EFFECT OF EDTA ON ACCUMULATION OF HEAVY METALS FROM MINING TAILINGS AND ITS RELATIONSHIP WITH ON GROWTH, PHYSIOLOGICAL RESPONSE AND ANTIOXIDANT ACTIVITY OF *GRINDELIA TARAPACANA* PHIL.

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Abstract. Wild plants that grow in areas contaminated with heavy metals can be used in phytoremediation due to the adaptation processes they use. In this work, the phytoremediation capabilities of *Grindelia tarapacana* in the presence of the chelating agent Ethylenediamine tetraacetic acid (EDTA) was evaluated. The effect of EDTA (50, 100, and 150 mg/kg) on the accumulation of heavy metals from mining tailings and its relationship with the physiological response and metabolism of greenhouse plants were tested. It was found that the biomass was increased in the treatment with 50 mg / Kg of EDTA. But the growth was decreased by up to 10% in treatments with higher concentrations. The photosynthetic pigments did not vary significantly in content. In the treatments with higher EDTA content, soluble carbohydrates were decreased significantly ($p < 0.05$), and soluble protein content changed significantly ($p < 0.05$). However, an opposite effect is observed for the antioxidant activity which increases with higher concentrations of EDTA in the substrate; consequently, lipid peroxidation also was decreased. The application of EDTA favored the accumulation of heavy metals in this species, in the order of roots> leaves> stems. Finally, it is concluded that *Grindelia* is a stabilizer and phytoextractor plant.
Keywords: *contaminated soil, antioxidative enzymes, phytoremediation, phytoextraction, toxicity*

Introduction

Phytoremediation is a technique that uses plant species to extract, volatilize, or stabilize heavy metals present in soils and water. Compared to other remediation techniques, this method requires lower resources while maintaining the integrity of soils due to their ecological safety (Chakravarty et al., 2017).

In this context, wild plant species growing around mines and mining tailings can be used as potential candidates for phytoremediation (Varun et al., 2015). These particular species may be well adapted to those polluted environments (Bech et al., 2002; Varun et al., 2015), therefore being capable of absorbing and accumulating appreciable amounts of heavy metals, specifically in the tissues of the tips of roots, stems, and spongy mesophyll cells (Islam et al., 2008). Additionally, due to their source area, these species may also require lower special care than crop plant species, such as frequent watering, fertilization, or pesticide treatments (Conesa et al., 2007).

However, the increased concentration of heavy metals in plants potentially results in decreased growth, physiological alterations, and oxidative stress. Consequently, cell and tissue damage (Gupta et al., 2013; Fryzova et al., 2017), is expressed as the increase in malondialdehyde, degradation of photosynthetic pigments contents; and the increase in compatible solutes such as soluble carbohydrates and proline. Other countable signals also include the increase in the synthesis of soluble protein and oxidative stress enzymes

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such as superoxide dismutase (SOD), peroxidases such as ascorbate peroxidase (APX), guaiacol oxidase (GPX), catalase (CAT), and glutathione reductase (GR) (Hameed et al., 2016; Khan and Khan, 2017).

The use of various chelating agents such as EDTA, diethylenetriaminepentaacetic acid (DTPA), Hydroxyethylethylenediaminetriacetic acid (HEDTA), Nitrilotriacetic acid (NTA) and different organic acids have been studied in field and laboratory conditions in order to improve the accumulation of metals by plants (Solhi et al., 2005). EDTA is considered one of the most effective chelators for mobilizing heavy metals, especially for Pb (Khalid et al., 2017), because of its ability to form ion-complex (Zhou et al., 2011; Dipu et al., 2012). Then, this chelating effect increasing the solubility of heavy metals present in soil solution, is a favorable process for remediation by plants (Chandra et al., 2018).

The genus *Grindelia*, from the Asteraceae family, is found in arid and semi-arid zones of Chile, Peru, Uruguay, southern Brazil and Argentina. Being most of its species found in regions whit annual rainfall lower than 250 mm (Castro et al., 1995). *Grindelia tarapacana* Phil. known as "chiri-chiri" is a perennial shrub with deep roots. It has been reported in other experiences because of its foliar bioaccumulation capacity for heavy metals, such as, Cu, Pb and Fe (Vásquez, 1998). Contrarily, studies on growth, physiological and metabolic factors associated with the ability of this plant for accumulation of heavy metals, are scarce. Therefore, the objective of this research was to evaluate the phytoremediation potential of *G. tarapacana* in the presence of increasing concentrations of the chelating agent EDTA.

Material and methods

Description of area for biological sample collection

Seeds of *G. tarapacana* and the mine tailings samples were collected from Kiowa mine, (south latitude 16 ° 32 '4.4 ", west longitude 71 ° 28' 23.7"), altitude 2450 m a.s.l. located in the province of Arequipa, Peru. All the tests were carried out in the Plant Physiology and Biotechnology laboratory, and greenhouse of the Faculty of Biological Sciences of Universidad Nacional de San Agustín de Arequipa.

Vegetal material

The seeds with healthy appearance, uniformity, and greater size were selected for the tests. First, the biological material was disinfected by soaking in commercial NaClO 5% (v/v) for 10 min and rinsed three times with distilled water, followed by a soaking process with distilled water at 40°C for three days (Hartman et al., 2002). Finally, the seeds were sown in beds with a substrate (sand: moss; 3:1 volume ratio), and were located in a growth chamber until the seedlings were 4-5 cm long.

Treatments of EDTA-Na

Substrates of treatments were prepared by mixing sand and soils (2:1) with 10 g of cattle manure and 20 g of moss per kg of substrate (Barrutia et al., 2009). The mine tailings were added to obtain its final concentration at 10%. After 10 days, fertilization was carried out by watering with 200 ml of Hoagland's solution ½, up to field capacity (FC). Then, the prepared substrate was watered for 15 days with distilled water in intervals of 3 days up to FC, and at the end of this period, the soil analysis was performed

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(*Table 1*). The substrate was placed in 1.5 kg pots and watered with distilled water, and the pots were kept in greenhouse conditions for two weeks prior to transplanting.

