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Distribution of chromium species in a Cr-polluted soil: Presence of Cr(III) in glomalin related protein fraction

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HIGHLIGHTS

- High concentrations of total Cr and total Cr(VI) were found in the studied area.
- *R. communis* and *C. maculatum* were the most abundant plants growing in the area.
- Glomalin related soil protein contributed to Cr(III) sequestration.
- Both plant roots accumulated more Cr than the shoots and were colonized by AMF

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ABSTRACT

The accumulation of Cr in soil could be highly toxic to human health; therefore Cr soil distribution was studied in rhizosphere soils from *Ricinus communis* and *Conium maculatum* and bare soil (BS) from an industrial and urban area in Argentina. Total Cr, Cr(VI) and Cr(III) concentrations were determined in 3 soil fractions: total, extractable and associated to total-glomalin-related protein (T-GRSP). BS had the highest total Cr and total Cr(VI) concentrations. Total Cr(VI) concentration from both rhizosphere soils did not differ from the allowed value for residential area in Argentina (8 μg Cr(VI) g^{-1} soil), while total Cr(VI) in BS was 1.8 times higher. Total Cr concentration in all the soils was higher than the allowed value (250 μg Cr g^{-1} soil). Extractable and associated to T-GRSP Cr(VI) concentrations were below the detection limit. Cr(III) bound to T-GRSP was the highest in the BS. These findings are in agreement with a long term effect of glomalin in sequestrating Cr. In both plant species, total Cr was higher in root than in shoot and both species presented arbuscular mycorrhizal fungi (AMF). As far as we know, this is the first study that reports the presence of Cr in T-GRSP fraction of soil organic matter. These findings suggest that Cr mycorrhizostabilization could be a predominant mechanism used by *R. communis* and *C. maculatum* to diminish Cr soil concentration. Nevertheless, further research is needed to clarify the contribution of native AMF isolated from *R. communis* and *C. maculatum* rhizosphere to the Cr phytoremediation process.

1. Introduction

Industrial development has helped to improve human's quality of life during the last decades. However, the lack of knowledge for appropriate industrial waste handling and final disposal has contributed to soil and water contaminations. The anthropogenic pressure generated by industrial activities triggers drastic deterioration of soil, producing a marked loss of stability, degradation and even desertification (Barea et al., 2007). Potentially toxic elements (PTE) such as As, Cu, Cd, Cr, Pb, and Zn, are one of the most common pollutants, mostly near industrial sites (Baena and Huertos, 2008; Järup, 2003; Meier et al., 2012a). High concentrations of PTE in soil have detrimental effects on ecosystems, and could become a risk to human health as they can enter to the food chain via agricultural products or contaminated drinking water. Hence, a proper management of PTE should be a priority associated to industrial activities (del Rio et al., 2002).

Chromium is used in several industrial processes (i.e. leather tanning, alloy and stainless steel production). Chemistry of Cr is quite complex; Cr is found in soils in two oxidation states, Cr(III) and Cr(VI). Cr(III) is non-toxic and not readily absorbed by plants; in contrast, Cr(VI) is highly toxic; it is a Class A carcinogen by inhalation and an acute irritating agent to living cells (Dhala et al., 2013; James, 1996; Khan, 2001). Cr(VI) is water soluble in the full pH range, while Cr(III) is prone to be adsorbed on soil surface or precipitate as chromium hydroxide in a slightly acidic and alkaline environment (Dhala et al., 2013; James, 1996; Khan, 2001). Hexavalent Cr exists in neutral-to-alkaline soils principally as a chromate anion (CrO_{4-}) or as

