Article

Phytoremediating potential of the chicura (*Ambrosia ambrosioides*) in soils contaminated by heavy metals

Ricardo Israel Ramírez Gottfried[1]
Mario García Carrillo[1§]
Vicente de Paul Álvarez Reyna[1]
Guillermo González Cervantes[2]
Vicente Hernández Hernández[1]

[1]Postgraduate in Agricultural Sciences-Antonio Narro Autonomous Agrarian University-Laguna Unit. Peripheral Raúl López Sánchez and Carretera Santa Fe s/n, Torreón, Coahuila, Mexico. CP. 27010. (Ricardo-gottfried@hotmail.com; vdpar-190754@hotmail.com; vickhdz.hdz@gmail.com). [2]INIFAP. Gomez Palacio, Durango. (gonzalez.guillermo@inifap.gob.mx).

[§]Corresponding author: mgc570118@hotmail.com.

Abstract

The objective of the present research work was to evaluate the phytoremediation potential of *Ambrosia ambrosioides* soil, determine the absorption of heavy metals (cadmium, copper and lead) in the plant under different concentrations 0, 20, 40, and 60 mg L^{-1} applied in the irrigation water The analyzes were performed by atomic absorption, and with the data obtained the translocation factor and bioconcentration factor were calculated. The development of the experiment and laboratory analysis were performed at the Autonomous Agrarian University Antonio Narro Laguna Unit in the year 2018. A 3-by-4 factorial design with 4 repetitions was used, with factor A being heavy metal and B being the concentration of metal, 12 treatments were taken in total. The results showed that in root, stem and leaf the higher concentrations occurred in the treatment of copper at 20 mg L^1 with values of 15 827.2, 13 030.9 and 4 979.4 mg kg^{-1} respectively. Copper was the metal that more absorbed the plant followed by cadmium and lead. The biological translocation factor indicated that cadmium is an element that the plant translocates towards its leaves more easily followed by copper. In *Ambrosia ambrosioides* the translocation of lead is null. The bioconcentration factor exceeded the comparative value of 1 in all treatments, this indicates that high phytoextraction was performed and that *Ambrosia ambrosioides* can be used in phytoremediation and soil health.

Keywords: absorption, accumulation, bioconcentration, translocation.

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Introduction

One of the most negative consequences of the industrial revolution has been the dispersion of pollutants in air, water and soil (Vullo, 2003; Becerril *et al*., 2007; Navarro *et al*., 2007). Soil is the most static environment where pollutants can remain for a long time, this long-term permanence is especially serious in the case of inorganic pollutants such as heavy metals that cannot be degraded (Becerril *et al*., 2007; Azpilicueta *et al*., 2010; Martí *et al*., 2011).

The United States Environmental Protection Agency has defined as possible dangerous elements Ba, Cd, Cu, Pb, Mn, Ni, Zn, Vn, Sn, its danger is potential and must be kept under control (Marrero *et al*., 2012). These metals when they exceed the maximum allowed limits cause negative effects on the physical, chemical and biological properties in soil (Trejo *et al*., 2015), the term used or used is ‘soil pollution’ (Prieto *et al*., 2009).

In the last decades of the 20th century, technologies based on the use of living organisms emerged to decontaminate soil and recover the affected ecosystems (Carpena and Bernal 2007). One of these technologies is the use of plant species for the removal of heavy metals from the soil or ‘phytoremediation’ (Covarrubias and Peña, 2017). This technique is based on the joint use of plants, soil amendments and agronomic techniques to eliminate, retain or reduce the toxicity of soil contaminants (Carpena and Bernal 2007; Delgadillo *et al*., 2011). An advantage of phytoremediation is that metals absorbed by plants can be extracted from harvested biomass and then recycled (Agudelo *et al*., 2005).

In recent years, interest has grown in plants that can accumulate and tolerate unusually high amounts of heavy metals, due to their potential utility for man as a tool in cleaning contaminated soil (Llugany *et al*., 2007). These types of plants called hyperaccumulators are relatively rare and are often found in remote geographical areas (Kidd and Monterroso, 2003). Its final concentration in aerial tissues depends on the metal and the species, reaching between 1 and 2% of its dry weight (Becerril *et al*., 2007; Diez *et al*., 2002).

