Synchrotron micro-scale measurement of metal distributions in *P. australis* and *T. latifolia* root tissue from an urban brownfield site

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Abstract:

Liberty State Park in New Jersey, USA, is a “brownfield” site containing various levels of contaminants. To investigate metal uptake and distributions in plants on the brownfield site, *Phragmites australis* and *Typha latifolia* were collected in Liberty State Park during the growing season (May - September) in 2011 at two sites with the high and low metal loads, respectively. The objective of this study was to understand the metal (Fe, Mn, Cu, Pb and Zn) concentration and spatial distributions in *P. australis* and *T. latifolia* root systems with micro-meter scale resolution using synchrotron X-ray microfluorescence (μ XRF) and synchrotron X-ray computed microtomography (μ CMT) techniques. The root structure measurement by synchrotron μ CMT showed that high X-ray attenuation substance appeared in the epidermis. Synchrotron μ XRF measurement showed that metal concentrations and distributions in the root cross-section between epidermis and vascular tissue were statistically different. Significant correlations were found between metals (Cu, Mn, Pb and Zn) and Fe in the epidermis, implying that metals were scavenged by Fe oxides. The results from this study suggest that the expression of metal transport and accumulation within the root systems may be element specific. The information derived from this study can improve our current knowledge of the wetland plant ecological function in brownfield remediation.

Keywords:

Phragmites australis

Typha latifolia

Trace metals

Synchrotron radiation technique

Brownfield

Introduction

Numerous studies have shown that soils/sediments in urban-industrial areas often contain mixed organic and inorganic contaminants (Feng et al., 1998, 2004; Zhang et al., 2009). “Brownfields”, which are abandoned industrial land, have presented environmental concerns for decades because one of the most pressing issues caused by “brownfield” is the presence of anthropogenic metal contamination (Gallagher et al., 2008; Koelmel and Amarasiriwardena,

2012). Restoration of “brownfield” sites with green technologies is a challenge (Feng et al., 2005; Weis and Weis, 2004). It is reported that plants can uptake metals from the contaminated soils through roots, translocate the metals to stems and leaves and accumulate these metals within the plant tissues (Lacerda et al., 1997; Qian et al., 2012; Rascio and Navari-Izzo, 2011; Williams et al., 1994). Therefore, metal distributions in the root tissue are the consequence of root metal uptake and transportation (Marschner, 2012; Merchant, 2010). Some metal ions can enter apoplast freely through root epidermis and passively diffuse through apoplast due to concentration gradient and evapotranspiration. At the same time, some metals can enter symplast through cell membranes at root epidermis under the assistance of selective transporters (Marschner, 2012; Taiz and Zeiger, 2010). Iron plaque, which is predominantly a layer of amorphous Fe hydroxide, is often observed on the surface of wetland plant root tissue. It can absorb or co-precipitate metals due to its negative-charged surface. Iron plaque was identified as a buffer or barrier that capable of enhancing or reducing plant metal uptake efficiency (Tripathi et al., 2014). The investigation of metal uptake by plants root system and distribution in the root tissues can provide useful information of understanding the function of plants for bioremediation and phytoextraction of metals in the contaminated soils.

Liberty State Park in northern New Jersey is a brownfield site with a portion of wetland. During the 19th and 20th centuries Liberty State Park in New Jersey was used as a railroad yard finally closing in 1969 (Gallagher et al., 2008). Soils in the park were severely contaminated because of the original filling materials and railroad operation over a century. After conducted corrective actions, such as clean soil capping and asphalt isolation, most part of the park was reopened to the public for recreation and was officially announced as Liberty State Park in 1976 (NJDEP, 1995; LSP, 2008). However, a 251 acre of brownfield was left unremediated in the middle of the park. Previous studies showed that soil metal concentrations in this portion of Liberty State Park exceeded both ecological and residential screening criteria (Gallagher et al., 2008; Qian et al., 2012). This site has remained isolated and much of the area has been re-colonized by various plant assemblages that represent unique associations of both endemic and nonnative species (Gallagher et al., 2008). The Liberty State Park management plans call for the restoration of approximately 44,500 m² (11 acres) of freshwater wetlands and the maintenance of approximately 60,700 m² (15 acres) of native urban wetlands (USACE, 2004). Thus, the site provides a unique laboratory for studying metal uptake and distribution in the plants as

