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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.

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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.

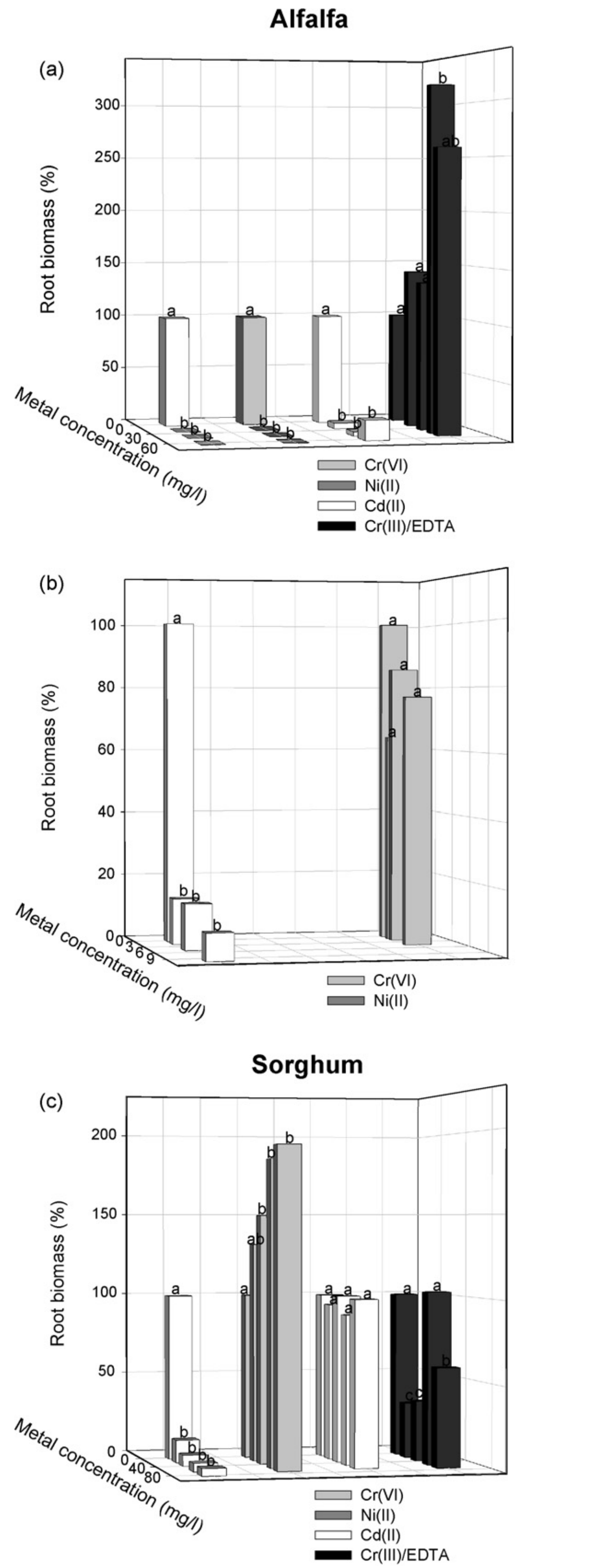

Fig. 1. Biomass of the root system for alfalfa (a and b) and for sorghum (c), after 30 days of growth in a hydroponic environment containing Cd(II), Ni(II), Cr(III)/EDTA or Cr(VI). Graphs (a) and (b) correspond to high and low metal concentrations respectively. Each bar represents an average of 30 plants; 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, $\alpha = 0.05$).

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