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**Synchrotron XRF measurement of metal distributions in *Phragmites australis* root system
in Yangtze River intertidal zone**

Huan Feng^{1*}, Weiguo Zhang², Yu Qian^{1#}, Wenliang Liu², Lizhong Yu², Shinjae Yoo³, Jun Wang⁴, Jia-Jun Wang⁴, Christopher Eng⁴, Chang-Jun Liu⁵, Ryan Tappero⁴

1. Department of Earth and Environmental Studies, Montclair State University, Montclair, New Jersey 07043, USA
2. State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, PRC
3. Computational Science Center, Brookhaven National Laboratory, Upton, New York 11973, USA
4. Photon Sciences Directorate, Brookhaven National Laboratory, Upton, New York 11973, USA
5. Biological Sciences Department, Brookhaven National Laboratory, Upton, New York 11973, USA

* Corresponding author.

E-mail address: fengh@mail.montclair.edu; Tel: 1-973-655-7549; Fax: 1-973-655-4072.

Current address: School of Ecology and Environmental Sciences, Yunnan University, Kunming, Yunnan 650091, PRC

Abstract:

This study investigates the distributions of Br, Ca, Cl, Cr, Cu, K, Fe, Mn, Pb, Ti, V and Zn in *Phragmites australis* root system and the function of Fe nanoparticles in scavenging metals in the root epidermis using synchrotron X-ray microfluorescence (XRF), synchrotron transmission X-ray microscope measurement (TXM) and synchrotron X-ray absorption near edge structure (XANES) techniques. The purpose of this study is to understand the mobility of metals in wetland plant root system after their uptake from rhizosphere soils. *Phragmites australis* samples were collected in Yangtze River intertidal zone in Jul 2013. The results indicate that Fe nanoparticles are present in the root epidermis and that other metals correlate significantly with Fe, suggesting that Fe nanoparticles play an important role in metal scavenging in the epidermis.

Keywords: Yangtze River intertidal zone; Metal uptake; Synchrotron X-ray radiation technique; *Phragmites australis*; Fe nanoparticles

Introduction

A wetland plant root system is an active site for transport of oxygen into the soil/sediment and absorption of water and nutrients in solution even against a concentration gradient (Enstone *et al.*, 2002). Many plants acquire metals from the rhizosphere and regulate their uptake properties within the root system (Hinsinger & Courchesne, 2008). Although the metal availability in the sediments is the source for plant uptake, metal properties and plant species are also factors affecting the metal transport in the plants. Other factors which complicate the synergistic interactions with the environmental parameters are factors such as soil pH, redox potential (E_h),

water availability, microbes and other biota, mineral and organic contents (Hinsinger & Courchesne, 2008) (Morrissey & Guerinot, 2009).

Natural processes that control the mobility of metals in soils and plants determine the biogeochemical cycle of trace elements. Because metals in the plant tissues are originated from rhizosphere through the root system absorption, investigation of metal uptake mechanisms, transport processes, function of Fe plaque controlling the metal mobility in plant root system is important and informative in understanding metal translocation and biogeochemical cycle in wetlands. In the rhizosphere, the role of Fe plaque, which forms on the surface of plant roots, in controlling metal biochemical cycle has been an issue of debate. Several early studies suggest that the Fe plaque serves as a barrier preventing heavy metals from entering plant roots (St-Cyr & Campbell, 1996; Bjørn *et al.*, 1998). However, others suggest that Fe plaque is not the main barrier (Ye *et al.*, 1998; Liu *et al.*, 2004). The Yangtze River estuary is one of the world largest estuarine systems and defined as a mesotidal estuary. Huge amounts of sediments ($\sim 4.86 \times 10^8$ ton y^{-1} during 1949-1984) discharged from the Yangtze River annually, resulting in an extensive intertidal zone in the Yangtze River estuary (Xiqing, 1998). The river's intertidal zone typically contains three distinct vegetation units seaward: a *Phragmites australis* zone, a *Scirpus mariqueter* and *Scirpus trigueter* zone, and bare unvegetated mudflats (Zhang *et al.*, 2001). *Spartina alterfloria* became a predominant species in the area after it was planted in the 1990s for promotion of sediment accretion and coastal defense.

