



Heavy metal (Pb, Zn) uptake and chemical changes in rhizosphere soils of four wetland plants with different radial oxygen loss

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Received 15 July 2009; revised 06 October 2009; accepted 16 October 2009

Abstract

Lead and Zn uptake and chemical changes in rhizosphere soils of four emergent-rooted wetland plants; *Aneilema bracteatum*, *Cyperus alternifolius*, *Ludwigia hyssopifolia* and *Veronica serpyllifolia* were investigated by two experiments: (1) rhizobag filled with “clean” or metal-contaminated soil for analysis of Pb and Zn in plants and rhizosphere soils; and (2) applied deoxygenated solution for analyzing their rates of radial oxygen loss (ROL). The results showed that the wetland plants with different ROL rates had significant effects on the mobility and chemical forms of Pb and Zn in rhizosphere under flooded conditions. These effects were varied with different metal elements and metal concentrations in the soils. Lead mobility in rhizosphere of the four plants both in the “clean” and contaminated soils was decreased, while Zn mobility was increased in the rhizosphere of the “clean” soil, but decreased in the contaminated soil. Among the four plants, *V. serpyllifolia*, with the highest ROL, formed the highest degree of Fe plaque on the root surface, immobilized more Zn in Fe plaque, and has the highest effects on the changes of Zn form (EXC-Zn) in rhizosphere under both “clean” and contaminated soil conditions. These results suggested that ROL of wetland plants could play an important role in Fe plaque formation and mobility and chemical changes of metals in rhizosphere soil under flood conditions.

Key words: lead; zinc; rhizosphere; wetland plant; radial oxygen loss

DOI: 10.1016/S1001-0742(09)60165-0

Introduction

In recent years, the possibility of using constructed wetlands for restoration of heavy metal-contaminated sediments and water bodies has received more and more attention (Ye et al., 2004). As a major component in constructed wetlands, wetland plants, such as *Typha latifolia* and *Phragmites australis*, are so metal-tolerant, fast growing and of high biomass that they are suitable to be selected as major species in constructed wetland systems (Ye et al., 1997a, 1997b).

Wetland plants can alter the biogeochemistry of various naturally waterlogged soils (Wright and Otte, 1999; Stoltz and Greger, 2002; Jacob and Otte, 2004). In order to tolerate anoxic wetland environment, many wetland plants have ability to transport O₂ from shoots to roots, which is not only used for respiration, but also diffused to the rhizosphere, termed radial oxygen loss (ROL) (Armstrong et al., 1967). Wetland plants can directly affect some metal mobility-regulating soil factors, such as redox potential (Eh) and pH, via ROL into the rhizosphere sediments (Wigand et al., 1997; Wright and Otte, 1999). But studies

dealing with ROL-induced changes in metal mobilization in rhizosphere soil produced different results. Some studies indicated that ROL could increase metal mobility by inducing sulfide dissolution, metal release and acid generation if O₂ was introduced to pyritic soils (Holmer et al., 1998; Vigneault et al., 2001; Jacob and Otte, 2004). Other studies reported that increased soluble metals concur with high Eh in the root zone of waterlogged soils when compared with unvegetated plots (Roane et al., 1996; Wright and Otte, 1999).

On the other hand, some studies showed that ROL could decrease metal mobility by causing iron-oxidation and co-precipitation of other metals (Roden and Wetzel, 1996). Another striking feature of ROL is the formation of root Fe plaques (Armstrong, 1967). The formation of Fe plaque is influenced by soil Fe availability and the oxidizing capacity of plant root (Hansel et al., 2002). Because of the high absorption capacity of Fe oxides, Fe plaque provides a reactive substrate for metal sequestration and translocation, such as As, Cu, Pb and Zn (Ye et al., 1998; Chabbi, 2003; Liu et al., 2005, 2008).

