In vivo Synchrotron Analysis of Thallium in Iberis Intermedia

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Introduction

Thallium (Tl) is a metal of great toxicological concern and its prevalence in the natural environment has steadily increased as a result of manufacturing and combustion practices. Due to its low natural abundance and the increasing demand, Tl is the fourth most expensive metal, thus, recovery and reuse could be a profitable endeavor. Thallium (Tl) is an element with greater toxicity to mammals than Hg, Cd, Pb or Cu (*1-3*) and is listed among the 13 priority metals by the USEPA (*4*). The toxicity of Tl was clearly recognized immediately after its discovery. Small doses (8 mg kg^{-1}) can be lethal to man (*5*) and the narrow margin between toxicity and therapeutic benefits stopped its original use in treating ringworm and other skin infections. Recently, more attention has been given to the occurrence of elevated concentrations of Tl in the environment (*6*). In the terrestrial environment, Tl concentrations in soils and plants, which are of concern for animal and human health, have been reported by various authors (*7-10*). Epidemiological studies in Europe have found significant positive correlations between Tl concentrations in the environment and the incidence of diseases of the circulatory system (*11*), and between Tl in soil and garden vegetables and a number of non-specific health effects for a study conducted in Germany (*12*). The major pathway of exposure to humans is the ingestion of vegetables grown in Tl-contaminated soils. Thallium can be readily taken up by plants because it is generally present in soil as thermodynamically stable Tl(I) and, as such, is an analogue of potassium.

Little is known regarding the chemical speciation of Tl in plants (*1*). However, this information is important because Tl has two oxidation states, monovalent Tl(I) and trivalent Tl(III), which differ in terms of toxicity and chemical reactivity. Trivalent Tl is approximately 50,000 times more toxic than Tl(I) and 43,000 times more toxic than Cd on a free-ion basis (*13*).

In this work we investigated *in vivo* Tl speciation and distribution in leaves of the Tl hyperaccumulator *Iberis intermedia*. This knowledge is valuable in view of the large potential of this plant for phytoremediation of Tl-contaminated soils as reported by Anderson et al. (*14*). The ability of *I. intermedia* to accumulate extremely large concentration of Tl in the above ground biomass (up to 13 429 mg kg^{-1}) makes this plant interesting in terms of phytomining. Currently, Tl is the fourth most expensive metal after Pt, Au and Pd. Determining the oxidation state of Tl in this plant is essential to assess disposal options and/or recycling possibilities of the metal-loaded plant biomass

Methods and Materials

Plant growth

I. intermedia seeds were initially germinated in a covered Petri dish on moist filter paper, and then transferred to pots and buried with approximately 1-cm of moistened soil. Plants (4-8) were grown in 1.5 L plastic pots of commercial potting soil at circumneutral pH spiked with Tl(I)Cl at rates of 0 (control), 10, or 20 mg kg^{-1}. The plants were grown in controlled environment growth chambers illuminated for sixteen hours per day to simulate approximately one-third sunlight with an average temperature of 25 ^{o}C. The *I. intermedia* plants were grown for ten weeks (reaching an average height of 23 cm) prior to examination by μ-XANES and μ-XRF.

X-ray Fluorescence and X-ray Absorption Near Edge Spectroscopies

The distribution and speciation of Tl were analyzed within the hyperaccumulator leaves by μ-XRF mapping and μ-XANES. Thallium (12 658 eV) L_{III}-μ-XANES spectra and μ-XRF maps were collected at beamline Sector 20-BM (Pacific Northwest Consortium - Collaborative Access Team (PNC-CAT)) at the Advanced Photon Source at Argonne National Laboratory, Argonne, IL. Energy calibration was accomplished by assigning the first inflection point of Au (L_{III}) foil to 11 919 eV and transmission data for a Tl(I)CO_3 standard was collected simultaneously with each scan to validate monochromator stability. The electron storage ring operated at 7 GeV with a top-up fill status. Scans were collected at ambient temperature in either fluorescence or transmission modes depending on the type of sample. Mapping data (μ-XRF) and μ-XANES spectra were collected in fluorescence mode with a solid-state 13-element detector. The μ-XRF mapping employed a 6 μm diameter spot size with a one second integration time per x-y step optimized with respect to Tl concentration and distribution within I. intermedia. Bulk XANES data were collected in transmission mode for reference standards and some plant analyses. Solid reference samples were examined in transmission mode by smearing a small amount of material on Kapton tape folded onto itself. XANES data were analyzed using WinXAS (*15*). The collected scans for a particular sample were checked for calibration, averaged, normalized, and the background was removed by spline fitting.

An obstacle in the μ-XRF and μ-XANES investigations was the awkwardness of situating a live plant in the beam path (Fig. 1). An attempt to determine metal speciation and distribution in live plant tissue by synchrotron methods has not been observed in the literature. However, this approach was deemed necessary to avoid pretreatment of the plant material that could cause artifacts both in terms of elemental distribution between the different plant tissues and in relation to the oxidation state of Tl. The isolated leaf was securely taped to a sample holder mounted at a 45^{o} angle relative to the electron beam on an x-y-theta step motor stage to raster the sample as the synchrotron beam passed through the leaf. Soil moisture within the pots was kept at field capacity during the μ-XRF and μ-XANES experiments. Other leaves that appeared to interfere with the beam path were removed with a scalpel.

