X-ray Imaging of Zinc Uptake and Accumulation in Hyperaccumulator Thlaspi caerulescens

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Abstract

The use of high energy x-ray fluorescence as a trace metal probe offers scientists an innovative tool for research. One application is the study of heavy metal accumulation in plant systems. The test species *Thlaspi caerulescens* has the ability to accumulate massive amounts of zinc, cadmium, and lead from contaminated soils as a form of bioremediation. In order to understand the efficiency of the plant, the distribution and relative concentration of zinc was determined for the hyperaccumulator *Thlaspi caerulescens* and a similar plant, *Thlaspi arvense*, a nonaccumulator. The leaves of the *Thlaspi* plants were scanned using x-ray microprobe fluorescence. An elemental distribution map of zinc concentration in the plant leaf was generated. *Thlaspi caerulescens* distributed zinc throughout the tissue of the plant leaf while *Thlaspi arvense* accumulated zinc in the veins of the plant with little translocation of zinc to surrounding tissues.

Introduction

The use of biological materials to clean up heavy metal contaminated soils has been focused on as an efficient and affordable form of bioremediation. Plants termed phytoremediators are capable of absorbing large amounts of heavy metals from the soil, and accumulating these metals in plant tissue (Brown, 1995; Lasat, 1996). Various plants have been studied for metal tolerance properties (Baker, 1977; Brooks, 1981; Salt, 1995; Ebbs, 1997). *Thlaspi caerulescens* is a hyperaccumulator occurring in natural or contaminated soils high in zinc, lead, and cadmium (Baker, 1994) that has been researched for its phytoremediation properties. Hyperaccumulators have the ability to accumulate large amounts of contaminants by translocating metals from root to shoot in the plant (Vasquez, 1992; Brown, 1995; Lasat, 1996). A nonaccumulator plant species normally absorbs 20-100 μg g^{-1} zinc, while *Thlaspi caerulescens* has been shown to accumulate up to 40,000 μg g^{-1} zinc in their shoots (Chaney 1993). The ability to uptake and accumulate large amounts of zinc without toxic effects make this plant an effective phytoremediator.

Thlaspi caerulescens is a small, slow growing plant with little biomass for harvesting. In order to maximize metal uptake from the soil and increase the efficiency of clean up, the exact location of heavy metal accumulation patterns must be determined. Past techniques used to study the uptake efficiency include radiotracer flux techniques, flame atomic absorption spectrometry, and Na_2S fixation and freeze substitution with EDAX (Vasquez, 1992; Brown, 1995; Lasat, 1996), which offer insight into plant uptake and heavy metal accumulation but damage the sample. One possible technique for studying heavy metal uptake is the use of synchrotron techniques using x-ray imaging to create an elemental distribution map of plant leaves. This nondestructive method can detect and pinpoint the distribution of heavy metals in plant systems. Using x-rays the plants can be tested at various stages of the

growth cycle since the leaves do not need to be removed from the plant. Vasquez (1992) with his use of x-ray microanalysis, is the only previous report on x-ray microanalytical data to my knowledge, but required controlled growth and extensive sample preparation. The technique of x-ray imaging offers a unique look at zinc uptake and accumulation in the hyperaccumulator *Thlaspi caerulescens*.

Materials and Methods

Plant Material

Thlaspi caerulescens seeds were obtained from plants located at a Zn/Cd mining site in Prayon Belgium (Vazquez 1992). Seeds were grown in a nutrient medium containing 1 μM Zn^{2+} for 31d. Plants were grown on 1-mm^2 nylon mesh covered with black polyethylene beads to minimize illumination of the growth solution (Lasat, 1996). Seedlings were then transferred to 5-L black plastic tubs containing 0, or 200 μM Zn^{2+} solution. After 28 d, plants were harvested for analysis. Seeds from *Thlaspi arvense* were obtained from the Crucifer Genetics Cooperative (University of Wisconsin, Madison). *Thlaspi arvense* seeds were grown in a nutrient medium containing 1 μM Zn^{2+} solution for 22 d. Seedlings were then transferred to 5-L black plastic tubs containing 10, or 50 μM Zn^{2+} solution. After 18 d, plants were harvested for analysis. Leaves were taken over a two week period for this experiment.

