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Mercury uptake and effects on growth in *Jatropha curcas*

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ABSTRACT

The use of metal-accumulating plants for the phytoremediation of contaminated soils is gaining more attention. Mercury (Hg)-contaminated soils from historical gold mines represent a potential risk to human health and the environment. Therefore, *Jatropha curcas* plant, that has shown its tolerance to these environments, is a species of particular interest to implement phytoremediation techniques in gold mining sites. In this work, the behavior of *J. curcas* was assessed in different hydroponic cultures fortified with Hg at concentrations of 5, 10, 20, 40, and 80 μg Hg/mL (T5, T10, T20, T40 and T80, respectively). After exposure, plant growth, net photosynthesis, leaf area, and Hg accumulation were determined and variables such as net Hg uptake, effective Hg accumulation, translocation and bioaccumulation factors were calculated. Accumulation of Hg in root and leaf tissues increased with respect to the Hg concentrations in the hydroponic culture, with statistically significant differences ($p < 0.05$) among treatments. Moreover, Hg concentration in roots was 7 and 12-fold higher in average than in plant leaves and shoots, respectively. Many effects were found in the development of plants, especially related with loss of biomass and leaf area, with significant growth inhibition related to control values (>50% with treatment T5). Moreover, percentage of inhibition was even higher (>60%) with same treatment for net photosynthesis. Finally, it should be highlighted that for T40 and T80 treatments, plant growth and photosynthesis were almost completely depleted (88%–95%).

Introduction

Mercury (Hg) contamination has become an environmental problem at the global scale (Schroeder and Munthe, 1998; Boening, 2002). Unlike the majority of the metals that function as nutrients, Hg has no known physiological importance and, hence, it is not metabolized by most organisms (Sahi et al., 2006). Currently, soil contamination generated by Hg constitutes a serious problem because it is still released onto the environment through different industrial processes, threatening the balance of ecosystems and human health (Diez, 2009; Moreno et al., 2005).

In Colombia, activities related to gold mining cause environmental impact due to uncontrolled use of Hg, introducing problems of water and fish contamination in different regions of the country (Marrugo-Negrete et al., 2008). The use of Hg has been increasing in gold mining, according to Telmer and Veiga (2008) who estimated that annual Hg emissions from artisanal gold miners in Colombia in 2007 were between 50 and 100 metric tons; whereas other studies reported that in 2009 Colombia imported 130 metric tons of metallic Hg (Cordy et al., 2011).

Soils disturbed by gold mining are characterized by having established plant coverage to a lower or higher degree with a

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unique diversity of species (Díaz and Elcoro, 2009). It has been demonstrated that plants have different natural properties that make them ideal to treat contaminated soils in a process denominated phytoremediation, which uses plants to reduce the concentrations or toxic effects of contaminants (Lomonte et al., 2010), given that they can accumulate the contaminant in their tissues, thus, inactivating them (Padmavathiamma and Li, 2007). It has been shown that the *Jatropha curcas* species presents good coverage and tolerance to the presence of Hg in soils from the El Alacrán mine, located in northeast Colombia. This plant has the capacity of accumulating Hg without reflecting visible phytotoxic effects (Marrugo-Negrete et al., 2015).

The phytotoxicity of Hg affects biomass and inhibits plant growth, while producing long-term effects on soil fertility (Sahi et al., 2006). Inhibition of nutrient and mineral absorption has also been reported (Cho and Park, 2000; Patra and Sharma, 2000), as well as reduced photosynthesis activity (Patra et al., 2004).

To evaluate the implementation of treatment systems through phytoremediation technologies employing *J. curcas*, it is necessary to evaluate and characterize the different processes associated to plant response against the contaminant. The aim of this work is to evaluate Hg resistance and bioaccumulation in hydroponically-grown *J. curcas* plant. The results of this study would be a pioneer contribution to enrich our knowledge on the tolerance, phytoremediation capacity and potential implementation of this plant in systems at real scale to treat soils contaminated with Hg.

1. Materials and methods

1.1. Plant growth and test set up

The experiment was conducted in the greenhouse at Córdoba University in Colombia, during a 21-day period. During the experiment the pH of the substrate was in a narrow range (5.5–5.6), and room conditions were maintained with a temperature of 27–30°C, and relative humidity between 55% and 65%, exposed to a 12-hr photoperiod.

