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Electron Microscopic Studies on Localization of Lead in Organs of *Typha angustifolia* Grown on Contaminated Soil

Thanawan Panich-pat[a], Peerasak Srinives[b,*], Maleeya Kruatrachue[c], Prayad Pokethitiyook[c], Suchart Upatham[d], and Guy R. Lanza[e]

[a] Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.
[b] Department of Agronomy, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.
[c] Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.
[d] Faculty of Science, Burapha University, Chonburi 20130, Thailand.
[e] Environmental Sciences Program, University of Massachusetts, Amherst, MA 01003, USA.

* Corresponding author, E-mail: agrpss@ku.ac.th

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ABSTRACT: A greenhouse study was conducted to observe the localization of lead in narrow-leaved cattail, *Typha angustifolia*. Light and transmission electron microscopic studies were performed on root, rhizome and leaf of the cattail grown in control (75 kg dry weight of soil with no added lead) and in the same weight of soil amended with 20,000 mg lead nitrate. At 15 and 90 days after planting, most lead was accumulated in root cells around vacuoles and slowly transported to leaves. In the lead-contaminated soil, parts of the root cell wall were damaged at the end of the experiment. Lead was deposited in the rhizome near the cell wall. Similar deposits were observed in the roots and rhizomes suggesting that lead was transported and localized in a similar area, whereas the leaf cells accumulated lead in the chloroplasts.

KEYWORDS: *Typha angustifolia,* narrow-leaved cattail, lead accumulation, TEM, ultrastructure.

INTRODUCTION

Lead is considered one of the most frequently encountered heavy metals of environmental concern and is the subject of much remediation research.[1] Severe lead contamination in soils may cause a variety of environmental problems, including loss of vegetation and groundwater contamination.[1] Lead can be absorbed by plants and cause reduction of growth and inhibition of cell division.[2] It is potentially damaging to water and soil resources, as well as a serious threat to human health. Many studies have shown that environmental remediation can be accomplished using plants such as corn[2], sunflower[2], brassica[3] and moss[4].

The transmission electron microscope (TEM) is a useful tool for analyzing metal and metal-composite specimens. Sharpe and Denny[5] used electron microscopy to study absorption and localization of lead in leaf tissue of *Potamogeton pectinatus* and found it mostly localized in the cell walls. Lead deposits appeared in two different forms: fine electron-dense granules along the middle lamella and in the outer wall and coarser and more distinct deposits in the inner cell wall towards the plasmalemma. No lead was deposited in the root up to a week after treatment. Skaar *et al*[4] demonstrated, through TEM studies in lead exclusion, lead accumulation in vacuoles, mitochondria, chloroplasts and nuclei of *Rhytidiadelphus squarrosus* from Trondheim, Norway. Brown and Buck[6] found that most lead and zinc were bound to the cell wall in *Grimmia donniana*.

Typha angustifolia (narrow-leaved cattail) grows easily in Thailand. This prolific plant plays an important role as shelter for different marsh-dwelling animals. It expresses high potential for remediation of metal-contaminated soil and in wastewater treatment.[7] Natural and artificially-planted aquatic treatment systems making use of cattails have been employed satisfactory in sewage treatment. Recently, wetlands have also been utilized to treat metal-contaminated effluent.[8] It was the purpose of this research to study localization of lead in root, rhizome and leaf of *T. angustifolia* grown in lead-contaminated soil.

MATERIALS AND METHODS

Soil and Plant Preparation

The soil used in this experiment was collected from Kamphaeng Saen District of Nakhon Pathom Province.

It was classified as sandy loam which is suitable for cattail growth. Soil was air-dried two days before use. The soil was mixed and put in 50 cm diameter buckets, 75 kg per bucket to attain a depth of 40 cm. Tap water was added until 10 cm above the topsoil layer. The average temperature throughout three months of study was 34±4 °C.

