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Influence of nutrient addition on growth and accumulation of cadmium and copper in *Lemna gibba*

Zeliha Leblebici*, Ahmet Aksoy and Fatih Duman

Erciyes University, Faculty of Art and Sciences, Department of Biology, 38039, Kayseri, Turkey
**E-mail: zleblebici@erciyes.edu.tr*

ABSTRACT

Aquatic plants have been identified as potentially useful for accumulating and bioconcentrating heavy metals. This study was developed to test the hypothesis that nutrient enrichment enhances the metal tolerance of floating macrophytes. Relative growth rates (RGR), photosynthetic pigments (chlorophyll a, b and carotenoid), malondialdehyde (MDA) content, and electrical conductivity (EC) were measured in *Lemna gibba* exposed to different cadmium and copper concentrations in laboratory conditions. Relative growth rates were negatively correlated with metal exposure, but nutrient addition suppressed this effect. Photosynthetic pigment levels were negatively correlated with metal exposures, and nutrient addition attenuated chlorophyll decrease in response to metal exposures. MDA content and EC also showed sharp increases at higher concentrations, indicating oxidative stress. This study indicates that nutrient enrichment increases the tolerance of *Lemna gibba* to metals, and that *Lemna gibba* is a suitable candidate for the phytoremediation of low-level copper and cadmium pollution.

Keywords: duckweed, nutrient enrichment, cadmium, copper, photosynthetic pigment, oxidative stress

1. INTRODUCTION

Over the past two decades, water contamination with heavy metals has become a severe problem. The presence of toxic metals in pond, ditch, and river water affects the lives and well-being of people who depend upon these sources for their daily water requirements (Rai *et al*., 2002). Consumption of aquatic foodstuffs contaminated with toxic metals may cause serious health hazards through food-chain magnification (Khan *et al*., 2000). A number of methods are available to remove toxic metals from water, including ion exchange, reverse osmosis, electrolysis, precipitation and adsorption. The latter is by far the most versatile and widely used. However, these methods have different efficiencies for different metals and can be prohibitively expensive, especially in large volumes, at low metal concentrations and in the presence of biota and suspended solids. The choice of purification method depends on the composition of the system, the pH of the water, redox conditions, and the nature of the pollutants.

It is well known that aquatic plants can accumulate metals taken in from the environment and concentrate them in trophic chains with accumulative effect (Outridge and Noller, 1991; Tremp and Kohler, 1995; Razinger *et al*., 2008). Significant copper accumulation has been observed in other aquatic plants such as *Ceratophyllum demersum* (Devi and Prasad, 1998), *Hydrilla verticillata* (Srivastava *et al*., 2006) *Lemna trisulca* (Prasad *et al*., 2001) *Vallisneria spiralis* (Vajpayee *et al*., 2005) and *Lemna minor* (Razinger *et al*., 2007; Razinger *et al*., 2009).

The use of aquatic macrophytes, such as water hyacinth, duckweed, and water lettuce, in wastewater treatment has attracted global attention in recent years (Reed *et al*., 1995; Gijzen and Khonner, 1997; Van der Steen *et al*., 1999; Vermaat and Hanif, 1998), as these plants can be grown on the surface of stabilization ponds, and may contribute to nutrient recovery from wastewater. Duckweed is a floating aquatic macrophyte belonging to the botanical family *Lemnaceae*, which can be found world-wide on the

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surface of nutrient-rich fresh and brackish waters (Zimmo, 2003).

Lee and Wang (2001) reported that an increase in nitrate concentration resulted in a significant increase in cadmium accumulation in *Ulva fasciata*. According to Hadad *et al*. (2007), nutrient enrichment either attenuated (chromium and zinc) or suppressed (nickel) root biomass decrease in response to metal exposure in *Salvinia herzogii*. Although heavy metal accumulation has been studied in *L. gibba* (El-Kheir *et al*., 2007; Demirezen, 2007; Demirezen *et al*., 2007), the effect of nutrient addition on this species' metal tolerance is unknown.

The aim of this study was to determine whether nutrient enrichment enhances the metal tolerance of floating macrophytes, which would therefore enable the growth of floating vegetation in constructed wetlands at metal concentrations that would otherwise inhibit plant viability.

