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Biochemistry response of *Paronychia microphylla* Phil., *Ambrosia artmisiodes* Phil. and *Croton ruizianus* Müll. Arg. against mercury for its bioprospecting in phytoremediation

Respuesta bioquímica de Paronychia microphylla *Phil.,* Ambrosia artmisiodes *Phil. y* Croton ruizianus *Müll. Arg. frente al mercurio para su bioprospección en fitorremediación*

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ABSTRACT

Mercury (Hg) is one of the most toxic heavy metals, and it is largely used in illegal gold extraction in Peru. We evaluated the tolerance to Hg in *Paronychia microphylla* Phil, *Ambrosia artemisioides* Willd and *Croton ruizianus* Müll. Arg. plants, using controlled concentrations (5, 10 and 80 mg Hg L^{-1}). Two groups were identified according to the antioxidant enzymatic and bioaccumulation response, but the changes in the soluble protein suggest other tolerance mechanisms in the species. *P. microphylla* has an accumulator performance (BCF = 1.16 at 10 mg Hg L^{-1}); in contrast, *C. ruizianus* has a exclusor behavior with low BCF and better antioxidant response. In that sense, the concentration of 5 mg Hg L^{-1} triggers the oxidative stress and increases the levels of malondialdehyde and proline, but when treatment concentration increases there is regulation in the antioxidant system. Among the three species, *C. ruizianus* Müll. Arg. has the better antioxidant response, but the lower bioaccumulation.

Keywords: Bioaccumulation; heavy metal; mercury; antioxidant enzymes; tolerance.

RESUMEN

El mercurio (Hg) es uno de los metales pesados más tóxicos y se utiliza principalmente en la extracción ilegal de oro en Perú. Se evaluó la tolerancia a Hg en en plantas de Paronychia microphylla *Phil,* Ambrosia artemisioides *Willd y* Croton ruizianus *Müll. Arg., utilizando concentraciones controladas (5, 10 y 80 mg Hg L^{-1}). Se identificaron dos grupos, según la respuesta antioxidante enzimática y de bioacumulación, pero los cambios en la proteína soluble sugieren otros mecanismos de tolerancia en las especies.* P. microphylla *tiene un desempeño acumulador (BCF = 1,16 a 10 mg Hg L-1); por el contrario,* C. ruizianus *tiene un comportamiento excluyente con bajo BCF y mejor respuesta antioxidante. En ese sentido, la concentración de 5 mg Hg L^{-1}, desencadena estrés oxidativo y aumenta los niveles de malondialdehído y prolina, pero cuando aumenta la concentración del tratamiento hay regulación en el sistema antioxidante. Entre las tres especies,* C. ruizianus *Müll. Arg. tiene la mejor respuesta antioxidante, pero la menor bioacumulación.*

Palabras clave: *bioacumulación; Metal pesado; mercurio; enzimas antioxidantes; tolerancia.*

Introduction

The increase in industrial activity, mining and intense and inappropriate use of pesticides are the main reasons for contamination by mercury (Hg), staying for long periods in the atmosphere and deposited in soils waters and sediments. The chemical form: Hg^{2+}, plays a key role in the cycle of this element and in toxicology, since it can be modified in organic chemical species, more toxic, which has become a

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concern for the degradation of ecosystems due to its harmful effects on the entire food chain and therefore adversely affecting the health of millions of people in the world (Sundseth *et al*., 2017).

Peru is the main gold producer in Latin America and currently 4th to worldwide, with a production of 150 tons in 2017, regions as Huancavelica, Puno, Cusco and the Peruvian Amazon in Madre de Dios, where informal mining (artisanal or small scale) is 8% of the production total annual gold, will expand rapidly, increasing the Hg load to the environment (Ramírez *et al*., 2020). Deleterious effects have been reported such as deforestation of 70.000 ha in the period of 1999-2016, and modifying the texture of upper layers of the soil presenting high concentrations of Hg (> 0.2 mg kg-1) that affects agriculture, coinciding with increasing mining (Fernandez, 2013). It has been estimated that since the 80's, more than 3,000 tons of mercury have been dumped in the Amazon rivers of the Peru, where 75% of the fishing is for subsistence, and the fishes have more than 0.3 ppm in their tissues, surpassing the permissible limits, in addition, it has been reported that hair samples from children contains Hg concentrations above 2.1 ppm (Fernandez, 2013). Thus, exposure to Hg is a constant concern in the Peruvian government agencies.

