



Full Length Article

Interaction between Arbuscular Mycorrhiza and Heavy Metals in the Rhizosphere and Roots of *Juniperus procera*

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ABSTRACT

The decline in the population of *Juniperus procera* Hochst. ex Enal. in the woodlands of Saudi Arabia appears to be associated to their poor regeneration in the natural habitat. This study was conducted to investigate the presence and interaction of arbuscular mycorrhiza fungi (AMF) in the roots and heavy metals; cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni), plumbum (Pb), uranium (U) and zinc (Zn) in the soil and roots of *J. procera*. Sampling was conducted in linear transaction, where a total of 120 individual trees were randomly selected. High values (59-79%) were recorded for root colonization with AMF in plant samples. Higher concentrations of Cd, Co, Cu, Pb, U and Zn in the soil and roots were found to be related to lower mycorrhiza infection, while Cr and Ni showed otherwise. In general, the concentration of heavy metal elements in the soil was significantly higher than that recorded in the roots of plant samples. AMF symbiosis probably affected heavy metal translocation from the soil into the roots. The presence of AMF and its relationship with heavy metals may give an insight into the dynamics of AMF infection in the plant. In addition, the roles played by AMF in the absorption of heavy metals in mycorrhizal plants are further discussed. © 2012 Friends Science Publishers

Key Words: Arbuscular mycorrhiza fungi; Heavy metal; *Juniperus procera*; Roots; Soil

INTRODUCTION

Plants can endure high level of metals in the soil with the help of some extrinsic mechanisms, which helps to reduce the absorption of metals by plant roots (Michalak, 2006). Amongst the mechanism, mycorrhizal action helps to augment plants' tolerance towards heavy metals (Turnau *et al.*, 2005) and can be exploited in remediating soils contaminated with heavy metals (del Val *et al.*, 1999). A mycorrhizal fungus is a symbiotic association in and on the roots of a host plant. Arbuscular mycorrhiza fungi (AMF) are the most common group of mycorrhizal fungi, which are obligatory (Javaid, 2007; Javaid *et al.*, 2007; Javaid & Riaz, 2008) and they are found in more than 80% of land plant families (Smith & Read, 2008). The fungus is supplied with soluble carbon sources by the host plants, whereas the fungus provides the host plant with a better ability to take up water and nutrients from the soil (Entry *et al.*, 2002; Javaid, 2009). Not only do fungal hyphae speed up the nutrition state of the host plant but they also relocate heavy metals to the plant (Li *et al.*, 2009) and protect the host plants from both abiotic (Augé, 2001; Javaid, 2007; Azcón *et al.*, 2009) and biotic stresses (Khaosaad *et al.*, 2007). It is hypothesized that the presence of heavy metal elements may

affect the colonization of AMF in the roots of *J. procera* and subsequently the heavy metal in the plant itself.

J. procera was once found to dominate a large part of woodlands in Saudi Arabia. Ongoing and persistent human influences have reduced these trees to isolated patches (IUCN, 2010). Fisher (1997) observed that the woodlands of Asir Highlands of Saudi Arabia, *J. procera* trees were generally seen to be reducing in numbers. The present population of *J. procera* in Saudi Arabia only represents a small fragment of the woodlands that once existed (Couralet, 2004). In the southwest region of Saudi Arabia, juniper forests have grown to be more at risk to deterioration because of its low natural regeneration capability and lack of plantation for regeneration (Al-Gamdi, 2006).

This study was carried out in an effort to determine the relationship between AMF with heavy metal concentrations in the soil as well as in the plant roots of *J. procera* in the southwest of Saudi Arabia.

MATERIALS AND METHODS

Sample collection: Rhizosphere soil was collected in AL-Sarawat, Saudi Arabia from four areas, namely AL-Janabin, Athroub, Shakran and Hazna. Their coordinates range from

latitude of N 19° 05' to 19° 53' and longitude of E 41° 32' to 41° 43'. In each area, soil and plant roots samples were selectively collected from six subareas 20 m apart. The soil samples were collected at about 30 cm depth. Six replicates were made for each area. The soil and root hairs were then brought back to the laboratory for further chemical analysis.