For each experimental unit, one seedling was transplanted for each pot; after 7 days, the EDTA treatments were applied at increasing concentrations of 50, 100, and 150 mg / Kg. The treatment with the substrate mentioned above and without application of EDTA was labelled as "Control"; and the treatment with field soil, "Blank". Then, the plants were grown in a greenhouse for 60 days. To avoid leaching and loss of nutrient and metals, a tray was placed under each pot, and the excess water was collected daily and returned to the pot (Ali et al., 2003). Finally, the plants were acclimatized to the substrate media in 7 days in a growth chamber (*Fig. 1*). with fluorescent lighting (40 Watts), the irradiance of 35 µmol m^{-2} s^{-1}, photoperiod 12 h / 12 h (D / N), the temperature of 22ºC and 18ºC (D / N) and relative humidity 40/70%.

Figure 1. *G. tarapacana plants with different EDTA treatments in acclimatization chamber*

Dry mass determination

The plants were carefully extracted from the pots. The roots, stems, and leaves of individual plants were separated, packaged, and labeled according to each treatment and repetition; for the specific case of roots, this material was carefully washed with distilled water, then all samples were put to desiccation at 70°C for 48 h in an oven to obtain the dry weight.

Physiological response

Malondialdehyde content (MDA)

The content of MDA or lipid peroxidation, was determined according to Chen et al. (2021). Briefly, 30 mg of fresh leaves were homogenized with 2 mL Trichloroacetic acid (TCA) 0.1% (p / v) and centrifuged at 5000 rpm for 10 minutes at 4°C. The supernatant (1 mL) was removed, and 2 mL of 20% TCA: 0.5% Thiobarbituric Acid was added. The sample was then heated in a water bath at 95°C for 30 minutes. The absorbance was measured at 440, 532, and 600 nm (UV-VIS Spectroquant Pharo 300 spectrophotometer).

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As the final step, the non-specific turbidity was corrected by subtracting the absorbance score obtained at 600 nm.

Photosynthetic pigments content

The content of chlorophylls and carotenoids was determined according to Steubing et al. (2001); for this purpose, 30 mg of fresh leaves were homogenized with 3 mL 85% acetone maintaining cold mortar. The homogenized sample was filtered with filter paper Whatman No. 2 and rinsed with 3 mL of cold acetone at 80%. The content of chlorophylls and carotenoids was calculated by using the absorbance of the extract measured with a UV-VIS spectrophotometer (Spectroquant Pharo 300) at 662 nm for chlorophyll A (chl a), 644 nm for chlorophyll B (chl b) and 440 nm for carotenoids (Nikolic et al., 2016).

Soluble Carbohydrate content

The total soluble carbohydrate content was determined according to Steubing et al. (2001). Briefly, 30 mg of fresh leaves were homogenized with 10 mL 5% TCA. The mixture was placed in a water bath for 3 h at 90°C, and it was centrifuged at 7500 rpm for 10 min. Then, 0.2 mL of the supernatant, 0.5 mL of 5% phenol, and 2.5 mL of concentrated sulfuric acid were mixed in a test tube. For the determination of soluble carbohydrates, the absorbance score data was obtained by spectrophotometry at 485 nm.

Soluble protein content

The total soluble protein content was determined as follows. Fresh leaves (30 mg) were homogenized with 2 mL of 100 mM potassium phosphate buffer pH 7.5. It was centrifuged at 6000 rpm for 30 min, at 4°C. A sample of 70 µl of supernatant and 2.5 mL of Bradford's reagent were mixed. The data from the analysis was obtained at an absorbance of 595 nm (Moreno et al., 2002).

Antioxidant enzymes activity

The extract for further activity determinations was obtained from 30 mg of fresh leaves. Briefly, the biological material was homogenized with 2 mL of 100 mM potassium phosphate buffer pH 7.5 and centrifuged at 6000 rpm for 30 minutes at 4°C (Moreno et al., 2002); then, the supernatant was isolated and stored for determination antioxidant enzymes.

Determination of SOD activity

SOD activity was determined according to Donahue et al. (1997), where 1188 uL of potassium phosphate buffer, 20 uL of 20 mM EDTA, 50 uL of methionine, 154 uL of Nitro blue tetrazolium (NBT), 200 uL of the supernatant, and 338 uL of riboflavin were mixed in a tube. The result of the reaction was measured at an absorbance of 560 nm.

Determination (estimation) APX activity

The APX activity was determined according to Zhao and Blumwald (1998). In a tube, 2 mL of potassium phosphate buffer, 80 uL of the supernatant, 80 uL of ascorbic acid, and 10 uL of H_2O_2 30% were mixed. The absorbance was measured at 290 nm for 1 minute.

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Determination (estimation) of GPX activity

GPX activity was determined according to Pinhero et al. (1997). In a tube, 2 mL of potassium phosphate buffer, 30 uL of the supernatant, 10 uL of Guaiacol, and 10 uL of H_2O_2 30% were mixed. Then, it was measured at 470 nm for 1 min.

Determination of CAT activity

The CAT activity was determined according to Pinhero et al. (1997). In a tube, 1 mL of 100 mM potassium phosphate buffer pH 7, 10 uL of the extract, and 10 uL of 30% H_2O_2 were mixed. It was measured at 240 nm for 1 min.

Determination (estimation) of GR activity

In a tube, 5 mL of potassium phosphate buffer 100 mM, pH 7.5, was mixed with 5 ml of DTT 1 mM and 5 ml of disodium salt of EDTA 1 mM. This solution was mixed with the extract containing PVPP, and the mix was incubated at 4ºC /1 hour and then centrifuged. The supernatant was mixed with 100 mM Phosphate Buffer,1 mM EDTA, 540 ul distilled water, and 100 ul of 2 mM NADPH. The absorbance was measured at 300-340 nm for 240 sec.

Metal absorption

Determination of the bioabsorption coefficient factor (BAC), bioconcentration factor (BCF) and translocation factor (TF).