exemplified by this urban brownfield since plants acquire metals from the rhizosphere soil and regulate their uptake within the root system (Hinsinger and Courchesne, 2008; McLaughlin et al., 1998). In order to understand the important role the plants play in metal uptake, translocation and accumulation in the plants, Qian et al. (2012) used bioconcentration factor (BCF), which is defined as a ratio of metal concentrations in the plant root to that in the soil, to evaluate metal uptake efficiency by plants in Liberty State Park. They found that the metal BCF varied among the metals and plant species.

Synchrotron X-ray microbeam techniques, such as synchrotron X-ray microfluorescence (μ XRF) and synchrotron X-ray computed microtomography (μ CMT), have important applications in high resolution study of metal transport and distribution in plants. The unique advantages of synchrotron-based techniques with high detection sensitivity and spatial resolution measurement have led to a better understanding of metal transport and distribution in plants (Feng et al., 2013; Martin et al., 2006; Punshon et al., 2009). In this study, we applied synchrotron CMT to show the root structure and XRF to map metal distributions in the wetland plant root system and assess the role of Fe plaque in metal accumulation in the roots.

1. Materials and methods

1.1 Study area

Two sites in Liberty State Park, Site TP-1 and Site TP-43, were selected for this investigation (Fig. 1). Site TP-1 in the northwest section of the study area was reported to have the lowest total soil metal load in the entire study area (Gallagher et al., 2008). The soil metal concentrations at Site TP-1 were found to be 19,400 $\mu\text{g/g}$ for Fe, 244 $\mu\text{g/g}$ for Mn, 124 ± 51 $\mu\text{g/g}$ for Cu, 453 ± 266 $\mu\text{g/g}$ for Pb and 309 ± 125 $\mu\text{g/g}$ for Zn, respectively (Qian, 2015). The dominant plant species at this site were *Typha latifolia* and *Phragmites australis*. Site TP-43 was located in the southwest section of the study area with relatively higher soil metal load (Gallagher et al., 2008). The site was within the wetland with standing water on the site under normal climatic condition. The soil metal concentrations at Site TP-43 were found to be 25,400 $\mu\text{g/g}$ for Fe, 312 $\mu\text{g/g}$ for Mn, 166 ± 72 $\mu\text{g/g}$ for Cu, 333 ± 132 $\mu\text{g/g}$ for Pb and 63.1 ± 15.6 $\mu\text{g/g}$ for Zn, respectively (Qian, 2015). This site was classified as successional northern hardwood, containing perimeter areas dominated by *Phragmites australis*.

1.2 Sample collection and preparation

Field work for the plant sample collection in Liberty State Park was conducted in May 2011 for *Typha latifolia* and *Phragmites australis* at Site TP-1 and *P. australis* at Site TP-43, respectively. There found no *T. latifolia* at Site TP-43 in May 2011. Because the synchrotron XRF analysis for *P. australis* collected at Site TP-1 in May 2011 was not a success, it was collected again in September 2011. These samples were collected using stainless steel spades and placed into large plastic containers and then transported immediately to Montclair State University for further treatment. The samples were cleaned by gently shaking off bulk soil with hands and rinsing off residual soils with deionized water. Some of the fresh root samples were processed immediately for synchrotron μ XRF analysis, while others were oven dried separately at 30°C for synchrotron μ CMT analysis. For synchrotron μ CMT analysis, a section of dry, clean root sample in a length of 2 cm was placed in a Kapton tube and put on a holding stand for the measurement. For synchrotron μ XRF analysis, the fresh root samples were suspended in an optimal cutting temperature (OCT) compound that does not infiltrate the specimen, and cooled at -20°C in a cryotome chamber (Cryostat CM1950, Leica Microsystems) (Feng et al., 2013). Once OCT solidified, the cryotome was used to cut a 30 μ m thin cross-section of the root sample. The thin section of the root samples were then mounted on a 25 mm $\times$ 76 mm quartz microscope slide (SPI Supplies[®]) and kept in a desiccator at NSLS X27A Beamline until synchrotron μ XRF analysis was conducted.