2. MATERIALS AND METHODS

2.1 Plant material and treatment conditions

Plants of species *Lemna gibba* L. were obtained from a pond Soysalli-Kayseri (38°23′500″N, 035°21′919″E, 1075 m), Turkey. The chemical composition of the pond water was (mean ± standard deviation): pH = 6.4 ± 0.1; conductivity = 92 ± 8 μS cm^{-1}; NH_4^+ −N = 0.021 ± 0.01 mg L^{-1}; NO_3^- −N = 0.02 ± 0.001 mg L^{-1}, NO_2^- −N = 0.002 ± 0.001 mg L^{-1}, SO_4^{2-} = 0.2 ± 0.03 mg L^{-1}. Before metal treatment, plants were acclimatized for 5 days in laboratory conditions in lake water (23°C and 14 h photoperiod, 350 μmol m^2 s^{-1}). Plants were later transferred to deionized water with nutrient and different metal concentrations. Plants were treated with different concentrations of copper (0, 1, 2, 5, 10 mg L^{-1}) and cadmium (0, 0.5, 1, 2, 4 mg L^{-1}) and maintained in double deionized water in 500 mL conical flasks under the aforementioned conditions for periods of 1, 3, 5 and 7 days. They were grown in deionized water added with the nutrients in concentrations that mimicked natural pond water. Maximum concentrations of copper and cadmium were taken from Wenhua (2007). Plant growth rates in response to metal exposures were compared with exposures enriched with 5 mg L^{-1} *P* (KH_2PO_4), 5 mg L^{-1} NO_3^- −N (KNO_3) and SO_4^{2-} (K_2SO_4). Flasks without metals grown alongside each set of experimental groups served as controls. The experiments lasted 7 days, when the plants exposed to the highest metal concentrations developed chlorosis and necrosis. After harvesting, plants were washed with double deionized water. Plants were placed on blotting paper and allowed to drain for 5 min before weighing. All treatments were carried out in triplicate.

L. gibba relative growth rates were calculated in each group according to Hunt's equation:

$$R = \ln W_2 - \ln W_1 / T_2 - T_1,$$

where R is the relative growth rate (gg^{-1} d^{-1}), W_1 and W_2 are the initial and final fresh weights, respectively, and ($T_2 - T_1$) is the experimental period (Hunt, 1978).

2.2 Cadmium and copper quantification

Harvested plants were washed thoroughly with double deionized water, blotted and oven dried 80°C. Each sample was then digested with 10 mL pure HNO_3, using a CEM-MARS 5 (CEM Corporation Mathews, NC, USA) microwave digestion system (maximum power: 1,200 W, power: 100%, ramp: 20:00 min, pressure: 180 psi, temperature: 210°C and hold time: 10:00 min). After digestion, the volume of each sample was adjusted to 25 mL using double deionized water. Determinations of cadmium and copper concentrations in all samples were carried out by inductively coupled plasma optical emission spectroscopy (Varian-Liberty II, ICP-OES) (Demirezen, 2007). Peach leaves (NIST, SRM-1547) and CRM 039-050 were used as reference material and also all analytical procedures were performed for reference materials. Samples were analysed in triplicate.

2.3 Plant growth parameters

Plant biomass was measured on the basis of fresh weight. Photosynthetic pigments of treated and untreated plants (100 mg) were extracted in 80% chilled acetone in dark. After centrifugation at 10,000 g for 10 min, absorbance of the supernatant was taken at 450, 645 and 663 nm. The content of chlorophylls and carotenoids were estimated by as previously described (Witham, 1971).

2.4 Lipid peroxidation and electrical conductivity

Lipid peroxidation was determined by estimation of the malondialdehyde (MDA) content following the protocol of Health and Packer (1968). MDA and EC content were not determined for the treatments