Previous studies have shown that wetland plants can absorb metals from the soils and store these metals in the plant biomass (Williams *et al.*, 1994; Weis & Weis, 2004; Gallagher *et*

al., 2008; Qian *et al.*, 2012). Wetland plants in freshwater system and salt water system may behave differently in metal uptake and transport. Even though in the same freshwater or salt water system, different species could also behave differently in metal uptake and transport. Recently, several studies indicate the presence of the Fe-nanocomplexes or Fe-nanoparticles on the root cross section (Pardha-Saradhi *et al.*, 2014; Fuente *et al.*, 2016). In general, these Fe-nanoparticles are amorphous iron oxyhydroxide and can further form larger nanocomplexes. (Pardha-Saradhi *et al.*, 2014). For example, *Imperata cylindrica* (L.) P. Beauv. is a hyperaccumulator plant. It was found that Fe deposited in the form of nanocrystals not only in the intercellular space, but also in the cells of the xylem, phloem and in the epidermal cells in root rhizomes and leaves (Rodríguez *et al.*, 2005; Fuente *et al.*, 2016). These iron nanocrystals are composed of jarosite, ferrihydrite, hematite and spinel phases (Fuente *et al.*, 2016). In this study, as synchrotron X-ray diffraction (XRD) measurement was not conducted on the samples in this study, there is no Fe-containing mineral information that can be reported. Nevertheless, synchrotron X-ray microbeam techniques, such as synchrotron X-ray fluorescence (XRF), have important applications in studying metal translocation and accumulation in plants with micrometer scale resolution (Martin *et al.*, 2006; Martin *et al.*, 2001; Naftel *et al.*, 2001; Sutton *et al.*, 2002; Zimmer *et al.*, 2011; Feng *et al.*, 2013; Feng, Zhang, *et al.*, 2015; Feng, Qian, *et al.*, 2015; Rouff *et al.*, 2013). Unlike conventional wet chemical analyses, the synchrotron-based techniques have demonstrated advantages in sample preparation and measurement. The high spatial resolution measurement provides high detection sensitivity and resolution of elemental distributions and leads to a better understanding of the chemical reaction mechanisms and fate of elements in the plants. This study aims at improving our current knowledge of metal uptake and accumulation in wetland plant roots with assistance of synchrotron radiation measurement

for better understanding the ecological function of wetland plants and the role of Fe plaque in metal transport. Wetland in Yangtze River intertidal zone is a unique test bed for the purpose of this study.

Materials and Methods

Sample collection and preparation

Field work for *Phragmites australis* sample collection was conducted in July 2013 at two sites (Site 1: 31°34'55.06" N, 121°54'10.32" E, and Site 2: 31°34'56.45" N, 121°54'12.62" E) on the north shore of Chongming Island within the Yangtze River intertidal zone, where *Phragmites australis* was an abundant species (Figure 1). These two sampling sites were very close to each other (~80 m apart). According to a previous study in this area, the metal concentrations in the sediments between these two sites in this area were essentially the same, which were reported to be Fe: 3.98±0.17 %, Cr: 91.7±0.6 mg g⁻¹, Cu: 44.4±4.7 mg g⁻¹, Mn: 987±3 mg g⁻¹, Pb: 28.9±0.5 mg g⁻¹, and Zn 119±13 mg g⁻¹ (Zhang *et al.*, 2009). The metal concentrations (Cr, Cu, Fe, Mn, Ti, V and Zn) except Pb in the study area were within the natural background levels (Zhang *et al.*, 2009). In this study, *Phragmites australis* samples were collected along with the soils using stainless steel spades, placed into large plastic containers and then transported to our laboratory at East China Normal University for further treatment. Bulk soils were easily removed from the plants by gentle shaking, rhizosphere soils were carefully removed by hands and the trace

residual soils on the roots were rinsed off with small amounts (<20 ml) of deionized water (Otte *et al.*, 1991).

During the sample preparation for synchrotron X-ray microfluorescence (μ XRF) measurement, the fresh root samples were suspended in an optimal cutting temperature (OCT) compound that does not infiltrate the specimen, and rapidly cooled to -20°C. Once OCT solidified, a cryotome (Cryostat CM1950, Leica Microsystems) was used to cut a 50 μ m thin section. The thin section of the root samples were then mounted on a 25 mm $\times$ 76 mm quartz microscope slide (SPI Supplies[®]) for synchrotron X-ray microfluorescence (μ XRF) analysis (Figure 2).

In order to examine possible difference in the root sections within the limited user time available at the beamline work station, root tip section of Sample 1-1 collected at Site 1 and root basal section of Sample 2-1 collected at Site 2 were chosen for synchrotron XRF analysis. For synchrotron transmission X-ray microscope measurement (TXM) and synchrotron X-ray absorption near edge structure (XANES) measurement to visualize Fe nanoparticles, each of the root samples was glued on the tip of a needle and then mounted on a stand (Figure 3). All the samples prepared for synchrotron radiation measurement were kept in our low temperature lab (4°C) or in a desiccator before the analysis.