1.3 Synchrotron computed microtomography (μ CMT) and X-ray microfluorescence (μ XRF) measurement

Three-dimensional (3D) visualization of *T. latifolia* and *P. australis* root structures was achieved using synchrotron X-ray computed microtomography (μ CMT) technique at the NSLS X2B Beamline of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (Upton, NY) (Jones et al., 2013). The tomography apparatus used a Si (1,1,1) monochromator to produce a monoenergetic beam of 10.0 keV. A beam size of about 6 mm $\times$ 6 mm was used to irradiate the root sample contained in a Kapton tube. The beam transmitted through the sample was detected with a CsI(Tl) scintillator. Light from the scintillator was magnified and then imaged using a CCD camera with dimensions of 1340 $\times$ 1300 pixels of 4 μ m size. The tomographic volume was produced from a collection of 1200 images taken in 0.15° steps. Metal (Fe, Mn, Cu, Pb and Zn) concentrations and distribution within the root tissue were investigated

using synchrotron μ XRF at NSLS X27A Beamline (Ablett et al., 2006). Briefly, this bend magnet beamline used Kirkpatrick-Baez (K-B) mirrors to produce a focused spot ($10\text{ }\mu\text{m} \times 10\text{ }\mu\text{m}$) of hard X-rays with tunable energy achieved via Si(111) or Si(311) channel-cut monochromator crystals. For synchrotron μ XRF imaging, the incident beam energy was fixed at 13.5 keV to excite all target elements simultaneously. The sample was oriented at 45° to the incident beam, and rastered in the path of the beam by an XY stage while X-ray fluorescence was detected by a 13-element Canberra Ge array detector positioned at 90° to the incident beam. Elemental maps were typically collected from a 1 mm^2 sample area using a step size of 10 or 20 μm and a dwell time of 7 sec. The fluorescence yields were normalized to the changes in intensity of the X-ray beam (I_0) and the dwell time. During the measurement, the X-ray influences were comparatively low and radiation damage effects were minimal.

1.4 Data analysis

The metal concentration from synchrotron μ XRF measurement was in the units of counts per second (cps). It was converted to the units of $\mu\text{g/g}$ by calibrating the samples against the NIST SRM 1832 and 1833 thin glass film on polycarbonate for x-ray fluorescence spectrometry, provided the root tissue density was $1.0\text{ }\mu\text{g/g}$. This calibration method did not account for differences in sample matrix and assumed that the absorption correction was not necessary, which works well for thin samples of uniform thickness. In data analysis, Pearson correlation analysis was performed on the data to examine the relationship between the metals (Fe, Mn, Cu, Pb and Zn) in the epidermis and the vascular tissue, respectively, in *T. latifolia* and *P. australis* collected at the two sites. Statistical *t*-test analysis was performed on the data to examine metal concentration differences between the epidermis and the vascular tissue of each plant root. Tukey Method ($p < 0.05$) was used for multiple comparison tests. To further examine the processes and mechanisms governing the metal transport and distribution, factor analysis was performed on the metals (Cu, Fe, Mn, Pb and Zn) (Gotelli and Ellison, 2004). Logarithmic transformation was performed on the data before the analysis to ensure a normal distribution. Varimax rotation was used to maximize the sum of the variance of the factor coefficients (Gotelli and Ellison, 2004).