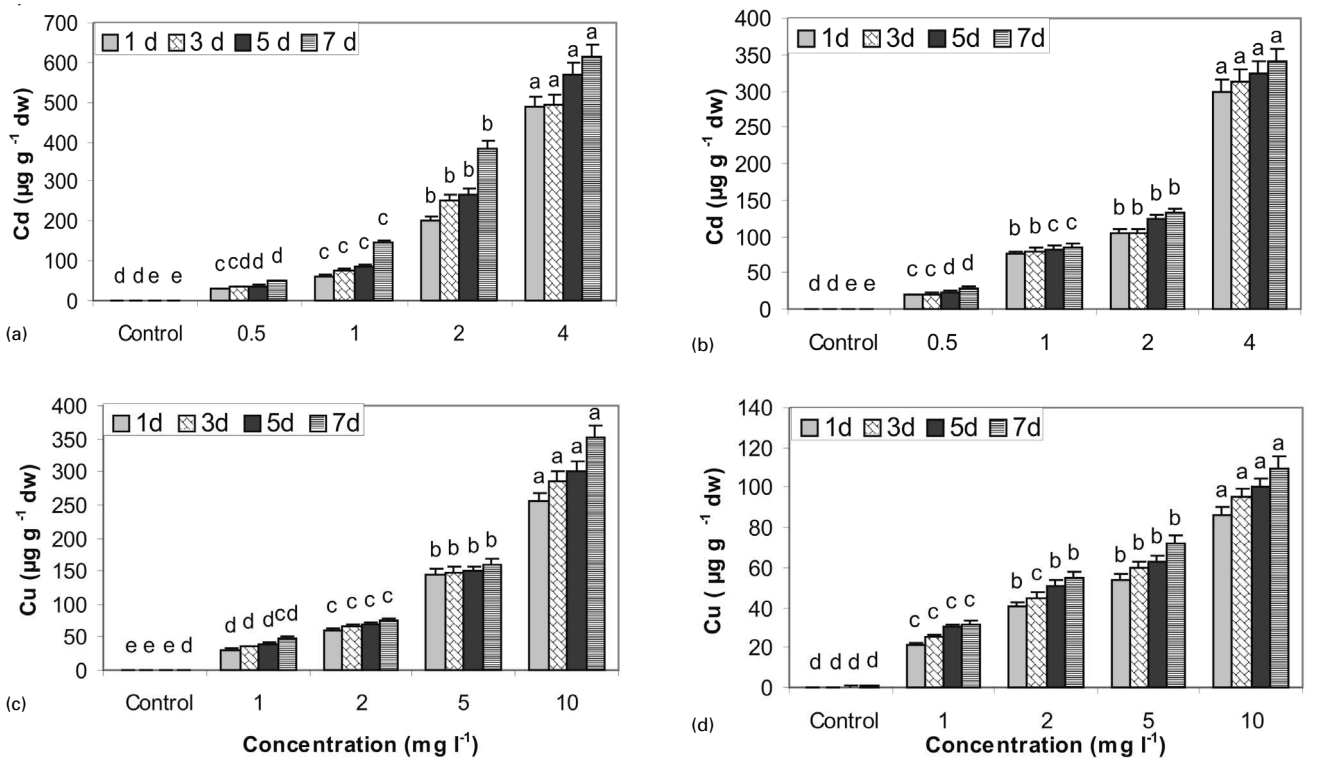

Figure 1 Accumulation of (**a**) Cd (**b**) Cd + nutrients (**c**) Cu and (**d**) Cu + nutrients by *Lemna gibba* exposed to different concentrations over various periods of time. All values are means of triplicates ± SD. ANOVA significance was set at $P \leq 0.01$. Different letters indicate significantly different values at a particular time point (DMRT, $P \leq 0.05$).

enriched with nutrients. Plant material (500 mg) was homogenized with 3 mL of 0.5% thiobarbituric acid in 20% trichloroacetic acid (w/v). The homogenate was incubated at 95°C for 30 min and reactions were stopped on ice. The samples were centrifuged at 10,000 g for 10 min and absorbance was recorded at 532 and 600 nm. The amount of MDA was calculated by subtracting non-specific absorbance at 600 nm from absorbance at 532 nm.

EC as a measure of ion leakage was determined according to Devi and Prasad (1998). Cadmium and copper exposed plants were washed with double deionized water and 500 mg of plant material was then transferred to 100 mL of deionized water for 24 h to facilitate maximum ion leakage. EC of the water was then recorded.

2.5 Statistical analysis

Two-way analysis variance (ANOVA) was done with all the data to confirm the variability of data and validity of results, and Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments. Statistical Package for the Social Sciences (SPSS) statistical program was used for statistical analysis.

3. RESULTS

3.1 Accumulation of heavy metals and effect on growth of plants

Plants accumulated high amounts of cadmium in a concentration-time dependent manner. The highest Cd accumulation was seen at a dose of 4 mg L^{-1}. The percent of the total Cd accumulated (615 µg g^{-1}) on day 7 at 4 mg L^{-1} was 79.1% on day 1, 80.6% on day 3, and 92.8% on day 5 (Figure 1a). In the groups enriched with nutrients, Cd accumulation at 4 mg L^{-1} was 87.9% on day 1, 90.8% on day 3, and 94.7% on day 5 of the total Cd accumulated (341.2 µg g^{-1}) on day 7 (Figure 1b). Maximum Cu accumulation was observed at a dose of 10 mg L^{-1}. The percent of the total Cu accumulated (351.6 µg g^{-1}) on day 7 at 10 mg L^{-1} was 72.7% on day 1, 81% on day 3, and 85.3% on day 5 (Figure 1c). In the groups enriched with nutrients, Cu accumulation at 10 mg L^{-1} was 78.1% on day 1, 86.3% on day 3, and 90.9% on day 5 of the total Cu accumulated (110 µg g^{-1}) on day 7 (Figure 1d).

