

THE USE OF THE AQUATIC PLANT *LEMNA GIBBA* IN WATER – TOXICITY CONTROL: INVESTIGATION OF CADMIUM EFFECTS THROUGH CHEMICAL MODIFICATIONS IN AQUATIC SYSTEM WITH PLANT

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Abstract:

The biological monitoring was developed in order to predict the toxicity of chemicals and to protect and preserve the biological integrity of natural systems.

Among species laboratory tests, aquatic plants have been used as tool for monitoring particularly metal contamination in aquatic ecosystem.

The objective of this laboratory study was to examine the response of an aquatic plant, *Lemna gibba*, to simulated cadmium discharges. The principal purpose of this study was to present the composition of nitrogen and phosphate in uncontaminated and contaminated growth medium of *Lemna gibba* and to present results that demonstrate the relationship of these nutrients to the concentration of cadmium adsorbed by *Lemna gibba*.

1. INTRODUCTION

Aquatic plants have been used as a tool for monitoring particularly metal water pollution (Guilizzoni, 1991; Mouvet, 1984). Significant correlations between metal in aquatic plants and aqueous concentrations were observed in field studies (Samecka-Cymerman & Kempers, 1996; Manny et al., 1991; Pip, 1990; Sprenger & McIntosh, 1989) and laboratory tests (Miretzky et al., 2004; Claveri et al., 1994). The final metal accumulated in plant is usually significantly larger than in the aqueous concentrations and this fact led investigators to be interested in their use as an indicator of the environment toxicity (Mal et al., 2002; Phillips, 1994; St-Cyr et al., 1994; Van Steveninck et al., 1992), in the transport of metals in macrophytes (Tripathi et al., 1995; Karez et al., 1990) and in their role in the biochemical cycles (Sinha et al., 2005; Jackson et al., 1994).

Among several species utilized for control of pollutant toxicity, *Lemna gibba* was used to study the effect of the herbicide glufosinate on the production of ammonia in this plant, which caused strong ammonia accumulation and induced a metabolic breakdown of the plant (Trogisch et al., 1989). This ammonia accumulation induced inhibition of nitrate uptake and its assimilation in a variety of aquatic plant (Guerrero et al., 1981).

In another context, investigations were described that in vitro and field conditions, product of aquatic plants, such as duckweed, to recover nutrients from the wastewater has promise as an alternative technology to convert nutrients into potentially useful products and prevent excessive nutrients of the aquatic environment (Cheng et al., 2002).

In ecotoxicological field, it is evident that in aquatic organisms, the sensitivity to heavy metals is related to the biochemical metal activity included as well, metal induced on nitrogen mechanism. Therefore, the objective of this laboratory study was to examine the effect of cadmium on nitrogen and phosphate contained in the medium of *lemna gibba*.

2. MATERIEL AND METHODS

Lemna gibba was cultured under sterilized conditions in a medium containing: 1.18 g.L⁻¹ Ca(NO₃)₂ 4H₂O; 0.05 g.L⁻¹ KNO₃; 0.049 g.L⁻¹ MgSO₄ 7H₂O; 6.8 mg.L⁻¹ KH₂PO₄; 3.037 mg.L⁻¹ FeSO₄ 7H₂O; 2.86 mg.L⁻¹ H₃BO₄; mg.L⁻¹; 1.55 mg.L⁻¹ MnSO₄ 7H₂O; mg.L⁻¹ 0.22 mg.L⁻¹ ZnSO₄ 7H₂O; 0.079 mg.L⁻¹ CuSO₄ 5H₂O; 0.078 mg.L⁻¹ NiSO₄ 7H₂O and 0.0179 mg.L⁻¹ Na₂WO₄ 2H₂O; pH = 5 ± 0.5.

The renewal of the medium plants was realized at each 10 days periods. Plants were visually followed during the culture for any decomposition products. Cultures and cadmium experiments were illuminated 16 h per day at 24 ± 2°C in thermostatically controlled growth chamber.

Plants were washed prior the incubations using diluted sodium hypochlorite solution to remove the possibility of the micro algae development. In each experiment, growth of new fronds of *Lemna gibba* were examined and ammonia-nitrogen, nitrite, nitrate, alkalinity, calcium and magnesium in the growth media were assayed according experiment durations: 2, 4, 6, 8 and 10 days.

In the experiment involving cadmium, the plants were exposed to concentrations of cadmium: 10⁻³; 10⁻² and 10⁻¹ mg.L⁻¹ for experiment duration of 10 days.

Statistical significance for all experiments was assessed by the analyse one-way ANOVA using analysis of variance. A statistically significant difference in nitrogen concentrations was reported for p values less than 0.05. Duncan's mutiple-range test was used to evaluate the mean difference among individual groups at a 0.05 significance level.