BAC, BCF, and TF were determined (*Eqs. 1-3*) to quantify the efficiency of heavy metal phytoextraction (Ebrahimi, 2013; Varun et al., 2015).

$$BAC = C\ stem \div C\ soil \tag{Eq.1}$$

$$BCF = C\ root \div C\ soil \tag{Eq.2}$$

$$TF = C\ stem \div C\ root \tag{Eq.3}$$

where: C is the heavy metal content for each case (Varun et al., 2015).

A number of 5 plants was used for each analysis.

Statistical analysis

The design applied was completely randomized. The statistical analysis was carried out by applying ANOVA, and the mean differences were established using the Tuckey test ($p < 0.05$). The statistical software STATISTICA version 10 was used, considering that all reported results correspond to the mean of five repetitions per treatment and controls.

Results

Soil characteristics

Table 1 presents the characteristics of the soil (Blank) and the substrate with tailings (Control). Concerning previous reports from the area where the soil material was obtained

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(INIA, 2017), both (blank and control) presented sandy texture, alkaline pH, and moderate salinity. The Electrical Conductivity (EC) for both controls was in the average of soils from the study area, with low organic matter content, low $CaCO_3$ content, low Cation exchange capacity (CEC) (meq / 100 g) score, similar nitrogen content, exchangeable cations (low Na, medium K, low Ca, low Mg), and high phosphorus content.

Table 1. *Physicochemical characterization of agricultural soil and substrates prepared at the beginning of the experiment*

Physicochemical parameters	Treatment	
	Blank	**Control**
pH	8.01	8.01
Calcium carbonate (%)	0.35	0.44
Cation exchange capacity (meq/100g)	5.8	6.2
Conductivity (dS/m)	2.52	2.76
Organic material (%)	1.39	1.44
Sand (%)	78.4	79.8
Silt (%)	16.5	14.2
Clay (%)	5.0	6.0
Nitrogen (%)	0.193	0.179
Available Phosphorus (ppm)	66.97	72.98
Potassium (meq/100g)	0.61	0.95
Calcium (meq/100g)	4.19	4.26
Magnesium (meq/100g)	0.83	0.84
Sodium (meq/100g)	0.17	0.15
Percentage of interchangeable sodium (%)	2.93	2.42
Heavy metal		
Total As (mg kg^{-1})		63.19893
Total Hg (mg kg^{-1})		0.02820
Total Cd (mg kg^{-1})		0.23285
Total Cr (mg kg^{-1})		11.83308
Total Cu (mg kg^{-1})		196.95120
Total Fe (mg kg^{-1})		4317.84000
Total Mn (mg kg^{-1})		62.33994
Total Ni (mg kg^{-1})		2.46189
Total Pb (mg kg^{-1})		261.44817
Total Zn (mg kg^{-1})		36.96600

Biomass

Treatments with mine tailings containing metals and metalloids (As and Hg) increased the biomass (dry matter) by 30% compared to the blank. However, increasing concentrations of EDTA and mine tailings in the substrate, resulted in a significant decrease of biomass ($p < 0.05$), being the lowest value obtained in the treatment with EDTA 150 mg / Kg (*Fig. 2*). Specifically, the biomass of *G. tarapacana* decreased 1, 12, and 30% compared to the control, being affected at the level of stems and roots with significant differences between each tissue.

Photosynthetic pigments

The levels of photosynthetic pigments in *G. tarapacana* leaves, treated with mine tailings in increasing concentrations of EDTA, are shown in *Fig. 3*. The content of Chl a and Chl b increased significantly ($p < 0.05$) as the concentration of EDTA increased up to

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100 mg / Kg. Carotenoids decreased significantly (p <0.05) in control compared to the blank, and the Chl a / Chl b ratio increased in the mine tailing treatments with EDTA (50 mg / kg). Furthermore, higher content of EDTA among treatments showed an increase in the Chl b content compared to the blank.

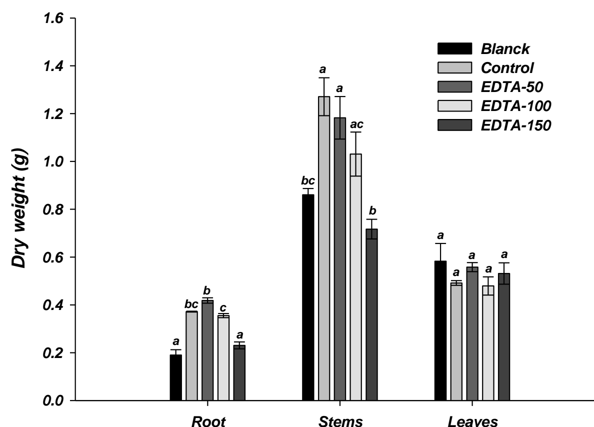

***Figure 2.** Biomass of G. tarapacana plants under EDTA treatments (50-100-150 mg Kg-1) after 60 days of cultivation; and biomass in Blank: no tailing; and Control: with tailing. The values correspond to the average of 5 repetitions per treatment; Different letters indicate significant differences evaluated by the Tukey test (p <0.05) after performing the ANOVA analysis*

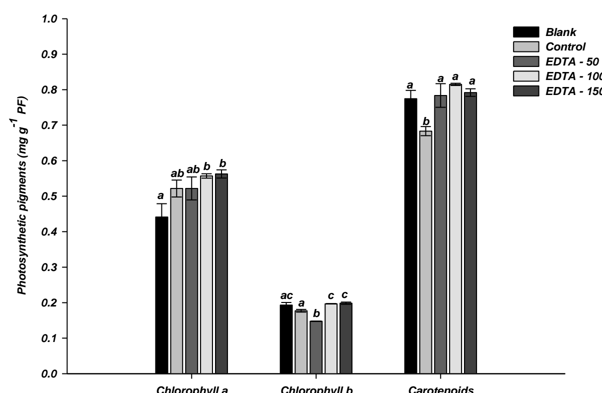

***Figure 3.** Effect of different concentrations of EDTA on the content of chl a, chl b and carotenoids in G. tarapacana leaves, after 60 days of cultivation. Blank: agricultural land, Control: substrate with tailings. The values correspond to the average of 5 repetitions. Different letters indicate significant differences according to Tukey's test (p <0.05)*

Carbohydrates and soluble protein

The mine tailing in the substrate and the application of EDTA significantly influenced (p <0.05) the content of carbohydrates and total soluble proteins in leaves (*Fig. 4*). The control and the treatment with 50 mg / kg of EDTA increased the concentration of soluble sugars by 42 and 20%, respectively, about blank. Higher concentrations of EDTA, 100 and 150 mg / kg decreased soluble sugar content by 2 and 25%, respectively, if compared to blank. Additionally, the protein content decreased in control and the treatments between 6.5-8% compared to blank.