Relative growth rates of *L. gibba* decreased in the presence of cadmium in a concentration dependent manner (Figure 2). However, the treatments enriched

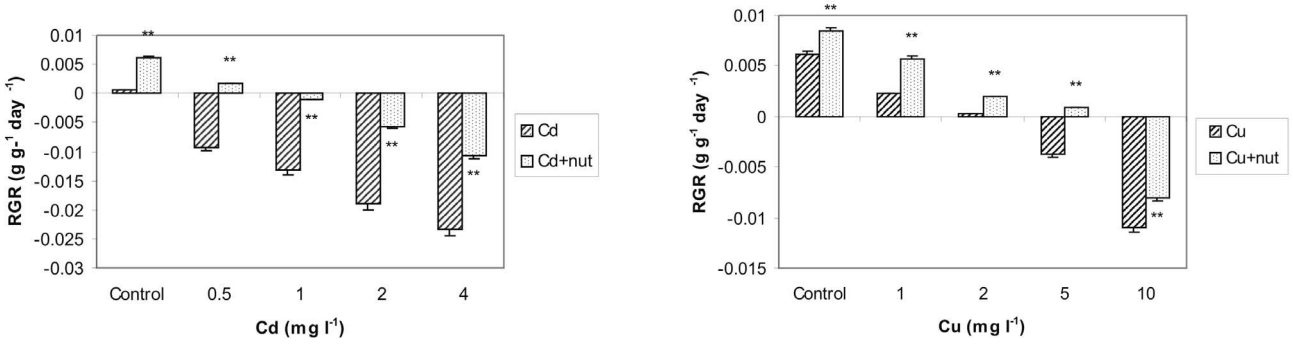

Figure 2 Relative growth rates of Cu and Cd treated plants (after 7 day). The bars represent standard deviation. ** ($P < 0.01$) indicate significant difference between the two treatment.

with nutrients did not show a similar correlation ($R = 0.980$). Relative growth rates of *L. gibba* decreased with copper in a concentration dependent manner (Figure 2), but the treatments enriched with nutrients did not show similar correlation ($R = 0.940$). Therefore, nutrient addition attenuated the decline in relative growth rates in response to metal exposure (Figure 2).

3.2 Effect of metals on photosynthetic pigments

Chlorophyll concentration in *L. gibba* was negatively correlated with cadmium and copper exposures (Tables 1 and 2). Nutrient enrichment attenuated the observed decrease in chlorophyll concentration by cadmium and copper exposures. Levels of chl *a* decreased in a cadmium concentration-dependent and time-depended manner, with a minimum value of 0.278 mg g^{-1} in the 4 mg L^{-1} Cd group. In the nutrient-enriched, 4 mg L^{-1} Cd group, chl *a* levels were reduced a minimum value of 0.349 mg g^{-1} fresh weight on day 7 (Table 1).

When *Lemna* fronds were exposed to 1 mg L^{-1} or higher concentrations Cu, a dose-depended decrease of chlorophyll pigments was also observed, with a minimum chl *a* value of 0.307 mg g^{-1} fresh weight on day 7 in 10 mg L^{-1} compared to 1.601 mg g^{-1} in controls (Table 2).

Table 1 Effect of different concentrations of Cd and nutrient on chlorophyll *a*, chlorophyll *b*, and carotenoid contents of *Lemna gibba*

