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Water Hyacinth: a Possible Indicator of Lead in Water Bodies

Jacinto D'água: um Possível Indicador de Chumbo em Corpos Aquáticos

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Abstract

During recent years, a great emphasis has focused on developing sensitive biomarkers as a useful tool for environmental studies. Studies have documented that several contaminants, both metal and organic compounds, are able to induce specific alterations in certain enzymes which are involved in cellular replication and generation perceptible in optical microscopy. These markers must be easy to handle and the evaluation technique must be cheap and quick. It is preferable that the markers can be used directly in the water body and that it can be maintained for a long time in the system. This work verified the hypothesis that *Echornia crassipes* could be used as a biomarker for lead fluctuations in water bodies. *E. crassipes* was grown in six different concentrations of lead (0.03; 0.05; 0.10; 0.20; 0.25 and 0.30 mg/L^{-1} Pb) and after 30 days' cultivation, the coif and the root's primary meristem were analyzed for chromosomal and structural abnormalities. Two hypotheses were raised and tested for the observed correlation between oxalate and metal concentration. A positive correlation was obtained between the abnormalities observed and the fluctuation in the metal concentration. An increase in the concentration of calcium oxalate raphids was also obtained, showing a positive correlation with the concentration of lead.

Keywords: Bioindicator. Heavy Metals. Pollution.

Resumo

Nos últimos anos, uma grande ênfase se concentrou no desenvolvimento de biomarcadores sensíveis como uma ferramenta útil para estudos ambientais. Estudos documentaram que vários contaminantes, compostos metálicos e orgânicos, são capazes de induzir alterações específicas em certas enzimas envolvidas na replicação e geração de células perceptíveis em microscopia óptica. Estes marcadores devem ser de fácil manejo e a técnica de avaliação deve ser barata e rápida. É preferível que os marcadores possam ser usados diretamente no corpo hídrico e que possa ser mantido por um longo período no sistema. Este trabalho verificou a hipótese de que o Echornia crassipes possa ser usado como biomarcador para flutuações de chumbo em corpos hídricos. O E. crassipes foi cultivado em seis concentrações diferentes de chumbo (0,03; 0,05; 0,10; 0,20; 0,25 e 0,30 mg/L^{-1} *Pb) e após cultivo por 30 dias, a coifa e o meristema primário da raiz foram analisados quanto as anormalidades cromossômicas e estruturais. Foram levantadas e testadas duas hipóteses para a correlação observada entre o oxalato e a concentração do metal. Obteve-se uma correlação positiva entre as anormalidades observadas e a flutuação na concentração do metal. Obteve-se ainda um aumento na concentração de ráfides de oxalato de cálcio, apresentando correlação positiva com a concentração de chumbo.*

Palavras-chave*: Bioindicador. Metais Pesados. Poluição.*

1 Introduction

Lead, a very toxic metal, is widely used in several industrial processes, and could be responsible for the death and /or lethal changes in the reproduction, growth and behavior of several species. Lead can be described as the most widespread toxic metal around the world. Naturally, many scientific papers have reported high levels of this pollutant metal. Sun *et al.* (2010), in his research on the western Hunan Province in China, found high levels of lead in surface water and in the soil used on agriculture and, consequently, the metal was found in large quantities in rice, the basis of the local food. In Europe, 27 urban topsoil samples (garden and lawn) were collected and analyzed in order to assess the metal concentration, and it was discovered that there was bioavailability in 67% of analyzed locations (ROUSSEL *et al.,* 2010). Equal contamination has been reported worldwide, Yugoslavia (DJURIĆ *et al.,* 2013); Mediterranean (BECHA, 2012) and Brazil (MACHADO *et al.,* 2013) are examples of these studies.

However, lead (Pb) contamination sources are often difficult to identify, because they depend on specialized and costly equipment (TAGAHU *et al.,* 2011). These restrictions and the necessity to measure contamination from lead and other toxic metals has led to a growing interest on developing sensitive bioindicators, such as plants (TAGAHU *et al.,* 2011), fishes (PEAKALL *et al.,* 2013), moss (STAFILOV, 2016) and even invertebrates (ARU *et al.,* 2016).