The term metal hyperaccumulator is currently used to assign plants that accumulate >10 mg kg^{-1} of Mn and Zn, >1 mg kg^{-1} of Co, Cu, Pb, Ni, As and Se and >100 mg kg^{-1} of Cd (Kidd *et al*., 2007). Currently, approximately 400 hyperaccumulatory species have been identified, of which most are endemic to serpentine soil and accumulate Ni (Diez *et al*., 2002). The genus *Ambrosia* L. belongs to the *Asteraceae* (*Compositae*) family and is made up of almost forty native species, mostly from North and South America.

They are usually annual herbs, bianules or perennials, exceptionally shrubs, with erect stems, branched in the upper half, alternate, opposite or sub-opposite leaves, whole, lobed or deeply divided, petioled or subsessile, with winged or aerophilous petiole (Morales *et al*., 2012).

Most of these species are little studied aromatic and medicinal plants, they are considered as weeds and, therefore, most of them have not been systematized (Cano, 2014).

Ambrosio ambrosioides is the most common shrub ragweed, it is found in areas such as roadsides, riverbanks, sandy soil and occasionally in rock crevices, its main use is for medicinal purposes (M. Turner *et al*., 1995; López, 2011; Gil. Salido *et al*., 2016). The objective of this research work was to determine the phytoremediation potential of chicura (*Ambrosia ambrosioides*) in soil contaminated by Cd, Cu and Pb. This plant is not reported in the literature as a hyperaccumulating or phytoremediating species.

Materials and methods

The development of this research, as well as the laboratory analyzes were performed at the Autonomous Agrarian University Antonio Narro Laguna Unit (UAAAN-UL) (25° 33' 12.53'' north latitude, 103° 22' 32.07'' west longitude) in the city of Torreón, Coahuila.

Collection of plants

The Lagunera Region is located in the north-center of Mexico, composed of five municipalities in the southwest of the state of Coahuila and 10 in the northeast of the state of Durango. The climate is dry desert with an average annual rainfall of 220 mm with an average altitude of 1 100 meters above sea level (Orona *et al*., 2006). In summer the climate varies from semi warm to warm-dry and in winter from semi-cold to cold, the rainy period includes from mid-June to mid-October (Nava and Cano, 2000). The plants used in this work were collected in the dry bed of the Nazas River in the area of the Cannon of Fernández State Park in the Lagunera Region. Plants between 20 and 30 cm tall were collected, transplanted in 600 cm^3 pots with soil from their area of origin and irrigated with distilled water. The total number of plants collected was 60.

Acclimatization of plants

After collected in the field, the plants were introduced for 21 days in a 200-square-meter greenhouse, semicircular with an arc-shaped roof covered in plastic, protected by shade mesh during the hottest seasons of the year, straight side walls, floor of gravel, with extractors and wet wall of the UAAAN-UL. In these 21 days the plants were irrigated every third day with 250 ml of Steiner nutrient solution (Steiner, 1984).

Treatments

In plastic bottles with a capacity of 20 liters, the Steiner solution mixture previously prepared in distilled water and high purity heavy metal standards (Cd, Cu and Pb) of one thousand parts per million of the Perkir Elmer brand was made in proportions which are presented in Table 1. The concentrations were 0, 20, 40 and 60 mg L^{-1} of each metal. 48 plants were transplanted into pots of 1 200 cm^3 of volume with a substrate composed of sand (80%) previously washed with 5% sodium hypochlorite (NaClO) and perlite (20%).

The sand is a chemically inert substrate that acts as a support for the plant, does not intervene in the process of adsorption and fixation of nutrients. Perlite was used to prevent sand compaction. Since the transplant was performed, every third day the plants were irrigated with 250 ml of solution of their respective treatment, in 21 days there was a total of 10 irrigations. At the end of the application of treatments the plants were extracted from each of the pots.

Table 1. Proportions used for the preparation of treatments.

