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Study of the heavy metal phytoextraction capacity of two forage species growing in an hydroponic environment

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ABSTRACT

Sorghum and alfalfa are two important forage crops. We studied their capacity for accumulating heavy metals in hydroponic experiments. Cadmium, nickel (as divalent cations) and chromium (trivalent and hexavalent) were added individually to the nutrient solution in a range of concentrations from 1 to 80 mg/l. Cr(III) was complexed with EDTA to increase its bioavailability. In alfalfa the increases in the concentration of Cr(III) and Cr(VI) favoured translocation of the metals to the upper parts of the plants, while with Ni(II) the level of translocated metal remained almost unchanged. In sorghum, both Cr(VI) and Ni(II) produced similar results to those in alfalfa, but increases in the concentrations of Cd(II) and Cr(III) in the solution lead to a higher accumulation of the metal at the root level. The concentrations referred to the dry biomass of alfalfa were 500 mg/kg (aerial parts) and 1500 mg/kg (roots) of Cr(III), simultaneously enhancing plant growth. Sorghum captured 500 and 1100 mg/kg (in aerial parts) and 300 and 2000 mg/kg (in roots) for Ni(II) and Cd(II) respectively, without significant damage to its biomass. The results show that alfalfa and sorghum can not only grow in the presence of high heavy metal concentration but also capture and translocate them to the aerial parts; because of these results special attention should be given to these crop plants for their possible use in phytoremediation of large contaminated areas but especially to avoid the possible introduction of the metals accumulated in aerial parts into the food chain when those plants grow in contaminated areas.

1. Introduction

The concentration of heavy metals in the soils and in the water sources has increased continuously through time due to diverse human activities [1–3]. Many of those metals in high concentrations can be harmful [4–7] and, in contrast to the situation with organic substances, the metals remain almost indefinitely in the environment unless a process is applied to remove them. Nickel and cadmium are heavy metals released as divalent cations into the environment by different industrial activities; except at high pH values those cations have high mobility and consequently high bioavailability. Chromium is a heavy metal with many technical applications in Argentina and for this reason it is one of the common heavy metal in polluted sites. Although the trivalent state has low solubility in non-acidic water, the acidification or its complexation can alter its mobility and bioavailability [8]. The hexavalent chromium is very soluble in a wide range of pH values and it is 100-fold more toxic than the trivalent chromium [9–11]. In addition, Cr(VI) is a recognized genotoxic human carcinogen.

The physicochemical removal strategies have high costs associated with them and they frequently cause unwanted collateral damages [12]. Phytoremediation, a technology based on the use of plants to uptake and accumulate metals from soils and water, has gained interest during the last decades since it means economic advantages compared with conventional treatments and in addition it minimizes the environmental impact [13,14].

The normal concentration of heavy metals in many terrestrial plants has been analyzed by different researchers throughout the years [15–17]. Some of those plants, referred to as hyperaccumulators, are capable of accumulating large quantities of these metals in their biomass [18,19]. Sorghum (*Sorghum bicolor* L.) and alfalfa (*Medicago sativa* L.), are two forage species adapted to climate conditions in Argentina, whose physiological characteristics (production of important root systems and high biomass harvests) make them very interesting for phytoremediation purposes. In this sense, previous studies have shown the possibility of utilizing them to

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remedy soils contaminated with organic [20–22] and inorganic pollutants, including heavy metals [23–28]. Nevertheless there are few results about their use under hydroponic conditions. The use of terrestrial plants instead of aquatic ones for phytoremediation has some advantages [29], such as lower water content in the terrestrial plants (which helps with the drying, compaction and incineration) and the fact that they have a big root system which allows for more surface area to capture the metals.

Precisely, the purpose of this study was to evaluate the metal extraction capacity of sorghum and alfalfa growing in hydroponic conditions, focusing the case of Cd (II), Ni(II), Cr(VI), and Cr(III), made partially soluble by complexing (simulating what occurs in nature) with EDTA. Our results show the phytoextraction potential of those plants and their possible use to clean up effluents or soils moderately contaminated with cadmium, nickel and chromium. On the other hand, since alfalfa and sorghum crops are intensively and widely used for animal-feeding among other applications in many countries, our results are a strong wake-up call for the risk to introduce those metals into the food chain through inadvertent contact of those plants with contaminated sites.