The Hg is not an essential element to plants, and its increasing concentration in soil and water has a considerable effect on the bioavailability of essential elements, thus affects plant growth and development, causing stress due to the toxicity and/or bioaccumulation of this element (Azevedo and Rodriguez, 2012). The availability of Hg for plants is usually limited due to the low solubility of this element in the soil solution and it has been described that Hg accumulates mainly in the roots, however the transfer and translocation exerted by the transpiratory current towards the offspring can occur, alternatively, plants can acquire atmospheric Hg through direct foliar absorption through stomata, thus, plants can act as an important pathway by which Hg can enter or exit terrestrial ecosystems (Bishop *et al*., 1998).

Similar to the toxicity described for other heavy metals, Hg has been reported to affect different physiological processes such as plant growth, root elongation, seed germination, seedling development, cell division, transpiration, photosynthesis, respiration, production of chlorophyll, lamellar organization of chloroplasts, phytohormonal balance, nutrient absorption, homeostasis, among others (Azevedo and Rodriguez, 2012). On the other hand, the toxicity of Hg induces the generation of free radicals via Reaction Haber Weiss-Fenton, triggering peroxidation lipid, promoting severe damage to the cell membrane, interference in enzymatic reactions and metabolic disorders, also apart of causing oxidative damage, the toxicity of Hg also resides in the ability of this metal to form inert substitution complexes with different biomolecules essential for cell proliferation and growth (Azevedo and Rodriguez, 2012).

It has been recognized the resilience of plants to cope with the heavy metal toxicity from different development is ecotypes and strategies extra and intracellular mechanisms related to avoidance, tolerance and detoxification. Hg toxicity can trigger a variety of metabolic responses leading to changes in plant growth and development (Xun *et al*., 2017). Since most heavy metals such as Hg, generate redox reactions, these can generate oxidative injuries due to the production of ROS and most of the time they are recycled at the biochemical level due to the activity of antioxidant enzymes and non-enzymatic antioxidant compounds (Xun *et al*., 2017). Among the major enzyme responses that has been described as mechanism tolerance are stimulating activity peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), and in regarding the non-enzymatic antioxidant components, an increase in the content of carotenoids, anthocyanins, flavonoids, polyamines, proline, nitric oxide, metallothioneins and phytochelatins, among others, has also been reported (Xun *et al*., 2017).

The characterization of different physiological and biochemical parameters serve as indicators of sensitivity or tolerance to metal toxicity in different groups of plants and is the starting point for the selection of tolerant genotypes for the purpose of phytoremediation (Llerena *et al*., 2021). The environmental collection of Hg in contaminated waters and soils is quite difficult, mainly economically, since currently there are different physical and chemical methods to remove heavy metals from the environment, involving prohibitive cost, intensive labor, alteration of soil properties and native soil microflora (Ali *et al*., 2013). An alternative for the elimination of the toxicity of heavy metals is to use phytoremediation techniques. Among the techniques of phytoremediation the most popular are phytoextraction and phytostabilization.

Phytoextraction involves the use of plants so that these accumulate the heavy metals in their aerial parts and across the phytostabilization is used to reduce the bioavailability and mobility of heavy metals and therefore the dispersion and potential transfer of heavy metals present in the soil towards the aquatic bodies and the trophic chain, being that in this strategy the plants accumulate metals in their roots (Ali *et al.*, 2013). Regardless of these two strategies, for a plant to be considered useful it must meet the following requirements: 1) have reasonable levels of accumulation, 2) tolerant, 3) be fast growing, 4) have high biomass, 5) rustic and competitive 6) have high rate of transpiration 7) dense root system, among others. Hyper-accumulating plants (species that accumulate metals above 10,000 mg kg DW^{-1}) meet the first two requirements well; but unfortunately, they grow slowly and do not accumulate biomass efficiently, in addition to that, the application of these plants is further limited because little is known about their agronomic characteristics, pest management, potential for improvement and physiology among others (Llerena *et al*., 2021).