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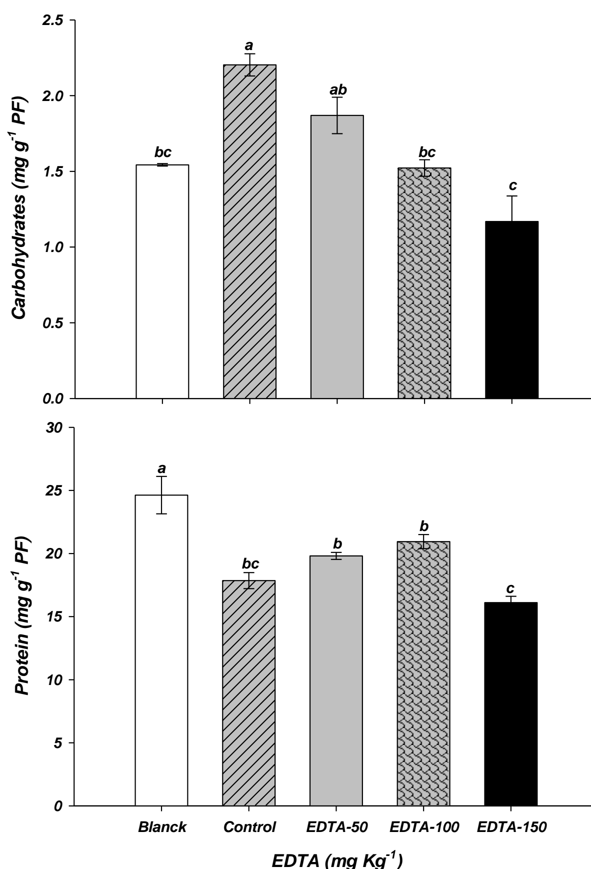

***Figure 4.** Effect of different concentrations of EDTA (mg Kg-1) on the content of carbohydrates (upper) and total soluble proteins (lower) in G. tarapacana leaves, after 60 days of cultivation. Blank: agricultural soil, Control: substrate with tailings. The values correspond to the average of 5 repetitions. Different letters indicate significant differences according to Tukey's test (p<0.05)*

Lipid peroxidation and antioxidant enzymes

The breakdown of the cell membrane by lipid peroxidation is measured by the detection of the MDA, which is present when the vegetal organism is exposed to conditions of high oxidative stress (Fryzova et al., 2017). From our results, no significant differences were found between the control and treatment with 50 mg / Kg of EDTA concerning the blank (*Fig. 5*). However, the treatments with 100 and 150 mg / Kg of EDTA showed significant differences ($p < 0.05$), having lectures of MDA for the treatment with 100 mg / kg in 236% and decrease in 36% with 150 mg / kg of EDTA to the blank.

The mine tailing in substrate and EDTA treatments affected significantly ($p < 0.05$) the SOD, APX, CAT, GPX and GR enzymatic activity (*Fig. 5*). In specific, the Superoxide dismutase activity in the treatment with EDTA at 150 mg / kg presented the highest SOD enzymatic activity (476%); meanwhile, the control presented the lowest activity (51.54%). The highest GPX enzymatic activity was obtained with the EDTA treatment (150 mg / kg, with 0.000300 µmol g protein^{-1} min^{-1}) up to 1034% compared to the control (with 0.000029 µmol g protein^{-1} min^{-1}). The least activity was presented in the blank.

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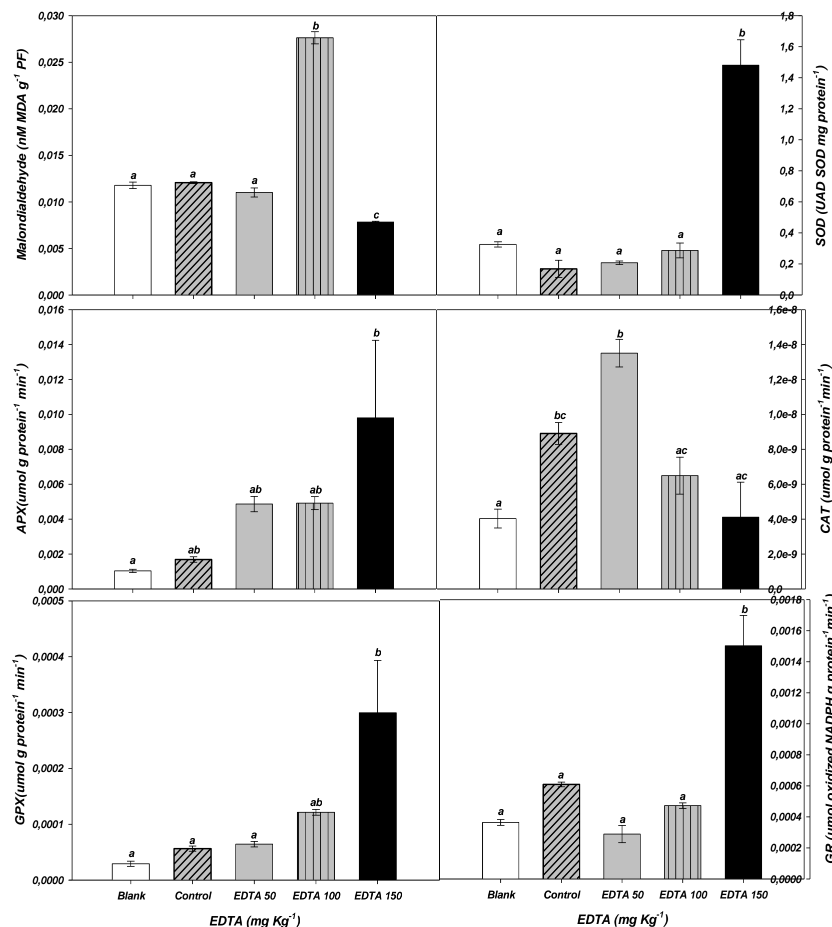