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moderately-to-scarcely soluble chromate salts (e.g. $CaCrO_4$, $BaCrO_4$, $PbCrO_4$) (Dhala et al., 2013; James, 1996). The differences between the biological and chemical properties of that two species of Cr make the determination of total Cr insufficient to evaluate the real pollution risk. Previous research showed that a variable fraction (typically <15%) of Cr(III) added to soils is oxidized to Cr(VI) (Barlett and James, 1979). Therefore, in order to prevent Cr(VI) lixiviation, remediation strategies should be planned in soils where Cr-wastes are disposed, even though no Cr(VI) is detected before its disposal. Chemical reduction of Cr(VI) to Cr(III) is the most common remediation strategy developed until now. Though the reduction–remediation strategy offers a rapid solution, it is very expensive for a large scale treatment and it does not assure that re-oxidation of Cr(III) to Cr(VI) will not occur (James, 1996; Panda and Sarkar, 2012). In contrast, phytoremediation, which uses higher plants and the associated soil microbes as decontaminating agents, is a less expensive, long-lasting and eco-friendly strategy to decontaminate PTE polluted soils (Meier et al., 2012a; Dhala et al., 2013; Ali et al., 2013). Arbuscular mycorrhizal fungi (AMF), which develop mutualistic associations with the roots of most terrestrial plants, allow plant a greater absorption of nutrients and water due to an increase in soil exploration area (Azcón-Aguilar et al., 1999). In this sense, it is worth exploring AMF in relation to their potential for improving phytoextraction and/or phytostabilization. Moreover, AMF contribute to soil aggregate stabilization by means of the network formed by the extra-radical hyphae and by a hydrophobic protein named glomalin (Cornejo et al., 2008; Vodnick et al., 2008; Wright and Upadhyaya, 1998). Glomalin, operationally defined as "glomalin related soil protein" (GRSP), appears to be a component of the hyphae and spore wall of AMF, likely released into the soil by mycelium turnover. GRSP has been reported as contributing to PTE sequestration (Cornejo et al., 2008; Vodnick et al., 2008; Aguilera et al., 2011; González-Chávez et al., 2004). For these reasons, AMF through GRSP may enhance phytostablization (mycorrzhizostabilization) processes (Khan, 2006; Meier et al., 2012b). The aim of this work was to study distribution of Cr species in soil and biomass from two plant species of higher occurrence in a Cr polluted area. Specifically: i) Cr(III) and Cr(VI) distribution in the bare soil and rhizospheres; ii) Cr bioaccumulation in plant tissues; iii) Cr in GRSP and vi) degree of mychorrizal colonization in roots; as a first approach to understand the *in-situ* distribution and accumulation of Cr species in a polluted area.

2. Materials and methods

2.1. Study area

In order to determine if the studied area had high Cr concentrations a preliminary sampling was done. The studied area belongs to Morón borough, an industrial/urban area with a highly dense population, in Buenos Aires Province, Argentina (34°39′0″S, 58°37′0″W). Morón stream flows to Reconquista River which ends at Rio de la Plata River (Fig. 1). Soil samples were taken at a distance of 1–3 m from the water flow along the west side of Morón stream from Martinez ditch to Roma Street Drainage (2 km long, Fig. 1). Preliminary analysis showed high concentrations of total Cr and total Cr(VI) in soils of the sampled areas.

2.2. Sample collection and analysis

The study area has a moderate plant cover, with plant cover and non-plant cover (bare soil-BS-) sections easily distinguishable from each other. The plants were mainly of herbaceous species (*Conium maculatum*, *Polygonum punctatum*, *Verbena granatensis*, *Rumex* sp., *Dipsacus fullonum*, *Brassica napus*) and the shrub *Ricinus communis*. Two plant species, the most abundant in the area, were sampled: *R. communis* and *C. maculatum*. Soil and plants were randomly sampled in a representative site of 2 km^2 from the studied area (site X, Fig. 1). Soil samples were collected at a 0–25 cm depth from *R. communis* and *C. maculatum* rhizospheres (just below the plant and adhered to the root) and from BS (an area where the nearest plant was at least 2 m apart). Five replicates were taken from each rhizosphere and BS; 5 replicates of both plant species for Cr biomass determination and 3 root replicates of both species for mycorrhizal colonization. Soil samples and plant material were dried in a muffle at 500 °C for 5–6 h to obtain the ash fraction. Organic matter was calculated as the difference between dry soil and its corresponding ashes (Davies, 1974). pH was determined in the supernatant of a mix soil:water 2:5 w:v after 60 min.

