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RESEARCH ARTICLE

Heavy metal bioaccumulation and morphological changes in *Vachellia campechiana* (Fabaceae) reveal its potential for phytoextraction of Cr, Cu, and Pb in mine tailings

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Abstract

Vachellia campechiana (Mill Seigler & Ebinger) is widely distributed in Mexico and is a dominant species of tailings in Huautla, in the state of Morelos, Mexico. Mining activities carried out in this region generated about 780 thousand tons of bioavailable heavy metal waste (HMs) that were deposited in the environment without any treatment. This study evaluates the bioaccumulation capacity and morphological changes of *V. campechiana* growing during 1 year in control or tailing substrates (treatments) under greenhouse conditions. The concentration of six HMs was also measured in roots, leaves, and seeds by atomic absorption spectrophotometry. Five metals showed a similar bioaccumulation pattern in the roots and leaves of *V. campechiana* grown in both substrates: Pb > Fe > Cr > Cu > Zn. The concentrations of Cr, Cu, and Pb were significantly higher in the roots and leaves of individuals growing on the exposed substrate. The presence of essential metals (Cu, Fe, Zn) was only recorded in the seeds, with similar concentrations in both treatments. Seventeen of 18 morphological characters evaluated in *V. campechiana* decreased in plants exposed to metals. Pb, Cu, and Fe showed a bioconcentration factor greater than one in roots and leaves. The translocation factor showed the following pattern: Cr > Cu = Pb. In conclusion, *V. campechiana* is a candidate species to phytoremediate environments contaminated with Pb, Cr, and Cu due to its ability to establish itself and turn into the dominant plant species in polluted sites, its ability to bioaccumulate non-essential metals in roots and leaves, and its high rate of HMs translocation.

Keywords Heavy metals · Mine tailings · Phytoremediation · Accumulator species · Translocation

Introduction

Mining generates waste in the form of gases, sewage, and/or tailings (Gutiérrez-Ruiz et al. 2007). As a result of the improper management of the waste derived from this activity, tailings have created environmental and health problems in Mexico (Mireles et al. 2012; Cortés-Jiménez et al. 2012; Mussali-Galante et al. 2013) due to their content of potentially

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toxic elements such as heavy metals (HMs), which harm living organisms by bioaccumulating in their tissues and damaging their genes and their ecosystems (Gutiérrez and Moreno 1995; Mussali-Galante et al. 2013).

The existence of plant species that tolerate high concentrations of metals in the soil has been reported before. These plants restrict the absorption of heavy metals and/or translocate them to the leaves, which allows them to maintain constant and relatively low concentrations of these pollutants in the aerial biomass, regardless of the concentration of metals in the soil (exclusion strategy). Other plant species actively absorb metals from the soil and accumulate them in non-toxic forms in the aerial biomass (accumulation strategy) (Brooks et al. 1977; Furini 2012; Marrero-Coto et al. 2012).

This differential response in the absorption of HMs present in the environment depends on the bioavailability of the metals, the retention capacity of the metals, the interaction between plants, roots, and metals, the plant metabolism, and the physicochemical properties of the soil, such as pH, organic matter content, and electrical conductivity (Kabata-Pendias 2000; Barceló and Poschenrieder 2003; Prieto et al. 2009; Rascio and Navari-Izzo 2011). It has been reported that the bioaccumulation of HMs by plants varies considerably across different taxonomic groups (Calow 1993).

Exposure to HMs can have various effects on plants, such as inhibiting seed germination, inhibiting root growth, causing alterations in the root biomass, hindering seedling development, causing micro-morphological alterations (stomata and trichomes), reducing the plant biomass (roots, stem, and leaves), and altering biochemical processes such as protein inhibition (Yadav 2010; Rengel et al. 2011; Tovar-Sánchez et al. 2019). Despite these adverse effects, some plant species have the ability to deal with the presence of HMs in the environment where they grow (Rascio and Navari-Izzo 2011). These species are known as accumulators and have the ability to establish themselves naturally in environments contaminated with HMs. They are fast growing species, with abundant aerial biomass, high capacity to absorb HMs from the soil, efficient translocation of HMs from root to leaf tissue, and high capacity to detoxify and retain large amounts of HMs in their leaf tissue (Rascio and Navari-Izzo 2011; Cappa and Pilon-Smits 2014; Shiqi et al. 2018). To determine if a species has these characteristics, it is necessary to evaluate how it responds to the effects of HMs when exposed to them.

The use of heavy metal accumulator plants has been a phytoremediation strategy in contaminated sites. These plants absorb the elements and remove them from mining waste, preventing their leaching to the water table. Phytoremediation uses plant species to immobilize and store HMs in different plant structures such as roots, stems, and leaves (Shiqi et al. 2018). Some indexes have been proposed in the literature that measure the capacity of plants to store HMs. For example, Yoon et al. (2006) proposed the bioconcentration factor (BCF) as a parameter that measures the efficiency of a plant species to accumulate metals from the soil in leaf tissue. They also proposed the translocation factor (FT), which indicates the efficiency with which metals are transported from the roots to the aerial parts of the plant. Using the latter index, Abhilash et al. (2009) reported that *Limnocharis flava* (L.) Buchenau (Limnocharitaceae) is an accumulator of cadmium (Cd).

About 450 species of plants have been recognized as accumulators of HMs, mainly Cd, nickel (Ni), copper (Cu), lead (Pb), and zinc (Zn) (Macnair 2003; Van der Ent et al. 2013). It is still important to characterize new plant species with the potential to bioaccumulate HMs and to be used in the phytoremediation of contaminated environments (Mendez and Maier 2008).

In Mexico, Huautla, located in the state of Morelos, was a mining area until 1988. It is estimated that there are in the area approximately 780,000 tons of waste in the form of tailings. These are rich in Pb, manganese (Mn), Cd, arsenic (As), Zn, Cu, iron (Fe), and chromium (Cr), all of which are bioavailable (Velasco et al. 2005; Mussali-Galante et al. 2013). Despite this, some species of plants, such as *V. campechiana* (Fabaceae), a shrubby species with different uses that is widely distributed in Mexico, mainly in arid and semi-arid areas, have established themselves in the Huautla area (Arce 2001; Rico 2001; Cervantes and Sotelo 2002; Armienta et al. 2008). Since it inhabits areas contaminated with HMs, *V. campechiana* could have characteristics that allow it to be considered as a plant with potential for phytoremediation; it has a high seed germination percentage, is fast growing, and has abundant leaf biomass. However, its possible role in the bioremediation of environments contaminated with HMs is still unknown. Therefore, the present study evaluated, under greenhouse conditions, the accumulation of HMs in the leaf and root tissue of *V. campechiana* individuals growing on a tailing substrate and a control substrate and the effect of this accumulation on seed germination and on the macro- and micromorphological leaf characters of the plants. The questions that guided this study were as follows: 1) Do individuals of *V. campechiana* growing on tailing substrate bioaccumulate HMs in root and leaf tissue? 2) Is the exposure time to HMs a factor that favors the bioaccumulation in root and leaf tissue of *V. campechiana*? 3) Does exposure to HMs promote changes in the macro- and micromorphological characters of this species? 4) Does the growing substrate (control, exposed) have an effect on the bioaccumulation levels of HMs and on seed germination? 5) Does the morphological and HMs bioaccumulation response (root, leaf, seeds) of *V. campechiana* plants chronically exposed to mine tailings make them useful for the phytoremediation of contaminated environments?

Methods

Study sites

This study was conducted in the municipality of Tlaquiltenango, in the state of Morelos. Mining activities were carried out in this area until 1988, mainly extracting Pb, Zn, and Ag. For decades, the waste generated from mining was deposited in the open, without any type of treatment. Three main tailings were formed, both of which are located in the town of Huautla, south of the municipality of Tlaquiltenango, Morelos, within the Sierra de Huautla Biosphere Reserve (REBIOSH). Tailing mine 1 (T1) is the largest and is located 500 m from the town (8° 26′ 36.37″ N and 99° 01′ 26.71″ W). Tailing mine 2 (T2) is located 1000 m from the town (18° 26′ 22.62″ N and 99° 01′ 51.71″ W). Tailings are rich in metals such as Pb, Mn, Cd, As, Zn, Cu, Fe, and Cr (Velasco et al. 2005). The following control sites were chosen: Quilamula (C1 site) located at 18° 30′ 52″ N and 98° 59′ 59″ W, at an altitude of 1100 m, and Ajuchitlán (C2 site), located at 18° 27′ 52″ N and 98° 58′ 53″ W, at an altitude of 1050 m. Both sites have very similar ecological and geographical characteristics to the exposed sites (Martínez-Pacheco 2008), but have no records of mining activity or anthropogenic metal contamination (Mussali-Galante et al. 2013). Both sites are more than 6 km in linear distance from the exposed sites. In general, Tlaquiltenango presents a natural richness of mineral soils (mainly sulfur minerals) of silver and lead. The most commonly found minerals are as follows: arsenopyrite (FeAsS), galena (PbS), acantite (Ag2S), and calclacita (Cu2S) (Volke et al. 2004, 2005; Secretaria de Economía 2011). Therefore, the soils of the region are naturally rich in minerals.

