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# Uptake of Lead by *Lemna gibba* L.: Influence on Specific Growth Rate and Basic Biochemical Changes

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In recent years heavy metals have become ubiquitous in the environment due to rapid industrialization and urbanization. Among the heavy metals, lead (Pb) content in water and soil are closely linked with leaded gasoline, lead-based paint, sewage sludges used as fertilizer or land disposal of sewage as well as industrial wastes (Zakrzewski 1991). The toxicity of lead to aquatic organisms is dependent on pH, water hardness, dissolved oxygen and its ionic form (Kramer and Allen 1991; Morel and Hering 1993). Several authors reported trace metal concentrations in aquatic plants (Hutchinson 1975; Welsh and Denny 1980). Submerged macrophytes, algae and bacteria are capable of biomagnifying metal ions several fold over the environment (Heisey and Damman 1982; Ilangovan *et al* 1992; Jain *et al* 1988; Buckley 1994). Removal of nutrients and certain heavy metals from waste water by *Lemna gibba* L. has been reported by Staves and Knaus (1985), Kwan and Smith (1991) and Buckley (1994). The main objective of the present investigation was to study the removal efficiency of lead under continuous and discontinuous illumination using *Lemna gibba* L.

## MATERIALS AND METHODS

All experiments were performed with *Lemna gibba* L. collected from Xochimilco Canal, Mexico City and held in outdoor reservoirs until used. All experiments were carried out in a greenhouse under controlled temperature (22.5 ± 1.5 °C) and light (210 $\mu E/m^2/s$). Before inoculation the plant fronds were rinsed with sterile water. Hoagland medium (diluted to 1:40 medium:sterile water) pH 6.7 was used as growth medium.

The maximum incubation time for all the experiments was 25 days. The prewashed *L. gibba* were inoculated with Hoagland medium containing various concentrations of Pb (30, 50, 100, 200, 300, 500 mg/l). The effects on growth were measured by calculating specific growth rate according to the method of Porath *et al* (1979), following the formula $R=\ln(Wt/Wo)/T$. Where ln=natural logarithm; Wo=weight of the plant at zero time of treatment; Wt=weight of the plant after treatment and T=number of days or hours. The plants were grown

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under continuous as well as discontinuous illumination (12 hr light and 12 hr dark) in metal-free polypropylene tanks (30.5 cm length x 25 cm width x 9 cm height). Total Pb content in the entire plant was determined (APHA 1992) using Plasma Emission Spectrometer (Perkin Elmer Model 4BM2281M). Perkin Elmer standard stock solution was used for calibrating the equipment with a detection limit of 0.02 µg/l. The control (lead free) and lead-treated plants were analyzed for total chlorophyll (Arnon 1949), total soluble starch (Mc Cready *et al* 1950), total soluble proteins (Lowry *et al* 1951), total soluble amino acids (Troll and Canan 1953), total soluble sugars (Dubois *et al* 1956) and total phenols (Swain and Hillis 1959). All experiments were carried out in triplicate and the results were analyzed using "t" test.