Preparation of root hairs: The clearing and staining of roots were carried out according to the methods stated by Brundrett *et al.* (1996). The root hairs were then ready for microscopic observation, where stained fungal structures can be easily recognized. The estimated density of AMF was calculated according to the equation below:

$$\frac{\text{Number of cells with AMF present}}{\text{Total cells}} \times 100 \quad (\text{Brundrett et al., 1996}).$$

Soil heavy metal analysis: Soil samples were digested according to the methods given by Ward *et al.* (1975). The solutions were then taken to near dryness over a water bath and the residues were dissolved, filtered through Whatman no. 42 filter paper and washed repeatedly with distilled water (Ramakrisnaiah & Somashekar, 2002). The solutions were then fed to an Atomic Absorption Flame Emission Spectrophotometer (Model AA670) for heavy metal analysis.

Root heavy metal analysis: The technique recommended by the Association of Official Analytical Chemists (1990) was used to analyze heavy metal in the roots. After the plant roots were digested, heavy metals in the root samples were determined with the Atomic Absorption Flame Emission Spectrophotometer (Model AA670).

Data analysis: The linear relationship between heavy metal in the soil and heavy metals in the roots with the percent of AMF infection was subjected to Pearson's Correlation Coefficient (r^2 value). The relationships between heavy metal in the soil and the percentage of colonization with AMF in the roots were ascertained by using regression analysis. Moreover, the relationships between the concentrations of heavy metal in the roots and the percentage of colonization with AMF were ascertained by regression analysis. The T-test was used to compare the concentration of heavy metal elements in the soil and in the roots of *J. procera*. All statistical analyses were performed using Statistix® Version 7.0 (Analytical Software, Tallahassee, Florida).

RESULTS AND DISCUSSION

Effect of AMF infection and heavy metal concentrations in the soil: In this study, mycorrhizal infection in the roots of *J. procera* was found to be high, ranging from 59 to 79%. Studies on grasses such as *Agrostis capillaries*, *Sesleria caerulea*, *Calamagrostis varia* etc., which are often found in Cd- Pb- and Zn-polluted mining soils showed high mycorrhiza infection between 50-81% (Leyval & Joner, 2001; Regvar *et al.*, 2006). Generally, the heavy metal

concentrations in the soil samples were low, where Cd concentration was found to be between 0.0-2.0 mg/kg, Co 12.0-24.0 mg/kg, Cr 36.0-56.0 mg/kg, Cu 29.0-46.0 mg/kg, Ni 25.0-42.0 mg/kg, Pb 10.0-23.0 mg/kg, U 33.0-60.0 mg/kg and Zn 59.0-72.0 mg/kg.

Mycorrhiza infection is affected by the concentration of the heavy metal elements in the soil. Different type of plant species, concentration of heavy metals in the soil and plant growth conditions can influence the performance of mycorrhizas (Janoušková *et al.*, 2007; Bai *et al.*, 2008; Jankong & Visoottiviset, 2008). AMF from different populations or geographical isolates has also revealed inconsistencies in their ability to withstand heavy metals/metalloids and related stress (Bai *et al.*, 2008).

Results showed that the heavy metal concentrations of Cd, Co, Cu, Pb, U and Zn As shown in (Fig. 1a, b, d & 2b, d) in the soil samples formed significantly negative associations ($P < 0.05$) with the percentage of AMF in the roots. Hildebrandt *et al.* (1999); found that there was higher mycorrhizal colonization of the roots of *Viola calaminaria*, which were cultivated in soils with high levels of Pb and Zn. On the other hand, there was a lower mycorrhizal colonization of the roots of maize (*Zea mays* L.) as reported by Chao and Wang (1990); when Cd, Cu, Cr, Ni, Pb and Zn were added to soil. In another study, Lingua *et al.* (2008) indicated that higher Zn treatment caused the number of arbuscules to decline. In barley, there was also a negative relationship between AMF colonization and the Zn concentrations when urban-industrial sludge was added to the soil (Boyle & Paul, 1988). del Val *et al.* (1999); found that Cd-, Pb- and Zn-contaminated sewage sludge hindered mycorrhizal colonization and development of external hyphae. In *S. canadensis*, Pb contamination negatively impacted the growth and production of mycorrhiza (Yang *et al.*, 2008). However, Tonin *et al.* (2001); found that the clover plant, *Trifolium repens* showed an increased mycorrhizal infection when the soil was contaminated with Cd and Zn.