Synchrotron transmission X-ray microscope measurement

Synchrotron transmission X-ray microscope analysis was conducted at the National Synchrotron Light Source (NSLS), X8C Beamline at Brookhaven National Laboratory (Upton, NY) and at the Advanced Photon Source (APS), 8BM Beamline at Argonne National Laboratory (Argonne, IL) that were equipped with a full-field transmission X-ray microscope (TXM) manufactured by Xradia, Inc. The newly developed TXM provides a large field of view ($40\text{ }\mu\text{m} \times 40\text{ }\mu\text{m}$), 30 nm resolution, local tomography, and automated maker-free image acquisition and alignment (Wang *et al.*, 2012; Wang *et al.*, 2014a). By tuning X-ray energy across the absorption edge of the element of interest, this TXM technique enables the obtaining of chemical information with high sensitivity (Wang *et al.*, 2014b). In order to investigate Fe distribution in the roots, the XANES data were collected by scanning the X-ray energy from 7092 eV to 7192 eV with a step size of 2 eV in this study. A stack of images was obtained by scanning the photon energy across the X-ray absorption edge of elemental Fe. A lens-coupled scintillator with a 2048×2048 pixel camera detector was used to record the images. With all 1024×1024 pixels for bin 2×2 , the full spectrum for each pixel was extracted. The XANES analysis was carried out using a customized program (Matlab, MathWorks, R2011b) developed in house (former Beamline X8C group, NSLS, BNL). Background normalization was made first for the TXM images with a unique background image collected at every energy. More information on TXM and XANES can be found in Wang *et al.* (2014b) and Wang *et al.* (2012). In addition, a tomographic dataset was achieved for each sample by collecting 361 projections at a rotation range between -90° to $+90^\circ$ (2×2 camera pixels with 10 s exposure time). The reconstruction and visualization of the experimental data were completed using proprietary software developed by Xradia, Inc.

Synchrotron X-ray fluorescence measurement

Metal concentrations and distributions in wetland plants were investigated using synchrotron μ XRF at NSLS X27A Beamline before the facility was shut down in 2014 (Ablett *et al.*, 2006). Briefly, this bend-magnet beamline uses Kirkpatrick-Baez (K-B) mirrors to produce a focused spot (10 by 10 μ 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 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 90° to the incident beam. Elemental maps were typically collected from a 1 mm² sample area using a step size of 10 - 20 μ m and a dwell time of 7 seconds. The fluorescence yields were normalized to the changes in intensity of the X-ray beam (I_0) and the dwell time. Points of interest on the images were selected for spectroscopic analysis. During the measurement, the X-ray influences were comparatively low and radiation damage effects were minimal. Data acquisition and processing were performed using IDL-based beamline software designed by CARS (University of Chicago, Consortium for Advanced Radiation Sources) and NSLS Beamline X26A. Further data analysis was conducted at Montclair State University and Brookhaven National Laboratory.

Data visualization, extraction and computation

The raw data from the synchrotron radiation measurements were processed at each beamline work station. Data visualization was achieved by IDL virtual machine using the software developed by CARS (University of Chicago, Consortium for Advanced Radiation Sources).

Data matrix extraction of the root epidermis and vascular tissue was made possible using Matlab (MathWorks) (Figure 4). The data statistical analysis was performed using Matlab (MathWorks) and Systat (Systat Software, Inc.).

Results

Visualization of Fe nanoparticle in root epidermis and metal distributions in root cross-section

The high resolution Fe XANES images show that Fe is present in the root epidermis as nanoparticles (Figure 5). The cross-section concentrations and distributions of Br, Ca, Cl, Cr, Cu, K, Fe, Mn, Pb, Ti, V and Zn in the *Phragmites australis* root samples from the epidermis to the vascular bundle are shown in Figures 6 and 7. It is seen that the distributions of these elements from the epidermis to the vascular bundle are apparently different. Most of these elements show very high concentrations in the epidermis, forming a nearly continuous, surficial rind (Figures 6 and 7). The average concentrations of Br, Ca, Cl, Cr, Cu, K, Fe, Mn, Pb, Ti, V and Zn in the epidermis and vascular bundle of each root collected at Sites 1 and 2 are summarized in Table 1. In general, the concentrations of these elements in the root epidermis are higher than that in the vascular bundle. This can contribute to the geochemical reactions at the rhizosphere soil-root interface, metal uptake from the soil to the root epidermis, and concentration gradient and horizontal transport from the epidermis to vascular bundle as well as the impact of Casparian band on metal aplastic transport. It is also seen that some localized areas in the epidermis with high Fe concentration usually have the highest metal concentrations