2. Materials and methods

2.1. Plant material and set up of the hydroponic cultures

Sorghum seeds (*Sorghum bicolor* L. variety *Hibrido forrajero*) were obtained from cultivating Pastizal Plus-INTA (1999) and alfalfa seeds (*Medicago sativa* L. variety *Bárbara SP*) were obtained from cultivating Cuenca del Salado Castelli-INTA station (1999). Seeds were superficially sterilized by contact with ethanol solutions 70% v/v for 1 min, followed commercial bleach 20% v/v for 30 min (under agitation in orbital shaker at 100 rpm). Finally the seeds were rinsed with sterile distilled water under agitation for 10 min, process repeated 5 times. The seeds were placed over moist filter paper disks in Petri dishes under sterile conditions and they were stored in the dark at room temperature for germination. The hydroponic units consisted of glass jars (7 cm in diameter × 14 cm height) containing Jensen medium as nutrient solution [30], with pH adjusted to 6.8. Plastic tripods (5 cm in diameter and 6 cm height) with openings at the top were placed inside the jars to hold the seedlings. The hydroponic units were aired through a system of flexible plastic tubes immersed in the solution, connected to air pumps. The jars were covered with a dark paper to prevent the development of photosynthetic algae. After 2 days of germination, 12 seedlings of sorghum or alfalfa with the same size were assembled in each hydroponic unit, letting the roots pass through the openings until they reached the solution. The volume of nutrient solution was kept at a constant value throughout the experiments, by adding sterile distilled water when necessary.

2.2. Uptake, distribution and accumulation of metals, Ni(II), Cd(II) Cr(III) and Cr(VI)

In order to evaluate the capacity of metal rhizofiltration of the plants, three hydroponic units per treatment were supplemented with Cd(II), Ni(II), Cr(III)/EDTA or Cr(VI) obtained from the following salts $CdSO_4$, $NiSO_4{\cdot}6H_2O$, $CrK(SO_4)_2{\cdot}12H_2O$ and $K_2Cr_2O_7$. The final concentrations in the hydroponic solutions were as follows: 20, 40, 60 and 80 mg/l for Cd and Cr(III), 20, 40, 60 and 70 mg/l for Ni and 2, 5, and 10 mg/l for Cr(VI). The adjusting of the pH value was made to 6.8 in all of the cases. In the treatments with Cr(III) the metal was previously complexed with EDTA (0.1144 g/l) in order to maintain it soluble in that pH condition.