It was the contrary with Cr and Ni (Fig. 1c & 2a) in the soil samples. At higher concentrations of Cr and Ni, mycorrhizal infection was significantly higher ($P < 0.05$). According to Sylvia *et al.* (1992); in the case of Cr and Ni, where percentage of mycorrhiza increased paralleled with the increase in the heavy metal, there is a possibility that high amounts of heavy metals in the soil may be prevented from causing harmful effect in the root with the help of AMF as they have the ability to tolerate heavy metals.

According to Colpaert *et al.* (2000); although copper and zinc are necessary for fungal and plant growth, they can become toxic at high concentrations. Nevertheless, some AMF can still be present even under highly contaminated soil (Rabie, 2005). AMF was reported to play a significant role in efforts to restore cadmium-contaminated soils and the plants can be protected from heavy metals (Kapoor & Bhatnagar, 2007).

Fig. 1: Relationship between the concentration of cadmium (a), cobalt (b), chromium (c) and copper (d) (mg/kg) in the soil with the percentage of mycorrhiza infection in the roots of *J. procera*

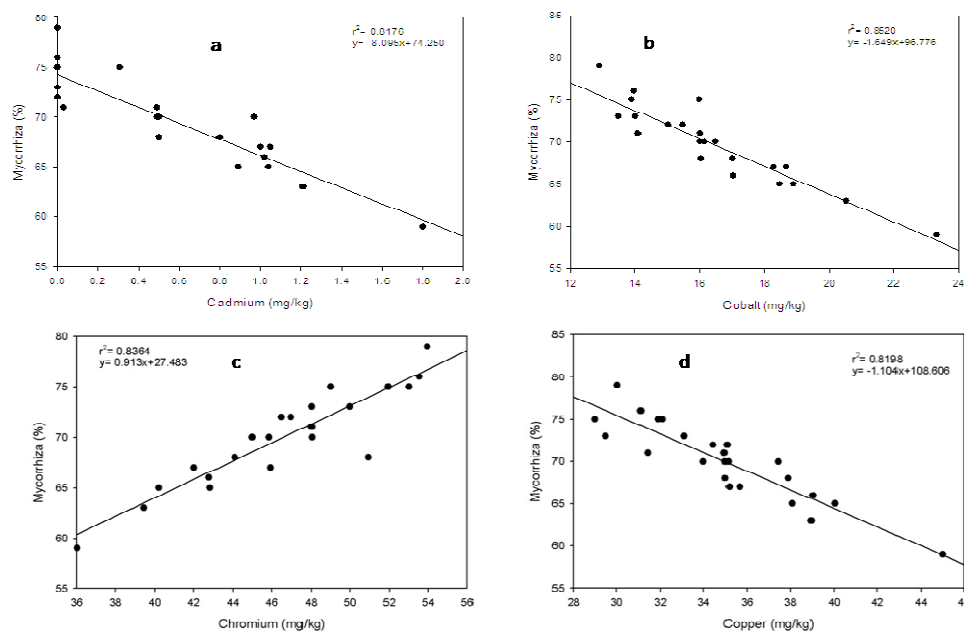
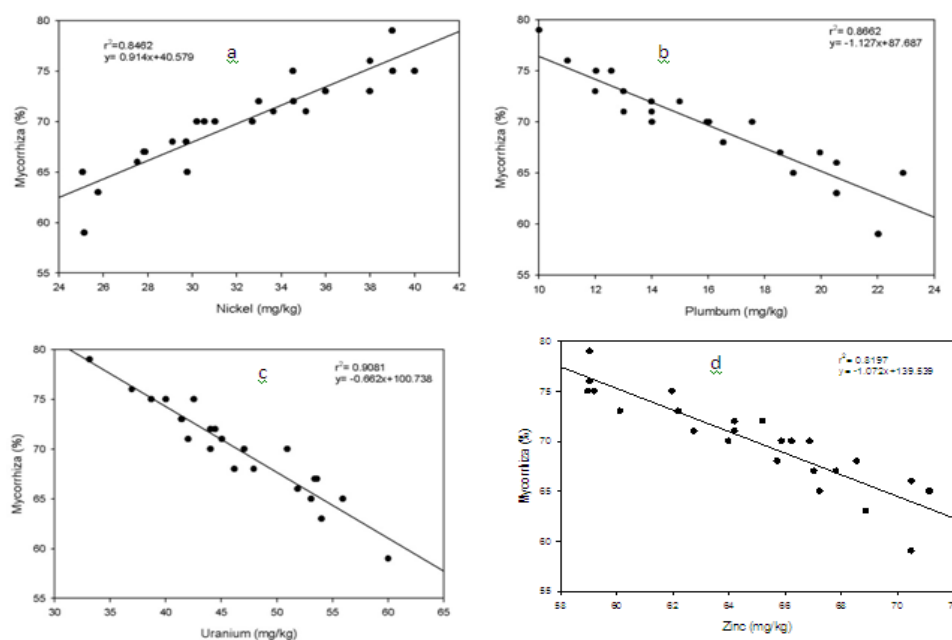