However, the search for plants to be used in phytoremediation systems, even though they are not hyper-accumulators, but with a great development of biomass, have been described as having the ability to accumulate metals in the aerial part and a developed biomass offers a high potential for phytoremediation. In Peru, there is little work on the physiological response and accumulation against Hg toxicity in species with potential for phytoremediation of soils and waters, considering the geography and mineral wealth of this Country, although the investigation of potential sources of germplasm and identification and selection of promising species tolerant in the field of technology and the phytoremediation has been developed. As mentioned above, the elimination of Hg toxicity from contaminated soils by the activity of the mining informally in Peru is one of the most important problems to be solved, using techniques of phytoremediation, which is why we have chosen to study endemic species: *Paronychia microphylla* (Caryophyllaceae), *Ambrosia artemisioides* (Asteraceae) *and Croton ruizianus* (Euphorbiaceae) as these grow in soils adjacent to tailings areas and abandoned mining veins (Banco Minero, Cobre, Coricancha and KIOWA in Arequipa-Peru) of polymetallic concentration (copper, silver and gold), presenting co-tolerance high concentrations of metals (Cu: 128,6-246,6; Pb: 200,6-252,7; Fe: 5603.5-14604.2 mg kg $soil^{-1}$, respectively) (Llerena *et al*., 2021).

It is known that certain types of plants growing in areas with high concentrations of heavy metals, have developed co and/or tolerance and avoidance mechanisms that will be dependent on the plant species, the heavy metal in particular and environmental conditions. As hypothesized that seedlings of *Paronychia microphylla*, *Ambrosia artemisioides and Croton ruizianus*, to being treated with different concentrations of Hg respond differently in their ability to bioaccumulate this metal in its offspring according to its degree of tolerance, by altering their content of proline, soluble protein, malondialdehyde and antioxidant enzyme activity. This work is the first to characterize preliminarily various biochemical markers, that we believe will serve to establish a point of departure in the vacuum of knowledge of the mechanism of tolerance by these plants against toxicity and accumulation of Hg and on the other hand, establishing tolerant genotypes of sensitives and possible strategic role in the field of phytoremediation (accumulator or stabilizer). As a consequence, this work may constitute a reference study that could form the basis for future studies on genetic transformation, to increase the capacity of accumulation and tolerance on the part of this species s for Hg and other metals of interest.

Material and methods

Plant material and growing conditions

The seeds of *Paronychia microphylla* Phil, *Ambrosia artemisioides* Willd and *Croton ruizianus* Müll. Arg. were collected in the "Malpaso" hill of the Yarabamba district (S16º37'38.03" O71º38'45.92"). Healthy seeds were washed with tap water and placed in a bath with distilled water for 24 h to discard possible inhibitors. The seeds were placed in Petri dishes containing paper towel discs, moistened with distilled water and kept in the dark at 25 ºC in a germination chamber. The germinated seeds were transplanted into pots (19×16 cm plastic pots) containing 1.5 kg of washed fine sand (sterilized) and previously prepared moss (3:1 v/v). Three seedlings per pot were placed for *Paronychia microphylla* Phil, 2 seedlings for *Ambrosia artemisioides* Willd. and 1 for *Croton ruizianus* Müll. Arg. These were grown under greenhouse conditions for 6 months,

and were watered to field capacity 2 times a week with distilled water and fertilized once a week with the diluted Hoagland nutrient solution (1/8 strength) conductivity of 1.32 mS cm^{-1} and pH = 6.8-7.0 set with HCl 1 N, containing: 147 mg L^{-1} $Ca(NO_3)_2$; 63.13 mg L^{-1} KNO_3;17 mg L^{-1} mM KH_2PO_4; 61.75 mg L^{-1} $MgSO_4$; 0.357 mg L^{-1} H_3BO_3; 0.226 mg L^{-1} $MnCl_2.4H_2O$; 0.0275 mg L^{-1} $ZnSO_4.7H2O$; 0.0125 mg L^{-1} $CuSO_4.5H_2O$; 0.0125 mg $L^{-1}Na_2MoO_4$; 1.25 mg L^{-1} Fe-EDTA. Subsequently, the plants were treated with different concentrations of mercury (5, 10 and 80 mg Hg L^{-1}), from mercury chloride salt ($HgCl^{+2}$) dissolved in Hoagland's nutrient solution with one application at field capacity (~200 mL). The pots without Hg served as a control. After 35 days of treatment, the seedlings were harvested, separating the roots and the shoot, to determine the mercury content. The protein content, levels of antioxidant enzymes, lipid peroxidation and proline were determined in the leaves of the species under study.