Treatment	Steiner solution volume (L)	Metal	Metal volume (mL)
T1	10	Cd	0
T2	9.8	Cd	200
T3	9.6	Cd	400
T4	9.4	Cd	600
T5	10	Cu	0
T6	9.8	Cu	200
T7	9.6	Cu	400
T8	9.4	Cu	600
T9	10	Pb	0
T10	9.8	Pb	200
T11	9.6	Pb	400
T12	9.4	Pb	600

Washed

The washing was done in order to remove any particles of soil and perlite that would have been left in the samples taken from the pots. It was carried out using a brush and a sponge in addition to common tap water. At the end all samples were rinsed three times with distilled water to avoid any contaminant that could affect the results.

Plant separation

Stage that consisted of separating leaves, stems and roots of each sample. The samples were placed in paper bags and labeled for identification.

Drying

The samples were left in the soil laboratory of the UAAAN-UL for a week at an average temperature of 30 °C, then they were placed in a Felisa drying oven at 65 °C for 24 hours.

Digestion preparation

The dry digestion method (DVS) was used, this methodology was performed in the Soil Department of the UAAAN-UL, the procedure was as follows: the process started with the milling of the plant that was crushed and ground in a mill electric until it is homogeneous and dusty. The milled sample was screened and the samples were placed in small plastic bags previously labeled. 1 g of sample was weighed in a 50 ml constant weight crucible to be introduced into a Furnace 1 500 brand flask where the plant samples were calcined at a temperature of 600 °C for 4 h.

Subsequently to the resulting ash in the crucible, 10 ml of 37% hydrochloric acid (HCL) was added, stirred for 10 s and allowed to stand for 20 min. The contents of the crucible were transferred to a 100 ml volumetric flask, to which 10 ml of cesium chloride (CsCl) was previously added, the content was packed with distilled water to complete 100 ml. The contents of the flask were filtered on filter paper by placing the residue in 100 ml plastic containers, the residue obtained in the can is used to determine the heavy metals.

Heavy metal determination

The quantification of heavy metals was performed on a Perkin-Elmer 2380 atomic absorption spectrophotometer. The spectrophotometer is an instrument used to determine at what wavelength the sample absorbs light and the intensity of absorption. All spectrophotometers consist of a light source, a wavelength selector, a transparent container in which the sample is deposited, a light detector and the meter, detect elements such as Ca, Mg, Na, K, Mn, Pb, Cd among others. A standard of 1 000 ppm of each metal was used for the calibration curve of the equipment and the corresponding lamp was placed.

Statistical analysis

In this study, the plant was considered as the experimental unit with destructive sampling, so the statistical analysis was a factorial design of A by B, where factor A corresponds to the heavy metal and B to its concentration (Table 2), with 4 repetitions and a total of 12 treatments.

Table 2. Identification of factor A and B.

Factor A		Factor B	
Cadmium	A1	0 mg L^{-1}	B1
Copper	A2	20 mg L^{-1}	B2
Lead	A3	40 mg L^{-1}	B3
		60 mg L^{-1}	B4

Each plant was dissected into three parts (leaf, stem and root) to determine the absorption of heavy metals. The means test was performed using the Tukey’s method and was carried out using the SAS version 9.0 program. Table 3 shows the different treatments applied.

Table 3. Applied treatments.

Interaction	Treatment
A1*B1	1
A1*B2	2
A1*B3	3
A1*B4	4

Interaction	Treatment
A2*B1	5
A2*B2	6
A2*B3	7
A2*B4	8
A3*B1	9
A3*B2	10
A3*B3	11
A3*B4	12

Results and discussion

Root

The root was the part of the plant where the greatest amount of heavy metals was absorbed (Table 4), a result similar to that of (Ortega *et al*., 2011; Carrión *et al*., 2012) who evaluated the phytoextractor capacity of various species and they found that the root is the part that holds more metals. This is because one of the main mechanisms of resistance and tolerance of heavy metal plants is the retention of the metal at the root (Barceló and Poschenrieder, 1992).

Table 4. Metal concentration in the root (mg kg^{-1}) under the different treatments.