(Figures 6 and 7). This could be attributed to the metal co-precipitation with Fe nanoparticles. According to a previous sediment study in this area, metal concentrations between these two sites are nearly the same (Zhang *et al.*, 2009)(Zhang et al., 2009). Therefore, the concentration differences in some elements between Sample 1-1 and Sample 2-1 as shown in Table 1 may not be simply caused by the concentration differences in the sediments. Usually, root tip is an active site for oxygen transport and nutrient uptake. For example, relatively higher metal (Ca, Cu, Fe, Mn, Pb and Zn) concentrations in new root tip than that in the main root base was found in *Spartina alterniflora* collected in the Yangtze River intertidal zone (Feng, Zhang, *et al.*, 2015). Therefore, although the possibility of spatial variation cannot be excluded, the difference between the root tip section and the root basal section may also cause the differences (Feng, Zhang, *et al.*, 2015).

Statistical analysis

1) Mann–Whitney U non-parametric test for *epidermis and vascular bundle*

Mann–Whitney U non-parametric test and two-sample t-test were performed on these two root samples to examine the concentration differences between these two root samples and between the epidermis and the vascular bundle in each sample. Logarithmic transformation was performed on the data before the analysis to ensure a normal distribution. The results show that except Ca, Cl, Cu and Mn there is no significant differences between these two samples in terms of element concentrations (Table 2). However, the concentration differences between the

epidermis and the vascular bundle are found in some elements in one or both of the roots (Table 3).

2) Pearson correlation analyses

To examine correlations between the elements (Br, Ca, Cl, Cr, Cu, K, Fe, Mn, Pb, Ti, V and Zn) in the epidermis and the vascular bundle in each root sample, Pearson correlation analysis was performed on the epidermis and vascular bundle data, respectively (Tables 4-5). Although the significant correlations ($p < 0.001$) vary between the elements in the epidermis and the vascular bundle, respectively, the results show that these elements are in general correlated significantly with Fe in the epidermis. To examine the function of Fe in metal scavenging, we treated the all data as one entity, i.e., no distinction between the sampling sites and the plant tissue compositions, and performed the linear regression between metals (Cr, Cu, Mn, Pb, V and Zn) and Fe (Figure 8). The significant ($p < 0.001$) correlations between the metals and Fe imply the scavenging of these elements by Fe nanoparticles.

Discussion

Soil rhizosphere is a favorable environment for microbial communities and provides essential nutrients for plant growth (Gilbert & Frenzel, 1998; Emerson *et al.*, 1999; Frenzel *et al.*, 1999;

King & Garey, 1999). Plants uptake nutrients including trace metals from the rhizosphere through their root system. During the uptake and transport processes, biogeochemical processes play an important role. Metal accumulation in the epidermis of the plant roots can be predominately controlled by geochemical mechanisms such as metal adsorption/desorption at soil/sediment-plant root interface, while high concentrations of metals in vascular bundle can be a result of symplastic or aplastic transport of these metals by their transport proteins. In metal uptake and transport through the roots, there are differences between the essential nutrients (e.g., Ca, Cu and Zn) and non-essential nutrients (e.g., Cr and Pb) (Feng, Qian, *et al.*, 2015; Feng, Zhang, *et al.*, 2015; Qian *et al.*, 2015). The defensive nature of the plants will not actively uptake and translocate non-essential metal nutrients to the root vascular tissues in a large quantity. In this study, synchrotron XRF micro-scale measurement demonstrated its unique role in showing high concentrations of Ca, Cu, Fe, Mn and Zn in the root system. As essential nutrients for the plant growth these elements can be taken up, transported and accumulated in both epidermis and vascular bundle. In contrast, the results from this study show that *Phragmites australis* does not actively uptake Cr and Pb from rhizosphere soil and translocate Pb and Cr within the plant tissues (Table 1).

