, 4th edn. CRC Press, Taylor & Francis Group, Boca Raton

Karimzadeh L, Heilmeier H, Merkel BJ (2012) Effect of microbial sideropore DFO-B on Cd accumulation by Thlaspi caerulescens hyperaccumulator in the presence of zeolite. Chemosphere 88:683–687

Keller C, Hammer D, Kayser A, Richner W, Brodbeck M, Sennhauser M (2003) Root development and heavy metal phytoextraction efficiency: comparison of different plant species in the field. Plant Soil 249:67–81

Kidd PS, Becerra-Castro C, Garcia-Lestón M, Monterroso C (2007) Aplicación de plantas hiperacumuladoras de niquel en la fitoextracción natural: et género *Alyssum* L. Ecosistemas 16:26–43

Kuffner M, Puschenreiter M, Wieshammer G, Gorfer M, Sessitsch A (2008) Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. Plant Soil 304:35–44

Li TQ, Di ZH, Islam E, Jiang H, Yang X (2011a) Rhizosphere characteristics of zinc hyperaccumulator Sedum alfredii involved in zinc accumulation. J Hazard Mater 185:818–823

Li TQ, Di Z, Yang X, Sparks DL (2011b) Effect of dissolved organic matter from the rhizosphere of the hyperaccumulator Sedum alfredii on sorption of zinc and cadmium by different soils. J Hazard Mater 192:1616–1622

Liang W, Wu ZB, Cheng SP, Zhou QH, Hu HY (2003) Roles of substrate microorganisms and urease activities in wastewater purification in a constructed wetland system. Ecol Eng 21:191–195

Lombi E, Zhao FJ, McGrath SP, Young SD, Sacchi GA (2001) Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. New Phytol 149:53–60

McGrath SP, Shen ZG, Zhao FJ (1997) Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. Plant Soil 188:153–159

Morera MT, Echeverria JC, Mazkiaran C, Garrido JJ (2001) Isotherms and sequential extraction procedures for evaluating sorption and distribution of heavy metals in soils. Environ Pollut 113:135–144

Nedelkoska TV, Doran PM (2001) Hyperaccumulation of Cadmium by Hairy Roots of *Thlaspi caerulescens*. Biotechnol Bioeng 67:607–615

Qiu RL, Zhao X, Tang YT, Yu FM, Hu PJ (2008) Antioxidative response to Cd in a newly discovered cadmium hyperaccumulator, Arabis paniculata. Chemosphere 74:6–12

Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallman K, Puschenreiter M (2013) The role of plant-associated bacteria in the mobilization and phytoextractiom of trace elements in contaminated soil. Soil Biol Biochem 60:182–194

Stepniewska Z, Wolińska A, Ziomek J (2009) Response of soil catalase activity to chromium contamination. J Environ Sci 21:1142–1147

Su H, Cai Z, Zhou Q (2013) Phytoremediation of cadmium contaminated soils. Advances and researching prospects, Mater. Sci. Forum 743–744:732–744

Sun RL, Zhou QX, Wei SH (2011) Cadmium accumulation in relation to organic acids and non-protein thiols in leaves of the new-found Cd-hyperaccumulator *Rorippa globosa* and the Cd-accumulating plant *Rorippa islandica*. J Plant Growth Regul 30:83–91

Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51:844–851

van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. Plant Soil 362:319–334

Wangeline AL, Valdez JR, Lindblom SD, Bowling KL, Reeves FB, Pilon-Smits EAH (2011) Characterization of rhizosphere fungi from selenium hyperaccumulator and nonhyperaccumulator plants along the eastern rocky mountain front range. Am J Botany 98:1139–1147

Wei SH, Zhou QX, Wang X, Zhang KS, Guo GL, Ma LQ (2005) A newly-discovered Cd-hyperaccumulator *Solanum nigrum* L. Chin Sci Bullet 50:33–38

Wei SH, Zhou QX, Saha UK (2008) Hyperaccumulative characteristics of weed species to heavy metals. Water Air Soil Pollut 192:173–181

Wei SH, Li YM, Zhan J, Wang SS, Zhu JG (2012) Tolerant mechanisms of *Rorippa globosa* (Turcz.) Thell. hyperaccumulating Cd explored from root morphology. Biores Tech 118:455–459

Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408

Wenzel WW, Bunkowski M, Puschenreiter M, Horak O (2003) Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. Environ Pollut 123:131–138

Whiting SN, Leake JR, McGrath SP, Baker AJM (2001) Zinc accumulation by *Thlaspi caerulescens* from soils with different Zn availability: a pot study. Plant Soil 236:11–18

Xie HL, Jiang RF, Zhang FS, McGrath SP, Zhao FJ (2009) Effect of nitrogen form on the rhizosphere dynamics and uptake of cadmium and zinc by the hyperaccumulator Thlaspi caerulescens. Plant Soil 318:205–215

Xiong JB, He ZL et al (2008) The role of bacteria in the heavy metals removal and growth of *Sedum alfredii* Hance in an aqueous medium. Chemosphere 70:489–494

Zhang X, Zhang S, Xu X, Li T, Gong G, Jia Y (2010) Tolerance and accumulation characteristics of cadmium in *Amaranthus hybridus* L. J Hazard Mater 180:303–308

Zhao FJ, Hamon RE, McLaughlin MJ (2001) Root exudates of the hyperaccumulator *Thlaspi caerulescens* do not enhance metal mobilization. New Phytol 151:613–620