2.3. Chromium determination

Total Cr was determined by atomic absorption spectrophotometry (Khan, 2001). Cr(VI) was determined by diphenylcarbazide photometric method; if Cr(VI) is present in the soil extract, the solution turns to pink when DFC is added (James et al., 1995). Total Cr and total Cr(VI), extractable Cr(III) and extractable Cr(VI), and total Cr and Cr(VI) bound to total GRSP (T-GRSP) concentrations were determined. Total Cr was extracted from soil ashes with an acid solution of 2 N HCl/1 N HNO_3 (1 g ashes: 50 ml acid solution) (Kumar et al., 1995). Total Cr(VI) was extracted from soil with an alkaline solution (NaOH/Na_2CO_3; pH = 12) within the first week after sampling in order to minimize changes in Cr redox status (1 g dry soil: 50 ml alkaline solution) (James et al., 1995). Extractable Cr(III) was obtained by incubating 2.5 g soil with 25 ml 0.5 M EDTA for 30 min (Honorato et al., 2001). Extractable Cr(VI) was obtained by incubating 2.5 g soil with 25 ml 10 mM phosphate buffer, pH = 7.2 (James and Barlett, 1983). Cr concentrations

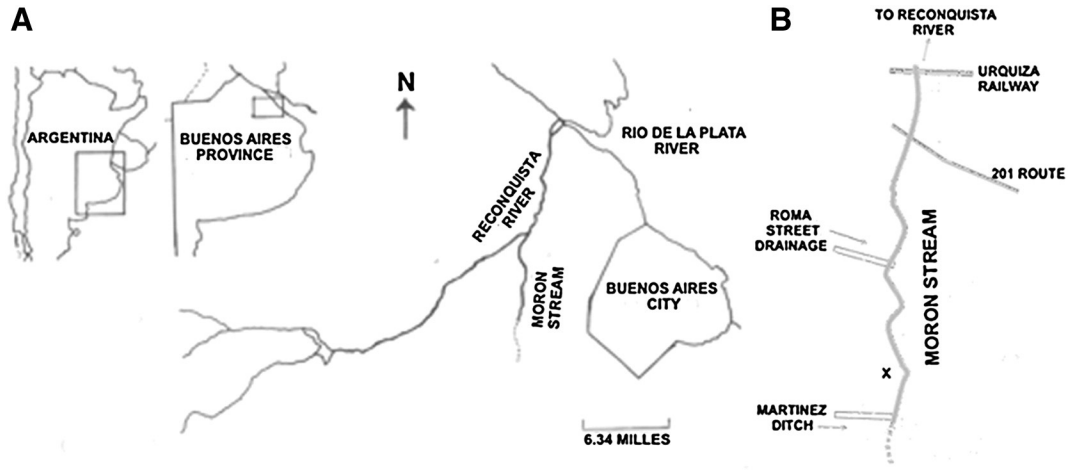

Fig. 1. Study area and site selection. A) Morón's stream general location. B) Sampled site specific location (X). Modified from Kuczynski (2007).

were expressed as μg Cr g^{-1} of dry soil. Total Cr/total Cr(VI) ratio was calculated. Cr associated to T-GRSP was extracted by 6 successive incubations with 50 mM citrate pH = 8 solution and autoclaving for 1 h at 121 °C, the solution was replaced each time (1 g soil: 8 ml 50 mM citrate) (Wright and Upadhyaya, 1998). For Cr(VI) determination in T-GRSP fraction, EDTA to a final concentration of 0.05 M was added to the citrate extraction solution in order to avoid Cr(VI) reduction (Chen et al., 2007). EDTA sequestrates manganese cations, preventing citric acid oxidation, since Cr(VI) can oxidize citrate and reduce to Cr(III) in the presence of manganese (Chen et al., 2007). For total Cr associated to T-GRSP determination, the total protein was precipitated with HCl and Cr concentration was measured. T-GRSP was quantified by Bradford's method (Wright and Upadhyaya, 1998). Cr concentration in the T-GRSP fraction was expressed as μg Cr g^{-1} soil or as μg Cr mg^{-1} of protein. Additionally, total Cr was extracted from root and shoot ashes with an acid solution of 2 N HCl/1 N HNO_3 (1 g ashes: 20 ml acid solution) (Kumar et al., 1995). Cr concentration in plant tissue was expressed as μg Cr g^{-1} dry weight (DW).

