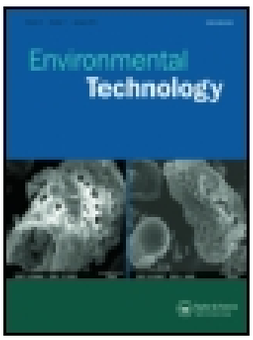

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Synergistic effect of chickpea plants and *Mesorhizobium* as a natural system for chromium phytoremediation

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ABSTRACT

The presence of chromium in soils not only affects the physiological processes of plants but also the microbial rhizosphere composition and metabolic activities of microorganisms. Hence, the inoculation of plants with Cr(VI)-tolerant rhizospheric microorganisms as an alternative to reduce Cr phytotoxicity was studied. In this work, chickpea germination was reduced by Cr(VI) concentrations of 150 and 250 mg/L (6 and 33%, respectively); however lower Cr(VI) concentrations negatively affected the biomass. On the other hand, its symbiont, *Mesorhizobium ciceri*, was able to grow and remove different Cr(VI) concentrations (5–20 mg/L). The inoculation of chickpea plants with this strain exposed to Cr(VI) showed a significantly enhanced plant growth. In addition, inoculated plants accumulated higher Cr concentration in roots than those noninoculated. It is important to note that Cr was not translocated to shoots independently of inoculation. These results suggest that *Mesorhizobium*'s capability to remove Cr(VI) could be exploited for bioremediation. Moreover, chickpea plants would represent a natural system for phytoremediation or phytostabilization of Cr *in situ* that could be improved with *M. ciceri* inoculation. This strategy would be considered as a phytoremediation tool with great economic and ecological relevance.

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Chromium; chickpea; phytoremediation; microsieve; Mesorhizobium; interaction

1. Introduction

Heavy metals contamination is actually a global problem, since they cannot be biologically degraded to less toxic products, and hence they persist in the environment. Among them, chromium (Cr) is one of the most toxic environmental pollutants that enter the agro-ecosystem through different sources, such as leather tanning, chromate preparation, and metal finishing. Cr is found in the environment mainly as trivalent Cr(III) and hexavalent Cr (VI) forms. Cr(VI) is more toxic than Cr(III) and produces mutagenic and carcinogenic effects [1]. Nevertheless, Cr(VI) is used in industry for metal plating, cooling tower water treatment, hide tanning and wood preservation. These anthropogenic activities have led to increasing levels of Cr contamination in the biosphere. The high concentration of Cr in soils severely affects the composition and metabolic activities of microbes [2,3] leading to losses in soil fertility [4], which also adversely affects the physiological processes of plants [5]. For instance, the accumulation of metals in plant organs to an undesired toxic levels shows limiting effects on photosynthesis and synthesis of chlorophyll pigments [6,7] and also inactivates plant protein synthesis, subsequently reducing crop yield [8,9].

For sustainable agriculture, the use of biofertilizers is very important for improving soil fertility and crop production [10]. In this context, the use of microorganisms which can maximize the ecological benefits and minimize the environmental hazard of chemical fertilizers is of great interest [11]. Hence, the inoculation of plants with rhizospheric microorganisms can be an alternative way to reduce Cr toxicity in plants, since they could reduce Cr levels present in soil or promote Cr adsorption and/or accumulation in roots, by changes in Cr speciation from available to nonavailable forms, avoiding translocation to the aerial part of plants. Thus, the combined use of plants and microorganisms, called 'rhizoremediation', to enhance the remediation of contaminated sites, has attracted the researchers' attention due to the biotechnological potential for metal removal.

Chickpea (*Cicer arietinum* L.) is the third most important grain legume in the world [12], being produced in over 45 countries, with a total of 12 million cultivated hectares, obtaining a total grain yield of 11 million tons [13]. This legume is generally inoculated with symbiotic N_2 fixer of *Mesorhizobium* genus [14]. Nodulating bacteria could protect plants against the toxic effects of Cr through adsorption/desorption mechanisms or by

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reducing Cr(VI) to the less toxic form Cr(III), through reductase enzymes [15]. However, little information is available about the effect of different Cr concentrations on chickpea growth and on plant-growth-promoting rhizobacteria. Thus, the aims of the present work were: (a) to evaluate Cr(VI) tolerance and Cr uptake capacity of chickpea plants, (b) to study the capability of their symbiont, to remove Cr(VI), and (c) to analyze the effect of Cr (VI) on chickpea–*Mesorhizobium* interaction and Cr accumulation in plants.