The effects of plants on the biogeochemistry of rhizosphere soils polluted by heavy metals also have received more attention (Shuman and Wang, 1997; Lin et al.,

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2003, 2004). Mechanisms underlying wetland plant-soil geochemical interactions in artificial soils (such as tailings) are under explored. The effect of wetland plants on rhizosphere is species specific (Wright and Otte, 1999; Stoltz and Greger, 2002), which possibly due to varied ability of root O₂ release (Brix, 1993). In order to explore the mechanism of wetland plants on biogeochemistry of rhizosphere soil, four emergent-rooted wetland plants (*Aneilema bracteatum*, *Cyperus alternifolius*, *Ludwigia hyssopifolia* and *Veronica serpyllifolia*) from uncontaminated sites were selected as they are: (1) fast growing; (2) widely distributed; (3) possess low nutrient requirement. The aims of this study were to investigate how the presence of wetland plants affect metal chemical forms under flooded condition; and to compare the changes of metal forms in rhizosphere soils of the four wetland plants with different ROL.

1 Materials and methods

1.1 Agar experiment for ROL rates of the four plants

Table 1 lists the four wetland species studied and the sites where the plants were collected. Tillers of *A. bracteatum*, *C. alternifolius*, *L. hyssopifolia* and *V. serpyllifolia* were all collected from “clean” (i.e., no heavy metal contamination) sites. Cuttings of the four vegetative species were rooted in sand and watered with 20% Hoagland solution (Hoagland and Arnon, 1938). In order to minimize carry-over effects resulting from growth in the field, a minimum of three successive vegetative generations, i.e., ramets were removed from the parents with at least three vegetative generations before use. Considering the different growth rates of the species studied, new uniform tillers (about 10 cm in height) from propagation were selected and transplanted to blackened boxes (containing 2 L of 10% Hoagland solution with additional 0.1% (W/V) agar, pH 5.5) for 30 days.

The dilute agar prevents convective movements in the solution (stagnant treatment), so that it may simulate the changes in gas composition found in waterlogged soils

Table 1 Species information for experiment

Species	Family	Site distribution of the species collected and general description
<i>Acorus tatarinowii</i> Schott	Araceae	Zhuhai, Guangdong, China; distributed in waterlogged sediment with Eh ranging from -80 to 20 mV
<i>Cyperus alternifolius</i> Linn.	Cyperaceae	Zhuhai, Guangdong, China; distributed in saturated sediment with Eh ranging from -20 to 70 mV
<i>Ludwigia hyssopifolia</i> (G. Don) Excel	Onagraceae	Sun Yat-sen University, Guangdong, China; distributed in waterlogged sediment with Eh ranging from -70 to 20 mV
<i>Veronica serpyllifolia</i> Linn.	Scrophulariaceae	Huadiwan, Guangdong, China; distributed in waterlogged sediment with Eh ranging from -100 to -50 mV

better than other methods used to impose root-zone O₂ deficiency in solution culture (Wiengweera et al., 1997).

Boxes were arranged in a random design and re-randomized every 7 days when all the solutions were renewed. Thirty days after incubation, the ROL from roots was measured. There were four replicates for each treatment. The rate of O₂ released through roots was estimated by colorimetric method with Ti³⁺-citrate solution (Kludze et al., 1994). Three-hundred milliliters of deoxygenated water was added to 17.7 g of sodium citrate to give a concentration of 0.2 mol/L sodium citrate solution. Thirty milliliters of 1.16 mol/L TiCl₃ was added to the sodium citrate solution, and the pH was adjusted to 5.6 by adding saturated Na₂CO₃. Forty milliliters of 10% Hoagland nutrient solution was poured into a 50-mL test tube, and N₂ gas was bubbled through the solution for 30 min to remove any dissolved O₂. Plant samples were washed, covered with parafilm at the bases, and inserted into the tubes. A 5-mL aliquot of Ti³⁺-citrate was then injected into each test tube with a syringe, followed immediately by layering the solution surface with 2 cm of paraffin oil to inhibit contamination by atmospheric O₂. Control treatment had no plant. The introduction of roots into Ti³⁺-citrate solution was sampled with a syringe through rubber tubing six hours later.

