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Effect of lead bioconcentration on leaf morphology and histology of aquatic macrophyte *Hydrocleys nymphoides* (*Willd.*)

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ABSTRACT: Health and environmental problems due to contamination are global concerns. Phytoremediation - a biological and sustainable technique - employs plant species to remove, slow down, integrate or decompose certain contaminants to purify soil and water. Our study consisted in contaminating specimens of aquatic macrophyte *Hydrocleys nymphoides* (Willd.) in the laboratory to test their effectiveness in absorbing and interacting with lead. We analyzed different histological planes of the section and characterized the morphological development of this species. We cultivated 30 plant specimens and 10 control specimens in hydroponic lead acetate solutions at two different concentrations each, 2 and 4 $mg.L^{-1}$. Statistical analysis revealed that even after contact with lead, specimens grew normally, increasing the number of leaves and flowers. They also presented greater stomatal development and spongy parenchyma thickening, corroborating that this macrophyte can effectively decontaminate water bodies contaminated with lead, constituting a low-cost and ecological alternative.

Keywords: Decontamination. Phytoremediation. Potentially toxic metals.

INTRODUCTION

Large industrial production generates huge amounts of waste every year (Sanchez, 2020). Potentially toxic metals are among the most harmful residues discarded in natural environments, such as soil and surface waters. These materials can bioaccumulate in living organisms, leading to several health issues (Shah *et al*., 2021).

As a consequence, many mechanisms and methodologies have been developed over the years to minimize the effects of such hazardous waste on its surroundings, promising a sustainable pathway towards better environmental quality, and an equilibrium between man and

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the environment. Phytoremediation employs trees, shrubs, creeping and aquatic plants and their microbiota to remove, degrade or isolate toxic substances from the environment (Lopes; Duarte, 2017). Pandey and Bajpai (2019) describe phytoremediation as an ecologically viable solution, since this technique uses accessible plants, making it a low-cost and effective tool to slow down the effects of contamination on soil and water.

Phytoremediation (phyto= plant; remediation= treatment) is a sustainable technique that applies elements of the local flora to treat and purify contaminated environments developed in 1991 (Hernández-Valencia *et al.,* 2017).

Lacerda *et al*. (2021), Lazaro (2020), Silva (2019), and Mota (2021) demonstrated that this technique yielded satisfactory results and proved that several plant species of high genetic potential can extract, stabilize or immobilize a series of contaminants of environmental concern, such as potentially toxic metals, oil, and domestic and industrial sewage. These authors stated that these plants can proactively absorb highly toxic elements, degrading, metabolizing, or slowing down substances threatening the environment and health of living beings. Once the plant extracts pollutants from the soil, it stores them within its structure. These substances are then transported from the roots to the leaves for subsequent treatment, where the plant metabolizes these pollutants, transforming them into less toxic or even inactive products (Souza *et al*., 2021; Henry-Silva; Camargo, 2018; Morita; Moreno, 2022).

1.1 MACROPHYTES IN THE TREATMENT OF POLLUTANTS

Aquatic vegetation performs essential functions for water resources equilibrium. Aquatic macrophytes contribute expressively to ecosystem biodiversity, especially the food chain, as they can create habitats for various aquatic organisms. Due to their adaptability, they act as protagonists in ecosystem colonization. They also play an expressive role in the nutrient cycle, oxygen balance, and water purification, absorbing excess nutrients such as nitrogen and phosphorus. They can survive in water or moist soil, developing quickly in lakes and watercourses (Haroon *et al*., 2021; Henry-Silva; Camargo, 2018).

They are considered invasive plants and compete directly with aquatic and photosynthetic microorganisms for sunlight and nutrients. They release volatile defenses that inhibit the growth of such organisms in aquatic environments. Aquatic macrophytes are divided into three main classes, according to the interaction between plant structure and water surface: submerged, floating, and emergent macrophytes (Xavier *et al.,* 2021).