Study species

V. campechiana is a shrub-like species (Fabaceae) commonly known as "cubata," reaching up to 4 m in height, with concave spines, usually reddish when young and brown when mature, spoon-shaped, and up to 3.5 cm long and 1.3 cm wide (Arce 2001). Its fruit is white, flattened, indehiscent, and brown-reddish, with no obvious margins. Seeds of ellipsoidal shape were light brown or yellowish, without aryl (Arce 2001). The seeds show physical latency; however, when this latency is broken, the germination percentage reaches 90% (Baskin and Baskin 2004). It is a kind of secondary vegetation of tropical deciduous forests that inhabits places with sub-humid or dry climates, at altitudes ranging from 1150 to 1450 m. It is an evergreen species that blooms in May–August and produces fruit in September–May. It is used as fuel, as material for fence posts and construction, and as a growing tutor for other plants to make agricultural tools. It is also a forage and medicinal species, used for kidney and stomach problems (Rico 2001; Cervantes and Sotelo 2002).

Seed collection

Seeds of *V. campechiana* were collected from individuals established in the two control sites (C1 and C2) and in the two exposed sites (T1 and T2) (Fig. 1). So that the germination analysis encompassed the genetic variability of the species, the seeds from sites C1 and C2 were considered as control seeds, while the seeds from T1 and T2 were considered exposed seeds. At each site, 20 individuals were randomly selected and 20% of the seeds were collected from them (Gold et al. 2004). The seeds were transported to the laboratory, where they were cleaned and selected, removing the seeds parasitized by insects.

Germination of *V. campechiana* and plant growing

To evaluate the germination percentage, *V. campechiana* seeds from the control and the exposed sites were subjected to mechanical scarification due to the presence of physical latency. Twenty-five seeds were sown in a Petri dish with agar (1%), using six replicates per treatment. This assay was monitored for 20 days.

After the seeds germinated, 72 seeds were transplanted into individual nursery polyethylene bags (15 L) with the treatment substrates (36 in tailing substrate and 36 in control substrate). Soil from Quilamula was used as control substrate; it was sieved with a stainless steel sieve number 35 (Fiicsa), with a 0.5 mm mesh, in order to obtain a particle size similar to that of the mine tailing. The mixture of residues from tailings 1 and 2 was considered the exposed substrate. All the plants were kept under greenhouse conditions; they were watered twice a day, three times a week, and the temperature ranged from 32 to 35 °C. The plants obtained were used to evaluate the bioaccumulation of HMs and measure the macro- and micromorphological characters of interest.

Evaluation of morphological characters

To assess the effect of the time of exposure to HMs on the morphological characters of *V. campechiana*, six individual plants were randomly selected per treatment (control and tailing substrates). Six leaves were randomly chosen from each individual, and the foliar macro- and micromorphological characters shown in Table 1 were measured every 2 months of exposition to treatments, over 1 year. The macromorphological characters were measured with a digital vernier (Stainless Hardened) and a digital scale (Acculab). For the micromorphological characteristics, a foliar epidermal impression was done using a replication technique with cyanoacrylate glue. Three slides with epidermal impressions of the abaxial part of the leaf were made per each individual plant. The slides were observed with an optical microscope (Leica) at 40X with bright field illumination (CC) and differential interference contrast (CDI).

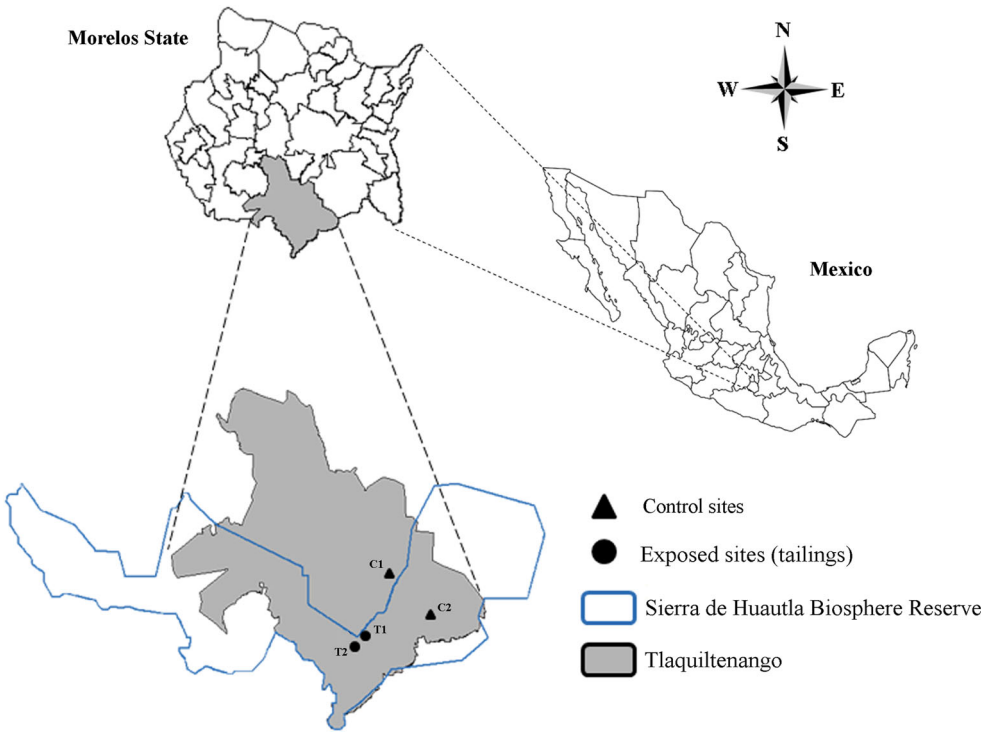

Fig. 1 Geographical distribution of the two study sites at the Sierra de Huautla Biosphere Reserve, Morelos, Mexico. Control site (black-filled up-pointing triangle). Exposed site (black-filled circle)

Three photomicrographs were taken at random from each slide. From the nine photomicrographs, an average of the number of stomata (E), epidermal cells (CE), and stomatal index (IE) was obtained for each individual plant. The stomatal index was calculated according to Salisbury (1968).

Table 1 Size and macro- and micro-morphological characters analyzed in *Vachelia campechiana*

Abbreviation	Character	Units
Size characters		
RL	Root length	cm
SL	Stem length	cm
FRB	Fresh root biomass	g
DRB	Dry root biomass	g
FLB	Fresh leaf biomass	g
DLB	Dry leaf biomass	g
Macro-morphological characters		
LBL	Leaf blade length	mm
WLB	Width of the leaf blade	mm
LP	Length of the petiole	mm
PD	Petiole diameter	mm
LIV	Length of the intermediate vein	mm
WIV	Width of the intermediate vein	mm
1/3AW	1/3 Apical width	mm
1/3 BW	1/3 Basal width	mm
LIL	Length of the intermediate leaflet	mm
WIL	Width of the intermediate leaflet	mm
CLB	Coverage of leaf blade	mm^2
Micro-morphological characters		
SI	Stomatal index	mm^2

Concentration of heavy metals in the substrate and plant tissue of *V. campechiana*

A total of 10 samples of the exposed substrate were analyzed to determine the concentration of metals (Cd, Cr, Cu, Fe, Mn, Pb, and Zn). The samples were dried and sieved following the method established by the Mexican standard NMX-AA-132-SCFI-2006. This process consists in adding 50 mL of CaCl2 (0.01 M) to 10 g of substrate. The sample is kept under stirring for 24 h and centrifuged at 1500 rpm for 15 min, recovering the supernatant by filtration. The concentration of metals in the substrate samples was determined by atomic absorption spectrophotometry using the flame method (GBC 908 A).

