Ultrastructural changes in leaf and root cells of *Lespedeza chinensis* and *Lespedeza davidii* after lead exposure

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Lead and lead compounds have been identified as a major hazardous chemical at 47% of the 1219 superfund sites on the USEPA's (U. S. Environmental Protection Agency) priorities list. Due to various anthropogenic activities, the concentration of lead in agricultural soil increased rapidly in many areas around the world. Consequently, as a low-cost, *in situ* 'green' technology for the progressive clean-up and ecological restoration of metal-polluted soils, phytoremediation has been exploited as a biological clean-up technique. Based on a field survey in a Pb/Zn mine, *Lespedeza chinensis* which has high biomass and grows fast, can accumulate large quantities of lead in their aerial parts and roots. At a subcellular level, lead has been found in general in cell walls, intercellular spaces and vacuoles. Small deposits were also found in dictyosomes, endoplasmic reticulum and dictyosome derived vesicles [1]. The knowledge of the subcellular localization and identification of lead can provide essential information on bioaccumulation mechanisms, detoxification and thus tolerance [2].

For the present study, we chose *Lespedeza chinensis* and *Lespedeza davidii* for comparison of their lead accumulation and tolerance mechanisms, as the latter is from the same genus but has higher biomass. The research into uptake and accumulation of lead by *Lespedeza chinensis* and *Lespedeza davidii* was carried out under gradient lead concentrations and Pb-EDTA exposure for 2 months. The concentration of Pb, K, Ca, Mg, Cu, Zn, Mn and Fe in leaves, stems and roots were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). The results showed that root has an ability to take up significant quantities of lead whilst restricting translocation to the aerial parts in both species. In the presence of EDTA in soil, lead concentration in leaf can be increased 20-40 times. For investigation of ultrastructural changes under lead exposure and subsequent subcellular localization of lead in leaf and root cells, *Lespedeza chinensis* and *Lespedeza davidii* seedlings were grown 2 months under $1mM.L^{-1}$ $Pb(NO_3)_2$ by hydroponic culture. Tissues of untreated controls and lead treated plants were fixed by high pressure freezing, cryosubstituted in 1% osmium tetroxide and 0.05% uranyl acetate dehydrate and embedded in Agar low viscosity resin. Ultrathin sections were collected on Formvar coated copper grids and viewed in a LEO 912 transmission electron microscope (ZEISS, Oberkochen) equipped with an in-column energy filter.

In root cell of *Lespedeza davidii* after 2 months lead treatment, malformed cells were observed and cell walls could be thickened unevenly and contain particularly distended areas which contain punctuate, crystalline and acicular electron-dense deposits (Fig 1, A-C). Additionally intracellular deposits were also distributed along the plasma membrane. In leaf cell of *Lespedeza davidii* after 2 months lead treatment, distended lipid granules and increased starch accumulation were found in the chloroplasts (Fig 1, D-F). The electron-dense deposits observed in cell walls, plasma membrane, chloroplasts, etc. are likely to be

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lead. Therefore, in the future work, electron energy loss spectroscopy (EELS) [3] will be used for subcellular localization of lead in cells, to provide important information for further understanding the mechanisms of lead tolerance and detoxification in *Lespedeza chinensis* and *Lespedeza davidii*.

1. Sharma, P., Dubey, RS. Lead toxicity in plants. Braz. J. Plant Physiol. 17(1) (2005) 35-52.
2. Hall, JL. Cellular mechanism for heavy metal detoxification and tolerance. J. Exp. Bot. 53(2002) 1-11.
3. Lütz-Meindl, U. Use of energy filtering transmission electron microscopy for image generation and element analysis in plant organisms. Micron 38(2007) 181-196.
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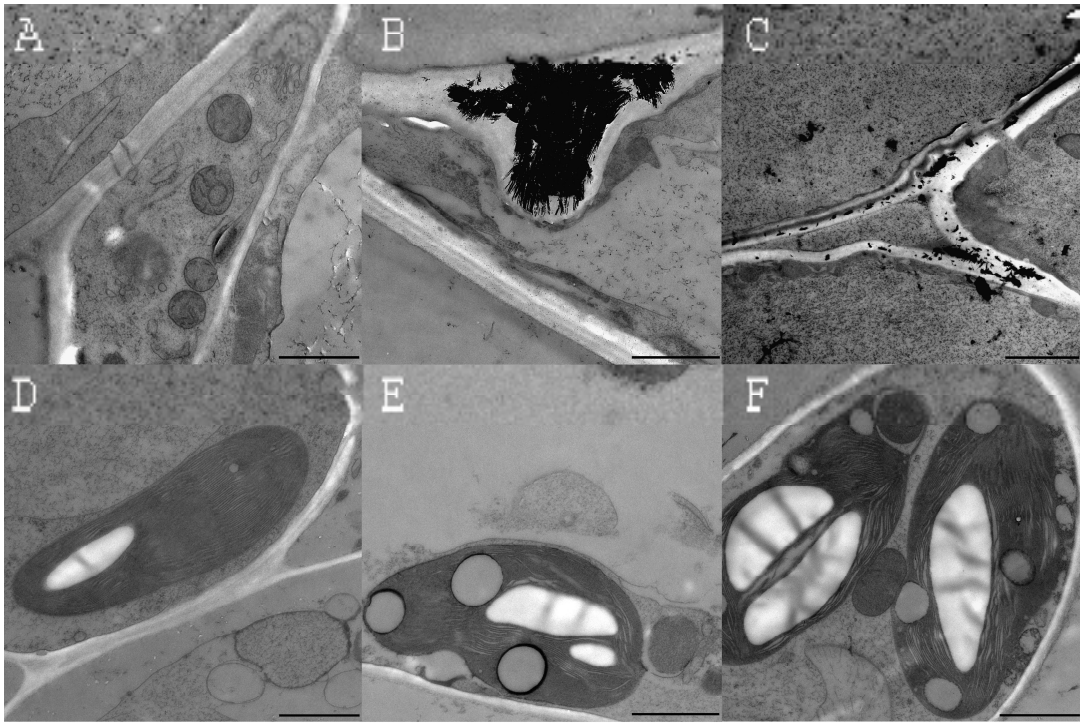

Figure 1. Tramsmission electron micrographs of root and leaf cells of *Lespedeza davidii* after Pb exposure. (A) Control of root cell in *Lespedeza davidii*, (B-C) Electron dense deposits in cell walls of root cells in *Lespedeza davidii* after 2 months Pb exposure, (D) Chloroplast of leaf cells in *Lespedeza davidii*, (E-F) Chloroplast in leaf cell of *Lespedeza davidii* after 2 months Pb exposure. A,B,D,E,F, Bar=1µm; C, Bar=2µm.

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