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REGULAR ARTICLE

Root porosity, radial oxygen loss and iron plaque on roots of wetland plants in relation to zinc tolerance and accumulation

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Abstract
Background and aims Wetland plants have been widely used in constructed wetlands for the clean-up of metal-contaminated waters. This study investigated the relationship between rate of radial oxygen loss (ROL), root porosity, Zn uptake and tolerance, Fe plaque formation in wetland plants.
Methods A hydroponic experiment and a pot trial with Zn-contaminated soil were conducted to apply different Zn level treatments to various emergent wetland plants.
Results Significant differences were found between plants in their root porosities, rates of ROL, Zn uptake and Zn tolerance indices in the hydroponic experiment, and concentrations of Fe and Mn on roots and in the rhizosphere in the pot trial. There were significant positive correlations between root porosities, ROL rates, Zn tolerance, Zn, Fe and Mn concentrations on roots and in the rhizosphere. Wetland plants with higher root porosities and ROL tended to have more Fe plaque, higher Zn concentrations on roots and in their rhizospheres, and were more tolerant of Zn toxicity.
Conclusions Our results suggest that ROL and root porosity play very important roles in Fe plaque formation, Zn uptake and tolerance, and are useful criteria for selecting wetland plants for the phytoremediation of Zn-contaminated waters and soils/sediments.

Keywords Aerenchyma · Heavy metal · Iron plaque · Radial oxygen loss (ROL) · Wetland plant · Rhizosphere

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Introduction

Industrial wastewaters containing elevated concentrations of heavy metals such as zinc (Zn) can pose environmental and health hazards. Constructed wetlands have been considered as an effective, low cost and practical approach for the clean-up of different kinds of wastewaters, including metal-contaminated waters (Ye et al. 2001, 2004; Sheoran and Sheoran 2006; Lizama et al. 2011). However, the effectiveness of wetland treatment systems varies in removing heavy metals from wastewater (Lin et al. 2010; Marchand et al. 2010). In constructed wetland systems, plants are one of the major components in the process of metal removal (Marchand et al. 2010). Different wetland plants have different levels or capacity for metal tolerance, accumulation and manipulation of the biogeochemistry of rhizosphere soils and sediments (Deng et al. 2006; Yang et al. 2010, 2012). Selection of appropriate plant species has been shown to be a key step to ensure successful removal of metals and metalloids from wastewaters and the phytoremediation of a contaminated soil/sediment (Weis and Weis 2004; Marchand et al. 2010). The degree of intrinsic metal tolerance differs between wetland plant species (Snowden and Wheeler 1993; Matthews et al. 2005; Deng et al. 2009). However, it is unique for some wetland plants in possessing constitutive metal tolerances throughout the species, irrespective of whether populations tested originate from metal-enriched sites or from 'clean' sites (Ye et al. 1997a, b, 1998; McCabe et al. 2001; Deng et al. 2006). A better understanding of the mechanisms related to the uptake of and tolerance to metals by wetland plants will help in selecting appropriate species for constructed wetland wastewater treatment systems. However, the relative roles of internal (e.g. root anatomical and physiological) and external (environmental) factors in the uptake and tolerance of heavy metals (e.g. Zn) in wetland plants showing a high level of metal tolerance are not clear. Furthermore, only a few emergent wetland species, such as *Phragmites australis*, *Typha latifolia* and *Scirpus validus*, among more than one thousand species of wetland plants, have been used widely in constructed wetlands (Sundaravadivel and Vigneswaran 2001; Weis and Weis 2004). It is therefore important to investigate the responses of a wider range of emergent wetland plants to determine their suitability for the phytoremediation of metals in constructed wetlands.

Waterlogging of the substratum, a typical characteristic of wetland ecosystems, often results in a deficiency of oxygen (O_2) and essential nutrients, low redox potential and an accumulation of phytotoxins such as ferrous ion (Fe^{2+}), manganese ion (Mn^{2+}), hydrogen sulfide (H_2S) and methane (CH_4) in sediments (Gambrell et al. 1991). To adapt to an anoxic environment, wetland plants have developed aerenchyma tissues containing enlarged gas spaces (Evans 2003), which can be expressed quantitatively as porosity (ratio of gas spaces to tissues volumes). Enlarged gas spaces can transport O_2 from aerial parts to roots for respiration and any excess O_2 may diffuse from roots into the rhizosphere zone, a process referred to as radial oxygen loss (ROL) (Armstrong 1979). ROL from roots is an essential process enabling wetland plants to tolerate a flooded, anoxic environment and to detoxify phytotoxins, such as Fe^{2+}, Mn^{2+}, and hydrogen sulphide (H_2S) by oxidation (Armstrong 1979; Chabbi et al. 2000; Armstrong and Armstrong 2005). Root porosity and rates of ROL have been reported to be markedly different between wetland plant species (Li et al. 2011) and also between different genotypes of the same species (Colmer 2003; Mei et al. 2009). Previous studies have shown that the tolerance of plants to flooding (Armstrong 1979; Chabbi et al. 2000), salinity (Rogers et al. 2008) and arsenic (As) exposure (Li et al. 2011) are positively related to ROL, amounts of aerenchyma tissues and/or root porosity. However, the specific relationships between the tolerance of wetland plants to other toxic metals (e.g. Zn) and ROL and/or root porosity remain unclear.

Emergent wetland plants oxygenate their rhizosphere via ROL and the oxidizing capacity of their roots to form an iron (Fe) oxyhydroxide plaque (Crowder and St-Cyr 1991; Mendellsohn et al. 1995). The effect of Fe plaque on the uptake of metals (e.g. Zn) has been found to depend on the amount of Fe plaque on root surfaces (Otte et al. 1989; Zhang et al. 1998; Chen et al. 2005; Hu et al. 2007). Some studies with rice also show that an enhancement of Fe plaque formation in the rhizosphere further reduces the accumulation of arsenic (As) and cadmium (Cd) in grains (Hu et al. 2007; Fan et al. 2010). Fe plaque may thus act as a barrier or a buffer to the uptake of heavy metals, probably due to adsorption and immobilization of metals (Taylor and Crowder 1983; Liu et al. 2004; Mei et al. 2012). However, little information is available on the relationships between ROL and Fe plaque on root surfaces and in the rhizosphere, with metal (e.g. Zn) uptake and accumulation among different wetland plants. We hypothesize that if a wetland species has a higher rate of ROL, it will possess a higher capacity to oxidize its rhizosphere. Consequently, more ferric hydroxide would be precipitated to produce higher degrees of Fe plaque on root surfaces and in the rhizosphere, which in turn would bind more Zn. Thus, a wetland species with a higher rate of ROL could have a greater ability to immobilize Zn on its roots and in the rhizosphere zone, thereby reducing Zn uptake by roots from sediment and its translocation from roots to shoot, so increasing Zn tolerance.