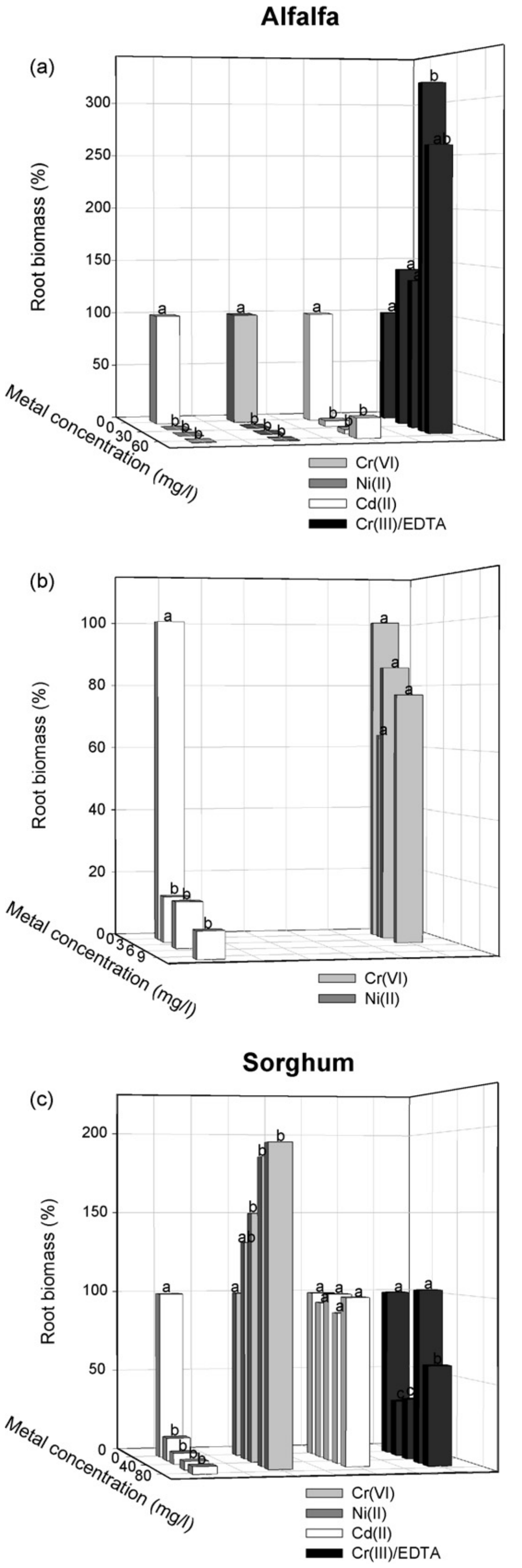


Fig. 1. Biomass of the root system for alfalfa (a and b) and for sorghum (c), after 30 days of growth in a hydroponic environment containing Cd(II), Ni(II), Cr(III)/EDTA or Cr(VI). Graphs (a) and (b) correspond to high and low metal concentrations respectively. Each bar represents an average of 30 plants; in each series with the same metal, data with the same letter indicates that no statistically significant differences among treatments were found (one factor ANOVA and LSD test, $\alpha = 0.05$).

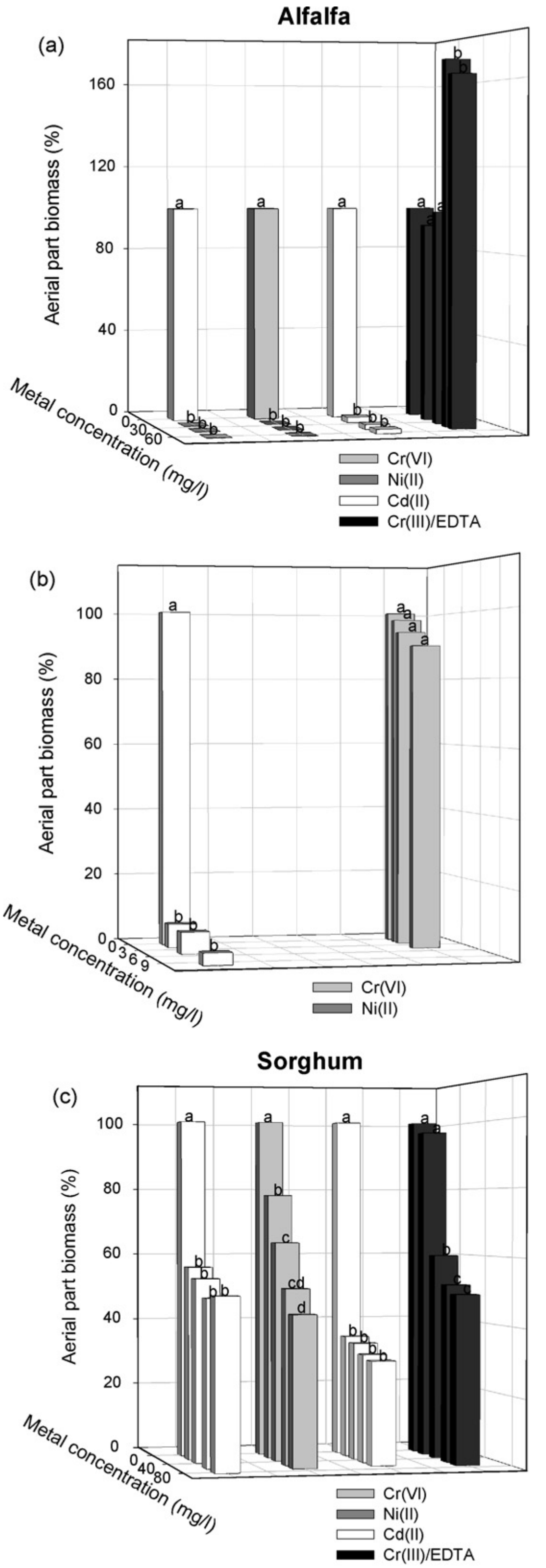


Fig. 2. Biomass of the aerial part for alfalfa (a and b) and for sorghum (c), after 30 days of growth in a hydroponic environment containing Cd(II), Ni(II), Cr(III)/EDTA or Cr(VI). Graphs (a) and (b) correspond to high and low metal concentrations respectively. Each bar represents an average of 30 plants; in each series with the same metal, data with the same letter indicates that no statistically significant differences among treatments were found (one factor ANOVA and LSD test, $\alpha = 0.05$).

