

Iron and lead sequestration at the soil-root interface

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Introduction

An understanding of metal complexation within the rhizosphere of aquatic plants is of ecological and nutritional importance since the chemical form of metals affects their bioavailability, toxicity, and mobility through food chains. Defining the mechanisms involved in metal accumulation and tolerance is essential for discerning physiological adaptations by plants to metal-contaminated soils, for defining the bioavailability of attenuated contaminants, and for exploiting specific plant attributes to maximize metal-binding efficiency. Observations of elevated metal concentrations within the root relative to shoot tissue suggest that wetland plants may adopt exclusion (external and/or internal) mechanisms to hinder translocation of metals to the aerial tissue [1]. Iron (hydr)oxide plaques on roots are suspected to limit the uptake of toxins [2]; however, mechanisms of contaminant immobilization are unresolved. To help elucidate metal precipitation processes at the soil-root interface, we examined the roots of *Phalaris arundinacea* (a common, indigenous aquatic plant) using scanning electron microscopy (SEM) and x-ray fluorescence microtomography (FCMT). We obtained mineralogical and structural information of root precipitates using x-ray absorption spectroscopy (XAS) and Raman spectroscopy.

Methods and Materials

P. arundinacea samples were obtained from a mine-waste-impacted wetland in the Coeur d'Alene Basin of northern Idaho [3]. Roots were preserved by freeze drying and storing them in a desiccator until time of analysis. Concentrations of metals within the plant portions were determined using a wet-digestion procedure consisting of a nitric acid extraction followed by successive additions of 30% hydrogen peroxide and heating. Supernatants were analyzed via inductively coupled plasma-optical emission spectroscopy (ICP-OES) with a 5% accuracy range (Thermo Jarrell Ash IRIS ICP-OES, Franklin, MA). Quality control was checked every 15 samples.

A number of microscopic and spectroscopic techniques were used to obtain elemental distributions, associations, and structural information on the root surface. SEN with energy dispersive spectroscopy was performed on freeze-dried, carbon-coated roots with an Amray 1600 scanning electron microscope and energy dispersive system. FCMT was performed on the undulator beamline 13-ID-C at the Advanced Photon Source, Argonne National Laboratory. For FCMT, the intact, unaltered root was mounted on the rotation axis of a x-y-theta stepping motor stage. Fluorescence data were collected with a solid-state energy dispersive x-ray detector that allows multiple elements to be detected simultaneously. The FCMT data were obtained by

translating the root through the x-ray beam and collecting fluorescence at each 2 μm step. At each position, the fluorescence signal from a given element is proportional to the integrated number of atoms of that element along the ray transected by the beam. The sample is then rotated by 0.5° about the vertical axis and the line scan repeated. The process is continued until the particle has been rotated through a total of 180° at the end of which a two-dimensional plane has been sampled. X-ray absorption near-edge structure (XANES) analysis was conducted on beamline 4-1 at the Stanford Synchrotron Radiation Laboratory (SSRL). The electron energy was 3 GeV with a current ranging from ~100 mA to ~50 mA. XANES spectra were analyzed using WinXAS (Ressler, 1997). Seven XANES spectra were averaged; the background subtracted using a low-order polynomial function and normalized by setting the total atomic cross-sectional absorption to unity. The first derivative of each spectrum was obtained using a Savitzky-Golay algorithm and Fe speciation determined by noting the main-edge peak position in the first-derivative curve. Raman spectra were collected using a Kaiser Hololab Raman microscope equipped with a 785 diode laser and a charge-coupled device detector having a 4 cm^{-1} resolution. The laser was operated at 1 mW average power at the sample to reduce sample degradation caused by laser heating. The laser was focused through a 100x objective to maximize signal intensity. A minimum collection time for all samples was 30 seconds per spectrum, and at least 30 spectra were averaged over a Raman shift of 100–3500 cm^{-1} .

Results

Consistent with previous investigations, concentrations of metals in the plant roots are several-fold higher than in shoots. Root tissue concentrations for Fe are 58,600 mgkg^{-1} and 1160 mgkg^{-1} for Pb. SEM discloses the proliferation of heterogeneous precipitates (or plaque) on the exterior of the roots (Figure 1). Coatings (which are composed primarily of Fe with lesser amounts of Pb) exist predominantly as casts or fillings in cavities of the epidermal cells.

Elemental distributions and associations for Fe and Pb were resolved by obtaining a cross-sectional image (a section) from the interior of intact roots through the epidermis and plaque using FCMT (Figure 2). Iron and lead are concentrated on the exterior of the root, forming a superficial rind on the root epidermis. XANES spectra of the plaque indicate a predominance of Fe(III) in an oxo or hydroxyl coordination environment (Figure 3).

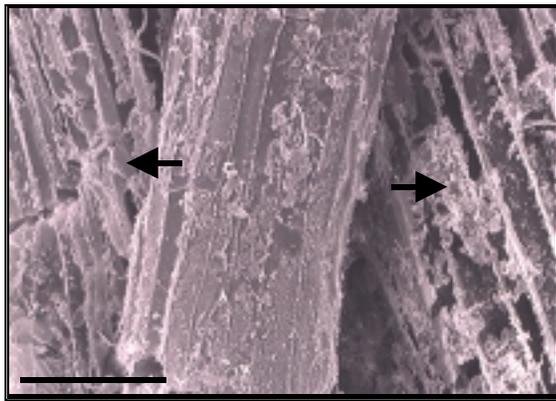


Figure 1: Scanning electron micrograph of *P. arundinacea* root sample denoting the extensive plaque that fills epidermal cells (arrows). Scale bar represents 100 μm .

