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L.Minor for Bio-Monitoring

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ABSTRACT: *Macrophytes can be used as biomonitoring tools as they accumulate various heavy metals intheirbiomass. In this study, the impact of copper on the growth of the Lemna minor. Its removal, was also studied with 1 mg/L of Cu in a quarter of Lesaint solution maintained at 6.1pH. In order to verify the weed's tolerance to Cu, photosynthesis was measured at the maximal concentration which caused no effect on the plant growth. The results showed that copper inhibited Lemnagrowth at concentrations ≥ 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 thephotosynthetic 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; Bio-indicator; Physiological modifications; Growth;Photosynthesis*

I. Introduction

Copper(Cu)isanessentialelementfororganisms 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 environmentalpollutioncausedbyanthropogenic activities (mining operations, manufacturing industries and agricultural technologies) which can modify the biogeochemical cycles of the metal.

Severalstudiesdemonstratedthatmanyspecies of duckweed, a group of free-floatingfreshwater plants ofthe family Lemnaceae, are able to absorb and accumulate high amount of copperin their biomass producinganinternalconcentration several fold greater than the nutrient medium (Jain et al., 1989; Zayed et al., 1998; Miretzkyetal.,2004;Ateretal.,2006).Thisaccumulation has, in some ways, a relationship with the tolerance phenomena which is defined as thecell 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.

Cupricionsareresponsibleformanyalterationsof plant cells and inhibition of enzymatic activities (Teisseire and Guy, 2000). They also cause significant changes in respiration,photosynthetic CO_2 fixation and photosynthetic pigmentsby increasing oxidation of chloroplast membranes (Prasad et al., 2001; Hattabet al., 2009). These physiological modifications, evaluated by biotoxicity tests, can be used as an indicator of metal pollution and offer data in biomonitoring (Movahedianet 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 (Zhouetal.,2008).Duckweedshavebeenwidely used in toxicity tests of different chemicals and effluents and particularly,Lemnaminorhasoften been selected to represent vascular aquaticplants in toxicity tests (Kanoun-Bouléet 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 themaximalconcentrationwhichcausesnoeffect on the plant growth. The results were compared with those of other studies traditionally reported in literature.

II. Materials And Methods

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

analysiswascarriedoutwithanatomicabsorption spectrophotometer which had a detection limitof 10^{-2} mg/L (PerkinElmer).
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 waterwas placed in an enclosure of which the atmosphere was renewed permanently (Gary, 1988). At this concentration, no morphological sign of toxicity wasobservedonLemnafronds.Controltreatment corresponded to the same essay withoutexposing plants to Cu. The infrared analyser used in differentialmodeallowed 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 (respiration1);thensuccessivelyexposedtothree lamps (1^{st}, $2^{nd}$and the 3^{rd}photosynthesis) and replaced finally in the darkness (respiration 2). Thedurationofessayswas100mincorresponding to a time of 20 min for each phase (2 phases of respiration and 3 phases of photosynthesis). Photosynthetic active irradiations of the 1^{st}, $2^{nd}$and 3^{rd}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.

TheCO_2flowallowedestablishingphotosynthesis and respiration regression equations.

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

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 (1 µmole CO_2 = 22.4×10 mL CO_2).
Brut photosynthesis (P_b) was expressed as follows:

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

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

$$\bar{}_{m} \quad \frac{p_1 + p_2}{2} \qquad (5)$$

P_3, P_4 and P_5 are the slopes of the regression equation of the 1st, 2nd and 3rd photosynthesis. Net and brut photosynthesis are expressed in µmol/m²s.

III. Results

Growth response to copper exposure

Data from experimental dose-response curve (Fig.1)showedthatLemnaminor,whenexposed 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, Cuconcentration of 0.2 mg/L was considered as the threshold toxicity in Lemna minor under conditions indicated above.