Figure 5. *Effects of EDTA in MDA content, activity of antioxidant enzymes SOD, APX, CAT, GPX and GR in G. tarapacana under substrates treatments with mine tailings (10%), treatments with EDTA (50 mg / kg, 100 mg / kg and 150 mg / kg), control without EDTA, and blank without mine tailing. Duration: 60 days. Values are means of 5 repetitions. Different letters indicate significant differences evaluated by the Tukey test ($p < 0.05$) after performing the ANOVA analysis*

Bioaccumulation of heavy metals

Table 2 presents the total concentration of metals absorption from the soil with mine tailing (10%) in root, stem, and leaves. Being from highest to lowest, Fe> Pb> Cu> Mn> As> Zinc> Cr> Ni> Cd> Hg. After applying the EDTA treatments, the highest absorption was found in root> leaves> stem. Accumulation varies by metal and EDTA treatments. The highest percentage of accumulation in the stem samples was, Pb> Cu> As> Zn> Ni with 209%, 135%, 134%, 60% and 32%, respectively for EDTA 150 mg / kg. The other metals presented accumulation scores lower than 20%. Furthermore, the metals Fe, Cu, and Pb were found at toxic levels in the leaves.

Table 3 shows the heavy metal bioaccumulation factors BAC, BCF and TF. The BAC values varied for Hg> Zn> Mn> Ni, with 5.86, 3.38, 1.81 and 1.80, respectively; and TF Hg> Zn> Ni> Mn, with 2.26, 1.85, 1.21 and 1.20, respectively. These values, according to the BAC> 1 and TF> 1 criteria, show this plant as a phytoextractor for these metals. Additionally, the BCF values show Cd> Cr> As> Cu> Pb> Fe with 8.82, 1.96, 1.7, 1.56, 0.91, 0.79, respectively, classifying this plant as a Phyto stabilizer.

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Table 2. *Improvement of the phytoremediation potential: concentration of metals in the soil and accumulation in roots, stems, and leaves of G. tarapacana (mg kg-1), treated with EDTA (50,100 and 150 mg kg-1), after 60 growing days*

	Treatments	Heavy metal									
		As	**Hg**	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
SOIL	**Blank**	2.99 ± 0.34	0.04 ± 0.02	0.04 ± 0.02	7.20 ± 0.20	21.8 ± 0.94	2600.9 ± 9.05	69.46 ± 0.95	2.91 ± 0.04	3.4 ± 0.93	19.43 ± 2.11
	Control	60.98 ± 1.15	0.04 ± 0.02	0.24 ± 0.14	6.38 ± 0.09	178.9 ± 1.60	4417.3 ± 3.16	65.55 ± 0.77	2.96 ± 0.03	266.0 ± 2.52	47.79 ± 0.79
	EDTA 50	62.55 ± 1.01	0.01 ± 0.01	0.21 ± 0.12	7.04 ± 0.01	186.4 ± 2.05	4509.3 ± 4.35	65.72 ± 0.89	2.41 ± 0.01	280.6 ± 3.13	41.39 ± 2.06
	EDTA 100	61.35 ± 0.67	0.07 ± 0.04	0.22 ± 0.12	4.41 ± 0.13	179.0 ± 2.17	4531.8 ± 6.73	69.61 ± 0.82	2.60 ± 0.03	260.5 ± 0.97	41.20 ± 0.80
	EDTA 150	56.81 ± 1.52	0.04 ± 0.02	0.23 ± 0.13	2.74 ± 0.33	161.1 ± 2.50	4568.4 ± 5.57	61.29 ± 0.79	2.41 ± 0.02	248.7 ± 3.14	50.54 ± 0.76
	F value	*	*	*	*	*	*	*	*	*	*
ROOT	**Blank**	12.88 ± 1.53	0.08 ± 0.05	0.45 ± 0.25	3.02 ± 0.02	31.8 ± 2.01	1209.5 ± 4.25	92.68 ± 1.94	2.59 ± 0.04	4.62 ± 1.42	29.95 ± 2.17
	Control	101.42 ± 1.29	0.14 ± 0.07	1.82 ± 0.37	5.82 ± 0.31	234.6 ± 2.66	3087.0 ± 6.67	78.12 ± 1.35	3.53 ± 0.03	255.13 ± 1.93	60.00 ± 1.46
	EDTA 50	64.46 ± 1.90	0.10 ± 0.05	1.14 ± 0.55	4.76 ± 0.32	145.9 ± 2.73	2057.5 ± 5.41	49.35 ± 2.61	2.84 ± 0.03	165.25 ± 4.29	50.00 ± 1.89
	EDTA 100	93.10 ± 0.74	0.11 ± 0.06	1.91 ± 0.99	5.61 ± 0.25	280.0 ± 3.24	3622.8 ± 4.95	81.78 ± 2.63	3.49 ± 0.04	238.04 ± 1.88	75.09 ± 1.37
	EDTA 150	96.61 ± 0.83	0.10 ± 0.05	1.45 ± 0.73	5.39 ± 0.10	246.1 ± 4.23	2774.1 ± 7.08	92.40 ± 0.91	3.59 ± 0.04	216.40 ± 0.91	75.93 ± 1.01
	F value	*	*	*	*	*	*	*	*	*	*
STEM	**Blank**	0.69 ± 0.10	0.04 ± 0.03	0.31 ± 0.17	1.45 ± 0.16	11.74 ± 0.90	79.42 ± 2.04	37.53 ± 0.95	1.12 ± 0.01	0.97 ± 0.03	29.01 ± 1.35
	Control	4.54 ± 0.78	0.05 ± 0.03	0.61 ± 0.35	1.77 ± 0.46	20.00 ± 0.51	140.60 ± 3.71	26.20 ± 1.37	1.28 ± 0.03	5.11 ± 0.77	42.86 ± 0.97
	EDTA 50	4.33 ± 0.09	0.04 ± 0.02	0.55 ± 0.32	1.70 ± 0.49	26.17 ± 1.19	164.49 ± 1.25	33.01 ± 0.77	1.31 ± 0.01	8.65 ± 1.05	44.39 ± 0.90
	EDTA 100	5.12 ± 0.19	0.06 ± 0.04	0.37 ± 0.20	2.12 ± 0.17	36.20 ± 1.20	198.84 ± 2.66	34.19 ± 0.67	1.72 ± 0.02	9.04 ± 0.67	55.02 ± 0.66
	EDTA 150	6.64 ± 0.85	0.04 ± 0.02	0.37 ± 0.20	1.89 ± 0.33	41.24 ± 0.61	167.78 ± 1.85	42.57 ± 1.01	1.97 ± 0.03	8.81 ± 0.87	53.42 ± 0.71
	F value	*	*	*	ns	*	*	*	*	*	*
LEAVES	**Blank**	5.55 ± 0.79	0.18 ± 0.09	0.33 ± 0.18	2.61 ± 0.25	35.18 ± 1.36	435.26 ± 2.05	41.85 ± 2.09	1.73 ± 0.01	11.33 ± 1.22	60.48 ± 0.96
	Control	1.46 ± 0.16	0.17 ± 0.09	0.23 ± 0.12	2.95 ± 0.42	26.78 ± 1.13	461.03 ± 1.30	65.75 ± 1.12	2.01 ± 0.01	3.87 ± 2.13	51.41 ± 0.72
	EDTA 50	6.21 ± 0.24	0.19 ± 0.10	0.40 ± 0.23	3.13 ± 0.07	47.35 ± 0.88	575.56 ± 3.11	59.98 ± 1.60	2.19 ± 0.04	16.47 ± 1.24	71.54 ± 1.00
	EDTA 100	6.22 ± 0.13	0.19 ± 0.11	0.47 ± 0.25	3.14 ± 0.09	49.61 ± 0.82	505.81 ± 0.94	50.53 ± 1.59	2.32 ± 0.01	14.95 ± 1.02	84.30 ± 0.89
	EDTA 150	7.36 ± 0.11	0.17 ± 0.09	0.41 ± 0.23	2.97 ± 0.40	68.74 ± 2.05	551.63 ± 3.14	68.73 ± 0.89	2.38 ± 0.04	19.00 ± 1.02	97.51 ± 0.77
	F value	*	ns	*	ns	*	*	*	*	*	*

