DOCTORAL THESIS

Earthworms and mycorrhizae in phytoremediation of Pb/Zn mine tailings: their effects on metal speciation, bioavailability and uptake by Leucaena leucocephala

Ma, Ying

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Earthworms and Mycorrhizae in Phytoremediation of Pb/Zn Mine Tailings: Their Effects on Metal Speciation, Bioavailability and Uptake by *Leucaena leucocephala*

MA Ying

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ABSTRACT

The present study aims to investigate the influence of earthworms and arbuscular mycorrhizae on lead and zinc speciation, bioavailability and uptake by *Leucaena leucocephala*, for phytoremediation of Pb/Zn mine tailings.

Pb/Zn mine tailings were found to be toxic to the earthworm *Pheretima*, unless the tailings were diluted by at least 20% with an artificial uncontaminated soil. LC_{50} values showed *Pheretma guillelmi* was the more tolerant species than *Pheretima sp.* Soil pH, EC and DTPA-Pb and -Zn significantly increased following inoculation with the earthworms, and the latter two parameters were increased by up to 48.2% and 24.8% respectively.

Single and sequential chemical extraction procedures were conducted to study Pb and Zn speciation and bioavailability as affected by earthworms in a plant-soil system. It was demonstrated that earthworms significantly increased Zn bioavailability due to decreased soil pH, increased DOC, and earthworm gut-related processes. However, Pb bioavailability in soils was not increased, which was probably due to the formation of insoluble complexes with phosphates to decrease reducible-Pb in soil.

The effects of earthworms on phytoextraction of Pb/Zn mine tailings were investigated in pot experiments using Indian mustard (*Brassica juncea*). It was found that earthworms increased metal bioavailability, largely through much higher extractable metals in earthworm casts. The results indicated that accumulator plants, in combination with earthworm inoculation, may be suitable for remediating low-level metal contamination. In mine tailings, however, excluder plants or plants that retain metals in roots are likely to be more useful for ecological restoration.

The effects of earthworms on the growth of *Leucaena leucocephala* on Pb/Zn mine tailings diluted with varying amounts of mineral soil were studied. *L. leucocephala* grew successfully on tailings with a 25% (w/w) soil amendment. The presence of earthworms improved the yield of plants by 10-30%. *P. guillelmi* increased bioavailable metal concentrations in the amended tailings, accompanied by a direct increase of metal uptake by the plants, and the increased rate of metal uptake into plants from tailings ranged from 16% to 53%.

Inoculation of arbuscular mycorrhizal fungi (AMF) was found to significantly improve growth and establishment of *L. leucocephala* on Pb/Zn mine tailings. Other soil microorganisms also facilitated plant growth, although the effect was less obvious. Plant root mycorrhizal infection was positively related to soil phosphatase activity and dehydrogenase activity. Higher root mycorrhizal infection increased Pb and Zn uptake by plants under lower metal concentrations, while it decreased Pb and Zn uptake under higher metal concentrations.

A synergistic effect between earthworms and arbuscular mycorrhizae on plant

growth was observed in higher metal concentrations. Earthworms considerably improved root mycorrhizal infection and somewhat stimulated plant root nodulation, while arbuscular mycorrhizae exerted positive effects on plant nodulation and nodule efficiency. It was demonstrated that earthworms stimulated plant growth mainly through enhancing soil microbial activity, improving soil nutrient availability, and increasing plant N, P uptake. Arbuscular mycorrhizae improved plant growth through stimulating root nodule efficiency and increasing plant N, P and K uptake. Uptake of Pb and Zn by *L. leucocephala* grown in Pb/Zn mine tailings was significantly enhanced by earthworms, most probably due to the increased plant dry weight production. However, AMF inoculation did not increase metal uptake. Arbuscular mycrrhizae were proved to protect plants from metal stress through restricting metal uptake to shoots and decreasing metal bioavailability in soil.

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