Measurements of the absorbance of the partly oxidized Ti³⁺-citrate solution were performed at 527 nm using a UV/Vis spectrophotometer (Perkin-Elmer 3, Germany). Released O₂ was determined by extrapolation of the measured absorbance to a standard curve previously obtained from a dilution series of the Ti³⁺-citrate solution being used. ROL (μmol O₂/(plant-hr)) was then calculated using the following equation (Kludze et al., 1994):

$$ROL = c \times (y - z) \quad (1)$$

where, c (L) is initial volume of Ti³⁺-citrate added to each tube; y (μmol Ti³⁺/L) and z (μmol Ti³⁺/L) are concentrations of Ti³⁺-citrate solution of control (without plant) and after 6 hr with plants, respectively.

1.2 Rhizobag experiment for analysis of chemical forms of Pb and Zn in rhizosphere and non-rhizosphere soils of wetland plants

Tillers of *A. bracteatum*, *C. alternifolius*, *L. hyssopifolia* and *V. serpyllifolia* were prepared as described in agar experiment above.

The “clean” soil was collected from a bamboo (Zhuyuan) garden (ZY, 0–20 cm depth) at the campus of Sun Yat-sen University. The contaminated soil was collected from a metal-contaminated site (0–20 cm depth) nearby Lechang (LC) Pb/Zn mine located at Shaoguan, Guangdong, China. The two soils were air-dried for 2 weeks, and then sieved through a 2-mm mesh. The characteristics of the two soils are listed in Table 2.

In order to separate rhizosphere from non-rhizosphere soil, a cylindrical water-permeable nylon bag (500 meshes, with a heat-sealed base) was placed in the center of a plastic pot (7.5 cm in diameter and 14 cm in length). Four hundred and fifty grams of soil was added to each rhizobag.

Table 2 Properties of the soils collected from Zhuyuan (ZY) and Lechang (LC) ($n = 3$)

Property	ZY	LC
pH (1:5)	7.3	7.4
Total N (mg/kg)	360	910
Organic C (mg/kg)	990	3006
Total P (mg/kg)	22	83
Available P (mg/kg)	10	21
Total Pb (mg/kg)	29	711
Total Zn (mg/kg)	62	580
DTPA-Pb (mg/kg)	1	12
DTPA-Zn (mg/kg)	3	15

Total Zn and total Pb were determined using 1:4 HNO₃/HClO₃ digestion; DTPA-Pb and DTPA-Zn were determined using 0.1 mol/L DTPA extraction.

The space outside the nylon bag was filled with 750 g of the same soil. In this system, the surfaces of the soil inside and outside the rhizobag were in the same level. Before planting, the two soils were stored in pots and submerged in water (2–3 cm above the soil surface) for a month. Two tillers of each plant mentioned above were transplanted into each rhizobag. There are four replicates for each treatment. For each treatment, soil in the pots was kept flooded with about 2 cm water. All plants were grown in a light- and temperature-controlled greenhouse at a regime of day (16 hr, 22–28°C)/night (8 hr, 16–22°C). All pots were arranged in randomized block design.

1.3 Analysis of plant and soil samples

The rhizobag experiment was carried out for 3 months since tillers were transplanted. After harvest, Fe plaque on root surfaces was removed using dithionite-citrate-bicarbonate (DCB) method (Taylor and Crowder, 1983). After DCB extraction, root and shoot tissues were oven-dried at 70°C to a constant weight. The dried tissues were weighed and ground into powder for metal analysis. After that, the soils in rhizobags and non-rhizosphere were air-dried, ground and passed through a 0.26-mm sieve.

Metal analysis of plant samples was carried out by acid digestion with a mixture of concentrated HNO₃ and concentrated HClO₄, at 5:1 (V/V) (Allen, 1989). For total Pb, Zn and Fe determination, the soil samples were digested in 4 mL of “aqua-regia” (HNO₃/HCl = 1/3, V/V). The forms of Pb and Zn in rhizosphere and non-rhizosphere soils were separated by a 5-step sequential extraction method (Tessier et al., 1979). A standard reference plant material (GSV-2) and a standard reference soil material (GSS-1) from the National Center for Standard Materials (Beijing, China) were used to verify the accuracy of metal determination. The recovery rates for all metals (Pb, Zn and Fe) analyzed were within (90 ± 10)%. Concentrations of Pb, Zn and Fe in digests of the soils, plants and DCB-extraction solutions of Fe plaque were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 2100, Perkin Elmer, USA) (Page et al., 1982).