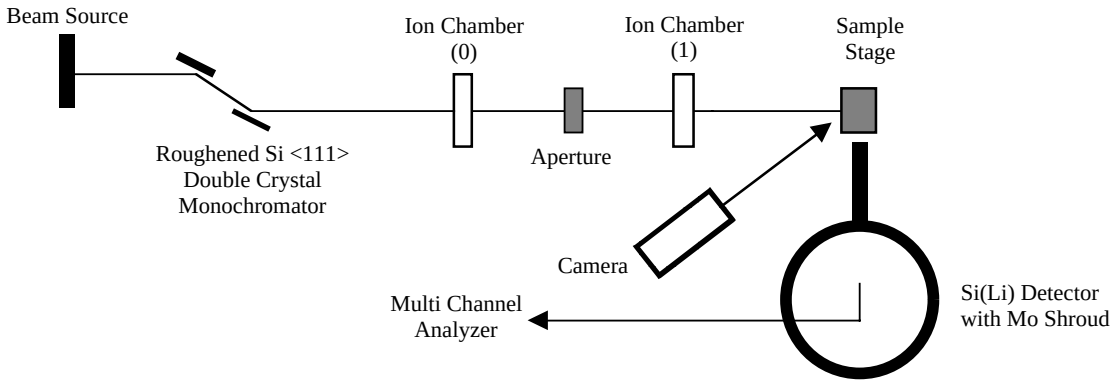

FIGURE 1. A schematic diagram of the experimental set-up in the B2 hutch at the CHESS lab. All images were generated using the equipment above.

X-Ray Set-up

The experiments were performed at the Cornell High Energy Synchrotron Source. The experimental set-up is shown in Fig. 1. The synchrotron produces a continuous white radiation beam of x-rays over a wide energy range. It is necessary to select the excitation energy needed to produce fluorescence in zinc. Zinc has a characteristic x-ray fluorescence emission energy of 9.66 keV, therefore an energy greater than this (10 keV) was selected to study zinc concentration in the *Thlaspi* sp. leaves. A silicon double-crystal monocromator was used to select an energy by adjusting the angle of the two silicon $\langle 111 \rangle$; crystals. A zinc foil was used to check the energy of the beam.

The beam passed through a gas ionization chamber which is used to measure the intensity of the beam. The beam passed through a Molybdenum aperture with varying hole sizes ranging from 1mm to 200 μm. The selected beam spot size controls both the point of fluorescence on the sample and the intensity of the beam. As beam width decreases, there is a decrease in intensity. To compensate for a lowered intensity, the length of time for each point exposure must be increased to allow for equal photon exposure. For example, as a beam is reduced from 1mm to 100 μm, the beam intensity is reduced to 1/100 This considerably increases the length of scan time needed to maintain equal exposure by x100. A second ion chamber was set up after the aperture to monitor radiation intensity at the sample.

A slide holder was made of aluminum to hold the interchangeable 2.5 x 4 cm slide frames containing leaf samples. The slide holder was mounted on a moving sample stage controlled with X and Z stepping motors. The sample was moved in relation to stationary beam to scan the entire leaf sample.

Fluorescence created from the beam striking the leaf was detected by a Si(Li) detector with a window area of 30 mm^2 and a resolution of 146 eV. A molybdenum aperture was used on the end of the detector to reduce background signals. All data collection and stage movement was controlled by computer using the SPEC program.

Plant Analysis

Mature plant leaves from each replicate were harvested 1 hour prior to analysis. Leaves were stored at 5° C between scans. Two plant species, *thlaspi caerulescens* grown in 0 and 200 μM Zn^{2+} solution and *thlaspi arvense* grown in 10 and 50 μM Zn^{2+} solution were scanned for zinc concentration. Two replicates from each of the four plants were scanned for zinc concentration. Each leaf was placed in a 2.5 x 4 cm slide frame to provide a secure frame for scanning.

In order to create an image of zinc concentration in the leaf, the x-ray beam was scanned over a grid of points on the leaf. Initially the spot size was 1mm then lowered to 500 μm to collect a coarse image of the leaf. As interesting features were found, the resolution of the image was increased by using a smaller aperture and a smaller step size. The time required to form an image increases rapidly with a finer resolution.

Results

Multiple images were created of zinc distribution in the *Thlaspi caerulescens* and *Thlaspi arvense* leaves. Scans were created from each of the four replicate leaves, *Thlaspi arvense* grown in a 10 μM Zn^{2+} solution and 50 μM Zn^{2+} solution and *Thlaspi caerulescens* grown in a 0 μM Zn^{2+} solution and 200 μM Zn^{2+} solution. The leaves were first scanned at low resolution with a 1 mm aperture to generate an image of the whole leaf as shown in Figs. 2 and 3. Each image created is a map of the relative distribution of zinc in the leaf, therefore the images can not be compared for zinc concentrations. The white areas indicate a high level of zinc concentration while black indicates zero concentration. Images from *Thlaspi arvense* grown in 10 μM Zn^{2+} solution and *Thlaspi caerulescens* grown in 0 μM Zn^{2+} solution had such low levels of zinc that the images did not have enough contrast to show zinc distribution.