In order to test this hypothesis, a hydroponic experiment for determining the rates of ROL, root porosity and tolerance to Zn of wetland plants and a rhizobag trial for determining the concentrations of Zn, Fe and Mn on root surfaces (plaque on roots) and in rhizosphere were conducted under glasshouse conditions. The present study therefore aimed to: 1) investigate the rates of ROL, root porosity and tolerance to Zn in 18 emergent wetland plants subjected to moderate and high levels of Zn contamination using hydroponic culture, and 2) determine the relationships between ROL and Fe plaque on root surfaces and in rhizosphere and Zn uptake and accumulation in 9 of the 18 wetland plants grown in a Zn-contaminated soil. Zinc is one of the commonest heavy metals in contaminated environments such as industrial wastewater and mine wastes (Ye et al. 2004), results from this study will be important when constructed wetlands are employed to treat industrial runoff water with high Zn loading or to phytostabilize Zn in mine tailings.

Materials and methods

Collection and preparation of plant samples

Eighteen emergent wetland plant species were collected from the non-contaminated sites listed in Table 1. Tillers of *Acorus tatarinowii*, *Alocasia cucullata*, *Alternanthera philoxeroides*, *Aneilema bracteatum*, *Fimbristylis monostachya*, *Hydrocotyle vulgaris*, *Rotala rotundifolia*, *Scirpus triqueter* and *Veronica serpyllifolia* were grown from vegetative propagation; the other nine species were germinated from seeds. Because of the differences in growth rates among these species, individuals of the same species with similar shoot height and root length were selected for a hydroponic experiment (18 species) and a pot trial (9 species). Plant cultivation was conducted in a glasshouse in a randomized block design. The glasshouse was illuminated with cool-white fluorescent tubes, supplying a photon flux density of 300 μmol m^{-2} S^{-1}, a relative humidity of 85 % and a light/dark cycle of 14 h day/10 h night. The day/night temperature regime was between 28/22 °C.

Hydroponic experiment with different levels of Zn

For each wetland species, nine replicates were prepared of a control (without any addition of Zn) and six replicates of two levels of Zn concentration (2 mg L^{-1} and 4 mg L^{-1}) supplied as $ZnSO_4 \cdot 7H_2O$. Uniform young plants were transferred to blackened pots (2 L in volume) containing 20 %-strength Hoagland's nutrient solution (Hoagland and Arnon 1938). The nutrient solution was changed every 3 days and the pH of the solution adjusted to 5.5 using HCl or NaOH. After 3 weeks of the experiment, all plants were harvested for the measurement of root length then rinsed thoroughly with deionized water. One third of the control plants and half of the Zn-treated plants were separated into below-ground (roots) and above-ground parts (shoots) for the determination of biomass and metal concentrations. Another one third of the control plants and the other half of the Zn-treated plants were used to measure ROL and the remaining one third of the control plants was used to measure root porosity (POR).

Rate of radial oxygen loss (ROL) was determined according to the method described by Kludze et al. (1994). All solutions were prepared under N_2 in order to remove dissolved O_2. Titanium trichloride (1.16 M, 30 mL) was added to a deoxygenated sodium citrate solution (0.2 M, 300 mL), and the pH adjusted to 5.6 by adding saturated sodium carbonate solution. Before the reactions, plant samples were washed carefully and inserted into tubes (80 mL) with roots completely immersed into 40 mL of 10 % deoxygenated Hoagland's solution. Subsequently, Ti^{3+}-citrate solution (5 mL) was injected into each tube with a plastic syringe, followed immediately by layering the solution surface with 20 mm of paraffin oil to hinder oxidation by ingress of atmospheric O_2. Tubes without plants were set up as blanks. All tubes, with and without plants, were kept under the same conditions, and all steps of the above operation were carried out in a sealed box filled with N_2 gas. After 6 h, the tubes were shaken gently and the solution sampled by a syringe through rubber tubing. The absorbance of the partly oxidized Ti^{3+}-citrate solution was measured by a UV-visible spectrophotometer (UV-1601, Shimadzu, Japan) at a wavelength of 527 nm. The amount of O_2 released was determined based on Ti^{3+}-citrate oxidation,

Table 1 Wetland plants employed in hydroponic culture and soil pot trial (only the species with * were used for pot trial)

Species	Family	Cotyledon	Site distribution of the species collected and general description
Acorus tatarinowii Schott*	Araceae	Dicotyledon	Zhuhai, Guangdong Province; distributed in waterlogged sediment with Eh from −80 to 20 mV
Alocasia cucullata (Lour.) Schott*	Araceae	Monocotyledon	Zhuhai, Guangdong Province; distributed in saturated soil with 0–50 mV.
Alternanthera philoxeroides (Mart.) Griseb.	Amaranthaceae	Dicotyledon	SYS University, Guangdong Province; distributed in semi-waterlogged soil.
Aneilena bracteatum (Clarke) O. Kuntze	Commelinaceae	Dicotyledon	SYS University, Guangdong Province; distributed in semi-waterlogged soil.
*Echinodorus amazonicus**	Alismataceae	Monocotyledon	Zhuhai, Guangdong Province; distributed in waterlogged sediment with Eh from −150 to −50 mV.
*Echinodorus baothii**	Alismataceae	Monocotyledon	Zhuhai, Guangdong Province; distributed in semi-waterlogged soil.
Eleocharis geniculata (L.) Romer & Schult.*	Cyperaceae	Monocotyledon	SYS University, Guangdong Province; distributed in saturated soil with 0–30 mV.
Emilia sonchifolia (Linn.) DC.	Acanthaceae	Dicotyledon	Zhuhai, Guangdong Province; distributed in waterlogged sediment with Eh from −30 to 20 mV.
Fimbristylis monostachya (Linn.) Hassk.	Cyperaceae	Monocotyledon	SYS University, Guangdong Province; distributed in saturated soil with Eh around 0–50 mV.
Hydrocotyle vulgaris L.*	Umbelliferae	Dicotyledon	Zhuhai, Guangdong Province; distributed in semi-waterlogged soil.
Ludwigia hyssopifolia (G. Don) Exell	Onagraceae	Monocotyledon	SYS University, Guangdong Province; distributed in saturated with 0–70 mV.
Myriophyllum aquaticum (Vell.) Verdc.	Haloragidaceae	Dicotyledon	Zhuhai, Guangdong Province; distributed in semi-waterlogged soil.
Panicum repens Linn.*	Gramineae	Monocotyledon	SYS University, Guangdong Province; distributed in saturated soil with −20–70 mV.
Paspalum scrobiculatum Linn.	Gramineae	Monocotyledon	Zhuhai, Guangdong Province; distributed in waterlogged sediment with Eh from −20 to 70 mV.
Philydrum lanuginosum Banks et Sol. ex Gaertner	Philydraceae	Monocotyledon	Zhuhai, Guangdong Province; distributed in saturated soil with −20–80 mV.
Rotala rotundifolia Buch.-Ham. ex Roxb.	Lythraceae	Dicotyledon	Zhuhai, Guangdong Province; distributed in semi-waterlogged soil.
Scirpus triqueter Linn.*	Cyperaceae	Monocotyledon	Zhuhai, Guangdong Province; distributed in saturated soil with −100–60 mV.
Veronica serpyllifolia Linn.*	Scrophulariaceae	Dicotyledon	Huadiwan, Guangdong Province; distributed in saturated soil with −100–50 mV.