To evaluate the concentration of metals (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) in the root and leaf tissue of *V. campechiana*, three samples were taken from six individuals per substrate (tailing and control), every 2 months of exposure to treatments over 1 year. To evaluate the concentration of metals in seeds of *V. campechiana*, three samples were taken from six individuals per substrate (tailing and control). An amount of 0.25 g of each plant structure were pulverized in containers previously washed with HNO_3.The samples were subjected to acid digestion in an Accelerated Reaction System Microwave (CEM® MARS-5) using 10 mL of HNO_3 (70%) in Teflon pumps. The samples were dissolved and filtered in distilled water to a final

volume of 50 mL until further analysis. A sample without tissue was processed simultaneously and was used as a control. The metals were analyzed by atomic absorption spectrophotometry using the flame method (GBC 908 A). The spectrophotometer was calibrated using standard solutions and known concentrations for each metal analyzed. The minimum detection limits (mg/L) of Cd, Cr, Cu, Fe, Mn, Pb, and Zn were 0.0004, 0.003, 0.001, 0.0015, 0.0015, 0.01, and 0.0005, respectively. The samples from the exposed and the control sites were processed simultaneously and in triplicate.

Statistical analysis

A two-factor analysis of variance (model I fixed effects, Zar 2010) was performed to assess the effect of the site (control or exposed), the treatment (mechanical scarification or no treatment), and interaction site × treatment on the germination of seeds of *V. campechiana*. A Tukey test was performed to find significant mean differences between sites and between treatments (Zar 2010). Students' *t* tests were performed to evaluate the effect of the site of origin of the seeds (control and exposed) on their biomass and cover.

Two-factor analysis of variance was used to determine the effect of exposure time (60, 120, 180, 240, 300, 360 days), treatment (control and exposed), and the interaction time × treatment on the variations of 18 morphological characters (17 macro and one micro). Afterwards, a Tukey test was carried out to determine significant differences between pairs of average values of morphological characters in both treatments (Zar 2010).

The same analysis was carried out to evaluate the effect of exposure time, treatment (control and exposure), and interaction time × treatment on the accumulation of Cr, Cu, Fe, Pb, and Zn (except for Cd, which was not detected) in the roots and leaves of individuals of *V. campechiana*. A Tukey test was also used to determine significant differences in the average concentration of each metal over time by plant structure analyzed, for both treatments (Zar 2010).

The Mann Whitney *U* analysis was used to assess the effect of seed origin (control or exposed substrate) on the accumulation of HMs in the testa and embryo. All analyses were performed using the STATISTICA 8 program (StatSoft 2004).

The capacity of *V. campechiana* to phytoextract HMs was evaluated using two indices: the bioconcentration factor (BCF), which determines the efficiency of the plant in the accumulation of substrate metals in its tissue (Yoon et al. 2006), and the translocation factor (FT), which measures the efficiency of the plant in the transportation of metals from the root to the aerial parts (Yoon et al. 2006). These indices are calculated as follows:

$$\mathrm{FBC} = \mathrm{Cfoliar}/\mathrm{Ctailing}$$

$$\mathrm{FT} = \mathrm{Cfoliar}/\mathrm{Croot}$$

where Cfoliar is the concentration of the metal in the leaf tissue, Ctailing is the bioavailable concentration in the tailing, and Croot is the concentration of the metal in the root tissue. It has been reported that if a plant has FT values > 1, the species is considered an accumulator of the analyzed metal (Yoon et al. 2006; Covarrubias and Cabriales 2017).

Results

Germination and morphological characteristics of *V. campechiana* seeds from the control site and the exposed site (tailing mine)

The germination experiment showed that the site of origin of the individual plants (control vs exposed) did not influence the germination percentage of the seeds. In contrast, the pre-germinative treatment did significantly change germination percentages. There was no significant effect of the interaction site × treatment on germination percentages (Table 2). The results indicate that, considering both sites of origin (control and exposed), only 12% of the seeds germinated when they did not receive pre-germinative treatment. In contrast, when the seeds were subjected to mechanical scarification, the percentage of seed germination increased to more than 90% in both sites (Table 2).

The site of origin of the individual plants did have an effect on seed biomass and surface area. The results indicate that *V. campechiana* seeds from the site exposed to heavy metals showed statistically lower values for both characters analyzed compared with seeds from the control site (Table 3).

Table 2 Seedling percentage of *Vachelia campechiana* from the control and the exposed site, under pregerminative treatments

Site	Treatment	Seedling (%)	ANOVA	
Control	No scarification	12.00 a	Site (S)	$F_{1,20} = 0.57$, ns
	Mechanical scarification	98.67 b	Treatment (t)	$F_{1,20} = 530.03$***
Exposed	No scarification	12.67 a	S × t	$F_{1,20} = 0.57$ ns
	Mechanical scarification	99.33 b		

Different lower case letters denote significant differences between treatments (Tukey $p < 0.05$). Average ± e.e.
*** = $p < 0.001$, ns = not significant differences

Table 3 Biomass (g) and coverage (mm^2) average (± e.e.) of *Vachellia campechiana* seeds from the control and the exposed sites

	Control		Exposed		Student *T*-test
Biomass	0.036 ± 0.0005	a	0.023 ± 0.0004	b	19.03***
Covarage	42.81 ± 0.40	a	37.26 ± 0.32	b	10.96***

Different lower case letters denote significant differences between sites*** = $p < 0.001$

Morphological and size changes in individuals of *V. campechiana* growing on tailing substrate or control substrate

In general, the results show that the time of exposure (t), the treatment (T), and the interaction between these two factors (t × T) had a significant effect on all the size and macro- and micromorphological characters analyzed during 360 days in individual plants of *V. campechiana* growing on a greenhouse under two different treatments (tailing substrate and control substrate). The treatment had no significant effect on the diameter of the petiole (DP), while the interaction between factors (t × T) had no significant effect on length (RL), dry biomass, and fresh root biomass (Table 4).

Size characters

All the evaluated characters of *V. campechiana* individuals grown on the control substrate showed a significant increase over time (360 days): root length (RL), stem length (SL), fresh root biomass (FRB), dry root biomass (DRB), fresh leaf biomass (FLB), dry leaf biomass (DLB). In contrast, in the plants grown on the tailing substrate, 50% of the characters (FRB, DRB, and DLB) showed a significant increase, while the remaining 50% of the characters (RL, SL, and FLB) showed no changes over time (Table 4).

The fresh and dried root biomass did not change significantly between both treatments, remaining unchanged in both treatments at the beginning and the end of the experiment. In contrast, the dry and fresh leaf biomass was significantly lower in plants grown on tailing substrate. In general, the RL did not change between treatments over time, while the SL was significantly lower in tailing substrate individuals (Table 4).

Macro-morphological characters

In the individuals of *V. campechiana* grown on the control substrate treatment, 54.5% of the evaluated characters had a significant increase over time (360 days): leaf blade length (LBL), petiole diameter (PD), length of the intermediate vein (LIV), leaf-shape characters such as the 1/3 apical width (1/3AW) and the 1/3 basal width (1/3BW), as well as coverage of leaf blade (CLB). Moreover, 27.3% of the characters decreased in size over time: width of the intermediate vein (WIV), length of the intermediate leaflet (LIL), and width of the intermediate leaflet WIL. The width of the leaf blade (WLB) remained more or less constant over time, while the length of the petiole (LP) showed oscillatory changes (Table 4). In the individuals established in the tailing substrate, 45.4% of the characters did not show changes over time: WLB, LP, WIV, WIL, and CLB. In contrast, 54.6% of the characters showed oscillatory changes over time: LBL, PD, LIV, 1/3BW, 1/3AW, and LIL (Table 4).

When comparing individuals from *V. campechiana* between treatments, the values of 63.6% of the analyzed characters decreased over time in the plants grown on the tailing substrate compared with the plants grown on the control substrate. Moreover, 27.3% of the characters showed an inverse pattern (WIV, LIL, and WIL), while the PD character did not change between treatments (Table 4).

Micro-morphological characters

With respect to micromorphology, the stomatal index (SI) decreased over time in individuals growing on the control substrate. Individuals growing on tailing substrate showed an inverse pattern. However, at the end of the treatment, there were no statistically significant differences in SI values between substrates (Table 4).

