

Boron toxicity in *Lemna gibba*

Toxicidad del boro en *Lemna gibba*

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ABSTRACT

Total soluble phenols and total chlorophylls content, changes of biomass and concentration factor in *Lemna gibba* exposed to different concentrations of boron were measured. Day six soluble phenols showed significant differences in treatment with 10 mg/L of boron. At day ten, chlorophylls content in treatment 2 mg/L concentration increased respect to other experimental groups and control group, there were no significant differences. Biomass of *Lemna gibba* decreased significant in treatments with 1.5 and 2.5 mg/L treatments of boron. The highest Bioconcentration factor was found in 0.25 mg/L of boron, this accumulation is not relevant. *Lemna gibba* showed tolerance in the presence of boron in concentrations used here. Suggesting that this aquatic macrophyte could be used for tertiary treatment and the possible renewal wastewater contaminated with boron.

Key words: Boron, chlorophylls, phenols, pollution, *Lemna gibba*.

RESUMEN

Se midieron fenoles solubles totales, contenido de clorofila total, cambios de biomasa y el factor de bioconcentración en *Lemna gibba* expuesta a diferentes concentraciones de boro. En el día seis los fenoles solubles totales mostraron diferencias significativas en el tratamiento con 10 mg/L de boro. Aunque el día 10 el contenido clorofila de la concentración de 2 mg/L se incrementó con respecto al control y a los otros grupos experimentales, no se encontraron diferencias significativas. La biomasa de *Lemna gibba* disminuyó en los tratamientos con 1.5 y 2.5 mg/L de boro. El factor de bioconcentración tuvo un valor máximo en la concentración de 0.25 mg/L de boro, esta acumulación no es relevante. *Lemna gibba* mostró tolerancia a la presencia del boro, en las concentraciones aquí utilizadas, esto sugiere que esta macrofita acuática podría ser usada para el tratamiento terciario y la posible renovación de aguas de desecho contaminadas con boro.

Palabras clave: Boro, clorofilas, fenoles, contaminación, *Lemna gibba*.

INTRODUCTION

Boron occurs naturally in soil and water as mineral salts (Nable, *et al.*, 1997). This element plays an important role in the synthesis of nucleic acids and cell division in all apical meristems. However, various anthropogenic sources of excess Boron may increase soil and wastewater to toxic levels for plants (Woods, 1994). The most important source of Boron is irrigation water. Others include wastes from surface mining, fly ash, and industrial chemicals. Route of entry into the aquatic environments is through weathering of borate-containing rock and release of borates in cleaning products through disposal to wastewater treatment systems (Nable, *et al.*, 1997). Boron is transformed in the environment depending of environmental conditions as pH, moisture level. Then changes in the specific form of Boron and its transport can occur. Natural weathering is expected to be a significant source of environmental Boron (IRIS, 2001).

When toxicants irreversibly alter plant communities, other organisms can be significantly and indirectly impacted by them (Powell *et al.*, 1996). This impact may persist long after the toxicants have dissipated (Sheehan & Locks, 1994; Harwell *et al.*, 1994). It has been observed that prolonged exposure to Boron could be toxic for plants and any living system (Barr *et al.*, 1996; Benderdour *et al.*, 1998).

Boron (B) levels in sewage water from Mexico City have progressively increased. In Valle del Mezquital in Mexico, it has been run a study about soil contamination and they found metals and toxic compounds in vegetables tissues, irrigated with wastewater. Most of compounds were Boron and Iron (Mendez, 1982). This element was selected for this study because, recently Boron levels in Mexico City's wastewater have increased for instance the tolerance limit (1 mg/L for human consumption and 0.75 mg/L for agriculture), it has been exceeded to up 2 mg/L at the "Gran Canal" (the main wastewater collection channel in Mexico City) and 2.96 mg/L at the Central Sewage System (Murray, 1995; ECETC, 1995; Jimenez *et al.*, 1997), and in Mexico, domestic wastewater is often used for irrigation.

Lemna gibba L. (duckweed) is widely distributed in Mexico, it is use as food in aquaculture (Arrivallaga & Arredondo, 1978). Duckweed is an important, fast growing tested organism. It is an aquatic plant and is relevant to many aquatic environments, including lakes, streams, and effluent. Additionally, duckweed is a vascular, flowering plant. It is known that under laboratory conditions its biomass can be doubled at 24-48 h under optimal nutrient supply, appropriate illumination and temperature of 25-29°C (Wang, 1990).

Lemna gibba is tolerant to heavy metals. Like cadmium and lead (Miranda *et al.*, 2000). Its absorption and bioaccumulation abilities could be used for removal of some toxic compounds

(Boyd & Walley 1972; Wang, 1990; Lemnatest, 2000). *Lemna gibba* can act as a pollution indicator, because it can degrade specific organic compounds and it is able to sequester heavy metals or radioactive ions in aqueous solution (Stomp *et al.*, 1993).