and ROL was calculated according to the following equation (Kludze et al. 1994; Li et al. 2011):

$$\text{Amount of ROL} \left(\text{mmol } O_2 \text{ plant}^{-1} \text{ h}^{-1}\right) = c \ (y{-}z)$$
$$\text{Rate of ROL} \left(\text{mmol } O_2{\bullet}\text{kg}^{-1} \text{ root d.w.d}^{-1}\right) = c \ (y{-}z)/g$$

Where c = initial volume of Ti^{3+}-citrate added to each tube, L; y = concentration of Ti^{3+}-citrate solution of control (without plants), mmol Ti^{3+} L^{-1}; z = concentration of Ti^{3+}-citrate solution after 6 h with plants, mmol Ti^{3+} L^{-1}; g = root dry weight of the plant, kg plant^{-1}.

Root porosity (POR) was measured by a pycnometer method (Jensen et al. 1969) and calculated using the following formula:

$$\text{Porosity } (\%, \text{ air space}) = \{[(\text{p and vr})-(\text{p and r})]/[(\text{p}+\text{r})]-(\text{p and r})]\} \times 100$$

Where r = mass of fresh roots, in g; p = mass of water-filled pycnometer, in g; p and r = mass of pycnometer with fresh roots and water, in g; p and vr = mass of pycnometer with vacuumed roots and water, in g.

Pot trials with sand and soil contaminated with Zn

The soil used in the pot trial was collected from an abandoned paddy field (0–20 cm) in Fankou (FK) Pb/Zn mine area located in Shaoguan city, Guangdong Province, China. The soil was thoroughly mixed, air-dried and ground to <2 mm. The physical and chemical properties of the soil were analyzed and presented as follows: pH (2.5:1 distilled water: soil, v/w) 5.6, organic matter (K_2CrO_7-H_2SO_4) 33.1 g kg^{-1}, total N (semi-quantitative titration) 1.63 g kg^{-1}, Olsen-P (0.5 M $NaHCO_3$) 12 mg kg^{-1}, available K (1.0 M NH_4OAc) 143.5 mg kg^{-1}, total Zn 775 mg kg^{-1}, total lead (Pb) 685 mg kg^{-1}, total Fe 18.1 g kg^{-1}, and total manganese (Mn) 138 mg kg^{-1}.

Sand is a common substrate used in constructed wetlands (Bubba et al. 2003) and so a rhizobag soil-sand combination incubation experiment was designed for the present study. Rhizobags, made of nylon netting with a mesh size of 40 μm, were 4 cm in diameter and 10 cm height filled with 0.3 kg of sand. The sand was collected from Huadiwan, Guangzhou, PR China and was not contaminated with heavy metals. Before use, the sand was washed, air-dried and sieved (<2 mm). The sand-filled rhizobags were placed in the centre of each soil pot (9 cm diameter x 11 cm height). Plants were transplanted into sand at the beginning of the pot experiment and the sand is here referred to as 'rhizosphere' material at the end of the study as it was totally permeated by roots. The rest of the pot outside the rhizobag was filled with 1 kg of air-dried FK soil with an inundation depth of *c.* 3–4 cm; this soil is here referred to as 'non-rhizosphere' (the comparable soil zone). This design successfully prevented roots and even root hairs from entering the adjacent non-rhizosphere soil zone, whilst permitting the transfer of microfauna and root exudates between the two compartments. This meant that although the soil was used to enclose the outside of the rhizobag, the rhizosphere was confined to the sand compartment and effectively separated from the non-rhizosphere soil compartment.

Nine wetland plant species with different rates of ROL, based on the results obtained from the above hydroponic culture experiment, were used for the pot trial. The heights of the nine wetland species ranged from 15 to 20 cm. For each species, two seedlings or tillers were transplanted into sand and were grown under the same glasshouse conditions as in the hydroponic experiment. Three replicates were prepared for each plant species. Rhizobag pots were arranged in a randomized design and their positions in the glasshouse were rotated regularly to ensure uniform growing conditions. At the end of 90 days, plants in each rhizobag pot were carefully removed from the sand, and the rhizosphere and non-rhizosphere materials separated. Plants were manually separated into roots and shoots, thoroughly rinsed with deionized water and used for the determination of biomass, concentrations of Zn, Fe and Mn in root and shoot tissues and on root surfaces.

At harvest, Fe plaque on fresh root surfaces or sand (the rhizosphere material) was extracted using dithionite-citrate-bicarbonate (DCB solution containing 0.3 M sodium citrate, 1.0 M sodium bicarbonate, with the addition of 1.5 g sodium dithionite) (Taylor and Crowder 1983; Otte et al. 1989). Roots or sand were immersed in 22.5 mL DCB solution and agitated for 3 h at room temperature. The extract was then filtered with quantitative filter papers, rinsed three times with deionized water that finally was added to the DCB extract. The resulting solution was made up to 100 mL with deionized water. After extraction with DCB, roots and shoots were oven-dried to constant weight at 60 °C for chemical analysis.