Heavy metal concentration in roots, leaves and seeds of *V. campechiana*

Roots

In general, the presence of Cr, Cu, Fe, Pb, and Zn was detected in the roots of individuals of *V. campechiana*, but Cd and Mn were not detected. The analysis of variance showed a significant effect of time (t), treatment (T), and interaction (t × T) on the bioaccumulation of four metals (Cr, Cu, Pb, and Zn) in the root of individuals of *V. campechiana*. In contrast, the aforementioned variables did not have a significant effect on the concentration of Fe in the roots (Table 5).

The bioaccumulation of Cr was only recorded in the roots of plants grown on tailing substrate, with a significant increase in the concentration with the time of exposure. The concentration of Cu in the roots was statistically higher in individuals growing in the control substrate, compared with those grown in tailing substrate. The concentration of Cu in the control substrate individuals remained constant over time, while plants growing in tailing substrate showed a significant increase in the concentration of Cu with the time of exposure. In general, there was no significant effect of time, treatment, and interaction (t × T) on the bioaccumulation of Fe. With respect to Pb, its concentration was significantly higher in

Table 4 Average (± e.e.) of macro- and micro-morphological characters from roots, stem and leaves from *Vachellia campechiana* growing for a year in greenhouse conditions on tailing substrate and reference substrate

Character	Time (days)	Treatment					ANOVA	
		Control		Exposed	SDT			
Size characters								
Root length								
	60	40.2 ± 2.7	a	35.9 ± 3.1	AB	ns	Time (t)	$F_{5,60} = 5.72$ ***
	120	40.7 ± 1.9	a	28.6 ± 1.2	A	***	Treatment (T)	$F_{1,60} = 30.89$ ***
	180	54.7 ± 3.7	ab	44.7 ± 5.4	B	ns	t × T	$F_{5,60} = 1.76$ ns
	240	50.9 ± 4.0	ab	39.4 ± 2.8	AB	ns		
	300	42.4 ± 3.0	a	36.4 ± 4.6	AB	ns		
	360	59.6 ± 4.7	b	36.2 ± 3.6	AB	**		
Stem lengh								
	60	42.2 ± 2.7	a	34.3 ± 3.9	A	ns	Time (t)	$F_{5,60} = 6.58$ ***
	120	59.5 ± 9.2	a	40.5 ± 5.6	A	ns	Treatment (T)	$F_{1,60} = 60.74$ ***
	180	55.2 ± 5.7	a	38.5 ± 3.8	A	*	t × T	$F_{5,60} = 4.48$ ***
	240	85.0 ± 8.2	b	45.2 ± 5.5	A	**		
	300	105.1 ± 6.9	c	42.7 ± 7.9	A	***		
	360	101.1 ± 17.3	c	36.8 ± 5.2	A	**		
Fresh root biomass								
	60	11.50 ± 9.29	a	5.86 ± 2.80	A	ns	Time (t)	$F_{5,60} = 16.80$ ***
	120	9.53 ± 3.99	a	6.15 ± 2.54	A	ns	Treatment (T)	$F_{1,60} = 13.67$ ***
	180	13.04 ± 7.78	a	5.46 ± 2.45	A	*	t × T	$F_{5,60} = 1.14$ ns
	240	40.3 ± 23.72	b	18.01 ± 4.65	B	*		
	300	35.19 ± 11.85	b	25.52 ± 13.69	B	ns		
	360	33.61 ± 8.19	b	29.37 ± 7.72	B	ns		
Dry root biomass								
	60	3.67 ± 2.35	a	2.21 ± 0.82	A	ns	Time (t)	$F_{5,60} = 20.05$ ***
	120	4.83 ± 2.07	a	3.08 ± 0.86	A	ns	Treatment (T)	$F_{1,60} = 21.95$ ***
	180	6.57 ± 3.87	a	3.05 ± 1.53	A	ns	t × T	$F_{5,60} = 2.48$ ns
	240	21.19 ± 11.39	b	8.03 ± 2.27	B	*		
	300	19.86 ± 6.13	b	12.56 ± 7.16	B	ns		
	360	17.80 ± 4.19	b	12.94 ± 3.26	B	ns		
Fresh leaf biomass								
	60	4.91 ± 1.46	a	3.20 ± 2.00	A	ns	Time (t)	$F_{5,60} = 6.51$ ***
	120	3.72 ± 1.46	a	1.19 ± 0.59	A	***	Treatment (T)	$F_{1,60} = 14.88$ ***
	180	3.55 ± 1.85	a	1.59 ± 1.35	A	ns	t × T	$F_{5,60} = 5.17$ ***
	240	12.83 ± 4.65	b	9.24 ± 3.14	B	**		
	300	39.64 ± 3.81	c	4.08 ± 1.47	A	**		
	360	14.04 ± 5.25	b	4.28 ± 0.94	A	**		
Dry leaf biomass								
	60	2.26 ± 1.13	a	1.27 ± 0.40	A	ns	Time (t)	$F_{5,60} = 5.43$ ***
	120	1.76 ± 0.50	a	0.64 ± 0.27	A	***	Treatment (T)	$F_{1,60} = 49.10$ ***
	180	1.53 ± 0.80	a	0.73 ± 0.62	A	ns	t × T	$F_{5,60} = 4.52$ ***
	240	5.84 ± 2.30	b	2.66 ± 0.67	B	**		
	300	7.84 ± 2.84	b	2.84 ± 2.33	B	**		
	360	6.75 ± 3.05	b	2.77 ± 0.43	B	**		
Macro-morphological characters								
Leaf blade lenght								
	60	47.38 ± 1.51	a	50.47 ± 2.59	A	ns	Time (t)	$F_{5,414} = 94.77$ ***
	120	42.34 ± 2.35	a	27.74 ± 2.10	B	***	Treatment (T)	$F_{1,414} = 258.79$ ***
	180	41.86 ± 2.71	a	19.64 ± 1.40	B	***	t × T	$F_{5,414} = 24.302$ ***
	240	75.72 ± 3.10	b	59.47 ± 2.33	C	***		
	300	79.59 ± 2.22	b	45.05 ± 1.33	D	***		
	360	80.54 ± 2.54	b	42.59 ± 1.89	D	***		
Width of the leaf blade								
	60	30.31 ± 0.47	ab	31.18 ± 1.63	A	ns	Time (t)	$F_{5,414} = 23.87$ ***
	120	27.25 ± 0.94	b	20.36 ± 0.98	AB	***	Treatment (T)	$F_{1,414} = 174.79$ ***
	180	31.35 ± 0.83	a	16.95 ± 0.61	AB	***	t × T	$F_{5,414} = 15.13$ ***
	240	36.51 ± 0.89	c	25.95 ± 0.58	C	***		
	300	32.88 ± 1.78	ac	27.60 ± 0.72	AC	***		
	360	31.98 ± 1.41	a	21.11 ± 0.73	B	***		
Length of the petiole								
	60	7.28 ± 0.26	a	8.95 ± 0.35	A	***	Time (t)	$F_{5,414} = 33.13$ ***
	120	6.27 ± 0.20	b	5.48 ± 0.38	B	ns	Treatment (T)	$F_{1,414} = 35.01$ ***
	180	7.63 ± 0.82	ad	4.91 ± 0.16	B	***	t × T	$F_{5,414} = 20.40$ ***

Table 4 (continued)