Although Fe is a nutrient for the plant growth, it shows a relatively high concentration in the epidermis, which can be attributed to the formation of Fe oxides due to redox reaction at soil/sediment – root interface in rhizosphere (Al-Sid-Cheikh *et al.*, 2015). In a previous study, synchrotron μ XANES measurement indicates that Fe speciation in *Typha latifolia* root epidermis was dominated by Fe³⁺ (Feng *et al.*, 2013). In this study, our synchrotron XANES measurement indicates that small Fe-oxide particles, or Fe nanoparticles, appear in the root epidermis (Figure

5). These Fe nanoparticles can be Fe-containing minerals and provide a reactive substrate to scavenge metals (Bargar *et al.*, 1997; Hansel *et al.*, 2001; Li *et al.*, 2015; Fuente *et al.*, 2016; Feng *et al.*, 2013; Feng, Qian, *et al.*, 2015; Feng, Zhang, *et al.*, 2015). Several early studies suggest that the Fe plaque on the surface of roots serves as a barrier preventing heavy metals from entering plant roots (St-Cyr & Campbell, 1996; Bjørn *et al.*, 1998). However, others suggest that Fe plaque is not the main barrier (Ye *et al.*, 1998; Liu *et al.*, 2004). In this study, significant correlations of trace metals (e.g., Cr, Cu, Mn, Pb, V and Zn) with Fe in the root system suggest that Fe nanoparticles can play a significant role in scavenging trace metals in the epidermis (Tables 4-5, Figure 8), although the mechanisms controlling the metal uptake, transport and accumulation in the root could be metal-dependent associated with different transport proteins. Because of the high adsorption capacity of Fe-oxides and large specific surface area (Bargar *et al.*, 1997; Eick *et al.*, 1999; Otte *et al.*, 1989; Otte *et al.*, 1991; Hansel *et al.*, 2001; Hansel *et al.*, 2002; St-Cyr & Crowder, 1990), Fe nanoparticles can provide a reactive substrate to scavenge metals for metal sequestration (Rodríguez *et al.*, 2005; Pardha-Saradhi *et al.*, 2014; Fuente *et al.*, 2016). Therefore, it is important to understand the function of Fe nanoparticles in controlling the mobility of metals in the plants. This study shows that the correlations of metals with Fe in the vascular bundle are relatively less significant than that in the epidermis (Tables 4-5). The metal uptake mechanisms by the roots and transport pathways within the plant tissues can be different among different metals, plant species and metal concentrations in soils/sediments (Gallagher *et al.*, 2008; Qian *et al.*, 2012; Qian *et al.*, 2015; Lyubenova *et al.*, 2013; Tripathi *et al.*, 2014; Feng, Qian, *et al.*, 2015; Feng, Zhang, *et al.*, 2015). The results from this study suggest that, after the metal uptake by *Phragmites australis* from the soil, transport of metals from the epidermis to the vascular tissue, and accumulation in the

Phragmites australis root system can vary from metal to metal, most likely due to differential expression of a number of different accumulation systems (Assunção *et al.*, 2008). In other words, the mechanisms and processes controlling metal transport and distributions between the epidermis and the vascular bundle in *Phragmites australis* could be different.

Conclusion

Synchrotron X-ray radiation measurement can provide new insights into the mechanisms taking place in plants during the course of metal uptake from the soils/sediments and transport in the plants at levels where interactions can be understood. This study investigates the concentrations and distributions of Br, Ca, Cl, Cr, Cu, K, Fe, Mn, Pb, Ti, V and Zn in *Phragmites australis* root system with micrometer scale resolution in order to understand the chemical mechanisms of metal uptake by plants and the transport pathways in the plants. The results are important to understand metal biogeochemical cycle and ecological function of the wetlands. As a complex biogeochemical process, the results show that the root epidermis can be an important environment that regulates metal biogeochemical cycling by forming less mobile metal-mineral species and metal complexes in the rhizosphere. Although this research concentrates on basic research, its outcomes have a potential application to the potential low-cost remediation effort (e.g., phytostabilization) to manage metal-contaminated sediments while performing wetland rehabilitation.

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Figure Legends

Figure 1. Map showing the sampling sites on the north shore of Chongming Island in the Yangtze River intertidal zone.

Figure 2 Optical images of *Phragmites australis* root cross-section: a) Sample 1-1 collected at Site 1, and b) Sample 2-1 collected at Site 2. The thickness of the cross-sections is 50 μm . These thin cross-section samples are prepared for synchrotron XRF measurement for metal concentrations and distributions. Areas in the frames are selected for synchrotron XRF measurement.

Figure 3 Image of *Phragmites australis* root samples collected at Site 2 mounted on the metal pin tips for synchrotron TXM measurement. Upper: Root sample 2E; and Lower: Root sample 2G.