The hyperaccumulator *Thlaspi caerulescens*, grown in 200 μM Zn^{2+} solution and the nonaccumulator *Thlaspi arvense* grown in 50 μM Zn^{2+} solution were scanned with a 200 μm

aperture to create images of higher resolution shown in Figs. 4 and 5.

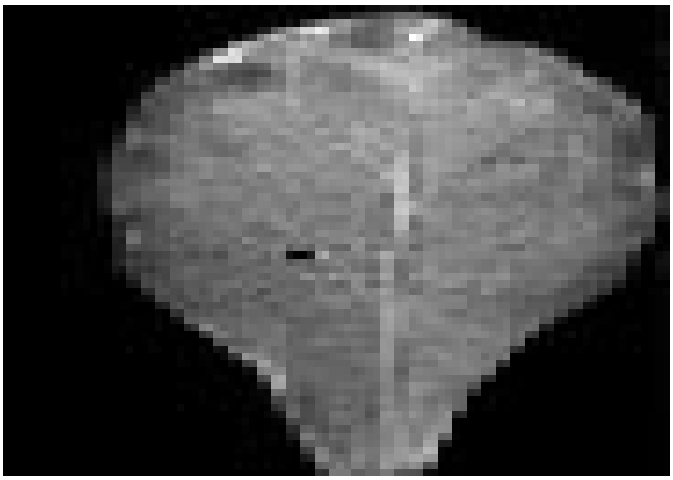

FIGURE 2. Image of Zinc distribution in the hyperaccumulator *Thlaspi caerulescens*, grown in 200 μM Zn^{2+} solution. The pixel size was created using a 1mm aperture with a pixel size of 1mm.

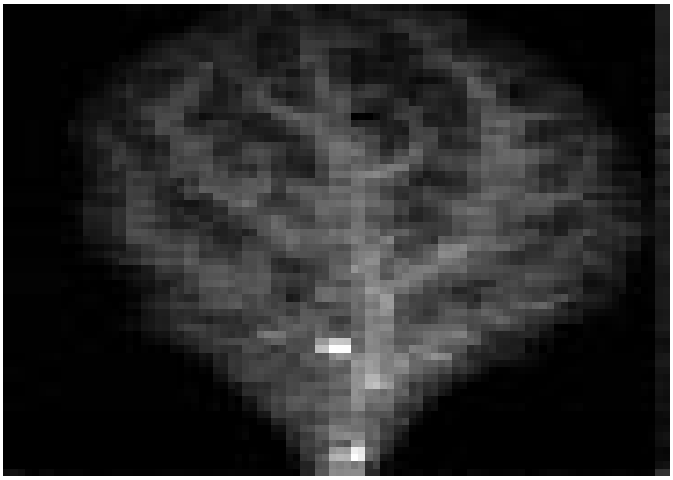

FIGURE 3. Image of Zinc distribution in the nonaccumulator *Thlaspi arvense,* grown in 50 μM Zn^{2+} solution. The mesh scan created using a 1mm aperture with pixel size of 1mm.

Discussion

The image of *Thlaspi arvense*, Fig. 2, shows zinc distribution primarily remains in the veins without translocation of zinc to surrounding tissues. The nonaccumulator is not able to translocate zinc to storage vacuoles, therefore it lacks the ability to detoxify zinc in the plant. The *Thlaspi arvense* can not tolerate large amounts of zinc and dies when grown in areas of high zinc concentration.

Thlaspi caerulescens shows a relatively even distribution of zinc throughout the leaf. The results confirm that zinc is translocated in the shoot and distributed evenly in the leaves of