2. Materials and methods

2.1. Plant material

Chickpea seeds (*Cicer arietinum* L.) of Chañaritos S156 variety, KABULI type, were used. These were provided generously by the Agronomic Engineer Julia Carreras (Córdoba National University). For different assays, the seeds were washed 7–10 times with sterile distilled water, and then they were placed in plates containing moistened filter paper with 6 mL of sterile distilled water. The plates were placed at 25 ± 2°C in the dark during 4 d until radicle emergence.

2.1.1. Effect of Cr(VI) on chickpea germination and development in early stages of growth

For germination assays, six seeds were washed, as previously described, and placed in plates containing moistened filter paper, with 6 mL of distilled water (control) or solutions with 20, 50, 100, 150 and 250 mg/L Cr(VI), which was added as $K_2Cr_2O_7$ (Sigma). After 4 d, germinated seeds, considering those with radicles of 2 mm or longer, were counted and the results were expressed as germination percentage. After 15 d, total biomass (dry weight) was registered. The assay was repeated in triplicate. Later, this experiment was repeated three times independently.

2.1.2. Chromium tolerance and uptake by chickpea plants

Each pregerminated seed (as was described in Section 2.1) was placed in pots containing 20 g of sterile perlite. These pots were placed in trays which contained Hoagland solution diluted (½) without (control) or with water solutions containing 10, 20 and 50 mg/L Cr(VI). When these solutions were totally consumed, Hoagland (½) solution and water were alternately added every two or three days, according to plant needs.

Pots without seeds and irrigated with solutions containing different Cr(VI) concentrations were used as controls to evaluate potential evaporation losses or adsorption to the matrix (perlite). Then, Cr(VI) was analyzed and these values were considered as 100% of Cr(VI), in order to estimate Cr (VI) uptake by plants. The pots were incubated in a chamber with controlled temperature (28 ± 2°C) under photoperiod regime [16 h light (200 µmol/m^2/s)/8 h dark] and relative humidity (70–80%). Dry weight (mg) of shoots and roots was registered after 30 d.

To determine residual Cr(VI) in pots containing perlite, 100 mL of distilled water was added to each one for Cr (VI) extraction. After 24 h an aliquot was used for its quantification by diphenylcarbazide (DPC) reaction in the acid solution, as described below.

2.2. Bacterial strain

Three simbiont strains of chickpea were used: *Mesorhizobium ciceri* (isolated from a commercial inoculant), *M. ciceri* UPMCa 7 T and *Mesorhizobium mediterraneum* UPMCa 36 T (provided by Dra. Susana Rosas, UNRC). The microorganisms were cultivated in the liquid YEM medium and maintained in the solid medium (YEMA) supplemented with Congo red (25 mg/L) [16].

2.2.1. Growth and Cr(VI) removal capability of Mesorhizobium strains

To analyze growth and Cr(VI) removal by the strains, Erlenmeyer flasks containing 20 mL of the YEM medium and supplemented with Cr(VI) (5, 10, 20 and 50 mg/L) were used. All flasks were inoculated with a bacterial culture previously grown in the YEM medium, to achieve an initial optical density (OD) of 0.1 at 600_{nm}, and then they were incubated in an orbital shaker at 200 rpm and 28 °C. Abiotic controls were performed using noninoculated media supplemented with 10 mg/L Cr(VI), and they were considered as 100%. Aliquots of 1 mL were withdrawn for bacterial growth evaluation and residual Cr(VI) determination (Section 2.4). Growth was monitored by measuring OD 600_{nm} and residual Cr(VI) was determined after centrifugation at 10,000 rpm, for 5 min. The experiments were conducted in triplicates.