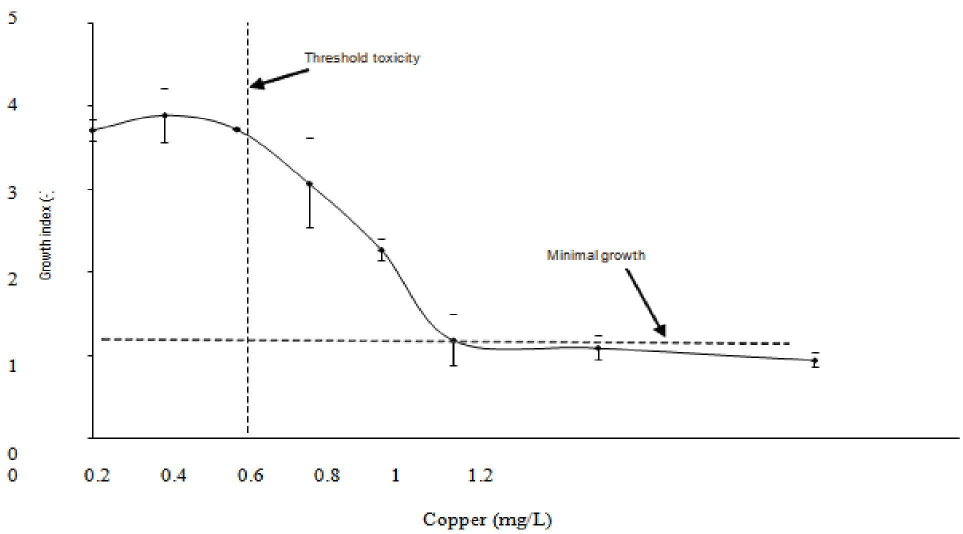

Fig. 1: Growth of Lemna minor fronds in the presence of copper in the nutrient medium. Vertical bars indicate standard deviation, n = 3 (Khellaf and Zerdaoui, 2009a)

removal of copper

Fig.2showstheconcentrationofcopperremoved by the plants after 96 h of exposure. The initial CuconcentrationintheCoïcandLesaintsolution was0.2mg/L.After1day,15%oftheinitialmetal 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 fromwater.

photosynthesis and respiration

Effect of copper at 0.2 mg/L on photosynthesis and respiration of Lemna minor is shown inFig.

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 of0.2

mg/Lofcupricions.Netphotosynthesisranged between 8.3and 12.2 μmol/m^2s for different

