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Inhibition of Selected Heavy Metals Translocation Through Mycorrhizal Fungi and Process Dependance on the Fungal Symbiont

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Abstract

The translocation of zinc, cadmium and lead ions in vegetative mycelia of four species of mycorrhizal fungi was studied. Strong accumulation of zinc, cadmium and lead in aerial mycelium of fungi growing in contaminated medium, and inhibition of the metals' translocation was found. These results were related to species of mycorrhizal fungus and the metal ions that were used for the contaminated medium.

Keywords: ectomycorrhiza, mycorrhizal fungi, heavy metals, inoculation, tolerance

Introduction

Toxicity of heavy metals for plants does not result exlusively from environmental pollution levels but is also dependant on many other factors such as their chemical form in subsoil composition and structure as well as the presence of other ions [2]. It also depends on those factors which may affect mineral transport from soil into plants. Among these, biotic factors need to be emphasized. These include bacteria and fungi,which on one hand mobilize the ions of some elements such as iron (siderophor) and phosphorus, and on the other limit translocation of zinc, cadmium or lead from soil into plants. Other literature [1,5,8,10,11] points to the fact that myccorhizal can play an important role in reducing soil contamination by heavy metals. One question that arises is does heavy metal translocation limit dependence of fungal symbiont on fungus species? The objective of the experiments was to determine the following: do the limits of translocation of the heavy metals zinc, cadmium and lead depend upon fungus species forming ectomycorrhizal symbiosis?

Materials and Methods

Material was obtained from vitro cultivated mycelium. The experiments were carried out on 4 fungi species out of which two: *Amanita muscaria* (L.) and *Amanita citrina* (Schaeff.) revealed the ability to form mycorrhizal in areas polluted by heavy metals producing industry. The two remainings are *Hebeloma crustuliniforme* (Bull), which was obtained from prof. Roman Pachlewski's colection; and *Xerocomus badius* (Fr.), isolated from fruit bodies in polluted stands in Kokotek by Lubliniec. The tissue method [13] employed for collection of fruit bodies in the period September-October 1995 allowed us to obtain pure cultures of mycelium extramatrical subjected to further experiments. In order to determine differences in metal translocation inside mycelium hyphae substrate "PP" (according to Pachlewski [13]) was prepared on Petri dishes separated by a dividing wall. The agar on one side of the dish was placed in addition to Zn, Cd and Pb, and on the other with no addition of metals. Subsequently, the mycelium extramatrical was sown transversely into the dividing wall according to diagram

(Fig. 1), trying to maintain a smooth continous spread. Then the dishes were incubated in darkness for 14 days in constant temperature of 240°C optimal for myccorhizal fungi. After that time mycelium was removed seperately from each side of the cell (removed without agar) and was scanned for metals content. The mycelium cultivated on the dish free from metals was cut in 1 cm slices and scanned for heavy metals concentration. As a means of control, sterile stripes of tissue paper dipped in distilled water were used. The stripes were placed on dishes transversely to the dividing wall (similarly as in the case of metals translocation inside mycelium, Fig. 1).

Two weeks after incubation the tissue paper was cut in 1 cm sectors and analyzed for heavy metals content.

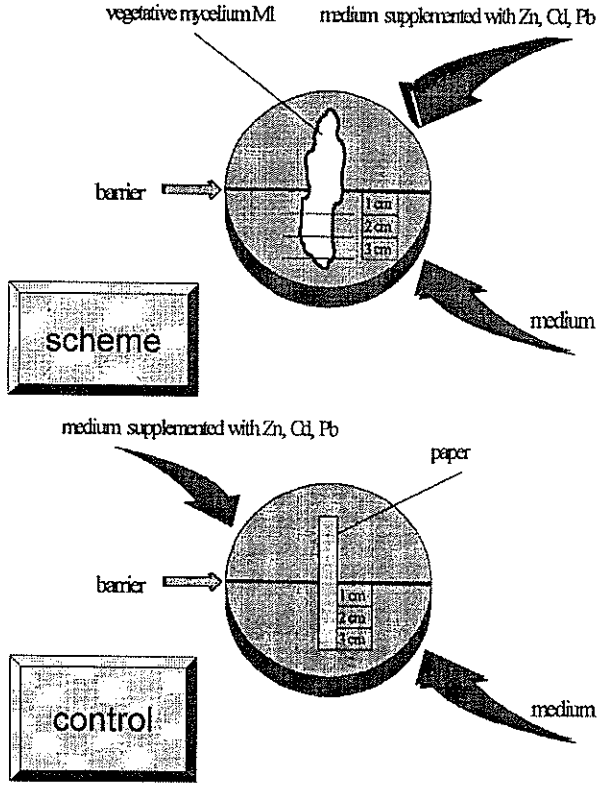


Fig. 1. Experimental schemes of metal translocation throughout mycelium and control tissue-paper.