Phenols and chlorophylls are widely distributed in plant tissues. One of the main roles of phenolic compounds in plants is defense (Iwanoska *et al.*, 1994; Daniel *et al.*, 1999). Phenolic compounds are produced as a response to the damage caused by a chemical stressor or a pathogen, since they work as a defense mechanism in plant metabolism (Daniel, *et al.*, 1999). Chlorophylls are important for plants metabolism, its degradation is a stress symptom (Powell *et al.*, 1996). Total chlorophylls content and soluble total phenols can be used as parameters of biochemical responses in toxicity tests.

The aim of this study was to determine total soluble phenols and total chlorophylls content, in duckweed exposed to B. Biomass changes and bioconcentration factor of B in *Lemna gibba* were also evaluated.

MATERIALS AND METHODS

Experimental design. *Lemna gibba* plants were obtained from Xochimilco's channels of water by (Caltongo's neighborhood in the southeast of Mexico City). Plants were rinsed with distilled water and cultured in polypropylene containers (30 cm length X 20 cm with X 10 cm height) using Hoagland's medium 1:40 in 1500 mL total volume and 150 g of fresh tissue for the first bioassay were assayed initial concentrations of 0, 2, 6 and 10 mg/L of B as boric acid during two weeks (Penningsfeld & Kurzman, 1975).

Experimental procedures. Total soluble phenols contents were determined by the Folin-Cicalteau method utilizing fresh tissue and using a spectrophotometer (Spectronic 2000, BAUSCH & LOMB USA) at a wavelength of 660 nm (Swain & Hillis, 1959).

Total chlorophylls content was evaluated by the colorimetric method from fresh tissue and using a spectrophotometer at a wavelength of 663 and 645 nm (Arnon, 1949). Determinations were done on days 1, 2, 6, 8, 10 and 14 once the experiment started.

Biomass and bioconcentration factor (BCF). Plants were cultured in polypropylene containers (7 cm length X 10 cm with X 10 cm height) using Hoagland's medium (1:40) in 500 mL total volume with 10 g of fresh tissue. Concentrations of 0.25, 0.75, 1.5 and 2.5 mg/L B as boric acid were used to find response from the plants exposed. For biomass determinations, samples were taken at days 0, 3, 6 and 10 from all fresh tissue according to Wang (1990). Then, samples were dried at 80°C for 72 h and dried tissue was measured in a digital balance. Bioconcentration factor was defined as the ratio of B concentration in the biomass (mg B/g dry weight *Lemna*

gibba) at the end of the experiment to the initial concentration of B in the feed solution in mg B/L solution (Jain *et al.*, 1990).

For B contents evaluation, plants were washed with tap water and rinsed with distilled water to remove any foreign material attached to the surface, dried at 80 °C for 48 h, and grounded in a porcelain mortar. Three replicates of soil plant samples (0.2 g) were digested in Teflon tubes with 5 mL concentrate HNO₃ into a microwave oven (Floyd INC 544). The resultant liquid was diluted to 25 mL with deionized water and analyzed for B using Plasma Emission Spectrometer (Perking Elmer Model 4BM 2281M). Xochimilco water samples were analyzed by the same method (APHA, 1992). Merck standard stock solution was used for calibrating the equipment with a detection limit 0.02µg/L.

The observed changes on the cultivated plants in different B concentrations were determined through simple observations such as changes in the surface from smooth to rouge and chlorosis (fronds turned yellow) followed by necrosis (dead fronds).

Three replicates for each experiment were done. Test containers were kept in a greenhouse under controlled temperature (25 - 29 °C) and light (210 µEm⁻²s) with 12/12 light/dark photoperiod (Miranda & Ilangovan, 1996).

Statistical analysis. Data obtained were statistically processed through an Analysis of Variance (ANOVA) followed a one-tailed Tuckey's test at the 0.05 level of significance in a Statgraphic program version 4.0.

RESULTS AND DISCUSSION

Total soluble phenols. Statistical analysis showed significant responses between 10 mg/L B and all concentrations on the sixth day ($P < 0.05$), that was the highest response (Fig. 1). Within the following days of the bioassay some variability in phenols production was observed without some other response to the presence of Boron. The highest response for total soluble phenols was only one day in one concentration, there were not significant statistics differences in others days ($P > 0.05$). However, visible damage was observed in border of leaves in 6 and 10 mg/L of B. Probably sampled times for phenols were not optimal to observe responses to B exposure. Even when phenols production is a response of damage in plants (Iwanoska *et al.*, 1994; Daniel *et al.*, 1999), probably for the case of *Lemna gibba* Boron didn't cause sufficient damage to respond with an increase of total soluble phenols, due to the role of Boron in plants (Woods, 1994; Nable, *et al.*, 1997).