	Cd				Cd + nutrient			
Kl *a*	1d	3d	5d	7d	1d	3d	5d	7d
Control	$1.63a \pm 0.1$	$1.60^{a} \pm 0.1$	$1.60^{a} \pm 0.1$	$1.60^{a} \pm 0.1$	$1.66^{a} \pm 0.1$	$1.62^{a} \pm 0.1$	$1.61^{a} \pm 0.1$	$1.61^{a} \pm 0.4$
0.5 mg L^{-1}	$1.10^{b} \pm 0.4$	$0.90^{b} \pm 0.2$	$0.64^{b} \pm 0.5$	$0.49^{b} \pm 0.5$	$1.33^{b} \pm 0.2$	$0.96^{b} \pm 0.5$	$0.72^{b} \pm 0.2$	$0.61^{b} \pm 0.2$
1 mg L^{-1}	$1.09^{b} \pm 0.5$	$0.80^{bc} \pm 0.1$	$0.56^{c} \pm 0.3$	$0.39^{c} \pm 0.6$	$1.30^{b} \pm 0.7$	$0.80^{bc} \pm 0.7$	$0.68^{b} \pm 0.4$	$0.50^{c} \pm 0.1$
2 mg L^{-1}	$0.99^{c} \pm 0.1$	$0.73^{c} \pm 0.3$	$0.39^{cd} \pm 0.2$	$0.29^{d} \pm 0.2$	$1.09^{c} \pm 0.3$	$0.75^{bc} \pm 0.6$	$0.63^{b} \pm 0.6$	$0.30^{d} \pm 0.5$
4 mg L^{-1}	$0.96^{c} \pm 0.2$	$0.69^{c} \pm 0.4$	$0.30^{d} \pm 0.1$	$0.27^{d} \pm 0.4$	$1.08^{c} \pm 0.4$	$0.50^{c} \pm 0.1$	$0.42^{c} \pm 0.2$	$0.34^{d} \pm 0.3$
Kl *b*								
Control	$0.86^{a} \pm 0.2$	$0.84^{a} \pm 0.1$	$0.85^{a} \pm 0.2$	$0.80^{a} \pm 0.2$	$0.91^{a} \pm 0.4$	$0.90^{a} \pm 0.2$	$0.87^{a} \pm 0.1$	$0.86^{a} \pm 0.1$
Cd 0.5 mg L^{-1}	$0.61^{b} \pm 0.5$	$0.49^{b} \pm 0.5$	$0.26^{b} \pm 0.4$	$0.23^{b} \pm 0.6$	$0.72^{ab} \pm 0.2$	$0.53^{b} \pm 0.4$	$0.35^{b} \pm 0.2$	$0.34^{b} \pm 0.4$
Cd 1 mg L^{-1}	$0.60^{b} \pm 0.6$	$0.44^{bc} \pm 0.3$	$0.20^{c} \pm 0.3$	$0.19^{c} \pm 0.4$	$0.62^{b} \pm 0.7$	$0.44^{bc} \pm 0.3$	$0.30^{c} \pm 0.3$	$0.30^{b} \pm 0.5$
Cd 2 mg L^{-1}	$0.55^{c} \pm 0.1$	$0.35^{c} \pm 0.2$	$0.20^{c} \pm 0.8$	$0.13^{d} \pm 0.8$	$0.60^{b} \pm 0.3$	$0.41^{c} \pm 0.8$	$0.28^{c} \pm 0.4$	$0.22^{c} \pm 0.6$
Cd 4 mg L^{-1}	$0.53^{c} \pm 0.2$	$0.23^{d} \pm 0.1$	$0.20^{d} \pm 0.1$	$0.13^{d} \pm 0.3$	$0.59^{c} \pm 0.4$	$0.28^{d} \pm 0.1$	$0.19^{d} \pm 0.1$	$0.20^{d} \pm 0.2$
Karotenoid								
Control	$1.00^{a} \pm 0.1$	$0.99^{a} \pm 0.2$	$0.98^{a} \pm 0.4$	$0.97^{a} \pm 0.4$	$1^{a} \pm 0.1$	$1.03^{a} \pm 0.2$	$1.02^{a} \pm 0.2$	$1.01^{a} \pm 0.4$
0.5 mg L^{-1}	$0.65^{b} \pm 0.1$	$0.62^{b} \pm 0.1$	$0.60^{b} \pm 0.2$	$0.56^{b} \pm 0.2$	$0.77^{b} \pm 0.2$	$0.76^{b} \pm 0.4$	$0.70^{b} \pm 0.4$	$0.65^{b} \pm 0.2$
1 mg L^{-1}	$0.63^{b} \pm 0.2$	$0.59^{b} \pm 0.3$	$0.54^{c} \pm 0.1$	$0.50^{c} \pm 0.3$	$0.74^{b} \pm 0.4$	$0.74^{b} \pm 0.3$	$0.63^{b} \pm 0.1$	$0.62^{b} \pm 0.4$
2 mg L^{-1}	$0.52^{b} \pm 0.4$	$0.51^{b} \pm 0.5$	$0.48^{c} \pm 0.4$	$0.41^{cd} \pm 0.1$	$0.68^{b} \pm 0.4$	$0.62^{b} \pm 0.1$	$0.52^{c} \pm 0.1$	$0.44^{c} \pm 0.1$
4 mg L^{-1}	$0.4^{b} \pm 0.1$	$0.39^{c} \pm 0.1$	$0.32^{d} \pm 0.6$	$0.27^{d} \pm 0.4$	$0.64^{c} \pm 0.2$	$0.55^{c} \pm 0.5$	$0.45^{d} \pm 0.5$	$0.38^{d} \pm 0.5$

All values are means of triplicates $\pm$ SD. ANOVA significance was set at $P \leq 0.01$. Different letters indicate significantly different values at a particular time point (DMRT, $P \leq 0.05$).