2.4. Mycorrhizal root colonization

Mycorrhizal colonization in *R. communis* and *C. maculatum* roots was determined by observation of stained roots adapted from Phillips and Hayman (1970) under a light microscope at 400× magnification. Briefly, roots were clarified with KOH 2.5%; *R. communis* roots were incubated for 20 min in the autoclave, and *C. maculatum* roots were incubated overnight at room temperature. Acidification was carried out by incubation of roots from both species overnight at room temperature. After acidification, roots were stained with cotton blue (0.5 g methyl blue: 1 L acid glycerin) for 15 min at 90 °C.

The intensity of the mycorrhizal root colonization (%M) was estimated as follows (Trouvelot et al., 1986):

$$\%M = (95*n°5 + 70*n°4 + 30*n°3 + 5*n°2 + 1)/\text{total } n°$$

where n° counts for the number of root pieces that have a certain degree of colonization (n°5 > 90%, n°4 > 50%, n°3 < 50%, n°2 < 10% and n°1 < 1%).

2.5. Statistical analysis

Differences between Cr concentrations in soils were analyzed with one-way ANOVA; differences between Cr concentrations in plant tissue were analyzed with paired *t*-test and differences between MC (%) was analyzed by *t*-test. All the analysis were made using INFOSTAT free edition. Multiple comparisons between medias were made with multiple range Tukey's test. Assumptions for homoscedasticity and normality were met for all the data sets analyzed, except for total Cr/total Cr(VI) ratio and μg Cr mg^{-1} protein. In order to meet homoscedasticity assumptions, natural logarithm was used to transform the data.

3. Results

3.1. Soil physicochemical analysis from the Cr-polluted site

Organic matter content (OM%) was lowest in BS and in *R. communis* rhizosphere soil, the difference between BS and *R. communis* rhizosphere soil was statistically significant while no difference was observed neither between BS and *C. maculatum* rhizosphere soil nor between *C. maculatum* and *R. communis* (Table 1). All the soils had basic pH values. The highest pH was recorded in *C. maculatum* rhizosphere soil and BS (*C. maculatum* = 8.2 ± 0.1; BS = 8.3 ± 0.2, respectively), which was significantly different from pH in *R. communis* rhizosphere soil (*R. communis* = 7.7 ± 0.1) (Table 1).

Table 1
Physico-chemical properties in rhizosphere and bare Cr polluted soils.

	Bare soil	*R. communis*	*C. maculatum*
OM (%)	6.2 ± 0.4[a]	7.8 ± 0.4[b]	6.7 ± 0.2[ab]
pH	8.3 ± .0.2[a]	7.7 ± 0.1[b]	8.2 ± 0.1[a]

One-way ANOVA, different letters indicate significant differences (Tukey's; p < 0.05). OM (%) = organic matter in soil. n = 5.

3.2. Total and extractable chromium (III) and (VI) in soils

Total Cr concentration in BS, *R. communis* and *C. maculatum* rhizosphere soil was 850 ± 90, 444 ± 60 and 617 ± 55 μg Cr g^{-1} soil, respectively. In all the cases, total Cr was over the allowed limit in Argentina for residential area (250 μg Cr g^{-1} soil Regulative order 831/93 law 24051; Fig. 2A, dashed line). Total Cr concentration was higher in BS than in *R. communis* rhizosphere soil, while it was not significantly different from *C. maculatum* rhizosphere soil (Fig. 2A). Total Cr(VI) concentration in BS was 14 ± 2 μg Cr(VI) g^{-1} soil and it was significantly higher than the allowed limit (8 μg Cr(VI) g^{-1} soil (Regulative order 831/93 law, 24051), Fig. 2B, dashed line). Total Cr(VI) concentration in both rhizosphere soils was 10.5 ± 1.6 and 7.5 ± 0.8 μg Cr(VI) g^{-1} soil, respectively. In both cases, the values were close to the allowed limit. Total Cr(VI) concentration found in BS was higher than in *C. maculatum* rhizosphere but not significantly different from that in *R. communis* rhizosphere (Fig. 2B), while total Cr and total Cr(VI) concentrations did not differ in both rhizosphere soils (Fig. 2A and B). As regards total Cr/total Cr(VI) ratio, it was the highest in *C. maculatum* rhizosphere soil, and it significantly differed from *R. communis* rhizosphere soil, while BS ratio was not significantly different from both rhizosphere soils (Fig. 2C).

