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Phytoextraction of heavy metals from a multiply contaminated dredged sediment by chicory (*Cichorium intybus* L.) and castor bean (*Ricinus communis* L.) enhanced with EDTA, NTA, and citric acid application

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ABSTRACT

The remediation of contaminated dredged sediments is necessary to eliminate the risk towards human beings or the environment when there is disposal on land. A greenhouse experiment was carried out to evaluate the chemically assisted phytoextraction to clean up dredged sediment contaminated with Cr, Cu, Pb, and Zn. The ability of castor bean and chicory to absorb, translocate, and accumulate metals from sediment to root and shoot was evaluated by applying EDTA (5 mM), NTA (5 mM), and citric acid (60 mM) to sediment, before the harvest. Citric acid 60 mM was the most effective treatment in increasing Cr, Cu, and Pb in castor bean and chicory shoot. Chicory could accumulate 1730 mg Cr kg^{-1} in shoot, and had greater values than one for the bioaccumulation and translocation factors when citric acid was added to the sediment. But, the Cr percentages removed per harvest of chicory were 0.05% and were lower for Cu, Pb, and Zn due to low biomass obtained. Citric acid-assisted phytoextraction with chicory can be a promising short time solution to reduce Cr concentration in sediment and reach the Cr level guide for industrial land use only if suitable agronomic practices could be implemented to increase crop yield.

KEYWORDS
Chelating agent; phytoextraction; heavy metals; dredged sediment

Introduction

Sediments are the principal compartment of aquatic systems where pollutants generated by human activities accumulate. Periodic dredging of the sediments is necessary for the deepening of ports and waterways, as well as for environmental restoration (Akcil et al. 2015). The disposal of contaminated sediments is seldom regulated and usually involves quick cheap solutions (*i.e.*, margins or open seas disposal) that may have harmful effects on human health and the environment (Magdaleno et al. 2008; Biruk et al. 2017). This is why remediation of dredged material is considered as a management option. Phytoextraction emerges as a cost-effective remediation technology, based on the ability of plant species to accumulate and remove contaminants (mainly metals) in soils, sediments, and water ways (Laghlimi et al. 2015). One of the main factors that restrict the effectiveness of this technique is the low availability of the metal in soil for its absorption by roots (Chaney 1988; Quartacci et al. 2006; Evangelou et al. 2007). Heavy metals can be solubilized by acidification of the soil and by the addition of amendments (*i.e.*, organic compounds) that form soluble metal complexes (Smolinska and Szczodrowska 2017). The use of natural and synthetic organic chelating agents has been investigated as a strategy to cause the desorption of the metals in the soil/sediment matrix, facilitating root absorption, transport in the xylem, and the translocation from the root to the aerial part of the plants (Mahmood, 2010).

Ethylene diamine tetraacetic acid (EDTA) is the chelating agent that has been widely used in phytoextraction studies owing to its high efficiency for the mobilization of many metals. However, EDTA and its metal complexes are persistent in the environment due to their low biodegradability (Shahid et al. 2014) and the leachates generated may be toxic for plants and soil microorganisms and/or pollute underground waters (Luo et al. 2006). Because of the reasons mentioned above, the use of biodegradable chelating agents such as ethylene diamine disuccinic acid (EDDS), nitrilo triacetic acid (NTA), and natural low molecular weight organic acids (LMWOA) has been proposed (*i.e.*, succinic acid, malic acid, oxalic acid, and citric acid). Most studies that investigated the efficacy of phytoextraction with LMWOA have been carried out with the same doses (lower than 10 mM) than those used with aminocarboxylic acids (*i.e.*, EDTA, EDDS, and NTA). For those doses, LMWOA generally have shown lower efficiency than aminocarboxylic acids to facilitate the phytoextraction process (Do Nascimento et al. 2006; Evangelou et al. 2006; Chen et al. 2012). In addition, to date, most of the research has been carried out on hydroponic crops (Hernández-Allica et al. 2007; Zaier et al. 2010) and in soils artificially contaminated with metals (Wu et al. 2004; Do Nascimento et al. 2006;

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Saima et al. 2010). Thus, results may be very different from those obtained in more realistic scenarios, such as in anthropogenically contaminated soils/sediments of fine textures, for which metals present generally very low availability.

The Matanza-Riachuelo River is located in the province of Buenos Aires (Argentina), and it is considered one of the most contaminated Rivers in Latin America. The river drains directly into La Plata River, a large binational estuary, which is the main source of drinking water for the City of Buenos Aires and its surroundings. This situation has led to the necessity to dredge sediments and its remediation.