Determination of the mercury concentration in the stem and root

Endogenous Hg was measured in the plant shoots by initial immersion in 0.1 M EDTA- Na_2 for 10 min and washing three times with distilled water to eliminate the metal on the surface of the samples. Subsequently, the samples were dried at 80 ºC for 72 h until finely ground and extracted according to Garay *et al.* (2003). Hg was determined in the digested pooled samples using flame atomic emission spectrometry (Perkin-Elmer 4000 equipment) with the following instrument set-up: Wavelength: 324 nm, Spectral Bandwidth: 0.2 nm, Lamp Intensity: 5 mA, Linear Range: 0.1-2.0 µg ml^{-1}. In each treatment the Hg concentration was determined in the pooled biomass of five shoots, representing one replicate. We used five replicates.

Determination of bioconcentration factor (BCF)

Indicates the efficiency of a plant species in accumulating a metal in its tissues from the surrounding environment (Ali *et al.*, 2013).

Determination of the translocation factor (TF)

Ability of the plant to transfer metals from the roots to the aerial parts of the plant (Ali *et al.*, 2013).

Lipid peroxidation

Lipid peroxidation was measured according to the method proposed by Heath and Packer (1968) In this method, the concentration of malondialdehyde (MDA) is measured as the end product of lipid peroxidation by reacting it with thiobarbituric acid (TBA). Fresh leaves samples (150 mg) were homogenized in a mortar with 1.5 ml of 0.2% trichloroacetic acid, then an equal aliquot of 20% TCA containing 0.5% TBA was added. Next, the solution is heated to 95 ºC for 25 minutes and then centrifuged for 1 minute at 1000 g. The absorbance was measured at 532 nm and the turbidity was corrected by reading the absorbance at 600 nm.

Proline concentration

Proline determination was carried out according to Bates *et al.* (1973). Fresh leaves (50 mg) were weighed and homogenized with 5 mL of 3% sulfosalicylic acid at room temperature. The homogenates were transferred to falcon tubes, and these were placed on a horizontal shaker, for 60 minutes at room temperature. It was then centrifuged at 6,000 rpm at 4 ºC for 30 minutes. 2 ml of the supernatant were placed in a test tube, 2 ml of the freshly prepared reaction mixture (2.5 g of ninhydrin dissolved in 60 ml of glacial acetic acid and 40 ml of 6M phosphoric acid) and 2 ml of acid warm glacial acetic. It was stirred vigorously for 15 seconds, avoiding direct incidence of light to the reaction mixture. The test tubes were covered with aluminum foil, and incubated vertically in a rack and subsequently boiled for one hour. Then it was quenched on an ice bath until reaching room temperature, to add 4 ml of toluene. It was stirred vigorously for 30 seconds and allowed to stand for 1 hour. The organic part of each sample was then collected with a pipette and its absorbance was read at 520 nm, using toluene as a blank. The proline concentration was determined from a standard curve.

Determination of total soluble proteins

The protein was determined by the method of Bradford (1976), using bovine serum albumin as standard and the results were expressed in mg g FW^{-1}.

Obtaining the crude extracts for the antioxidant enzyme assay (POD, CAT and APX)

The crude enzymatic extracts for the determination of catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) activity were obtained by grinding 300 mg of fresh leaves (keeping the mortars on ice, throughout the process) with addition of 2 ml of homogenization buffer. The homogenization buffer for POX and CAT had the following composition (Peixoto *et al*., 1999): 0.1 M phosphate buffer (pH 6.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF) and polyvinylpyrrolidone (PVPP) 1% (w/v), and the homogenization buffer for APX (Nakano and Asada,1981): 50 mM phosphate buffer (pH 7.0), 1 mM ascorbate and 1 mM EDTA, followed by a centrifugation of 12000 g for 15 minutes at 4 ºC.