The cultures were allowed to grow for 30 days in a greenhouse under the following conditions: 16 h photoperiod, light intensity at 500 μmoles/m^2 at 26–28 °C. After that period, the plantlets were harvested for biomass and chemical analyses and the levels of metals in the solution quantified.

2.3. Analytical methods

After the end of the assay, the contents of the metals studied in the roots and aerial parts as well as growth parameters of alfalfa and sorghum plants were analyzed. The plant roots were thoroughly rinsed in abundant distilled water and were separated from the aerial parts in order to determine their respective dry weights (oven dried at 60 °C, until constant weight). Metal contents in plant tissues was determined after by acidic digestion with $HNO_{3\ (c)}/H_2SO_{4\ (c)}$ (initial volume ratio of 4:1). Later volumes of $HNO_{3\ (c)}$ were added until total oxidation of the organic matter [31]. The measurement of the metal content in the extracts was accomplished through AAS (using a Shimazdu AA 6650F Atomic Absorption Spectrophotometer).

2.4. Data analysis

The growth of the plants was evaluated in relative terms with respect to the control (in the absence of heavy metals) referring it the base value of 100%. Metal concentration into the plants was recorded as mg of metal per kilogram of dry biomass. To quantify the translocation of heavy metals from the roots to the harvestable aerial parts it was used the translocation factor (TF) [32], which is calculated as the ratio of the metal concentration in the shoots to the metal concentration in the roots, on dry biomass bases.

In the statistical analysis of the data a single factor ANOVA was used, while, for the comparisons of the measurements between treatments a least significant difference (LSD) test was used with a confidence level of 95%, according to suggestions by previous researchers [33].

3. Results and discussion

3.1. Plant growth

The effects the tested metals, Ni(II), Cd(II), Cr(III)/EDTA and Cr(VI) had on alfalfa and sorghum corresponding to root and aerial parts biomass are shown in Figs. 1 and 2, respectively.

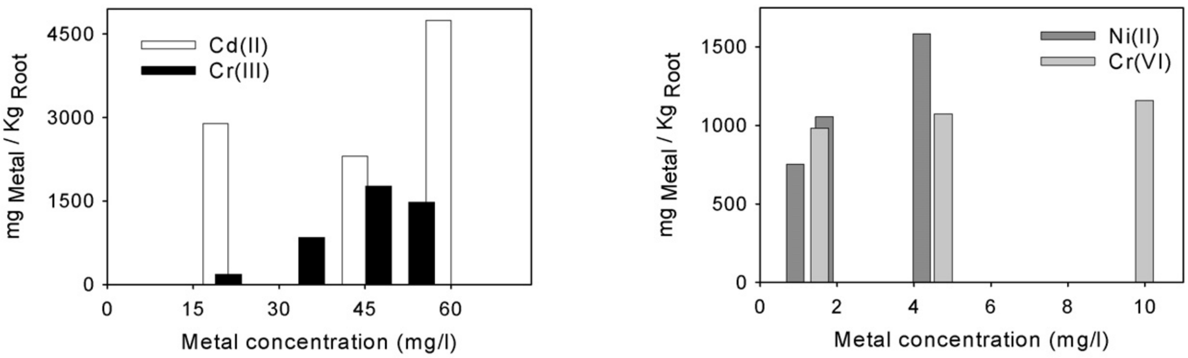
Only in the experiments with Cr(VI) and Ni(II), the concentrations containing between 20 and 80 mg/l produced a complete deterioration of the alfalfa seedlings. Results from experiments conducted with a lower concentration range, between 0 and 10 mg/l, as shown in Figs. 1(b) and 2(b).

As can be noted in the previous figures, Cr(VI) is the element which caused the highest damage (90–95% biomass reduction, compared to the control) at the root level of both plants and even with the lower concentrations. This result coincides with that found for *Phaseolus vulgaris* L. [34], where damage to the cellular tissue was noted, as well as alterations in their essential mineral content.

The presence of Cr(III)/EDTA inhibited the development of root growth in sorghum (up to 60% for the highest concentration); Cd(II) did not affect the growth in the complete range of evaluated concentrations, and on the other hand, the presence of Ni(II) stimulated the growth in a manner proportional to the solution concentration (up to 190% for 70 mg/l).