T. angustifolia was collected from Laem Phak Bia Environmental Research and Development Project (LERD), Petchaburi Province, Thailand. Plants can be divided into leaves, rhizomes and roots. Leaves were the above-ground portion. Rhizomes were the enlarged underground stems below the leaf portion. Roots were distinguished from rhizomes by their thread-like and irregular branching. Each plant was approximately 1.0 –2.0 cm in diameter and was cut to a length of 45 cm prior to planting three plants in each experimental bucket.

Lead Treatment

$Pb(NO_3)_2$ was dissolved to prepare a solution of 20,000 mg/L concentration (20 g in 1 L deionized water). One liter of this solution was placed in each treatment bucket right after planting the cattail. The experimental period lasted a total of 90 days. Selected plants from the control (0 mg/L) and treated buckets were examined under a light microscope and a transmission electron microscope on day 15 and day 90 after planting.

Histological Study

Fifteen days after planting, samples were taken from both the control and treated plants. Roots, rhizomes, and leaves were separated and cut into small pieces. They were fixed in FAA (37% formalin, glacial acetic acid, 70% ethyl alcohol) for 24 hr, dehydrated through a graded series of tertiary butyl alcohols (TBA; 50, 70, 85, 95, and 100%) and embedded in Paraplast®(Tycohealthcare, New York, USA). Sections were cut to 10-12 mm thick on a rotary microtome, stained with safranin-fast green and observed under an Olympus®(E for L International, Tokyo, Japan) light microscope.

Ultrastructure Study

For ultrastructural study, samples were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7) for 3 hr, and postfixed in 2% osmium tetroxide for 3 hr. They were then dehydrated through a graded series of increasing ethanol concentrations (30, 50, 70, 80, 90, and 100%), and embedded in Spurr's resin. Ultrathin sections were cut with a diamond knife and stained in 10% uranyl acetate and lead citrate, then examined under a transmission electron microscope (Hitachi H-7000, Hitachi, Ibaraki, Japan).

Results and Discussion

Light Microscopic Study

Root Section

Figure 1A shows a cross-section of *T. angustifolia* root in the control. The epidermal cells have developed root hairs. Just beneath the epidermis is the outermost layer of the cortex. One of its functions is to repair the epidermis when it is destroyed. The spaces between the cells, designated as intercellular spaces, furnish an excellent aerating system to secure the supply of oxygen to all living cells and facilitate removal of waste carbon dioxide arising from respiration.

The stele is the central cylinder surrounded by the pericycle or a row of parenchyma cells. The xylem occupies the center of the stele. The phloem is made-up of large, thin-walled sieve tube cells and smaller companion cells. The water absorbed from the soil moves across the epidermis, cortex, and pericycle, and then enters the xylem.

Light microscopy did not reveal histological alteration in the roots of control plant (Fig 1A) and lead-treated plants (Fig 1B).

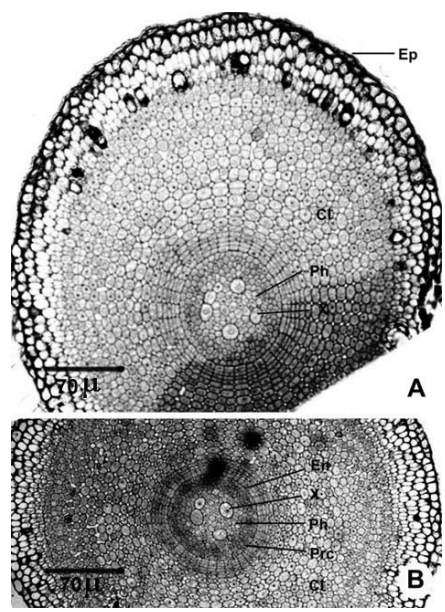

Fig 1. Light micrographs in cross-section of *T. angustifolia* root.
A. Control : cattail root at medium magnification showing epidermis (Ep), cortex (Ct), xylem (X) and phloem (Ph).
B. Treated plant : cattail root at medium magnification showing a normal appearance of cortex (Ct), endodermis (En), pericycle (Prc), xylem (X) and phloem (Ph).