Determination of catalase activity

Catalase activity was determined by adding 100 µL of crude enzyme extract to 2.9 ml of reaction buffer (50 mM K phosphate buffer pH 7.0 and 12.5 mM hydrogen peroxide). At the end of the first minute of reaction, at 25 ºC, the decrease in absorbance at 240 nm was measured. The enzymatic activity was calculated using the molar extinction coefficient of 36 M^{-1} cm^{-1} (Anderson *et al*., 1995) and expressed in µmol min^{-1} mg of $protein^{-1}$.

Determination of peroxidase activity

Peroxidase activity was determined by adding 100 µL of crude enzyme extract to 2.9 mL of reaction buffer (25 mM phosphate buffer (pH 6.8), 20 mM pyrogallol and 20 mM hydrogen peroxide). The increase in absorbance during the first minute of reaction at 420 nm, at 25 ºC, made it possible to determine the production of purpurogallin. The activity was calculated using the molar extinction coefficient of 2.47 mM^{-1} cm^{-1} (Chance and Maehly, 1955) and expressed in µmol min^{-1} mg of $protein^{-1}$.

Determination of ascorbate peroxidase activity

Ascorbate peroxidase activity was determined by adding 100 µL of crude enzyme extract to 2.9 ml of reaction buffer (50 mM phosphate buffer (pH 6.0), 0.8 mM ascorbate and 1.0 mM hydrogen peroxide). The decrease in absorbance at 290 nm at 25 ºC was determined during the first minute of reaction (Nakano and Asada, 1981). Enzyme activity was calculated using the molar extinction coefficient of 2.8 mM^{-1} cm^{-1} and expressed in µmol min^{-1} mg of $proteins^{-1}$.

Statistic analysis

A completely randomised design (3 × 3) was applied, where the first factor is the number of species, and the second, the number of Hg treatments, including the control. A Kruskall-Wallis test was performed ($\alpha = 0.05$), using the statistical software R (R Core Team, 2020). For the biochemical analyses (enzymatic activity (CAT, POX e APX, soluble proteins and malondialdehyde) we analysed 3 Hg treatments (Control, 5, 10, 80 mg Hg L^{-1}) each one with five biological replicates.

Results and discussion

Accumulation of mercury in plants

The results obtained indicate that when *Paronychia microphylla* seedlings (Figure 1) are treated with 5 and 10 mg Hg L^{-1}, the accumulation of this metal occurs mainly in the shoots (5.87 mg Hg kg^{-1}). However, when the seedlings are treated with 80 mg Hg L^{-1}, this metal accumulates at the root level (25.27 mg kg^{-1}).

Likewise, in *Ambrosia artemisioides* seedlings, it is observed that when treated with 5, 10 and 80 mg Hg L^{-1} in the substrate, the accumulation of this metal occurs mainly in the roots (0.6, 2.67 and 33.63 mg Hg kg^{-1} respectively) compared to the control (Figure 1). This is probably due to the fact that the root system serves as a partial barrier to mercury transport to the leaves (Calgaroto *et al*., 2010). These results are similar to other studies conducted in cucumber seedlings (Cargnelutti *et al*., 2006), wheat plants (Sahu *et al*., 2012), and *Pfaffia glomerata* seedlings (Calgaroto *et al*., 2010) where the highest accumulation of Hg occurred in the roots.

