LEAD HYPERACCUMULATION BY *SESBANIA DRUMMONDII*

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ABSTRACT

Sesbania drummondii is a leguminous plant commonly found in the southeastern United States. Our studies have shown that seedlings of *Sesbania drummondii* can hyperaccumulate lead (Pb) in a controlled hydroponic environment. The addition of a chelating compound (EDTA) further increases the amount of Pb that a seedling can accumulate. Pb-treated seedlings were analyzed with scanning and transmission electron microscopes equipped with energy dispersive X-ray spectrometers. Pb deposits were observed in concentric rings around the stele and on sub-epidermal cortical cells, and on the surface of the epidermis. The lead appeared to be localized within and on the cell wall. Phosphorus was present whenever lead was detected, suggesting the lead precipitated as a lead phosphate.

Key words: *EDTA, hyperaccumulation, lead, phytoremediation, Sesbania drummondii*

INTRODUCTION

Over the past century, mining, manufacturing, and many other industrial processes have all been major contributors to extensive soil pollution (Cunningham et al., 1995). As a result, there is an extreme interest burgeoning in the industry to find a cost-effective method of remediating these contaminated soils. Among the forefront in technologies being examined is phytoremediation. Phytoremediation is the use of plants to remove or stabilize a harmful contaminant in the environment. Current research, such as the work done by Blaylock et al. in 1997, shows that some plants can accumulate high levels of normally toxic metals in their tissues and still survive. Harvest of these plants then provides a means of removing the metals from the environment.

In this work, we sought to evaluate whether *Sesbania drummondii* would be suitable for use in the phytoremediation of lead (Pb). We chose *S. drummondii* because it is a relatively large plant found growing naturally on a site contaminated with Pb, along with other inorganic and organic contaminants.

For *Sesbania* to be useful in phytoremediation, it must not only accumulate large amounts of Pb from soil, but also translocate the Pb to aerial parts for harvest. Pb, however, is not very soluble in soil, and translocates poorly from roots to shoots (Huang et al., 1997). One way to increase Pb solubility is to lower the pH. Decreasing the soil pH has also been shown to increase the amount of metal that a plant can accumulate (Huang et al., 1997). Alternatively, chelating agents such as EDTA have the potential to increase the bioavailability of metal in the soil solution and may increase the amount of metal accumulated in plant tissue (Huang et al., 1997).

Although our ultimate goal is to determine if *Sesbania* can remove Pb from soil at contaminated sites, we have begun by evaluating

the ability of *Sesbania* seedlings to accumulate Pb from solutions. Here we show that *Sesbania* seedlings do accumulate Pb, that Pb deposits form within the cell walls of these plants, and that a metal ion chelator can increase the uptake of Pb by the seedlings.

MATERIALS AND METHODS

Germination of Sesbania drummondii *Seedlings*

Seeds were first scarified in 85% sulfuric acid for 15 minutes and washed under running tap water for 30 minutes. Seeds were then surface sterilized by treatment with 0.2% mercuric chloride, followed by two distilled water rinses. The surface-sterilized seeds were aseptically placed in 1.5% water agar, six to a petri dish, and allowed to germinate for one week in a night/day incubator at 25° C. Seedlings were then transferred to solutions of modified Hoaglands medium containing various concentrations of Pb.

Solution Preparation

Modified Hoaglands medium/Pb solutions were produced by adding up to 1000mg/L Pb as lead nitrate (Sigma), and then adjusting the pH to 5.8 with 1M HCl. EDTA was added to solutions after Pb additions, and the pH was adjusted to 6.8 to completely dissolve the EDTA.

Concentration of Pb in Seedlings

Seedlings were placed into tubes containing their respective concentrations of Pb. They were then permitted to grow for a period of two weeks in a night/day incubator at 25°C. Seedlings were harvested by washing with distilled water and then stored in -20°C freezer until analysis. Analysis of metals was performed using inductively coupled spectrophotometry (ICP).

Transmission Electron Microscopy (TEM)/ Scanning Electron Microscopy (SEM)

Samples of *Sesbania* were fixed using glutaraldehyde followed by osmium tetroxide for the TEM work. For the SEM work, samples were quick-frozen in liquid nitrogen slush, then freeze-dried under vacuum. Pb in samples was identified using energy-dispersive X-ray analysis.

RESULTS/DISCUSSION

Sesbania drummondii seedlings were able to accumulate significant amounts of Pb under hydroponic conditions. After five days of treatment with 1000 mg/L $Pb(NO_3)_2$ at pH 5.8, Pb levels in the root tissue of seedlings amounted to more than 7.2% Pb per gdw of tissue.

As expected, decreasing the pH increased the seedlings' ability to accumulate Pb per gdw. However, at lower pHs, plants exhibited signs of stress, including stunted growth and chlorotic leaves. The optimum pH of the solutions appeared to be 5.8.

Most of the Pb accumulated by the seedlings remained in the roots. Using the SEM, Pb deposits were observed to form concentric rings in the root; they formed around the stele, in the cortex beneath the epidermis, and on the root epidermal surface. Outside of the root, significant deposits were only observed in the lower stem, where they were present on pith parenchyma cells. The TEM was used to determine the exact location of deposits within

cells. In both root and stem cells, deposits were clearly located within the cell wall. Using energy dispersive X-ray spectrometry, these deposits were found to contain phosphorous as well as Pb, suggesting that the Pb precipitated as a phosphate.

The addition of a chelator, EDTA, was investigated as a means of increasing total Pb accumulation in seedlings. The presence of small amounts of EDTA was capable of increasing the amount of Pb that the seedlings could accumulate. The greatest increase occurred with 100 mmol EDTA, which led to the accumulation of 16% more Pb than in the absence of EDTA.

CONCLUSION

Sesbania drummondii is able to tolerate, accumulate, and to some extent, translocate Pb ions from solution. If in further studies *Sesbania* proves capable of hyperaccumulating Pb from soils, it could become a valuable tool for the phytoremediation of Pb-contaminated sites.

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