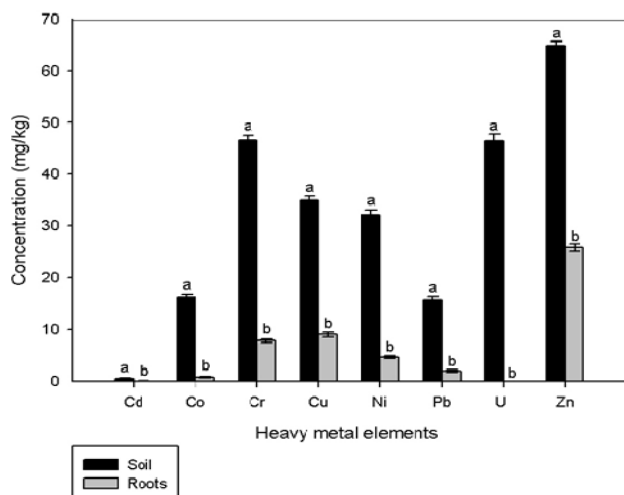
Fig. 2: Relationship between the concentration of nickel (a), plumbum (b), uranium (c) and zinc (d) (mg/kg) in the soil with the percentage (%) of mycorrhiza infection in the roots of *J. procera*



Heavy metal concentration in roots and soil: Fig. 3 shows that the amount of heavy metal concentrations recorded from the soil was higher than in the roots. All heavy metal elements showed a significantly lower amount in the roots than compared to the surrounding soil ($P < 0.05$). Generally, the uptake of heavy metal elements (Cd, Co, Cr, Cu, Ni, Pb, U & Zn) by *J. procera* was 60 to almost 100% lower than the amount of elements in the soil. Although Cd

concentration in both the soil (0.5250 ± 0.1044 mg/kg) and roots (0.0775 ± 0.0193 mg/kg) were relatively low, they were significantly different ($P < 0.05$). Cobalt concentrations in the roots were lower by about 95% than compared to the amount found in the soil. In comparison to the soil, results showed that Cr, Ni and Pb were similarly lower by 83, 86 and 87%, respectively. In addition, U concentration in the soil was recorded at around 46 mg/kg but very little traces

Fig. 3: Comparison between the concentration (mg/kg) of heavy metal elements in the soil and in the roots of *J. procera* (Paired T-test; $P < 0.05$)



of U (less than 0.01 mg/kg) were found in the roots of *J. procera*. Uranium was amongst the lowest amount of element absorbed by *J. procera*. On the other hand, Cu and Zn in the roots were recorded to be significantly lower (around 4- & 3-folds, respectively) than that in the soil ($P < 0.05$).