Figure 4 Extracted areas in Sample 1-1 collected at Site 1: (a) epidermis and (b) vascular bundle; and Sample 2-1 collected at Site 2: (c) epidermis and (d) vascular bundle. Data extracted from these areas were used for the data analyses.

Figure 5. Synchrotron transmission X-ray microscope measurement (TXM) for Fe XANES below and above Fe edge on two *Phragmites australis* root samples collected at Site 2.

Synchrotron X-ray absorption near edge structure (XANES) measurement confirms the presence of Fe nanoparticles in the epidermis of *Phragmites australis* root: (a) and (b) Fe XANES image taken at 7092 eV (below Fe^{2+} pre-edge); (c) and (d) Fe XANES image taken at 7192 eV (above Fe^{3+} pre-edge). Black dots in the circles in images (c) and (d) are Fe nanoparticles. The “shadows” in (a) and (b), where Fe nanoparticles are found, indicates the absorption of other metals, such as Mn, Cu and Ni, whose pre-edges are below Fe pre-edge. The results suggest the metal scavenging by Fe nanoparticles.

Figure 6. Metal concentrations and distributions in the root tip cross-sections of Sample 1-1, *Phragmites australis*, collected at Site 1. The first row: Br, Ca, Cl and Cr; the second row: Cu, K, Fe and Mn; and the third row: Pb, Ti, V and Zn.

Figure 7. Metal concentrations and distributions in the root basal cross-sections of Sample 2-1, *Phragmites australis*, collected at Site 2. The first row: Br, Ca, Cl and Cr; the second row: Cu, K, Fe and Mn; and the third row: Pb, Ti, V and Zn.

Figure 8. Results of the linear regression between metals (Cr, Cu, Mn, Pb, V and Zn) and Fe in the *Phragmites australis* root samples collected at Sites 1 and 2.

Fig 1

(a)

(b)

Fig 2

Fig 3

(a)

(b)

(c)

(d)

Fig 5

Phragmites, Br Ka

Phragmites, Ca Ka

Phragmites, Cl Ka

Phragmites, Cr Ka

Phragmites, Cu Ka

Phragmites, K Ka

Phragmites, Fe Ka

Phragmites, Mn Ka

Phragmites, Pb La1

Phragmites, Ti Ka

Phragmites, V Ka

Phragmites, Zn Ka

Phragmites, Br Ka

Phragmites, Ca Ka

Phragmites, Cl Ka

Phragmites, Cr Ka

Phragmites, Cu Ka

Phragmites, K Ka

Phragmites, Fe Ka

Phragmites, Mn Ka

Phragmites, Pb La1

Phragmites, Ti Ka

Phragmites, V Ka

Phragmites, Zn Ka

Fig 8

Table 1 Summary of Br, Ca, Cl, Cr, Cu, Fe, K, Mn, Pb, Ti, V and Zn average concentrations (mean±S.D.) in units of counts per second (cps) and range of variations in the epidermis and vascular bundle □
in Sample 1-1 and Sample 2-1 collected at Site 1 and Site 2, respectively

Sample ID	Layer	Statistics	Br	Ca	Cl	Cr	Cu	Fe	K	Mn	Pb	Ti	V	Zn
Sample 1-1	Epidermis n=1237	Mean±S.D.	41.1 ± 27.3	196 ± 388	79.4 ± 28.4	11.9 ± 23.0	36.8 ± 20.9	1900 ± 7267	227 ± 139	73.7 ± 75.9	13.9 ± 21.2	54.3 ± 379	14.5 ± 63.9	35.8 ± 34.8
		Range	-44.2 - 154	14 - 6621	5.8 - 223	-33.1 - 417.0	-11.6 - 183	29 - 92133	-1.5 - 1695	-1.4 - 881	-45.4 - 161	-48.7 - 8857	-62.0 - 1423	-15.7 - 369
Sample 1-1	Vascular tissue n=568	Mean±S.D.	34.3 ± 24.7	92 ± 43	87.0 ± 33.4	8.6 ± 10.3	29.7 ± 15.0	113 ± 106	200 ± 91	58.2 ± 28.4	10.2 ± 18.5	8.2 ± 12.5	7.4 ± 10.0	25.8 ± 15.2
		Range	-24.2 - 129	11 - 356	45.1 - 230	-28.3 - 38.9	-11.7 - 93.6	10 - 907	54 - 527	4.8 - 180	-40.4 - 66.1	-25.6 - 115	-29.5 - 34.6	-19.3 - 69.5
Sample 2-1	Epidermis n=364	Mean±S.D.	40.1 ± 21.6	173 ± 257	71.2 ± 17.6	10.1 ± 9.8	29.6 ± 12.4	1500 ± 3077	192 ± 58	25.1 ± 26.9	9.5 ± 18.6	40.2 ± 102	11.2 ± 18.6	34.7 ± 19.6
		Range	-26.5 - 105	27 - 4242	45.1 - 140	-17.4 - 45.3	-2.6 - 92.0	22 - 30495	21 - 379	-8.8 - 320	-34.2 - 78.3	-16.4 - 1322	-18.0 - 205	-9.8 - 122
Sample 2-1	Vascular tissue n=174	Mean±S.D.	37.9 ± 22.8	149 ± 46	72.5 ± 17.5	7.7 ± 9.6	26.5 ± 13.1	84 ± 97	199 ± 57	17.8 ± 11.4	8.0 ± 17.7	13.0 ± 65.7	6.2 ± 12.3	26.9 ± 14.1
		Range	-21.1 - 116	44 - 293	46.0 - 127	-19.5 - 29.3	-13.3 - 61.1	0 - 1105	47 - 386	-11.3 - 46.2	-42.2 - 53.5	-12.7 - 838	-14.6 - 122	-2.6 - 62.5