The objective of this study was to compare the potential use of castor bean (*Ricinus communis* L.) and chicory (*Cichorium intybus* L.) plants for the EDTA, NTA, and citric acid enhanced by phytoextraction of Cr, Cu, Pb, and Zn from contaminated dredged sediment of Matanza-Riachuelo River.

These particular species were selected for their rapid growth, high biomass production, because they are tolerant to heavy metals (Souza et al. 2013; Miniño et al. 2014), and they survive under a wide range of temperatures and soil types (Aksoy 2008). The choice of the species was based on the fact that both species grow in agricultural and industrial areas near the Matanza-Riachuelo River and other rivers and streams of the province of Buenos Aires; thus, they have adaptative strategies to those types of polluted environments. Despite the use of hyperaccumulating species is usually recommended in phytoremediation schemes (Li et al. 2018), their less biomass production limits the overall amount of heavy metals extracted by plant shoots per harvest (Luo et al. 2006). So, the use of fast-growing high-biomass native or already established plants is also a crucial factor for obtaining high metal accumulation.

Materials and methods

Sediment sampling

Samples of freshwater sediments were dredged from Matanza-Riachuelo River (34° 39′29″S 58° 22′52″W). The sampling site is located in the most industrialized area of Argentina (tanneries, metallurgical factories, electroplating, food, paints, textiles, meat processing plants, chemical, petrochemical plants, gas stations, among others) with a high population density (more than 5 million people).

Surface freshwater sediments were collected using a shovel and transferred to plastic buckets for transportation. The sediment was air dried, large objects were removed manually for grounding and afterward it was passed through a 2 mm plastic sieve. The main physical-chemical characteristics are shown in Table 1. The sediment texture (USDA classification) was silt-loam. The near neutral pH value (6.9 units), the low electrical conductivity (EC) (less than 2 dS m^{-1}), the high N content (0.34%) and extractable P (23.3 mg kg^{-1}) showed adequate characteristics for the growth of plants. The high organic matter (OM) content of the sediment (13.4%) is related to the high organic load of industrial and domestic effluents that are clandestinely spilt into the river (Magdaleno et al. 2008). The concentrations of Cu and Zn in sediment (Table 2) were higher than the maximum levels allowed for agricultural and urban use of soil (HWAA, Federal Hazardous Waste Act of Argentina 24,051, Regulatory Decree No 831/93, Annex II). On the other hand, Cr is the only metal that would impede the industrial use of the sediment disposal site, in accordance with the federal law.

Table 1. Selected physical-chemical properties of sediment.

		Method
Sand (%)	6.2	Hidrometer measurement (Gee and Bauder 1986)
Silt (%)	25.3	Hidrometer measurement (Gee and Bauder 1986)
Clay (%)	68.5	Hidrometer measurement (Gee and Bauder 1986)
pH	6.9	Sediment to water ratio (1:2.5) (McLean 1982)
EC (dS m^{-1})	1.66	Saturated paste method (Rhoades 1982a)
CEC (cmol$_c$ kg^{-1})	28.0	Ammonium acetate, pH 7 (Rhoades 1982b)
OM (%)	13.4	Loss on ignition (Davies 1974)
N (%)	0.34	Kjeldahl (AOAC (Association of Official Analytical Chemistry) 1975)
P extractable (mg kg^{-1})	23.3	(Bray and Kurtz 1945)
Ca (%)	1.26	EPA 3050B
Fe (%)	3.44	EPA 3050B

EC: electrical conductivity; CEC: cation exchange capacity; OM: organic matter.

Table 2. Pseudototal metal concentrations (mg kg^{-1}) in dredged sediment (method EPA 3050B) and maximum permitted levels for different uses of the soil in Argentina (HWAA 24,051 decree regulatory No 831/93, Annex II).

	Cr	Cu	Pb	Zn
Mean value (mg kg^{-1})	1010 ± 23	309 ± 12	488 ± 10	1023 ± 36
Limit value of metals in soils (HWAA, decree 831/93 Annex II)				
Agricultural use	750	150	375	600
Residential use	250	100	500	500
Industrial use	800	500	1000	1500

Pot experiment

A pot experiment was used to test the effect of the addition of EDTA (disodium salt), NTA (disodium salt), and citric acid (CA) on the phytoextraction of metals by castor bean (*Ricinus communis* L.) and chicory (*Cichorium intybus* L.). A completely randomized design was performed: four treatments (EDTA as a positive control, NTA, CA, and a control with no chelant addition) were applied to each species, with four replicates per treatment. The bioassay was carried out during September in a greenhouse with no light or temperature control, that is to say, under the natural fluctuations of the season. The average temperature was 14.8 °C (maximum: 19.2 °C; minimum: 10.1 °C) and the average photoperiod was 11 h, according to the National Meteorological Service.