3. RESULTS AND DICUSSION

3.1 Nitrate quantification

Initial laboratory test showed (table 1) a decrease in ammonia-nitrogen concentrations after 10 days cadmium-experiments using plant cultured in sterilized medium but not washed by sodium hypochlorite and ethanol solutions prior its culture. The development and growth of new fronds were completely suppressed by 10⁻¹ mg.L⁻¹ or more of cadmium. It is well known (table 1) that by inhibition of growth, cadmium caused a significant (p = 0.036 < 0.05) reduction of ammonia present in the culture medium which reached respectively: (42.44; 69.50 and 74.41)% with 10⁻³, 10⁻² and 10⁻¹ mg.L⁻¹ Cd. Ammonia-nitrogen accumulated in control medium within 10 days was significantly great relative to the initial in nutrient solutions (N-NH₄ = 0 mg.L⁻¹, see material and methods) and probably not comparable to that in natural complex water composition. The presence of different aquatic micoorganisms in the experimental solutions and principally symbiotic bacteria are confirmed evidence of a great levels of ammonium which is obviously one of the final bacteria metabolism principally of excretion. The decrease of ammonium in the water is attributed solely to an interaction of cadmium with pathways for the ammonia micoorganism production.

Table 1. Inhibition by cadmium of the ammonia-nitrogen contained in incubation medium; experiment without sterilization treatment of plant, each value of ammonia-nitrogen concentrations represents the means ($n = 4$) \pm standard error; experimental period of 10 days

Cd ⁺⁺ (mg.L ⁻¹)	0	10 ⁻³	10 ⁻²	10 ⁻¹
pH ₀	5.59	5.09	5.49	5.0
pH ₁₀	6.22	6.79	6.79	6.53
Inhibition of growth (%)	08.15	42.44	69.50	74.41
N-NH ₄ ⁺ (mg.L ⁻¹)	7.74 \pm 1.2	4.47 \pm 0.9	2.36 ^a \pm 1.8	1.98 ^a \pm 1.0

pH₀ = mean value at time zero

pH₁₀ = mean value at the end of experiment duration 10 days

^(a): mean values of NH₄ in cadmium experiment significantly different from those of the control ($p < 0.05$)

In cadmium experiments using sterilized plant, the aqueous residual nitrate concentrations are illustrated in figure 1.

Nitrate consumption indicates a significant decrease ($p = 0.017 < 0.05$) with increasing cadmium concentration from 0 to 10⁻¹ mg.L⁻¹. Effectively, nitrate remaining increased to 385.17 \pm 22.95 mg.L⁻¹ which was significantly higher ($p < 0.05$) than value for the control, N-NO₃ = 158.83 \pm 32.09 mg.L⁻¹, and parallel, results in table 2 mentioned an obvious correlation between rate inhibition of growth and the reduction of nitrate uptake. But, the range of nitrate concentrations (figure 1) was similar for both cadmium concentrations: 10⁻³ mg.L⁻¹ Cd (N-NO₃ = 269.12 \pm 16.09 mg.L⁻¹) and 10⁻² mg.L⁻¹ Cd (N-NO₃ = 265.80 \pm 24.12 mg.L⁻¹). Influence of cadmium was pronounced with 10⁻¹ mg.L⁻¹ and the metabolic nitrate uptake was clearly affected by its toxicity.

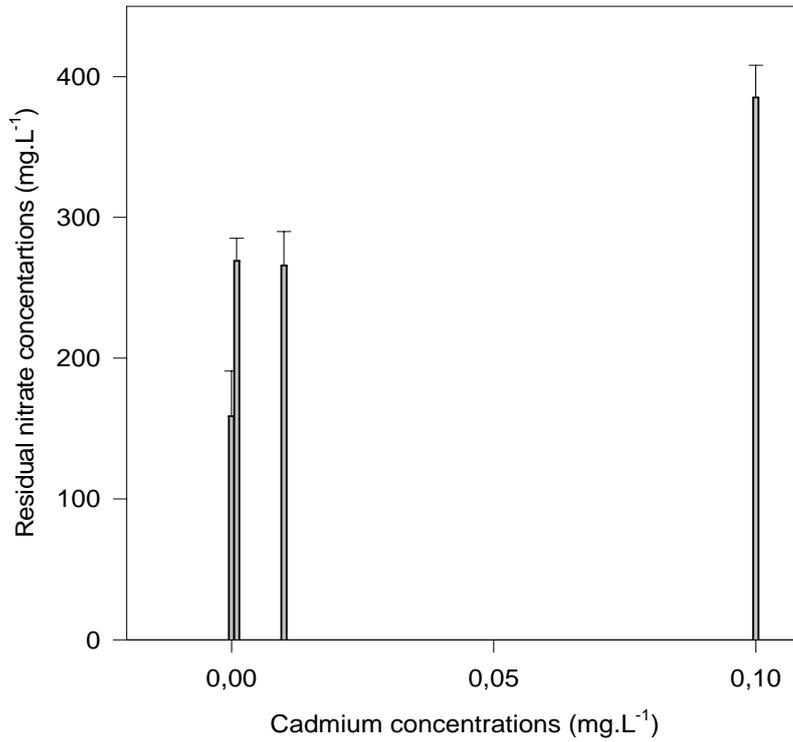


Figure 1 Influence of cadmium on residual nitrate-nitrogen of *Lemna gibba* content medium after 10-days experiments. Experiments with sterilized plant. Mean values \pm standard errors of the means, n = 4