Table 2 Effect of different concentrations of Cu and nutrients on chlorophyll *a*, chlorophyll *b*, and carotenoid contents of *Lemna gibba*

	Cu				Cu + nutrient			
Kl *a*	1d	3d	5d	7d	1d	3d	5d	7d
Control	$1.63^{a} \pm 0.1$	$1.60^{a} \pm 0.2$	$1.60^{a} \pm 0.4$	$1.60^{a} \pm 0.1$	$1.66^{a} \pm 0.2$	$1.62^{a} \pm 0.1$	$1.61^{a} \pm 0.1$	$1.61^{a} \pm 0.2$
1 mg L^{-1}	$1.13^{ab} \pm 0.3$	$1.01^{b} \pm 0.1$	$0.75^{b} \pm 0.1$	$0.53^{b} \pm 0.3$	$1.41^{ab} \pm 0.2$	$1.06^{b} \pm 0.5$	$0.97^{b} \pm 0.4$	$0.76^{b} \pm 0.3$
2 mg L^{-1}	$1.01^{b} \pm 0.2$	$0.80^{b} \pm 0.3$	$0.73^{b} \pm 0.2$	$0.52^{b} \pm 0.4$	$1.02^{b} \pm 0.1$	$0.92^{b} \pm 0.2$	$0.92^{b} \pm 0.6$	$0.73^{b} \pm 0.5$
5 mg L^{-1}	$0.76^{b} \pm 0.4$	$0.73^{b} \pm 0.4$	$0.68^{b} \pm 0.4$	$0.46^{c} \pm 0.2$	$1.00^{b} \pm 0.3$	$0.90^{b} \pm 0.3$	$0.78^{c} \pm 0.7$	$0.73^{c} \pm 0.3$
10 mg L^{-1}	$0.75^{b} \pm 0.2$	$0.62^{c} \pm 0.2$	$0.53^{c} \pm 0.6$	$0.30^{d} \pm 0.8$	$0.99^{b} \pm 0.5$	$0.76^{c} \pm 0.6$	$0.77^{c} \pm 0.3$	$0.55^{d} \pm 0.1$
Kl *b*								
Control	$0.86^{a} \pm 0.4$	$0.84^{a} \pm 0.1$	$0.84^{a} \pm 0.1$	$0.80^{a} \pm 0.4$	$0.91^{a} \pm 0.3$	$0.90^{a} \pm 0.1$	$0.90^{a} \pm 0.4$	$0.88^{a} \pm 0.3$
1 mg L^{-1}	$0.62^{b} \pm 0.2$	$0.56^{b} \pm 0.3$	$0.36^{b} \pm 0.3$	$0.32^{b} \pm 0.2$	$0.77^{ab} \pm 0.1$	$0.58^{b} \pm 0.3$	$0.45^{b} \pm 0.1$	$0.41^{b} \pm 0.1$
2 mg L^{-1}	$0.46^{bc} \pm 0.1$	$0.38^{c} \pm 0.4$	$0.36^{b} \pm 0.2$	$0.31^{b} \pm 0.3$	$0.56^{b} \pm 0.2$	$0.50^{bc} \pm 0.4$	$0.41^{b} \pm 0.2$	$0.40^{b} \pm 0.5$
5 mg L^{-1}	$0.42^{bc} \pm 0.3$	$0.36^{c} \pm 0.6$	$0.35^{b} \pm 0.4$	$0.28^{c} \pm 0.5$	$0.55^{b} \pm 0.4$	$0.49^{bc} \pm 0.5$	$0.40^{b} \pm 0.3$	$0.31^{c} \pm 0.6$
10 mg L^{1}	$0.38^{c} \pm 0.1$	$0.35^{c} \pm 0.7$	$0.27^{c} \pm 0.1$	$0.12^{d} \pm 0.5$	$0.55^{b} \pm 0.3$	$0.40^{c} \pm 0.2$	$0.34^{c} \pm 0.1$	$0.20^{d} \pm 0.4$
Karotenoid								
Control	$1.0^{a} \pm 0.4$	$0.99^{a} \pm 0.2$	$0.98^{a} \pm 0.2$	$0.97^{a} \pm 0.1$	$1.01^{a} \pm 0.4$	$1^{a} \pm 0.2$	$1^{a} \pm 0.2$	$1^{a} \pm 0.2$
1 mg L^{-1}	$0.62^{b} \pm 0.2$	$0.61^{b} \pm 0.6$	$0.56^{b} \pm 0.3$	$0.44^{b} \pm 0.2$	$0.76^{ab} \pm 0.2$	$0.67^{b} \pm 0.4$	$0.67^{b} \pm 0.7$	$0.62^{b} \pm 0.4$
2 mg L^{-1}	$0.56^{b} \pm 0.8$	$0.42^{c} \pm 0.1$	$0.37^{c} \pm 0.4$	$0.31^{c} \pm 0.3$	$0.66^{b} \pm 0.1$	$0.58^{bc} \pm 0.1$	$0.54^{c} \pm 0.1$	$0.52^{c} \pm 0.2$
5 mg L^{-1}	$0.54^{b} \pm 0.1$	$0.33^{c} \pm 0.5$	$0.33^{c} \pm 0.1$	$0.28^{c} \pm 0.4$	$0.64^{b} \pm 0.3$	$0.54^{bc} \pm 0.2$	$0.48^{c} \pm 0.2$	$0.39^{cd} \pm 0.5$
10 mg L^{-1}	$0.42^{c} \pm 0.3$	$0.29^{d} \pm 0.4$	$0.24^{d} \pm 0.5$	$0.24^{d} \pm 0.2$	$0.65^{b} \pm 0.1$	$0.45^{c} \pm 0.4$	$0.33^{d} \pm 0.2$	$0.25^{d} \pm 0.6$