Each plastic pot was filled with 500 g (dry weight basis) of dried and sieved sediment and placed in an individual tray to avoid any free drainage. The water content was maintained at 80% of the field capacity of the sediment by the addition of deionized water and left to equilibrate for 7 days (Chen et al. 2004). Then, pots containing dredged sediment were sown with three chicory or castor bean seeds. After the germination period of 20 days, the plants were thinned to 1 per pot. The pots were watered daily with deionized water (80% sediment field capacity). To avoid nutrient deficiencies during plant growth, they were fertilized once a week with a nutrient solution containing 200 mg N, 72 mg P, and 180 mg K per liter of solution. After a growth period of 8 weeks, 30 mL of the chelating agents solutions were applied to the sediment surface. The doses tested were for CA: 60 mmol kg^{-1} (CA 60) and for NTA and EDTA 5 mmol kg^{-1} (NTA 5 and EDTA 5 treatments, respectively). These doses were defined in a previous exploratory leaching essay where various chelating agents and doses were tested (Bursztyn Fuentes et al. 2015). Harvesting of the plants was performed two days after the addition of the chelating agents. The application time was also defined in a previous essay where chelates degradation was evaluated (Bursztyn Fuentes et al. 2015). The biomass was washed with tap water to remove the sediment adhered to the plants and then rinsed with deionized water. The root and shoot were separated from the plants (stems + leaves), dried in an oven at 70 °C for 48 h and then dry weights were recorded.

The dried material was ground and digested with HNO_3 and $HClO_4$ (Miller 1997). The concentrations of the metals in the plant digests were determined using air-acetylene flame atomic absorption spectrometry (Perkin Elmer Model AAnalyst 200), using external standards prepared from stock solutions of the metals (1000 mg kg^{-1}, Merck). Calculation of the limits of detection (LOD) and limits of quantification (LOQ) was based on the expression 3s/b and 10s/b respectively, where s is the standard deviation of the blank and b is the slope of the calibration graph. The respective LOD and LOQ values for Cr, Cu, Pb, and Zn were 0.05, 0.04, 0.04, and 0.05 mg kg^{-1} and 0.16, 0.14, 0.14, and 0.15 mg kg^{-1}, respectively. Correlation coefficients (R^2) of the calibration curves were better than 0.997 for all metals studied. After the plants were harvested, the pH of the pot sediments (ratio 1:2.5 sediment: water) was determined by potentiometric analysis using a glass electrode (McLean 1982).

Analysis of data

The data presented in this study were the average values obtained from the four replicates of each treatment. Statistical analysis was performed with InfoStat® software. Data were analyzed using an analysis of variance (ANOVA), and the means were compared using Tukey Test ($\alpha = 0.05$). When the set of data did not meet the normality and/or homoscedasticity assumptions, the statistical analysis was performed using Kruskal–Wallis Test.

To evaluate the effect of treatments on the concentration of metals in chicory and castor bean, two indices were calculated: the bioaccumulation factor (BAF) expressed as the ratio of the metal concentration in shoot (stem + leaves) and the concentration of the metal in the sediment ($C_{shoot}/C_{sediment}$) and the translocation factor (TF) expressed as the ratio of heavy metal concentration in the shoot to the metal concentration in the root (C_{shoot}/C_{root}), TF provides an indication of internal heavy metal transportation. To evaluate the efficiency of the phytoextraction process, we calculated the phytoextraction rate (PR) and the time duration of the phytoremediation process (t):

$$\mathrm{PR} = \frac{C_{shoot} * M_{shoot} * N}{C_{sed} * M_{sed}} * 100$$

To estimate the time t (years) needed to decrease the concentration of each metal in the soil to suitable levels for agricultural, urban and industrial use (according to National Act), we applied the theoretical equation proposed by Robinson et al. (2006):

$$t = \frac{(C_{sed} - C'_{sed})}{C_{shoot} * M_{shoot} * N},$$

where C_{shoot} is the metal concentration in shoot of plant (mg kg^{-1}), M_{shoot} is yield (t ha^{-1}) of aboveground biomass of plant, C_{sed} is the metal concentration (mg kg^{-1}), in sediment, M_{sed} is the mass of sediment (t ha^{-1}), C'_{sed} are the grams of metal per hectare, metal target level according to HWAA (1993), and N is the number of harvest per year.

