

Effect of chromium contaminated soil on arbuscular mycorrhizal colonisation of roots and metal uptake by *Plantago lanceolata*

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Abstract

Industrial practices are the primary causes for the accumulation of chromium in the environment, an element considered as a toxic heavy metal when present in high concentrations. The beneficial contribution of arbuscular mycorrhizal fungi (AMF) to plant nutrition and growth has been acknowledged, however, results of heavy metal uptake by plants under mycorrhizal symbiosis vary. The AMF *Glomus intraradices* (BEG 72) was used with *Plantago lanceolata* as a host plant in three experiments. In the first one, devised to assess the plant tolerance to Cr(III) in the soil, four levels of chromium concentration were applied in a sterile soil mix, placed in pots with inoculated and non inoculated plant treatments. Plant survival, shoot weight and AMF root colonisation were measured. In the second experiment which was designed in order to determine the effect of the symbiosis on the chromium uptake, similar treatments were used, and in addition, the heavy metal plant tissue content was measured and the bioconcentration factors calculated. In the third experiment the chromium uptake from an industrial chromium waste contaminated soil was assessed using treatments with and without the AMF. Results showed that chromium has a severe impact on the survival of non inoculated plants, however, plants inoculated with AMF in moderately contaminated soil, perform in terms of growth and survival rate, as well as the non inoculated plants in soil with no chromium added, suggesting a buffering effect of the AMF by decreased intake of the toxic element in the roots and its translocation to the shoot.

Additional key words: *Glomus intraradices*, heavy metal tolerance, phytoremediation.

Resumen

Efecto de la contaminación del suelo con cromo en la colonización de las raíces con micorrizas arbusculares y en la absorción de metales pesados de *Plantago lanceolata*

Prácticas industriales, tales como el cromado, el curtido de pieles, tratamientos de la madera y la minería, son las principales causas de la acumulación en el medio ambiente del cromo, un elemento considerado como metal pesado tóxico cuando está presente en grandes concentraciones. Se ha estudiado ampliamente la contribución de los hongos de la micorriza arbuscular (HMA) a la nutrición vegetal y al crecimiento de las plantas y se han descrito distintos efectos de la micorrización en la captación de metales pesados por las plantas. El HMA *Glomus intraradices* (BEG 72) fue utilizado con *Plantago lanceolata* como planta hospedante para evaluar la respuesta a la contaminación con Cr(III). Los ensayos se realizaron en suelo esterilizado al que se añadieron tres concentraciones de Cr(III) (100 mg kg⁻¹, 200 mg kg⁻¹, 400 mg kg⁻¹) y en un suelo industrial contaminado con vertidos de cromo. Los resultados mostraron que la acumulación de cromo tiene graves repercusiones en la supervivencia de las plantas no micorrizadas, sin embargo, las plantas inoculadas con el HMA en un suelo moderadamente contaminado, tienen un crecimiento y una tasa de supervivencia comparables a los de las plantas no inoculadas creciendo en suelo no contaminado. La colonización con *G.intraradices* ejercería un efecto amortiguador de la toxicidad del cromo disminuyendo su absorción por las raíces y su translocación hacia el tallo.

Palabras clave adicionales: fitoremediación; *Glomus intraradices*, tolerancia a metales pesados.

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Introduction

Chromium is one of several heavy metals that cause severe environmental contamination in soil, sediments and groundwater (Pawlisz *et al.*, 1997). Wastes coming from chromium related industries, such as tanneries, electroplating and mining activities contribute to most of the chromium contamination; however, in many countries tanning industry is the main recognized Cr polluting industry (Apte *et al.*, 2005; Tariq *et al.*, 2005).

Chromium salts, in particular chromium sulphate, are the most widely used tanning substances today (Bajza and Vrcek, 2001). During the tanning process, the leather takes up about 60% to 80% of applied chromium and the rest of metallic salts are usually discharged into waste waters (Mant *et al.*, 2005). The determination of Cr levels in environmental samples is of great concern because chromium toxicity is well known (Barnhart, 1997; Barceloux, 1999). The two main oxidation states of chromium, Cr(III) and Cr(VI), are habitually present in natural waters but they significantly differ in biological, geochemical and toxicological properties (Sule and Ingle, 1996). Whereas Cr(III), over a narrow concentration range, is considered to be less toxic, Cr(VI) salts have severe toxic effects on humans (Kornhauser *et al.*, 2002). The range of toxicity for most agronomic plants ranges from 5 to 100 mg kg⁻¹ of available Cr in soil (Ghosh and Singh, 2005). Cr(III) was chosen as the contaminant for this study because it is found in polluted effluents of tanneries and in waste. The treatment of contaminated water effluents of these industries leave a sludge with a high concentration of Cr(III). The sludge is collected and disposed on land, where a subsequent soil treatment system needs to be implemented to reduce the impact of the contamination. Cr(III) is less toxic than Cr(VI) and many remediation practices end at the reduction of Cr(VI) to Cr(III) by physicochemical processes. Besides the processes involving soil treatments and land filling, the final restoration step involves the revegetation of the polluted site. Also high levels of chromium can be found in agricultural soils that have been fertilised with composted urban wastes. In both cases the bioavailability of the heavy metal in the soil is of great concern because plants may take up excessive amounts and pass it into the food chain.