Character	Time (days)	Treatment					ANOVA	
		Control		Exposed	SDT			
	240	6.69 ± 0.18	d	6.02 ± 0.13	B	*		
	300	6.47 ± 0.21	d	5.70 ± 0.17	B	**		
	360	6.23 ± 0.23	b	4.69 ± 0.15	B	***		
Petiole diameter								
	60	0.54 ± 0.01	a	0.66 ± 0.03	A	ns	Time (t)	$F_{5,414} = 56.17$ ***
	120	0.43 ± 0.02	b	0.48 ± 0.03	B	ns	Treatment (T)	$F_{1,414} = 3.69$ ns
	180	0.49 ± 0.02	b	0.54 ± 0.03	BC	ns	t × T	$F_{5,414} = 6.36$ ***
	240	0.73 ± 0.02	c	0.70 ± 0.03	A	ns		
	300	0.61 ± 0.02	ac	0.58 ± 0.03	C	ns		
	360	0.63 ± 0.03	ac	0.57 ± 0.03	C	ns		
Length of the intermediate vein								
	60	15.73 ± 0.39	a	18.09 ± 0.57	A	**	Time (t)	$F_{5,414} = 53.79$ ***
	120	12.57 ± 0.43	b	10.12 ± 0.53	B	***	Treatment (T)	$F_{1,414} = 124.27$ ***
	180	14.57 ± 0.40	c	7.99 ± 0.24	C	***	t × T	$F_{5,414} = 23.79$***
	240	17.47 ± 0.40	d	14.48 ± 0.34	D	***		
	300	16.23 ± 0.54	d	13.87 ± 0.33	D	***		
	360	16.26 ± 0.71	d	10.95 ± 0.34	B	***		
Width of the intermediate vein								
	60	4.81 ± 0.12	a	4.95 ± 0.35	A	ns	Time (t)	$F_{5,414} = 14.88$ ***
	120	3.61 ± 0.12	b	5.48 ± 0.38	B	***	Treatment (T)	$F_{1,414} = 120.96$ ***
	180	4.16 ± 0.12	c	4.91 ± 0.16	B	ns	t × T	$F_{5,414} = 9.42$ ***
	240	3.67 ± 0.14	b	6.20 ± 0.13	A	***		
	300	3.48 ± 0.18	b	5.70 ± 0.17	B	***		
	360	3.30 ± 0.15	b	4.69 ± 0.15	B	***		
1/3 apical width								
	60	28.23 ± 0.70	a	28.54 ± 1.54	A	ns	Time (t)	$F_{5,60} = 24.32$ ***
	120	27.64 ± 0.88	b	18.83 ± 0.99	B	***	Treatment (T)	$F_{1,60} = 212.06$ ***
	180	29.64 ± 0.83	c	16.49 ± 0.57	B	***	t × T	$F_{5,60} = 16.17$***
	240	36.06 ± 0.90	d	25.02 ± 0.46	C	***		
	300	31.08 ± 1.03	c	27.97 ± 0.74	A	*		
	360	31.25 ± 1.23	c	21.03 ± 0.77	C	***		
1/3 basal width								
	60	27.09 ± 0.73	a	27.23 ± 1.63	A	ns	Time (t)	$F_{5,60} = 31.75$ ***
	120	22.83 ± 0.79	b	17.20 ± 0.99	B	***	Treatment (T)	$F_{1,60} = 225.14$ ***
	180	27.46 ± 0.76	ac	14.40 ± 0.47	C	***	t × T	$F_{5,60} = 14.37$ ***
	240	33.51 ± 0.77	d	23.61 ± 0.49	A	***		
	300	30.89 ± 1.09	cd	24.17 ± 0.50	A	***		
	360	30.13 ± 1.23	cd	18.48 ± 0.58	B	***		
Length of the intermediate leaflet								
	60	3.04 ± 0.08	a	3.28 ± 0.21	A	***	Time (t)	$F_{5,414} = 46.39$ ***
	120	2.39 ± 0.05	bc	2.74 ± 0.99	B	***	Treatment (T)	$F_{1,414} = 28.41$ ***
	180	2.68 ± 0.14	ab	2.18 ± 0.07	C	**	t × T	$F_{5,414} = 13.69$ ***
	240	2.14 ± 0.08	c	2.69 ± 0.08	B	***		
	300	2.19 ± 0.11	c	2.69 ± 0.09	B	***		
	360	2.25 ± 0.06	c	2.23 ± 0.04	C	ns		
Width of the intermediate leaflet								
	60	0.66 ± 0.03	a	1.46 ± 0.17	A	***	Time (t)	$F_{5,414} = 53.04$ ***
	120	0.51 ± 0.02	b	0.83 ± 0.02	B	***	Treatment (T)	$F_{1,414} = 387.34$ ***
	180	0.49 ± 0.02	b	0.59 ± 0.02	B	***	t × T	$F_{5,414} = 7.86$ ***
	240	0.47 ± 0.02	bc	0.76 ± 0.02	B	***		
	300	0.42 ± 0.02	bc	0.73 ± 0.03	B	***		
	360	0.39 ± 0.02	c	0.60 ± 0.03	B	***		
Coverage of leaf blade								
	60	383.38 ± 8.62	a	402.92 ± 17.6	A	ns	Time (t)	$F_{5,414} = 77.64$ ***
	120	343.39 ± 13.4	a	237.39 ± 14.5	B	***	Treatment (T)	$F_{1,414} = 302.93$ ***
	180	361.29 ± 16.5	a	180.56 ± 7.37	B	***	t × T	$F_{5,414} = 23.77$ ***
	240	553.85 ± 18.5	b	421.48 ± 13.2	B	***		
	300	555.00 ± 14.6	b	367.39 ± 8.86	B	***		
	360	555.25 ± 18.2	b	285.27 ± 13.1	B	***		
Micro-morphological characters								
Stomatal index								
	60	36.72 ± 0.61	a	23.66 ± 0.44	A	***	Time (t)	$F_{5,414} = 13.55$ ***
	120	34.19 ± 0.58	b	33.45 ± 0.69	B	ns	Treatment (T)	$F_{1,414} = 95.42$ ***

Table 4 (continued)

Character	Time (days)	Treatment					ANOVA	
		Control		Exposed	SDT			
	180	30.01 ± 0.53	c	32.59 ± 0.44	BC	***	t × T	$F_{5,414} = 47.25$ ***
	240	33.08 ± 0.53	b	29.21 ± 0.56	C	***		
	300	32.01 ± 0.62	bc	27.87 ± 0.43	C	***		
	360	30.31 ± 0.67	c	31.05 ± 0.67	C	ns		

Different lower case letters denote significant differences between control individuals during treatment time (Tukey $p < 0.05$)

Different upper case letters denote significant differences between exposed individuals during treatment time (Tukey $p < 0.05$)

SDT = statistical differences between treatments, *nd* = not detected, *ns* = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

the roots of the plants growing in tailing substrate compared with the plants growing in the control substrate. The concentration of Pb in individuals growing in the control substrate did not change significantly over time, while the roots of the plants growing in tailing substrate showed an increase in the concentration of Pb over time. Similarly, the concentration of Zn in the roots of the plants growing in tailing substrate was significantly higher than in the plants growing in the control substrate. The plants growing in the control substrate showed a significant reduction in the concentration of Zn over time; in the plants growing in the tailing substrate, the concentration remained constant (Table 5).

Leaves

In general, the presence of Cr, Cu, Fe, Pb, and Zn was detected in the leaves of individuals of *V. campechiana*, but the presence of Cd was not. The analysis of variance showed a significant effect of time, treatment, and interaction of factors on the concentration of Cr and Zn in the leaves of individuals of *V. campechiana*. Cu did not respond to the interaction of factors (t × T), while Fe did not respond to time of exposure or interaction (t × T), and Pb did not respond to time of exposure (Table 5).

The results indicate that the bioaccumulation Cr occurred only in the leaves of plants grown on the exposed substrate, with a significant increase in the concentration with the time of exposure. The concentration of Cu in leaf tissue was statistically higher in individuals growing in the control substrate compared with those grown in the tailing substrate. The concentration of Cu in the control substrate plants remained constant over time, while plants growing in tailing substrate showed a significant increase in Cu with time of exposure. Moreover, there was no significant effect of time and interaction of factors (t × T) on the bioaccumulation of Fe in leaf tissue. In contrast, there was a significant effect of the treatment on the bioaccumulation of Fe. The Tukey test ($P < 0.05$) showed significant differences in the concentration of Fe at 300 and 360 days, with higher concentrations in the plants growing in the control substrate.

With respect to Pb, its concentration was significantly higher in the leaves of plants growing in exposed substrate compared with plants growing in the control substrate. However, the concentration of this metal remained constant over time in plants growing in the control substrate. Moreover, the concentration of Pb showed a tendency to increase over time in the tailing substrate plants, it being significantly higher at 360 days.

Similarly, the concentrations of Zn in the leaves of plants growing in exposed substrate were significantly higher than in those growing in the control substrate. The concentration of Zn showed a significant decrease over time in the plants growing in the control substrate, while concentration remained constant (Table 5) in the plants growing in exposed substrate.

Seeds

Regarding the concentration of heavy metals in the seeds of *V. campechiana*, there was bioaccumulation of Cu, Fe, and Zn in the testa and embryo, but no bioaccumulation of Cr, Cd, and Pb.