Results and discussion

Plant growth

There were no visible symptoms of heavy metal toxicity in the plants during growth. However, after the addition of citric acid to the sediment, both species showed symptoms of chlorosis and necrosis in leaves. Chlorosis and necrosis were also reported by Aderholt et al. (2017) and Kidd and Proctor (2001) who attributed it either to the solubilization and uptake of toxic levels of Al at low pH or to the oxidative stress caused by hydrogen toxicity, respectively. The values of dry weight on chicory and castor bean (Figure 1) did not show significant differences between treatments. The absence of alterations in the growth is attributed to the short period of exposure (2 days) of the plants to the chelating agents. Miniño et al. (2014) found similar results for *Ricinus communis* with the application of EDTA (2 mM) and CA (2 mM) to an artificially contaminated soil with 600 mg Pb kg^{-1}. Evangelou et al. (2006) worked with doses of CA of 62.5 mM in *Nicotiana tabacum* and they did not observe effects on biomass production either, but they did observe chlorosis and necrosis in leaves. Gramss et al. (2004) observed these symptoms in *Brassica chinensis* L. with the addition of a high dose of citrate (83 mM) to the culture medium, suggesting that the citrate destroyed the physiological barriers in the roots that control the flow of solutes, thus allowing access of metal complexes from the soil solution to the roots. In contrast, Quartacci et al. (2005) reported that the addition of 10 mM NTA reduced 33% of the dry weight of shoots of *Brassica juncea*, whereas the addition of CA did not affect biomass production. Research shows that the effects of adding chelating agents to soil/sediment on plant biomass is poorly understood (Khan et al. 2016) and hence difficult to predict, as they depend on the chelator, the dose used, the form of application, the contact time with the soil, forms of metals in the soil, and on the plant species.

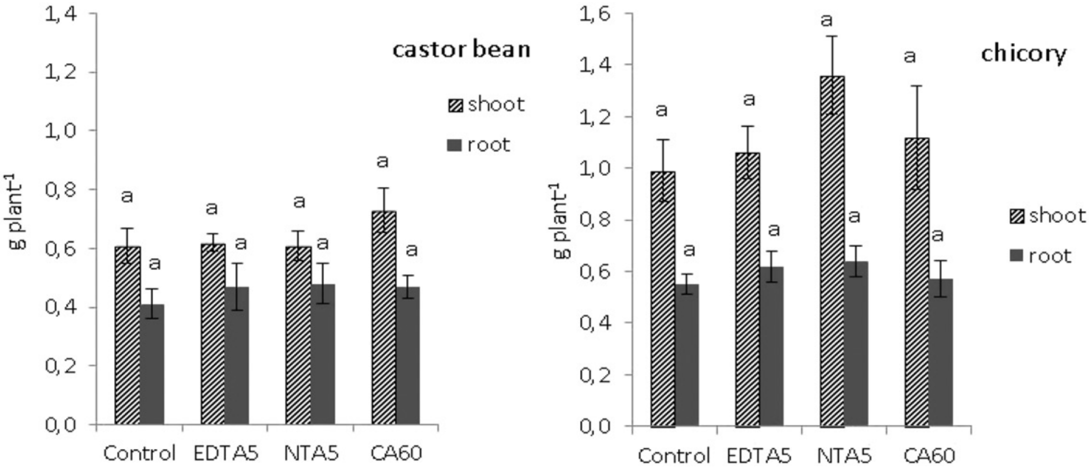

Figure 1. Dry weight of roots and shoots (g plant^{-1}) of castor bean and chicory for each treatment. Different letters indicate significant differences between treatments for each organ. Values are the mean of four replicates ± SD.