Chemical analysis

Oven-dried plant shoot or root samples were ground using a Retsch grinder (Type: 2 mm, made in Germany), and Zn in plant tissue was extracted by digesting the sample with nitric (HNO_3) and perchloric ($HClO_4$) acids (4:1, v/v). The concentrations of Zn in plants tissues, as well as Zn, Fe and Mn in DCB-extracts, were determined by Inductively-Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (Optima 2000DV, Perkin Elmer, USA) (Page et al. 1982). For quality assurance, blanks and standard plant materials [GBW-07603 (GSV-2) China Standard Materials Research Center, Beijing, PR China] were employed. Average recovery rates for all metals (Zn, Fe and Mn) were within the range of 90±10 %.

Statistical analysis

Zn tolerance was quantified by a tolerance index (TI) calculated from a comparison between the elongation of the longest root on each plant in treatments with and without Zn additions (Wilkins 1978).

$$\text{Tolerance index}(\%) = \frac{\text{root elongation in solution with Zn}}{\text{root elongation in solution without Zn}} \times 100$$

Data on plant performances were tested for their normality and variance prior to a one-way analysis of variance (ANOVA), as no data transformation was needed. If the differences among plant species for each Zn treatment, or among different Zn treatments for each plant species, were significant at 5 % level, the least significant difference (LSD) was calculated as the *post hoc* test to determine where differences lay. All statistical analyses were performed using the SPSS 11.0 statistical package.

Results

Rates of ROL and porosity of roots in the hydroponic experiment

After growing in Hoagland's solutions for 3 weeks, both root porosities and rates of ROL varied significantly among the 18 wetland plants tested, even in the control without Zn addition (Table 2). In the control, root porosities ranged from 9 % (*M. aquaticum*) to 28 % (*E. amazonicus*), and the rates of ROL from 86 (*M. aquaticum*) to 872 (*E. baothii*) mmol O_2 • kg^{-1} root d.w. d^{-1}. Compared with the control, the rates of ROL of all species were reduced with increasing amounts of Zn treatment. The reduction (in terms of % of the control) was significantly different (P<0.01) among the 18 wetland species, ranging from 24.4 % (*F. monostachya*) to 66.4 % (*A. bracteatum*) in the 2 mg Zn L^{-1} treatment and from 56.2 % (*A. cucullata*) to 78.7 % (*H. vulgaris*) in the 4 mg Zn L^{-1} treatment.

Root elongation of the wetland plants in the hydroponic experiment

The root elongations of most plants in the Zn treatments were significantly less than in the control. Tolerance indices of the 18 species varied from 32 % to 108 % in the hydroponic cultures with 2 mg Zn L^{-1} and from 14 % to 59 % in the 4 mg Zn L^{-1} treatments, indicating that the reduction was more significant as Zn concentration increased (Table 2).

Concentrations of Zn in shoot and root tissues in the hydroponic experiment

Zinc concentrations of the 18 species grown in the control, 2 and 4 mg Zn L^{-1} treatments ranged from 43 to 356, 53 to 1,216, 74 to 3,196 mg kg^{-1} in shoots, respectively, and the matching values in roots were 55 to 658, 763 to 13,576, 2,026 to 16,440 mg kg^{-1} (Table 4). The concentrations of Zn in root tissues of plants exposed to Zn in hydroponic culture increased significantly (P<0.05). Zinc concentrations in root tissues were significantly higher than those in shoot tissues (P<0.05).

Table 2 Porosity (%), rate of ROL (mmol $O_2 \cdot kg^{-1}$ root d.w. d^{-1}), root elongation (cm) and tolerance indices (TI, % of control) of 18 species of wetland plants grown in hydroponic culture at different Zn concentrations: CK (control, without Zn), 2 and 4 mg Zn L^{-1} (as $ZnSO_4 \cdot 7H_2O$) treatments, for 3 weeks (mean ± S.E., *n*=3)

Species	Porosity (%)	ROL (mmol $O_2 \cdot kg^{-1}$ root d.w. d^{-1})			Root elongation (cm)			Zn TI (%)	
	CK	CK	Zn2	Zn4	CK	Zn2	Zn4	Zn2	Zn4
A. tatarinowii	14±0.4	349±6 a	158±10 b	151±22 b	4.38±0.29 a	3.12±0.15 b	2.00±0.05 c	71	46
A. cucullata	19±0.6	365±7 a	213±14 b	160±17 c	15.35±1.31 a	7.95±0.72 b	4.18±0.45 c	51	27
A. philoxeroides	15±2.1	302±18 a	178±22 b	76±14 c	4.97±0.39 a	3.42±0.28 b	2.22±0.26 c	68	44
A.a bracteatum	13±1.5	265±9 a	89±7 b	67±16 b	12.72±0.88 a	6.22±0.35 b	4.95±0.25 b	49	39
E. amazonicus	28±0.6	835±11 a	363±25 b	327±37 b	18.23±1.67 a	16.93±0.71 a	10.07±0.86 b	93	55
E. baothii	27±0.9	872±17 a	398±18 b	184±23 c	9.40±0.47 a	8.07±0.35 b	3.79±0.20 c	86	47
E. geniculata	17±0.6	489±11 a	248±17 b	177±46 b	4.28±0.67 a	2.55±0.10 b	1.98±0.11 c	54	42
E. sonchifolia	16±1.9	459±13 a	151±26 b	103±17 b	6.67±0.23 a	2.33±0.07 b	2.07±0.16 b	36	32
F. monostachya	11±0.9	193±7 a	146±12 b	71±9 c	3.47±0.22 a	2.05±0.05 b	1.50±0.09 c	59	43
H. vulgaris	13±1.7	202±6 a	64±8 b	43±13 b	4.55±0.73 a	2.33±0.20 b	1.48±0.14 b	51	32
L. hyssopifolia	13±0.6	248±8 a	148±17 b	68±13 c	16.17±0.13 a	8.75±0.23 b	5.52±0.59 c	54	34
M. aquaticum	9±1.2	86±7 a	55±7 b	22±5 c	13.82±1.19 a	5.38±0.19 b	1.98±0.12 c	32	14
P. repens	13±0.6	211±6 a	97±9 b	48±7 c	6.12±0.52 a	3.42±0.37 b	1.82±0.21 c	56	30
P. scrobiculatum	17±0.5	473±4 a	203±14 b	131±10 c	3.63±0.09 a	2.35±0.08 b	2.12±0.12 b	65	59
P. lanuginosum	14±0.9	276±5 a	136±11 b	100±14 b	6.32±0.42 a	2.40±0.37 b	2.00±0.15 b	38	32
R. rotundifolia	15±0.6	454±5 a	240±33 b	124±23 c	6.18±0.16 a	3.67±0.10 b	2.00±0.23 c	60	33
S. triqueter	14±0.3	428±8 a	235±17 b	101±17 c	5.05±0. 08 a	2.50±0.13 b	2.42±0.28 b	49	47
V. serpyllifolia	21±0.6	580±7 a	289±32 b	143±28 c	4.55±0.15 a	4.97±0.29 a	2.50±0.11 b	108	54

Different letters after mean ± SE within the same plant species indicate significant differences in ROL or root elongation among three Zn treatments [CK, Zn2, Zn4] at $P<0.05$ according to one−way ANOVA followed by LSD test

Plant growth, uptake and immobilization of Zn, Fe and Mn in the soil pot trial

Significant differences in the biomass of the nine species were found when grown in FK soil (Table 4). Among the nine species, *P. repens* showed the highest biomass in both shoots and roots and *E. geniculata* the lowest.