1.4 Statistical analyses

All results were presented as arithmetic mean with standard error. Data was analyzed using ANOVA, LSD-test and/or *T*-test as available in the SPSS statistical package. All figures and tables were plotted using PC-based excel.

2 Results

2.1 ROL rates of four plants

Under deoxygenated conditions, the rates of ROL of the four plants grown in deoxygenated solution were in the order of *V. serpyllifolia* ((3.11 ± 0.05) μmol O₂/(plant·hr)) > *C. alternifolius* ((2.75 ± 0.23) μmol O₂/(plant·hr)) > *L. hyssopifolia* ((1.33 ± 0.08) μmol O₂/(plant·hr)) > *A. bracteatum* ((1.20 ± 0.06) μmol O₂/(plant·hr)). The ROL rate of *V. serpyllifolia* was significantly higher than other three plants ($P < 0.05$). There was no significant difference in rates of ROL between *L. hyssopifolia* and *A. bracteatum*.

2.2 Soil properties

The contaminated LC soil was neutral (pH 7.4) and contained mainly Zn and Pb. The ZY soil was uncontaminated with pH 7.3 and hence was used as a control soil for plant culture study. LC soil has much higher organic matter contents and total P concentrations than ZY soil (Table 2).

2.3 Biomass of plants grown in two soils

There were significant differences in biomass of the plants grown in the ZY and LC soils, except shoot biomass of *A. bracteatum*, and root biomass of *V. serpyllifolia* (Fig. 1). Shoot and root biomass of *L. hyssopifolia* and shoot biomass of *V. serpyllifolia* in the ZY soil were significant higher than those in the LC soil, while shoot and root biomass of *C. alternifolius* and root biomass of *A. bracteatum* in LC soil were significant higher than those in ZY soil ($P < 0.05$).

2.4 Metal concentrations in shoot, root tissues and Fe plaque (DCB-extractions) of four plants

The concentrations of Zn and Pb in shoot and root tissues and Pb, Zn and Fe in Fe plaque of the four plants grown in ZY and LC soils for 3 months were shown in

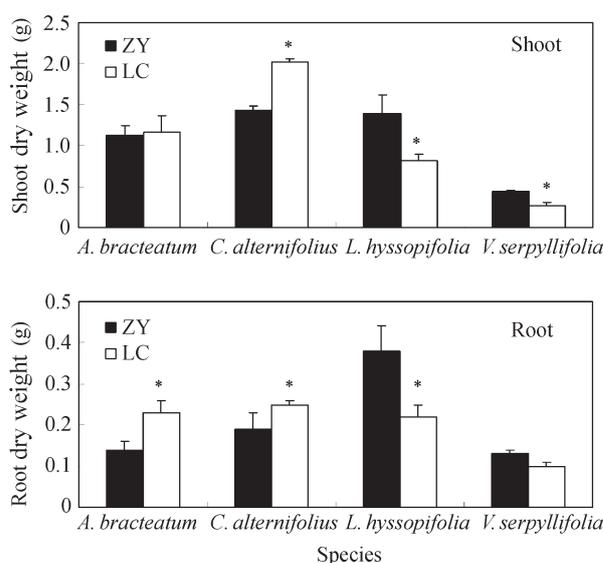


Fig. 1 Biomass of shoot and root of *A. bracteatum*, *C. alternifolius*, *L. hyssopifolia*, and *V. serpyllifolia* grown in ZY and LC soils (mean ± SE, $n = 4$). Asterisk in the same plant show significant differences ($P < 0.05$) according to *T*-test.

Table 3. In general, concentrations of Pb and Zn in the plants grown in the LC soil were higher than those in the ZY soil, and the metal concentrations in shoots and roots were significant differences among the plants ($P < 0.05$). The concentrations of Pb and Zn in shoot and root tissues and Pb, Zn and Fe in Fe plaque of *A. bracteatum*, *L. hyssopifolia* and *V. serpyllifolia* grown in the LC soil were significantly higher than those in ZY soil, especially in root tissues ($P < 0.05$). *V. serpyllifolia* grown in LC soil accumulated significantly higher Pb (34 mg/kg) in root tissues and Zn (261 mg/kg) in shoot tissues, and Fe (657 mg/kg) in Fe plaque than the other species. However, *C. alternifolius* grown in LC soil did not accumulate significantly high Pb (2.2 mg/kg) and Zn (37 mg/kg) in its shoot tissues comparing with that in ZY soil.