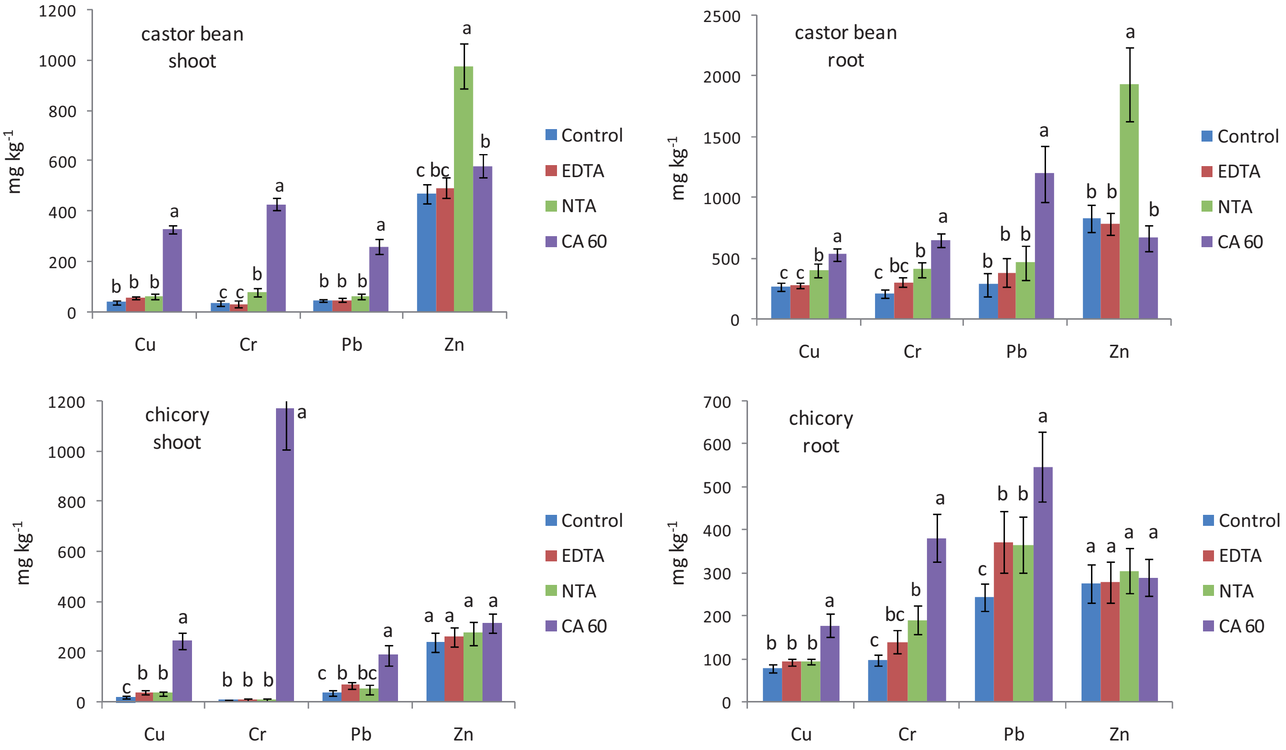

Figure 2. Metal concentrations (mg kg^{-1}) in shoots and roots of chicory and castor bean. Values are the mean of four replicates ± SD. Different letters indicate significant differences between treatments ($p < 0.05$) for each metal.

Metal concentrations in plants

Figure 2 shows the concentrations of metals in the aerial part and root of castor bean and chicory. The accumulation of metals from the soil/sediment in the shoots of the plants involves at least three stages. The first stage consists of the transport of the metal from the soil (by diffusion or mass flow) to the surface of the root. The second stage is the transport of the metal from the root surface to the root cells, and the third one consists of the release of the metal into the xylem (Xu et al. 2007). Therefore, the efficiency of a chelating agent in favor of the absorption and translocation of a metal depends on many physical-chemical and biological factors (*i.e.*, metal solubility, chelating agent, and plant species).

The concentrations of Cr, Cu, and Pb in root and shoot of castor bean and chicory (Figure 2) in the CA60 treatment were significantly higher ($p < 0.05$) than in the other treatments. Concentrations of Cu and Pb in shoots of chicory and castor bean increased by 112% and 105% for Cu and 718% and 333% for Pb, respectively compared to the control. On the other hand, NTA5 was the only treatment that presented a significant increase of the Zn concentration in root (138%) and shoots (921%) of castor bean with respect to the control. For both species, concentrations of Cu, Cr, and Pb in root were substantially higher than those in shoots (15–17% for castor bean and 8–25% for chicory). Although castor bean plants exhibited significant

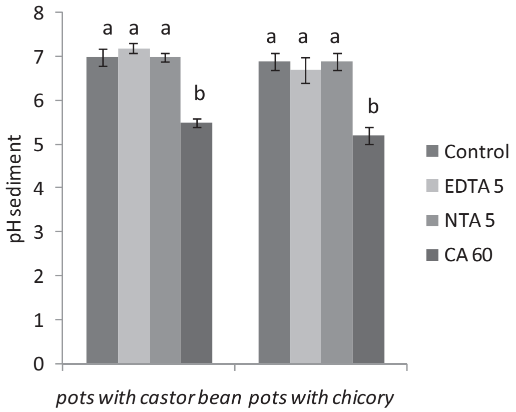

Figure 3. pH of the sediments for each post-harvest treatment in pots with castor bean and chicory. Values are the mean of four replicates ± SD. Different letters indicate significant differences between treatments ($p < 0.05$).