There were no significant differences in the concentration of Cu and Fe between the testa and the embryo of the seeds from control sites, but there were significant differences in the concentration of Zn between these seed structures, with the highest concentration of this metal found in the embryo. The seeds from exposed sites did not show significant differences in the concentration of the three metals evaluated between the testa and the embryo. There were significant differences in the concentration of Cu and Zn in the seed testa between sites (control vs exposed), with Cu showing the highest concentration values in the testa of seeds from control sites. In the case of Zn, the highest values were found in the testa of seeds from the exposed sites. In the case of the embryo, no significant differences were found in the concentration of the metals evaluated in the seeds from both sites. Finally, no significant

Table 5 Average values (± e.e) of heavy metal concentration (mg/Kg) in roots and leaves of *Vachelia campechiana,* growing in reference substrate and tailing substrate during 1 year treatment

	Treatment									
	Root					Leaf				
Time (days)	Control		Exposed			Control		Exposed		
Chrome (Cr) 0.003 DL (mg/L)										
60	nd		0.55 ± 0.003	A	*	nd		0.92 ± 0.031	A	*
120	nd		0.54 ± 0.036	A	*	nd		1.01 ± 0.033	A	*
180	nd		0.68 ± 0.033	B	*	nd		1.24 ± 0.07	AB	*
240	nd		0.67 ± 0.097	B	*	nd		1.55 ± 0.027	B	*
300	nd		0.81 ± 0.030	C	*	nd		1.99 ± 0.083	C	*
360	nd		0.83 ± 0.032	C	*	nd		2.75 ± 0.156	D	*
ANOVA	Time (t)		$F_{5,60}$ = 4.87 ***			$F_{5,60}$ = 23.40 ***				
	Treatment (T)		$F_{1,60}$ = 880.11 ***			$F_{1,60}$ = 726.21 ***				
	t × T		$F_{5,60}$ = 4.89 ***			$F_{5,60}$ = 23.40 ***				
Cooper (Cu) 0.001 DL (mg/L)										
60	0.55 ± 0.010	a	0.25 ± 0.008	A	*	0.51 ± 0.011	a	0.34 ± 0.008	A	*
120	0.54 ± 0.011	a	0.26 ± 0.008	A	*	0.54 ± 0.004	a	0.35 ± 0.011	A	*
180	0.50 ± 0.030	a	0.29 ± 0.009	AB	*	0.55 ± 0.016	a	0.39 ± 0.008	B	*
240	0.51 ± 0.017	a	0.32 ± 0.009	BC	*	0.52 ± 0.005	a	0.35 ± 0.007	A	*
300	0.56 ± 0.012	a	0.34 ± 0.012	CD	*	0.52 ± 0.013	a	0.39 ± 0.006	B	*
360	0.54 ± 0.024	a	0.37 ± 0.012	D	*	0.53 ± 0.006	a	0.38 ± 0.003	B	*
ANOVA	Time (t)		$F_{5,60}$ = 7.26 ***			$F_{5,60}$ = 4.54 ***				
	Treatment (T)		$F_{1,60}$ = 853.62 ***			$F_{1,60}$ = 734.84 ***				
	t × T		$F_{5,60}$ = 2.73 ***			$F_{5,60}$ = 2.06 ns				
Iron (Fe) 0.005 DL (mg/L)										
60	2.62 ± 0.049	a	2.06 ± 0.252	A	ns	2.33 ± 0.141	a	1.37 ± 0.341	A	ns
120	2.68 ± 0.099	a	1.92 ± 0.162	A	ns	2.58 ± 0.189	a	1.90 ± 0.266	A	ns
180	2.65 ± 0.136	a	3.17 ± 0.800	A	ns	2.55 ± 0.187	a	1.96 ± 0.155	A	ns
240	2.75 ± 0.046	a	1.52 ± 0.379	A	ns	2.40 ± 0.052	a	1.68 ± 0.251	A	ns
300	2.38 ± 0.137	a	1.96 ± 0.195	A	ns	2.59 ± 0.126	a	1.68 ± 0.160	A	*
360	2.66 ± 0.088	a	2.00 ± 0.211	A	sn	2.47 ± 0.141	a	1.46 ± 0.218	A	*
ANOVA	Time (t)		$F_{5,60}$ = 0.62 ns			$F_{5,60}$ = 0.53 ns				
	Treatment (T)		$F_{1,60}$ = 3.22 ns			$F_{1,60}$ = 20.80 ***				
	t × T		$F_{5,60}$ = 0.66 ns			$F_{5,60}$ = 0.15 ns				
Lead (Pb) 0.01 DL (mg/L)										
60	1.49 ± 0.102	a	2.44 ± 0.132	A	*	1.49 ± 0.078	ab	3.74 ± 0.139	A	*
120	1.37 ± 0.176	a	3.22 ± 0.252	BC	*	1.47 ± 0.043	ab	3.53 ± 0.098	A	*
180	1.60 ± 0.022	a	2.83 ± 0.121	AB	*	1.44 ± 0.105	ab	3.80 ± 0.111	A	*
240	1.57 ± 0.035	a	3.32 ± 0.054	BC	*	1.45 ± 0.085	ab	3.88 ± 0.176	A	*
300	1.41 ± 0.137	a	3.64 ± 0.236	CD	*	1.72 ± 0.079	b	4.06 ± 0.117	A	*
360	1.44 ± 0.113	a	4.25 ± 0.146	D	*	1.25 ± 0.075	a	4.75 ± 0.185	B	*
ANOVA	Time (t)		$F_{5,60}$ = 3.60 ***			$F_{5,60}$ = 2.23 ns				
	Treatment (T)		$F_{1,60}$ = 199.92 ***			$F_{1,60}$ = 564.25 ***				
	t × T		$F_{5,60}$ = 4.70 ***			$F_{5,60}$ = 3.97 ***				
Zinc (Zn) 0.0005 DL (mg/L)										
60	0.13 ± 0.003	a	0.48 ± 0.038	A	***	0.28 ± 0.001	a	0.19 ± 0.001	A	***
120	0.19 ± 0.003	b	0.63 ± 0.094	B	***	0.16 ± 0.001	b	0.16 ± 0.013	A	ns
180	0.13 ± 0.003	a	0.68 ± 0.014	B	***	0.08 ± 0.0003	c	0.21 ± 0.020	A	***
240	0.09 ± 0.001	c	0.76 ± 0.056	C	***	0.12 ± 0.0009	d	0.15 ± 0.006	A	**

Table 5 (continued)

	Treatment									
	Root					Leaf				
Time (days)	Control		Exposed			Control		Exposed		
300	0.06 ± 0.002	c	0.47 ± 0.022	A	***	0.13 ± 0.002	d	0.19 ± 0.024	A	*
360	0.10 ± 0.0006	c	0.47 ± 0.015	A	***	0.11 ± 0.0009	d	0.21 ± 0.011	A	***
ANOVA	Time (t)		$F_{5,60}$ = 2.54 *			$F_{5,60}$ = 7.29 ***				
	Treatment (T)		$F_{1,60}$ = 166.52 ***			$F_{1,60}$ = 12.51 ***				
	t × T		$F_{5,60}$ = 2.00 ns			$F_{5,60}$ = 8.37 ***				

Different lower case letters denote significant differences between control individuals during treatment time

Different upper case letters denote significant differences between exposed individuals during treatment time

LD = detection limit, *nd* = not detected, *ns* = not significant

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

differences were found between sites in the concentration of Fe in the testa and embryo of seeds (Table 6).

The translocation factor and heavy metal enrichment in the roots and leaves of *V. campechiana* plants growing in substrate exposed to metals

Enrichment (BCF) of Cu, Fe, and Pb was recorded in the roots and leaves of *V. campechiana* plants growing in tailing substrate. In contrast, no enrichment of Cr and Zn was detected in any of the plant structures analyzed (Table 7). In roots, the metal enrichment factor showed the following pattern: Pb > Cu > Fe. In leaf tissue, the pattern of the enrichment factor was as follows: Cu > Pb > Fe (Table 7). The pattern of the translocation factor (FT) was as follows: Cr > Pb = Cu > Fe > Zn (Table 7).