Zinc accumulated in plant tissues when grown in FK soil, with Zn concentrations of the nine species ranging from 72 to 517 mg kg^{-1} in shoots and 157 to 808 mg kg^{-1} in roots (Table 4). Concentrations of Zn, Fe and Mn on root surfaces ranged from 82 to 279, from 2,330 to 13,125 and from 69 to 384 mg kg^{-1}, respectively and on sand surfaces 126–350, 8,527–23,308 and 417–1,078 mg kg^{-1}, significantly higher than the root values (Table 4). Iron concentrations were also higher than for Zn or Mn on both root and sand surfaces. Zinc concentrations in plants followed the order of root tissues > root surfaces > shoot tissues.

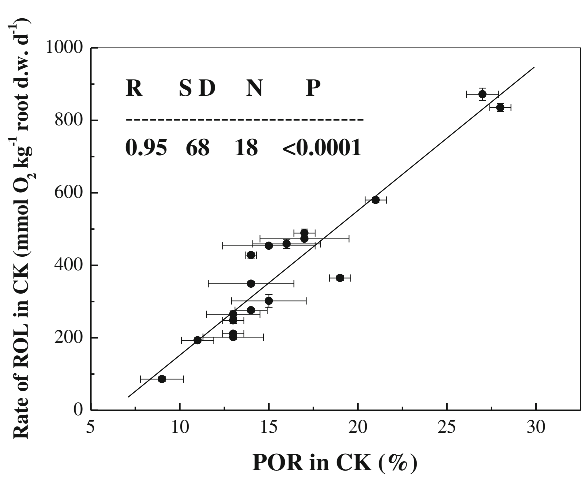

Fig. 1 Correlations between rates of radial oxygen loss (ROL) and root porosity (POR) of the control wetland plants (CK) in hydroponic culture

Correlations between ROL rates, porosity, Zn tolerance and Zn, Fe and Mn on roots and in the rhizosphere

ROL rates in all plants grown in the control solution were significantly and positively correlated with root porosities (Fig. 1). Significant correlations were also found between the rates of ROL/porosities of control plants and Zn tolerance indices in plants grown in the 2 mg Zn L^{-1} and 4 mg Zn L^{-1} treatments (Fig. 2). Positive correlations were also found between the rates of ROL in Zn-treated plants and their Zn tolerance indices in both 2 mg Zn L^{-1} and 4 mg Zn L^{-1} treatments (Fig. 2). These results suggested that the rates of ROL and porosities had significant impacts on Zn tolerance indices in the hydroponic culture. ROL from roots also affected the concentrations of Zn, Fe and Mn on root surfaces (plaque on root surface) and on sand surfaces (plaque in rhizosphere). Significant correlations were found between the rates of ROL of the nine plants grown in the control solution and the concentrations of Zn, Fe and Mn on root surfaces of the plants grown in FK soil, as well as their concentrations of Zn and Fe on sand surfaces (the rhizosphere material) (Fig. 3). Positive correlations were observed between Zn concentration and Fe or Mn concentration

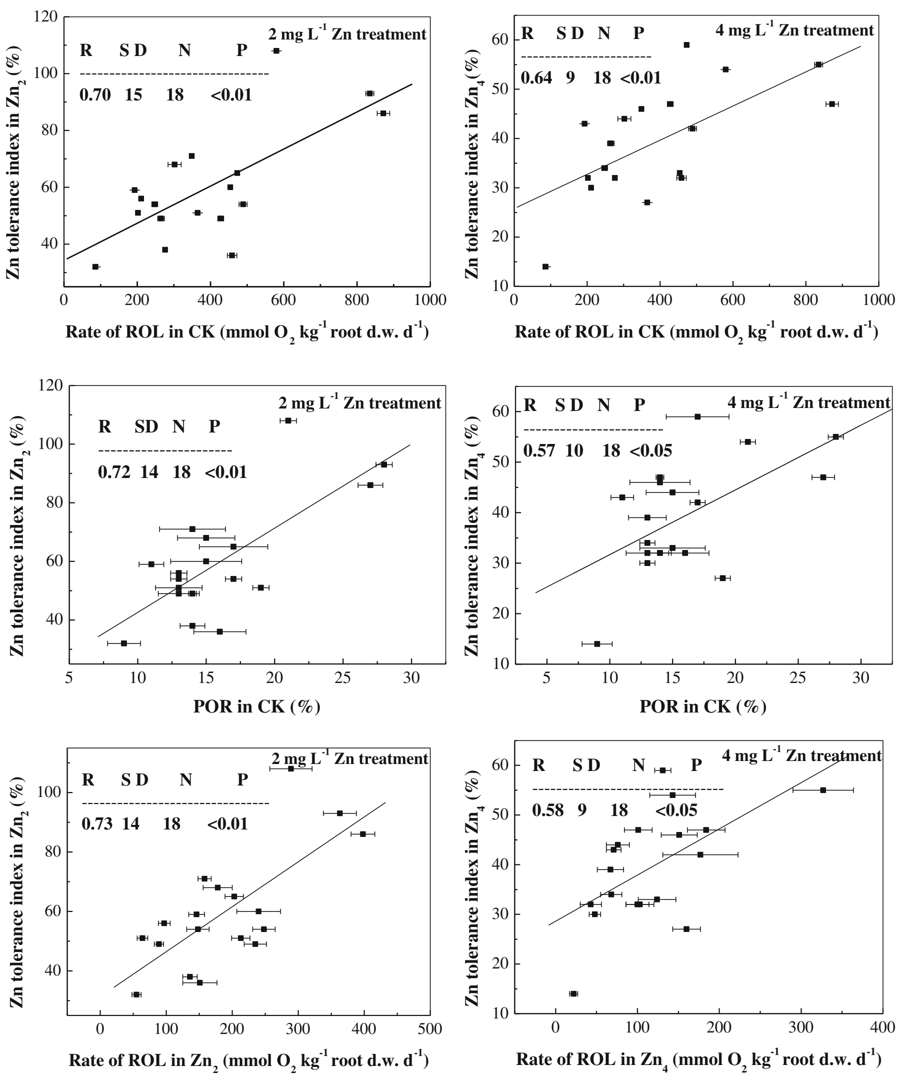

Fig. 2 Correlations between Zn tolerance index of 18 species of wetland plants grown in 2 mg L^{-1} (*left panels*) or 4 mg L^{-1} (*right panels*) Zn treatments and rate of radial oxygen loss (ROL) and porosity (POR) of the control plants (CK), as well as rate of ROL in Zn-treated plants in hydroponic culture

on root surfaces, as well as between Zn and Fe concentration on sand surfaces (Fig. 4).