Table 2. Results of Mann-Whitney U non-parametric test and two-sample *t*-test to examine metal concentration difference between the two root samples collected at Sites 1 and 2, respectively.

Type of test	log (Br)	log (Ca)	log (Cl)	log (Cr)	log (Cu)	log (Fe)	log (K)	log (Mn)	log (Pb)	log (Ti)	log (V)	log (Zn)
Mann-Whitney U non-parametric test												
<i>p</i> -value	0.530	0.000	0.000	0.023	0.000	0.810	0.069	0.000	0.040	0.002	0.122	0.461
Two-sample <i>t</i> -test												
<i>p</i> -value	0.526	0.000	0.000	0.036	0.000	0.227	0.048	0.000	0.147	0.037	0.034	0.321
Bonferroni Adjusted <i>p</i> -value	1.000	0.000	0.000	0.429	0.000	1.000	0.575	0.000	1.000	0.443	0.409	1.000
Dunn-Sidak Adjusted <i>p</i> -value	1.000	0.000	0.000	0.354	0.000	0.955	0.445	0.000	0.852	0.363	0.340	0.990

Table 3. Results of Mann-Whitney U non-parametric test and two-sample *t*-test to examine metal concentration difference between the epidermis and the vascular bundle in the roots collected at Sites 1 and 2, respectively.

Sample ID	Type of test	log (Br)	log (Ca)	log (Cl)	log (Cr)	log (Cu)	log (Fe)	log (K)	log (Mn)	log (Pb)	log (Ti)	log (V)	log (Zn)
Sample 1-1 at Site 1	Mann-Whitney U non-parametric test												
	<i>p</i> -value	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.152	0.000	0.109	0.000
	Two-sample <i>t</i> -test												
	<i>p</i> -value	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.323	0.000	0.019	0.000
	Bonferroni Adjusted <i>p</i> -value	0.001	0.000	0.000	0.159	0.000	0.000	0.003	0.000	1.000	0.000	0.230	0.000
	Dunn-Sidak Adjusted <i>p</i> -value	0.001	0.000	0.000	0.148	0.000	0.000	0.003	0.000	0.991	0.000	0.207	0.000
Sample 2-1 at Site 2	Mann-Whitney U non-parametric test												
	<i>p</i> -value	0.204	0.308	0.390	0.155	0.036	0.000	0.087	0.006	0.318	0.000	0.000	0.000
	Two-sample <i>t</i> -test												
	<i>p</i> -value	0.207	0.879	0.384	0.119	0.082	0.000	0.176	0.049	0.313	0.000	0.000	0.000
	Bonferroni Adjusted <i>p</i> -value	1.000	1.000	1.000	1.000	0.986	0.000	1.000	0.585	1.000	0.000	0.000	0.001
	Dunn-Sidak Adjusted <i>p</i> -value	0.938	1.000	0.997	0.781	0.643	0.000	0.902	0.451	0.989	0.000	0.000	0.001

Table 4. Pearson correlation matrix of elements in the roots (i.e., epidermis and vascular bundle). The sample was collected at Site 1. Parameters in the bold face show significant correlatoin ($p < 0.001$).