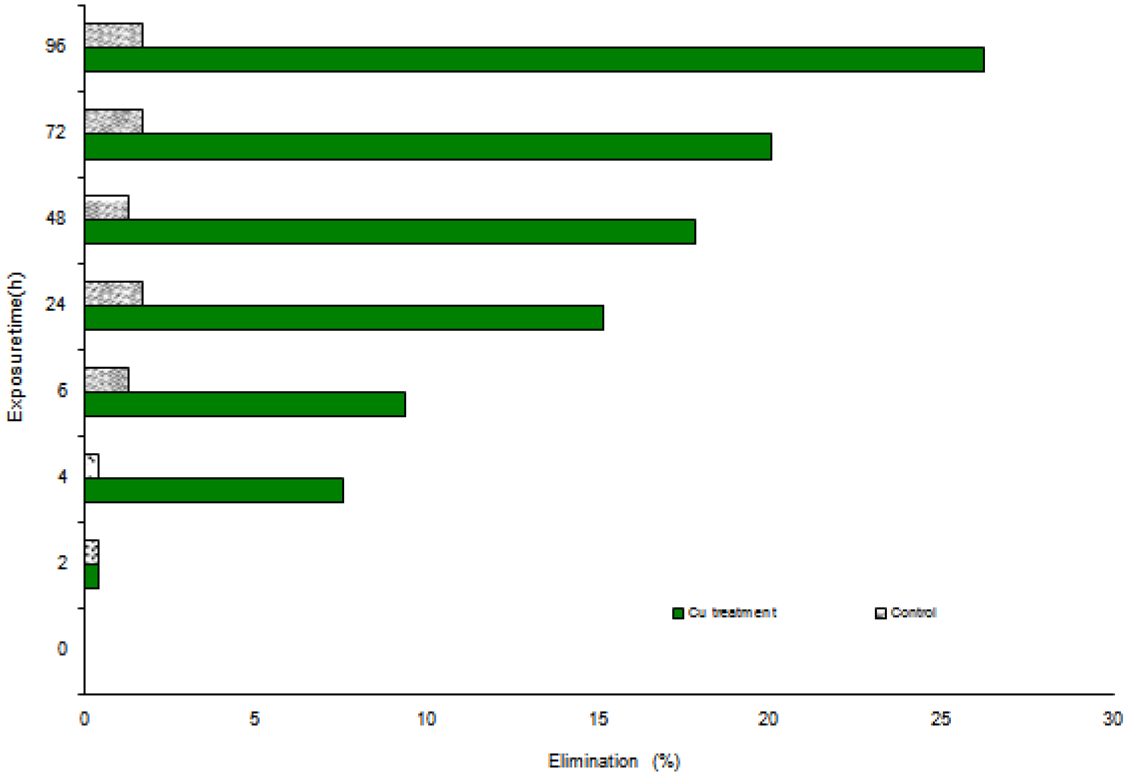

Fig. 2: Elimination of copper from the nutrient medium in the presence of L. minor.

luminous energies used in this study. Compared to control, net photosynthesis wasapproximately 3timeshigher.Brutphotosynthesiswasonly2times of the control because of the inhibition of the gas exchanges in dark. However, for an irradiation ≥ 495 μmo/m^2s, it was noticed that saturation was mainly observedinthecaseofbrutphotosynthesis(Fig.4).

IV. Discussion

Copper is considered to be one of the most toxic tracemetalstoplants,althoughitisrequiredasan essentialelementformetabolicandphysiological processes (Xia and Tian, 2009). Dewezetal. (2005) explainedsome hypothesis concerning the mechanism of Cu toxicity on the plant growth. Copperwasrecognizedtobeastronginhibitorof 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 toxicityof