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Table 3. *Evaluation of the phytoremediation potential of Grindelia tarapacana exposed to mining tailings (10%) and increasing concentrations of EDTA (50, 100 and 150 mg kg-1): Characteristics of metal accumulation, BAC (Cstem/ Csoil), BCF (Croot / Csoil) and TF (Cstem/ Csoil), after 60 days of growth*

Treatment	Factors	Heavy metals									
		As	Hg	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Blank	BAC	2.07 ± 0.06	5.35 ± 0.02	15.67 ± 0.03	0.56 ± 0.04	2.15 ± 0.01	0.20 ± 0.00	1.14 ± 0.03	0.98 ± 0.01	3.69 ± 0.05	4.69 ± 0.04
	BCF	4.31 ± 0.03	1.96 ± 0.00	11.11 ± 0.01	0.42 ± 0.01	1.46 ± 0.03	0.47 ± 0.00	1.33 ± 0.01	0.89 ± 0.00	1.32 ± 0.06	1.55 ± 0.06
	TF	0.48 ± 0.01	2.72 ± 0.01	1.41 ± 0.00	1.35 ± 0.13	1.48 ± 0.02	0.43 ± 0.00	0.86 ± 0.02	1.10 ± 0.01	3.11 ± 0.78	3.01 ± 0.14
Control	BAC	0.10 ± 0.01	5.49 ± 0.02	3.50 ± 0.00	0.74 ± 0.13	0.26 ± 0.01	0.14 ± 0.00	1.40 ± 0.02	1.11 ± 0.00	0.03 ± 0.01	1.97 ± 0.00
	BCF	1.66 ± 0.01	3.45 ± 0.01	7.61 ± 0.28	0.91 ± 0.04	1.31 ± 0.00	0.70 ± 0.00	1.19 ± 0.01	1.19 ± 0.00	0.96 ± 0.01	1.26 ± 0.01
	TF	0.06 ± 0.01	1.59 ± 0.05	0.46 ± 0.02	0.81 ± 0.11	0.20 ± 0.00	0.19 ± 0.00	1.18 ± 0.01	0.93 ± 0.00	0.04 ± 0.01	1.57 ± 0.01
EDTA 50	BAC	0.17 ± 0.00	17.14 ± 0.04	4.48 ± 0.00	0.69 ± 0.08	0.39 ± 0.01	0.16 ± 0.00	1.41 ± 0.02	1.45 ± 0.01	0.09 ± 0.01	2.80 ± 0.05
	BCF	1.03 ± 0.01	6.97 ± 0.01	5.35 ± 0.03	0.68 ± 0.04	0.78 ± 0.00	0.46 ± 0.00	0.75 ± 0.03	1.18 ± 0.01	0.59 ± 0.01	1.21 ± 0.05
	TF	0.16 ± 0.00	2.46 ± 0.00	0.84 ± 0.00	1.01 ± 0.05	0.50 ± 0.00	0.36 ± 0.00	1.88 ± 0.05	1.23 ± 0.00	0.15 ± 0.01	2.32 ± 0.05
EDTA 100	BAC	0.18 ± 0.00	3.76 ± 0.00	3.87 ± 0.00	1.19 ± 0.02	0.48 ± 0.01	0.16 ± 0.00	1.22 ± 0.02	1.55 ± 0.01	0.09 ± 0.01	3.38 ± 0.03
	BCF	1.52 ± 0.00	1.62 ± 0.00	8.82 ± 0.02	1.27 ± 0.02	1.56 ± 0.00	0.80 ± 0.00	1.17 ± 0.02	1.34 ± 0.00	0.92 ± 0.01	1.82 ± 0.00
	TF	0.12 ± 0.00	2.32 ± 0.01	0.44 ± 0.00	0.94 ± 0.00	0.31 ± 0.00	0.19 ± 0.00	1.04 ± 0.01	1.16 ± 0.00	0.10 ± 0.01	1.86 ± 0.01
EDTA 150	BAC	0.25 ± 0.01	5.86 ± 0.01	3.36 ± 0.01	1.78 ± 0.06	0.68 ± 0.01	0.16 ± 0.00	1.82 ± 0.01	1.80 ± 0.01	0.11 ± 0.01	2.99 ± 0.02
	BCF	1.70 ± 0.03	2.58 ± 0.01	6.20 ± 0.03	1.97 ± 0.21	1.53 ± 0.00	0.61 ± 0.00	1.51 ± 0.00	1.49 ± 0.01	0.87 ± 0.01	1.50 ± 0.00
	TF	0.14 ± 0.01	2.27 ± 0.01	0.54 ± 0.00	0.90 ± 0.12	0.45 ± 0.0	0.26 ± 0.00	1.20 ± 0.01	1.21 ± 0.00	0.13 ± 0.01	1.99 ± 0.01
Treatment	*F value*	*	*	*	*	*	*	*	*	*	*
Factors	*F value*	*	*	*	ns	*	*	*	*	ns	*
Interaction	*F value*	*	*	*	*	*	*	*	*	*	*