2.5 Root-induced changes of Pb and Zn chemical forms in rhizosphere soils of four plants

The distributions of Pb and Zn forms in rhizosphere and non-rhizosphere soils of the four plants are presented in Table 4. In both soils, Zn was predominately presented in residual form (RES), followed by Fe-Mn oxides form

(OX), organic form (OM), carbonate form (CAR) and exchangeable form (EXC). In the case of Pb, RES was dominated form in the ZY soil, followed by OX, OM and CAR form. While OX was dominant form in the LC soil, followed by RES, OM and CAR forms. The concentrations of extractable Pb in both soils were too low to be detected.

Compared with non-rhizosphere soils, all plants studied had obvious effects on rhizosphere metal form. In ZY soil with low Zn, all the four plants increased significantly EXC-Zn in rhizosphere soils, the increment ranging from 0.2% (*C. alternifolius*) to 1.63% (*V. serpyllifolia*) ($P < 0.05$). However, in LC soil with high Zn, the plants tested, except *C. alternifolius*, reduced differently EXC-Zn in rhizosphere soils, the decrease ranging from 0.02% (*L. hyssopifolia*) to 0.06% (*V. serpyllifolia*). As for Pb, the four plants reduced differently CAR-Pb in rhizosphere soils collected from ZY and LC. Especially in ZY soil with low Pb, CAR-Pb in rhizo-soil was significantly reduced by the four plants, the decrease ranging from 3.91% (*V. serpyllifolia*) to 8.3% (*A. bracteatum*). All plants increased significantly OM-Pb in rhizo-soil in both the ZY and LC soils.

Table 3 Metal concentrations (mg/kg dw) in shoot, root tissues and in Fe plaque of plants grown in the ZY and LC soils for 3 months

Species+ soil	Shoot		Root		Fe plaque		
	Pb	Zn	Pb	Zn	Pb	Zn	Fe
<i>A. bracteatum</i> + ZY	BDL	46 ± 2 de	1.9 ± 0.4 f	65 ± 2 e	BDL	15 ± 2 cd	82 ± 5 e
<i>C. alternifolius</i> + ZY	BDL	30 ± 3 e	5.9 ± 0.9 e	44 ± 2 f	BDL	10 ± 2 d	201 ± 10 cd
<i>L. hyssopifolia</i> + ZY	3.7 ± 0.3 c	64 ± 5 d	6.9 ± 0.4 e	85 ± 2 e	BDL	11 ± 3 d	37 ± 3 f
<i>V. serpyllifolia</i> + ZY	2.0 ± 0.4 c	157 ± 6 b	6.5 ± 1.1 e	202 ± 10 cd	BDL	36 ± 9 b	131 ± 24 d
<i>A. bracteatum</i> + LC	11 ± 2 b	66 ± 2 d	324 ± 34 b	235 ± 5 c	3.1 ± 0.6 d	17 ± 5 c	355 ± 33 b
<i>C. alternifolius</i> + LC	2.2 ± 0.5 c	37 ± 2 e	177 ± 25 d	154 ± 12 d	7.7 ± 1.3 c	10 ± 2 d	284 ± 34 c
<i>L. hyssopifolia</i> + LC	10 ± 3 b	103 ± 3 c	600 ± 48 a	315 ± 25 b	19 ± 1 b	19 ± 2 c	609 ± 47 a
<i>V. serpyllifolia</i> + LC	34 ± 5 a	261 ± 9 a	262 ± 9 cd	555 ± 21 a	29 ± 3 a	80 ± 21 a	657 ± 47 a

Values in the same column with different letters are significant differences at the level of $P < 0.05$ according to Least Significant Difference (LSD) test. Data are presented as mean ± SE ($n = 4$). BDL: below detection limit.