Root		Br	Ca	Cl	Cr	Cu	Fe	K	Mn	Pb	Ti	V	Zn
Epidermis (n = 1237)	Br	1.000											
	Ca	0.155	1.000										
	Cl	0.580	0.137	1.000									
	Cr	0.081	0.285	-0.002	1.000								
	Cu	0.460	0.349	0.467	0.264	1.000							
	Fe	0.133	0.453	0.093	0.399	0.451	1.000						
	K	0.415	0.627	0.528	0.295	0.636	0.545	1.000					
	Mn	0.281	0.640	0.260	0.446	0.618	0.726	0.761	1.000				
	Pb	0.131	0.128	0.130	0.114	0.200	0.230	0.225	0.225	1.000			
	Ti	0.090	0.823	0.037	0.280	0.280	0.404	0.448	0.484	0.122	1.000		
	V	0.089	0.820	0.035	0.288	0.289	0.402	0.458	0.503	0.124	0.985	1.000	
	Zn	0.272	0.524	0.233	0.425	0.633	0.744	0.719	0.867	0.203	0.443	0.462	1.000
Vascular bundle (n = 568)	Br	1.000											
	Ca	0.290	1.000										
	Cl	0.510	0.569	1.000									
	Cr	0.047	-0.002	0.057	1.000								
	Cu	0.335	0.387	0.538	0.038	1.000							
	Fe	0.182	0.313	0.405	-0.008	0.308	1.000						
	K	0.520	0.733	0.881	0.057	0.554	0.447	1.000					
	Mn	0.393	0.695	0.671	0.018	0.483	0.428	0.798	1.000				
	Pb	-0.038	-0.027	0.028	-0.079	0.025	0.148	0.016	0.010	1.000			
	Ti	0.054	0.009	-0.010	-0.082	0.021	0.139	-0.006	0.018	0.064	1.000		
	V	0.021	-0.042	-0.015	0.052	-0.014	0.001	-0.030	-0.046	0.024	0.102	1.000	
	Zn	0.228	0.247	0.391	-0.020	0.305	0.245	0.414	0.355	0.030	0.034	-0.047	1.000

Table 5. Pearson correlation matrix of elements in the roots (i.e., epidermis and vascular bundle). The sample was collected at Site 2. Parameters in the bold face show significant correlatoins ($p < 0.001$).

Root		Br	Ca	Cl	Cr	Cu	Fe	K	Mn	Pb	Ti	V	Zn
Epidermis (n = 364)	Br	1.00											
	Ca	0.02	1.00										
	Cl	0.29	0.18	1.00									
	Cr	-0.02	-0.11	-0.03	1.00								
	Cu	0.10	-0.01	0.06	0.06	1.00							
	Fe	0.20	-0.04	-0.11	0.27	0.34	1.00						
	K	0.35	0.15	0.57	0.05	0.19	0.17	1.00					
	Mn	0.17	-0.02	-0.04	0.19	0.23	0.74	0.12	1.00				
	Pb	0.05	-0.03	-0.05	0.17	0.11	0.34	0.09	0.20	1.00			
	Ti	0.04	-0.03	-0.11	0.21	0.39	0.53	0.12	0.33	0.26	1.00		
	V	0.02	0.00	-0.14	0.15	0.28	0.46	0.11	0.31	0.24	0.86	1.00	
	Zn	0.18	0.02	0.03	0.20	0.35	0.66	0.24	0.53	0.32	0.51	0.44	1.00
Vascular bundle (n = 174)	Br	1.000											
	Ca	0.326	1.000										
	Cl	0.360	0.557	1.000									
	Cr	0.025	0.048	-0.008	1.000								
	Cu	0.352	0.343	0.309	0.011	1.000							
	Fe	-0.012	0.060	0.045	0.032	-0.094	1.000						
	K	0.447	0.784	0.745	-0.009	0.457	0.112	1.000					
	Mn	0.240	0.186	0.296	0.081	0.265	0.003	0.207	1.000				
	Pb	0.199	0.112	0.080	0.075	0.205	0.199	0.098	0.118	1.000			
	Ti	-0.025	-0.077	-0.021	0.068	-0.106	0.834	-0.003	-0.031	0.166	1.000		
	V	-0.067	0.055	0.014	-0.003	-0.113	0.629	0.037	-0.012	0.215	0.742	1.000	
	Zn	0.391	0.239	0.263	0.024	0.233	-0.003	0.312	0.049	0.027	-0.041	-0.023	1.000