On the other hand, *Croton ruizianus* Müll. Arg. presents low levels of metal translocation even at high Hg concentrations. It suggests a well adapted root system. On the other hand, Hg has high solubility in water and easiness to shift into vapour, these properties could allow a better translocation

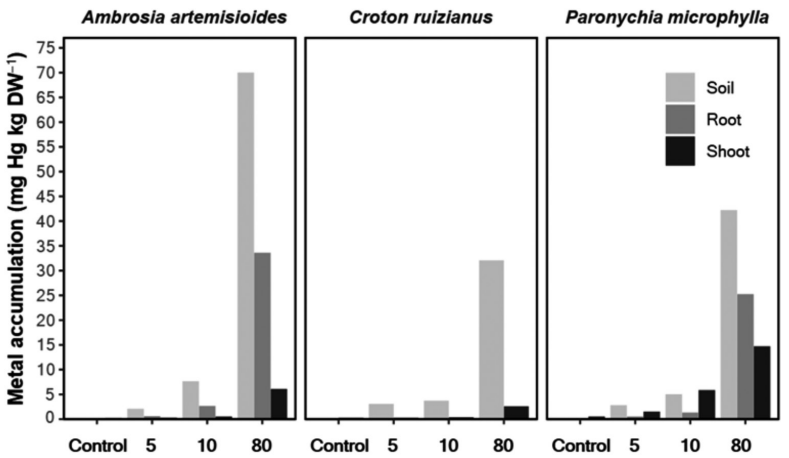

Figure 1. Mercury distribution in plants against mercury contaminated soils.

or a direct absorption of the gaseous form into the shoot (Azevedo and Rodriguez, 2012).

Bioconcentration (BCF) and Translocation Factor (TF) of Hg in *P. microphylla*, *A. artemisioides* and *C. ruizianus* seedlings.

The BCF factor in *P. microphylla* seedlings (Table 1) has a value >1 when treated with 10 ppm of Hg. On the other hand, the FT of the same species with 5 and 10 mg Hg L^{-1}, has values> 1, compared to the treatment with 80 mg Hg L±$^{-1}$, which allows us to deduce that this plant behaves as an accumulator of this metal up to the concentration from 10 mg Hg L^{-1} and as an exclusive plant at 80 mg Hg L^{-1}. In a different way, the parameters of BCF and FT in *A. artemisioides* (Table 1), reflect values <1 under concentrations of 5, 10 and 80 mg Hg L^{-1}, which indicates that it is an exclusive plant.

Effect of Hg on Proline content

Mercury significantly ($p < 0.05$) affected the proline content (Figure 3B) in *P. microphylla* Phil leaves and *A. artemisioides* Willd, but has little effect on *C. ruizianus*. Higher content of this amino acid is observed when treated with 5 mg Hg L^{-1}, however at higher concentrations (10 and 80 mg Hg L^{-1}) *P. microphylla* Phil and *A. artemisioides* Willd experience a drop in their proline values. These results are similar to studies with mercury-treated rice plants by Wang *et al.* (2008), where they discovered an increase and subsequent decrease in proline.

Effect of Hg on the content of soluble proteins

The soluble protein content of *P. microphylla* and *A. artemisioides* was not significantly affected ($p > 0.05$), but there was a significant increase ($p<0.05$) in *C. ruizianus* at higher Hg doses (Figure 2A). The changes observed in *P. microphylla* at the concentration with 5 mg Hg L^{-1}, could indicate that the mercury would be interfering in the de novo synthesis of proteins or causing an increase in the breakdown of proteins into amino acids. It could also be due to a higher generation of ROS, therefore more oxidative stress that could have resulted in a decrease in the level of protein content through oxidative damage (Cargnelutti *et al.*, 2006). These findings are similar to the studies carried

Table 1. Bioconcentration Factor (BCF) and Translocation Factor (TF) under mercury treatment.

Specie	Treatmentmg Hg L^{-1}	BCF	TF
Ambrosia artemisioides	5	0.15	0.53
	10	0.07	0.21
	80	0.09	0.18
Croton ruizianus	5	0.09	n.d.
	10	0.1	0
	80	0.08	0
Paronychia microphylla	5	0.54	2.96
	10	1.16	4.45
	80	0.35	0.58

n.d. = not determined.

out in *Mentha arvensis* plants (Manikandan *et al*., 2015) and cucumber seedlings (Cargnelutti *et al*., 2006), who suggest a greater increase in protein content at concentrations of 15 mg Hg L^{-1}, but it decreases slightly with higher doses, due to the high accumulation of Hg content in leaf tissues.

