GROWTH, PHOTOSYNTHESIS AND RESPIRATORY RESPONSE TO COPPER IN *LEMNA MINOR*: A POTENTIAL USE OF DUCKWEED IN BIOMONITORING

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ABSTRACT
Aquatic macrophytes are known to accumulate various heavy metals in their biomass. This accumulation is often accompanied by physiological changes which can be used in biomonitoring for aquatic pollution. In this study, the impact of copper (Cu) on the growth of the duckweed *Lemna minor*, followed by its removal, was studied with 0.1–1.0 mg/L of Cu in a quarter Coïc and Lesaint solution at pH=6.1. In order to verify duckweed tolerance to Cu, photosynthesis was measured at the maximal concentration which caused no effect on the plant growth. The results showed that copper inhibited *Lemna* growth at concentrations $\geq$ 0.3 mg/L. At 0.2 mg/L, the final biomass was approximately four times greater than the initial biomass. Analysis of metal concentration in water showed that *Lemna minor* was responsible for the removal of 26% of Cu from the solution. In the presence of Cu, respiration was reduced, while photosynthesis increased considerably. Net photosynthesis approximately increased three times compared to the control. Copper was responsible for 130-290% increase in the photosynthetic activities. These results suggested that *Lemna minor* could be a good tool for the evaluation of copper pollution in biomonitoring programs.

Key words: Aquatic pollution; Bioindicator; *Lemna minor*; Physiological modifications; Growth; Photosynthesis

INTRODUCTION
Copper (Cu) is an essential element for organisms and is involved in numerous physiological processes (Teisseire and Guy, 2000). However, it is toxic at higher concentrations by causing deleterious effects to human, animals and plants (Vinodhini and Narayanan, 2009). Excess of Cu may reach living organisms as a result of environmental pollution caused by anthropogenic activities (mining operations, manufacturing industries and agricultural technologies) which can modify the biogeochemical cycles of the metal.

Several studies demonstrated that many species of duckweed, a group of free-floating freshwater plants of the family *Lemnaceae*, are able to absorb and accumulate high amount of copper in their biomass producing an internal concentration several fold greater than the nutrient medium (Jain *et al*., 1989; Zayed *et al*., 1998; Miretzky *et al*., 2004; Ater *et al*., 2006). This accumulation has, in some ways, a relationship with the tolerance phenomena which is defined as the cell capability to protect plant tissues against injury caused by the metal (Sabreen and sugiyama, 2008). At metal concentration greater than the tolerated concentration, toxicity symptoms and physiological changes are induced.

Cupric ions are responsible for many alterations of plant cells and inhibition of enzymatic activities (Teisseire and Guy, 2000). They also cause significant changes in respiration, photosynthetic CO_2 fixation and photosynthetic pigments by

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increasing oxidation of chloroplast membranes (Prasad *et al*., 2001; Hattab *et al*., 2009). These physiological modifications, evaluated by biotoxicity tests, can be used as an indicator of metal pollution and offer data in biomonitoring (Movahedian *et al.*, 2005).

Sedentary macrophytes as bioindicators have some advantages such as high tolerance to aquatic metal pollution, convenience for sampling, large individuals and easy to realize laboratory raise (Zhou *et al*., 2008). Duckweeds have been widely used in toxicity tests of different chemicals and effluents and particularly, *Lemna minor* has often been selected to represent vascular aquatic plants in toxicity tests (Kanoun-Boulé *et al*., 2009). This genus is an invasive plant wild-growing in European regions and other Mediterranean countries.

The main objective of the present study was to evaluate the effects of elevated Cu levels on *Lemna minor* growth and photosynthesis. The specific objectives were to: (a) determine the growth of plants in experimental Cu treatments ranging from 0.1 to 1.0 mg/L, (b) assess Cu removal percentage in the presence of duckweed and (c) verify the plant tolerance to Cu by measuring net photosynthesis and respiration at the maximal concentration which causes no effect on the plant growth. The results were compared with those of other studies traditionally reported in literature.

MATERIALS AND METHODS

Plant culture

The plants originated from the Rhônes Alpes area of France, were cultured in laboratory as described in our previous study (Khellaf and Zerdaoui, 2009a).