Table 3. Bioaccumulation (BAF) and translocation (TF) factors of metals for each treatment.

Treatment	$\mathrm{BAF}_{\mathrm{Cu}}$	$\mathrm{BAF}_{\mathrm{Cr}}$	$\mathrm{BAF}_{\mathrm{Pb}}$	$\mathrm{BAF}_{\mathrm{Zn}}$	$\mathrm{TF}_{\mathrm{Cu}}$	$\mathrm{TF}_{\mathrm{Cr}}$	$\mathrm{TF}_{\mathrm{Pb}}$	$\mathrm{TF}_{\mathrm{Zn}}$
castor bean								
Control	0.1 b	0.0 b	0.1 b	0.5 b	0.2 b	0.2 b	0.2 a	0.6 b
EDTA5	0.2 b	0.0 b	0.1 b	0.5 b	0.2 b	0.1 b	0.1 a	0.6 b
NTA 5	0.2 b	0.1 b	0.1 b	1.0 a	0.2 b	0.2 b	0.1 a	0.5 b
CA 60	1.1 a	0.4 a	0.5 a	0.6 b	0.6 a	0.7 a	0.2 a	0.9 a
chicory								
Control	0.1 b	0.0 b	0.1 b	0.3 a	0.3 b	0.1 b	0.2 ab	0.9 a
EDTA5	0.1 b	0.0 b	0.1 b	0.3 a	0.4 b	0.1 b	0.2 ab	0.9 a
NTA 5	0.1 b	0.0 b	0.1 b	0.3 a	0.4 b	0.1 b	0.1 b	0.9 a
CA 60	0.8 a	1.2 a	0.4 a	0.3 a	1.4 a	3.1 a	0.3 a	1.1 a

In same column, different letters indicate significant differences between treatments for each plant species.

accumulation of these metals in the roots, limited translocation capacity suggests that only a small fraction of the metal ions (which can be translocated) are absorbed into the root cells, the rest may be physically adsorbed into the cell walls (negatively charged) of the root or may be sequestered and immobilized in the cell vacuoles (Lasat 2002). In contrast, the translocation capacity of Zn was substantially higher than for all other metals (57% for castor bean and 87% for chicory). Several works have shown the high mobility of Zn in plants, which favors the movement of metal in vascular tissues (xylem) and its translocation to aboveground tissues. Conflicting reports state that Zn uptake is an active or passive process. Independently of the type of uptake kinetics of Zn, as a fraction of this metal occurs bound to light organic compounds in xylem fluids, its high mobility in the plants is recognized (Tsonev and Cebola Lidon, 2012).

One of the most important results of this bioassay was the high Cr concentration induced by citric acid (CA60) in shoots (1173 mg kg^{-1}) of chicory with respect to the control (7.7 mg kg^{-1}). Under natural conditions (without addition of chelating agent) Cr is mostly immobilized in the roots and scarcely translocated to stems and leaves (Zayed and Terry 2003) with a $\mathrm{Cr}_{\mathrm{root}}/\mathrm{Cr}_{\mathrm{leaves}} \sim 100$ ratio. In our study, this ratio had values substantially lower for both species (12.6 and 1.5 for chicory and castor bean, respectively). Jean et al. (2007) showed that citric acid (two applications of 10 mM) was more effective than EDTA (10 mM) in increasing the uptake of Cr by *Datura innoxia*, due to the greater effectiveness of citric in solubilizing Cr from the soil.

The main factors that control the availability of metals in the soil are pH, CEC, and OM content (Chen et al. 2012). In our experiment, the addition of NTA and EDTA did not modify the pH of the sediment, whereas the addition of 60 mM citric acid significantly decreased ($p < 0.05$) the pH (−1.5 units) with respect to the control (Figure 3). The increase of sediment acidity would lead to desorption of the metals associated with the solid phases of the sediment and thus would facilitate the formation of soluble metal complexes (Ebbs and Kochian 1998). Chen et al. (2003) showed that the adsorption of Cd and Pb in the soil decreased due to a reduction of pH in the presence of citric acid and that this ligand could alleviate the toxicity of metals to radish, and stimulate their translocation from root to shoot. Aderholt et al. (2017) also found that the incorporation of citric acid and the consequent decrease in pH (from 6.8 to 4.0–4.5) improved Pb solubilization and uptake. In parallel, Jean et al. (2007) asserted that the effectiveness of the chelators in solubilizing metals is not directly related to their complexation constants. This can be explained by their ability to solubilize the mineral matrix containing the metals.