Effect of Hg on Malondialdehyde (MDA) content

When treated with Hg for a period of 35 days, the *P. microphylla*, *A. artemisioides* and *C. ruizianus* seedlings showed significant differences ($p < 0.05$) between species in the MDA levels. This indicates that the treatments (5, 10 and 80 mg Hg L^{-1}) with mercury induced lipid peroxidation, in relation to the control. This is reflected in the high levels of malondialdehyde (MDA) (Figure 3A). Despite not presenting significant differences ($p > 0.05$) between the treatments, it should be noted that the lowest levels of MDA occurred with 10 mg Hg L^{-1}. Our results show that the mercury treatments produced greater oxidative damage to *Paronychia microphylla* seedlings than to *Ambrosia artemisioides* seedlings. These results coincide with many other investigations carried out on rice (Wang *et al*., 2008), *Pfaffia glomerata* (Calgaroto *et al*., 2010), and cucumber seedlings (Cargnelutti *et al*., 2006).

Effect of Hg on peroxidase enzyme activity (POX)

Peroxidase activity in *P. microphylla*, *A. artemisioides* and *C. ruizianus* subjected to different concentrations of Hg for 35 days, shows significant differences ($p < 0.05$) depending on the species. The highest POX activity was presented in the *C. ruizianus* seedlings treated with 5 mg Hg L^{-1} (Figure 2C). However, the POX activity levels do not show significant differences ($p > 0.05$) between the treatments in relation to the control, but at concentrations of 5 and 80 mg Hg L^{-1}, it is evident a decrease in POX activity for *A. artemisioides* and *P. microphylla*, with respect to the control. These results are similar to other studies carried out in wheat plants (Sahu *et al*., 2012). These authors have observed a progressive increase in POX activity treated at concentrations of up to 20 µM Hg, and an additional increase of this metal (25 µM of Hg^{2+}) caused the activity of this enzyme to begin to decrease.

Effect of Hg on catalase enzyme activity (CAT)

The levels of CAT in seedlings of *P. microphylla*, *A. artemisioides* and *C. ruizianus* cultivated in greenhouse conditions, and subjected to different

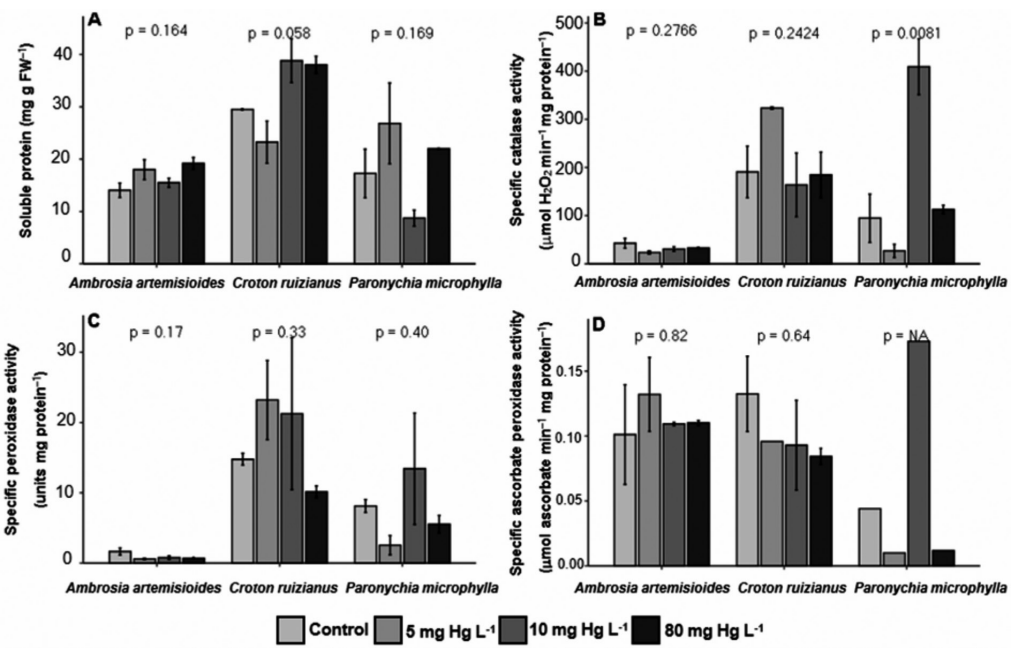

Figure 2. Enzymatic response to mercury stress. p-values correspond to the Kruskal- Wallis test