2. Results and discussion

2.1 Root structure visualization and metal distributions in roots

As shown in **Fig. 2**, synchrotron μ CMT visualization of the plant root structure shows high X-ray attenuation occurring in epidermis of the root tissue. Rhizosphere is a favorable environment for microbial communities that enhance the biogeochemical reactions in wetland plant root system (Gilbert and Frenzel, 1998). Metal availability for plant uptake is dependent on soil pH, redox potential (pE), water availability, microbes and other biota, mineral and organic contents, and is complicated by synergistic interactions between these variables (Dzantor and Beauchamp, 2002; Martin et al., 2003, 2006; Morrissey and Guerinot, 2009; Naftel et al., 2001). However, the synchrotron μ CMT measurement in this study could not identify the chemical composition of the high attenuation substances (**Fig. 3**). Metal (Cu, Fe, Mn, Pb and Zn) concentrations and distributions made from synchrotron X-ray microfluorescence (μ XRF) measurement show differences from epidermis to vascular tissue with relatively higher concentrations in the epidermis than that in the vascular tissue (**Fig. 4**). High concentration of Fe in the root epidermis is found and can be attributed to the formation of Fe plaque due to redox reaction at the soil-root interface in rhizosphere (Hansel et al., 2001, 2002; Otte et al., 1989; St-Cyr and Crowder, 1990). In the epidermis, Fe must be included in the high attenuation substance shown in **Figs. 2** and **3**, which is supported by the information from synchrotron μ XRF measurement that indicates high Fe concentration in the epidermis (**Fig. 4**). As an Fe species, it has been reported that Fe plaque in the plant roots is predominantly Fe oxides (Feng et al., 2013; Hansel et al., 2001; St-Cyr and Campbell, 1996). Because other trace metals (e.g., Cu, Mn, Pb and Zn) were also found in the epidermis (**Fig. 4**), the results suggest that these trace metals could be associated with Fe plaque or Fe-oxides and included in this high attenuation substances in the epidermis due possibly to scavenge by Fe plaque.

2.2 Metal concentration difference between epidermis and vascular tissue in roots

In order to understand the metal transport and accumulation in the root system and, in the meantime, avoid processing massive data, two subareas were selected within the plant root cross-section of each species, one in the epidermis and the other in the vascular tissue as indicated in the optical images shown in **Fig. 4**. Each subarea contains 80 to 200 data points and represents a range of metal concentrations for statistical analysis. The average concentrations of metals (Cu, Fe, Mn, Pb and Zn) in each subarea in the epidermis and vascular tissue of those two species, *Phragmites australis* and *Typha latifolia*, are summarized in **Table 1**. In general, metal concentrations in the epidermis were higher than that in the vascular issue. In this study,

Student *t*-test was performed to examine the difference in metal (Cu, Fe, Mn, Pb and Zn) concentrations between the epidermis and the vascular tissue. As shown in **Table 1**, the results show significant differences ($p < 0.01$) in metal (Cu, Fe, Mn, Pb and Zn) concentrations between the epidermis and the vascular tissue in each of the plant roots. The difference in metal concentrations between the epidermis and the vascular tissue can be explained by different mechanisms. In the epidermis, metal adsorption/desorption at the soil-plant root interface, metal uptake and transport by the plants and metal scavenge by Fe-Mn oxides are the major controlling mechanisms (Bargar et al., 1997; Feng et al., 2013; Hansel et al., 2001; Liu et al., 2004), while metal accumulation in vascular tissue can be dominantly controlled by the biological (or biochemical) processes such as metal symplastic or apoplastic transport in the roots (Baxter et al., 2008; Lyubenova et al., 2013; MacFarlane and Burchett, 2002).