Bioaccumulation and translocation factor

Plant exhibiting BAF and TF values greater than one are suitable for phytoextraction (Domen et al. 2008). In the control treatment, BAF and TF (Table 3) showed very low values (lower than one), indicating that the plants were not adequate for sediment remediation. Zn was the metal that exhibited the highest TF for both plant species (except Cu and Cr in CA60, in chicory).

Several authors have shown the high effectiveness of EDTA in desorbing soil metals and increasing their transfer to roots and shoots of plants (Shahid et al. 2014). In contrast, our results have shown that EDTA did not favor the absorption of metals by roots (except for Pb in castor bean) or the translocation of metals from the roots to the shoots in any of the species with respect to the control. Nowack et al. (2006) have shown that Ca, which is always present in soils at high concentrations (see Table 1), can greatly reduce the mobilization of heavy metals by EDTA. On the other hand, the addition of CA (60 mM) significantly increased the BAF and TF values of Cu, Cr, and Pb in chicory with respect to the rest of the treatments. In CA60 treatment, the values of $\mathrm{BAF}_{\mathrm{Cr}}$ and $\mathrm{TF}_{\mathrm{Cr}}$ were higher than one (1.2 and 3.1, respectively), and the Cr concentration in shoots was higher than 1000 mg kg^{-1}, showing that chicory is a plant species that accumulates Cr. However, the CA60 treatment showed a high concentration of Pb (1200 mg kg^{-1}) in the castor bean roots, with a low translocation to the aerial part ($\mathrm{TF}_{\mathrm{Pb}} = 0.2$). Although chelating agents are proposed to induce phytoextraction of metals, our results show that citric acid could be an effective amendment for the phytostabilization of Pb with castor bean or other phytostabilizing plants that should tolerate high concentrations of heavy metals and immobilize contaminants in the roots, with minimal translocation to harvestable biomass. It is interesting to highlight that despite the castor bean is tolerant to the complex

Table 4. Values of the phytoextraction rate (PR) of metals for each treatment.

			PR			
Species	Treatment	Yield t d wt./ha	Cr	Cu	Pb	Zn
Chicory	Control	0.99	0.0003 c	0.0023 d	0.0028 c	0.0084 d
	EDTA5	1.07	0.0004 bc	0.0048 c	0.0054 b	0.0098 c
	NTA5	1.36	0.0005 b	0.0056 b	0.0052 b	0.0133 a
	CA60	1.12	0.0471 a	0.0324 a	0.0156 a	0.0125 b
Castor bean	Control	0.61	0.0008 c	0.0337 c	0.0021 b	0.0102 c
	EDTA5	0.62	0.0007 c	0.0361 c	0.0023 b	0.0109 c
	NTA5	0.61	0.0017 b	0.0699 a	0.0029 b	0.0211 a
	CA60	0.73	0.0113 a	0.0499 b	0.0142 a	0.0151 b

In same column, different letters indicate significant differences between treatments for each plant species.

contaminated sediment where they can naturally grow, this species does not seem to be that tolerant to the soil conditions modified by the chemical treatments, especially CA. Thus, agronomic practices become a relevant issue to promote plant's health in a phytostabilization scheme.

It is important to mention that the period of 2 days of exposure was selected arbitrarily as a compromise between removal efficiency and phytotoxic effects. We are aware that longer exposure periods could have definitely promoted higher variations in metals accumulation and bioconcentration and translocation factors. Though sequential additions of CA could have been essayed (given that this LMWOA has short degradation times), phytotoxic effects are a limitation: the high doses of CA applied in this article compared to the ones used in many papers, jeopardized the health of the plant.

Efficiency of phytoextraction process

The aim of phytoextraction is to reduce the levels of metals in contaminated soil or sediment to acceptable levels within a reasonable time frame. The process depends on the ability of the selected plants to accumulate high levels of metals in above-ground tissue and has a high yield of biomass under the specific climate and soil conditions (Begonia et al. 2002).

Although the PR obtained in this experiment were extremely low, differences between plants and between chelators were observed (Table 4). The highest PR_{Zn} were obtained in the NTA5 treatment for both species. The application of citric acid (60 mM) to the sediment increased significantly the PR of all metals with respect to the Control, by chicory and castor bean. Despite the high concentration of metals in shoots of chicory, in the CA60 treatment (Figure 2), it was observed that PR values were very low due to the low aerial biomass production of the plants obtained in this experiment. For example, only 0.05% Cr removal of the sediment was obtained by chicory harvest. Even if four crops were harvested per year, it would take 285 years to reduce the Cr concentration in the sediment to the value suitable for industrial land use (according to HWAA 1993).