## RESULTS AND DISCUSSION

*Lemna gibba* L. is one of the most commonly available and widely distributed aquatic macrophytes in Mexico. *L. gibba* has been reported to resist elevated temperature (30-35°C) with high photosynthetic rates (Wedge and Burris 1982). The effects of continuous and discontinuous illumination on specific growth rate and Pb uptake of *Lemna* plants are illustrated in Figures 1 and 2, respectively. Maximum specific growth rate was observed at initial 10 days of growth after inoculation in the nutritive medium. High lead concentrations (200 to 500 mg/l) in the medium inhibited significantly the specific growth rate (R) of *Lemna* under continuous (Fig. la) as well as discontinuous illumination (Fig. lb). However, low concentrations of Pb (30-50 mg/l) did not inhibit the specific growth rate of the plant under continuous illumination at 7 days of growth when compared to discontinuous illumination. Diluted (1:40) Hoagland nutrient medium did not affect the solubility of Pb. The rate of absorption of lead was found to be maximum at 72 hr of growth. However, significant Pb uptake was noticed during the initial 24 hr of growth. From Figure 2a and b it is evident that the rate of Pb absorption by *Lemna* (from 24 to 72 hr) was significant under discontinuous illumination than continuous illumination (Table 1). Similarly the increased absorption under discontinuous illumination by *L. gibba* resulted in reduced residual concentration in the medium (Table 1). Reduced frond size and chlorosis were observed at the concentration of 500 mg/l of Pb than at 30 and 50 mg/l. The mean light: dark ratio was 1:1 and showed a high rate of Pb absorption than a 24 hr light period with light intensity of 120 $\mu E/m^2/s$ at 25°C. Wedge and Burris (1982) reported that saturated light intensity (300-600 $\mu E/m^2/s$) did not allow photoinhibition but enhanced photosynthesis was observed in *Lemna*. Similar behavior was also observed in our studies in terms of higher chlorophyll *a* concentration under continuous light (Fig. 3a) than at discontinuous light condition (Fig. 3b). No significant chlorotic symptoms were noticed in the plants treated with 30, 50, 100 and 200 mg/l of Pb, but were prominent at 500 mg/l, resulting in reduced total chlorophyll concentration (Fig.3a, b).