All the values are means of triplicates $\pm$ SD. ANOVA significance was set at $P \leq 0.01$. Different letters indicate significantly different values at a particular time point (DMRT, $P \leq 0.05$).

3.3 Effect on malondialdehyde content and electrical conductivity

Both MDA and EC content increased gradually with increasing concentrations of both Cd and Cu, though Cd had a more significant effect (Figures 3 and 4). Maximum increases in MDA and EC (11-fold and 8-fold higher than control, respectively) were observed in 4 mg L^{-1} Cd treated plants on day 7 (Figure 3). Maximum increase in MDA and EC (5-fold and 2-fold higher than control, respectively) were observed after 7 days exposure to 10 mg L^{-1} Cu (Figure 4).

4. DISCUSSION

In the present study, high accumulations of Cd and Cu were observed in *L. gibba* over 7 days. Nutrient enrichment resulted in decreased accumulation of Cd and Cu in growing plants over the time. Growth rates of *L. gibba* decreased with increasing cadmium and copper concentrations. However, nutrient enrichment led to increased growth rates at Cd and Cu concentrations that impaired growth in the non-enriched groups. Cd had a stronger effect on growth rates

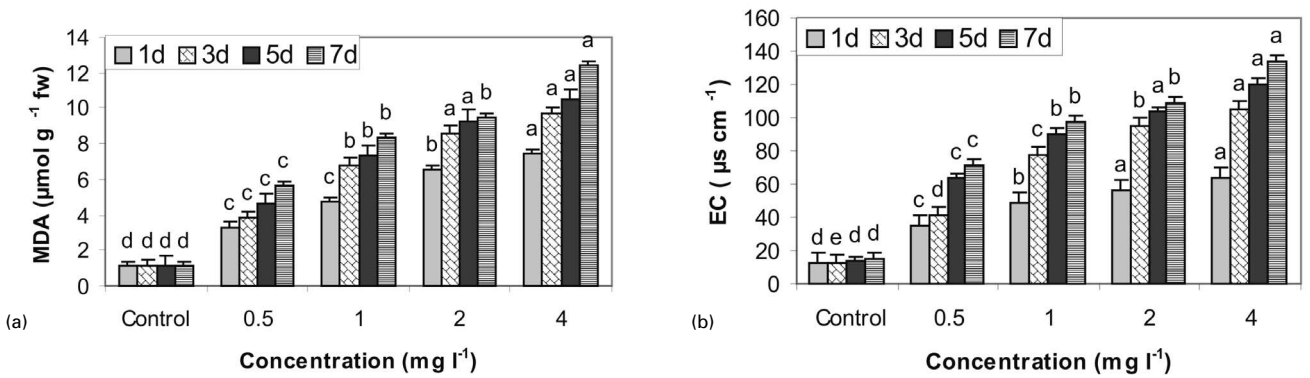

Figure 3 Effect of different concentrations of Cd on MDA content (**a**) and EC (**b**) of *L. gibba*. All the values are means of triplicates $\pm$ SD. ANOVA significance was set at $P \leq 0.01$. Different letters indicate significantly different values at a particular time point (DMRT, $P \leq 0.05$).