The amount of extractable Cr(VI) was below the detection limit (1.28 μg available Cr(VI) g^{-1} soil), though all the soil samples turned to pale pink when DFC was added, indicating the presence of extractable Cr(VI). In accordance with total Cr(VI) distribution, where BS had the highest Cr(VI) concentration (Fig. 2B), the pink color observed in the extraction solution of extractable Cr(VI) was stronger in BS samples than in *C. maculatum* and *R. communis* rhizosphere soils, suggesting a higher amount of the metal in BS (data not shown). The distribution pattern of extractable Cr(III) was similar to that of total Cr in soils; the highest Cr(III) concentration was determined in BS soils (Fig. 2D, white bars). Extractable Cr(III) was below 5% in all the soils and the value was similar between soils (Fig. 2D, gray bars).

3.3. Chromium in glomalin-related soil protein

No Cr(VI) was detected associated to T-GRSP. Cr(III) concentration associated to T-GRSP was highest in BS (42 ± 2 μg Cr g^{-1} soil), whereas it was similar in both *C. maculatum* (31 ± 3 μg Cr g^{-1} soil) and *R. communis* (31 ± 2 μg Cr g^{-1} soil) rhizosphere soils (Fig. 3A, white bars). Cr(III) associated to T-GRSP referred to total Cr in soils did not show significant differences among all the soils, it ranged from 5.6% (BS) to 6.6% (*R. communis* rhizosphere soil) (Fig. 3A, gray bars). Cr(III) concentration per mg of T-GRSP was higher in BS with respect to *C. maculatum* and *R. communis* rhizosphere soils (BS = 6.8 ± 0.9, *C. maculatum* = 4.1 ± 0.3 and *R. communis* = 3.5 ± 0.5 μg Cr mg^{-1} T-GRSP; Fig. 3B white bars) and T-GRSP concentration was highest in *R. communis* rhizosphere and lowest in BS (BS = 6.5 ± 0.6, *C. maculatum* = 7.6 ± 0.5 and *R. communis* = 8.8 ± 0.5 mg T-GRSP g^{-1} soil; Fig. 3B gray bars).

3.4. Chromium in C. maculatum and R. communis root and shoot tissues

Total Cr quantification in *R. communis* and *C. maculatum* root and shoot tissues was determined. In both plant species, Cr concentration in root was higher than in shoot. In *R. communis* roots, Cr concentration was 13 times higher, while in *C. maculatum* roots was 28 times higher than that in shoots of the same species (Fig. 4A and B, respectively).