Discussion

Effects of Zn on root elongation and ROL

The growth of wetland plants (e.g. root elongation) is usually depressed when stressed by high levels of toxic elements (Ye et al. 1997a, b; Matthews et al. 2005). The present study clearly showed that root elongation of most wetland species was reduced when grown in the presence of elevated Zn concentrations compared to the control solution, but the degree of reduction differed between plant species, suggesting that the different wetland plants tested have different Zn tolerances.

ROL has been considered as a byproduct of root oxygenation, varying considerably between species or cultivars of different wetland plants (Van Bodegom et al. 2005; Mei et al. 2009; Li et al. 2011). In the present study, significant differences in rates of ROL were found between the 18 species under control conditions ($P<0.01$), ranging from 86 to 872 mmol $O_2 \bullet kg^{-1}$ root d.w. d^{-1}. In addition, the rates of ROL of the 18 species reduced considerably with increasing Zn stress and the

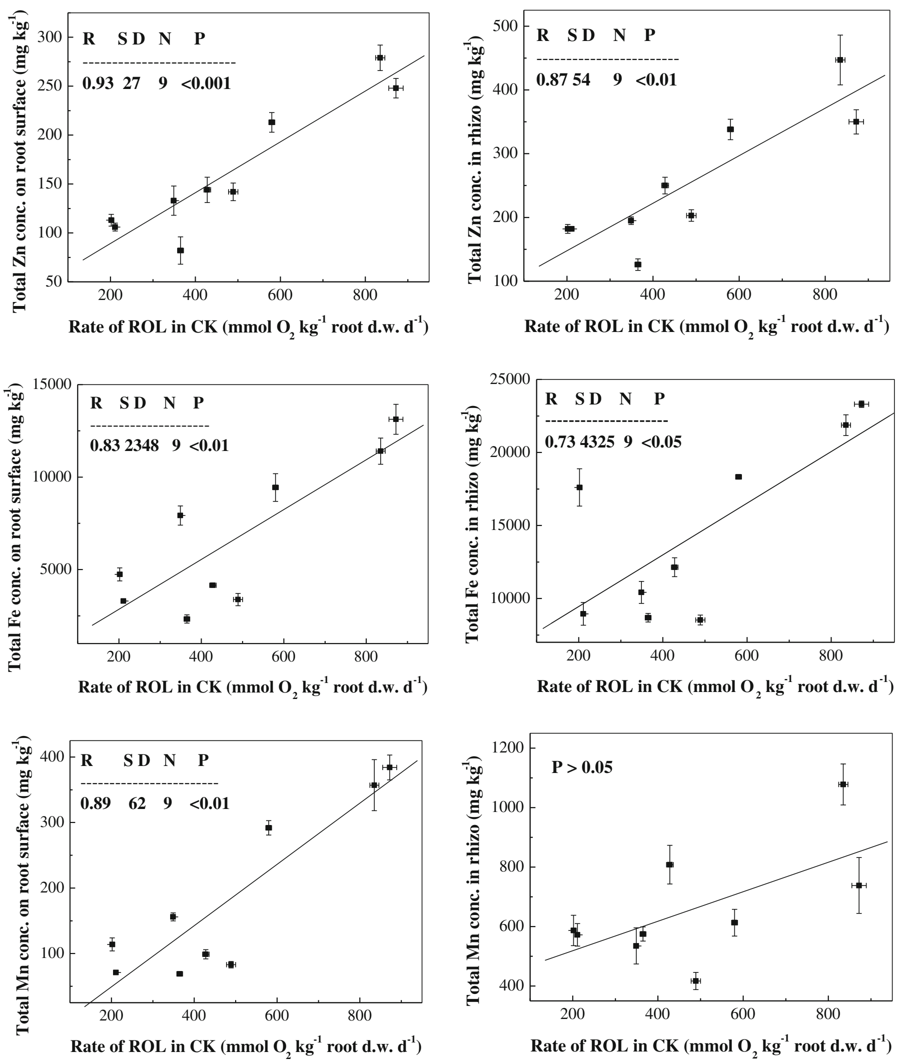

Fig. 3 Correlations between total concentrations of Zn, Fe and Mn on root surfaces (*left panels*) or on sand surfaces (rhizosphere) (*right panels*) of nine species of wetland plants grown in FK soil having 775 mg Zn kg^{-1} and rates of radial oxygen loss (ROL) of the control plants grown in hydroponic culture

effects of Zn on ROL differed markedly between them. It is suggested that reduction in ROL by Zn exposure may be partly due to changes in root porosity resulting from damage to root tissues, such as aerenchyma. Liu et al. (2009) also reported that the biomass of wetland plants is positively correlated with ROL. Moreover, the concentrations of photosynthetic pigments and the efficiency of the photosynthetic process, an important source of oxygen for ROL, decrease proportionately in mangrove plants when exposed to Zn (MacFarlane and Burchett 2002). These results suggest that a decrease in biomass, particularly that of leaves, will reduce photosynthesis and consequently lead to decreases in the ROL from entire roots in all wetland plants.

Root porosities of the 18 wetland plants grown in the control solution ranged from 9 % to 28 % (Table 2), which indicated that root porosity differed greatly between the wetland plant species tested. A highly significant correlation ($P<0.001$) was found between ROL and root porosities (Fig. 1), which suggests that higher root porosities facilitate more oxygen loss from roots to the rhizosphere. Similarly, Van Bodegom et al. (2005) showed that ROL rates are mainly determined by factors such as the amount of aerenchyma tissue in shoots and roots, root respiration and the presence of a ROL barrier in the basal root zones, reducing oxygen diffusion into the rhizosphere.