Previous studies at Liberty State Park, New Jersey, showed that metals could be translocated from the plant roots to the aerial parts although the concentrations in above-ground tissues were at least an order of magnitude lower than that in the root tissue (Gallagher et al., 2008; Qian et al., 2012). Various mechanisms regulating cytoplasmic metal concentration have been put forward, of which chelation and sequestration of metals by particular ligands are important mechanisms used by plants to deal with metal stress (Brune et al., 1994; Palmer and Guerinot, 2009). In an earlier study of plant uptake of metals in Liberty Study Park, Qian et al. (2012) found that metal uptake and bioaccumulation in the plants increased with increasing soil metal concentration. Low organic matter content was in favor of bioaccumulation of Cu in the roots, while low pH was generally in favor of bioaccumulation of Zn in the roots (Qian et al., 2012). It is known that adequate amount of metals in soils are essential nutrients for the plant growth. After uptake of these metals as the nutrients, the plants translocate the metals from epidermis to vascular tissue and further to stem and leaf (Gallagher et al., 2008; Qian et al., 2012). However, high metal concentrations are toxic to the plants. Plants that are metal excluders can restrict metal entrance based on their tolerance and even hypertolerance strategies. These plants can retain and detoxify most of the toxic metals in the root tissues with a minimized translocation to the leaves (Deng et al., 2007; Hall, 2002; MacFarlane and Burchett, 2002). For those metals (Pb) that are not essential nutrients for the plant growth, the defensive nature of the plants will not actively translocate these metals to the root vascular tissues in a large quantity (Lyubenova et al., 2013; Verbruggen et al., 2009). Therefore, the differences found in this study

reflect the nature of these metals as essential or non-essential nutrients for the plants and the metal uptake mechanisms and transport pathways can be metal-dependent (Cheng, 2003; Lasat, 2002).

2.3 Correlation analysis of metals in roots

Table 2 shows the results of Pearson correlation analysis of the metal (Fe, Mn, Cu, Pb and Zn) concentrations in the epidermis and vascular tissue in each root sample. At Site TP-1, metals (Fe, Mn, Cu, Pb and Zn) in *Phragmites australis* and *Typha latifolia* show significant correlations in the epidermis ($p < 0.05$), but no such significant correlation found in the vascular tissue ($p > 0.05$). At Site TP-43, metals (Fe, Mn, Cu, Pb and Zn) in *Phragmites australis* roots also show significant correlations ($p < 0.05$) in the epidermis. In the vascular tissue, Cu and Zn show significant correlations ($p < 0.05$) with Fe. In addition, Cu also shows significant correlations with Mn and Zn (**Table 2**).

In the rhizosphere, the role of Fe plaque, which forms on the surface of plant roots, in regulating metal cycle has been an issue of much debate. Several studies suggest that the Fe plaque on the surface of roots serves as a barrier preventing heavy metals from entering plant roots (St-Cyr and Campbell, 1996; Sundby et al., 1998). However, others suggest that Fe plaque is not the main barrier (Ye et al., 1998; Liu et al., 2004). Understanding the function of Fe oxides in controlling the mobility of metals in plants is important in phytoextraction of metals from the contaminated soils (Tripathi et al., 2014). In this study, a strong association of metals (Cu, Mn, Pb and Zn) with Fe was found in the root epidermis (**Fig. 5-7**). This could be a consequence of metal scavenges by Fe plaque or formation of Fe-Mn oxides (Bargar et al., 1997; Eick et al., 1999; Hansel et al., 2001; Ye et al., 1998). There were no such relationships found in the vascular tissue. The results suggest that, after the metal uptake by the plants from the soil, transport of metals from the epidermis to the vascular tissue, and accumulation in the root system can vary from metal to metal, most likely due to differential expression of a number of different accumulation systems with distinct metal-affinity patterns (Assunção et al., 2008). Some metals may share the same transport pathways while others may not. The results from this study suggest that Fe-Mn oxides or Fe plaque appeared to play a role in governing the metal uptake at root-soil interface and metal transport and accumulation in the epidermis. Because of the high adsorption capacity of Fe-oxides, Fe plaque can be considered as a reactive substrate for metal sequestration

(Feng et al., 2013; Hansel et al., 2001, 2002; Otte et al., 1989, 1991; Sundby et al., 1998; St-Cyr and Crowder, 1990, St-Cyr and Campbell, 1996) . Although Fe oxide species vary in their formation pathways and activities towards metals, our results demonstrate that the root epidermis in *Typha latifolia* and *Phragmites australis* is an area of forming Fe plaque that can scavenge other metals such as Cu, Pb and Zn.