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Discussion

Heavy metals cause toxicity and adverse effects on mineral nutrition, photosynthesis and cell metabolism, decreasing growth (Antoniadis et al., 2017). However, *G. tarapacana* had a favorable response to the mine tailings by increasing its growth and tolerance to high concentrations of metals (As, Fe, Cu and Pb). Response which has also been reported in *Poa annua, Helianthus annuus* and other species (Nouri et al., 2009; Vamerali et al., 2010; Varun et al., 2015).

The results show that theTreatments with mine tailings increased the biomass compared to the blank (*Fig. 2*), this may be because some metals can stimulate cell proliferation (Kadukova and Kavulicova, 2010). The biomass was decreased at higher concentrations of EDTA, altering the root/shoot ratio (0.18) to the blank (0.13) (*Fig. 2*). This response would be consistent with descriptions from Hadi et al. (2010) in *Zea mays* treated with Pb + EDTA + AG3 and Greman et al. (2001) in *Brassica rapa* and *Trifolium pratense* treated with low concentrations of EDTA. This response may be attributed to the effect of how higher concentrations of EDTA increase solubility, breaking the physiological barriers in the roots, and a further indiscriminately translocation of heavy metals as described in *Chrysanthemum coronarium* and *Vigna radiata* (Luo et al., 2006); in consequence, reducing the activity of "Plant Growth Promoting Rhizobacteria," dubbed PGPRs, involved in stem growth (Ali et al., 2003; Bahadur et al., 2017).

In this study, the toxic concentrations of heavy metals in the mine tailings did not affect the content of chlorophylls in leaves but decreased carotenes (*Fig. 3*). The EDTA treatments increased the contents of Chl a and Chl b and carotenes. This response is because a fraction of the heavy metals binds to EDTA. Another fraction forms complexes with the xylem pathway and cell walls of the leaf mesophyll, reducing their mobility and preventing toxicity and cell damage (Zhao et al., 2010; Tian et al., 2011).

The results show how mine tailings increase the content of total soluble sugars in *G. tarapacana*. This trend coincides with reports from studies on *Oryza sativa* (Mishra and Dubey, 2013), *Pisum sativum* (Devi et al., 2007), and *Cucumis sativus* (Burzynsky and Klobus, 2004), where the increase in sucrose synthase and invertase acidic activity was also found. Toxic concentrations of heavy metals affect the light phase of photosynthesis and alter the carbon fixation process, controlled by several enzymes, regenerating the Calvin-Benson Cycle, in consequence affecting plant growth (Verma and Duvey, 2001; Burzynski and Klobus, 2004). At higher EDTA concentrations, total sugar levels decreased. This can be explained because of the degradation of starch and the synthesis of sucrose as crucial processes in the release of energy, originating hexoses to be metabolized in the glycolytic pathway, together with the pentose phosphate, which is strongly involved in tolerance processes to ROS caused by heavy metals (Devi et al., 2007; Rosa et al., 2009; Van den Ende and Valluru, 2009).

The soluble protein content of *G. tarapacana* decreases in the presence of mine tailings. The most significant effect was observed in the treatments with the highest concentration of EDTA (150 mg / L). This response would be related to the toxic content of Fe, Cu, and Pb in leaves, which could be associated with the greater protease activity (Palma et al., 2002; Hasan et al., 2017) or various structural alterations caused by protein denaturation (John et al., 2009; Hassan et al., 2017). This result coincides with investigations in *Digitaria sanguinalis*, where the soluble protein content decreased significantly by exposure to Pb and less effective with Cd and Ni (Ewais, 1997). However, in *Lemna minor*, an increase in soluble protein content was also observed by the effect of

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Cu and Cd (Hou et al., 2007); furthermore, a similar response was observed in pea plants exposed to Ni (Gajewska and Sklodowska, 2006; Romero-Puertas et al., 2007).

The results evidence the negative relationship between lipid peroxidation and antioxidant enzyme activity. In circumstances of low activity of antioxidant enzymes, high lipid peroxidation was found, and vice versa. This could be explained as the response to heavy metals. High concentrations of metals increase the content of MDA (Kumar and Prasad, 2018). Toxic concentrations of heavy metals produce alterations in cell permeability due to membrane decomposition related to lipid peroxidation that increases MDA formation, indicating high oxidative stress (Fryzova et al., 2017). Similar effects have been observed in plants treated with Cu and Pb, such as *Ceratophyllum demersum* (Devi et al., 2007), *Salsola passerina, Chenopodium album* (Hu et al., 2012) and *Lemna minor* (Hou et al., 2007). Similar responses were found with Pb and Cd in *Zea mays* (Gupta et al., 2013) and *Triticum aestivum* (Dey et al., 2007).