Metal	Concentration				A
	0 (mg L^{-1})	20 (mg L^{-1})	40 (mg L^{-1})	60 (mg L^{-1})	
Cadmium	14.3 e	860.4 de	4 195.3 b	3 841.1 bc	2 227.8 b
Copper	1 063.4 de	15 827.2 a	2 386.8 cd	1 592.8 de	5 217.5 a
Lead	214.5 e	300 e	252.5 e	231.5 e	249.6 c
B	430.7 c	5662.5 a	2 278.2 b	1 888.4 b	

Means with different letters are statistically different (Tukey's $p\leq 0.05$); CV= 28.36%; A= heavy metal; B= concentration; CV= coefficient of variation.

For the heavy metal factor, copper was the metal that most absorbed the root of the plant 5 217.5 (mg kg^{-1}), the one with the lowest absorption was lead 249.6 (mg kg^{-1}). In the concentration factor it was observed that in 20 (mg L^{-1}) it was where the highest accumulation was 5 662.5 (mg kg^{-1}) and the lowest in 0 (mg L^{-1}) 430.7 (mg kg^{-1}), in factors 40 (mg L^{-1}) and 60 (mg L^{-1}) found no significant difference. Copper-20 (mg L^{-1}) (treatment 6) was where the greatest heavy metal accumulation was found in the root 15 827.2 (mg kg^{-1}) followed by accumulation in cadmium-40 (mg L^{-1}) (treatment 3). The lowest accumulation occurred in cadmium-0 (mg L^{-1}) (treatment 1).

Leaf

The plant leaf showed the lowest heavy metal accumulations (Table 5). This is because many species tolerate high concentrations of metals in the soil because they restrict their absorption and translocation towards the leaves, which allows them to maintain constant concentrations (Kidd *et al*., 2007).

Table 5. Accumulation of metals in the leaf (mg kg^{-1}) under the different treatments.

Metal	Concentration				A
	0 (mg L^{-1})	20 (mg L^{-1})	40 (mg L^{-1})	60 (mg L^{-1})	
Cadmium	49.7 e	164.1 e	1 037.3 ed	429.3 e	420.1 b
Copper	1 823.2 cd	4 979.4 a	3 362 b	2 679.9 bc	3 211.1a
Lead	589 e	547.6 e	833.9 de	872.5 de	710.7 b
B	820.6 c	1 897 a	1 744.4 ab	1 327.2 bc	

Means with different letters are statistically different (Tukey's $p \leq 0.05$ CV= 24.26%. A= heavy metal; B= concentration; CV= coefficient of variation.

For the heavy metal factor, copper was the one that most absorbed leaf 3 211.1 (mg kg^{-1}), cadmium and lead showed no significant difference. In the concentration factor it was observed that in 40 (mg L^{-1}) it was where the greatest accumulation of metals 1 744.4 (mg kg^{-1}) was presented, the lowest was presented in the concentration of 0 (mg L^{-1}) with 820.6 (mg kg^{-1}). Copper-20 (mg L^{-1}) (treatment 6) and copper-60 (mg L^{-1}) (treatment 8) is where the largest heavy metal accumulations were found on leaf 4 979.4 and 3 362 (mg kg^{-1}) respectively. The lowest accumulation in the leaf was presented in cadmium-0 (mg L^{-1}) (treatment 1) and cadmium-20 (mg L^{-1}) (treatment 2) with 49.7 and 164.1 (mg kg^{-1}), respectively.

Stem

For the heavy metal factor, copper was the one that most absorbed the stem of the plant 4 912 (mg kg^{-1}), the metal that had the lowest absorption was cadmium 243.7 (mg kg^{-1}). In the concentration factor it was observed that in 20 (mg L^{-1}) the highest accumulation was 4 466.2 (mg kg^{-1}) and the lowest in 0 (mg L^{-1}) 164.1 (mg kg^{-1}). The concentrations of 40 and 60 (mg L^{-1}) showed no significant difference. In the treatments, copper-20 (mg L^{-1}) (treatment 6) presented the greatest accumulation of heavy metal 13 030.9 (mg kg^{-1}) followed by accumulations in copper-40 (mg L^{-1}) (treatment 3) and copper-60 (mg L^{-1}) (treatment 4). The lowest accumulation was presented in cadmium-0 (mg L^{-1}) (Table 6).

Table 6. Accumulation of metals in the stem (mg kg^{-1}) under the different treatments.