Total chlorophylls content. Chlorophylls content at 2 mg/L B concentration increased in day 10 (Fig. 2), however differences were not significant comparing with all concentrations ($P < 0.05$) and plants recovered at 10 day. This suggests that chlorophylls production in *Lemna gibba* was not affected by B exposure in the concentrations used. Experimental groups observed changes at the surface from smooth to wrinkled, yellowish appearance (chlorosis) and necrosis since day 3. Control groups (without Boron) *Lemna gibba* did not show any visible damage. According

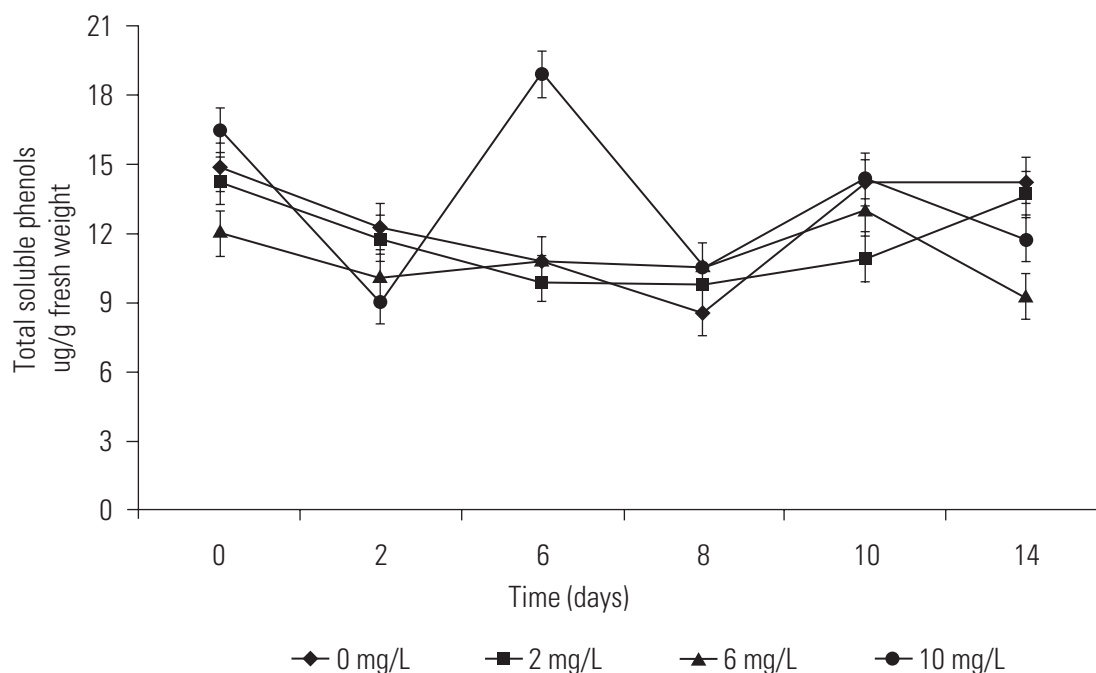


Figure 1. Total phenols vs time of Boron exposition in *Lemna gibba* concentrations in mg/L.