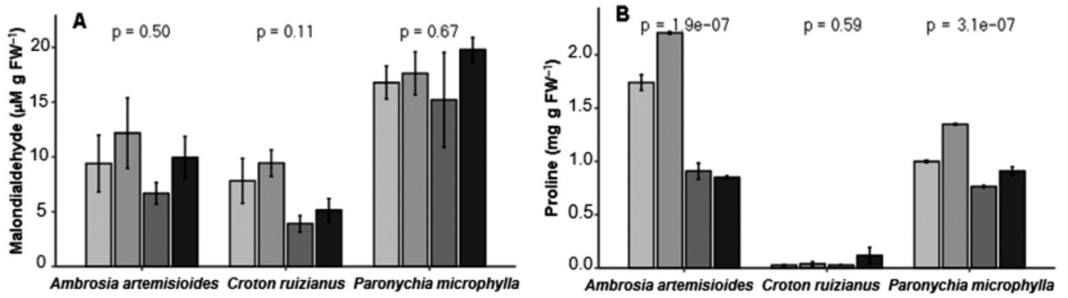

Figure 3. Lipid peroxidation and proline content under growing mercury concentrations. p-values correspond to Kruskal-Wallis test.

concentrations of Hg for 35 days, show significant differences ($p < 0.05$) depending on the species. The highest catalase activity occurred in *Paronychia microphylla*, reaching a maximum of 409.15 µmol H_2O_2 min^{-1} mg $protein^{-1}$, in the seedlings treated with 10 mg Hg L^{-1}. Furthermore, our results indicate that there are significant differences ($p < 0.05$) between the treatments, observing low levels of catalase in the seedlings treated with 5 and 80 mg Hg L^{-1} in (Figure 2B) in *P. microphylla*, *A. artemisioides*, but was higher for *C. ruizianus.* As in the present study, an increasing trend in CAT activity when treated with Hg has been reported in *M. arvensis* plants (Manikandan *et al*., 2015) and wheat plants (Sahu *et al*., 2012) , but as the concentration of this metal increases, the enzymatic activity decreases.

Effect of Hg on ascorbate peroxidase (APX) enzymatic activity

C. ruizianus and *A. artemisioides* showed similar APX activity, since the statistical analysis indicates that there are no significant differences ($p < 0.05$) in the expression of this enzyme, with respect to the control, for both species (Figure 2D). Although there are no differences between APX levels between species, it should be noted that *A. artemisioides* seedlings show the highest values of enzymatic activity. On the contrary, *P. microphylla* seedlings show higher values when they are subjected to 10 mg Hg L^{-1}, with respect to the seedlings treated with 5 and 80 mg Hg L^{-1}, which show relatively low levels of APX. This low enzymatic activity was also observed in *Triticum aestivum* (Sahu *et al*., 2012) and *Mentha arvensis* (Manikandan *et al*., 2015) by increasing the concentration of Hg.

Concluding remarks

The present work is the first to study the mechanisms of Hg tolerance of *P. microphylla*, *A. artemisioides* and *C. ruizianus*. The plant survival was 100%. The ability to accumulate mercury varies significantly between species, developing different mechanisms to respond to stress caused by heavy metals. The results of this study showed that *P. microphylla*, *A. artemisioides* and *C. ruizianus* have different tolerance mechanisms, based on accumulation and exclusion strategies, respectively. Taken together, our data show evident oxidative damage to membrane lipids, changes in proline and protein content. Decreased POX, CAT and APX activities in *P. microphylla* and *Ambrosia artemisioides* seedlings by exposing them to high concentrations of mercury. Although *C. ruizianus* has the better biochemical and antioxidant response, but has a poor bioaccumulation. Finally, *P. microphylla* and *A. artemisioides* can be used for phytoextraction and phytostabilization, due to their ability to accumulate and reduce the concentration of Hg in contaminated soils.

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