Discussion

Bioaccumulation of heavy metals in *V. campechiana*

There are few studies that evaluate the transport and accumulation of metals in the seeds of wild plant species for ecotoxicological purposes. The present study found that *V. campechiana* seeds from both the control and the exposed sites accumulated only essential metals (Cu, Fe, and Zn) and that the concentration of metals in the embryo of seeds did not differ between sites. In contrast, the bioaccumulation of metals in the testa of seeds from the control site had a higher concentration of Cu and Zn. Our results are similar to those reported by Tyler and Zohlen (1998) for several herbaceous species, *Achillea millefolium* L. (Asteraceae), *Arctium tomentosum* Mill. (Asteraceae), *Arenaria serpyllifolia* L. (Caryophyllaceae), *Cerastium semidecandrum* L. (Caryophyllaceae), *Filipendula ulmaria* (L.) Maxim. (Rosaceae), *Hypericum maculatum* Crantz. (Hypericaceae), and *Laserpitium latifolium* (L.) (Apiaceae), the seeds of which bioaccumulate high concentrations of essential metals such as Mn, Cu, Fe, and Zn. Furthermore, the present study agrees with the study by Waters and Sankaran (2011), who reported an increase in the transport of micronutrients, mainly Fe and Zn, to the seeds of plants with nutritional potential.

It has been documented that the transport of minerals to the reproductive parts and to the seeds is carried out through the phloem (Zhang et al. 2007); in the leaves, it is carried out through the xylem. Recent studies have shown that the nicotianamine (NA) molecule transports various metals

Table 6 Average values (± e.e) of heavy metal concentration (mg/Kg) in testa and embryos of *Vachelia campechiana* seeds from the exposed and control sites

	Control		Exposed		*U* Mann Whitney Test	
	Testa	Embryo	Testa	Embryo	Testa	Embryo
Cu	0.40 ± 0.16	0.20 ± 0.10	0.0005 ± 0.00	0.06 ± 0.02	2.38 *	1.19 ns
Fe	0.66 ± 0.05	0.89 ± 0.15	0.56 ± 0.09	0.61 ± 0.03	0.57 ns	1.19 ns
Zn	0.30 ± 0.05	0.58 ± 0.03	0.80 ± 0.18	0.55 ± 0.13	− 2.78 **	− 0.66 ns

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, *ns* not significant

Table 7 Heavy metal enrichment (FBC) and translocation values (FT) of Cr, Cu, Fe, Pb, and Zn in roots and leaves of *Vachelia campechiana* from individuals growing on tailing substrate during treatment

	Concentration (mg/Kg)					
Time (days)	Tailing	Root	Leaf	FBC root	FBC leaf	FT
Chrome (Cr)						
	–	0.55	0.92	–	–	1.67
	–	0.54	1.01	–	–	1.87
	–	0.68	1.24	–	–	1.82
	–	0.67	1.55	–	–	2.31
	–	0.81	1.99	–	–	2.46
	–	0.83	2.75	–	–	3.31
$\bar{x}$ ± d.e						2.24 ± 0.61
Copper (Cu)						
	0.04	0.25	0.92	6.25	23.00	1.36
	0.04	0.26	1.01	6.50	25.25	1.35
	0.04	0.29	1.24	7.25	31.00	1.34
	0.04	0.32	1.55	8.00	38.75	1.09
	0.04	0.34	1.99	8.50	49.75	1.14
	0.04	0.37	2.75	9.25	68.75	1.03
$\bar{x}$ ± d.e				7.62 ± 1.16	39.42 ± 17.37	1.22 ± 0.15
Iron (Fe)						
	0.4	2.06	1.37	5.15	3.43	0.67
	0.4	1.92	1.90	4.80	4.75	0.99
	0.4	3.17	1.96	7.93	4.90	0.62
	0.4	1.51	1.68	3.80	4.20	1.11
	0.4	1.96	1.68	4.90	4.20	0.86
	0.4	2.00	1.46	5.00	3.65	0.73
$\bar{x}$ ± d.e				5.28 ± 1.55	4.34 ± 0.49	0.83 ± 0.19
Lead (Pb)						
	0.23	2.44	3.74	10.61	16.26	1.53
	0.23	3.32	3.53	14.43	15.35	1.06
	0.23	2.83	3.80	12.30	16.52	1.34
	0.23	3.22	3.88	14.00	16.87	1.20
	0.23	3.64	4.06	15.83	17.65	1.12
	0.23	4.25	4.75	18.48	20.65	1.12
$\bar{x}$ ± d.e				15.08 ± 2.31	17.41 ± 1.99	1.23 ± 0.18
Zinc (Zn)						
	2.14	0.48	0.19	0.22	0.09	0.40
	2.14	0.63	0.16	0.29	0.07	0.25
	2.14	0.68	0.21	0.32	0.10	0.31
	2.14	0.76	0.15	0.36	0.07	0.20
	2.14	0.47	0.19	0.22	0.09	0.40
	2.14	0.47	0.21	0.22	0.10	0.45
$\bar{x}$ ± d.e				0.27 ± 0.05	0.086 ± 0.01	0.33 ± 0.09

through the phloem, including Cu, Fe, and Zn, towards reproductive structures, including seeds (Grillet et al. 2014). This suggests that *V. campechiana* bioaccumulates and translocates essential metals such as Cu, Fe, and Zn, since these metals participate in the development of seeds and seedlings when germination begins (Stacey et al. 2008; Grillet et al. 2014).

All the analyzed metals (Cu, Fe, Zn, Cr, and Pb) bioaccumulated in the root and leaf tissue of

V. campechiana, except for Cd. Three of these metals are considered essential (Cu, Fe, and Zn); the other two are considered non-essential (Cr and Pb). The accumulation pattern was as follows: Pb > Fe > Cr > Cu > Zn. It was also found that the accumulation of metals, mainly Cr and Pb, was greater in plants growing on exposed substrates than in control plants. This coincides with what has been found in other phylogenetically related plant species, such as *Acacia robeorum* Maslin. (Fabaceae), which has been reported to bioaccumulate metals such as Al, Fe, Mn, Cu, Zn, and Mo. These results suggest that the bioaccumulation of HMs in the leaf tissue of plant species inhabiting sites contaminated with heavy metals could be a detoxification strategy (He et al. 2012).

In the present study, only plants growing in substrate exposed to HMs accumulated Cr in root and leaf tissue, the latter being where Cr accumulated in greater amounts. The absorption of Cr through the roots may be due to the union of organic acids with the insoluble metals found in the soil, making them available for plant absorption (Panda and Choudhury 2005). The accumulation of Cr occurs when it is immobilized in vacuoles in the root cells, which makes it less toxic to the plant (Shanker et al. 2005). Moreover, the high concentration levels of Cr in the leaf tissue of *V. campechiana* is supported by the study conducted by Skeffington et al. (1976) on the *Hordeum vulgare* L. (Poaceae) species. The authors describe that Cr can enter the aerial parts of the plant through molecules that transport essential elements such as Fe, S, and P and that it can be translocated to the leaves through the xylem.

With respect to Pb, the present study showed that its concentration in root and leaf tissue increased over time in plants growing on tailing substrate, with the highest concentration found in the leaves. Similar results have been described for other shrub-like species similar to *V. campechiana*; for example, *Brickellia veronicifolia* (Kunth) A.Gray. (Asteraceae) accumulates Cd, Cu, Ni, and Pb in its leaf tissue, with Pb showing the highest concentration (Hernández-Acosta et al. 2009). Salas-Luévano et al. (2009) found high concentrations of Pb in the leaf tissue of *Buddleja scordioides* Kunth. (Scrophulariaceae), *Mimosa aculeaticarpa* Ortega. (Fabaceae), and *Acacia schaffneri* (S.Watson) F.J.Herm. (Fabaceae). This could be due to the fact that Pb inhibits the transport of essential metals such as Cu, Fe, and Zn (Patra et al. 2004), which increases in concentration over time and thus its translocation to the aerial parts of the plant.

In *V. campechiana*, low concentration levels of Cu, Fe, and Zn were found in the root and leaf tissue. The explanation of these results could be that the absorption of Pb replaces that of Cu, Fe, and Zn, probably modifying the activity and permeability of the membranes, making them unavailable for absorption and transport within the plant (Patra et al. 2004).

Changes in the morphological characters of the roots, stems, and leaves of *V. campechiana* due to exposure to heavy metals

Most ecotoxicological studies analyze the effect of metals on the morphology of herbaceous species, with few studies focusing on shrub- or tree-like plants. In addition, most of these studies focus on three morphological characters: root length, root biomass, and leaf biomass (Prasad et al. 20011; Maldonado-Magaña et al. 2011). The development of effective phytoremediation strategies for contaminated sites requires the study of plant species with different life forms. Herbaceous life forms, for example, have a short life cycle that can last up to 24 months; shrubs and trees, by contrast, have a long life cycle that can last several years, and this influences their efficiency in the accumulation of HMs.