2.3. Inoculation assays

Pregerminated chickpea seeds were placed in pots containing 120 g of sterile perlite and soil (1:1). For the inoculation assay, bacterial inoculum was obtained by growing *M. ciceri* in the YEM medium during 72 h at 28°C and 200 rpm. Then, the culture was centrifuged at 10,000 rpm for 15 min and the pellet was suspended in saline solution (NaCl 0.9%) to reach an OD 600_{nm} of 1.0. Total colony-forming units (CFU)/mL were calculated by the drop count plate method [17]. Aliquots of 1 mL of this suspension (10^9 CFU) were used to inoculate each chickpea seed. The different treatments were: (a) control

plants (noninoculated, without Cr(VI)), (b) plants noninoculated plus Cr(VI) 60 or 120 mg/kg, (c) inoculated plants without Cr(VI), and (d) inoculated plants plus Cr(VI) 60 or 120 mg/kg. The pots were incubated in a chamber with controlled temperature (28 ± 2°C) under photoperiod regime [16 h light (200 µmol/m^2/s)/8 h dark] and relative humidity (70–80%). The plants were irrigated with the Hoagland (½) medium with nitrogen (noninoculated plants) and modified Hoagland (½) solution, without nitrogen (inoculated plants) during the first 6 d; then, water was used for irrigation. After 45 d, plants were harvested and dry weight (mg) of both shoots and roots was registered. Moreover, in inoculated plants nodules were counted. Shoot and root tissues were dried at 70–80 °C and these samples were used for total Cr quantification. Bioconcentration factor (BCF) and translocation factor (TF) were calculated as follows: BCF = Cr concentration tissue/Cr concentration soil. TF = Cr concentration shoots/Cr concentration roots (Cr concentration in shoots was always below the detection limit (<3 mg/kg); therefore its value was considered as 3 mg/kg).

2.4. Chromium quantification

Cr(VI) was determined after the reaction with DPC in the acid solution at 540_{nm}. The reaction mixture contained 500 µL of sample, 500 µL of H_2SO_4 0.2 N and 200 µL of DPC (5 mg/L) in a final volume of 5 mL which was obtained by adding distilled water, according to the APHA (1989) modified method [18]. The OD data were converted to Cr(VI) concentrations using a calibration curve from 0 to 10 mg/L, with a r^2 of 0.988.

For total Cr determination in plant tissues, dried shoots and roots samples were first digested with HNO_3, and total Cr accumulated was analyzed by atomic absorption spectrometry using a Perkin Elmer AAnalyst 400 (AAS).

2.5. Statistical analysis

All experiments were performed three or four times in independent assays. To determine the statistical difference between at least one pair of means, the analysis of variance test (ANOVA) was used. When the assumptions of homogeneity of variance (*Levene* test) and normality (*Shapiro-Wilk* test) were not checked, corresponding transformations were performed using the appropriate functions. To determine significant differences between treatments, *Tukey* test was applied, with a significance level of 0.05 ($p < .05$). The statistical program used was InfoStat (2012e version).

Table 1. Effect of different Cr(VI) concentrations on seed germination and seedling total biomass, after 15 d of treatment.

Cr(VI) Concentration (mg/L)	Germination (%)	Total biomass (mg)
Control	99 ± 1	375 ± 29
20	99 ± 1	303 ± 34*
50	98 ± 1	270 ± 25*
100	97 ± 3	260 ± 17*
150	94 ± 2*	186 ± 10*
250	67 ± 5*	37 ± 15*

Note: Data represent means ± standard error. Asterisk (*) represents significant differences with respect to the control value ($p < .05$).

3. Results and discussion

3.1. Effect of Cr(VI) on chickpea germination and development at early stages of growth

To analyze the effect of Cr(VI) on germination, seeds were exposed to different concentrations of this heavy metal (Table 1). Germination was not significantly affected for concentrations up to 100 mg/L ($p > .05$), however higher concentrations (150 and 250 mg/L) significantly decreased the germination percentage (6 and 33%, respectively) compared to control ($p < .05$).

The seedlings showed a decrease in total biomass in a concentration-dependent manner. The Cr(VI) concentrations between 20 and 100 mg/L produced a reduction of total biomass around 20–25%, whereas 150 and 250 mg/L Cr(VI) reduced the biomass 46 and 90%, respectively ($p < .05$). In general, the radicles length was more affected by Cr(VI) than hypocotyls length, in all treatments (data not shown).