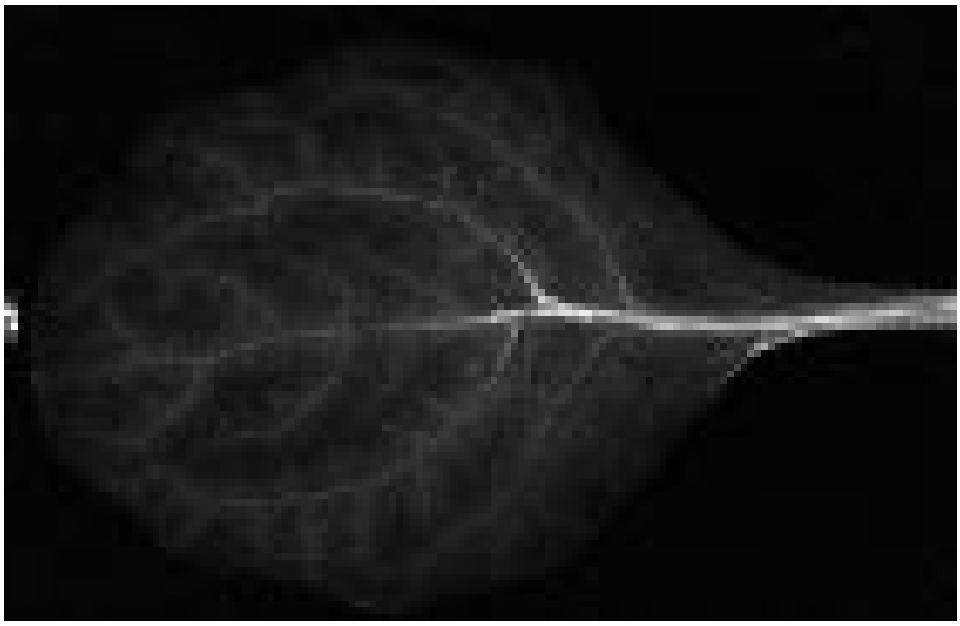

FIGURE 4. Image of zinc distribution in the nonaccumulator *Thlaspi arvense*, grown in 50 μM Zn^{2+} solution. The mesh scan was created using 200 μm aperture with a pixel size of 200 μm.

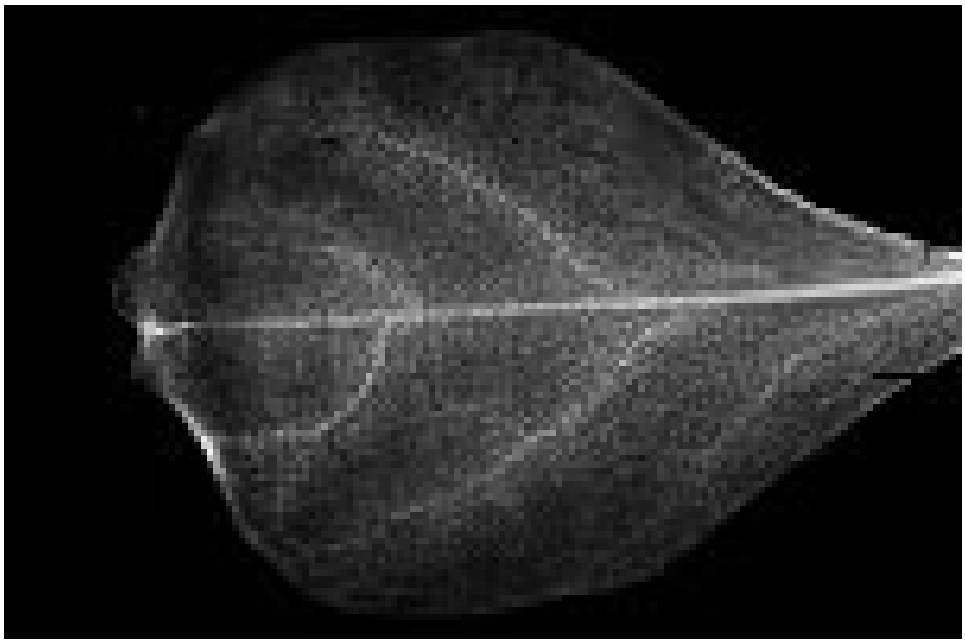

FIGURE 5. Image of zinc distribution in the hyperaccumulator *Thlaspi caerulescens*, grown in μM Zn^{2+} solution. The mesh scan was created using 200 μm aperture with a pixel size of μm.

the hyperaccumulator as hypothesised by Vasquez (1992), Brown (1995), and Lasat (1996). The success of *Thlaspi caerulescens* as a hyperaccumulator is based on the plants ability to

detoxify zinc by storing heavy metals in vacuoles. (Brooks, 1981; Vasquez, 1992; Lasat 1996) It is not known exactly where the zinc is stored or what ligands are formed with zinc in the cells.

Further areas of study may include studying *Thlaspi caerulescens* grown in a range of zinc concentrations to determine the efficiency of the plant as a phytoremediator in lower zinc levels. Also, since the technique of x-ray fluorescence is non-destructive, it would be possible to look at one plant leaf over a period of time to study time dependent uptake. One other area of study would be to analyze the roots of the two species of *Thlaspi* vs. the shoots of both species. The nonaccumulator should have a higher concentration of zinc in the roots, while the hyperaccumulator should have a higher concentration of zinc in the shoots.

These questions may be addressed with further use of x-ray imaging. The mechanisms of heavy metal transport, specifically the translocation in the xylem (and possibly phloem), transport across the leaf-cell plasma membrane and storage in the leaf-cell vacuole (Lasat, 1996) can be further studied with higher beam resolution. Currently, it is possible to achieve beam widths as low as 10 μm. With smaller beam sizes to create high resolution scans, it is possible to study zinc in relation to cell physiology as well as complex ligand structures.

Future research can benefit greatly by integrating the use of x-ray imaging. This innovative tool offers scientists a versatile tool in performing research. The use of synchrotron technology can lead to major advances in the field of biology and a new multi-disciplinary field of study.

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