Table 4 Distribution of Pb and Zn forms (% of total Pb and Zn content) in the rhizosphere and non-rhizosphere soils of plants grown in the ZY and LC soils

Species+soil	EXC		CAR		OX		OM		RES	
	R	NR	R	NR	R	NR	R	NR	R	NR
Pb										
<i>A. bracteatum</i> + ZY	BDL	BDL	4.44	12.74**	32.34	31.66*	14.48	15.83*	48.75	39.77*
<i>C. alternifolius</i> + ZY	BDL	BDL	6.53	12.50*	32.65	32.03	15.92	16.41	44.90	39.06*
<i>L. hyssopifolia</i> + ZY	BDL	BDL	4.69	9.20**	33.59	35.06*	17.97	11.49**	43.75	44.25
<i>V. serpyllifolia</i> + ZY	BDL	BDL	5.81	9.72*	29.46	31.94	17.83	12.04**	46.90	46.30
<i>A. bracteatum</i> + LC	BDL	BDL	17.74	20.59*	36.48	30.23**	22.30	21.08	23.48	28.10**
<i>C. alternifolius</i> + LC	BDL	BDL	16.69	21.84*	37.56	29.56**	21.03	21.02	24.72	27.59
<i>L. hyssopifolia</i> + LC	BDL	BDL	18.63	21.00*	35.27	35.02	22.80	19.06**	23.29	24.92
<i>V. serpyllifolia</i> + LC	BDL	BDL	17.66	22.96**	36.67	30.28*	21.90	20.30	23.77	26.46*
Zn										
<i>A. bracteatum</i> + ZY	1.56	0.52*	5.60	8.74**	21.69	25.52*	12.43	8.03**	58.72	57.18
<i>C. alternifolius</i> + ZY	1.31	1.11	5.55	8.82**	23.22	25.07*	13.12	8.36**	56.79	56.64
<i>L. hyssopifolia</i> + ZY	1.72	0.77**	3.35	7.73**	22.00	25.35*	15.06	8.69**	57.88	57.46
<i>V. serpyllifolia</i> + ZY	2.44	0.81**	4.78	8.37*	20.56	24.86	14.35	8.13**	57.87	57.84
<i>A. bracteatum</i> + LC	0.08	0.14**	4.25	4.63	27.57	31.32*	15.40	10.28*	52.70	53.64
<i>C. alternifolius</i> + LC	0.14	0.13	4.30	4.52	28.75	30.83	14.88	9.96**	51.94	54.56
<i>L. hyssopifolia</i> + LC	0.09	0.11	4.62	4.74	27.74	31.55**	16.92	11.96**	50.62	51.65
<i>V. serpyllifolia</i> + LC	0.06	0.12**	4.35	4.93*	29.70	30.57*	15.98	10.32**	49.91	54.07*

R: rhizosphere; NR: non-rhizosphere; EXC: exchangeable form; CAR: carbonate form; OX: Fe-Mn oxides form; OM: organic form; RES: residual form; BDL: below detection limit.

Data are presented as mean ± SE ($n = 4$); * and ** significance at $P < 0.05$ and $P < 0.01$, respectively (T -test).

3 Discussion

The result from visual observation showed that Pb and Zn in the LC soil did not cause any visible toxic symptoms to the four studied plants. Previous studies also showed that high concentrations of Pb and Zn did not cause any toxic symptoms or retarded growth in *T. latifolia* (Taylor and Crowder, 1983; Ye et al., 1997a, 1997b). Baker (1981) suggested two basic strategies of metal tolerance in plants: (1) the “excluded” strategy, in which shoot concentrations of heavy metals are maintained at a constant low level when grown in heavy metal-contaminated soils; (2) the “accumulator” strategy, in which metals are actively concentrated within plant tissues over the full range of soil concentrations, implying that internal metal detoxification tolerance mechanism may exist.