The oxidative stress caused by heavy metals has a first line of defense in the antioxidant enzymes SOD, APX, GPX, CAT, and GR (Gratao et al., 2005; Hameed et al., 2016). The results in this study show how SOD decreased in the control, but increased with EDTA. This increase in SOD activity in *G. tarapacana* leaves could be related to defensive mechanisms against oxidative stress, probably related to the specific concentration of metals such as Fe, As, Cu, and Pb (Gratao et al., 2005; Antoniadis et al., 2017).

The APX activity increased in *G. tarapacana* due to the mine tailings in the substrate. Similarly, high APX enzymatic activity was found in the presence of Cd, Cu, Zn, Al or Ni in *Avena sativa, Pisum sativum, Phaseolus vulgaris, Cucumis sativus* (Yruela, 2009). From the results, the APX activity increased in conditions of mixed metals compared to a separate application. This coincides with other experiences, such as in the case of *Ceratophyllum demersum*, where Cd and Zn was applied in mixed and separate solution (Chibuike and Obiora, 2014). And with other experiences where APX activity was higher with the treatment of 150 mg / Kg of EDTA, due to the higher availability of heavy metals (Raza et al., 2021).

The GPX activity in *G. tarapacana* was low, differing of what has been found in other studies, where under exposition to Pb and Cu, *Lupinus luteous, Triticum aestivum, Helianthus annuus*, and *Macrotyloma uniflorum*, showed higher activity of GPX (Jouili and Ferjani, 2003; Reddy et al., 2005; Dey et al., 2007; Mourato et al., 2009).

The results show how CAT activity in *G. tarapacana*, increased its activity in treatments with mine tailing; on the contrary, higher concentrations of EDTA decreased its activity for the blank. This would be explained by the fact that CAT activity has been reported as efficient at low concentrations of heavy metals (Gratao et al., 2005). In addition, the CAT activity has evident variation under the effect of the different heavy metals under study, also depending on the species and type of tissue. The activity of this enzyme in the presence of Cd was reported to decrease in *Phaseolus vulgaris, Lemna minor* and *Capsicum spp*., but increased in the presence of Cu and Ni in *Agropyrum repen, Raphanus sativus, Saccharum officinarum*, and *Helianthus annuus* (Jouili and El Ferjani, 2003). Finally, in experiences with *Glicine maxima*, the treatments with Cd have not presented changes in the activity (Gratao et al., 2005).

The GR activity in *G. tarapacana* increased by the effect of the mine tailings in the substrate. Our results are consistent with what has been described by Gratao et al. (2005), where the exposition to heavy metals increased GR activity, specifically in the presence of Pb (*Oryza sativa*), Hg (*Arabidopsis thaliana*), Cd (*Phaseolus vulgaris, Solanum

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tuberosum), Ni (*Coffea arabica*), and As (*Brassica napus*) (Zuccarelli and Freschi, 2018). However, the GR activity reached its maximum activity with the highest EDTA, increasing the availability of heavy metals (Raza et al., 2021).

The bioaccumulation of heavy metals varies among the different organs of the plant, being influenced by the type of metal and the concentration in the soil (Varun et al., 2015). The greatest accumulation of heavy metals in *Grindelia* occurred in the roots. This response was consistent with the structure and function of roots in various species (Islam et al., 2008). In this case, the roots act as selective barriers, decreasing toxicity in the apoplastic and symplastic pathways (Jhon et al., 2009). Additionally, the combinations of mine tailings with EDTA reduced root growth due that EDTA facilitates the penetration of some metals into the root tissue, reducing growth (Evangelou et al., 2007; Antoniadis et al., 2017).

The results indicate differences in the accumulation of heavy metals in the stem. Which is explained by the different translocation indices (ITF). In *Grindelia*, ITFs were improved by EDTA, showing differences among the different metals. These variations may be related to concentration, mobility, speciation, and competition for cell transporters, with essential mineral elements such as K, Ca, Mg, P, Fe, and Zn (Ke et al., 2007; Antoniadis et al., 2017). This coincides with other experiences in different species of grasses (Chami et al., 2015; Varum et al., 2015).

The phytoremediation capacity as phytoextractor or Phyto stabilizer species depends on the values obtained for the Bio adsorption coefficient (BAC), Bioconcentration Factor (BCF), and Translocation Factor (TF) (Vamerali et al., 2010; Varum et al., 2015). In this study, *G. tarapacana* showed an accumulative capacity for not exceeding 1000 mg / Kg of dry weight, but it has shown phytoextraction capacity for Hg, Zn, and Mn; and Phyto stabilization capacity for Cd, Cr, As, Cu, Pb, and Fe. The growth response, tolerance, and bioaccumulation of this species, suggest its potential as a phytoremediation species of Fe, Cu, and Pb using EDTA, as it has been described in the Asteraceae family (Nouri et al., 2009; Doncheva et al., 2012) and other plant species (Luo et al., 2006; Meers et al., 2007; Vamerali et al., 2010; Chami et al., 2015).

Conclusion

The growth response showed decrement due to treatment with heavy metals and the application of EDTA in higher concentration. The availability of heavy metals increased by effect of EDTA, improving its absorption by the roots, translocation and accumulation in stems and leaves. The activity of antioxidant enzymes increased in the presence of heavy metals and was affected by the concentration of EDTA. The bioaccumulation of heavy metals establishes the potential use of *G. tarapacana* as an accumulator species. Furthermore, this species responded as Phyto-extractor for Hg, Zn and Mn; and as a Phyto stabilizer for Cd, Cr, As, Cu, Pb and Fe. Finally, the growth responses, physiology and antioxidant activity under high concentrations of heavy metals, suggest the potential use of this specie in phytoremediation for Cu and Pb metals. We recommend evaluating the use of EDTA in combination with phytohormones and microorganisms to improve plant growth and increase phytoremediation capacity.

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