Metal	Concentration				A
	0 (mg L^{-1})	20 (mg L^{-1})	40 (mg L^{-1})	60 (mg L^{-1})	
Cadmium	108.9c	138.8 c	460.8 c	266.2 c	243.7 b
Copper	183.3 c	13 030.9 a	3429 b	3 005 b	4 912 a
Lead	200 c	228.9 c	440.1 c	1 119.6 c	497.2 b
B	164.1 c	4 466.2 a	1 443.3 b	1 463.6 b	

Means with different letters are statistically different (Tukey's $p \leq 0.05$); CV= 32.38%. A= heavy metal; B= concentration; CV= coefficient of variation.

The results indicate that at the root, stem and leaf the highest concentrations of heavy metals were presented in copper at 20 mg L^{-1}, this is due to the fact that copper is an essential element for plants, is required for its growth and participates in reactions of oxidation-reduction (Hernández *et al*., 2012; León and Sepulveda, 2012). In copper a downward absorption trend was observed in concentrations greater than 20 (mg L^{-1}) in the same way in cadmium this behavior occurred in concentrations greater than 40 (mg L^{-1}) this is because some plants develop precise strategies to survive in soil with high level of metals (Becerril *et al*., 2007).

The lowest concentrations were presented in cadmium at 0 (mg L^{-1}). In a study where *Ambrosia ambrosioides* was exposed to lead solutions of 0.25, 0.5 and 1.5 (mg L^{-1}), it was found that the greatest absorption of the metal was at the root 4 638 (mg kg^{-1}) followed by the stem 520 (mg kg^{-1}) and leaf 484.38 (mg kg^{-1}). It was concluded that the higher the concentration of the metal, the greater the absorption in the plant (Contreras *et al*., 2016).

The results on the stem and leaf agree with those of (Contreras *et al*., 2016) since lead absorption showed similar behavior. At the root of the plant a decay in absorption was shown from the concentration of 40 mg kg^{-1}. Lead was the only heavy metal that showed this behavior.

Biological translocation factor (FTB) in plant

The biological translocation factor associates the concentration of root metal and the concentration of leaf metal, the comparative value is 1. A value greater than 1 means that the plant translocates the metal from its root to its leaves (Argota *et al*., 2014). Table 7 shows the FTB for *Ambrosia ambrosioides*.

Table 7. Biological translocation factor in *Ambrosia ambrosioides*.

Metal	Concentration				A
	0 (mg L^{-1})	20 (mg L^{-1})	40 (mg L^{-1})	60 (mg L^{-1})	
Cadmium	0.35d	5.23 b	4.07 bc	9.74 a	4.85 a
Copper	0.53 d	3.41c	0.47 d	0.21 d	1.15 b
Lead	0.43 d	0.49 d	0.35 d	0.26 d	0.38 c
B	0.44 c	3.04 a	1.63 b	3.4 a	

Means with different letters are statistically different (Tukey’s $p \leq 0.05$); CV= 24.25%. A= heavy metal; B= concentration; CV= coefficient of variation.

For the heavy metal factor, cadmium was the metal with the highest FTB 4.85, followed by copper 1.15 and lead 0.38. This factor indicates that cadmium is the heavy metal that the plant translocates more easily. Copper exceeds the comparative value of 1, so this metal is also translocated by the plant. Lead is an element that the plant cannot translocate. In the concentration factor it was observed that in 60 (mg L^{-1}) the highest coefficient 3.4 was presented, the lowest coefficient was presented in the concentration of 0 (mg L^{-1}) 0.44, this indicates that at a higher concentration of heavy metals the plant translocates them more easily. Cadmium at 60 (mg kg^{-1}) (treatment 4) is where the highest translocation coefficient was found 9.74. Copper at 60 (mg kg^{-1}) (treatment 8) was where the lowest FTB 0.21 was obtained. Most of the treatments were statistically similar and less than 1, which indicates that the plant in most of the treatments does not translocate the metal from the root to its leaves.