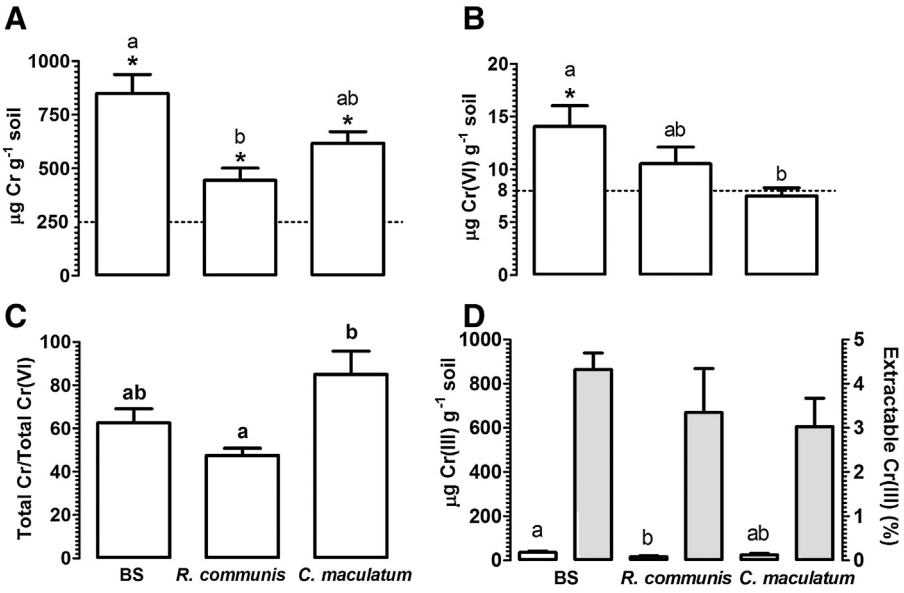

Fig. 2. Total Cr (A), total Cr(VI) (B), total Cr/total Cr(VI) ratio (C) and extractable Cr(III) (D), in *R. communis* and *C. maculatum* rhizosphere and bare soil. One way ANOVA, different letters indicate significant differences (Tukey's $p < 0.05$). Statistical differences between means and the allowed value for residential areas in Argentina (dashed line in A and B, Regulative order 831/93 law 24051) was evaluated by one sample *t*-test and marked by an asterisk (*). Gray bars correspond to extractable Cr percentage referred to total Cr concentration (D). BS = bare soil. n = 5.

3.5. AMF root colonization in plants growing in Cr polluted soils

The fact that Cr was found in the GRSP fraction strongly suggested that *R. communis* and *C. maculatum* roots were colonized by AMF. This assumption was corroborated when roots from both plant species were analyzed for AMF (Fig. 5). *R. communis* mycorrhizal colonization (MC) was 51 ± 13%, *C. maculatum* MC was 25 ± 12% (Fig. 5). *R. communis* roots presented vesicles (Fig. 5A) and arbuscules (Fig. 5B) whereas *C. maculatum* presented mostly arbuscules (Fig. 5D).

4. Discussion

Total Cr and total Cr(VI) concentrations were lower in rhizosphere soils than in BS, suggesting that both plants — *R. communis* and *C. maculatum* — may have the potential to diminish Cr soil concentration (Fig. 2A and B).

Cr(III) concentration, when it was referred to total Cr, did not differ in all the soils (Fig. 2D, gray bars), as it was expected, since total Cr distribution was similar to extractable Cr(III) distribution (Fig. 2A vs. 2D white bars). This result suggests that *R. communis* and *C. maculatum* do not have a fundamental role in total Cr and extractable Cr(III) balance at these Cr concentrations. Since Cr(III) has beneficial effects while Cr(VI) is carcinogenic, an elevated total Cr/total Cr(VI) ratio is desired, indicating that most of the Cr is as Cr(III). Interestingly, rhizosphere soil of *C. maculatum* had the highest total Cr/total Cr(VI) ratio while *R. communis* had the lowest (Fig. 2C).

Soil pH is a major variable controlling the balance between Cr species. Reduction of Cr(VI) by organic matter or other electron donors is favored in soils with pH < 6, and both oxidation and reduction may be inhibited in soils with pH > 6 (James, 1996; Bolan et al., 2014). The balance observed between insoluble and soluble forms of Cr in all the soils, may be related to soil pH.

GRSP contribution to PTE sequestration has already been reported (Cornejo et al., 2008; Vodnick et al., 2008; González-Chávez et al., 2004). The proportion of Cr(III) extracted from GRSP, regarding total Cr in soil, was similar to the proportions reported for Zn and Pb (Cornejo et al., 2008; Vodnick et al., 2008; González-Chávez et al., 2004). GRSP was found in BS. GRSP is synthesized by HMA and since HMA grow in symbiosis with plant roots it suggests that the origin of the GRSP found at BS was from HMA that once colonized the plant roots growing at the sampled BS. Moreover, preliminary analysis showed that BS had HMA spores, though the amount of spores per g of soil was lower than in both rhizosphere soils (BS: 4, *R. communis* 10 and *C. maculatum* 8 spores g^{-1} soil). The similar values of Cr associated to T-GRSP, referred to total Cr in soil, between BS and rhizosphere soils are in agreement with a long term effect of GRSP in sequestrating PTE. In this sense, GRSP contribution to Cr sequestration (and other PTEs) continues even though there are no longer plants at the site (Aguilera et al., 2011).