The effect of arbuscular mycorrhiza (AM) in plant growth and nutrition has been well documented (Smith and Read, 2008), however, the effect of the symbiosis in the uptake of heavy metals can vary depending on

the fungal isolate, the plant and the metal concerned (Orlowska *et al.*, 2005). In the present work we studied the effect of *Glomus intraradices* (BEG72) on the growth and chromium uptake of *Plantago lanceolata* grown on soil spiked with Cr(III) and on soil obtained from a heavily contaminated site. *Plantago lanceolata* was selected as a model plant because it is strongly mycorrhizal, common to a wide range of habitats and has been suggested as a potential indicator of soil toxicity (Djingova *et al.*, 2003).

Material and methods

Mycorrhizal inoculum

The AM fungus *G. intraradices* used was originally isolated from a citrus orchard, with high pH and high levels of calcium carbonate (Camprubí and Calvet, 1996) and is registered in the International Bank for the Glomeromycota (Canterbury, UK) with the reference BEG 72. The fungus was multiplied in pot culture with leek as a host plant and Terragreen[®] as the growth substrate. Inoculum from the pot culture was a mixture of spores, mycelia and leek roots in Terragreen[®], containing approximately 100 spores per 10 g.

Host plants

Seeds of *Plantago lanceolata* were surface sterilised in a 10% solution of HClO₄ for 10 min and washed three consecutive times with sterile water for 10 min. They were then germinated in two seed trays with sterile sand, in one of them the inoculum was disposed as a layer underneath the seeds to establish the symbiosis. Seeds were grown for three weeks before transplanting.

Experimental design

The first experiment was devised to assess the plant tolerance to Cr(III) in the soil. A sterile soil: quartz sand: peat (3:2:1) mix was spiked with chromium chloride (CrCl₃), added as a water solution to each 1-L pot to achieve a soil concentration of 100, 200 and 400 mg kg⁻¹ of chromium respectively. The levels of chromium were chosen according to the British soil guideline values for chromium contamination (DEFRA,

2002), where the maximum level allowed for the agricultural use of soil is 100 mg kg^{-1} , the maximum level for residential use is 200 mg kg^{-1} and above this level and up to $5,000 \text{ mg kg}^{-1}$ the soil can only have an industrial use. The experiment had three heavy metal treatment levels and two inoculation treatments: inoculated with *G. intraradices* (BEG72) and non-inoculated. Each combination of treatments was replicated 7 times. Each experimental unit was a pot where three *P. lanceolata* plants were transplanted 3 weeks after germination. Plant survival was recorded every week. Four weeks after transplant the plants were harvested and the shoot weight and the arbuscular mycorrhizal root colonisation were measured.

The objective of the second experiment was the determination of the effect of the symbiosis on chromium uptake. Plants were inoculated and grown in the same substrate as the previous experiment spiked with 100 and 200 mg kg^{-1} of Cr(III) for 8 weeks. There were three heavy metal levels (none, 100 mg kg^{-1} and 200 mg kg^{-1}) and two inoculation treatments: inoculated with *G. intraradices* (BEG72) and non-inoculated. There were 7 replicates per treatment consisting in a pot with three plants each. After 8 weeks growth, the plants were harvested and the shoot dry weight along with the mycorrhizal root colonisation were measured. The dried shoots were milled and mineralised in a mixture of HNO_3 . The heavy metal content was determined by atomic absorption spectrometry. Bioconcentration factors (BCF) were calculated by dividing the heavy metal concentration in the above-ground plant tissue by the total heavy metal concentration in the soil (McKone and Maddalena, 2007).