Plant toxicity test and metal removal

The effect of copper on the growth of duckweed was assessed according to the test protocols derived from the standard draft guideline 221 of the Organization for Economic Cooperation and Development (OECD, 2002). The details of data analysis were the same as those described in our previous study (Khellaf and Zerdaoui, 2009a).

Water samples (1 mL) were regularly drawn in order to ascertain the Cu concentration removed from the solution. The metal concentration analysis was carried out with an atomic absorption spectrophotometer which had a detection limit of 10^{-2} mg/L (Perkin Elmer).

The elimination percentage of Cu was calculated according to Khellaf and Zerdaoui (2009b):

$$\text{Elimination}(\%) = \frac{C_0 - C_f}{C_0} \times 100 \qquad (1)$$

in which: C_0 and C_f are initial and remaining concentrations of metal in the medium (mg/L).

Photosynthesis experiment

The essays were investigated using an infrared gas analyzer (IRGA) in a closed system with an airflow of 1.1×10^{-2} mL/min (the capacity of the circuit and the room of assimilation was of 11 litres). A 42-cm^2 frond area (previously exposed to 0.2 mg/L of Cu during 4 days) contained in a crystallising cup with a low volume of water was placed in an enclosure of which the atmosphere was renewed permanently (Gary, 1988). At this concentration, no morphological sign of toxicity was observed on *Lemna* fronds. Control treatment corresponded to the same essay without exposing plants to Cu. The infrared analyser used in differential mode allowed the direct measurement of the difference in CO_2 concentration between the entry and the exit. These measurements were taken with regular time intervals. The CO_2 concentration in the air (370 mg/L) was used for the calibration of the apparatus.

The plants were initially placed in the darkness (respiration 1); then successively exposed to three lamps (1st, 2nd and the 3rd photosynthesis) and replaced finally in the darkness (respiration 2). The duration of essays was 100 min corresponding to a time of 20 min for each phase (2 phases of respiration and 3 phases of photosynthesis). Photosynthetic active irradiations of the 1st, 2nd and 3rd lamps were 337, 495 and 756 μmol/ m^{2}s, respectively.

The CO_2 flow (N), absorbed or rejected, was calculated according to Garry (1988):

$$N = -Qe.\ \Delta c \ \ [\text{mL/min}] \qquad (2)$$

Where:

Qe=the airflow passed in the enclosed,

Δc=the difference in CO_2 concentration between the entry and the exit.

The CO_2 flow allowed establishing photosynthesis and respiration regression equations.

Net photosynthesis, P_n, was calculated as follows (Papazoglou *et al.*, 2005):

$$P_n = P.N. \frac{1}{0.0224.60.S} \quad [\mu mol/m^2s] \qquad (3)$$

Where:

P=the slope of the regression equation of the function

$\Delta c = f(t)$,

S=the total frond area,

N=the CO_2 flow (1µmole CO_2 = 22.4×10^{-3} mL CO_2).

Brut photosynthesis (P_b) was expressed as follows:

$$P_b \text{ (1st lamp)} = (P_1 + P_3).N$$
$$P_b \text{ (2nd lamp)} = (P_m + P_4).N \qquad (4)$$
$$P_b \text{ (3rd lamp)} = (P_2 + P_5).N$$

Where:

P_1 and P_2 are the slopes of the regression equation of the 1st and 2nd respiration,

$$P_m = \frac{P_1 + P_2}{2} \qquad (5)$$

P_3, P_4 and P_5 are the slopes of the regression equation of the 1^{st}, 2^{nd} and 3^{rd} photosynthesis.

Net and brut photosynthesis are expressed in µmol/m²s.

RESULTS

Growth response to copper exposure

Data from experimental dose-response curve (Fig. 1) showed that *Lemna minor*, when exposed to Cu concentrations from 0.3 to 1.0 mg/L, exhibited significant inhibition of growth. For Cu concentration of 0.5 mg/L, duckweed growth was inhibited by 70% (indicated on the figure by the broken line) representing the minimal growth index attained under our experimental conditions.

On the other hand, at concentration ranging from ~ 0 (control) to 0.2 mg/L, the growth index was optimal. Based on these results, Cu concentration of 0.2 mg/L was considered as the threshold toxicity in *Lemna minor* under conditions indicated above.

