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Application of manure and compost to contaminated soils and its effect on zinc accumulation by *Solanum nigrum* inoculated with arbuscular mycorrhizal fungi

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The use of S. nigrum in combination with manure appeared as an effective method for the stabilisation of a metal contaminated soil.

Abstract

Zn accumulation in *Solanum nigrum* grown in naturally contaminated soil in the presence of different types of organic amendments was assessed. Under the same conditions, the response of the plant to inoculation with two different isolates of arbuscular mycorrhizal fungi (AMF) (*Glomus claroideum* and *Glomus intraradices*) was also evaluated. *S. nigrum* grown in the non-amended soil always presented higher Zn accumulation in the tissues, with the addition of amendments inducing reductions of up to 80 and 40%, for manure and compost, respectively, and enhancing plant biomass yields. The establishment of *S. nigrum* in the Zn contaminated soil combined with the application of amendments led to a 70−80% reduction in the amount of Zn leached through the soil. The use of *S. nigrum* in combination with manure appeared as an effective method for reducing the effects of soil contamination, diminishing Zn transfer to other environmental compartments via percolation.

Keywords: Solanum nigrum; Arbuscular mycorrhizal fungi; Zinc; Phytoremediation; Organic amendments

1. Introduction

The remediation of metal contaminated sites often involves expensive and environmentally invasive, civil engineering based, practices. This may prohibit their use at many sites, where consequently no action is taken to adequately assess and, when necessary, reduce the risks to human health and the environment. Without some remediation effort, the soil from these contaminated areas remains exposed to human contact and to erosion that may carry contaminants off site.

Phytoremediation can be defined as the combined use of plants, soil amendments and agronomic practices to remove pollutants from the environment or to decrease their toxicity (Salt et al., 1998). Because the costs of growing a crop are minimal when compared to those of soil removal and replacement, the use of plants to remediate hazardous soils is seen as having great potential (Chaney et al., 1997). Plants are used to cover the soil surface in order to prevent erosion, reduce water percolation, and serve as a barrier to prevent direct contact with the soil and, in some cases, to remove the contamination via extraction of the metal from the soil and incorporation in the plant tissues (Berti and Cunningham, 2000). Addition of organic matter (OM) amendments, such as compost or manure, is an inexpensive and common practice to facilitate re-vegetation of contaminated soils. The effects of OM amendments on heavy metal bioavailability depend on the nature of the organic matter, and on the particular soil type and metals concerned (Clemente et al., 2005), usually involving the formation of insoluble contaminant species, less likely to leach through the soil profile (Berti and Cunningham, 2000). Organic

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amendments can decrease heavy metal bioavailability, shifting them from ‘‘plant available’’ forms to fractions associated with OM, carbonates or metal oxides (Walker et al., 2004), with consequent reductions in the metal uptake by the installed plants. For example, Walker et al. (2004) reported lower Zn tissue concentration in *Chenopodium album* L. plants grown in compost and manure amended soil and Clemente et al. (2005) found the same behaviour for *Brassica juncea* (L.) Czern. grown in OM amended contaminated soil. However, no reduction in the accumulation of Zn was demonstrated for *Agropyron elongatun* and *Trifolium repens* growing in Zn/Pb mine tailings amended with pig manure (Ye et al., 1999).

Some microorganisms can also facilitate the re-vegetation of polluted soils. Arbuscular mycorrhizal fungi (AMF) can enhance plant growth and improve plant reproduction (Smith and Read, 1997). Mycorrhizal plants are usually more competitive and more tolerant to environmental stresses than non-mycorrhizal ones (Sylvia and Williams, 1992). The contribution of AMF to plant adaptation in highly metal contaminated sites varies. Citterio et al. (2005) reported enhanced growth and metal root to stem translocation of *Cannabis sativa* plants inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae*, while Huang et al. (2002) reported an exclusion strategy, showing lower Zn accumulation in arbuscular mycorrhizal *Zea mays*.

Solanum nigrum L. (black nightshade) was found to be present in a large amount in a contaminated sediment, containing up to 1130 mg Zn kg^{-1} tissue (Marques et al., 2003). Previous laboratory studies showed that *S. nigrum* was able to accumulate up to 3800 mg Zn kg^{-1} dry tissue in the roots, when grown in sand spiked with 100 mg Zn kg^{-1} (Marques et al., 2006a), and up to 1622 mg kg^{-1} of Zn, when grown in naturally contaminated soil (with ca. 400 mg Zn kg^{-1}) (Marques et al., 2006b), with no visible toxicity signs. Furthermore, inoculation with the AMF *Glomus intraradices* and *Glomus claroideum* resulted in an increase of the Zn accumulation in the tissues of up to 122% (Marques et al., 2006b).

A greenhouse experiment was carried out to assess the influence of the addition of OM amendments on the growth and metal uptake and accumulation by *S. nigrum* in Zn contaminated soils, and to evaluate the effect of the application of different AMF on the bioavailability of Zn. The production of percolates and the corresponding Zn contents was also considered.