J. curcas seeds were collected from plants found in soils that were uncontaminated with Hg. The seeds were washed with tap water and submerged in a 0.3% (*v*/*v*) sodium hypochlorite solution for 5 hr (Peña-Pontón, 2009). Finally, the seeds were rinsed with deionized water and, prior to the germination process, the seeds were kept refrigerated at 10°C for 2 months (De Argollo and Campos, 2007).

The seeds were cultivated in 16-ounce capacity pots with 13.0 cm height, which contained nutrient solution (Hoagland) and moist perlite as support material (700 g per pot), prior sterilization with water at 100°C for 2 hr. Seedlings used in testing were selected from groups of plants grown from seeds planted and cultivated ahead of time to provide sufficient seedlings at the 2- to 4-true leaf stage of growth (typically 1 week post-emergence) and of uniform size to initiate the test for a given test species on the same day. After germination of the seeds, the seedlings were kept for 1 week in nutrient solution; thereafter, and for another week, each pot was treated with 80 mL of Hg as $Hg(NO_3)_2$ at concentrations of 5, 10, 20, 40, and 80 μg Hg/mL (*i.e.*, T5, T10, T20, T40 and T80, respectively, in triplicate). A control treatment (T0) was conducted also in triplicate with the same nutrient solution, free of Hg, as adopted from Zornoza et al. (2010). Details of initial experimental conditions in each pot are shown in Table 1.

1.2. Analysis of the Hg content

The total Hg content in plant tissues (root and leaf) was carried out by taking 0.5 g of the plant material, and subjecting it through assisted digestion *via* microwave with an HNO_3/H_2O_2 acid mixture in 5:2 mL proportions, respectively (Jedrzejczak et al., 1996). The Hg analysis was carried out through cold-vapor atomic absorption spectroscopy (CV-AAS) by using a Thermo Scientific iCE3000 series analyzer.

For the analytic control of quality, a tomato-leaf reference material was evaluated in triplicate (CRM 1753a, 34 ng/g), total Hg recovery percentages were of 98.5% ± 0.35%. The detection limit was of 14 ng Hg/g dry weight (dw), calculated as the mean plus three times the standard deviation (SD) (Buccolieri et al., 2006).

1.3. Biomass estimation

The plants were harvested at the end of the test; biomass was determined by drying the samples in a stove with an integrated timer (Binder 5°C–300°C) within 4 days at 40°C, then the material was weighed in grams on an analytic scale (OHAUS Corp., Adventure, model AP2140).

1.4. Determination of the leaf area

The leaf area for each plant was determined immediately after the plants were harvested; this procedure was performed through leaf area digital determiner (DDA) software (Ferreira et al., 2008), where the leaves of each plant were separated and then scanned with a table-top scanner (Canon Pixma Mp250). The DDA software took the shapes of the leaves and digitizes and processes them through the determination of the number of pixels occupied by the leaves; thereafter, this is joined to the area occupied by each pixel converting by square centimeters.

1.5. Evaluation of net photosynthesis (Pn)

Pn was determined on the leaf surface for each treatment in triplicate before harvesting the plants. An infrared gas analyzer (IRGA) (LiCor 6400 XT or CIRAS-2 PP SYSTEMS or LCI-Pro+ (ADC — UK)) was used for this purpose to establish that the same photosynthetically active radiation (PAR) pattern in each treatment received a 1000-μmol photon (m^2/sec) light radiation, adjusted based on the PAR of the site where the measurements are made, and CO_2 supply of 350 μmol CO_2 (m^2/sec); readings were made between 11:00 AM and 01:00 PM.

1.6. Calculations and statistical analysis

Some parameters were calculated to study the behavior of plant. The net Hg content (C_{Hg} (μg) in plants and in shoots was calculated according to Eq. (1) (Moreno-Jiménez et al., 2007):

$$C_{Hg} = C_{Hgt} \times (TDW)_t \quad (1)$$

Table 1 – Values of variables related to Hg accumulation and Hg removal in the hydroponic experiment.