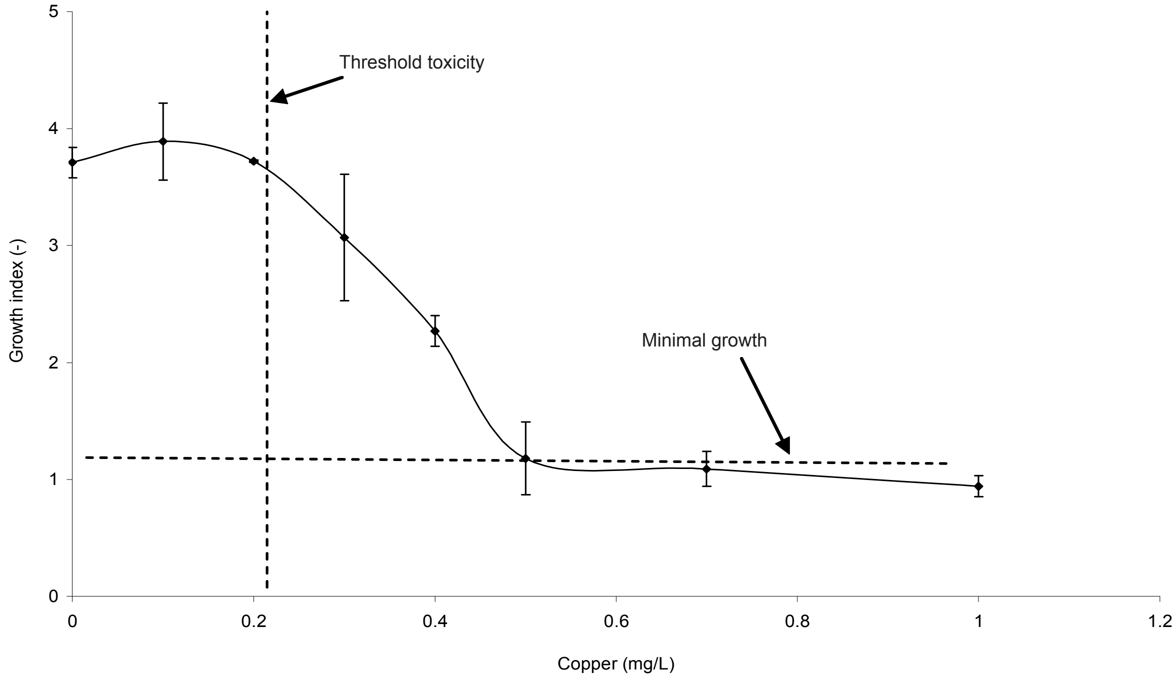

Fig. 1: Growth of *Lemna minor* fronds in the presence of copper in the nutrient medium. Vertical bars indicate standard deviation, n = 3 (Khellaf and Zerdaoui, 2009a)

Removal of copper

Fig. 2 shows the concentration of copper removed by the plants after 96 h of exposure. The initial Cu concentration in the Coïc and Lesaint solution was 0.2 mg/L. After 1 day, 15% of the initial metal concentration was removed and after 4 days, *Lemna minor* had removed 26% of Cu from the nutrient medium. The control treatment showed that the aquatic plants were responsible for the disappearance of amount of Cu from water.

Photosynthesis and respiration

Effect of copper at 0.2 mg/L on photosynthesis and respiration of *Lemna minor* is shown in Fig. 3. Dark respiration was inhibited in the presence of Cu; the 1st and 2nd respiration slopes were respectively 0.41 and 0.20, whereas those of the control were 1.70 and 1.66 (Table 1). However, CO_2 assimilation increased in the presence of 0.2 mg/L of cupric ions. Net photosynthesis ranged between 8.3 and 12.2 μmol/m^{2}s for different luminous energies used in this study. Compared to control, net photosynthesis was approximately 3 times higher. Brut photosynthesis was only 2 times of the control because of the inhibition of the gas exchanges in dark. However, for an irradiation ≥ 495 μmo/m^{2}s, it was noticed that saturation was mainly observed in the case of brut photosynthesis (Fig. 4).