Rhizome Section

Rhizomes are horizontal stems which grow below or at the surface of the soil. Like any stems, they have buds which developed into branches. In addition, adventitious roots are present. Figure 2A shows a cross-section of *T. angustifolia* rhizome. The outermost layer of the rhizome is the epidermis. The somewhat circular structures scattered throughout the rhizome are vascular bundles responsible for conduction. Each vascular bundle consists of xylem toward the inside and phloem toward the outside. The ground parenchyma is the tissue between the vascular bundles. The cells in this region are large, thin-walled, and loosely arranged.

Light microscopy was not able to observe histological alteration between the rhizomes of control plants (Fig 2A) and lead-treated plants (Fig 2B).

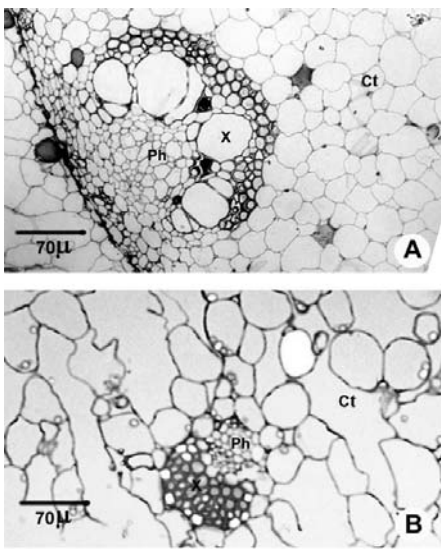

Fig 2. Light micrographs in cross-section of *T. angustifolia* rhizome.
A. Control : cattail rhizome at medium magnification showing cortex (Ct), xylem (X) and phloem (Ph).
B. Treated plant : cattail rhizome at medium magnification showing a normal appearance of xylem (X) and phloem (Ph).

Leaf Section

Figure 3A shows a cross-section of *T. angustifolia* leaf. The three tissues which make up a foliage leaf are epidermis, mesophyll, and veins. Each leaf surface is covered by an epidermis made up of closely fitting, interlocked, transparent epidermal cells and guard cells. The space between the adaxial and abaxial epidermis is occupied by mesophyll cells. The mesophyll cells toward the adaxial surface are cylindrical in shape and known as palisade cells, whereas those toward the abaxial surface are rounded and called spongy cells.

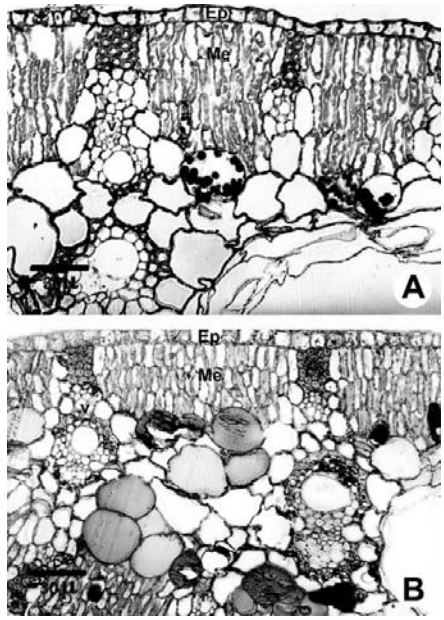

Fig 3. Light micrographs in cross-section of *T. angustifolia* leaf.
A. Control : cattail leaf at medium magnification showing epidermis (Ep), mesophyll (Me) and vein (v).
B. Treated plant : cattail leaf at medium magnification showing a normal appearance of epidermis (Ep), mesophyll (Me) and vein (v).

The veins (vascular tissue) form the structural framework to conduct water, minerals, and food. Each vein consists of conducting tissue surrounded by a bundle sheath. Two conducting tissues are present in a vein, viz. xylem lays toward the adaxial epidermis to conduct water and minerals, phloem lays toward the abaxial side to conduct photosynthates and foods.

Again, light microscopy was unable to observe histological alteration in the leaf tissue of control plants (Fig 3A) and lead-treated plants (Fig 3B).