Lead obtained the lowest values in this factor, as indicated by Diaz *et al*. (2001) who mentions that translocation in lead is generally limited. Tolerance to potentially toxic elements in plant organisms is the result of an evolutionary process that gives different plant species the ability to grow and develop in environments with high concentrations of potentially toxic elements (González *et al*., 2008). Capacity demonstrated by *Ambrosia ambrosioides*.

Bioconcentration factor (BCF) in plant

The bioconcentration factor is the ability of a plant to accumulate soil metals, a coefficient that is defined as the ratio of the concentration of the metal in the plant with respect to the soil. A value greater than 1 implies that it is feasible to use the species in phytoextraction (Hernández and Romero, 2012). If the coefficient is greater than one, it can be said that the metals absorbed by the plants can be extracted from the harvested biomass and then recycled (Agudelo *et al*., 2005). Table 8 shows the BCF for *Ambrosia ambrosioides*. Treatments 1, 5 and 9 are excluded because in these the concentration of heavy metal applied was 0 (mg L^{-1}).

Table 8. Bioconcentration factor in *Ambrosia ambrosioides*.

Metal	Concentration			A
	20 (mg L^{-1})	40 (mg L^{-1})	60 (mg L^{-1})	
Cadmium	58.17 de	142.33 cd	75.61 cd	92.04 b
Copper	1 691.87 a	237.63 b	173.58 bc	701.03 a
Lead	53.82 de	38.16 e	37.06 e	43.02 c
B	601.29 a	139.38 b	95.42 c	

Means with different letters are statistically different (Tukey's $p\leq$ 0.05); CV= 13.79%. A= heavy metal; B= concentration; CV= coefficient of variation.

For the heavy metal factor, copper was the one with the highest bioconcentration factor 701.03, the lead factor was the lowest 43.02. The concentration factor indicates that in 20 (mg L^{-1}) the highest 601.29 was presented; the lowest in the concentration of 60 (mg L^{-1}), 95.42. In the treatments copper at 20 (mg L^{-1}) (treatment 6) was the one that obtained the highest bioconcentration factor 1691.87, the minor presented in lead-60 (mg L^{-1}) (treatment 12) 37.06.

The heavy metal factors and concentration, as well as all the treatments presented in Table 8 exceeded the comparative value of 1, which indicates the feasibility of *Ambrosia ambrosioides* to be used in phytoremediation techniques. The term metal hyperaccumulator is currently used to designate plants that accumulate >10 (mg kg^{-1}) of Mn and Zn, >1 (mg kg^{-1}) of Co, Cu, Pb, Ni, As and Se and >100 (mg kg^{-1}) of Cd (Kidd *et al*., 2007). They have gained relevance in recent decades, due to their potential use in metal phytoextraction techniques in contaminated soils (González and Zapata, 2008).

Ambrosia ambrosioides absorbed enough heavy metals showing that it is a hyper-accumulating plant of cadmium, copper and lead, it can be used in soil phytoremediation techniques. Some of the effects of cadmium on plants are reduction in growth and elongation of the roots, inhibition of stomatic opening, inhibition of chlorophyll synthesis and photosynthesis (Pernia *et al*., 2008).

Copper is a toxic metal when it is found in tissues at concentrations greater than those necessary for plant growth (>30 mg kg^{-1}) (León and Sepúlveda, 2012). The accumulation of Pb ions in plants can cause multiple effects, direct and indirect. It can cause effects on metabolism that have an impact on growth, photosynthesis and nutrient absorption (Díaz *et al*., 2001). *Ambrosio ambrosioides* had no visible symptoms of phytotoxicity in any treatment.

Conclusions

Copper is the heavy metal that *Ambrosia ambrosioides* absorbed most. Copper at a concentration of 20 (mg L^{-1}) had the highest accumulation in root, stem and leaf with 15 827.2, 13 030.9 and 4 979.4 (mg kg^{-1}) respectively. Cadmium and copper showed a tendency to decrease in their accumulation when applied at concentrations greater than 40 and 60 (mg L^{-1}) respectively. The biological translocation factor indicated that cadmium is the heavy metal that the plant translocates towards its leaves more easily. Lead is a metal that the plant cannot translocate. The bioconcentration factor in cadmium, copper and lead indicates that high phytoextraction was carried out when all the treatments exceeded the comparative value of 1.

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