Cr concentration was higher in root than in shoot from both *R. communis* and *C. maculatum*. A higher Cr concentration in root than in shoot tissues has already been reported for plants adapted to Cr-polluted areas (Khan, 2001). Moreover, tolerance to high levels of Cd

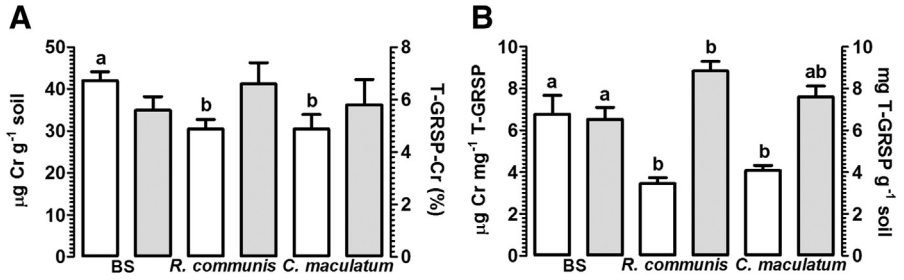

Fig. 3. Total Cr associated to T-GRSP in rhizosphere and bare soils. One way ANOVA, different letters indicate significant differences (Tukey's $p < 0.05$). A) Gray bars correspond to the percentage of Cr associated to GRSP referred to total Cr concentration; B) gray bars correspond to mg GRSP per g of soil. BS = bare soil. n = 5.

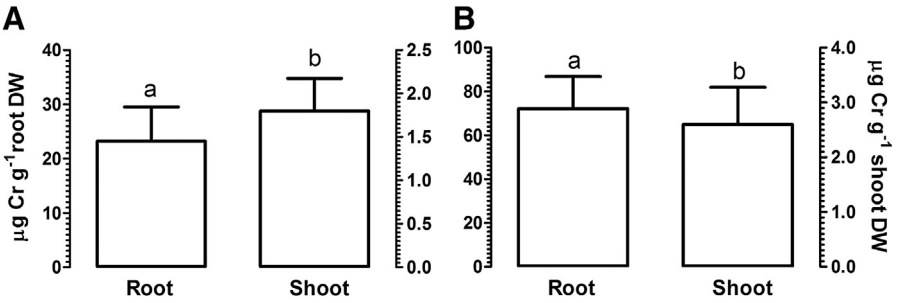

Fig. 4. Cr contents in root and shoot tissues of *R. communis* (A) and *C. maculatum* (B) growing in a Cr(III) and Cr(VI) polluted soil. Paired *t*-test, different letters indicate significant differences DW = dry weight. n = 5.

and As, and to Cd, Cu, Pb and Zn has already been reported for *R. communis* and *C. maculatum*, respectively. Cd concentration was higher in *R. communis* roots than in shoots; no PTE determinations were made for *C. maculatum* (Gulezian et al., 2012; Shi and Cai, 2009; Tamás and Kovács, 2005). Some plant species, called hyperaccumulators, can concentrate PTE in their aboveground tissues to levels far exceeding those in the soil or in the nearby non-accumulating plants (Ali et al., 2013). Ali et al. (2013) reviewed PTE concentrations which were suggested to use as criteria to determine if a given plant is a hyperaccumulator. The concentration reported for Cr was 1000 μg g^{-1} DW, which is more than 100 times higher than the concentrations determined in shoots from both plants analyzed in this study (Fig. 4), thus none of the plant species evaluated could be considered a Cr hyperaccumulator according to these criteria. The higher Cr concentration and the presence of AMF structures found in roots from both plant species (Fig. 5), together with the presence of Cr in the GRSP fraction, suggest that Cr mycorrhizostabilization could be a predominant mechanism used by *R. communis* and *C. maculatum* to diminish Cr soil concentration rather than phytoextraction. Phytoremediation is defined as the use of higher plants and the associated soil microbes as decontaminating agents (Ali et al., 2013). PTE phytoextraction and stabilization are both mechanisms used by plants and their associated microbes for phytoremediation. Mycorrhizostabilization refers to a phytostabilization process enhanced by the presence of AMF (Khan, 2006). The symbiosis between AMF and the plant root is a dynamic relationship where both members interact regulating the colonization process (Parniske, 2008). Therefore, mycorrhizostabilization may be a mechanism "chosen" by the plant to prevent Cr uptake, thus mitigating the toxic effect. In this sense, it would be interesting to study the potential of the AMF isolated from Cr contaminated rhizosphere soils in assisting *in situ* biorremediation.