Start of the experiment				End of the experiment					Net uptake (μg/(g·day))	Hg translocation (μg/(g·day))		EA_{Hg} (%)	F_b			T (%)
V_i	Treat	m_i	C_i	M_l	M_r	M_s	M_{sh}	M_w		T_{leaves}	T_{shoot}		Root	Leaf	Shoot	
80	T5	400	5 (0.6)	51.1	343.0	81.1	132.2	475.1	20.2	10.8	6.2	119	0.86	0.13	0.33	28
80	T10	800	10 (1.1)	55.3	332.0	112.2	167.5	499.5	28.7	15.7	10.7	62	0.41	0.07	0.21	34
80	T20	1600	20 (2.3)	63.0	169.7	101.5	164.6	334.2	46.1	42.2	25.1	21	0.11	0.04	0.10	49
80	T40	3200	40 (4.6)	72.6	118.1	109.7	182.2	300.4	88.2	103.7	59.2	9	0.04	0.02	0.06	61
80	T80	6400	80 (9.1)	200.1	125.2	135.5	335.6	460.8	170.3	357.4	138.3	7	0.02	0.03	0.05	73

V_i: initial Hoagland solution (mL); m_i: initial mass of Hg in nutrient solution (μg); C_i: initial concentration of Hg in each pot (μg Hg/mL), between the parentheses is the Hg concentration (in μg Hg/g substrate) considering 700 g of perlite; *M*: total mass absorbed in the different parts of the plant (M_l: leaves; M_r: roots; M_s: stems; M_{sh}: shoots and M_w: whole plant); EA: effective Hg accumulation (μg/g/d); F_b: bioaccumulation factor; *T*: translocation.

where C_{Hgt} (μg/g) is the concentration of Hg uptake for the total biomass (TDW, grams in dw) after hydroponic culture (t = 7 day).

EA_{Hg} is the effective Hg accumulation, $[Hg]_t$ is the total Hg accumulation in leaves or shoot. $[Hg]_{added}$ (μg) is the Hg added in nutrient solution.

$$EA_{Hg}\% = 100 \times \frac{[Hg]_t}{[Hg]_{added}} \quad (2)$$

The following variables in Eq. (3) were calculated (Zornoza et al., 2010) to estimate Hg removal (Hg μg/plant) in shoots and accumulation by the plants growing under different treatments.

$$F_b = \frac{C}{C_s} \quad (3)$$

where, F_b is the bioaccumulation factor, *C* (μg/g dw) is the Hg concentration in shoots, roots or leaves (*C*), and C_s (μg/g dw) is the available Hg concentration in soil.

The translocation (*T*) of Hg is expressed as Eq. (4):

$$T = \frac{A_{ts}}{A_{tp}} \quad (4)$$

where, A_{ts} (μg) is the total Hg accumulated in shoots; A_{tp} (μg) is the total Hg accumulated in whole plant.

The values of the figures are mean values of three independent repetitions; significant differences among treatments were analyzed *via* one-way analysis of variance (ANOVA), having a significance level of $p < 0.05$. Data were analyzed by using the SAS Plus 4.1 program.

2. Results

2.1. Mercury accumulation in plants

Accumulation of Hg in root and leaf tissues of *J. curcas* plants increased with respect to the Hg concentrations in different treatments, showing statistically significant differences ($p < 0.05$) among them (Fig. 1). Concentration of Hg in all the treatments in the roots was around 7 times higher in average than in plant leaves, and 12 times that of the shoots. At the end of the experiment, the Hg concentration in the roots of plants treated with the lower Hg doses (*e.g.*, T5 and T10) was 12 times that of the leaves and 20 times that of the shoots. However, at higher treatments (T40 and T80), this ratio is close to one since the leaves accumulated between 725 and 2500 μg Hg/g, while the roots accumulated up to 3100 μg Hg/g (Fig. 1).

The bioaccumulation factor in the roots (F_b in roots, Table 1), expressed as the ratio metal concentration in the roots to that in soil, decreases when the Hg dose increasing. A similar trend was observed for the calculation of bioaccumulation factor in leaves and shoots (Table 1). On the other hand, the percentage of Hg translocated to the aerial part of the plant from the whole plant ranged from 28 to 73, and the highest value was seen for plants grown in the presence of T80. Maximum Hg translocation per unit of time was found at the highest treatment for both leaves and shoot and the net uptake is around 170 μg/(g·day) (Table 1).