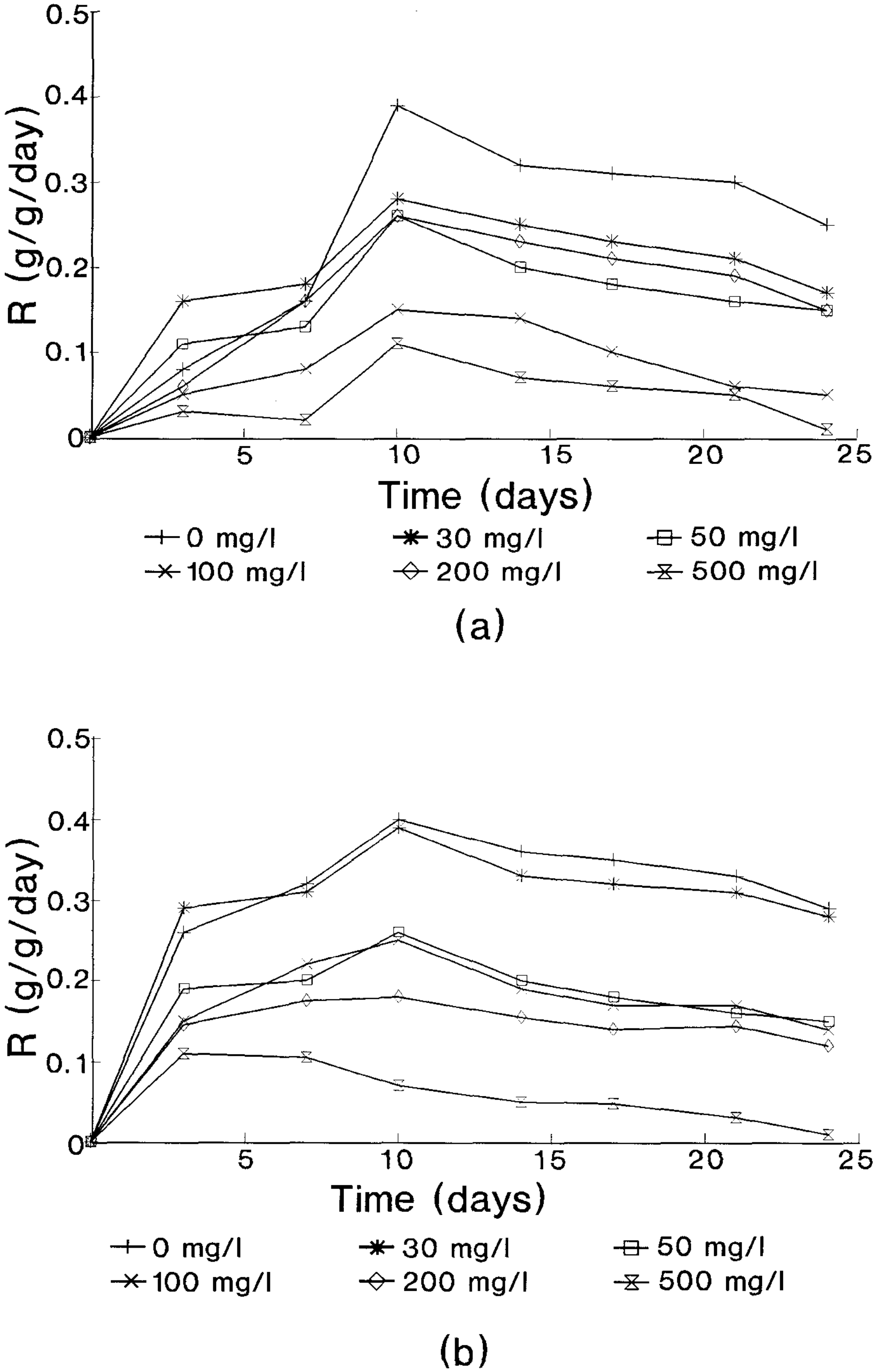



**Figure 1.** Specific growth rate (R) of *Lemna gibba* L. grown on Hoagland medium with different concentrations of lead under a) continuous and b) discontinuous light illumination.