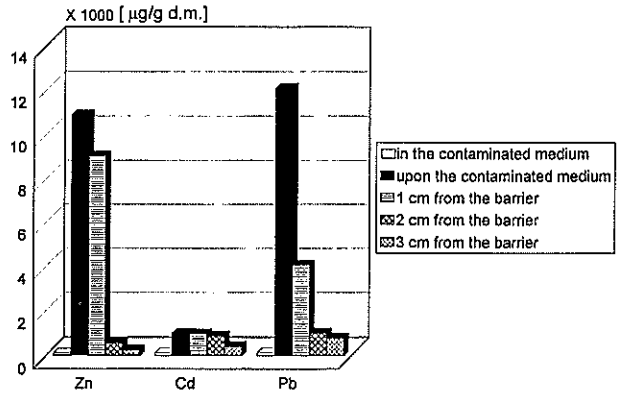


Fig. 2. Decrease of zinc, cadmium and lead concentrations in the mycelium of *Amanita citrina* with distance from the barrier.

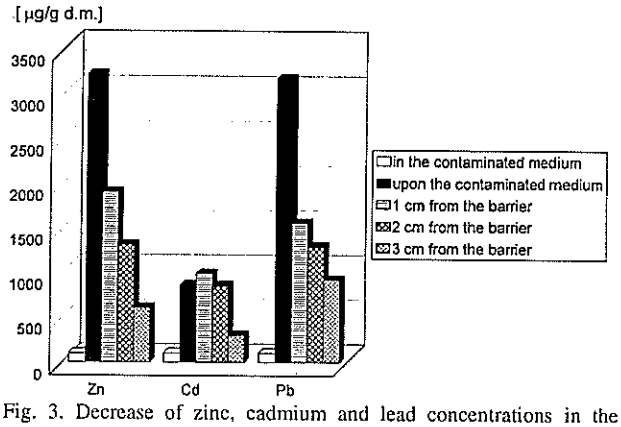


Fig. 3. Decrease of zinc, cadmium and lead concentrations in the mycelium of *Amanita muscaria* with distance from the barrier.

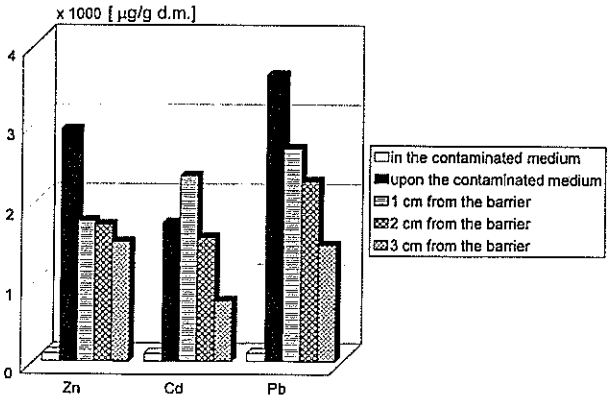


Fig. 4. Decrease of zinc, cadmium and lead concentrations in the mycelium of *Hebeloma crustuliniforme* with distance from the barrier.

Results

The results are illustrated in Figures 2-6. Strong accumulation of heavy metals in mycelium collected from contamined agar was recorded. In spite of the same dose of metal for contaminating all substrates, metal accumulation content depended on fungus species and type of contaminating metal. Lead accumulation in mycelium cultivated on contaminated agar was about 3000 (g Pb/g of dry mass) *X.badius*, *H. crustuliniforme* and *A. muscaria* and as much as 12000 (g Pb/g *A.citrina.* Mycelium *H.crustuliniforme* collected for cadmium contaminated agar accumulated twice as much of this metal in comparison to other surveyed species. And quite different was the reaction of strains on zinc accumulation. *A. muscaria* and *H.crustuliniforme* accumulated about 3000 (g Zn/g of dry mass), while *X.badius* and *A.citrina* had 10,000 (g Zn/g). All the strains showed a significant drop in accumulated metal ions the farther from the dividing wall. Immobilization of heavy metals uptake was the largest in *A.citrina*, while zinc content from about 1000 (g dropped to 250 g) at the distance of 3 cm away from the dividing wall. *H.crustuliniforme* had the most difficulty accumulating the heavy metal ions where inhibition at the distance 3