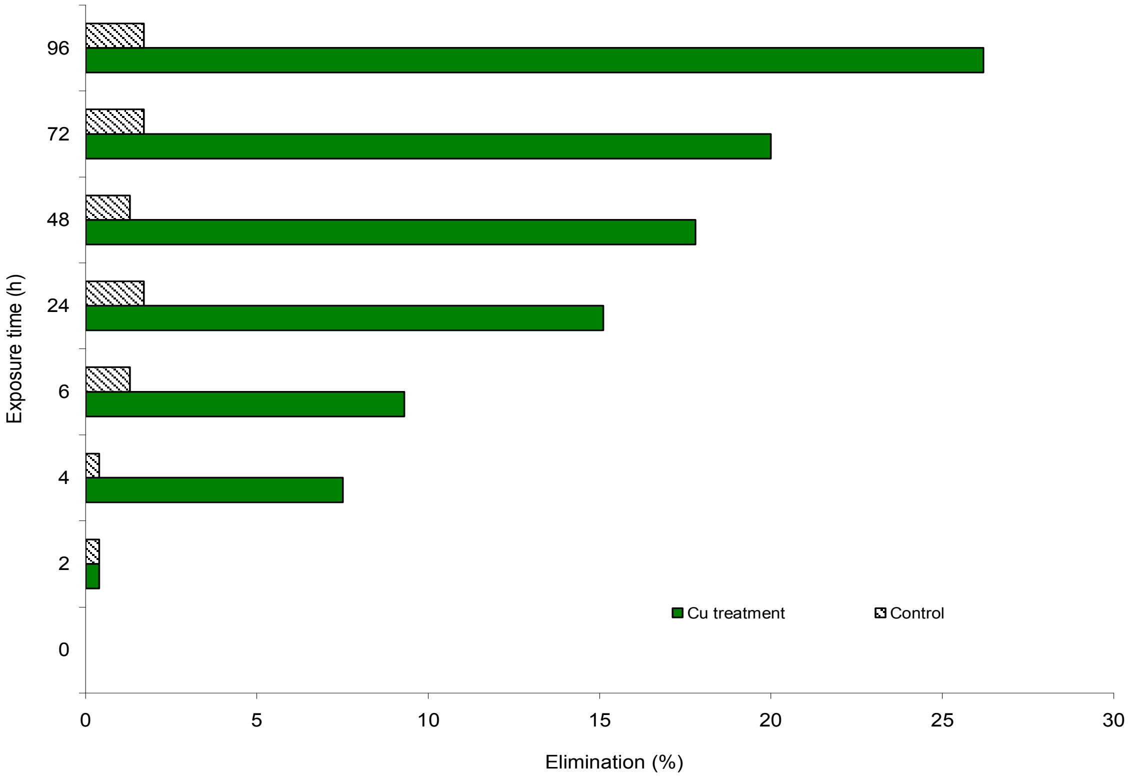

Fig. 2: Elimination of copper from the nutrient medium in the presence of L. minor.
The initial Cu concentration was 0.2 mg/L

DISCUSSION

Copper is considered to be one of the most toxic trace metals to plants, although it is required as an essential element for metabolic and physiological processes (Xia and Tian, 2009). Dewez *et al.* (2005) explained some hypothesis concerning the mechanism of Cu toxicity on the plant growth. Copper was recognized to be a strong inhibitor of photosystem II (PSII) electron transport activity associated to the water splitting system; by this effect, the metal may alter the energy storage via photosynthesis which causes the decrease of biomass growth. However, some plant species tolerate this element at concentrations higher than those used in medium cultures. Our study indicated that, *Lemna minor* was sensitive to copper for concentrations ≥ 0.3 mg/L and the threshold toxicity was 0.2 mg Cu/L. Published quantitative data for the threshold toxicity of