In the present study, seventeen of 18 morphological characters of *V. campechiana* decreased in plants exposed to HMs with respect to control plants. These results show that the HMs interfere in the plant growth, causing a reduction in the *V. campechiana* growth. Similar results were reported by Tovar-Sánchez et al. (2018), who observed a decrease in the morphological values of the roots and leaves of *Zea mays* L. (Poaceae) associated with sites contaminated by tailings in Taxco de Alarcón, GRO, Mexico. Furthermore, Hernández-Lorenzo (2015) found a decrease in the leaf morphological values of *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C.Johnst. (Fabaceae) associated with tailings in Huautla, MOR, Mexico. In other shrub-like species [*Salix viminalis* L. (Salicaceae), *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae), *Albizia lebbeck* (L.) Benth. (Fabaceae), *Acacia holosericea* Cunn. ex G.Don. (Fabaceae), *Leucaena leucocephala* (Lam.) de Wit. (Fabaceae) and *Vachellia farnesiana* (L.) Wight & Arn. (Fabaceae)] exposed to Cr and Pb, there have been reports of a decrease in root length and leaf biomass (Panda and Patra 2000; Prasad et al. 2001; Iqbal et al. 2001; Suseela et al. 2002; Shanker 2003; Shanker et al. 2005; Maldonado-Magaña et al. 2011). Some studies have reported that the reduction in the plant morphological characters may be due to the fact that HMs could interfere with metabolic processes and are potentially toxic. Resulting in growth abnormalities (Bini et al. 2012) as a weak plant growth, yield depression, and may be accompanied by disorders in plant metabolism such as reduction of the meristematic zone (Maleci et al. 2001). In addition, other studies have found that nonessential HMs such as Cr and Pb inhibit the mitosis process in root cells, reducing the extension of this tissue. For example, it has been reported that nonessential HMs such as Cr and Pb inhibit the mitosis process in root cells, reducing the extension of this tissue. Prasad et al. (2001) reported a toxic effect of Cr, which inhibited primary root growth and suppressed the growth of new secondary roots. Studies conducted by Shanker et al. (2005) found that Cr inhibits root

growth in *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae), *Triticum aestivum* L. (Poaceae), and *Vigna radiata* (L.) R.Wilczek. (Fabaceae) Furthermore, the absorption of Pb causes a decrease in the rate of root growth and affects the root branching pattern (Maldonado-Magaña et al. 2011). For example, in *Picea abies* (L.) H.Karst. (Pinaceae), the development of secondary roots is particularly sensitive to exposure to Pb (Godbold and Kettner 1991; DalCorso 2012).

Changes in the leaf morphology of plant species that are exposed to pollution with HMs can be explained by the biochemical changes that form part of the adaptive mechanisms developed by plants to tolerate or bioaccumulate these metallic elements in their tissues (Meharg 1994). The bioaccumulation of HMs in leaf tissue triggers a detoxification mechanism in which metals bind to a ligand (chelation) such as sulfhydryl, phosphate, carboxyl, and hydroxyl groups and to peptides such as phytochelatins and metallothioneins (Rauser 1995; Cobbett 2000). The HMs are surrounded by ligands, forming a complex that is immersed in a chemical interaction that keeps it in electronic balance while it is transferred to inactive cell compartments, mainly vacuoles (Yong-Eui et al. 2004; Yang et al. 2005; Rodríguez-Serrano et al. 2008; Lin and Aarts 2012).

In the present study, the stomatal index decreased in individuals of *V. campechiana* grown in substrate exposed to HMs. Similar results were reported for *P. laevigata* exposed to HMs (Hernández-Lorenzo 2015). Thakur (1990) mentioned that the decrease in the number of stomata per unit area (mm^2) prevents excessive perspiration in plants and increases stomatal resistance. Although few studies have focused on the stomata of plants exposed to metals, it has been reported that occlusive cells are sensitive to chemical stress, so that the position and number of stomata can change or a mechanism for fall of leaves could be developed to protect the plant against the effects of metals (Zimmermann 2001).

With respect to the seeds of *V. campechiana*, the size and biomass of the seeds was smaller in the sites exposed to metals. However, germination was not affected, with similar values in both treatments. This could be due to the fact that seeds from sites exposed to HMs only accumulated essential elements (Cu, Fe, and Zn), which are involved in the germination process and the development of seedlings.

Potential use of *V. campechiana* as a phytoremediation species in sites contaminated with metals

V. campechiana is a shrub species that is distributed in disturbed places such as sites contaminated by HMs. This species commonly shares its habitat with *V. farnesiana*, a taxonomically close species that has been reported to accumulate HMs as As, Cu, Mn, Ni, Pb, V, and Zn (Maldonado-Magaña et al. 2011; Alcantara-Martinez et al. 2016; Cervantes-Ramírez et al. 2018). However, the phytoremediation potential of *V. campechiana* is still unknown. In general, it has been documented that the plant species used to phytoremediate environments contaminated by HMs belong to the following families: Asteraceae, Brassicaceae, Caryophyllaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae (Prasad 2003; Mahar et al. 2016), these being mostly herbaceous species. The results obtained in the present study suggest that *V. campechiana* has phytoremediation potential for sites contaminated by Cr, Cu, and Pb. The reasons that justify this suggestion are as follows: 1) *V. campechiana* is an accumulator species of Cr, Cu, and Pb. Olguín and Sánchez-Galván (2012) and Ali et al. (2013) propose that a plant can be considered an accumulator if its translocation factor (TF) is equal to or greater than 1. In the present study, in spite of *V. campechiana* growing under experimental conditions, it showed TF values greater than one for Cr (2.24), Pb (1.23), and Cu (1.22). These levels are similar to the TF found in species considered as accumulators that grow directly in contaminated environments such as *Gentiana pennelliana* Fernald [(Gentianaceae) (TF Zn = 1.2)], *Cyperus esculentus* L [(Cyperaceae) (TF Pb = 1.6, TF Zn = 1.1)], *Phyla nodiflora* (L.) Greene [(Verbenaceae) (TF Cu = 12.0, TF Zn = 1.1)], *Rubus fruticosus* L [(Rosaceae) (TF Cu = 5.6), *Sesbania herbacea* (Mill.) McVaugh [(Fabaceae) (TF Cu = 4.0)], and *Limnocharis flava* (L.) Buchenau [(Alismataceae) (TF Cd = 1.3)] (Yoon et al. 2006; Abhilash et al. 2009). 2) The bioaccumulation factor (BCF) for Cu in roots and leaves was 7.6 and 39.4, respectively. The study by Lin et al. (2003) supports the results obtained in the present work. They reported bioaccumulation of Cu in *Helianthus annuus* L. (Asteraceae), with a concentration that was 2 to 10 times higher than the concentration of Cu in the soil. With respect to Pb, it showed a BCF in roots of 15.1 and of 17.4 in leaf tissue. 3) In *V. campechiana*, the accumulation of Cr and Pb increased over time (12 months) in leaf tissue; thus, long-term studies could record a higher accumulation of Cr and Pb. 4) Seeds do not accumulate nonessential metals such as Cr and Pb, and seed germination is not affected by exposure to heavy metals. Unlike other species such as *Sanvitalia procumbens* Lam. (Asteraceae), the germination percentage of seeds from sites exposed to HMs decreases compared with the germination percentage of seeds from control sites (Rosas-Ramírez 2018). 5) Throughout the study (12 months), no mortality was recorded in the plants exposed to HMs. 6) The root biomass of *V. campechiana* plants was not significantly affected by the bioaccumulation of heavy metals.

Conclusion

In the present study *V. campechiana* chronically exposed to mine tailings in greenhouse/experimental condition showed

high levels of HMs translocation, its ability to bioaccumulate non-essential metals in roots and leaves and changes in the macro- and micromorphological characters. These findings suggest that this plant may be a suitable candidate for use in phytoremediation studies in contaminated environments mainly for Cr, Cu, and Pb. Future studies in situ are necessary for complementing the role of *V. campechiana* as a phytoremediation plant. Also, we consider that conducting ecotoxicological studies on new plant species with different life forms provides useful information to arrive at effective phytoremediation strategies for soils contaminated with HMs. A detailed characterization of the effects of these xenobiotics on the exposed plants would allow the selection of the species whose macro- and micro morphological characters are affected the least by exposure to HMs. Furthermore, different species can extract different metals, so it is necessary to combine plant species to develop more effective strategies that can be used in environments polluted with more than one HMs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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