Seed germination is the first physiological process that could be affected by Cr(VI) exposure. For this reason, the capability of seeds to germinate in presence of the contaminant is indicative of their tolerance level [19]. In our study, the highest concentration of Cr(VI) used, reduced a 33% the germination. It has been reported that Cr(VI) affects α and β amylase activities, enzymes involved in starch hydrolysis, resulting in an impaired supply of sugars to the developing embryo axis [7,20]. Moreover, it has been observed that the activity of proteases is increased by the Cr treatment, which could contribute to reduce seed germination [21]. The germination of several plant species such as *Beta vulgaris*, *Raphanus sativus*, *Daucus carota*, *Solanum melongena*, *Solanum lycopersicum* and *Brassica oleracea* var. *acephala*, was decreased between 13 and 30% when 100 mg/L Cr(VI) was used [22,23], whereas in our study the germination percentage was not significantly affected by this concentration. In this context, chickpea would be more tolerant at this metal concentration than the mentioned species. The tolerance level depends on physical and chemical properties of the metal ions as also on the seed coats, which would

determine the extent of heavy metal reaching the embryo tissues [21].

Regarding the effect of Cr(VI) on biomass, Ahmad et al. [24] reported that Cr(VI) could produce a reduction of dry weight as a consequence of the low production, translocation, and distribution of the assimilates at different parts of plants, disrupting their essential metabolic processes. In concordance with our results, several authors described that roots were more affected by Cr (VI) than shoots in *Zea mays* L., *Sorghum*, *B. vulgaris, Solanum lyicopersicum*, among others, probably because the roots are the first organ to come in contact with the contaminant [22,25]. The chickpea growth reduction in the presence of Cr(VI) observed in our work could be related to the inhibition of root cell division and elongation, as a consequence of collapsed tissue, and therefore the inability of roots to absorb nutrients and water [7,26].

3.2. Evaluation of Cr(VI) tolerance and uptake capability of chickpea plants

Based on the results obtained about Cr(VI) effect on germination and development at the early stages of growth, concentrations lower than 100 mg/L were selected to evaluate the tolerance of chickpea plants and Cr(VI) uptake capability.

Figure 1 shows that Cr(VI) significantly decreased dry weight of shoots and roots. When plants were grown in the presence of 10 mg/L Cr(VI), dry weight of shoots and roots decreased 50% and 37%, respectively, while for 20 mg/L treatment this reduction was higher (around 60%) for both tissues. Cr(VI) 50 mg/L totally inhibited plant development. This demonstrated the toxic effect of this metal on chickpea plants growth. Similarly, Maiti et al. [27] reported a linear decrease in plant biomass, as well as in root or shoot dry weight of maize plants by exposition to increasing Cr concentrations.

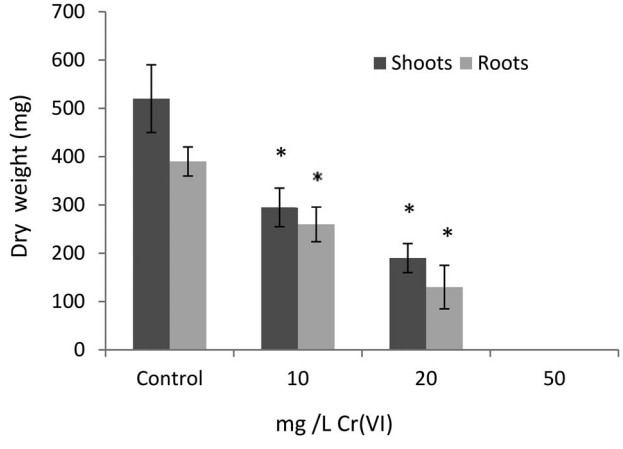

Figure 1. Shoot and root dry weight of chickpea plants, after 30 d exposition to different Cr(VI) concentrations. (*) Asterisks indicate statistically significant differences with respect to the control ($p < 0.05$).

Dasgupta et al. [28] reported that biomass reduction depends not only on metal concentration but also the exposition time. In our study, chickpea germination was not significantly affected by Cr(VI) concentrations up to 100 mg/L (Table 1), however, lower concentrations (50 mg/L) completely inhibited plant development. These results could be due to higher contact of pregerminated seeds with Cr solutions compared with the germination assay in plates, which could cause more toxicity affecting plant ability to survive and normally grow.