Zinc in shoot and root tissues and Zn, Fe and Mn on roots and in the rhizosphere

Concentrations of Zn in shoot and root tissues varied greatly between species when exposed to Zn treatments in both the hydroponic culture and the soil pot trial (Tables 3 and 4), suggesting different abilities in Zn accumulation. Concentrations of Zn in root tissues in both culture conditions were significantly higher than those in shoot tissues ($P<0.05$). This may be due to the operation of an 'excluder' strategy in which the concentrations of heavy metals in the shoots of plants are maintained at a constant low level when grown in heavy metal-contaminated soils (Baker 1981). The low Zn concentration in shoots, such as in *E. geniculata* and *S. triqueter* (72–80 mg kg^{-1}), also explains why plants grown in FK soil in the present study did not show any visible Zn toxicity symptoms. However, even *V. serpyllifolia* having the highest accumulation of Zn in shoots (up to 517 mg Zn kg^{-1}) of the 18 species investigated in the present study still did not show any signs of Zn toxicity. These findings indicate that internal Zn detoxification tolerance

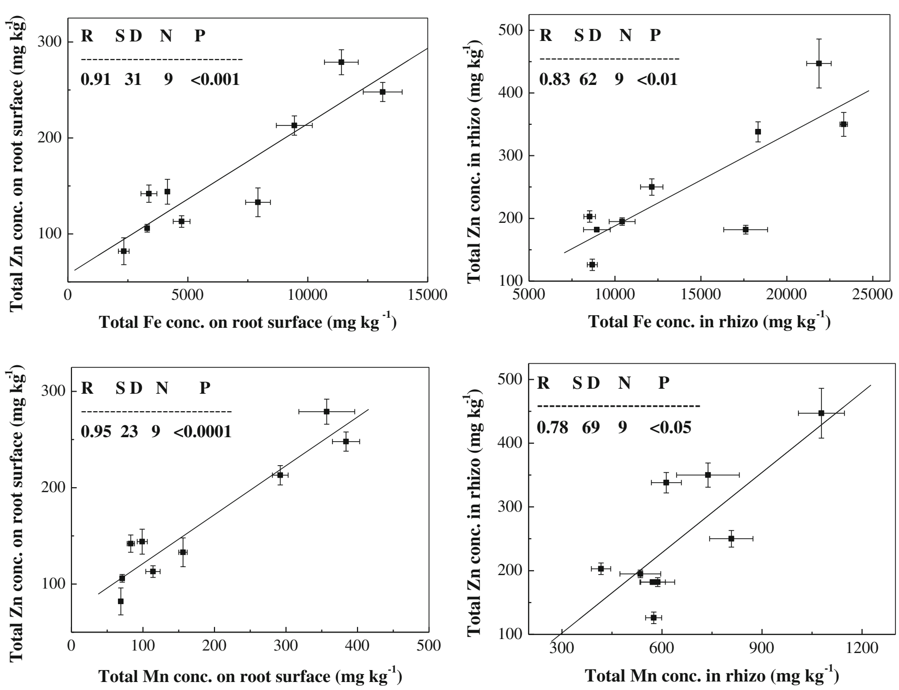

Fig. 4 Correlations between total Fe or Mn concentrations and total Zn concentrations on root surface (*left panels*) or on sand surfaces (rhizosphere) (*right panels*) of nine species of wetland plants grown in FK soil (775 mg Zn kg^{-1} in soil)

mechanisms may exist in some wetland plants, such as *V. serpyllifolia,* in addition to the operation of an 'excluder' strategy. Baker (1981) also suggested that internal detoxification tolerance mechanisms might exist in plants when grown in heavy metal-contaminated soils.

Our results showed that the concentrations of Fe were higher than those of Mn both on root surfaces and in the rhizospheres (Table 4). Similar results have been reported by Ye et al. (2001) and Hu et al. (2007). It is possible that Fe oxides and hydroxides may precipitate at lower redox potentials than Mn oxides at any pH values, or at a lower pH than Mn under fixed Eh conditions (St-Cyr and Crowder 1990).

Correlations between rates of ROL, porosity, Zn tolerance and Zn, Fe and Mn on roots and in the rhizosphere

ROL and root porosities of plants in both the control and Zn solutions were positively correlated with Zn tolerance indices of plants in the two Zn treatment solutions ($P<0.05$) (Fig. 2), indicating the importance of these two root properties in the expression of Zn tolerance. The data shown in Figs. 3 and 4 suggest that an increase in oxygen release from roots will stimulate the formation of Fe plaque, due to the oxidation of Fe(II) to Fe(III) on root surfaces or in the rhizosphere. The formation of Fe plaques then increases the adsorption of Zn on to root surfaces and in the rhizosphere. An increase of Zn adsorption on root surfaces due to Fe plaque has previously been reported in other wetland plants, such as *Aster tripolium, Typha latifolia* and *Juncus effusus* (Otte et al. 1989; Ye et al. 2001). This phenomenon has also been observed for other metals and metalloids, such as Cu (Greipsson 1995) and As (Li et al. 2011). The formation of Fe plaque is influenced by the availability of Fe in soil and the oxidizing capacity of plant roots (Hansel et al. 2002). The mobility and availability of many trace and toxic metals and metalloids to plants growing in wetland soils are often controlled by redox potential and the associated rhizosphere pH (Gambrell et al. 1991). These two properties in turn are influenced by the rate of ROL

Table 3 Zinc concentration (mg kg^{-1} dry w.t.) in shoots and roots of 18 species of wetland plants grown in hydroponic culture with different Zn contamination: CK (control, without Zn), 2 and 4 mg Zn L^{-1} treatments for 3 weeks (mean ± S.E., *n*=3)