2.2. Effect of Hg on biomass

Growth of *J. curcas* plants was evaluated with respect to biomass accumulation (Fig. 2). When compared with the control treatment, it can be noted that as Hg concentrations increase the biomass decreases, showing statistically significant differences among treatments ($p < 0.05$), suggesting that reduction exists in the development of plants planted in each level of Hg concentration.

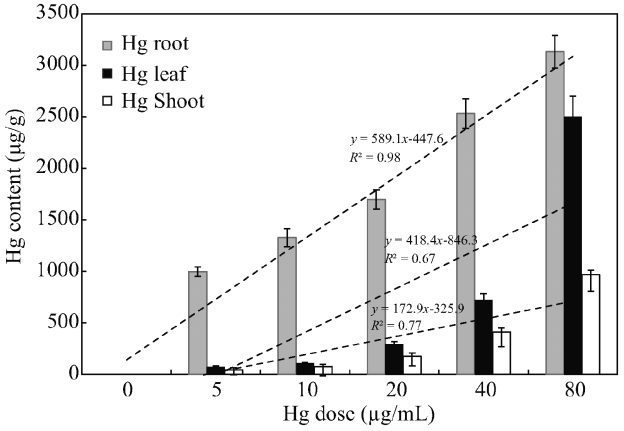

Fig. 1 – Effect of Hg supply on Hg accumulation in roots and leaves of *J. curcas*. Values are represented as the mean ± S.E. (n = 3). S.E.: standard error.

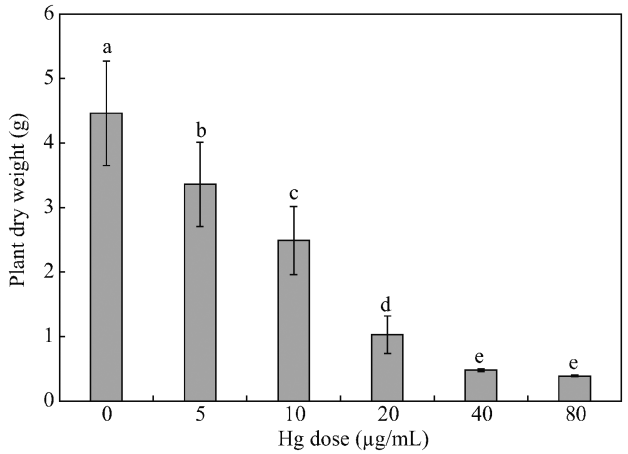

Fig. 2 – Effect of Hg on the biomass of *J. curcas* plants. Vertical bars indicate (±) standard error of the mean (n = 3). Significant ($p < 0.05$) differences across samples following analysis of variance test are indicated by different letters.

2.3. Effects of Hg on leaf area

As can be seen, the leaf area decreases as the dosage of Hg increases with respect to the control (Fig. 3), showing statistically significant differences among treatments ($p < 0.05$). The leaf area reduces its surface area for 53% related to control when the plants were subjected to T10 in the hydroponic culture medium. For higher Hg concentration treatments (T20, T40 and T80), the percentage of inhibition of leaf growth related to untreated soil (T0) was in the range of 88%–95%.

2.4. Effects of Hg on Pn

Results of Pn are shown in Fig. 4. Highly significant differences among the different treatments could be observed ($p < 0.05$). These results indicate that *J. curcas* endured decrease of Pn upon increasing Hg concentrations in the different treatments. In the control it was maintained at 7.2 μmol CO_2 (m^2/sec), and then a dosage of T5 leads to 5.4 μmol CO_2 m^2/sec; as of the dosage of T10, the assimilation capacity of the CO_2 molecules is reduced by over

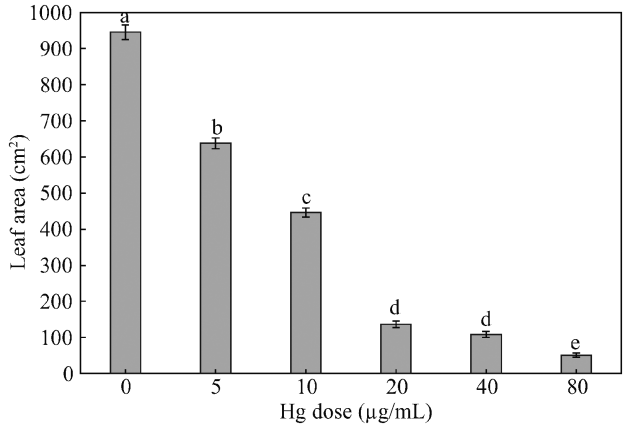

Fig. 3 – Effect of Hg on leaf area of *J. curcas* plants. Values represent the mean ± S.E. (n = 3). Different lower-case letters indicate statistical significance ($p < 0.05$). S.E.: standard error.