Studies have documented that several contaminants, being both from metal and organic compounds, are able to induce specific alterations in certain enzymes, which are directly involved in cellular replication and perceptible generation in optical microscopy (MALAR *et al.,* 2014).

The integrity and functioning of the DNA has been investigated in many organisms under different conditions of contamination (ZHOU *et al.,* 2016). The use of biomarkers as a measureable biological response to the contamination of toxic metals is very important to simplify and reduce costs of toxicological monitoring, even in aquatic environments (ERK *et al.,* 2016).

Amongst the water environments bioindicators, aquatic macrophytes are the best signs of contamination, due its dependency on this kind of environment as well as its sessile life, which can easily be monitored (EL-KHATIBA, 2013).

Khan *et al.* (2009) attested to the *Eichhornia crassipes* ability in accumulating metals, in his work with lead in wetlands for the removal of metals from industrial effluents. Chigbo *et al.* (1982) also analyzed the *Eichhornia crassipes* as a pollution monitor for the simultaneous accumulation of arsenic, cadmium, lead and mercury. The ratio of arsenic and mercury concentrations in the leaves and stems was 2:1. Cadmium and lead showed a concentration ratio in leaves and stems around 1:1.

2 Material and Methods

2.1 Specimens used in the experiment

The plants used in this study were manually collected in a protected area, washed and maintained in reservoirs with 50 L of Clark Solution (CLARK, 1975) for clones' production, which were used in the experiments.

2.2 Experimental design

We chose an experimental design with six runs assembled in triplicate, totaling 18 reservoirs, being that each one received a volume of 30 L of solution with the lead acetate $Pb{\cdot}(C_2H_3O_2)_2$ prepared in deionized water. A control group was also mounted in triplicate.

For the study we used the concentrations of 0.03; 0.05; 0.25 and 0.30 mgL^{-1} Pb, with the intention of keeping the plants within the concentration range which allow them to survive.

2.3 Cultivation conditions

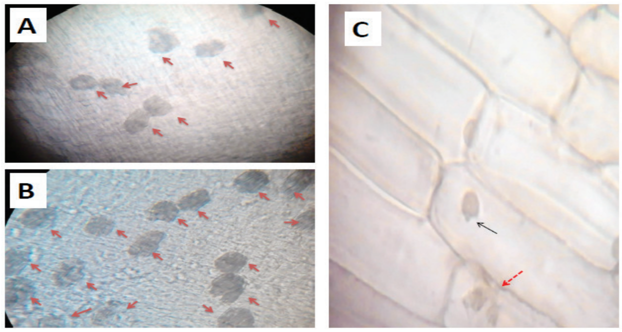
The experiment was conducted outdoors, in a period of 30 days, in two moments: during the summer period and during the winter period. There is no control of climatic factors in order to get the experiment as close to the reality as possible.

2.4 Cellular abnormalities

It was made the counting of the micronuclei in an optical microscopy (400x increase), using the coif of meristem and the meristem, colored with a Propionic carmine. (Figure 1C).

The pictures were taken with the Samsung DV180 16.2 MP 5x Optical Zoom digital camera coupled to the ocular microscope.

Figure 1 - Coif in random microscopic fields. A: treatment sample with 0.25 mg L^{-1} Pb (100 x increase). B: sample of 0.3 mg L^{-1} Pb (100x increase); calcium oxalate raphids are arrows in "A" and "B"; The micronucleus is represented by the black arrow in "C" (400x increase), and the dotted arrow are degenerate nucleus



Source: Research data.

In order to quantify the calcium oxalate crystals, the coif was observed in three random microscopic fields, with three repetitions, 100x increase (Figure 1, A and B).

For the quantification of the nuclear abnormalities, it was counted 50 cells (400x increase, Figure 01-C) in three random replications.

2.5 Scanning electron microscope images

For image acquisition, we used a scanning electron microscope (SEM) model TM3000, which presented: 15kV with an operating beam, tungsten filament supply, backscattered electron detector with vacuum chamber and charge reduction attached to an energy dispersive spectrometer (EDS) SwiftED3000 with silicon detector SDD 30 mm^2 and 161 eV resolution (Cu-Kα) and multichannel analyzer 2048 channels (10 eV/ channel), both of the Hitachi brand.

