

DOCTORAL THESIS

Enhanced phytoextraction of metal contaminated soils using beneficial microorganisms

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**Enhanced Phytoextraction of Metal Contaminated
Soils Using Beneficial Microorganisms**

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**A thesis submitted in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy**

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ABSTRACT

The present study aims to investigate the effects of inoculation of beneficial soil microorganisms, such as plant growth promoting rhizobacteria (PGPRs) and arbuscular mycorrhizal fungi (AMF), on enhancing phytoextraction of higher plants on heavy metal contaminated sites.

The inocula of both PGPRs and AMF were successfully established in clean soil and metal contaminated soil, although the elevated metal concentrations in soil exerted toxic effects on the introduced microorganisms. Survival and growth of soil bacteria declined according to the increase of metal concentrations. The order of metal toxicity to N-fixing bacterium (*Azotobacter chroococcum*) was: Cd > Cu > Zn > Pb.

It was found that the mechanisms of bacterial tolerance to metal toxicity might involve either extracellular binding or intracellular metal sequestration by metal-induced heat stable proteins (HSPs). The capacity of bacterial sorption of metal ions from solution could be described well by the linearized Freundlich and Langmuir adsorption isotherm. *A. chroococcum* (Gram negative) tended to sorb more metal ions than did *Bacillus megaterium* (Gram positive). An extra synthesis of heat stable proteins (HSPs) was induced in the bacterial cells of both Gram positive and Gram negative strains when exposed to metal ions. The concentration of HSPs in the cells usually increased with the increase of metal concentrations.

It was demonstrated that the inoculation of bacteria or/and mycorrhizae could alter metal speciation and bioavailability in soils through a soil column experiment and greenhouse study. Bacterial inoculation significantly increased fractions of DTPA-extractable, HOAc-soluble and water-soluble metals (Zn, Cu and Cd). This can be attributed to the decrease in soil pH due to the secretion of proton, amino acids and

organic acids through metabolic activities of bacteria. However, the bioavailability of Pb was markedly decreased due to microbial inoculation, since Pb ions can be easily bound on surface of bacterial cells and mycorrhizal hyphae. In addition, the inoculation of P-solubilizing bacteria and AMF resulted in an increase of available phosphate in soil solution, which can form precipitation of Pb-phosphate therefore reduced Pb bioavailability.

The inoculation of beneficial microorganisms significantly stimulated the growth of host plants, because these microbes were able to i) increase nutrient availability and soil fertility in the rhizosphere; ii) produce metabolites and other biologically active substances, which are beneficial to plant growth; and iii) exert a protection effect on the host plants through alleviating the metal toxicity. Although inoculation of PGPRs and/or mycorrhizal fungi in most cases led to a decrease of metal concentrations in the shoot tissue, the total amount of heavy metals removed by plants were still significantly increased due to a higher harvestable biomass compared to the control. In addition, it was observed that an intercropping system with the high-yielding mycorrhizal plants grown alongside accumulator/hyperaccumulators (non-mycorrhizal) could be a new alternative for improving the efficiency of phytoremediation. Based on the results of this study, beneficial microorganisms such as PGPRs and AMF may play a significant role in phytoremediation of metal contaminated soil.

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