Effect of AMF infection and heavy metal concentrations in *J. procera* roots: The concentration of heavy metal elements in the root samples of *J. procera* were found to be positively (Cr & Ni) and negatively (Cd, Co, Cu, Pb, U, Zn) related to the percent of mycorrhiza infection found in the roots. The results were in accordance to that recorded between heavy metals contents in the soil samples and AMF infection. In the root samples, Cd concentration ranged from 0.0-0.4 mg/kg, Co 0.0-2.5 mg/kg, Cr 4-10 mg/kg, Cu 5.0-13.0 mg/kg, Ni 2.0-7.0 mg/kg, Pb 0.0-5.0 mg/kg, U 0.0-0.02 mg/kg and Zn 18.0-34.0 mg/kg. Results showed that an increase in AMF infection decreased the uptake of heavy metals such as Cr and Ni (Fig. 4c, 5a), while Cd, Co, Cu, Pb, U and Zn were found to increase proportionately with AMF infection [(Fig. 4a,b & d), 5b,c & d].

The concentrations of heavy metal elements in *J. procera* roots were either non-existing or at very low levels. Cd, Co, Cr, Cu, Ni, Pb, U and Zn concentrations in the roots of *J. procera* were lower than compared to that in the soil samples. However, different findings were found for different plants. In ryegrass (*Lolium perenne* L.), AMF transferred a lower amount of Cd, Ni and Zn in the plant from the soil (Takács *et al.*, 2001). On the contrary, Carvalho *et al.* (2006); found that when the salt marsh plant, (*Aster tripolium* L.) were grown in soil with high levels of Cu, AMF augmented the concentration of Cu within the plant. Besides that, Chen *et al.* (2005); reported that mycorrhizal colonization was observed to increase the entry

of Pb into plant roots of *Kummerowia striata*, *Ixeris denticulate*, *Lolium perenne*, *Trifolium repens* and *Echinochloa crusgalli*. Qureshi *et al.* (2001); found that the uptake of U by plant roots was greatly influenced by the AM fungus, which showed that AMF translocate higher amounts of U in mycorrhizal roots. Our results showed otherwise, where U concentrations in the roots were low.

In *Pisum sativum*, the presence of AMF reduced the deleterious effect when Cd was added to the soil (Rivera-Becerril *et al.*, 2002). It is believed that Cd transfer to the root cells is reduced as a result of the AMF having negatively charged surfaces, which adsorb the metal (Joner *et al.*, 2000) or that Cd transfer from the soil to the roots is effective but its translocation to the shoots is hindered by AMF (Joner & Leyval, 1997). In addition, *Viola calaminaria* showed greater endurance to pollution with Zn and Pb when colonized by AMF as reported by Hildebrandt *et al.* (1999). Nevertheless, Chen *et al.* (2005) reported that mycorrhizal population in either soil or culture solution can be affected positively, negatively, or neutrally by heavy metals.

Various factors can affect the function of AMF on the absorption of heavy metals by plants. These include the variety of the heavy metal and its amount, the physicochemical characteristics of the medium, the association between the AMF isolate and the plant, as well as the plants' growth and developmental state plant and fungi species present and environmental conditions (Leyval & Joner, 2001).

CONCLUSION

It is concluded that AMF in both the soil surrounding *J. procera* trees and in the roots of *J. procera* increased with an increase in Cr and Ni levels, while the percentage of AMF infection reduced when the concentration of Cd, Co, Cu, Pb, U and Zn increased. Heavy metal concentrations in the roots were lower as compared to that in the soil. AMF was found to significantly affect the uptake of heavy metals from the soil to the roots in *J. procera*. Although, the heavy metal elements in the soil are extremely low, a low absorption of metals by the roots could have affected the growth and development of these trees, hence the increase in reports regarding the deterioration of *J. procera* trees. The presence of AMF in the roots may have hindered the absorption of the heavy metals but it could have also prevented the uptake of necessary nutrients for the growth and development of *J. procera*.