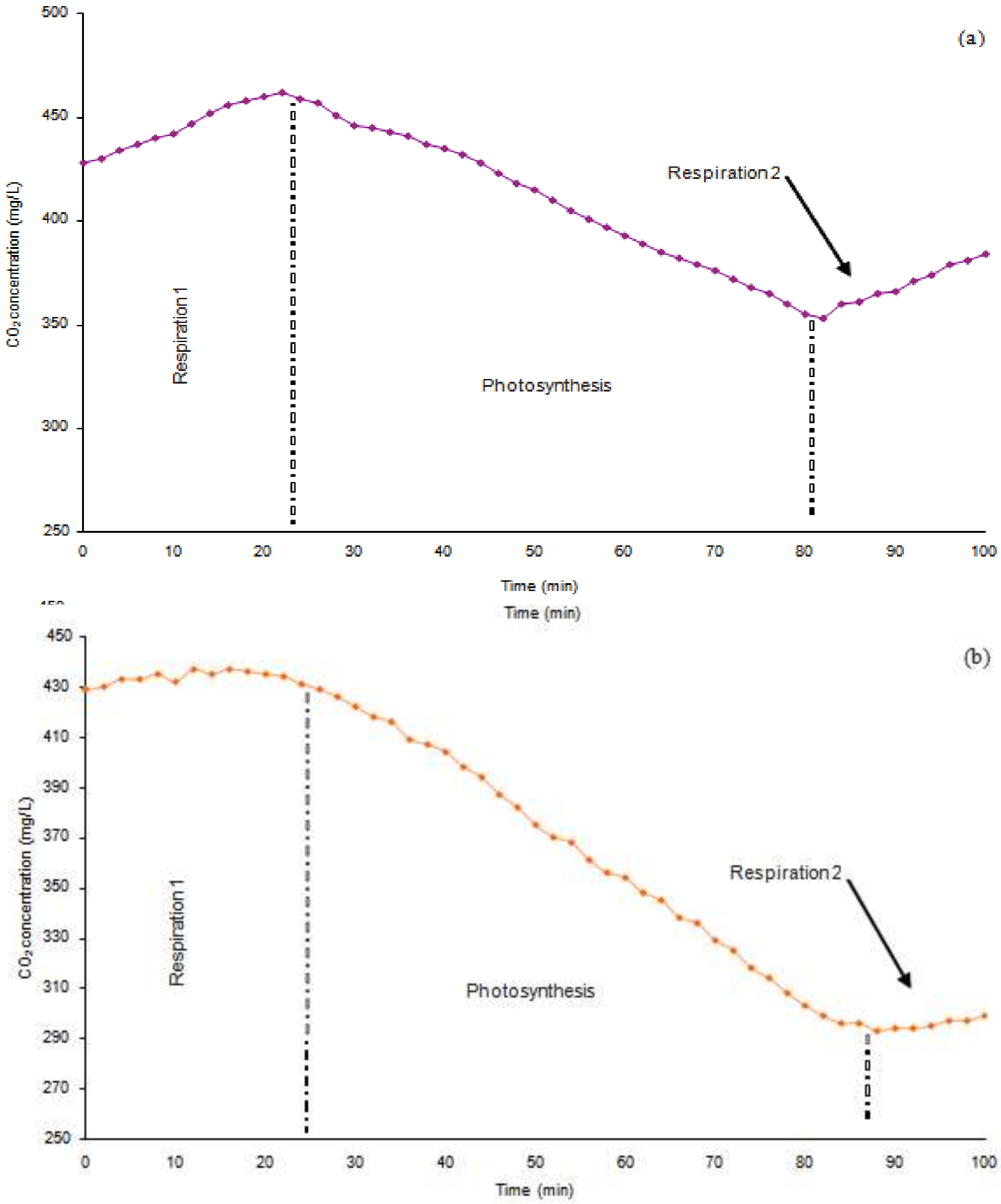

Fig. 3: CO_2 concentration as a function of time; (a) plants 96 h after exposure to 0.2 mg Cu/L and (b) control values are means of 2 essays

metalionsonduckweedspeciesareveryvariable (Table2).Thisiscausedbythedifferentduckweed species used and by the different test conditions, especially concerning the nutrient media as well as by the methods of evaluation (Appenrothet al., 2010).

TheCuremovaltreatmentshowedthattheaquatic 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 demonstratedthatduckweedspecies(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 Lemnagibba) were able to accumulate elevated amount of Cu in their tissues (Jain et al., 1989; Zayed et al., 1998;Ateret al., 2006; Megateliet 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 ofCu.Photosyntheticactivitiesincreasedinthepresence of 0.2 mg/L of cupric ions for different luminous energies. However, according to the study of Oletteet al. (2008), copper used as $CuSO_4$ (pesticide) inhibited the photosyntheticactivities of Lemna minor at concentrations of 12, 24, 40 and100µg/L.Foranexposuretimeof7days,the metal element present in the medium inhibited the photosynthesis of the aquatic plants by 0.4%; this inhibition reached 8% in the presence of100 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 Lemnatrisulca(another species of duckweed). Theseresultsagreewiththoseofthepresentstudy. The presence of 0.2 mg/L of Cu in the nutrient medium increased considerably the absorptionof

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	Ateret al., 2006
Lemnagibba	White nutritive Solution; pH=6.8	0.5 mg/L	Ateret al., 2006
Lemnagibba	Hoagland medium; pH=6.5	10^{-4} mg/L	Megateliet al., 2009
Lemna minor	1/10 Hoagland Solution; pH=6	1.6 mg/L	Kanoun-Bouléet al., 2009