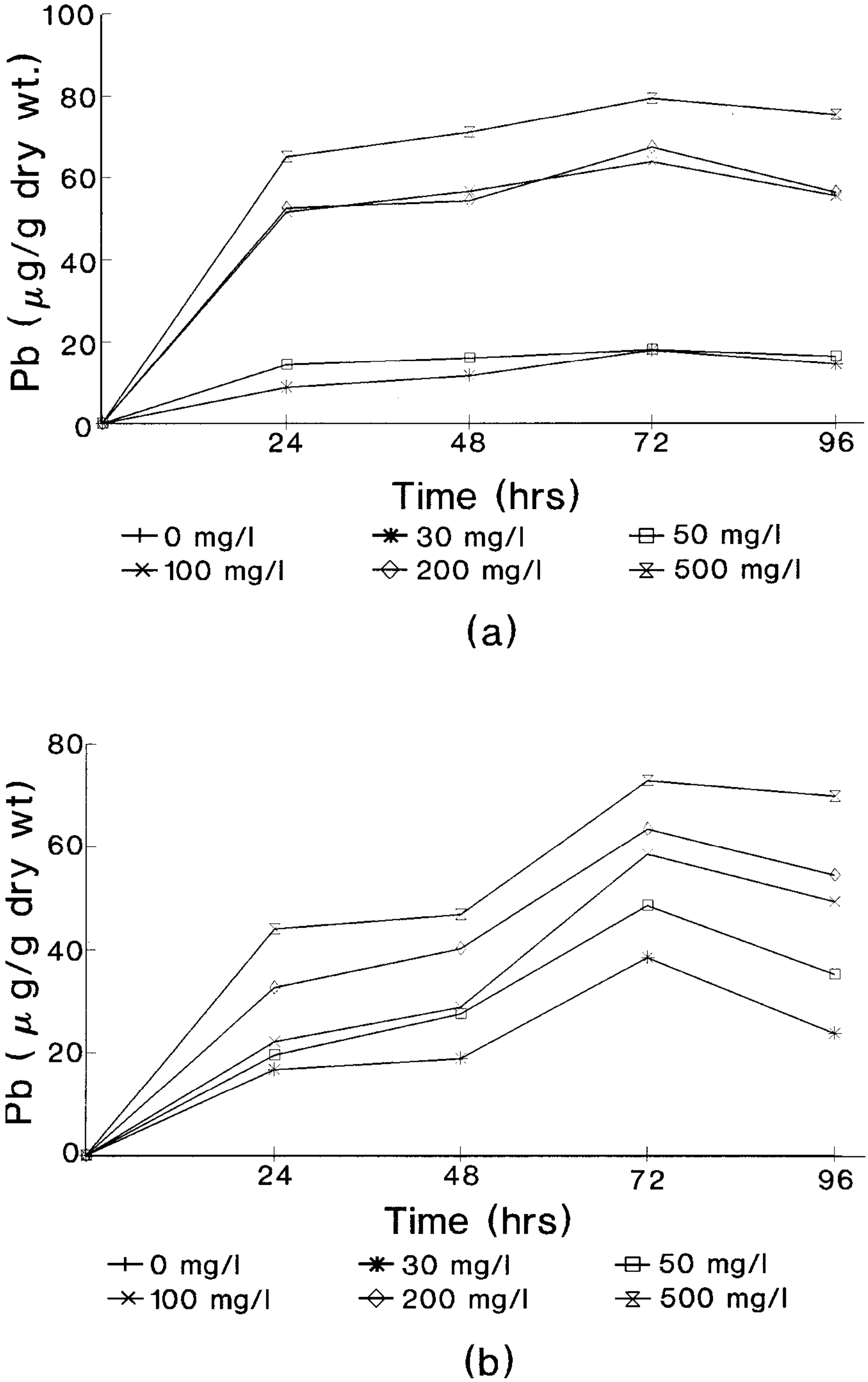



**Figure 2.** Rate of lead uptake by *Lemna gibba* L. under a) continuous and b) discontinuous light illumination.

**Table 1.** Mean rate of absorption of lead (mg Pb/g dry wt/hr) under continuous and discontinuous illumination by *Lemna gibba* L. and residual Pb concentration in the growth medium.

| Illumination | Lead concentration (mg/l) 30 | 50 | 100 | 200 | 500 |
|---|---|---|---|---|---|
| | ($\mu$g Pb/g dry wt./hr) | | | | |
| Continuous | 18.8 | 77.4 | 253.8 | 308.0 | 297.0 |
| Discontinuous | 450.7+ | 602.8+ | 758.4+ | 643.1+ | 602.4+ |
| | *Mean residual lead concentration in Medium ($\mu$g Pb/l) | | | | |
| Continuous | 11.2 | 19.5 | 28.9 | 59.4 | 146.5 |
| Discontinuous | 3.1+ | 4.6+ | 9.4+ | 16.1+ | 42.3+ |

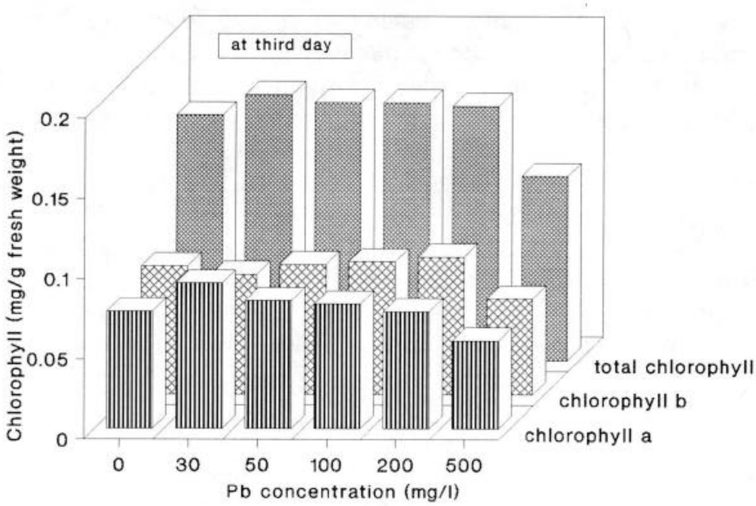
+Significant at < 0.5
*Mean residual Pb concentration obtained after 72 hr of growth

Based on our experiments at optimum levels of temperature and light, the rate of Pb absorption was more prominent when plants were exposed to discontinuous light than continuous illumination. Accumulation of Pb in the plant cells could affect the physiology of photosynthesis of the plant. It is well documented in the literature that heavy metals (Pb, Cd, Cu, Zn etc) affect the photosynthetic carbon fixation and permeability of membranes (Gorgham 1950; Filbin and Hough 1979).