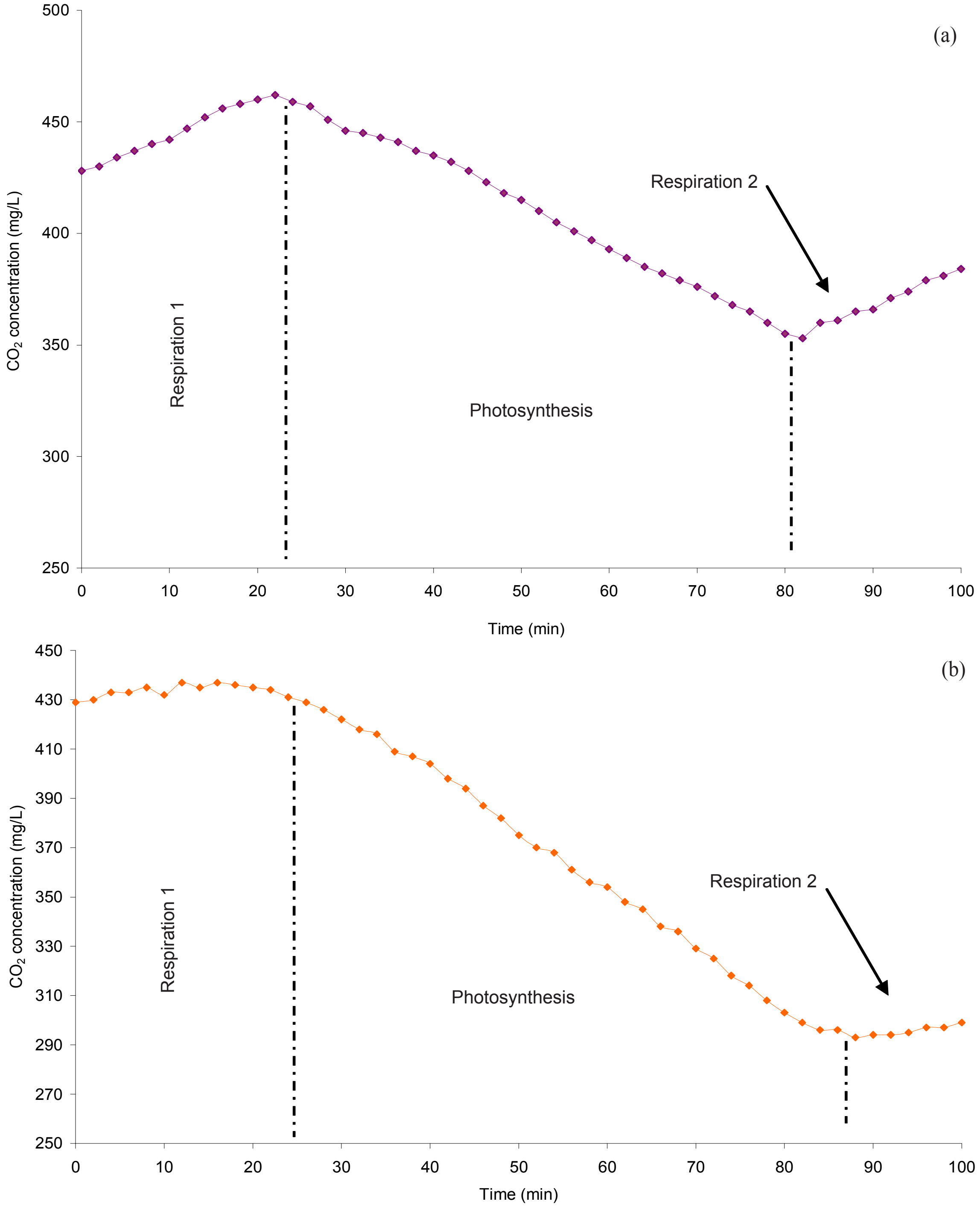

Fig. 3: CO_2 concentration as a function of time; (a) plants 96 h after exposure to 0.2 mg Cu/L and (b) control values are means of 2 essays

metal ions on duckweed species are very variable (Table 2). This is caused by the different duckweed species used and by the different test conditions, especially concerning the nutrient media as well as by the methods of evaluation (Appenroth *et al*., 2010).

The Cu removal treatment showed that the aquatic plants were responsible for the disappearance of Cu from water. From earlier results, it seems that metal removal from the medium was due to an accumulation in plants; several studies demonstrated that duckweed species (particularly

Table 1: Slope values of photosynthesis and respiration curves

Gas exchange	Slope values (mg/L. min)			
	Control	R^2	Plants exposed	R^2
Respiration 1	1.70	0.99	0.41	0.80
Respiration 2	1.66	0.97	0.20	0.84
Photosynthesis 1	- 1.50	0.99	- 1.73	0.99
Photosynthesis 2	- 2.18	0.99	- 2.54	0.99
Photosynthesis 3	- 1.83	0.98	- 2.53	0.99

The plants were exposed to 0.2 mg/L during 4 days. 1, 2 and 3 correspond to the 1st, 2nd and 3rd lamp in photosynthesis essays. Different slope values are means of 2 values; error was < 10%. R2 is the coefficient of determination.

