Polish Journal of Environmental Studies Vol. 12, No. 6 (2003), 723-726

Positive Aspects of Interaction Between Plants and Mycorrhizal Fungi Originating from Soils Polluted with Cadmium

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Received: 16 January, 2003
Accepted: 7 April, 2003

Abstract

A laboratory study was carried out to evaluate the protective role of ectomycorrhizal fungi against contamination of plants growing in soil treated with cadmium at a dose of 150 μg Cd/ g soil. An alginate-immobilized inoculum of mycorrhizal fungi was used to introduce the fungi to the soil. The impact of fungi was examined in terms of changes in cadmium levels in inoculated and non-inoculated seedlings of *Pinus sylvestris* L. It was found that the concentration of cadmium in plants inoculated with fungi was significantly lower than in non-inoculated seedlings. We also observed that the total concentration of cadmium in contaminated soil inoculated with fungi was lower than in non-inoculated soil.

Keywords: mycorrhizal fungi, symbiosis, cadmium

Introduction

Contamination of the natural environment by industry causes many adverse changes in ecosystems. Emitted with dusts, large amounts of heavy metals such as lead, cadmium and zinc are accumulated and high concentrations of these metals disturb biological processes in both soil and living organisms. One of the most dangerous elements is cadmium. It has no essential biological function, and even at low concentrations it is highly toxic to all living organisms. Cadmium pollution of the environment has been rapidly increasing in recent decades as a result of rising consumption of Cd by industry. Sources of contamination by Cd are the mining and smelting of Pb and Zn, atmospheric pollution from metallurgical industries, the disposal of wastes containing Cd, sewage sludge application to land and the burning of fossil fuels [1].

Considerable attention has been focused on the potential role of mycorrhizal fungi in protection of plants against heavy metal toxicity. A beneficial role of mycorrhizal fungi in delivering water and mineral salts to plants has been widely reported. It has been shown that plants with well-developed mycorrhiza grow better than plants lacking mycorrhiza, especially under difficult conditions, such as in saline soil and in soils which are nutrient deficient or polluted by industrial wastes.

Accumulation of heavy metals in plants depends on many factors, including the transport of metals from the soil to the plant. Among these factors bacteria and fungi have received special attention. Mycorrhizal fungi directly link soil and roots and can be of great importance in heavy metal availability and toxicity to plants.

Currently, numerous studies are being conducted on the isolation of mycorrhizal strains, especially their ecotypes, that are the most effective in counteracting the toxicity of heavy metals in soils. In most studies showing the beneficial role of ectomycorrhizal fungi the protective mechanisms involve the prevention of translocation of heavy metals into the host [2,3].

The tolerance of ectomycorrhizal fungi to heavy metals varies. In growth studies on agar plates and in liquid culture *Laccaria laccata* (Scop.:Fr) proved sensitive at 10

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ppm to Cu and Al. In contrast, *Thelephora terrestris* was able to grow in the presence 500 ppm Cu and 1000 ppm Zn [4]. Natural resistance of microorganisms to heavy metals has implications for the selection of appropriate ectomycorrhizal fungi for use of protection of plants in heavy metal contaminated sites. However, neither selection based on *in vitro* growth trials nor selection of presumably adapted fungi will guarantee success.

The aim of this work was to find out whether, and to what extent, mycorrhizal fungi from soils contaminated with different amounts of heavy metals can affect translocation of cadmium from the soil to seedlings of *Pinus sylvestris* L. under controlled conditions.

Materials and Methods

The experiments were carried out under laboratory conditions in soil with the following characteristics: 80% sand, 12% clay, 8% silt, organic C 12.6%, 8.7 me/100g cation exchange capacity (CEC), pH 6.5.