To investigate Cr(VI) uptake capacity of chickpea plants, perlite was used as support, because it is inert, and therefore it does not absorb/adsorb Cr(VI). The quantification of Cr(VI) in perlite after 30 d of plants growing, with initial concentrations of 10 and 20 mg/L Cr(VI), showed that they were capable to uptake 50% and 35% of Cr(VI), respectively. With higher concentrations (50 mg/L Cr(VI)), despite plants development was completely inhibited, the Cr(VI) present in the support decreased 6%, which may be due to Cr(VI) adsorption to chickpea seeds (data not shown). The results demonstrated that chickpea plants were capable to uptake this heavy metal.

Phytoremediation can be used as a tool to exploit the metal-accumulation capability of plants to remediate contaminated soils through an economic and ecologically healthy approach [29]. However, considering that chickpea is an important leguminous crop, it is important to determine its accumulation in different parts of plants since this metal could be incorporated into the food chain if it is translocated to grain.

3.3. Growth and removal capability of Mesorhizobium *strains*

Chickpea is usually inoculated with *Mesorhizobium* strains as a biofertilizer with the purpose of increasing the yield production. On the other hand, the inoculation with bacterial strains has been used to reduce the toxic effect and accumulation of heavy metals on several plants species [30]. For this reason, three *Mesorhizobium* strains were used to study their capabilities to grow and remove Cr(VI), in order to select the most suitable one for the inoculation assays. *M. ciceri*, *M. ciceri* UPMCa7 T and *Mesorhizobium mediterraneum* UPMCa36 T were capable to grow in the presence of Cr(VI) 5–20 mg/L, whereas 50 mg/L Cr(VI) significantly affected bacterial growth. When Cr(VI) removal was analyzed, *M. ciceri* emerged as the strain that achieved a higher removal

capability compared with the other strains, thus it was selected for further assays and these results are shown in Figure 2(A) and (B).

This strain reached high removal percentages (95%, 85% and 75%) after 11 d for 5, 10 and 20 mg/L Cr(VI), respectively (Figure 2(B)). Removal capability was strongly reduced when Cr(VI) concentrations of 50 mg/L was added, which was coincident with the low growth detected.

Cr(VI) removal by *Rhizobium* strains such as *R. leguminosarum*, *Mesorhizobium amorphae* and *Mesorhizobium* RC3 has been described [5,31,32]. Different mechanisms can be involved in the microbial removal, such as bioadsorption, Cr(VI) reduction and intracellular accumulation. However, the reduction of Cr(VI) to Cr(III) is the main mechanism described in bacteria. In the environment, Cr(VI) can be reduced to Cr(III), either by abiotic ways or by enzymes called chromate reductases [15]. Cr(III) is relatively harmless due to its low solubility, and therefore this form is 1000 times less toxic than Cr (VI) [33]. Therefore, the presence of chromate reductase enzymes for Cr(VI) to Cr(III) reduction, in *M. ciceri,* would reduce the phytotoxic effects of Cr(VI) on plants development.

3.4. Interaction studies between chickpea plants and M. ciceri

Different values of allowable Cr concentration have been proposed in many countries; nonetheless concentrations of 50–600 mg/kg are often found in contaminated soils [34]. Thus 60 and 120 mg/kg Cr(VI) were selected for interaction studies. Figure 3 shows that the presence of Cr(VI) in the medium of noninoculated plants significantly reduced shoots and roots dry weight compared to control conditions (without Cr(VI)) ($p < .05$). When plants were inoculated and treated with Cr(VI) (60 and 120 mg/kg), *M. ciceri* produced a significant positive effect on shoots and roots biomass compared with noninoculated plants exposed to Cr(VI).

Similarly, Wani et al. [3] reported that chickpea plants grown with 136 mg/kg Cr(VI) showed a poor growth and reduced biomass. However, when plants were inoculated with *Mesorhizobium* strain RC3, dry weight and protein concentrations were increased. Also, Wani and Khan [35] indicated that the inoculation of wheat with Cr-resistant bacterial strains improved the general plants state as well as growth parameters.

In these sense, diverse plant-growth-promoting bacteria (PGPB) have been reported by their capability to expedite the plant growth and development against various environmental stresses, including metal stress [36]. On the other hand, plants inoculated with PGPB exhibiting Cr(VI) removal capability have shown better adaptation when they grew in chromium-contaminated soils, therefore these beneficial bacteria could induce changes in plant metabolism (extensive proliferation of roots mediated by phytohormone production, for better nutrient absorption, presence of chromate reductases, increased bacterial siderophore-mediated metal uptake, phosphate solubilization and upregulation of genes involved in stress mitigation) thereby the plants become more tolerant to chromium stress [29].

