SUMMARY

Dissertation title: Bioremediation for heavy metal contaminated brownfields

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Summary

Metals are natural constituents of the Earth's crust, some of them being essential elements for life. Human activity has altered the distribution and speciation of these metals, generating ecological imbalances by increasing toxicities in ecosystems prone to anthropogenic sources. The development of eco-friendly technologies for soil decontamination is of considerable importance in the achievement of environmental pollution control. Specifically, brownfields contaminated with heavy metals are causing concern, because the biogeochemical behavior of metals is influenced by the soil characteristics of the polluted sites. Heavy metals are considered persistent hazardous pollutants of environmental concern, the toxicity of which depends on the chemical speciation of the metal. For the prediction of ecotoxicological effects that might be exhibited by the presence of heavy metals is necessary to determine the bioavailability of the respective metals in the brownfield subjected to investigations.

With regard to this issue, a field site located in the North-Western part of Romania, close to a non-ferrous metallurgical plant, was chosen as study area. Soil samples were collected and a chemical assessment was conducted for the evaluation of environmental risks associated to metal contents in soil. The investigation revealed elevated metal concentrations which often exceeded the alert and intervention thresholds, and indicated concerning metal contents in the bioavailable fractions. In addition, the soil parameters were severely affected by the metal contamination, suggesting disturbances in soil function and quality. Since natural recovery requires extended periods of time and conventional methods are extremely expensive, the objective of the present study was to create a bioremediation system to promote development of a self-sustaining ecosystem under conditions present at the investigated site.

Ectomycorrhizal (ECM) symbiosis of metal resistant organisms can represent a beneficial association for the improvement of soil quality in order to re-establish vegetation in brownfields. The soil limitations in water and nutrient supply can be

overcome by ECM fungi which are able to colonize the roots of the trees even in harsh environments, while the host tree provides the required carbohydrates for the fungal growth. To anticipate the chances of such organisms to develop in the soil conditions of the investigated site, a biological experiment was designed. Pine (*Pinus sylvestris*) growth in the presence of the ECM fungi *Paxillus involutus* and *Pisolithus tinctorius* was tested in regard to exposure at different heavy metal concentrations. The organisms were chosen based on their reported ability to develop in heavy metal contaminated sites, and because they are natural ECM symbiosis partners. The main purpose of the experiment was to investigate the possible defense strategies involved in ECM metal tolerance. Different developmental features were observed in relation to differing metal concentrations, and the physiological modifications were linked to induced metal stress.

Bioremediation is an eco-friendly approach for decontamination of metal polluted environments. Initially, the decontamination strategies were focused on the removal of heavy metals from the soil using hyperaccumulator plant species, which are able to take up high contents of metals through phytoextraction abilities. Due to issues raised with biomass storage of hyperaccumulators, altering the chemical form of the metals to less mobile chemical forms is presently investigated as a better alternative. The goal of this remediation strategy is to decrease the metal toxicity in the soil by reducing the mobility and bioavailability of the heavy metals. Phytostabilization through metal resistant plant species can achieve these demands by restricting the metal movement in the rhizosphere and limiting the metal translocation to shoots.

Mycorrhized pine seedlings have been investigated by assessing lead uptake and distribution within plant tissues. Electron microscopy revealed metal accumulation on the surface of the colonized root, and a possible immobilization strategy by adsorption onto root surface, followed by metal precipitation. Metal accumulations were present also inside the root and inside the needle crown of the seedlings. The reduced number and sizes of metal deposits found in the needle compared to the ones found in the root was related to phytostabilization mechanisms, which have restricted the metal transport from root to shoot. An elemental microanalysis was performed to gain information on the composition of metal accumulations, and the results indicated a lead biomineralization as metal defense mechanism.

The biomineralization potential was discussed in terms or bioreduction, biosorption and bioaccumulation processes that might have been involved by the investigated organisms. The formation of biominerals was investigated by electron diffraction, which revealed pyromorphite minerals present within plant tissue. The structures were extracellularly embedded in the plant cell wall with indications for mineralization during cell wall growth. This study is the first evidence on biogenic chloropyromorphite mineralization as a defense mechanisms of pine seedlings exposed to lead. The identification of different phases of the minerals between root and needles of the same seedling were discussed with regard to tolerance and detoxification mechanisms. A hypothetical pathway was created in order to describe the transport of lead ions along the path from soil through water, mycorrhiza and plant biomass. This includes inter- and intracellular defense mechanisms towards lead. Since the bioremediation system proved its efficiency in the designed conditions, the ecological implications of ECM associations have been highlighted for afforestation strategies of metal polluted sites.