The third experiment was done to assess the chromium uptake from a soil obtained from a contaminated site. The soil was analysed and the bioavailability of the chromium was determined. Plants were germinated in a tray as before and were transplanted in the contaminated soil in 1-L pots. There were 7 replicates of two treatments; contaminated soil with mycorrhiza and without mycorrhiza inoculation. In each pot/replicate there were three plants. Each plant was treated separately when determining shoot weight and heavy metal concentration; however, the root system of each pot was considered a whole unit when measuring AM colonisation and heavy metal concentration. Plants were grown for 8 weeks.

Plant data from all experiments were analysed using a one way or two way analysis of variance, according to the experimental design.

Results

The first experiment showed Cr plant toxicity. *Plantago lanceolata* survival and growth was severely hindered when chromium was added to the soil (Fig. 1 and Table 1). Non-inoculated plants transplanted to soil with 400 mg kg^{-1} of added Cr did not survive more than two weeks. The addition of 400 mg kg^{-1} of chromium precluded plant growth when the plant was not inoculated; inoculated plants also had a low survival rate, and a reduced growth. At 200 mg kg^{-1} of added Cr, inoculated plants had a higher survival rate than non-inoculated plants, however, when assessing plant growth there were no differences between inoculation treatments. At 100 mg kg^{-1} of added Cr, there were no differences in plant survival between inoculated and non-inoculated plants, however, growth of AM inoculated plants was significantly better. AM inoculation increased plant tolerance to the added chromium. At 400 mg kg^{-1} of added Cr the inoculated plants that survived grew poorly and the extent of root colonisation decreased with the addition of the heavy metal. Both survival and plant growth were improved by inoculation with AMF at moderate levels of added Cr (100 mg kg^{-1} and 200 mg kg^{-1}).

The inoculation with *G. intraradices*, in the second experiment, improved considerably the survival of

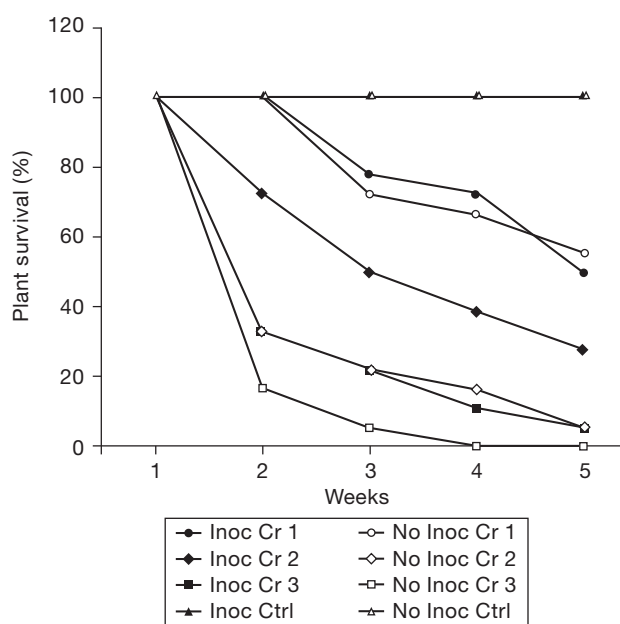


Figure 1. Evolution of *Plantago lanceolata* survival inoculated or non-inoculated with *Glomus intraradices* in soil spiked with chromium. (Cr1: 100 mg kg^{-1} Cr; Cr2: 200 mg kg^{-1} Cr, Cr3: 400 mg kg^{-1} Cr).

Table 1. Shoot dry weight and root arbuscular mycorrhizal (AM) colonisation of *Plantago lanceolata* inoculated with *Glomus intraradices* and non-inoculated grown in soil with added chromium (4 weeks after transplant). Data are means of all the plants that survived in each treatment \pm SD (standard deviation)

Inoculation treatment	Cr added to the soil (mg kg ⁻¹)	Shoot dry weight (g)	% root AM colonisation
Non-inoculated	0	1.11 \pm 0.08	0
Non-inoculated	100	0.55 \pm 0.12	0
Non-inoculated	200	0.20 \pm 0.21	0
Non-inoculated	400	NM ^a	0
<i>G. intraradices</i>	0	1.43 \pm 0.08	32
<i>G. intraradices</i>	100	1.02 \pm 0.14	15
<i>G. intraradices</i>	200	0.69 \pm 0.16	6
<i>G. intraradices</i>	400	0.65 \pm 0.24	5

^a NM: non measurable.

plants grown in soil with added Cr (Table 2). No significant growth increase was observed at 100 mg kg⁻¹ of Cr, however, at 200 mg kg⁻¹ of Cr non inoculated plants did not survive eight weeks in the contaminated soil. Uptake of chromium measured in leaves and shoots was higher in non-inoculated plants than in plants forming the symbiosis. The uptake increased with increasing levels of available Cr in the soil, accordingly, the bioconcentration factor for Cr was lower for plants inoculated than for plants non-inoculated. Because of the high mortality of non-inoculated plants grown at 200 mg kg⁻¹ of chromium, no Cr uptake data could be measured for this treatment.