2.4 Factors governing metal transport and distributions in roots

Factor analysis was performed on metal (Cu, Fe, Mn, Pb and Zn) data to examine the factors governing the metal accumulation and distribution in the plant roots (Gotelli and Ellison, 2004). All the plants were analyzed together with separation of metal concentrations in the epidermis from that in the vascular tissue. For the epidermis, there are two factors which have eigenvalue greater than 0.5 and account for 77% of the total variance (**Table 3**). Factor 1 with high loadings of Zn (0.892) and Cu (0.884) and a moderate loading of Fe (0.647) explains 40% of the variation. This factor reflects the association of Zn and Cu with Fe in the epidermis of the plants, implying the adsorption of Cu and Zn on Fe oxides. In another words, a certain amount of Zn and Cu could be scavenged by Fe plaque in the epidermis during the transport. Factor 2 has high loadings of Mn (0.890) and Pb (0.883) and a moderate loading of Fe (0.521), and accounts for 37% of the variation (**Table 3**). This factor suggests that, as a non-essential nutrient, Pb uptake, transport and accumulation in the epidermis are different from the other nutrient metals (Cu and Zn) and can be controlled by Fe-Mn oxides (Feng et al., 2013; Hansel et al., 2001, 2002). In the vascular tissue, the first three factors with eigenvalue greater than 0.5 account for 86% of the total variance (**Table 3**). Individually, Factor 1 has a high loading of Fe (0.923), a moderate loading of Zn (0.622) and a negative moderate loading of Mn (-0.642) (**Table 3**). This factor is mainly an Fe factor and suggests that Fe and Zn may share some similar mechanisms and pathways in the vascular tissue, but the mechanism is exclusive to Mn to a certain extent. Factors 2, which accounts for 22% of the variation, has a high negative loading of Pb (-0.960) and is essentially a Pb factor, suggesting that transport of Pb into the vascular tissue is different from the other metals and this factor plays a negative role in the transport (**Table 3**). Factor 3 is characterized by a high loading of Cu (0.925), a moderate loading of Zn (0.589) and a negative moderate loading of Mn (-0.511), and explains 30% of the total variance (**Table 3**). This factor suggests that Cu and Zn may share the same mechanism in transport into the vascular tissue as

nutrients required by the plants. In the meantime, the same mechanism plays a moderate negative role in transporting Mn. This analysis suggests that although Cu, Zn and Mn are essential nutrients for plant growth, the transport mechanisms are different. Overall, the results indicate that the mechanisms controlling metal transport from the epidermis to the vascular tissue can be very different and governed by individual factors or transport proteins specifically for an individual metal.

3. Conclusions

This study demonstrates that synchrotron X-ray microbeam techniques have important applications in studying metal spatial distributions in *Phragmites australis* and *Typha latifolia* with a micro-scale resolution. Application of such state-of-the-art technologies can result in high-resolution information on spatial distribution of metals on wetland plants and their association with Fe plaque with very high sensitivity. The results from this study indicate that metal transport from the epidermis to the vascular bundle and metal distributions in the root tissues differ significantly, which depend on the metals and the plant species. As essential nutrients for plant growth, Cu and Zn are actively taken up by the roots and may share same transport pathways and similar mechanisms. Iron (Fe) and Mn, besides acting as essential nutrients for the plants, can form Fe plaque and Fe-Mn oxides that plays a major role in governing other metal transport in the plants by scavenging the other metals in the epidermis. In this study, *Phragmites australis* and *Typha latifolia* showed concentration dependent, metal preference patterns with regard metal accumulation, most likely due to differential expression of different uptake and transport systems with distinct metal-affinity patterns. As a result of the complex biogeochemical process, this study suggests that uptake of metals by the plant root system, or stabilization of metals within the plants provides a potential approach for brownfield remediation and wetland rehabilitation. Therefore, the results from this research will allow us to make broad inferences about the relevant plant uptake mechanisms. In other words, the sequestration of metal contaminants in the wetland plant root system suggests a potential low-cost remediation method (phytostabilization) to manage metal-contaminated sediments for brownfield remediation while performing wetland rehabilitation.