Submerged aquatic macrophytes include plants living underwater. They are usually rooted in the bottom soil with the vegetative parts predominantly submerged. However, some species are submerged plants with floating leaves. Floating macrophytes include plants growing in the aquatic environment with roots in water and floating leaves. A common floating species is *Eichhornia crassipes*, popularly known as water hyacinth. Such species are not affected by changes in water levels, depth, or substrate properties (Adeniji, 1979; Xavier *et al*., 2021). On the other hand, the emergent macrophytes are rooted in sediments beneath the water with petiole and leaves in the air, above the water surface. These plants do not depend on water for support and present a high growth rate and biomass production (Symmoens *et al*., 1981; Xavier *et al*., 2021).

The water poppy belongs to *Alismataceae* family, *Hydrocleys genus* (Olson, 2009). This species presents fundamental phytoremediation properties: great reproduction of new specimens and growth rate. The water poppy *(Hydrocleys nymphoides*), like water hyacinth, is also considered an invasive plant. This species floats on heart-shaped fleshy leaves connected to the roots by long petioles. Depending on where it grows, the water poppy can present lemon-yellow flowers with 3 petals and a brownish-red center, besides numerous stems in black or brown with 3-7 cm. Its flowers open two hours after dawn and last one day (Canalli, 2017; Nxumalo, 2016).

Aquatic macrophytes are representatives of the kingdom *Plantae* that can extract and/or absorb organic, inorganic, and metal compounds in water bodies. They can efficiently treat several pollutants in the aquatic environment, absorbing compounds at various concentrations in their plant tissues (Rezania *et al*., 2015; Smith, 1979).

Metals interact with plant species in different ways. They can affect the development or the photosynthetic process — possibly due to changes in cell membrane permeability — disturb enzymatic activity and oxidative stress, induce an increase in stomata number, and darken the root system (*Steliga; Kluk*, 2020; Pereira *et al*., 2010).

Several factors may explain the mechanisms of interaction between a plant and a particular contaminant, including organ development, color, and growth inhibition (Munsell, 1994).

This study aimed to describe how Hydrocleys nymphoides acts as a bioindicator intervening in environmental problems, specifically bodies of water contaminated by lead, in order to use the macrophyte for phytoremediation. This heavy metal offers several risks to human health, mainly in the interaction of trophic chain.

2 MATERIAL AND METHODS

2.1 COLLECTING AND CULTIVATING PLANT SPECIMENS

The 30 aquatic plant specimens were collected at São João river, near the MA-201, in Rio São João, São José de Ribamar, Maranhão, Brazil (2°33'19.39" S44°8'7.88"W) (Figure 1). We collected leaves, flowers, and roots.

Figure 1. São João river, Source: Google Maps, 2022.

The initial plant cultivation was carried out in the laboratory of the Department of Chemistry of the Federal Institute of Education, Science and Technology of Maranhão (IFMA), Campus São Luís - Monte Castelo, Brazil. The specimens were treated and grown hydroponically — soil-free cultivation technique, where the roots receive a balanced nutrient solution, with tap water and all the essential nutrients for plant development provided by the Microbe Lift, BLOOM & GROW ALL-IN-ONE.

The hydroponic cultivation site was set in a greenhouse exposed to sunlight, covered with sombrite 70%, built with PVC pipes and plastic shelves, with 2 meters high, 1 meter wide with 1 meter radius, in IFMA *Campus* São Luís - Monte Castelo.

2.2 MORPHOHISTOLOGICAL ANALYSIS

For morphological analysis, we used one leaf from each of the 10 specimens. all collected from the 4th node. Paradermal and transverse cross-sections measures were obtained by hand using a razor blade. The sample slides were prepared with glycerine and analyzed under a transmission light microscope, Zeiss AXIO Scope A1. The slides were photographed considering the 40x objective and 10x eyepiece (400x magnification). The results were submitted to variance analysis for statistical tests.