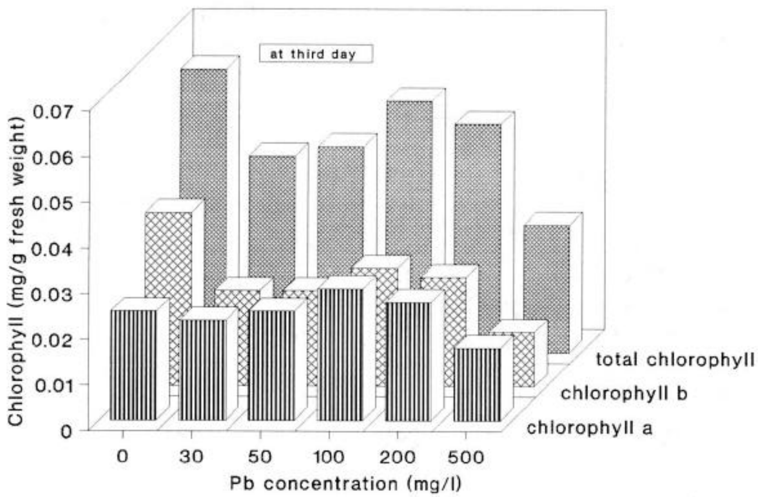
**Table 2.** Influence of Pb absorption on the biochemical components of *Lemna gibba* L.

| Concentration of Pb (mg/l) | Total phenols | Total soluble starch | Total free sugar | Total soluble proteins | Total free amino acids |
|---|---|---|---|---|---|
| | | ($\mu$g/g fwt) | | | |
| Control | 44.0±0.45 | 35.1±2.15 | 48.0±0.41 | 28.5±0.60 | 11.3±0.15 |
| 50 | 46.0±0.81 | 16.0±0.75 | 53.0±0.26 | 11.4±0.35 | 11.7±0.17 |
| 100 | 47.1±0.52 | 15.6±0.95 | 57.0±0.30 | 5.8±0.20 | 15.0±0.47 |
| 200 | 50.0±0.37 | 14.8±1.21 | 59.0±0.45 | 3.2±0.36 | 18.0±0.26 |
| 300 | 52.0±0.25 | 14.3±1.27 | 72.0±1.01 | 1.5±0.41 | 39.1±0.37 |
| 500 | 54.0±0.26 | 7.0±0.36 | 68.0±0.70 | 1.3±0.26 | 31.8±0.36 |

Results are means ± standard deviation
All the basic biochemicals were analyzed after three days of treatment with lead. The values are the average of three different samples.




**(a)**




**(b)**

**Figure 3.** Effect of lead on chlorophyll pigment concentration in *Lemna gibba* L. under continuous (a) and discontinuous light illumination (b).

The effect of Pb concentrations ranging from 200 to 500 mg/l on *L. gibba* increased the Pb accumulation in the plants and exhibited increased loss of total soluble starch concentration with a significant accumulation in early photosynthetic intermediates like solubles sugars.

Similarly, Pb decreased the concentration of total soluble proteins (Table 2). From the results it is evident that the response of *Lemna* to Pb showed a high rate of Pb absorption under discontinuous light conditions and that excess cellular accumulation of Pb could affect the physiological metabolism of the plant. From these results we suggest that *Lemna gibba* L. can be used in tertiary treatment plants to remove heavy metals like Pb efficiently and that it is important to harvest these plants at their maximum specific growth period to avoid the toxicity of lead to the plant itself.

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