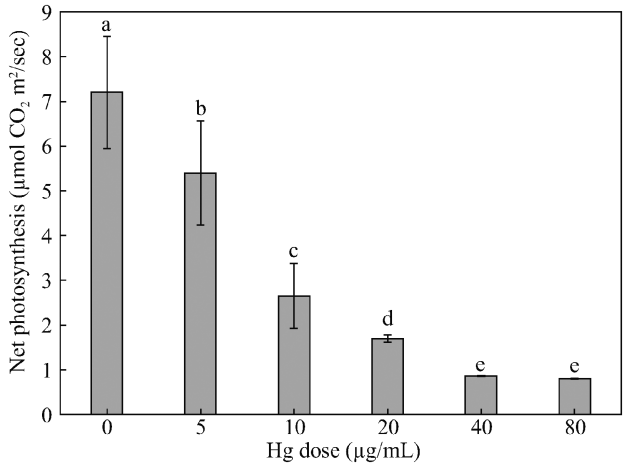

Fig. 4 – Effect of Hg on net photosynthesis in *J. curcas*. Vertical bars indicate standard error of the mean (n = 3). Significant ($p < 0.05$) differences across samples following ANOVA test are indicated by different letters. ANOVA: analysis of variance.

60% per every square centimeter over the leaf surface; achieving a maximum for T40 and T80 (no significant differences) in the range of 88% to 89%, respectively.

3. Discussion

Mercury levels obtained in the different plant tissues (Fig. 1) demonstrate that roots accumulate higher amounts of Hg, similar as reported previously (Molina et al., 2006; Wang and Greger, 2004; Sahi et al., 2006; Zornoza et al., 2010). This behavior is probably because the roots are directly exposed to the Hg present in the substrate, with a large amount of Hg adhering to them in the cell walls to, thus, avoid toxic effects in the upper parts of the plant, especially the development of necrosis and chlorosis in leaves (Lindqvist et al., 1991; Qian et al., 2009). Moreover, a linear relationship was seen between the Hg supplied to hydroponic cultures and Hg concentration in the roots (R^2 = 0.98; $p < 0.05$), in the leaves (R^2 = 0.67, $p > 0.05$), and in the shoots (R^2 = 0.77, $p > 0.05$). Furthermore, the slope for the root was 3–4 times that of the shoots (Fig. 1). In comparison with a previous study using *J. curcas*, performed with spiked soils (Marrugo-Negrete et al., 2015), a much higher accumulation was obtained here. In fact, Hg was continuously uptaken in the hydroponically-grown plants during the 7 days of exposure, and the accumulation of Hg was linear over the concentration range tested. The amount of Hg retained in the roots grown in perlite is about 1000-fold higher than values obtained in similar experiment with spiked soils (at 4 weeks). A higher bioavailability is probably the main reason for the apparently larger accumulation of Hg in the hydroponically-grown plants. Similar trends regarding bioaccumulation and translocation were reported in hydroponic experiments of white lupin (*Lupinus albus* L.), with the Hg dose increasing, the percentage of Hg translocated to the shoots decreased lightly, although no significant differences were found in a wide Hg range (2.50 to 10 μmol/L Hg) (Zornoza et al., 2010). In comparison with other plants previously studied, *J.*

curcas showed a higher translocation since its shoot-to-root distribution of Hg (ratios $[Hg]_{shoot}/[Hg]_{root}$) is higher than the values found in *Rumex induratus and Marrubium vulgare* grown directly in perlite as substrate (Moreno-Jiménez et al., 2007) with similar Hg supply. Ratios obtained with tomato cultured in perlite–vermiculite (Cho and Park, 2000) and for water-cultured rice (Du et al., 2005) with lower Hg supply were also lower. Much lower ratios were reported in plants grown directly in nutrient solution, such as willow (Wang and Greger, 2004) or water spinach (Göthberg et al. 2004). Better translocation factors were obtained in our work in comparison with studies performed with *Pteris vittata* and *Nephrolepis exaltata* (Chen et al. 2009), grown in a hydroponic system, where the roots of both cultivars accumulated large amounts of Hg, but exhibited limited Hg translocation to shoots. In sum, *J. curcas* has a high capacity for bioaccumulation, phytotranslocation and phytoremediation of heavy metals (Yadav et al., 2009; Jamil et al., 2009; Gao et al., 2010; Marrugo-Negrete et al., 2015), and this plant crop has been shown also to help restore damaged and contaminated ground (Debnath and Bisen, 2008), as well as a good tolerance to copper and lead (Gao et al., 2008, 2009).