Electron Microscopic Study

Root Section

Control. The cytoplasm of parenchyma cells from the root zone contains numerous vacuoles, mitochondria, and ribosomes. The nucleus often has a distinct nucleolus. The cell wall is a primary one (Fig 4A).

Day 15. Treated *T. angustifolia* showed parenchyma cells with what appears to be lead granules around the vacuoles (Fig 4B). The cell wall was normal in appearance. Mitochondria and ribosomes were observed in the cytoplasm (Fig 4C).

Day 90. Treated parenchyma cell contains partially degenerated cell wall. Some lead granules were still accumulated around the vacuoles (Fig 4D).

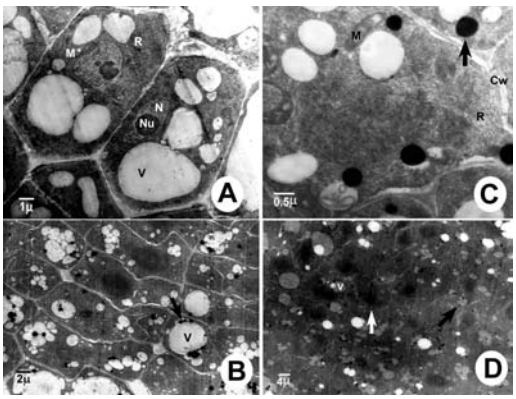

Fig 4. Transmission electron micrographs of *T. angustifolia* root.
A. Control: cattail root parenchyma cells consist of vacuoles (V), mitochondria (M), ribosome (R), nucleus (N), nucleolus (Nu).
B. Treated plant: cattail root parenchyma cell at day 15 showing lead granules (arrow) around vacuoles (V)
C. Treated plant: cattail root parenchyma cell at day 15 showing a normal cell wall (Cw), mitochondria (M), ribosomes (R). Lead granules (arrow) were accumulated near the cell wall.
D. Treated plant: cattail root parenchyma cell at day 90 showing the degenerated cell wall (white arrow). Some lead granules (black arrow) accumulated around the vacuoles (V).

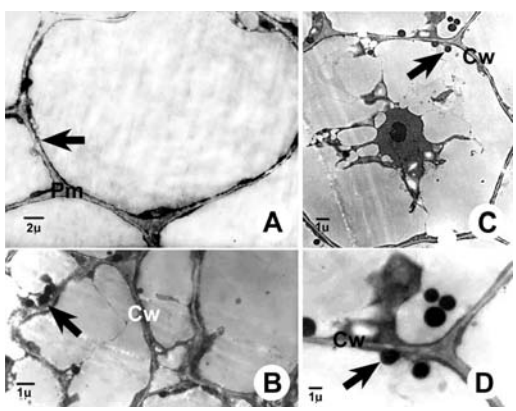

Fig 5. Transmission electron micrographs of *T. angustifolia* rhizome.
A. Control: cattail rhizome parenchyma cell consists of normal cell wall (arrow) and plasmalemma (Pm).
B. Treated plant: cattail rhizome parenchyma cell at day 15 showing lead granule (arrow) accumulation near the cell wall (Cw).
C. Treated plant: cattail rhizome parenchyma cell at day 90 showing lead granule (arrow) accumulation near the cell wall (Cw).
D. Treated plant: cattail rhizome parenchyma cell at day 90 showing lead granules (arrow) (0.1 mm in diameter) which are spherical in shape.

Rhizome Section

Control. The ultrastructure of parenchyma cells of the rhizome showed normal cell wall and plasmalemma (Fig 5A).

Day 15. The ultrastructure of treated *T. angustifolia* rhizome showed lead granule accumulation near the cell wall (Fig 5B).

Day 90. There was lead granule accumulation in the cytoplasm and near the cell wall (Fig 5C). These granules were small and spherical in shape (Fig 5D).

Leaf Section

Control. The ultrastructure of palisade cells showed vacuoles, mitochondria, and numerous chloroplasts within the cytoplasm (Fig 6A).