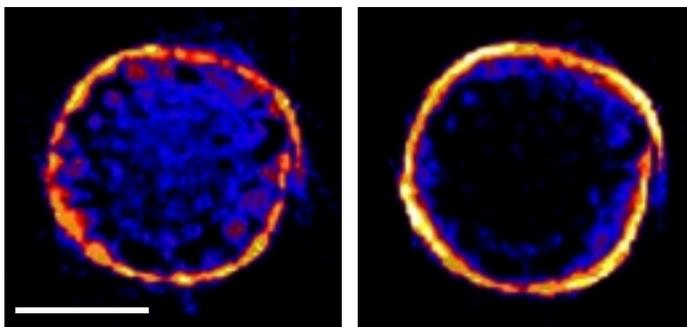


Figure 2: Fe and Pb distributions on and within grass roots as depicted by fluorescence microtomography. The spatial distribution of Fe (left) and Pb (right) within a cross section of the grass root is illustrated. Scale bar represents 300 μm .

Raman analyses of plaque precipitates reveal diffuse peaks indicative of short-range-order minerals. Following dehydration, however, distinct peaks were obtained analogous to those for hematite ($\alpha\text{-Fe}_2\text{O}_3$). It is therefore apparent that root plaques contain a preponderance of short-range-order hydrated iron oxides, [e.g., ferrihydrite ($\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O}$)]. The large surface area of hydrated iron oxides (which is often in excess of $200 \text{ m}^2 \text{ g}^{-1}$) provides a reactive substrate for contaminant sequestration. Moreover, Pb has a specific and high affinity for iron (hydr)oxides and would thus explain the absence of Pb within shoot and internal root tissue.

Discussion

Physiological processes within aquatic plants allow for the production of heterogeneous redox microenvironments and the subsequent aforementioned mineralogical assemblage. Concentration gradients allow for metal diffusion toward the root surface and potential uptake and subsequent toxicity to plants and biota. A physiological adaptation of aquatic plants allows for the absorption of $\text{O}_{2(\text{g})}$ in aerial tissue followed by translocation and release of oxygen into the rhizosphere [4, 5]. Rapid Fe oxidation allows for

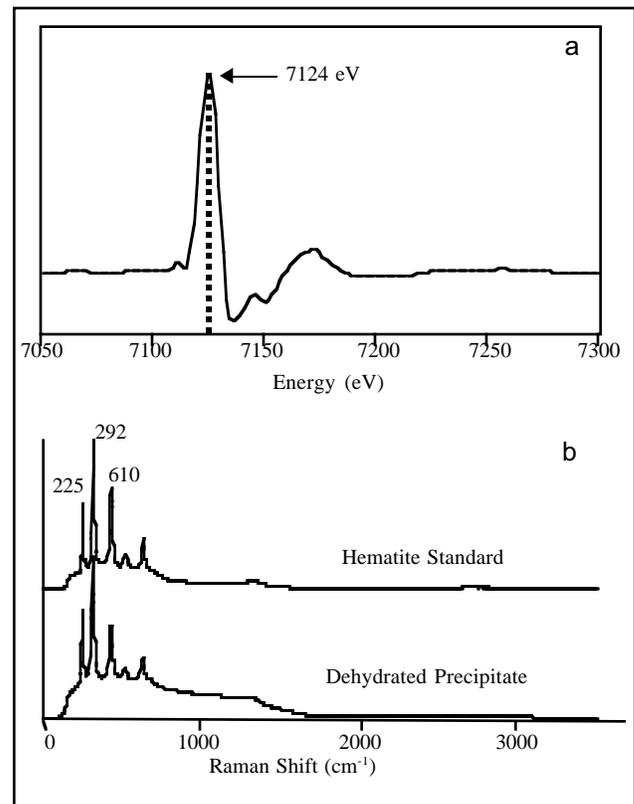


Figure 3: Mineralogical identification of iron plaque. (a) Iron first-derivative XANES spectra of *P. arundinacea* roots revealing a predominance of Fe(III) within the plaque. (b) Raman spectra of dehydrated plaque precipitate and analogous hematite standard.

homogeneous Fe (hydr)oxide plaque precipitation on the root epidermis (Figure 2). Due to the high surface area of short-range-order Fe hydroxides, metal attenuation (e.g., Pb) ensues and hence protects the plant from metabolic impairment (Figure 2).

External exclusion of Fe and Pb at the root surface explains the anomalously high concentrations of metals associated with aquatic plants and thus provides insight into the biogeochemical cycling of these metals within anaerobic systems. We have illustrated the sequestration mechanisms at the soil-root interface resulting from a substrate-induced anoxic-oxic boundary. Plant roots may thus provide a template for iron (hydr)oxide precipitation and subsequent metal attenuation. As such, minimal concentrations of detrimental metals are absorbed and translocated within the root or aerial plant tissue and subsequently introduced to the food chain. Consequently, the retention of contaminants on the surface of plant roots is an important control in stabilizing otherwise mobile and toxic metal species. Conversely, plant uptake of nutrients such as phosphate may be impeded by competitive sorption onto the plaque. Accordingly, these results have profound consequences on understanding and predicting metal and nutrient mobility, cycling, and bioavailability within anaerobic ecosystems.

Acknowledgments

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