2.6 Fourier transform infrared spectroscopy methodology

The Fourier transform infrared spectroscopy (FTIR) spectrum was obtained with a FTIR SPECTRUM 2000 PERKIN-ELMER spectrometer. The transmission of Mid Infrared (MIR) spectra was obtained with the resolution 4 cm^{-1}, gain 1, spectral range 4000 to 400 cm^{-1} and 40 scans. The Photoacoustic Spectroscopy (PAS) spectra was obtained with the accessory PAS MTEC Model 300, as follows: purging with helium gas, spectral region 4000-400 cm^{-1} resolution 4, gain 7 on the amplifier, speed 0.05 cm/s, 32 scans.

The samples coifs and root ends were prepared for obtaining the spectrum MIR transmission 14 as KBr pellets (1: 400 mg).

2.7 Statistical analysis

The data was analyzed using the software XLSTAT 2015 for the Pearson correlation table and the Principal Component Analysis.

3 Results and Discussion

The data demonstrated that the main abnormalities observed in the *E. crassipes* coif tissue, which were exposed to lead, were the presence of calcium oxalate shaped in raphids, micronuclei, degenerate nucleus, unreduced nuclei and the presence of anucleate cells (Figure 2). There was also a positive correlation between the concentration of lead and the abnormalities. This correlation is shown in Figure 3.

Figure 2 - Spider plot of abnormalities observed in *Eichhornia crassipes* treated with Pb. A: Experiment realized in summer, May/2013; B: Experiment realized in winter, Feb/2014

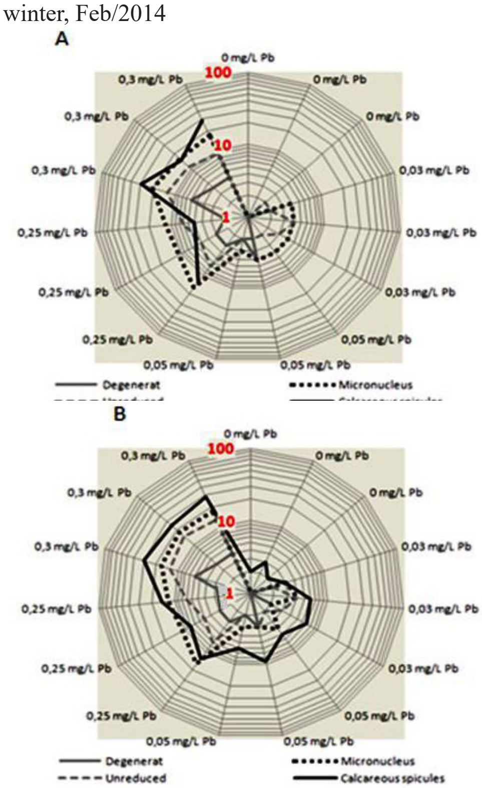


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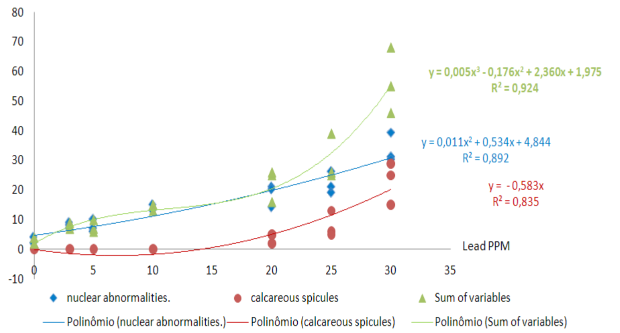
Figure 3 - Correlation observed between abnormalities observed in *Eichhornia crassipes* treated with Pb



Source: Research data.

The Pearson correlation revealed that micronuclei and unreduced nuclei have a high correlation (0.92), and micronucleus and degenerated nucleus have a correlation of 0.80, allowing to group them in the analysis as a single factor, here called as nuclear abnormalities.