Day 15. Lead granules were accumulated in the chloroplasts. No lead granules were within the nucleus and mitochondria (Fig 6B).

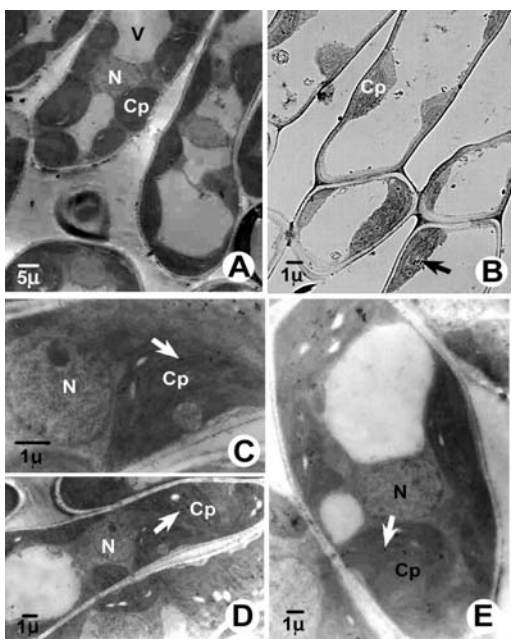

Fig 6. Transmission electron micrographs of *T. angustifolia* leaf.
A. Control: cattail leaf palisade cells consist of vacuoles (V), chloroplast (Cp), and nucleus (N).
B. Treated plant: cattail leaf palisade cell at day 15 showing lead granule (arrow) accumulation in the chloroplast (Cp).
C. Treated plant: cattail leaf palisade cell at day 90 showing nucleus (N), chloroplast (Cp), and lead granule (arrow) accumulation in the chloroplast.
D, E. Treated plant: cattail leaf palisade cell at day 90 showing lead granule (arrow) accumulation in the lamellae of the chloroplast (Cp). No lead granules were observed in nucleus (N).

Day 90. The cell wall appeared normal. The organelles were present in the cytoplasm and appeared normal (Fig 6C). No lead granules were found in the mitochondria, but they were deposited in the lamellae of chloroplasts (Fig 6D, 6E).

Discussion

The treated plants grew well without showing toxic symptoms. It is likely that the plants tolerate the heavy metal or that they have the ability to exclude heavy metal. The limits of useful magnification are determined by the instrument's resolving power. For the light microscope study, the highest magnification used was 40X, which could not identify lead deposits, while electron microscopic study has enormous powers of resolution. Thus, the study under light microscopy showed no histological alteration in root, rhizome, or leaf in treated plants. Cumming and Taylor [9] suggested that exclusion of metal from the symplasm might be achieved by modifications of the rhizosphere which reduce the available concentration or the activity of free metal ions at the plasma membrane - soil solution interface. Ye *et al* [8] studied zinc, lead and cadmium tolerance, uptake and accumulation by *T. latifolia* and found metal tolerance and metal exclusion in this species may be related to its oxygen transport capability, radial oxygen loss from the roots, and the capability to modify its rhizosphere. In the ultrastructural study, the pattern of lead deposition was small and spherical in shape. Most of the lead accumulated in leaf cells, especially in chloroplasts, and vacuoles. At 90 days, treated plants contained partially degenerated cell wall at the root section. Skaar *et al*[4] studied lead accumulation within nuclei of moss leaf cells and found that the nuclear membrane was damaged as the nucleus contained many electron-dense particles. In this experiment, we found no effect of lead on nuclei in any parts of *T. angustifolia*.

In rhizome, lead was found deposited near the cell wall. Likewise, Malone *et al*[10] used the electron microscope to study localization of lead accumulated by corn plants (*Zea mays* L.) and found lead deposited in the root system. In our experiment, the accumulated lead in roots caused damage in part of the cell wall by fusing it with the plasmalemma. However, in the rhizome and leaf, lead granules only accumulated near the cell wall and in the chloroplasts. This study has shown that *T. angustifolia* accumulated lead from the soil.

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