Results and Discussion

Initial fluorescence data for energies 200 eV above the Tl edge indicated the presence of Tl and Ca in *I. intermedia* grown in Tl-spiked soil, with Ca being the lightest detectable element for our experimental conditions. Obviously other elements including K were present in the leaves; however, K could not be definitely differentiated from the Ca peak. Analysis of plant tissue, via total digestion, for Tl concentrations yielded two expected trends. Firstly, Tl concentrations in *I. intermedia* shoots increased as Tl concentrations in soil increased, from 6.8 mg kg^{-1} DW in the control treatment to 3450 and 5670 mg kg^{-1} DW in the soil spiked with Tl at 10 and 20 mg kg^{-1}, respectively. Secondly, Tl concentrations in *I. intermedia* were greater in mature (basal) leaves than young (apex) leaves. Although roots and stem represented almost 50% of the total weight of the plant, they contained only about 5% of the total Tl accumulated by the plant. The majority (95%) of Tl was stored in the leaves. Therefore, phytoextraction technologies must maximize the harvesting of this part of the plant, and removal of the root system may not be necessary.

Fig. 1. Experimental setup of I. Intermedia in hutch.

Fig. 2 shows the μ-XRF maps for Tl and Ca for the area encompassing the main central vein of a mature *I. intermedia* leaf. It is evident from Fig. 2 that Tl and Ca are closely associated and localized within the vascular system of the leaf. Identical results were obtained for other leaves and sections of leaves including minor veins between the main central vein and the edge of the leaf. Regardless of leaf maturity, Tl and Ca were localized to veins (primary and smaller secondary veins) within the leaves of *I. intermedia*. Upon collection of initial μ-XRF maps during the experimental run, Tl μ-XANES spectra were collected to speciate, *in vivo*, the form of Tl in the plant. It is worthwhile noting that this *in vivo* synchrotron research method could only be accomplished on a bending magnet (BM) beamline rather than an insertion device (ID) beamline, which has approximately 1000 times greater intensity and would certainly disintegrate the leaf tissue. By examining "hotspots" (color concentration gradient of μ-XRF map, light colors represent highest concentrations) via μ-XANES, we collected 50 spectra at 50 separate x-y coordinates along the central vein of a particular leaf by moving the sample motor stage linearly by a simple algebraic autosampler sequence program.

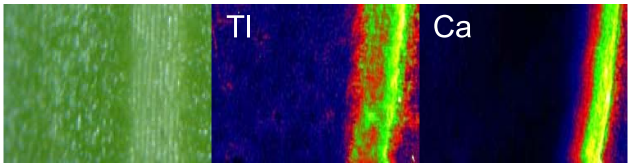

Fig. 2. μ-XRF distribution of Tl and Ca in *I. Intermedia* leaf. (scan dimensions 660 μm x 500 μm)

To aid in the speciation of Tl in *I. Intermedia* for data collected during the μ-XANES experiments, bulk XANES spectra (Fig. 3) of Tl reference materials were collected in transmission mode. The edge energies for Tl(I) and Tl(III) measured in our study were 12 660 eV and 12 683 eV, respectively. Also shown in Fig. 3 are XANES spectra for results of Tl speciation in two μ-XANES investigations and one bulk XANES sample of *I. intermedia*. The bulk XANES sample was prepared and examined by placing approximately 10 leaves from one plant in a stack, so as to align the main central vein of each leaf. The bundle of leaves were taped together, using Kapton film, and placed in line with the electron beam for bulk XANES analysis in transmission mode. The spectra shown in Fig. 3 for the μ-XANES samples are the collection, reduction, and average of 50 individual μ-XANES scans in close proximity on the same central vein of two separate leaves from different plants grown in 20 mg kg^{-1} spiked soil. The reference spectrum for aqueous $Tl(I)NO_3$ is shown in Fig. 3 as the best representation of the data collected for the speciation of Tl in the plant tissues. The XANES results indicate the Tl speciation in *I. intermedia* is aqueous Tl(I).

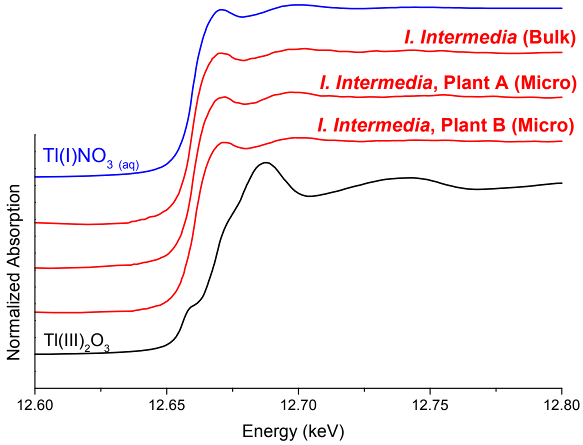

Fig. 3. XANES analysis of Tl in I. Intermedia.

Determining the speciation and localization of Tl in the hyperaccumulator *I. intermedia* to be similar to aqueous Tl(I) within the vascular system of the leaves will aid in the next step of harvesting Tl for beneficial reuse. Thallium(I) has a very low stability constant with both organic and inorganic ligands (*1,16*) and therefore should be easily extractable from the leaves. As economic demand for Tl increases, *I. intermedia* phytomining in Tl-contaminated environments could be environmentally sound and financially feasible.

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