In alfalfa the presence of Cd(II) significantly reduced root biomass production (up to 82%), while Cr(III)/EDTA strongly stimulated the production (258% when a solution concentration of 53 mg/l was supplied). The presence of Ni(II) did not appear to

(a) alfalfa



(b) sorghum

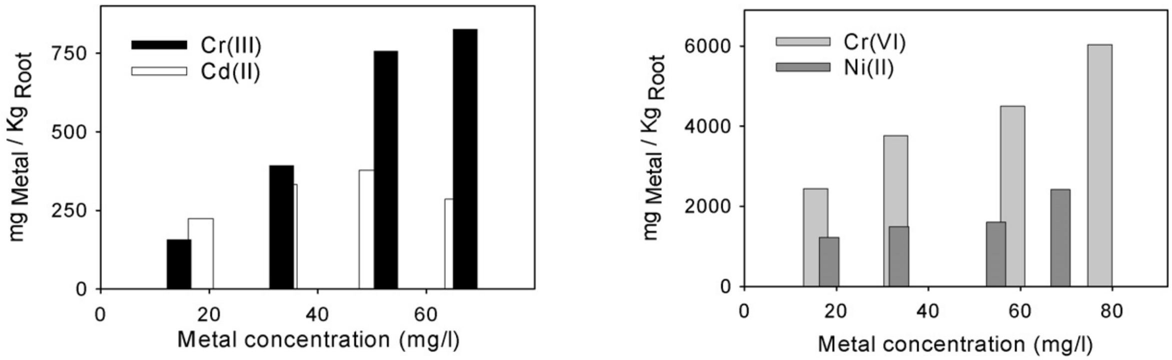


Fig. 3. Metal uptake by roots in alfalfa (a) and sorghum (b).

affect in any significant manner the alfalfa roots in this range of concentration (20–70 mg/l), within the significant level determined ($\alpha = 0.05$).

Different from many reports in which the influence of Cr(III) was analyzed in conditions where most of it is precipitated, this study added EDTA as a complexing agent, which was able to maintain the metallic ion soluble during the experiments. As a function of discriminating the true effects of Cr(III), in parallel experiments it was proved that the addition of EDTA in general negatively affected the development of those two plants (data not shown). Notwithstanding, the positive effects of Cr(III) partially neutralize these difficulties for sorghum. In the case of alfalfa those negative effects are completely overcome by the positive effects produced by the addition of Cr(III).

As can be noted in Fig. 2, all metals affected the development of the aerial parts of the sorghum and the greatest decreases noted were of 70% for cadmium and 50% for all the other metals.

In the alfalfa tests, Cd(II) also reduced by a significant factor the biomass production of the aerial parts (up to 98%). This coincides with the observations of this plant growing in clay soils [26], even though the damage noted in this case was not so significant (probably due to less bioavailability and exposure time). The reduction of growth caused by the cadmium seems to have a direct relationship not only with the reduction of chlorophyll content, which was verified in our research (from 3.15 μg total chlorophyll/mg fresh weight for the control to 1.64 μg total chlorophyll/mg fresh weight for the maximum concentration of cadmium evaluated), following the described methods [35] but also with the photosytem I activity, tested by other researchers [36,37].