Shahid *et al.* (2011) used *Vicia faba* in a genotoxicity test, this was performed in order to assess the lead contaminated soil. Seedlings of *V. faba* were exposed for 6 h in controlled hydroponic conditions for 5 µM of lead nitrate, it resulted in a weak correlation between the total lead concentration absorbed by the roots and the genotoxicity (R^2=0.65), which was significantly below the correlation found in our study (R^2 = 0.92).

There are some adaptations present in macrophytes, which can facilitate the entry of heavy metals, such as an increase of the tissue responsible for capturing gases and an improved transpiration ability (MARTINS, 2005; ALI *et al.*, 2019).

The main component analysis revealed that the micronuclei, unreduced nuclei and raphids are the most representative abnormalities (adding up to 87.59%) (Table 1). It also exposed that anucleate cells have a low representation in the sample (2.20%).

Table 1 - Summary for the variables and contributions of variables (%) in the main component analysis

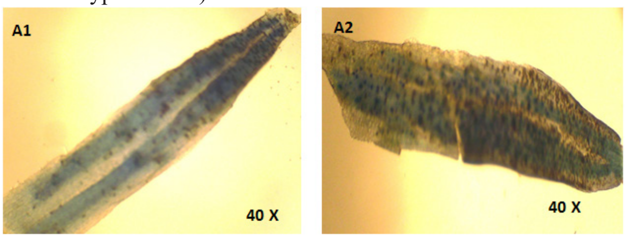
Summary for all the variables					
	Degenerate	Anucleated	Micronucleus	Unreduced	Calcareous spicules
R^2	0,560	0,119	0,904	0,791	0,623
F	24,206	2,560	178,700	72,111	31,433
Pr > F	< 0,0001	0,126	< 0,0001	< 0,0001	< 0,0001
Contributions of variables (%) in Principal component analysis:	20,340	2, 198	26,079	25,060	26,322

Source: Research data.

Kiran and Sahin (2005) report that increased lead concentration reduced the root cell division of *Lens culiaris*, besides producing several mitotic abnormalities such as c-mitosis, lagging chromosomes, multipolar anaphases and chromosome bridges.

Guerra (1988) describes that lagging chromosomes and multipolar anaphases generate the presence of cells with micronuclei. It is also known that irregularities in mitosis generate anucleated and non-reduced cells, as well as observed in our work.

Figure 4 - Optical microscopy of the *E.crassipes* root end submitted to different lead concentrations (A1: 0.05 mg L^{-1} Pb. A2: 0.3 mg L^{-1} Pb). The dark spots correspond to raphids of calcium oxalate (metilene stained blue after treatment with sodium hypochlorite)



Source: Research data.

Anatomically, plants not shown perceptible change to the naked eye, but when analyzing the coif and the roots (Figure 4), it becomes clear the corresponding accumulation and the increased concentration of lead in solution. Statistically, there is a positive correlation ($R^2 = 0.62$) between the calcium oxalate concentration and the Pb levels used in the experiment (Table 1).

Our results are new in the area, and demonstrated an intense accumulation of calcium oxalate, being that the dose is dependent on the lead contamination. As far as we know the approach realized had not yet been made.

In taller plants (vascular), the growth occurs from young tissues in which cell units still do not differ and are called meristems. The process is determined by external factors such as the weather or the availability of nutrients, and by endogenous factors such as the production of plant hormones (e.g., auxin or acid gibberellic) (VALIO, 1979).

Shahida *et al.* (1993) describes that most of the heavy metals are bound to the negative charges of the root cell walls is complexed with phytochelatins (small peptides rich in cysteine) and stored in the vacuoles.

Calcium is absorbed in the divalent form (Ca^{2+}) and has low mobility in plant, thus the absorption region is restricted at the roots ends. The calcium is transported by the xylem, also in a divalent form (KERBAUY, 2004).

One of the functions of calcium is to be a macromolecules structural component, establishing stable, but reversible intermolecular bonds, on the cell wall and plasma membrane. In the middle lamella it binds to polygalacturonic acids R-groups (pectin) into a more or less exchangeable form. With the increase in the supply of calcium for many plant species, the proportion of Ca oxalate also increased. Most of the Ca is located in the vacuoles, in order to maintain the low concentration of Ca, it is compartmentalized in the vacuole in the form of raphids (KERBAUY, 2004).