The morphological analysis consisted in observing and quantifying the dimensions (length and width) of each leaf and the petiole length. We also looked for possible signs of toxicity on plants in contact with lead compared with control specimens. We tested two different lead acetate concentrations: C1 (10 specimens exposed to 2 mg.L^{-1} of lead acetate) and C2 (10 specimens exposed to lead acetate 4 mg.L^{-1}). The control group (C) consisted of 10 specimens without contact with the contaminant.

2.3 PREPARING THE LEAD SOLUTION

Lead solutions were prepared at 2.0 and 4.0 mg.L^{-1} (C1 and C2, respectively) from the dilution of a 500 mg.L^{-1} stock solution prepared from lead acetate salt 99.99% pure (ISOFAR®). We prepared 3L of both C1 and C2. A control group (C) was prepared with ten plant specimens, totaling 30 experimental units.

The solutions were stored in polyethylene basins with 8L capacity, identified by concentration (C, C1, and C2). The plants were exposed to lead for 15 days with constant pH monitoring, using pH-indicator strips of McolorpHast.

2.1.1 Waste treatment

The plant specimens were washed two times with hot water at approximately 100ºC and the residues of the lead solution were treated by precipitation. Lead was precipitated as PbCl2, and separate for discard.

3 RESULTS AND DISCUSSION

3.1 MORPHOLOGY OF *Hydrocleys nymphoides*

All 30 specimens blossomed during lead contamination test (Figure 2). This means both C1 and C2 presented good development after being exposed to lead. Good flower development means good plant health and good interaction with the environment, as the plant is able to survive in an environment in contact with lead where other plants would possibly not be able to due to its toxic effects (Rodrigues *et al.,* 2016).

Figure 2. Macrophyte *Hydrocleys nymphoides* flower development in a hydroponic greenhouse in São Luís, MA

Although we observed signs of toxicity in C1 and C2, such as changes in the leaves, petiole, and roots, the specimens presented good development, as shown in Table 1. Usually, the leaves are more exposed to air and therefore, more sensitive to changes. Changes in environmental conditions, such as stress caused by exposure to potentially toxic metals (Pb, Cd, Cu & Zn), result in leaf morphological and anatomical responses (Li *et al*., 2007; Shi; Cai, 2009).

Roots of the macrophyte Hydrocleys nymphoides in contact with lead kept a uniform appearance of tissue, color and measures. Alterations are common among species sensitive to these pollutants, and this result reveals a good lead tolerance. Vaculík *et al*. (2012) stated that normally, roots are the first plant organs affected by potentially toxic metals contamination.

The specimens developed well and the leaves, roots, and petiole remained in good condition throughout their life cycle. Our findings contrast the results by Wolff *et al*. (2009) — who analyzed the morphology of macrophyte Salvinia auriculata and observed lesions and marginal necrosis in leaves exposed to zinc at concentrations of 7,5 mg.L^{-1} and 10 mg.L^{-1} — and Zhou *et al.* (2020), who tested E. crassipes to treat lead but observed expressive leaf damage. Water pH also contributes to a regular behavior and development of the macrophytes. Values between 5 - 6 promote an ideal environment for good development. Plants are considered tolerant to potentially toxic metals when they are able to survive in environments with high concentrations of these elements (Rodrigues *et al*., 2016), such as the macrophyte *Hydrocleys nymphoides*. Statistical analysis showed no significant differences ($p>0.05$)

between the average dimensions of the control group and C1 and C2 groups — exposed to lead acetate. Our findings show this species can tolerate lead contamination and survive with no significant modifications required or damage observed.