Lemna minor and *Lemna gibba*) were able to accumulate elevated amount of Cu in their tissues (Jain *et al*., 1989; Zayed *et al*., 1998; Ater *et al*., 2006; Megateli *et al*., 2009) inducing an abatement of Cu concentration in water. Our result confirmed that *Lemna minor* showed a potential of phytoremediation of contaminated waters charged with low concentrations of Cu.

Photosynthetic activities increased in the presence of 0.2 mg/L of cupric ions for different luminous energies. However, according to the study of Olette *et al*. (2008), copper used as $CuSO_4$ (pesticide) inhibited the photosynthetic activities of *Lemna minor* at concentrations of 12, 24, 40 and 100 µg/L. For an exposure time of 7 days, the metal element present in the medium inhibited the photosynthesis of the aquatic plants by 0.4%; this inhibition reached 8% in the presence of 100 µg/L of Cu. Prasad *et al*., (2001) demonstrated that 1 and 10 µM of Cu present in a Hoagland solution increased (160 and 120% of the control) the concentration of photosynthetic pigments of *Lemna trisulca* (another species of duckweed). These results agree with those of the present study. The presence of 0.2 mg/L of Cu in the nutrient medium increased considerably the absorption of

Table 2: Threshold toxicity* of copper in duckweed

Duckweed species	Experimental conditions	Threshold toxicity	Reference
Lemna minor	Tap water, pH=7.2	1 mg/L	Jain *et al*., 1989
Lemna minor	1/4 Hoagland Solution; pH=6	< 5 mg/L	Zayed *et al*., 1998
Lemna minor	Inorganic growth Medium; pH=6.5	< 0.25 µM	Teisseire and Guy, 2000
Lemna minor	White nutritive Solution; pH=6.8	< 0.5 mg/L	Ater *et al*., 2006
Lemna gibba	White nutritive Solution; pH=6.8	0.5 mg/L	Ater *et al*., 2006
Lemna gibba	Hoagland medium; pH=6.5	10^{-4} mg/L	Megateli *et al*., 2009
Lemna minor	1/10 Hoagland Solution; pH=6	1.6 mg/L	Kanoun-Boulé *et al*., 2009

*Threshold concentration is Cu concentration at which no growth inhibition is observed in duckweed biomass.