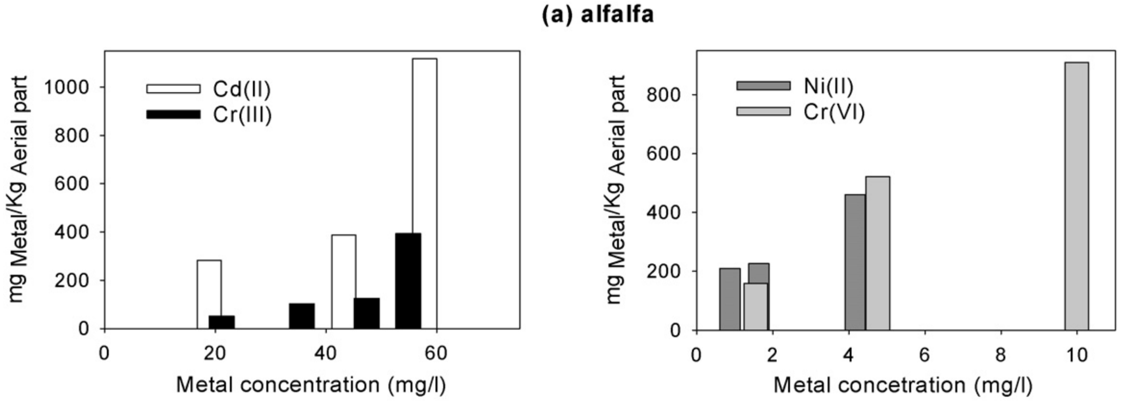
As part of our study, the plants were in contact with the nutritive solution containing the heavy metals from the beginning of their growth (two days after germination) and it is possible that they were more vulnerable to the metallic stress due to this. The aforementioned coincides with the information recorded for *P. coccineus* [37], where Cd(II) affected the young plants more than those which were more mature. Notwithstanding, the fact that the alfalfa was able to withstand the exposure to cadmium is a relevant result in our study, since in other tests [38] only a very small concentration of Cd(II) was needed, such as 10^{-2} M (approximately 1 mg/l) of Cd(II), to cause their complete deterioration.

The presence of Cr(VI) also significantly affected the aerial parts of the alfalfa (maximum inhibition of 96% for 10 mg/l). This observation coincides with that reported for *Nymphaea alba* L. [39], where concentrations between 5 and 10 mg/l produced a chlorophyll content reduction and a reduction of the activities of diverse enzymes. Opposite of what was observed for Cr(VI) yet very similar to the findings for roots, Cr(III)/EDTA was able to strongly stimulate the growth of the aerial parts of alfalfa up to 160%; on the other hand, the presence of Ni(II) did not affect it significantly ($\alpha = 0.05$).

3.2. Heavy metal uptake

The quantities (in mg of metal per kg of dry biomass) of cadmium, chromium and nickel accumulated in the tissue of the alfalfa and sorghum plants, grown in a hydroponic environment containing different concentrations of Cd(II), Cr(VI), Ni(II) or Cr(III)/EDTA are shown in Figs. 3 (roots) and 4 (aerial parts).

For alfalfa, as the concentrations of all the metals in solution increased so did the quantities of these taken up into the plants, in the root system as well as in the aerial parts, with the exception of the treatment with Cr(VI) in which the roots maintained a constant metal concentration value. The maximum concentrations reached in the aerial parts were around 500 mg/kg for Cr(III)/EDTA and Ni(II) and of 1000 mg/kg for Cd(II) and Cr(VI). Results for the roots



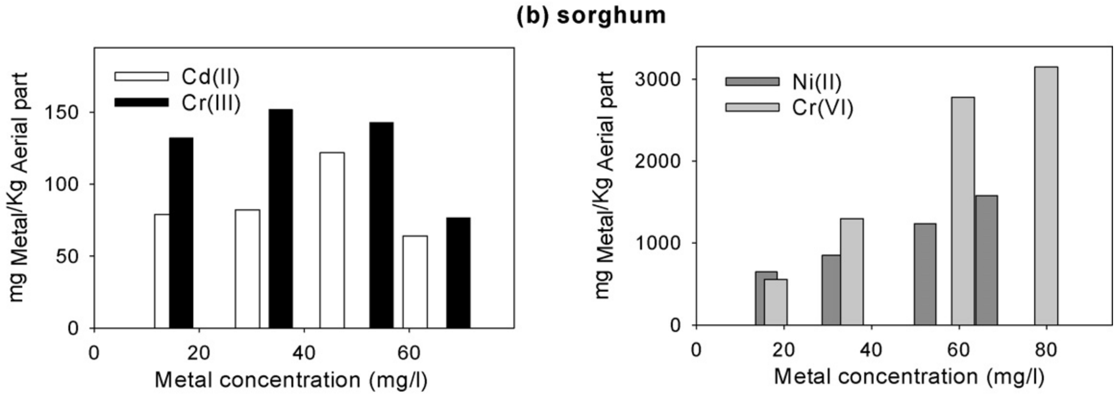


Fig. 4. Metal uptake by aerial parts in alfalfa (a) and sorghum (b).

were 1000 mg/kg of Cr(VI), 1500 mg/kg of Ni(II) and Cr(III)/EDTA and 4500 mg/kg for Cd(II). Similar values were previously found for alfalfa growing in the soils [28]. High concentrations of cadmium can be introduced into the plant as stable complexes that this metal can form with chloride [40,41].