Table 1. Morphological measurements: petiole and leaves (cm)

Measurements	C	C1	C2	P-value	MSD	CV%
Petiole length	18,4 a	19.00 a	21.44 a	0.06	1.55	12.13
Leaf width	8.56 a	9.06 a	9.18 a	0.58	2.02	16.47
Leaf length	9.24 a	9.08 a	9.58 a	0.56	9.58	35.45

3.2 *Hydrocleys nymphoides* HISTOLOGY

The results showed a significant difference in the lacunar parenchyma (PL) between the control plants (C) and the lead-contaminated plants (C1 and C2), evidenced by the value ($p< 0.05$) observed in Table 2. The thickening of the spongy parenchyma in lead-contaminated plant individuals (C1 and C2) compared to the control (Figure 3) reveals that the macrophyte *Hydrocleys nymphoides* adapted to the stress caused by the contaminant. Other studies (Costa *et al*., 2009; Souza *et al.,* 2010) also describe the development of adaptation mechanisms when in contact with the contaminant, mostly involving the mesophyll.

Table 2. Tissue properties observed in cross-section

Tissues	C	C1	C2	CV%	MSD	p-value
ADT	7.78 b	10.15 a	11.83 a	11	2.18	0.15
ABT	7.04 b	10.22 a	11.53 a	8.59	1.64	0.13
PPT	63.74 b	63.36 b	66.5 a	0.89	1.153	0.14
LPT	153.06 b	250.66 a	281.5 a	8.7	39.77	0.02

ADT = adaxial epidermis thickness, ABT = abaxial epidermis thickness, PPT = palisade parenchyma thickness, LPT = lacunose parenchyma thickness.

Many phytoremediation studies investigating plant tolerance and behavior when contaminated by potentially toxic metals have described anatomical changes according to contaminant concentration and plant stress (Da Paz Schiller *et al.,* 2018; Morita; Moreno, 2022; Lazaro, 2020).

Pereira *et al*. (2011) also observed spongy parenchyma thickening in macrophyte *Eichhornia crassipes*, when in contact with arsenic. This mechanism promotes the formation of intercellular spaces to retain carbon dioxide. The thicker the spongy parenchyma, the more CO_2 is retained (Castro *et al.,* 2009). Enhanced CO_2 flow improves macrophytes photosynthesis, endorsing plant reproduction (Taiz *et al*., 2017)

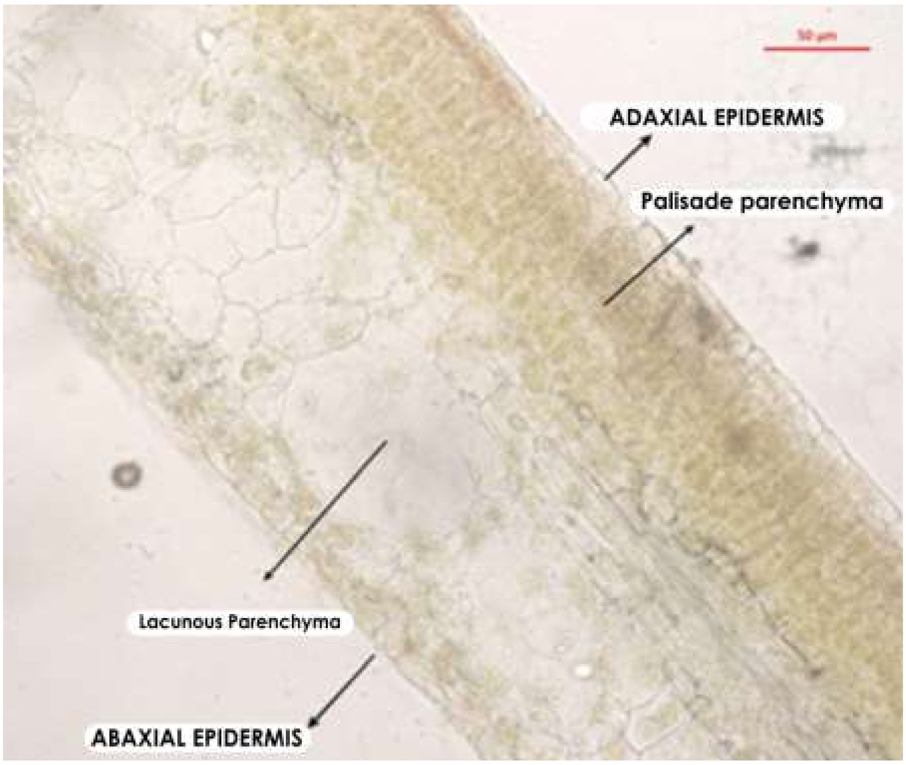

Figure 3. Cross-section of macrophyte tissues seen under a microscope.