In the third experiment plants were grown in a natural soil contaminated with Cr, from a metal plating factory. The soil analytical parameters are summarised in Table 3. Plant available Ca, Mg, Na, K and Cr were measured after extraction with ammonium acetate. The total amount of Cr, extracted with a mixture of nitric and hydrochloric acid (aqua regia) was also determined. Available Cr measured as extractable Cr with

ammonium acetate represented less than a 10% of the total Cr present in the soil. After eight weeks growth, the survival of plants inoculated with *G. intraradices* was 57%, whilst only 24% of the non-inoculated plants survived, and growth of non-inoculated plants was very poor (Table 4). The uptake of Cr was higher in non-inoculated than in inoculated plants. In both treatments the bioconcentration factor was lower for shoots than for roots. The relationship between the BCF of non mycorrhizal versus mycorrhizal plants was maintained at 3.5 (\pm 0.5) when comparing similar tissues. However, because of the higher biomass produced by mycorrhizal plants, total Cr in the shoots was higher in mycorrhizal plants than in non-inoculated plants.

Discussion

The use of AMF inoculation could be a possible strategy to accelerate the re-vegetation process in areas polluted by heavy metals. Few works (*e.g.* Jordao *et*

Table 2. Survival (%), shoot dry weight, Cr uptake (mg kg⁻¹) and root arbuscular mycorrhizal (AM) colonisation of *Plantago lanceolata*, grown at two levels of added chromium, inoculated and non-inoculated with *Glomus intraradices*, six weeks after transplant. Data are means of all plants that survived in each treatment \pm SD

Inoculation treatment	Cr addition level	Survival (%)	Shoot dry weight (g)	Cr in leaves and shoots (mg kg ⁻¹)	Root AM colonisation
Non-inoculated	No Cr	100	0.32 \pm 0.036	19	0
Non-inoculated	Cr1 (100 mg kg ⁻¹)	14	0.21 \pm 0.036	85	0
Non-inoculated	Cr2 (200 mg kg ⁻¹)	0	NM ^a	NM	0
<i>G. intraradices</i>	No Cr	100	0.51 \pm 0.036	14	25
<i>G. intraradices</i>	Cr1 (100 mg kg ⁻¹)	100	0.21 \pm 0.036	52	36
<i>G. intraradices</i>	Cr2 (200 mg kg ⁻¹)	85	0.20 \pm 0.036	187	41

^a NM: non measurable.

Table 3. Soil characteristics of the contaminated site

Parameter	
Texture (USDA)	Sandy loam
pH (H ₂ O)	8.73
Electric conductivity (dS m ⁻¹)	2.87
Ca (mg kg ⁻¹)	18,197
Na (mg kg ⁻¹)	4,477
K (mg kg ⁻¹)	240
Mg (mg kg ⁻¹)	490
N% (Kjedhal)	0.10
P (mg P ₂ O ₅ kg ⁻¹) (Olsen)	73
Total Cr (mg kg ⁻¹) (extractable with HNO ₃ + HClO ₄)	870
Available Cr (mg kg ⁻¹) (extractable with CH ₃ COONH ₄)	50

al., 1997; Farmer *et al.*, 2006) have dealt with chromium toxicity although it is a common occurrence among many industrial activities, linked to tanneries and to metal plating with chromates, therefore the contaminated sites are not widespread, although they are abundant worldwide and especially in many Mediterranean countries where leather industries are important. The data presented in this paper clearly demonstrates the importance of AMF for plant survival and growth. *Plantago lanceolata* is a well studied species, tolerant of many environmental stresses, that is strongly mycorrhizal and has been considered useful to conduct toxicity tests. In non contaminated soils *P. lanceolata* can survive and grow without the symbiosis, however, when Cr is added to the soil, the survival of *P. lanceolata* decreases rapidly and most of non-inoculated plants

Table 4. Survival, plant growth and Cr uptake of *Glomus intraradices* inoculated and non inoculated *Plantago lanceolata* grown in the contaminated soil, 8 weeks after transplant. Data are means of all the plants that survived in each treatment ± SD (standard deviation)