Materials: Four species of ectomycorrhizal fungi *Laccaria laccata* (Scop.:Fr), *Rhizopogon luteolus* (Corda) Th.M, *Xerocomus badius* (Fr.) Kuhn, and *Suillus luteus* (Fr.) S.F.Gray orginally isolated from soil around steelworks in Miasteczko Śląskie were used for the experiments. Fungi were collected in September 2001. Pure cultures were obtained from fruiting bodies according to Pachlewski's [5] method. Inocula were produced by submerged aerobic culture in medium according to Kuek [6]. For production of inocula, colonies were first established on agar plates. From 3 plates colonies were removed and placed in 250 ml Erlenmayer flasks containing 50 ml of medium for two weeks. Inocula were prepared by immobilization of mycelium in 4% sodium alginate. Broth culture of mycelium was mixed with alginate soluble in hot water and cooled to 30°C. Then the solution containing mycelium and alginate was dropped into a sterile solution of 0.2 M $CaCl_2$. The beads formed were washed using sterile distilled water. Finally, beads of inocula were thoroughly mixed with soil contaminated with Cd at a dose of 150 µg Cd/g soil. As a control soil inoculated only with alginate beads was used. After three weeks of incubation the content of water-soluble cadmium was measured in the samples. For this purpose 5 g of soil was suspended in 100 ml of distilled water and shaken one hour. Then soil suspensions were filtrated through filter paper and the concentration of cadmium in the filtrate was determined.

To show the accumulation of cadmium by different fungi the total amount of cadmium in soil samples was also measured. For this 1g of soil (inocula were gently removed) was mineralized in conc. HNO_3 and the amount of cadmium in suspension soil was calculated.

The efficiency of the chosen fungal strains in protection of plants against contamination by cadmium was tested under laboratory conditions using seedlings of *Pinus sylvestris*. The experiments were conducted from February to May 2002. Twenty 1-month-old seedlings growing in soil were inoculated with each strain of mycorrhizal fungi by pouring 100 ml of appropriate fungi inoculum to the sterile soil. Seedlings were treated twice a week with water containing 20 µg Cd/ml. Plants were kept in a plant growth cabinet at 23±2°C, 14h of light, light source: high frequency fluorescent lamps "Flora", light intensities: 150 micromoles/m^2/s (13,000 lux), 70% relative humidity. After 3 weeks, when the ectomycorrhizal association had not yet formed, and after 3 months when the ectomyccorhizal association was observed, the amount of cadmium in the plants was tested. Seedlings were removed from the soil and separated into the above- and belowground parts. Then the content of cadmium in both parts of plants was measured. To determine cadmium in the plants the samples were dried to constant weight and mineralized in conc. HNO_3 in a microwave oven (MLS-1200 MEGA, Milestone). Cadmium concentration was analyzed by anodic stripping voltametry using a microtrace analyzer AMAK (Enter,

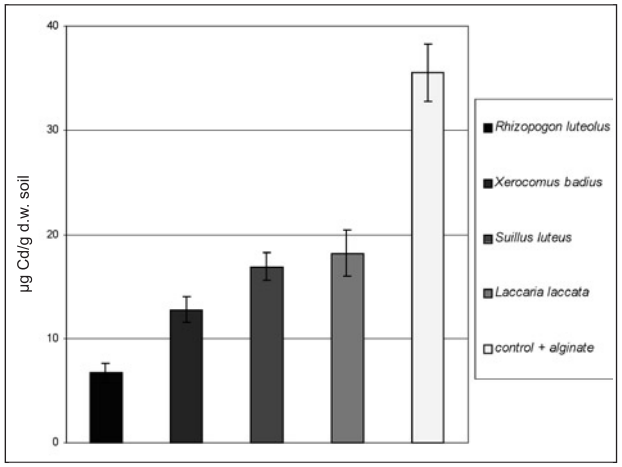

Fig.1 Content of water-soluable cadmium in soil after 3 weeks incubation with mycorrhizal fungi.

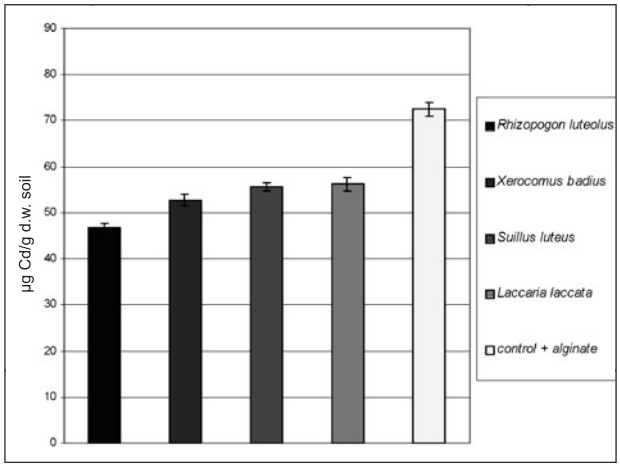

Fig.2 Total content of cadmium in soil after incubation period.