Table 2 reveals significant differences between the controls and C1 and C2. However, statistical analysis ($p>0.05$) suggests a significant level of lead tolerance. Pereira *et al.* (2014) stated that the lack of major changes in leaf epidermis and palisade parenchyma of both contaminated plants (C1 and C2) indicates good leaf tissue development without high levels of lead toxicity, like no color changes and no tissue damage. Showing the same appearance as the control plants (C).

Macrophyte anatomy alterations suggest a significant interaction between plant and contaminant and possible stress in plant mechanism.

Statistical analysis showed no significant differences ($p >0.05$) between contaminated plants and the control for almost all average stomatal dimensions. The exception was the abaxial stomata width ($p<0.05$). However, the average dimensions shown in Table 3 reveal that the plants at C1 and C2 concentrations presented higher values than the control specimens (C), which may be due to the macrophyte tolerance to the contaminant.

The best abaxial development observed at C2 concentration (Table 3) may be associated with high CO_2 absorption and retention. This mechanism improves the development of plant specimens and represents a high tolerance to lead contamination (Pereira, 2010; Fahn, 1990; Rogiers; Hardie; Smith, 2011).

Table 3. Stomatal properties: average dimensions of the abaxial epidermis (µm)

Measurements	C	C1	C2	P-value	CV%	MSD
Width	9.29 b	9.66 b	15.46 a	0.01	11.86	1.25
Length	18.67 b	19.33 b	21.78 a	0.07	9.68	1.77

Lira (2020) observed that the leaf epidermis presented smaller stomata at lower lead concentrations. As lead levels increase, so do the stomata. Lead can reduce CO_2 absorption, altering the plant mechanism by reducing the stomata. Therefore, our results are associated with *Hydrocleys nymphoides* response to high lead concentrations. The plants changed their stomata to increase CO_2 efficiency (Schlegel; Godbold; Hüttermann, 1987; Ekmekci *et al*., 2009).

The absence of significant modifications in leaf epidermis and palisade parenchyma in lead presence resulted in good leaf tissue development without evidence of toxicity. This corroborates the understanding of macrophytes interaction capacity in contact with the contaminant.

Table 4. Stomatal properties: average dimensions of the adaxial epidermis (µm)

Measurements	C	C1	C2	P-value	CV%	MSD
Width	10.57 b	6.44 c	13.72 a	0.057	10.85	1.01
Length	20.54 a	17.19 b	20.30 a	0.123	9.18	1.39

4 FINAL CONSIDERATIONS

The present study shows the effects of lead bioaccumulation on the macrophyte *Hydrocleys nymphoides* grown in a hydroponic solution that can be used as a low-cost decontamination of water bodies. The plant exhibited good resistance and anatomical aspect during contact with lead. This species was able to grow, increasing the number of leaves and flowers, and also inhibiting signs of toxicity in the roots. Histological analysis revealed that these plants adapt their structure when exposed to lead. Thickening of the spongy parenchyma and increased stomata number evidence the interaction plant-metal and the great resistance to lead observed for this species.

Results showed that the macrophyte *Hydrocleys nymphoides* acts as a bioindicator of sites contaminated by metals, reducing lead contamination levels by bioaccumulation. The great resistance of this species to potentially toxic metals makes it a sustainable and economically feasible alternative to treating water bodies contaminated by lead.

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