The present study showed that *A. bracteatum*, *C. alternifolius* and *L. hyssopifolia* maintained low concentrations of Pb (2.2–11 mg/kg) and Zn (30–157 mg/kg) in shoots so that toxicity symptoms did not occur. But, for *V. serpyllifolia*, the highest accumulations in shoots were exhibited by Pb and Zn at 34 and 261 mg/kg respectively. The present studies suggested that internal Pb and Zn detoxification tolerance mechanisms might exist in *V. serpyllifolia*, in addition to the exclusion strategy. The present results also showed that the plants differ widely in their ability to accumulate Pb and Zn (Table 3). For example, among the four plants, *C. alternifolius* accumulated the lowest Pb (2.2 mg/kg) and Zn (37 mg/kg), while *V. serpyllifolia* accumulated the highest Pb (34 mg/kg) and Zn (261 mg/kg) in their shoot tissues when grown in the LC soil.

ROL has been considered as a by-product of root oxygenation and varies considerably among wetland species (Peter et al., 2005). Peter et al. (2005) measured ROL in five wetland species, *Calamagrostis epigejos*, *Carex flacca*, *Juncus articulatus*, *Samolus valerandi* and *Schoenus nigricans*, and found that ROL of these species were ranged from 0.6 $\mu\text{mol O}_2/(\text{plant}\cdot\text{hr})$ (*C. epigejos*) to 4.1 $\mu\text{mol O}_2/(\text{plant}\cdot\text{hr})$ (*S. valerandi*). The present results also showed the significant differences in ROL among the four wetland species studied, from the lowest 1.20 $\mu\text{mol O}_2/(\text{plant}\cdot\text{hr})$ for *A. bracteatum* to the highest 3.11 $\mu\text{mol O}_2/(\text{plant}\cdot\text{hr})$ for *V. serpyllifolia*. Peter et al. (2005) suggested that rates of ROL were mainly determined by three interacting factors: (1) the presence of aerenchyma in shoots and root, (2) root respiration, and (3) the presence of a barrier in the basal root zones that would block oxygen diffusion into the rhizosphere.

ROL is also considered as the most important biotic factor controlling Fe plaque formation (Hansel et al., 2002). However, so far, the effect of Fe plaque on the oxygen dynamics, especially ROL of roots of wetland plants, is not clear. The present results show that *V. serpyllifolia*, with the highest ROL, formed the highest degree of Fe plaque on root surface among the four plants when grown in LC soil (Table 3). This suggests that Fe plaque on root surface could not effectively inhibit ROL from the roots. Møller and Sand-Jensen (2008) investigated the effect of Fe plaque on ROL of *Lobelia dortmanna* L.

in hydroponic solutions. The results showed that the Fe plaque on root surface could divert a larger proportion of downward oxygen transport to root meristems in *L. dortmanna* and reduce the ingress of phytotoxins from the sediment. Therefore, the presence of Fe plaque on root surface of wetland plants might play an important role in diminishing root anoxia and improving survival in reduced sediment.

Due to the high adsorption capacity of Fe hydroxides, Fe plaque can provide a reactive substrate for Zn sequestration (Otte et al., 1989; Zhang et al., 1998). The present results showed that Fe plaque on root surface of *V. serpyllifolia* could immobilize more Zn than that of the other three plants both in the ZY and LC soils. However, the capacity to immobilize Zn is limited and may relate to some other factors, such as the extent of plaque (Otte et al., 1989; Zhang et al., 1998), the concentrations of metal and pH in the culture solution or rhizosphere, and the age of roots (Ye et al., 1997a; Batty et al., 2000). As for Pb, present results indicated that the outer cortex of roots, not Fe plaque, may act as a major barrier for Pb uptake and translocation (Table 3), as it was also reported that most of the absorbed Pb could accumulated in the outer cortex of roots of wetland plants (Ye et al., 1998; Vesik et al., 1999). In addition, Fe plaque may also act as an effective Fe reservoir to increase Fe concentrations in active cells and then ameliorate metal toxicity (Ye et al., 1998).