Poland). Mineralization and determination of cadmium concentration were performed according to the producer's instruction [7,8]

Statistical analysis of data was performed using the STATISTICA v.5.0 software program. Comparisons between the sampling dates were done using Tukey's HSD Post Hoc test. Results are expressed as the means of three separate experiments with triplicates in each experiment.

Results

Figure 1 shows the water-soluble amount of cadmium in soil contaminated with cadmium at a dose of 150 µg Cd/g d.w. soil. In soil with alginate inoculum (without ectomycorrhizal fungi) a decrease of water-soluble content of cadmium to 35 µg Cd/g d.w. of soil was observed. Significant differences of Cd content were observed in soils treated with ectomycorrhizal inoculum. In the soil inoculated with *Rhizopogon luteolus* the Cd content was only 6.7 µg/Cd g d. w. of soil. In the case of *Xerocomus badius* concentration of cadmium was higher at 12.8 µg/g d.w of soil. Least effective was the inoculum prepared from *Laccaria laccata*, but still the Cd content was only half of that in the control soil (Fig. 1).

Figure 2 shows the total amount of cadmium in soil samples. Significant differences ($p>0.005$) in samples with different fungi have been observed. In the control soil the total amount of Cd was 72.5 µg/g d.w. of soil whereas in soil inoculated with *Laccaria laccata* it was 56.2 µg/Cd g d. w. of soil and in the case of *Rhizopogon luteolus* 46.7 µg/Cd g d. w. of soil.

We observed the protective role of the fungi used. The content of cadmium in aboveground parts of 3 weeks old seedlings growing in soil inoculated with fungi without ectomycorrhizal association was significantly lower than in the control plants (Fig. 3). In Fig. 4 the localization of cadmium in 3-month-old seedlings of *Pinus sylvestris* is shown. In the control plants there was no significant difference ($p<0.005$) between the concentration of cadmium in above- and belowground parts. The amount of cadmium in belowground parts was 107 µg Cd/ g dry mass of plant and in aboveground parts 95.7 µg Cd/ g dry mass of plant. In plants growing in soil inoculated with fungi, just like after 3 weeks but still more clearly, the accumulation of cadmium in aboveground parts was significantly lower ($p<0.005$) than in belowground parts. For example, the content of Cd in the roots of seedlings inoculated with *Rhizopogon luteolus* was 120 µg Cd/ g dry mass of plant whereas in aboveground parts it was 35.7 µg Cd/ g dry mass. In the case of *Xerocomus badius* the concentration of Cd was 52 µg Cd/ g dry mass in aboveground and 118 µg Cd/ g dry mass in belowground parts, respectively (Fig. 4).

Discussion

We observed that in control soil and in soil inoculated only with alginate beads the water-soluble content of Cd was significantly lower than the total amount of Cd added (Fig. 1). It seems that this decrease of Cd concentration was caused by the complexation of cadmium with soil organic matter.

The concentration of cadmium in aboveground parts of *Pinus sylvestris* mycorrhized with fungi was significantly lower than in non-mycorrhized seedlings. Fungi have protected shoot tissue against cadmium accumulation. The protective role of fungi was observed especially when the association seedling-fungi was formed. A beneficial role of ectomycorrhizal fungi has been reported earlier by many investigators [9-11]. The mechanism suggested for the protective effect of the fungus is the prevention of translocation of heavy metals into the host. Galli et al. [2] have shown that in *Picea abies* mycorrhized by *Laccaria laccata* most Cd was bound in the cell walls of the fungus. Colpaert and Van Assche [12], studying metal uptake and accumulation in the mycelium in axenic culture contaminated with heavy metals, found that the fungus/soil