Furthermore, the total number of nodules significantly decreased with increasing Cr(VI) concentrations (25% and 60%) for 60 and 120 mg/kg Cr(VI), respectively ($p < .05$) (Figure 4). This heavy metal could negatively affect the nodulation process and the functionality of nodules, since the enzymes activity involved in biological nitrogen fixation could be diminished. In this sense, the Cr(VI) application in *Cyamopsis tetragonoloba* (L.) Taub. plants, negatively affected nitrogen metabolism by inhibiting the activity of several enzymes (nitrogenase, nitrate reductase, nitrite reductase, glutamine synthetase and glutamate dehydrogenase), possibly as a result of its interference with the key enzymes of nitrogen metabolism and photosynthetic pigments [37].

3.4.1. Total Cr determination in plant tissues

In view of the fact that chickpea and its symbiont were capable to independently remove different Cr(VI) concentrations we analyzed the effect of the inoculation on total Cr bioaccumulation in plant tissues, since it is a crop of agronomic and nutritional value. For this reason, Cr determination in plant tissues, as well as the translocation to grain is of great importance to human life. Bioaccumulation includes all processes responsible for the uptake of available metal ions by living cells. It includes biosorption (or passive uptake) and intracellular accumulation and bioprecipitation mechanisms [38].

Table 2 shows total bioaccumulated Cr in roots and Cr accumulated in shoots of plants growing at different conditions. As expected, the total Cr concentration in plants growing in the control condition (without Cr(VI)) was below the sensitivity of the detection technique (<3 mg/kg).

Noninoculated plants exposed to Cr(VI) bioaccumulated the metal in roots, and this accumulation was dependent of Cr(VI) concentration, since total Cr detected was practically twice for a double Cr concentration. Nevertheless, when plants were inoculated, total Cr bioaccumulation was significantly increased (75 and 62%) for treatments with 60 and 120 mg/kg Cr(VI), respectively. These results are in agreement with BCF values, which were higher in inoculated than noninoculated plants, however, these values were always <1 . In this sense, Retno et al. [39] also demonstrated that the

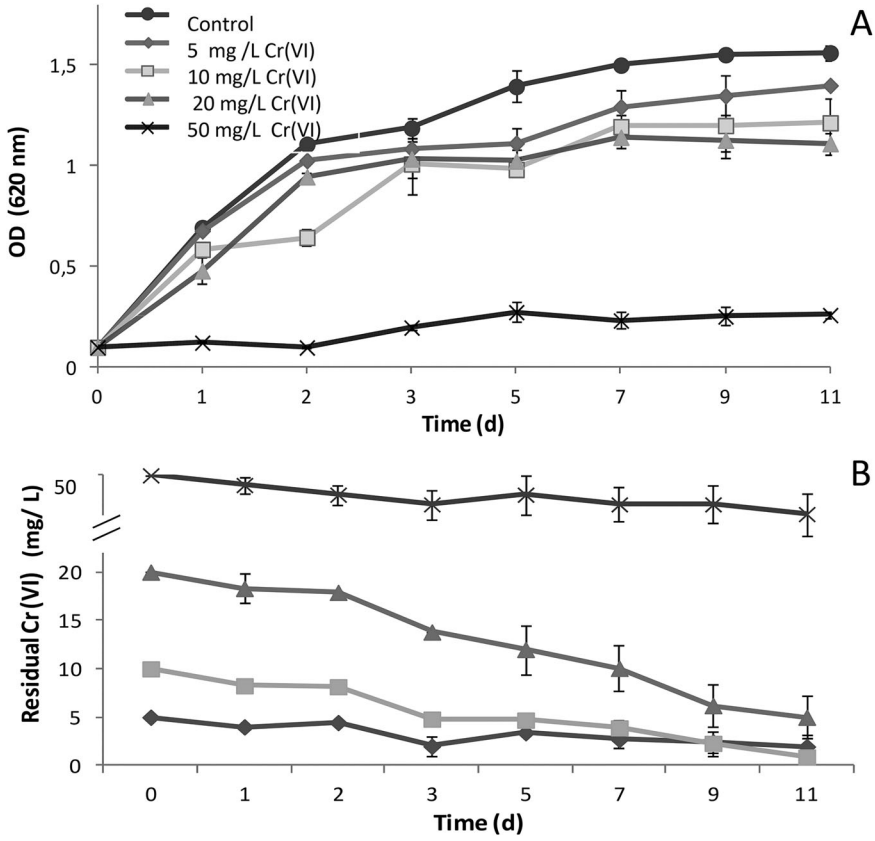

Figure 2. Growth (A) and removal capability (B) of different Cr(VI) concentrations by *M. ciceri* in the YEM liquid medium.