Table 5 shows the time (t) that remediation for each species would require in a hypothetical scenario using the concentrations of metals in the aerial biomass of the plants obtained in our experiment, the yields of the literature, and the agronomic management of each species. The calculation of t was based on the following considerations: (1) Chicory seeds sowing can be done throughout the year. The harvest occurs after about 8 weeks of planting. Li and Kemp (2005) have shown that chicory requires a high level of fertility for maximum yield and that under optimal growth conditions 9 $t\ ha^{-1}$ of dry matter per harvest can be obtained. It was assumed that chicory may be cultivated four times each year ($N = 4$) and that the active rooting zone, corresponding to the uppermost 20 cm of the sediment layer, giving a total sediment mass of 2760 $t\ ha^{-1}$ (assuming a sediment bulk density of 1.38 $t\ m^{-3}$); (2) Castor bean can only be sown in early spring, that is, it is cultivated once a year ($N = 1$), and in the agroclimatic conditions of the study area, it can be obtained 20.6 $t\ ha^{-1}$ of material dry (Wassner 2013).

Table 5. Time (years) needed to decrease the concentration of each metal until reaching the levels of hazardous waste for agricultural, residential and industrial use of the soil for each treatment and plant species.

	Chicory				Castor bean			
Treatment	Cr	Cu	Pb	Zn	Cr	Cu	Pb	Zn
t (Years) agricultural use								
Control	2590	606	396	135	930	512	324	120
EDTA5	1846	318	221	125	1046	366	305	114
NTA5	1954	349	296	117	437	338	234	58
CA60	17	49	81	103	81	65	58	97
t (Years) residential use								
Control	7600	797	–	167	2715	673	–	145
EDTA5	5395	418	–	154	3060	481	–	141
NTA5	5712	459	–	145	1276	444	–	72
CA60	50	65	–	127	236	85	–	120
t (Years) industrial use								
Control	2090	–	–	–	750	–	–	–
EDTA5	1490	–	–	–	845	–	–	–
NTA5	1578	–	–	–	353	–	–	–
CA60	14	–	–	–	65	–	–	–

(–) indicates that the concentration of the metal in the sediment was lower than the guide level of the regulation (HWAA, Decree 831/93).

Table 5 shows that for both species, it would take about 100 years for the sediment to be used for agricultural use, Zn being the metal that requires the most years to reach the level established by the National Act (600 mg Zn kg^{-1} see Table 2). To reach the urban use of the soil, the time required for the reuse of the sediment depended on the plant species used. However, none of them proved efficient for the remediation. In contrast, it was observed that the reuse of the dredged sediment disposal site for industrial use is achievable within a reasonable time. Cr is the only metal that has a concentration in the sediment (1010 mg kg^{-1}, see Table 2) greater than the maximum level established for industrial use of the soil (800 mg kg^{-1}). The use of chicory and citric acid (60 mM) proved to be the best combination, with the estimated remediation time being 14 years. Our research shows that the selection of the phytoextractor species should not be based solely on the production of aerial biomass and the concentration of the metal in the higher tissues, but also on the possible agronomic management of the crop. These factors will determine the best land use option for the remediation process to take place within a reasonable time.

Conclusions

The results of this study support the use of a high dose of citric acid (60 mM) for the phytoextraction of Cu, Cr, and

Pb from the multiply metal contaminated dredged sediment compared to EDTA and NTA 5 mM.

Citric acid was the most effective chelating agent in increasing the concentration of Cu, Cr, and Pb in root and in the aerial part of chicory and castor bean. On the other hand, NTA5 was the only treatment that produced a significant increase in Zn concentration in the castor bean tissues with respect to the control. Both in the sediment with and without the addition of chelating agents, the accumulation of metals by castor bean was higher in the roots than in the aboveground tissues. However, while castor bean is tolerant to the complex contaminated sediment where it can naturally grow, this species does not seem to be that tolerant to the soil conditions modified by the chemical treatments, especially CA. Thus, agronomic practices become a relevant issue to promote plant's health in a phytostabilization scheme.

Chicory had BAF and TF of Cr greater than one and very high concentration of the metal in the harvestable biomass, showing that chicory may be a good candidate for the phytoextraction of Cr assisted with the addition of a high dose of citric acid. However, the net Cr removal of the sediment was very low due to the low biomass of the plants obtained in our experiment. We speculate that the time required to reuse sediment deposited on land for industrial use could be achieved in a few years if growing conditions of the chicory crop were improved. Further research may be necessary to investigate the physical, chemical, or biological properties that are adversely affecting the normal development of plants.

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