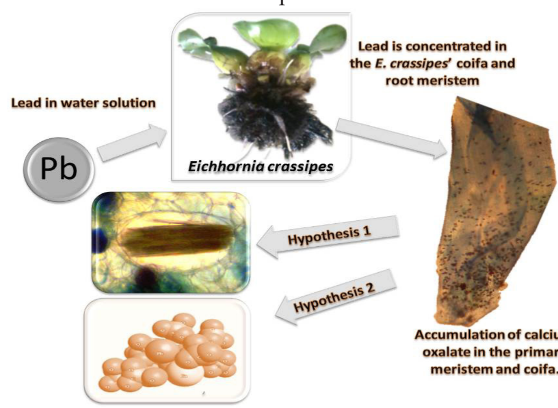
The aquatic plants ability in removing heavy metals in solutions is well documented, and in most cases the metals are concentrated in roots and the translocation process into the aerial parts is usually slow (SHARID *et al.,* 2011; UPADHYAY; PAME, 2019; THANPATTI *et al*., 2020).

Suggested that the coif being the outermost tissue of the plant is in direct contact with the environment, if it is contaminated, their cells will be the first to respond to the pressure from the toxic environment. For this reason, the results were much more expressive in the coif and roots of the *E. crassipes*, not showing modifications in the aerial parts.

The results observed in this work reported the relationship between Pb and Calcium and suggested that the Ca could be acting as chelator of heavy metals in *E. crassipes*. In this way it would become inert in the process and remain accumulated in the vacuoled of the coif cells.

A second hypothesis explaining an observed relationship would be that lead inactivates the proteins responsible for the mobilization of Ca from the vacuole to the xylem, promoting its accumulation in the form of raphids (Figure 5).

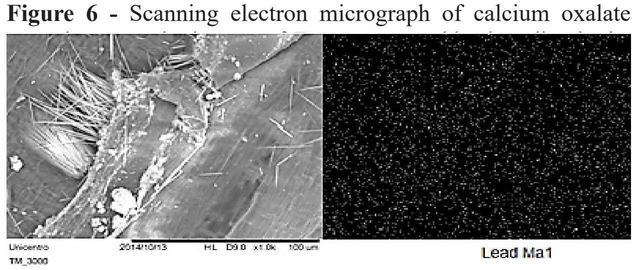
Figure 5 - Hypothetical model to explain the correlation between lead and calcium oxalate crystals in plant. A - the lead dissolved in water; B - *E.crassipes* absorbs lead through roots; C - Lead interferes with replicating cells, on the coif and in the primary meristem, and these abnormalities accumulate calcium oxalate crystals above the common; D - hypotheses 1: the lead binds to calcium oxalate and makes it accumulate in the cells of the coif; E - says that lead inactivates the proteins responsible for the mobilization of Ca from the vacuole to the xylem, promoting its accumulation in the form of raphids



Source: Research data.

The hypotheses were tested through scanning electron microscopy (Figure 6) and also mapped the presence of Pb in the calcium oxalate samples, our results showed no presence of Pb in crystals, but detected lead scattered on the sample, these results suggest that Pb was not accumulating in the calcium oxalate but in the proteins responsible for its metabolism.

Figure 6 - Scanning electron micrograph of calcium oxalate



Source: Research data.

4 Conclusion

It was possible to check a positive correlation between the amount of lead acetate present in an aquatic environment and the amount of *raphids* and nuclear abnormalities observed in the coif and in the meristem of the *E. crassipes'* roots. The presence of micronucleus, degenerate cell, anucleated cell and cell with reduced nucleus can be used as indicators of lead contamination. The presence of *raphids* was the most relevant factor in *Eichhornia crassipes* submitted to concentrations of 0.03; 0.05; 0.25 and 0.30 mgL^{-1} lead. It can be inferred that lead, at the tested concentrations, inactivated the proteins responsible for the metabolism of calcium oxalate, causing its accumulation in the meristematic tissues of the roots and coif.

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