cm away from the dividing wall depended on the metal 2-3 times. Restriction of heavy metals transfer was not observed in Fig. 6. In tissue paper of agar contamined ions Zn and Cd metals accumulation was not observed. Restriction of metal ions transfer with the distance from the dividing wall was not observed. Only in the case of lead was some drop recorded. Restriction of translocation in this case is not so evident as in mycelium and can be caused by the low lability of that ion.

Discussion

Ectomycorrhizas may restrict translocation of heavy metals from soil to plant on three levels:

- Through mantel mycelium of the mycorrhizas, e.g. citric acid, malonic acid or specific proteins metallothioneins [7].
- Through ions binding in hyphae in the so-called interface matrix [3].
- The metals can be metabolically removed by fungus or transformed to non-toxic form inside the hyphaes [6,8,12].

Bücking and Heyser [1] studied the zinc infection of the roots of *Pinus silvestris* found the dependence between

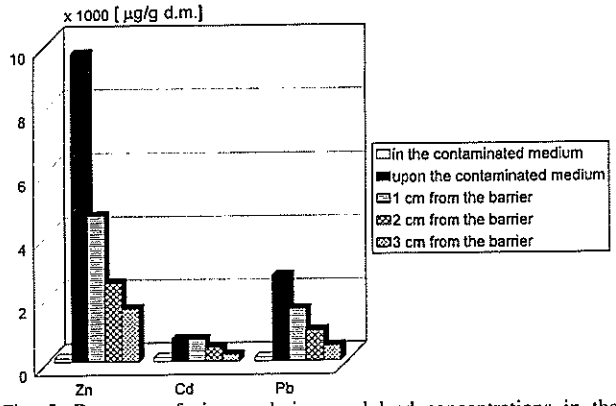


Fig. 5. Decrease of zinc, cadmium and lead concentrations in the mycelium of *Xerocomus badius* with distance from the barrier.

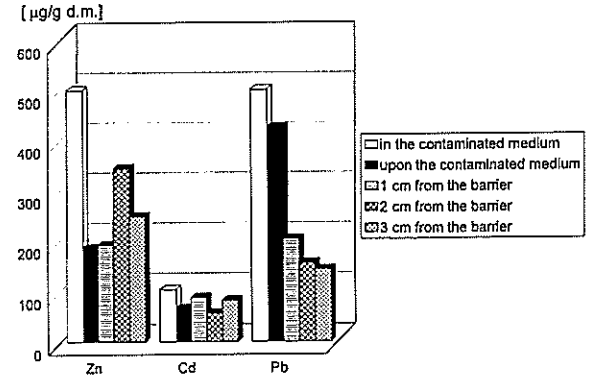


Fig. 6. Decrease of zinc, cadmium and lead concentrations in the control tissue-paper.

zinc accumulation in roots of mycorrhizal fungus. Assuming that fungus accumulation in mycorrhizas retards its transfer according to the authors, this observation is in agreement with the finding of this study.

Fungus species can also have an influence on a given metal accumulation on the surface of mycorhizas, but it seems also to be connected with host plant species. Brown and Wilkins [4] studying elevated tolerance on high zinc concentrations confirmed its accumulation outside hyphal mantle while its content in mycorrhizosphere remained at a low level. Similarly Kahle [9] confirms zinc, lead, cadmium accumulation on the outer parts of tree roots of *Betula* and *Salix* however shows another mechanism of trees detoxication from *Acer, Fagus, Picea*. Heavy metal ions are immobilized in these species in mycelium itself due to binding proteins. The study aimed to differentiate the capability to immobilize translocation and accumulation of heavy metals by myccorhizas fungi. The differences are most probably caused by synthesis of different binding proteins. The degree of heavy metals accumulation in mycelium extramatrical of mycorrhizal symbionts depended on fungus species and the type of metal ions contaminating the substrate. Mycelium extramatrical was assesed in this study and two stages of heavy metals immobilization were noted (immobilization in interface matrix and inside mycelium structure). The retention capacity of the mycelium of the determined fungi can help to select such fungus species for artificial inoculation of seedlings which are most adaptable to forestation in areas degraded by heavy metals.

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