Except for nickel for which the translocation into the aerial parts of the plant was not altered by the change of concentration of the metal in the solution, for the rest of the metals the translocation increased as the concentration increased.

For sorghum, increases in the concentrations of the metals in solution led to a higher accumulation of these at the root level. The concentration also increased in the aerial portions for the tests containing Ni(II), Cr(VI) and Cd(II) (concentration<60 mg/l); nonetheless, for Cr(III)/EDTA (concentration<60 mg/l) the metal concentration in the aerial parts remained at an almost constant and independent level regardless of the applied dose.

The maximum concentrations reached in this study for the aerial parts was around 500 mg/kg for Cd(II) and Cr(III)/EDTA, 1100 mg/kg for Ni(II) and of 3000 mg/kg for Cr(VI); while in the roots the values obtained were 300, 800, 2000 and 5800 mg/kg for Cd(II), Cr(III)/EDTA, Ni(II) and Cr(VI), respectively.

Unlike the alfalfa results, only in the treatments with Cr(VI) the increase in solution concentration favoured their translocation to the aerial parts of the plants.

In the presence of Ni(II), the sorghum plants behaved in a similar way to the alfalfa plants and the amount translocated remained practically constant for all the concentrations tested.

Based on the analysis of the total quantity of metal absorbed by alfalfa and sorghum, their potential capacities for the extraction and bioaccumulation of these were evaluated. The numeric values obtained were between 400 and 500 of Cr(III) for both tests; 1600 and 250 mg/kg of Cd(II), 600 and 2000 mg/kg of Ni(II), and 1000 and 3000 mg/kg of Cr(VI) respectively for the total biomass of alfalfa and sorghum.

In alfalfa, the increases in the concentration of Cr(VI), Cd(II) and Cr(III)/EDTA, favoured the translocation of the metal to the aerial parts of the plants.

The maximum values for the translocation factor (TF) found in this plant were 0.79 for Cr(VI), 0.24 for Cd(II), 0.27 for Cr(III)/EDTA and 0.29 for Ni(II).

In sorghum and for all the metals evaluated, increases in their concentration in a solution lead to a greater accumulation at the root level. Nonetheless, with respect to the possibility that translocation would occur, the actual situation is very different to that of alfalfa; only in the case of Cr(VI) increases in the metal solution concentration lead to higher translocation of this metal, reaching a value of 0.52 of the aforementioned coefficient. For the rest of the metals (Ni(II), Cd(II) and Cr(III)), these values were 0.77, 0.32 and 0.39 respectively. Therefore, in this test, the maximum efficiency of translocation is that of Ni(II).

4. Conclusions

This research study found that alfalfa as well as sorghum is able of growing in liquid environments that are supplemented with chromium (in both of its most important oxidation stages), nickel and cadmium. In general, sorghum was less affected by the presence of the metals. The greatest inhibition in the root system development were caused by Ni(II) and Cr(VI) while Cr(III) had a positive effect on the growth of both plants. In all the tests for both plants, there was low but significant extraction of metals, reaching 400 and 500 mg/kg of Cr(III), 1600 and 250 mg/kg of Cd(II), 600 and

2000 mg/kg of Ni(II), and 1000 and 3000 mg/kg of Cr(VI) respectively for alfalfa and sorghum total biomass. In general terms, the translocation to the aerial parts was much more effective for alfalfa than for sorghum, being the exception Cr(VI) and Ni(II) which were efficiently translocated for plant species.

The results found in this study show that while they may not be hyperaccumulator species, both plants could be important in the use of metal phytoextraction processes, such as those tested in this study. Notwithstanding, and even more importantly, alfalfa and sorghum are two very important forage plants in Argentina and they are incorporated in great quantities into the food chain. According to our results, they are not only capable of growing in the presence of high concentrations of these metals but that they are also able to incorporate them into their biomass and translocate them into the aerial portions of the plant. Even more, according to our experiments the amount of metals present in the plants largely overcome the allowed values in animal foods which are 10, 400 and 1000 mg/kg for Cd, Ni and Cr respectively. That is why, our results mean a strong wake up call to prevent the inadvertent contact of these plants with contaminated environments which could lead to the incorporation of heavy metals into the food chain.

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