inoculation with a rhizobacteria (I30) increased Cr concentration in the roots of maize plants.

It is important to note that in inoculated and noninoculated plants exposed to both Cr(VI) concentrations, the metal was not detected in shoot tissues, therefore, TF were very low. These data clearly reflect that Cr is mainly retained in roots and the low possibility of Cr accumulation in seeds.

Similar to our work, other crops such as *Arachis hypogea* and *Zea mays*, growing in the presence of Cr,

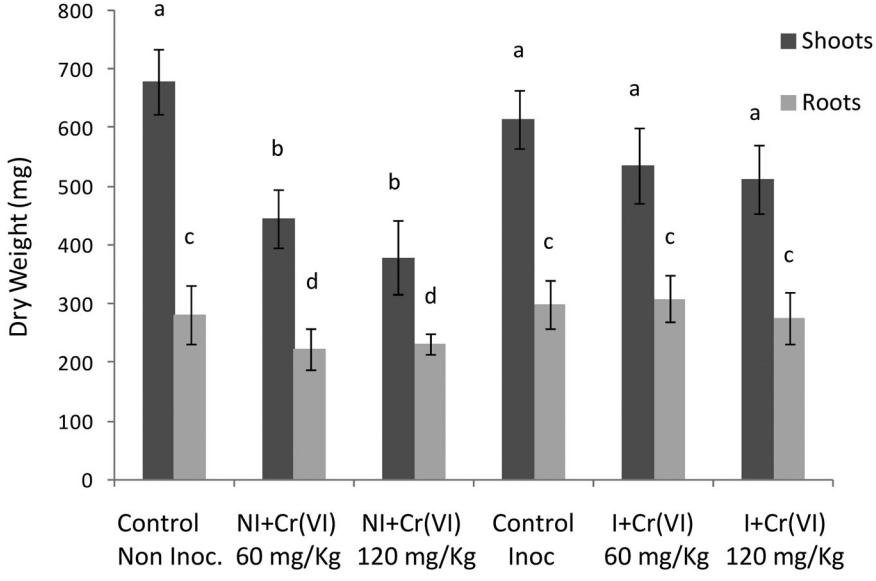

Figure 3. Shoots and roots dry weight of chickpea plants growing during 45 d in presence of Cr(VI) and inoculated with *M. ciceri*. Different letters indicate significant differences between different treatments ($p < 0.05$). NI: noninoculated. I: inoculated.

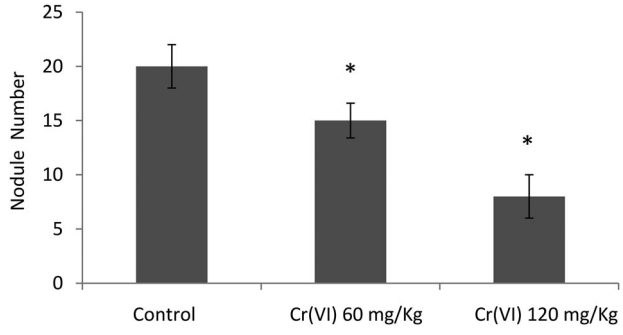

Figure 4. Nodule number (per plant) of inoculated plants with *M. ciceri* in presence of different Cr(VI) concentrations. Asterisks (*) indicate statistically significant difference with respect to control plants without the contaminant ($p < 0.05$).

accumulated higher concentrations of the metal in roots than in aerial parts [7,28,40,41]. This high metal accumulation in roots could be due to the presence of different functional groups with high exchange capacity, such as carboxyl and hydroxyl derived from different cell wall polysaccharides. These groups could transform the roots in an efficient matrix for cation exchange, reducing Cr translocation to aerial parts [42]. Other mechanism of Cr accumulation in roots could be related with its capability to reduce Cr(VI) to Cr(III). Cr(III) can be retained by the cells of root cortex as nonsoluble compounds, such as Cr-EDTA, Cr-phytochelatins and/or Cr-metallothioneins. These complexes may be immobilized in the vacuoles of root cells, as less toxic forms [43]. In this sense, Qiu et al. [44] demonstrated that root cell walls and vacuoles were the main plant subcellular compartments for Cr accumulation in rice seedlings.