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Fig. 4: Relationship between the concentration of cadmium (a), cobalt (b), chromium (c) and copper (d) (mg/kg) in the roots of *J. procera* with the percentage (%) of mycorrhiza infection in the roots of *J. procera*

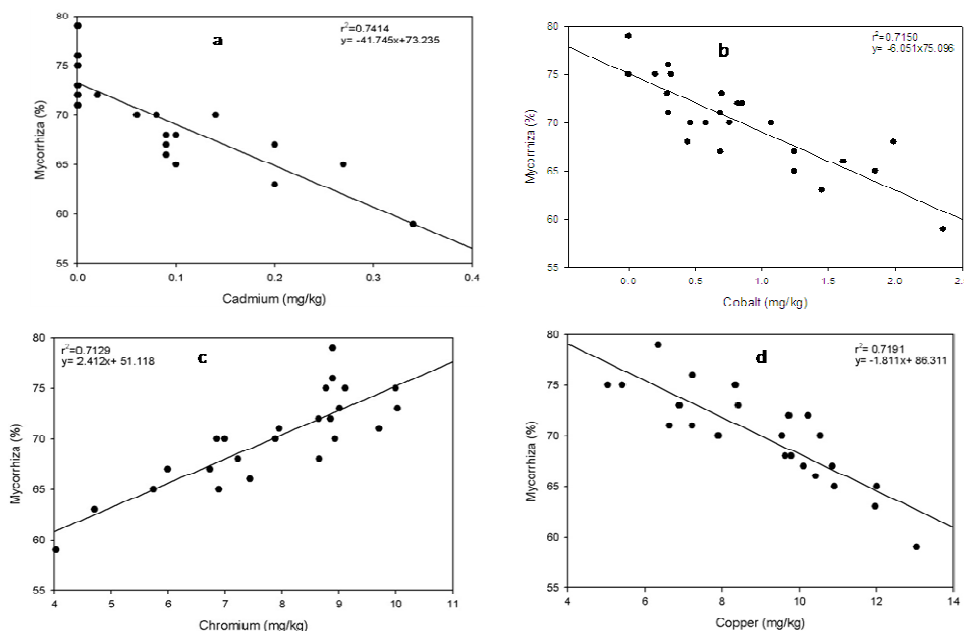
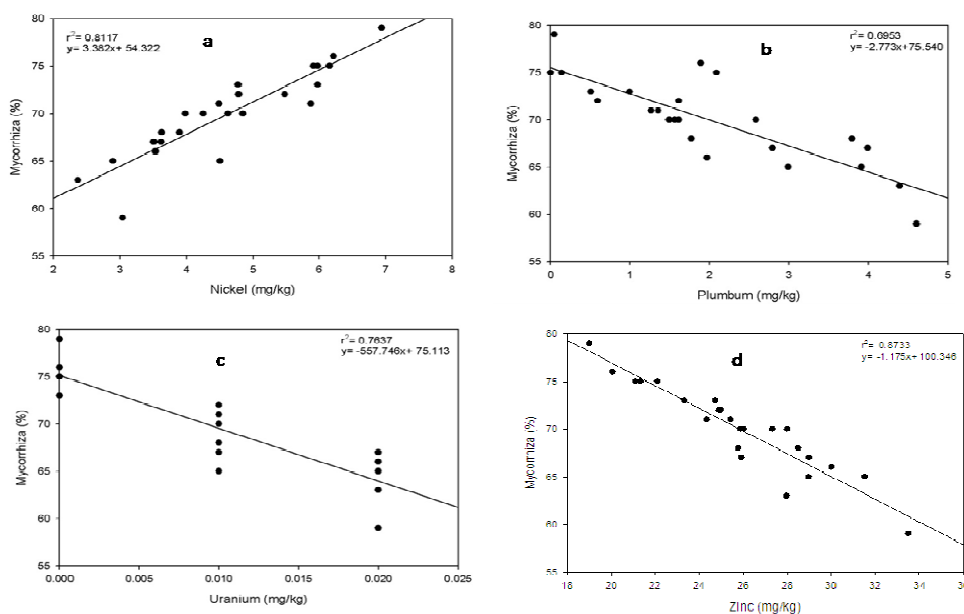


Fig. 5: Relationship between the concentration of nickel (a), plumbum (b), uranium (c) and zinc (d) (mg/kg) in the roots with the percentage (%) of mycorrhiza infection in the roots of *J. procera*



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