As demonstrated in Table 4, it was found that the chemical forms of Pb and Zn in rhizosphere soils differed remarkably from non-rhizosphere soils of the plants. In ZY soil with low Zn and Pb, EXC-Zn in rhizosphere was significantly increased while CAR-Zn in rhizosphere was significantly decreased. *V. serpyllifolia*, with the highest ROL, possess the highest EXC-Zn (2.44%) in rhizosphere soil among the four plants. A possible explanation for these results could be the decreasing pH in the rhizosphere of the plants studied. Shuman and Wang (1997) have reported that the pH in rice rhizosphere soil was lower than that in the non-rhizosphere soil. Iron oxidation by root ROL of the plants studied, H^+ produced by Fe^{2+} and sulfide oxidation were consumed by carbonates, thus CAR-Zn was dissolved. Zinc is an essential element to plants. In order to take up more Zn from ZY soil, the activities of roots transferred less available form (CAR-Zn) to easily available form (EXC-Zn) in rhizosphere. Tao et al. (2003) reported that the activities of corn in rhizosphere soil transfer less available form (CAR-Cu, OX-Cu, RES-Cu) to easily available form (EXC-Cu) of Cu. For Pb, CAR-Pb in rhizosphere was significantly decreased while RES-Pb in rhizosphere was significantly increased, which suggested the transformation of Pb from unstable forms (CAR-Pb) to more stable forms (RES-Pb) and the decrease of Pb mobility in rhizosphere of the plants grown in ZY soil. In LC soil with higher Pb and Zn, the transformation of Zn from unstable forms (EXC-Zn, CAR-Zn) to stable forms (OX-Zn, OM-Zn) was remarkably higher in rhizosphere than non-rhizosphere soils. Similar results also found in the transformation of Pb. *V. serpyllifolia*, with the highest ROL, showed the lowest EXC-Zn (0.06%) in

rhizosphere soil among the four plants. The reason for the transformation of Pb and Zn in rhizosphere of the plants grown in LC soil was mainly due to root ROL of the plants evaluated. ROL of roots of wetland plants increased Eh in rhizosphere soil (Armstrong, 1967; Bravin et al., 2008), which were concurrent with Fe and Mn oxidation in rhizosphere (Hansel et al., 2002). The present studies also showed that *V. serpyllifolia*, with the highest ROL, had the highest Eh in rhizosphere soil among the four plants (data not shown). Lin et al. (2004) found the increment of OX-Pb in rice rhizosphere. In addition, the activities of wetland plant roots could activate some metal elements such as Fe and Mn, which increased the amorphous Fe in rhizosphere (Lin et al., 2004; Ren et al., 2007). Therefore, Fe/Mn oxidation might play an important role in transformation of OX in rhizosphere soil.

Besides affecting Eh via ROL into the rhizosphere soil, root secretion and microbial activities of wetland plants may accumulate more organic matter and thereby facilitated the desorption of Pb and Zn, which increased OM-Pb and OM-Zn of rhizosphere soils of the plants when grown in LC soil. In summary, present results showed that Pb mobilities in rhizosphere soil of the four plants grown in the LC and ZY soils were decreased. In ZY soil with lower Zn, the mobility of Zn in rhizosphere of the plants was increased, while in LC soil with higher Zn, the mobility of Zn in rhizosphere was decreased.

4 Conclusions

This study clearly demonstrated that the wetland plants have significant effects on the mobility and chemical forms of Pb and Zn in rhizosphere soils. These effects varied with different metal elements and their concentrations in soil. For Pb, as a non-essential element, the wetland plants are able to decrease its mobility in both “clean” soil (with lower Pb) and polluted soil (and higher Pb); while for Zn, as an essential element, the plants are able to increase its mobility in “clean” soil (with lower Zn), but decrease its mobility in polluted soil (with higher Zn).

Among the four plants studied, *V. serpyllifolia*, with the highest ROL, formed the highest degree of Fe plaque on root surface, immobilized the highest concentration of Zn in Fe plaque, and had highest effects on changes of Zn form (EXC-Zn) in rhizosphere in both “clean” and polluted soils. These results suggest that ROL may play important roles in Fe plaque formation and mobility and chemical forms of metals in rhizosphere soil under flood conditions.

The present results are significant in elucidating the transformation of Pb and Zn in rhizosphere soil of wetland plants with different ROL. Further research, particularly using *in situ* techniques, is required to clarify the relationship between ROL and the availability of Pb and Zn in rhizosphere soil of wetland plants.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 30570345, 30770417), the Guangdong Natural Science Group Foundation (No.

06202438) and the Specialized Research Fund for the Doctoral Program of Higher Education, China (No. 20558097).

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