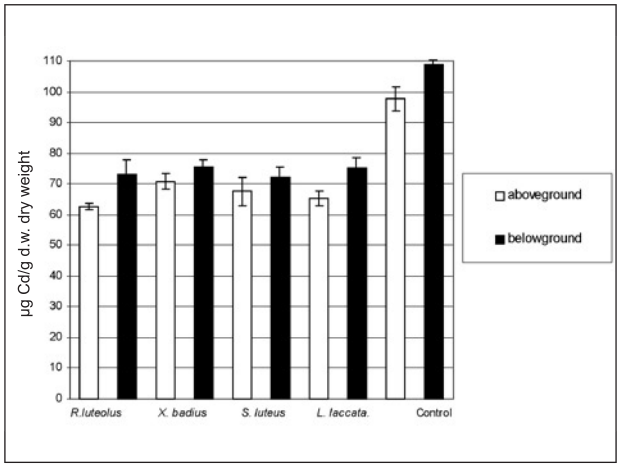

Fig.3 Content and localization of cadmium in 3-week-old seedlings inoculated with fungi.

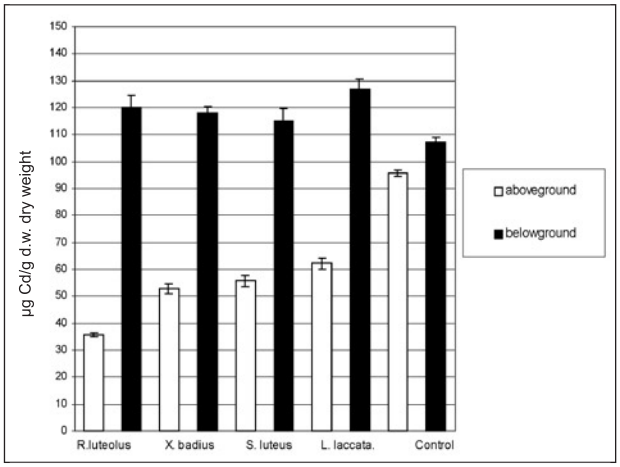

Fig.4 Content and localization of cadmium in 3-month-old seedlings inoculated with fungi.

concentration ratios were around 200 and 80 for Cd, 40 and 30 for Zn of non-tolerant and metal-tolerant isolates of *Suillus bovinus*, respectively. In *Rhizopogon roseolus* and *Pinus sylvestris* associations, Cd and Al accumulated in the fungal mantles and their concentrations decreased along the Hartig net towards the root interior [13-15].

Protection of plants against heavy metals is by the physical barrier or mantle [16] and may include metabolic processes such as intracellular metal accumulation and the extracellular precipitation of metals by metabolites in exudates [17]. Turnau et al. [18] reported that *Pisolithus tinctorius* bound Cd, Cu and Fe in the outer pigmented layer of the cell wall. Krupa et al. [19] observed that *Amanita muscaria*, *Amanita citrina* and *Hebeloma crustiliniforme* strongly accumulated zinc and lead and inhibited the translocation of these metals from soil to seedlings of *Pinus sylvestris*. Ectomycorrhizal fungi often bind heavy metals to cell wall components such as chitin, cellulose derivatives and melanin [10].Tam [20] observed that in *Pisolithus tinctorius* Cu and Zn were bound by extrahyphal slime and polyphosphate. Concentrations of heavy metals were usually found to be little altered in roots of mycorrhizal birch, pine and spruce but were high in extramatrical hyphae of the symbionts *Amanita*, *Paxillus*, *Pisolithus*, *Rhizopogon*, *Scleroderma* and *Suillus* spp. [21].

Heavy metals are also metabolically removed by fungi from soil and transformed into non-toxic forms inside hyphae [11,22]. Morselt [23], using histochemical methods, showed the occurrence of metallothioneins, thiol-containing metal binding proteins, in pure cultures of *Pisolithus tinctorius* treated with heavy metals.

On the basis of our results we confirmed an essential role of ectomycorrhizal fungi in protecting plants against contamination by cadmium. A key point for understanding the interactions between heavy metals and mycorrhizae is to take into account the functional diversity of mycorrhizal fungi and their ability to accumulate heavy metals. Fungi used in our experiments were isolated from highly contaminated sites, indicating that these fungi evolved a heavy metal-tolerance and they may play a role in the phytoremediation of polluted soils.

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