2. Materials and methods

2.1. Preparation of soil and amendments

The soil used in this study was collected in the banks of a stream located in a metal contaminated site in Northern Portugal – Esteiro de Estarreja. The characteristics of the site, for which Zn is a major contaminant, have been described by Marques et al. (2006b). Soil was collected randomly from the banks of the contaminated stream, to a 20 cm depth, in the dry season. The amendments used in this study were a compost resulting from the co-composting under controlled and reproducible conditions of biosolids – sludges from a domestic wastewater treatment plant – with crushed pine tree bark or sawdust, via aerobic stabilisation followed by maturation – Agronat [20% organic carbon, 35% humic compounds, with a granulometry of 1–1.5 mm (from Serviços Municipalizados da Maia, Maia, Portugal)] – and compressed cow and horse manure – Biorex [43% organic carbon, 40% humic compounds, with a granulometry of 3.5–3.8 mm (from Crimolara, Lisboa, Portugal)]. The soil and the amendments were grinded to <2 mm, sterilised at 120 °C for 70 min in 2 consecutive days and dried in an oven at 40 °C for 4days.

In order to obtain more information on the behaviour of *S. nigrum* when exposed to higher toxicity, soil with a second level of Zn contamination was prepared by adding a solution of $ZnSO_4$ to half of the mass of soil collected for the experiments, up to a concentration of 500 mg Zn kg^{-1} dry soil. The portion of metal-treated soil was wetted for 1 week by adding deionised water to maintain 60% of the water holding capacity; the soil was then dried in the greenhouse for approximately 2 weeks. This spiked soil was subjected to three cycles of wet and dry processes before amendments were added (Blaylock et al., 1997).

2.2. Preparation of mycorrhizal inocula

The two isolates of AMF used in this study have been isolated from heavy metal contaminated soils in central Europe and are in the AMF collection of the Department of Mycorrhizal Symbioses from the Institute of Botany, Academy of Sciences of the Czech Republic. Each of the two mycorrhizal fungi isolates was individually grown in zeolite (clinoptilolite 1.0–2.5 mm, Chemko, Slovakia) for 12 months prior to the beginning of the experiment in multispore pot cultures with both *Z. mays* L. and *Trifolium pratense* L. as host plants under the same greenhouse conditions. An inoculum suspension of each isolate was prepared by wet sieving (710 mm) 450 cm^3 of zeolite from pot cultures with deionised water to a final volume of 150 ml, and was used to inoculate the pots of each Zn treatment. Each pot of the mycorrhizal treatments received 10 ml of the inoculum suspension containing colonised root fragments, hyphae and spores. The suspension was pipeted at 2 cm below the soil surface. Pots from control treatments received the same volume of the inoculum suspension autoclaved twice (121 °C for 25 min).

2.3. Experimental design

The experiment was a factorial design with two matrix Zn levels [the local contaminated soil from Estarreja (L) and the same soil spiked with extra Zn (L + Zn)], three amendment treatments [no amendment, compost (C) and manure (M)] and three AMF treatments [no AMF, *G. claroideum* (Gc) and *G. intraradices* (Gi)]. Each treatment was replicated five times. Dried compost was mixed with the soil at a rate of 10% (w/w), which is the rate commonly used in land application of compost for agricultural soils (Shiralipour et al., 1992) and dried manure was added at a rate of 5% (w/w), which corresponds to a similar addition of organic carbon as in the other treatment. Microbial populations from the local (L) soil were reintroduced to the autoclaved soil by adding 10 ml of filtrate (Whatman No. 1) to each pot. The filtrate was obtained from 200 g of non-sterile local soil (L) shaken for 2 h in 2 l of sterile deionised water (Oliveira et al., 2006). Manure (M) and compost (C) filtrates were also prepared using the same procedure, and respectively added to the pots that received the amendment treatment.

After addition of the amendments and soil and amendments’ filtrates, the soils were subjected to a 1-week equilibrium period (Cao and Ma, 2004).

S. nigrum L. seeds (obtained from Instituto Botânico do Porto) were surface sterilised with 0.5% NaOCl for 10 min and were subsequently washed with sterilised water. Seeds were then germinated in the naturally contaminated Estarreja soil in the greenhouse. Three weeks after seeding, three equally developed seedlings were transplanted into plastic pots containing 300 g of one of the sterilised matrixes: soil alone, soil with compost 10% (w/w) and soil with manure 5% (w/w) – for the local (L) and the extra-Zn spiked soils (L + Zn). Pots were randomised on the greenhouse, process that was repeated every 3 weeks. After sowing, seedlings were reduced to four per pot. Pots with the combinations of both soils and the amendment treatments, but with no plants were also placed in the greenhouse during the experiment. The pots were maintained in a controlled growth room (12 h photoperiod, 450 μmol m^{-2} s^{-1} photosynthetically active radiation, 18–38 °C temperature range, 16–71% relative humidity range) and watered every 2 days at 40% of the field capacity.