Species	Shoot			Root		
	CK	Zn2	Zn4	CK	Zn2	Zn4
A. tatarinowii	46±8 b	54±4 b	97±4 a	265±6 c	4410±102 b	6133±122 a
A. cucullata	105±12 c	1216±46 b	1839±235 a	92±3 c	4978±52 b	6273±72 a
A. philoxeroides	98±7 c	648±7 b	1239±94 a	406±7 c	3697±87 b	6865±74 a
A. bracteatum	82±3 c	840±73 b	1145±76 a	651±15 c	6108±127 b	6758±139 a
E. amazonicus	62±5 c	394±11 b	598±8 a	203±3 c	6824±14 b	9022±82 a
E. baothii	356±13 c	664±16 b	1775±139 a	568±8 c	4496±183 b	6137±57 a
E. geniculata	76±2 b	392±7 a	409±20 a	88±3 c	2380±79 b	3549±69 a
E. sonchifolia	113±5 c	682±46 b	1041±115 a	413±35 c	2309±103 b	3856±153 a
F. monostachya	43±5 b	53±4 b	74±8 a	55±3 c	763±85 b	2026±79 a
H. vulgaris	194±4 b	958±20 b	1264±50 a	440±8 c	2939±80 b	4184±80 a
L. hyssopifolia	119±5 c	1101±105 b	3196±171 a	398±104 c	8321±307 b	10826±258 a
M. aquaticum	144±6 b	387±23 a	464±35 a	744±53 c	13350±533 b	16440±398 a
P. repens	60±4 c	215±5 b	314±5 a	216±9 b	2133±98 a	2354±52 a
P. scrobiculatum	84±6 c	814±52 b	1569±160 a	183±3 c	1286±56 b	4149±504 a
P. lanuginosum	70±2 c	218±9 b	520±27 a	152±9 c	3610±185 b	5975±80 a
R. rotundifolia	59±2 c	502±23 b	667±43 a	360±14 c	6641±154 b	7467±158 a
S. triqueter	53±4 b	131±4 b	484±87 a	256±17 c	1891±75 b	3122±23 a
V. serpyllifolia	215±5 c	1177±191 b	1762±71 a	658±7 b	13576±443 a	14201±182 a

Different letters after mean ± SE within the same plant species and same plant tissue indicate significant differences in shoot or root concentrations among three different Zn treatments [CK, Zn2 and Zn4] at $P<0.05$ according to one-way ANOVA followed by LSD tests

Table 4 Biomass (g pot^{-1}) of shoot and root, Zn concentrations (mg kg^{-1} d.wt) in shoot tissues, root tissues, plaque on root surface ($Plaque_{root}$) and plaque in rhizosphere ($Plaque_{rhizo}$), Fe and Mn concentrations (mg kg^{-1} d.wt) in $Plaque_{root}$ and $Plaque_{rhizo}$ of the nine wetland species grown in FK soil for 3 months (mean ± S.E., n=3)

Species	Biomass		Zn concentration				Fe concentration		Mn concentration	
	Shoot	Root	Shoot	Root	$Plaque_{root}$	$Plaque_{rhizo}$	$Plaque_{root}$	$Plaque_{rhizo}$	$Plaque_{root}$	$Plaque_{rhizo}$
A. tatarinowii	3.9±0.1 b	0.79±0.17 bc	88±7 e	442±27 d	133±15 de	195±6 d	7924±518 d	10417±754 cd	156±6 c	535±61 d
A. cucullata	2.9±0.6 c	0.81±0.03 bc	81±10 e	157±16 f	82±14 f	126±9 e	2330±223 f	8682±294 d	69±3 d	575±24 cd
E. amazonicus	3.7±0.4 bc	0.74±0.17 bc	154±4 d	604±34 bc	279±13 a	447±39 a	11402±705 b	21870±712 a	357±39 a	1078±69 a
E. baothii	2.6±0.2 cd	0.89±0.05 bc	188±8 c	606±21 bc	248±10 b	350±19 b	13125±813 a	23308±220 a	384±19 a	738±94 c
E. geniculata	0.32±0.04 e	0.22±0.03 c	72±5 e	373±21 d	142±9 de	203±9 d	3379±332 ef	8527±333 d	83±5 d	417±29 d
H. vulgaris	1.8±0.1 d	0.62±0.04 bc	412±23 b	245±17 e	113±6 e	182±7 d	4740±351 de	17605±1281 b	114±10 cd	587±51 c
P. repens	7.6±0.5 a	2.3±0.7 a	149±15 d	579±20 c	106±4 ef	182±3 d	3303±53 ef	8947±777 d	71±3 d	572±38 c
S. triqueter	2.7±0.2 cd	0.61±0.08 bc	80±3 e	663±29 b	144±13 d	250±13 c	4151±47 e	12142±650 c	99±7 d	808±65 bc
V. serpyllifolia	2.5±0.3 cd	1.0±0.1 b	517±10 a	808±39 a	213±10 c	338±16 b	9440±749 c	18328±137 b	292±11 b	613±45 cd

Different letters after mean ± SE in the same column indicate significant differences in each parameter among the nine wetland plant species grown in flooded soil at $P<0.05$ according to one-way ANOVA followed by LSD tests

from roots (Mei et al. 2012; Yang et al. 2012). Yang et al. (2010) found that *V. serpyllifolia*, having the highest ROL of the four wetland plants studied, forms the greatest extent of Fe plaque on root surfaces and immobilizes the highest Zn concentration in Fe plaque. This also has the greatest effect in reducing the bioavaibility of Zn in rhizosphere soil. Iron plaque might also act as an effective Fe reservoir to increase Fe concentrations in active cells and ameliorate Zn toxicity (Ye et al. 1998). The present study together with previous results suggest that ROL from roots has important roles in Fe plaque formation and in the mobility and bioavailability of Zn, both on root surfaces and in the rhizosphere, and is thus involved in the detoxification of Zn. This contention is further supported by the present findings that species with higher rates of ROL tend to have a greater Zn tolerance as measured by root elongation.

The effects of Fe plaque on metal adsorption, uptake and accumulation have been reported but most previous studies only focus on Fe, Mn and metals and metalloids (As, Cd, Zn, Cu, Ni) on root surfaces (Ye et al. 1998; Liu et al. 2004, 2006). Our results show that the concentrations of Fe, Mn and Zn in the plaque in the rhizosphere were significantly higher than those on root surfaces (Table 4), suggesting that Fe plaque in the rhizosphere may have a more important role than that on root surfaces. Therefore, more in-depth studies on the role of Fe plaque are clearly needed both in the rhizosphere and on root surface.

Conclusions

This study has revealed that wetland plants possessing high porosity and high ROL from their roots tend to have high Fe, Mn and Zn concentrations on root surfaces and in their rhizosphere. They also have high Zn tolerance indices, suggesting that the porosity and ROL of roots may play important roles in detoxifying Zn in wetland plants. Oxidation in the rhizosphere resulting from ROL will lead to the precipitation and immobilization of Zn on root surfaces and in the rhizosphere zone, thus decreasing metal translocation from roots to shoots. The results suggest that the wetland plants with higher root porosity/ROL have higher ability in treating Zn (or other metals) contaminated waters and sediments/soils and the rate of ROL and root porosity could be useful indices for selecting wetland plants for the phytoremediation of heavy metals.

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