Table 2 Inhibition rate of growth and nitrate uptake within experimental period of 10-days; experiments with sterilized plant

Cadmium concentrations (mg.L ⁻¹)	10 ⁻³	10 ⁻²	10 ⁻¹
Inhibition of growth (%)	40.59	52.77	83.65
Inhibition of nitrate uptake (%)	58.99	61.23	90.26

3.2 Phosphate quantification

Concerning the phosphate quantification (Figures 2,3) show the change of phosphate compound content in the culture medium of *Lemna gibba* exposed to 10^{-3} mg.L⁻¹ (figure 2) and 10^{-2} mg.L⁻¹ of cadmium for 2, 4, 6, 8 and 10 days experiment duration.

It seems that the increasing of phosphate in cadmium experiments is induced by the inhibition of phosphate uptake process. The increase was of 45% and 58% respectively at Cd concentrations of 10^{-3} mg.L⁻¹ (Figure 2) and 10^{-2} mg.L⁻¹ (Figure 3).

The inhibitory effect induce various metabolic in plant and the phosphate absorption is broken. This result suggests that phosphatase may take part in the inhibition of the enzyme activities by cadmium stress and it could not be excluded that other enzyme reactions was also affected such as oxidative processes.

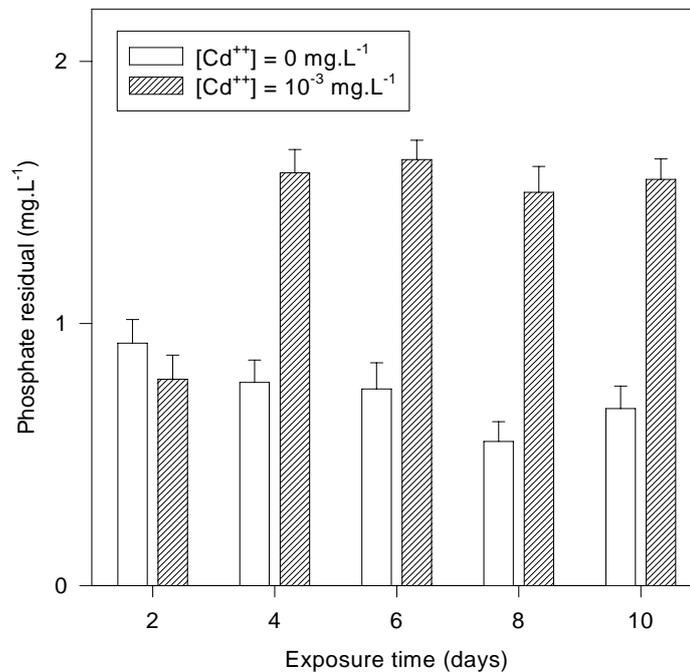


Figure 2 Residual phosphate in the culture medium of *Lemna gibba* exposed to cadmium, Mean values \pm standard error, n = 4

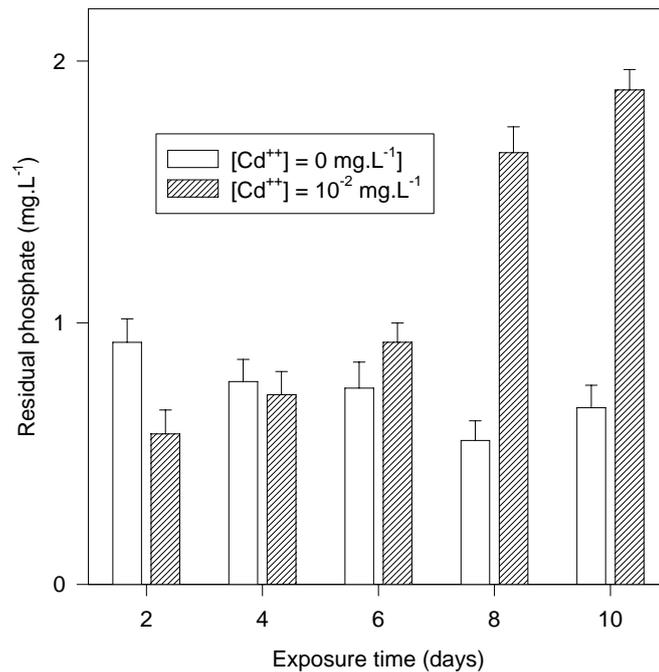


Figure 3 Residual phosphate in the culture medium of *Lemna gibba* exposed to cadmium, Mean values \pm standard error, n = 4

4. Conclusion

- a-** The reduction rate of the nitrate uptake process was dependent of the increasing cadmium concentrations. It's therefore suggested that the decreasing of nitrate concentrations in the growth medium could constitute a tool for detecting water metal contamination,
- b-** The cadmium inhibitory effect induce various metabolic in plant and the phosphate absorption is broken.
- c-** Ammonia in the experimental solutions of bioassays without sterilization of plant results from microorganism's excretion.

5. References

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