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Figure caption

Fig. 1 Map showing the study area in Liberty State Park. Sites TP-1 and TP-43 were selected for this study. The vegetation assembling patterns: SNH, successional northern hardwood; SSB, successional shrubland; SOF, successional old field; MS, maritime shrubland; MG, maritime grasslands; CRM, common reed/mugwort; FFW, floodplain forested wetlands; SSW, shrub swamp wetland; SEM, shallow emergent marsh; CRW, common-reed-dominated wetland.

Fig. 2 Three-dimensional tomographic root structure images of *Typha latifolia* and *Phragmites australis* from synchrotron μ CMT measurement. Upper panel: synchrotron X-ray images of the original roots in Kapton tubes. Lower panel: reconstructed root images from synchrotron μ CMT measurement. The samples were collected at Site TP-1 in May 2011.

Fig. 3 Cross-section images of the plant root tissue from synchrotron μ CMT measurement for a) *Typha latifolia* root, and b) *Phragmites australis* root. It is seen that high X-ray attenuation occurs in epidermis associated with the root tissue structure. Pixel size is 4 μ m. The samples were collected at Site TP-1 in May 2011.

Fig. 4 Two-dimensional (2D) maps show the information of a cross-section of *Phragmites australis* root sample collected at Site TP-1 (a), *Typha latifolia* root sample collected at Site TP-1 (b) and *Phragmites australis* root sample collected at Site TP-43 (c) in September 2011. Optical image shows the root tissue structures of *Phragmites australis*. The framed areas in epidermis and vascular tissue of *Phragmites australis* root thin section (30 μ m in thickness) were selected for statistical analysis. Images from synchrotron radiation measurement show Cu, Fe, Mn, Pb and Zn concentrations and distributions in the root tissue.

Fig. 5 Relationship of Cu, Mn, Pb and Zn with Fe in the epidermis of *Typha latifolia* root collected at Site TP-1 in May 2011. Significant correlation between metals (Cu, Mn, Pb and Zn) and Fe indicates metal scavenge by Fe plaque and formation of Fe-Mn oxides.

Fig. 6 Relationship of Cu, Mn, Pb and Zn with Fe in the epidermis of *Phragmites australis* root collected at Site TP-1 in September 2011. Significant correlation between metals (Cu, Mn, Pb and Zn) and Fe indicates metal scavenge by Fe plaque and formation of Fe-Mn oxides.

Fig. 7 Relationship of Cu, Mn, Pb and Zn with Fe in the epidermis of *Phragmites australis* root collected at Site TP-43 in May 2011. Significant correlation between metals (Cu, Mn, Pb and Zn) and Fe indicates metal scavenge by Fe plaque and formation of Fe-Mn oxides.

Fig. 1

fig. 2

Synchrotron X-ray image

Reconstructed image

Typha latifolia

Phragmites australis

fig. 3

Typha latifolia

Phragmites australis

fig. 4

a Site TP-1 *Phragmites australis* Fe

b Site TP-1 *Typha latifolia* Fe

c Site TP-43 *Phragmites australis*

fig. 5

fig. 6

fig. 7

Table 1
Average concentrations ($\mu\text{g/g}$) of Cu, Fe, Mn, Pb and Zn in the representatives areas within the epidermis (E) and vascular tissue (V) of *P. australis* and *T. latifolia* roots collected at Sites TP-1 and TP-43, respectively.