Reduction of *J. curcas* biomass when subjected to different Hg concentrations may depend on the Hg accumulated by the plants; thereby, the cells might have to spend additional energy to confront the high Hg concentration within the plant (Sahi et al., 2006). Another effect of Hg may be due to the inhibition of aquaporins, which are proteins that facilitate water transport within the plant, hence, when inhibited they do not have the capacity to efficiently intake and transport nutrients — an effect reflected on the growth of the plant (Cárdenas-Hernández et al., 2009). At T10 treatment, a high biomass reduction (56%) of *J. curcas* has occurred; these values agreed with those reported for *Brassica juncea* (Shiyab et al., 2009) and *Brachiaria dictyoneura* (Morales and Gallego, 2013), which accumulates Hg in their tissues. Finally, a significant reduction in biomass (about 90%) was noticed in the highly contaminated T40 and T80 soils as compared to T0.

Few studies have been conducted with respect to the influence of heavy metals on plant leaf area, specifically referring to Hg, a relation that remains almost constant with respect to biomass (Figs. 2 and 3). The leaf area is a physiological parameter that permits estimating plant response to different stimuli, both biotic and abiotic and it is closely related to the interception of light, photosynthetic capacity, accumulation of dry matter, metabolism, growth, and performance (Severino et al., 2004). It is likely that reduction of leaf area is a strategy that permits the *J. curcas* species to reduce water loss through transpiration due to the inhibition of formation of aquaporins that enable water transport. Heavy metals may alter physiological processes of plants and can have negative effects on their growth and development (Cárdenas-Hernández et al., 2009). Mercury reduces biomass and leaf area and also affects photosynthesis (Fig. 4). Some studies have shown that light and dark photosynthesis reactions are altered (Patra et al., 2004). Reduction in CO_2 assimilation when increasing Hg concentration where *J. curcas* plants grow may be because the metal inhibits other processes subsequent to photosynthesis, affecting various processes of the root metabolism, causing inhibition in water and nutrient intake (Tamás et al., 2008). Consequently, photosynthesis reduction is related to the physiological processes that determine hydric regulation of the plant (Santala and Ryser, 2009), given that aquaporins are inhibited. In fact, aquaporins not only control water transport from the roots to the leaves in the transpiration stream (Cárdenas-Hernández et al., 2009), but also regulate other processes like transport of assimilates in the phloem, opening and closing of stomata on leaves, leaf movement, and control of cytoplasmic homeostasis (Chaumont et al., 2005). It is possible that the *J. curcas* species avoids water loss in vapor form by reducing the entrance of the CO_2 molecule upon limiting stomata opening, that directly affects the plant growth.

4. Conclusions

The present study shows that when Hg is completely available (as in our hydroponic experiments), it is mainly retained in the roots of *J. curcas*, with levels in the non-aerial part of the plant around 7 and 12-fold higher in average than in leaves and shoots, respectively. In fact, absorption of Hg in both roots and shoots of plants increased linearly with respect to the Hg concentrations in different hydroponic treatments, showing statistically significant differences ($p < 0.05$). Furthermore, biomass, leaf area and Pn area also affected at low Hg doses. In sum, the results show that Hg affects significantly the growth of *J. curcas* when Hg concentrations increase in the hydroponic culture. Hence, results suggested that the species can grow normally, without demonstrating low performance at the lowest Hg concentrations (*i.e.*, T5: 5 μg Hg/mL), given that their growth is affected above such concentration. The results of this study expand our knowledge of the Hg tolerance and absorption capacity of this plant, but further research is needed to verify whether the present results are maintained under different field conditions, especially in soils affected by artisanal and small-scale gold mining.

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