R. communis is a perennial shrub belonging to the Euphorbiaceae family, native from Mediterranean Europe, Southern Africa and India, while *C. maculatum* belongs to the *Apiaceae* family, is an annual or biennial (perennial in favorable conditions) growing herb, native from temperate regions of Europe, West Asia and North Africa. Both plants are spread worldwide (López et al., 1999) and have potential biotechnological uses. The use of castor oil extracted from *R. communis* seeds as biodiesel is being studied (Berman et al., 2011; Scholza and da Silva, 2008). Furanocoumarins are organic chemical compounds produced by some plants with antibacterial, antifungal, antitumoral and spasmolytic properties. Al-Barwani and Eltayeb (2004) reported an increased production of furanocoumarins in *C. maculatum* leaves and seeds when exposed to PTE. Some furanocoumarins, such as bergapten and psoralen, are used to treat several skin disorders such as vitiligo and psoriasis (Hönigsmann, 2013; Ozçelik et al., 2004). Since the Cr concentration determined in *R. communis* and *C. maculatum* aerial tissues was below to that reported for Cr hyperaccumulator plant species, it could be an alternative to use the harvested tissues to develop biotechnological products. In this sense, it will be worthy to determine Cr concentration in *R. communis* seeds and to study the biological properties of furanocoumarines extracted from *C. maculatum* leaves and seeds.

5. Conclusion

Total Cr and total Cr(VI) concentrations were lower in *R. communis* rhizosphere soils than in BS; *R. communis* and *C. maculatum* roots had more Cr concentration than the shoots, and both plants presented AMF structures. Importantly, Cr was found in all T-GRSP fractions, reporting for the first time the presence of Cr in T-GRSP fraction. These findings suggest that Cr mycorrhizostabilization could be a predominant mechanism used by *R. communis* and *C. maculatum* to diminish Cr soil concentration. Nevertheless, further research is needed to clarify the contribution of native AMF isolated from *R. communis* and *C. maculatum* rhizosphere to the Cr phytoremediation process.

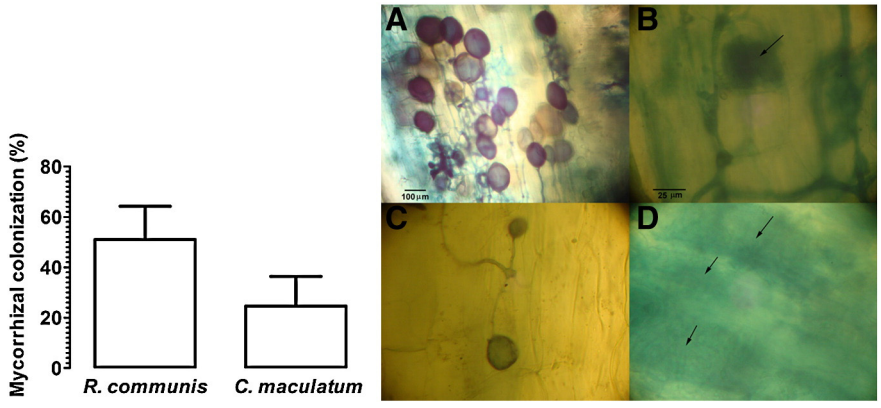

Fig. 5. Mychorrhizal root colonization in *R. communis* and *C. maculatum* isolated from Cr(III) and Cr(VI) polluted soils. T-test, no difference was observed (p = 0.2099). n = 3. Photographs: upper panel: *R. communis* vesicles (A) and arbuscules (B); lower panel: *C. maculatum* vesicles (C) and arbuscules (D). A: 100×; B, C and D: 400×.

Conflict of interest

The authors of the manuscript entitled "**Distribution of chromium species in a Cr-polluted soil: presence of Cr(III) in glomalin related protein fraction**" declare no conflict of interest.

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