Site	Species	Sample size	Element	Epidermis (mean $\pm$ s.d.)	Vascular bundle (mean $\pm$ s.d.)	<i>p</i> value
Site TP-1	<i>P. australis</i>	E = 208	Fe	474 $\pm$ 913	19.3 $\pm$ 5.2	0.000
			Mn	27.4 $\pm$ 15.9	20.5 $\pm$ 8.9	0.000
		V = 81	Cu	18.7 $\pm$ 11.2	10.0 $\pm$ 3.6	0.000
			Pb	23.9 $\pm$ 19.8	14.2 $\pm$ 7.4	0.000
			Zn	65.2 $\pm$ 51.8	11.0 $\pm$ 4.4	0.000
	<i>T. latifolia</i>	E = 155	Fe	686 $\pm$ 1120	25.0 $\pm$ 7.2	0.000
			Mn	33.6 $\pm$ 26.0	11.2 $\pm$ 4.5	0.000
		V = 110	Cu	16.0 $\pm$ 7.5	9.35 $\pm$ 3.44	0.000
			Pb	21.3 $\pm$ 16.1	12.6 $\pm$ 6.9	0.000
			Zn	43.9 $\pm$ 36.1	13.9 $\pm$ 4.9	0.000
Site TP-43	<i>P. australis</i>	E = 144	Fe	265 $\pm$ 479	36.5 $\pm$ 9.1	0.000
			Mn	2.51 $\pm$ 1.33	1.88 $\pm$ 0.76	0.000
		V = 120	Cu	17.4 $\pm$ 6.4	20.6 $\pm$ 5.6	0.000
			Pb	6.09 $\pm$ 3.09	4.91 $\pm$ 2.29	0.001
			Zn	64.4 $\pm$ 29.2	39.2 $\pm$ 15.8	0.000

Statistical analysis shows significant difference ($p < 0.01$) in the concentrations between epidermis and vascular tissue.

Table 2 Pearson correlation between metals in epidermis (E) and vascular tissue (V).

Site	Species	Sample size		Epidermis (E)					Vascular bundle (VB)				
				Fe	Mn	Cu	Pb	Zn	Fe	Mn	Cu	Pb	Zn
Site TP-1	<i>P. australis</i>	E = 208	Fe	1.000					1.000				
			Mn	0.564	1.000				-0.010	1.000			
		V = 81	Cu	0.678	0.465	1.000			0.036	0.022	1.000		
			Pb	0.864	0.574	0.655	1.000		0.004	0.109	-0.022	1.000	
			Zn	0.735	0.460	0.810	0.706	1.000	0.106	0.169	0.168	0.146	1.000
	<i>T. latifolia</i>	E = 155	Fe	1.000					1.000				
			Mn	0.660	1.000				-0.038	1.000			
		V = 110	Cu	0.535	0.694	1.000			-0.019	0.147	1.000		
			Pb	0.850	0.595	0.426	1.000		0.115	-0.298	0.019	1.000	
			Zn	0.881	0.756	0.721	0.723	1.000	0.181	0.029	0.004	0.186	1.000
Site TP-43	<i>P. australis</i>	E = 144	Fe	1.000					1.000				
			Mn	0.472	1.000				0.011	1.000			
		V = 120	Cu	0.370	0.263	1.000			0.195	0.186	1.000		
			Pb	0.459	0.457	0.227	1.000		-0.035	-0.066	0.038	1.000	
			Zn	0.284	0.256	0.466	0.227	1.000	0.387	0.048	0.550	-0.093	1.000

Bold face indicates a significant correlation level at 5% two-tailed significance.

Table 3 Results of factor analysis.

Epidermis			Vascular tissue			
Latent roots (Eigenvalues)			Latent Roots (Eigenvalues)			
1	2		1	2	3	
2.598	1.283		3.019	0.684	0.615	
Rotated loading matrix (VARIMAX, Gamma = 1.000000)			Rotated loading matrix			
	1	2		1	2	3
logZn	0.892	0.045	logFe	0.923	0.135	0.115
logCu	0.884	0.100	log Mn	-0.642	-0.3	-0.511
logFe	0.647	0.521	log Zn	0.622	0.189	0.589
logMn	0.044	0.890	log Pb	-0.195	-0.96	-0.189
logPb	0.166	0.883	log Cu	0.164	0.162	0.925
Percent of total variance explained						
1	2		1	2	3	
40.5	37.1		34.3	21.8	30.2	
Eigenvalue is set at 0.5 as a cut off value.						

Eigenvalue is set at 0.5 as a cut off value.

Site TP-43,
Phragmites australis

Cu

Fe

Mn

Pb

Zn