The inoculation of chickpea plants with *Mesorhizobium* besides stimulating plant growth also enhanced metal accumulation in roots. These results are similar to those described by Luo et al. [45], who described that plant-growth-promoting *Bacillus* sp. increased biomass production as well as manganese and cadmium uptake by sweet sorghum. It is well known that rhizobacteria influence growth, yield and nutrient uptake of plants growing in a contaminated soil by different mechanisms. They may help plants by increasing supply of nutrients, such as phosphorus, sulfur, iron and copper, and producing plant hormones. They may also reduce toxic effects of heavy metals on plants through the secretion of acids, proteins and other chemicals that serve as an effective metal sequestering [46]. In this regard, PGPB increase the bioavailability of chromium in soils for phytoextraction by producing various primary and secondary metabolites, such as siderophores and organic acids [47]. In addition, bacterial biosurfactants also increase the phytoavailability of metals, including chromium, as the they help in releasing metals that are strongly bound to soils [47,48].

In spite of adsorption to cell surface or Cr uptake into the cells are among the potential mechanisms involved in Cr accumulation by plants, more studies should be performed to determine the main mechanism involved in chickpea plants. Furthermore it is important to note that the presence of *M. ciceri*-enhanced Cr accumulation in roots, however, no translocation to aerial parts was observed, suggesting that the accumulation of Cr in grains would not be probably. This result is of great value considering the potential impact of Cr in the food chain.

Therefore, these bacterial strains do not only facilitate protection and promotion of plant growth from harmful impact of metal toxicity, but also can facilitate the remediation of metal-contaminated soils when they are used as bioinoculants [36].

In this context, the application of PGPB in phytoremediation, which may be directed chiefly to either accumulating of toxic metal species in plant tissues through phytoextraction in moderately contaminated soils or to mitigate the metal-generated toxic effects on plants through phytostabilization in extremely polluted sites, has gained wider acceptability due to its excellent performance in augmenting the remediation efficiency as well as growth of plants [49].

For this reason, the combined use of plants and growth-promoting rhizobacteria could have a synergistic

Table 2. Total Cr bioaccumulation in shoots and roots in inoculated and noninoculated plants treated with different Cr(VI) concentrations.

	Total Chromium (mg/kg)			
Cr(VI) concentration	Shoots	Roots	Bioconcentration factor	Translocation factor
0	ND	ND	–	–
60 mg/kg				
Noninoculated	ND	20.5 ± 2.3	0.34	<0.14
Inoculated	ND	36.0 ± 1.8 (*)	0.60	<0.08
120 mg/kg				
Noninoculated	ND	38.5 ± 3.1	0.32	<0.078
Inoculated	ND	62.5±2.5 (*)	0.52	<0.048

Note: ND: Not detectable below the detection limit (<3 mg/kg).
Asterisk (*) represents significant differences between inoculated and noninoculated ($p<.05$).

effect that allow increasing yield crops and enhance phytoremediation of Cr-contaminated soils, using an environmentally and economically sustainable technology.

4. Conclusions

Cr(VI) negatively affected chickpea plants development. However inoculation with *M. ciceri* enhanced plant growth and Cr accumulation in roots. The present observations suggest that the capability of *Mesorhizobium* to remove Cr(VI) could be exploited for bioremediation and to enhance the legume productivity in Cr-contaminated soils. In such soils, in which the metal content exceeds the limit of plant tolerance, it may be possible to inoculate plants with bacteria possessing metal detoxifying characteristics as well as plant-growth-promoting properties thereby stabilizing the vegetation and remediating these metal-polluted soils. Thus, chickpea plants would represent a natural system able to phytoremediate or phytostabilize Cr *in situ*, and this potential could be enhanced when plants are inoculated with *M. ciceri* strain. This strategy would be considered as a bioremediation tool with great economic and ecological relevance.

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