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

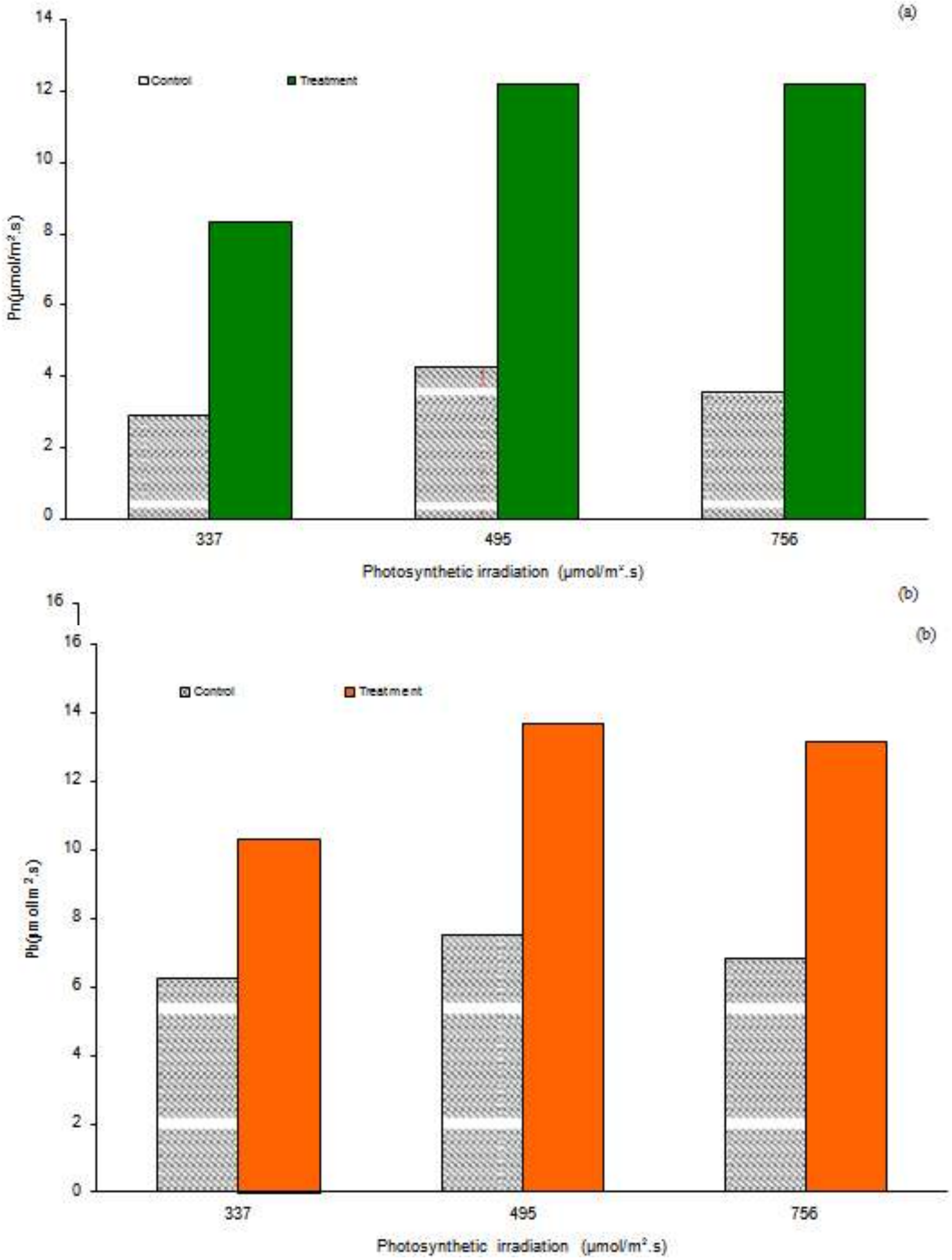

Fig. 4: Variation of (a) net photosynthesis and (b) brut photosynthesis, with the luminous energy. Treatment corresponds to plants exposed to 0.2 mg/L of Cu. Net and brut photosynthesis were calculated using the values on Table 1.

CO_2 by duckweed. Net photosynthesis increased approximatelythreetimescomparedtothecontrol. 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 elementincellularmetabolismandacatalytic 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 increaseinphotosyntheticactivitieswasobserved for different luminous energies. However, foran

irradiation≥495µmo/m^{2}s,saturationwasmainly 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.

AccordingtoWedgeandBurris(1982)theenergy saturationforLemnaminorrangedintheinterval 300-600 µmo/m^{2}s. Our results showed that the energy saturation for the species used in this study corresponded to a photosynthetic active irradiation of 495-750µmo/m^{2}s.

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 ourstudy 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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