Parameter	Non-inoculated	Inoculated with <i>G. intraradices</i>
Survival (%)	24	57
Shoot and leaves weight (g)	0.42 ± 0.37	3.99 ± 0.91
Roots weight (g)	0.58 ± 0.30	3.40 ± 0.91
AM root colonisation (%)	0	49
Cr (mg kg ⁻¹ in leaves and shoots)	163 ± 94	37 ± 12
Cr (mg kg ⁻¹ in roots)	2,150 ± 139	217 ± 68
BCF ^a (leaves)	2.6 ± 0.62	0.75 ± 0.25
BCF ^a (roots)	17.2 ± 6.82	4.34 ± 1.37

^a BCF: bioconcentration factor.

do not survive at concentrations higher than 100 mg kg⁻¹ of Cr(III) (Fig. 1). Although Cr(III) is not in this ionic form extremely toxic as a heavy metal, the addition of Cr as CrCl₃ might have accumulative effects both increasing soil salinity and heavy metal toxicity. *Helianthus annuus*, a plant described as hyperaccumulator of several heavy metals (Shahandeh and Hossner, 2000), although with a low tolerance to Cr, was shown to have an increased Cr tolerance and uptake through inoculation with *G. intraradices*. Davies *et al.* (2001), working with a range of both Cr(III) and Cr(VI) concentrations, found that although mycorrhizas reduced *H. annuus* plant stress due to Cr toxicity, the symbiosis enhanced Cr uptake both in shoots and roots. Ghosh and Singh (2005) found that non-hyperaccumulator plants tend to avoid Cr uptake and translocation, and the higher concentration of Cr is found in roots, rather than leaves or stems.

Our results show that mycorrhizal plants take up less Cr than non-mycorrhizal plants, both in the shoots and also in the roots, confirmed by the BCF values found in our experiments, therefore AMF seem to be acting as a barrier decreasing the Cr uptake. Khan (2001) also found that plants growing in an area where soil was contaminated with Cr were always mycorrhizal and found no correlation between extractable Cr in the soil and Cr in the shoots. Orłowska *et al.* (2005) found a negative correlation between the mycorrhizal root colonisation of *P. lanceolata* and the concentration of lead (Pb) and Zinc (Zn) in the shoots of plants grown in heavy metal contaminated soil. The effect of AMF on the uptake of heavy metals by plants is not uniform, as both increases and decreases have been reported. The outcome probably depends on the selected plant and also on the species and strain of the fungus used. Recently Rivera-Becerril *et al.* (2002, 2005) demonstrated a Cd-stress buffering effect of mycorrhizal colonisation on pea plantlets. In line with these results, in our experiments plants inoculated with AMF in moderately contaminated soil, grow just as well as non-inoculated plants in soil with no chromium added. The majority of plants without AMF do not survive transplanting into contaminated soil. Those that survive grow badly, and accumulate higher concentrations of chromium, both in the shoots and also in the roots. Similarly to other reports (Khan *et al.*, 2000; Orłowska *et al.*, 2005) the heavy metal was strongly retained in the root system, both in mycorrhizal and non-mycorrhizal plants. The effect of AMF in decreasing heavy metal stress has been assigned to the selective immo-

bilisation of the toxic metal within the root tissues that are colonised by the fungus (Kaldorf *et al.*, 1999) or to the high metal sorption capacity of the extraradical mycelium of the AMF (Joner *et al.*, 2000). Plants already have mechanisms that decrease the translocation of heavy metal into shoots, and AMF might be modifying these mechanisms. Ouziad *et al.* (2005) analysing differential gene expressions in AM colonized tomato grown under heavy metal stress found that genes related to the tolerance to heavy metal stress (methallothionein Lemt2 and a broad range metal transporter LeNramp1) were up-regulated in non-mycorrhizal plants grown in contaminated soil but not in mycorrhizal plants. This effect might be due to a decrease in the heavy metal concentration in the plant roots cells and also to a different allocation of the metal particles within the root cell influenced by the fungus colonisation. Our results with Cr are consistent with a buffering effect of the AMF, increasing the tolerance to the heavy metal contamination while decreasing the intake of the contaminant in the roots and the translocation to the shoots. Christie *et al.* (2004) have also shown an alleviation effect of AMF in front of Zn toxicity, decreasing the Zn translocation towards the shoot. They suggested a direct effect of the AMF through adsorption and binding of the heavy metal in the mycorrhizosphere and an indirect effect through the improvement of the plant nutrition. The use of mycorrhizal plants as a tool in phytoremediation strategies needs further research to understand the mechanisms involved in the plant's protection against metal toxicity. These research efforts will help to integrate this biotechnology in agricultural and environmental engineering processes.

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