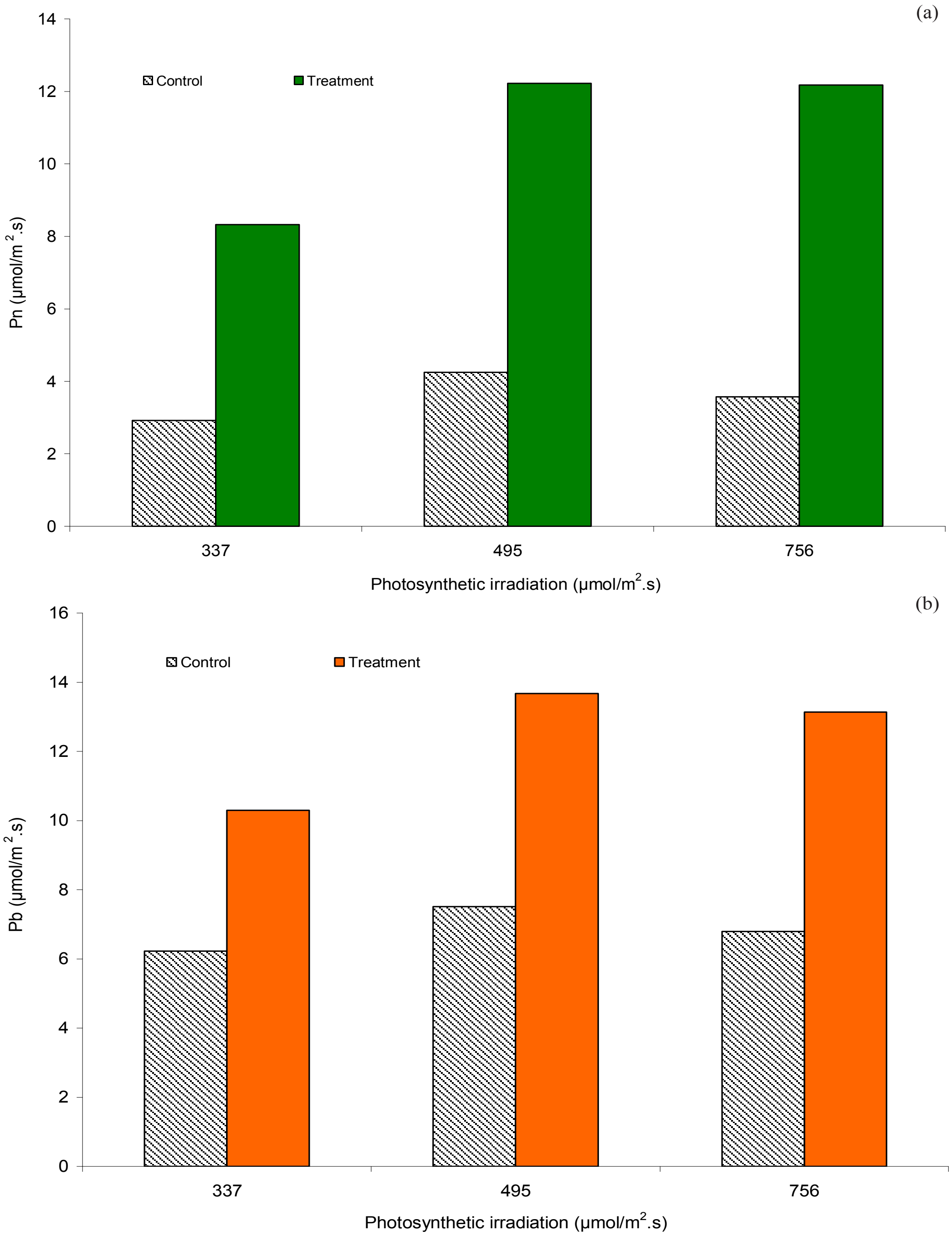

Fig. 4: Variation of (a) net photosynthesis and (b) brut photosynthesis, with the luminous energy. Treatment corresponds to plants exposed to 0.2 mg/L of Cu. Net and brut photosynthesis were calculated using the values on Table 1.

CO_2 by duckweed. Net photosynthesis increased approximately three times compared to the control. Copper was responsible for 130-290% increases in the photosynthetic activities. This effect might be explained by an increase in electron transport in photosynthetic systems. Copper is an essential element in cellular metabolism and a catalytic component of proteins and enzymes (Teisseire and Guy, 2000). It is plausible that this element was responsible for the synthesis of plastocyanin (Bertrand and Poirier, 2005).

Additionally, this study demonstrated that the increase in photosynthetic activities was observed for different luminous energies. However, for an irradiation ≥ 495 μmo/m²s, saturation was mainly noticed in the case of brut photosynthesis (Fig. 4). Beyond that, the capacity of absorption of photons exceeds the capacity of their use. The reactions of CO_2 assimilation become limiting and photosynthetic activities present a maximal intensity.

According to Wedge and Burris (1982) the energy saturation for *Lemna minor* ranged in the interval 300-600 μmo/m²s. Our results showed that the energy saturation for the species used in this study corresponded to a photosynthetic active irradiation of 495-750 μmo/m²s.

Finally, it can be concluded that among the tools used to study effects of toxic elements on plants, growth and photosynthesis are often proposed as simple, rapid and sensitive methods. Based on these methods, the results of our study showed that *Lemna minor* was sensitive to copper for concentrations ≥ 0.3 mg/L. For lowest concentrations, plant growth was optimal and photosynthetic activities increased by 290% under elevated luminous energy. The duckweed species could survive in contaminated medium (≤ 0.2 mg/L) and could detect sensitively metal concentration ≥ 0.3 mg/L. It was concluded that *Lemna minor* could be a good candidate for the evaluation of metal pollution in biomonitoring programs for risk assessments and toxic effect prediction.

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