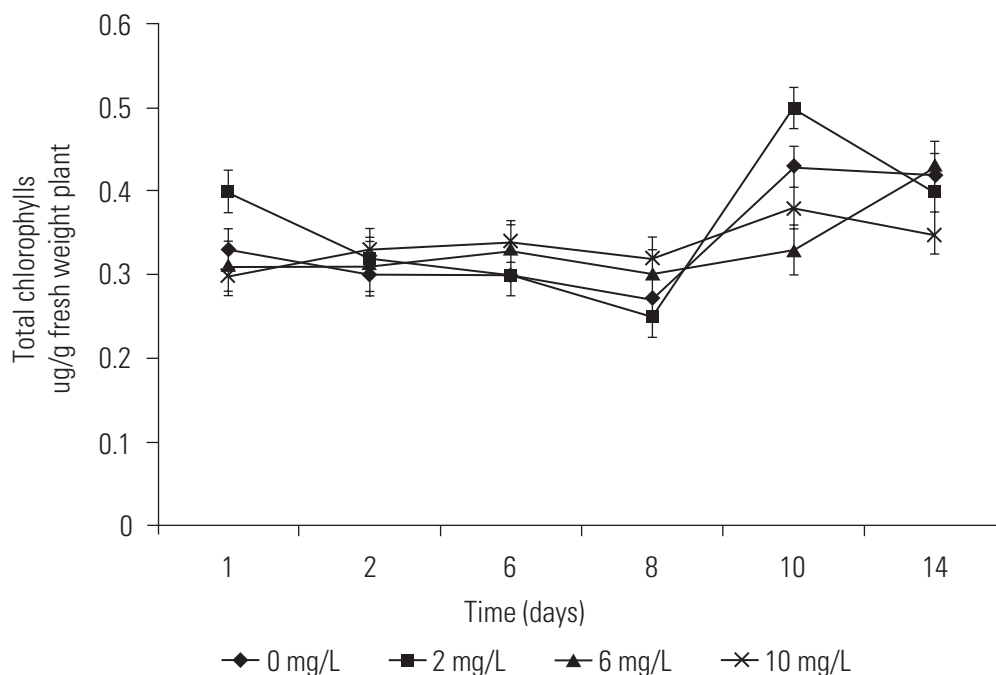


Figure 2. Total chlorophylls vs time of Boron exposition in *Lemna gibba* concentrations in mg/L.

with other works *Lemna gibba* is described as tolerant to environmental toxicity, to a big variety of contaminants compounds. On the other hand, the plant is described as sensitive to toxicity, suggesting that it may be highly adaptive, due to its fast growth rate (Wang, 1990).

Biomass effects. Table 1 shows effects of B in biomass. The highest significant decrease of biomass was observed in concentrations 1.5 and 2.5 mg/L B s at days 6 and 10 ($P < 0.05$). While in concentrations 0.25 mg/L and 0.75 mg/L B there were not significant changes ($P > 0.05$). These results showed an inverse relationship between biomass, Boron concentration and exposure time.

Bioconcentration factor (BCF). Table 2 shows B (BCF) for *Lemna gibba* in the present study and refers to tissue: water concentration ratio after 9 days of B exposure under the defined experimental conditions.

The results showed that at the lowest concentrations (0.25 and 0.75 mg/L) of B, the plant absorbs higher amount of this element but when the concentration of B increases, over these concentrations this produces a saturation response avoiding B to get inside the tissue. Stomp *et al.* (1993), probed that this aquatic macrophyte is able to sequester heavy metal in aqueous solution. In the conditions here used this didn't happen. One reason

Table 1. Biomass (g dry weight) of *Lemna gibba* exposed to Boron.

Boron (mg/L)	Time (days)			
	0	3	6	10
0.0	2.0 ± 0.0	2.4 ± 0.02	2.6 ± 0.02	2.8 ± 0.01
0.25	2.0 ± 0.0	2.0 ± 0.06	2.0 ± 0.07	1.8 ± 0.03
0.75	2.0 ± 0.0	2.0 ± 0.07	1.8 ± 0.03	1.8 ± 0.03
1.5	2.0 ± 0.0	1.9 ± 0.02	1.8 ± 0.03*	1.6 ± 0.06*
2.5	2.0 ± 0.0	1.8 ± 0.03	1.6 ± 0.06*	1.5 ± 0.10*

Significant differences *

Table 2. Boron concentration factor (BCF) for *Lemna gibba* after 9 days of exposure to B feed solutions.

B concentration (mg/L)	BCF
0.25	107.6
0.75	2.98
1.5	1.57
2.5	0.89

could be the role of B in cell division and in all apical meristems (Woods, 1994).

There are relatively few data for comparative studies on different species. King and Coley (1985) compared the sensitivity of *Lemna gibba*, *Lemna minor* L. and *Lemna perpusilla* T. to aqueous extracts or natural and synthetic oils, as well as to coal distillate, they reported that *Lemna gibba* was the most resistant among the three species. This could be a reason why this specimen of aquatic macrophyte showed resistance to concentrations of Boron used here. It also has been reported that time exposure is most important than concentration in Boron tolerant plants (Camacho *et al*; 2002), therefore more studies would be done in order to confirm the tolerant condition of *Lemna gibba*.

CONCLUSIONS

Significant response was found in total soluble phenols content at 10 mg/L B concentration and on the sixth day. However, the highest response for total soluble phenols was only one day in one concentration in other days there were not changes, suggesting that phenols production was not affected by B concentration used. Chlorophylls production in *Lemna gibba* was not affected by B exposure in the concentrations used. Boron exposure produces changes in biomass of *Lemna gibba* in concentrations 1.5 and 2.5 mg/L of B at days six and ten. This research demonstrates that *Lemna gibba* is tolerant to B exposure at concentrations used here. This aquatic macrophyte could be used as a third treatment through a bed of *Lemna gibba* growing in ponds and possible renovation for contaminate wastewater with Boron.

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