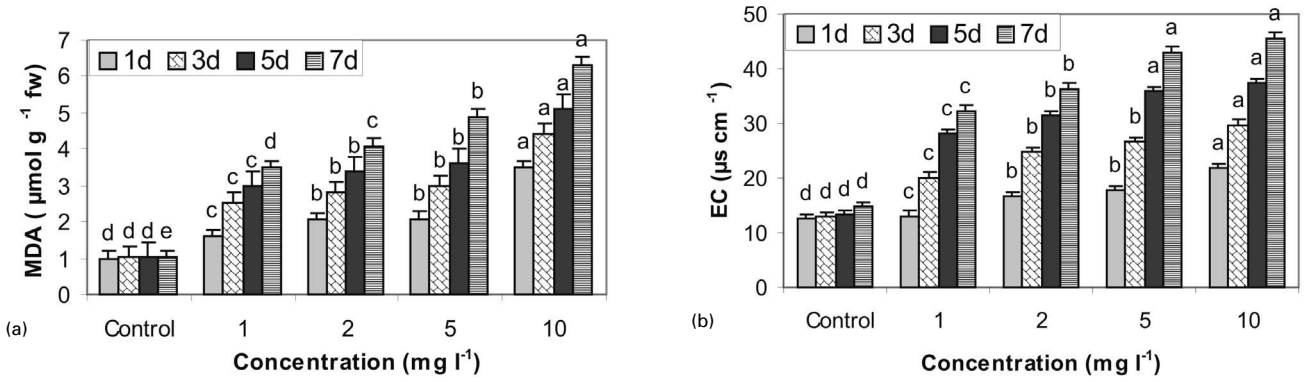

Figure 4 Effect of different concentrations of Cu on MDA content (**a**) and EC (**b**) of *L. gibba*. All the values are means of triplicates ± SD. ANOVA significance was set at $P \leq 0.01$. Different letters indicate significantly different values at a particular time point (DMRT, $P \leq 0.05$).

than Cu. Low concentrations of Cu actually increased plant growth rates, while higher concentrations had negative effects.

Appenroth *et al*. (2008) investigated the modification of chromate toxicity by sulfate in duckweeds. The authors explained that sulfate influences the toxicity of chromate mainly by chromate uptake, with negligible impact on other physiological processes. Rahman *et al*. (2008) reported on the uptake of arsenate in *S. polyrrhiza* and its interactions with PO_4^{3-} and Fe ions. Their study found that arsenate uptake in *S. polyrrhiza* occurred through the phosphate uptake pathway and by physico-chemical adsorption on Fe-plaques of plant surfaces as well. Similarly, Hadad *et al*. (2007) found that nutrient enrichment enabled *Salvinia hergozii* growth at Zn and Ni exposures that impaired growth in plants without nutrient addition. Göthberg *et al*. (2004) found high metal concentrations in *Ipomea aquatica* cultivated for human consumption in freshwater courses near Bangkok receiving variable amounts of cultural nutrient loads. The authors proposed fertilization as a means to attenuate metal accumulation. Their experimental work, in agreement with our findings, showed that nutrient enrichment led to increased *I. aquatica* tolerance to cadmium, lead and mercury. Lead and mercury accumulation decreased with increasing concentrations of nutrients, as was observed cadmium and copper in the present study.

Chlorophyll concentrations in *L. gibba* were negatively correlated with Cd and Cu accumulation, but nutrient enrichment mitigated the decrease in chlorophylls in both cases. Cd, as a non-essential metal ion, was found to have more of a toxic effect on pigments in *L. gibba* fronds than Cu did. Even at low concentrations, *e.g.* 0.5 mg L^{-1}, Cd had adverse effects on photosynthetic pigment levels. Cd ions may inhibit the formation of chlorophylls by interfering with protochlorophyllide reduction and the synthesis of aminoevulinic acid, or may interfere with various steps of the Calvin cycle, resulting in the inhibition of photosynthetic CO_2 fixation (Mohan and Hosetti, 1997; Weigel, 1985). In addition, Cd could damage the chloroplast envelope and thylakoid *via* increased production of free radicals (Halliwell and Gutteridge, 1984). In fronds treated with Cu, a concentration of 1 mg L^{-1} was sufficient to cause a decrease in pigment molecules, indicating that although copper is an essential micronutrient for the growth and development of plants, it could be a strong inhibitor of photosynthesis at high levels (Frankart *et al*., 2002; Vavilin *et al*., 1995). The loss in chlorophyll content could be due to peroxidation of chloroplast membranes or replacement of magnesium in chlorophyll molecules by Cu ions (Mal *et al*., 2002; Sandmann and Boger, 1980). Razinger *et al*. (2007) investigated antioxidative responses of duckweed (*Lemna minor* L.) to short-term copper exposure. Their study found that the intensity of delayed chlorophyll fluorescence decreased indicating reduced photosynthesis of plants under $CuSO_4$ stress.

MDA is the decomposition product of polyunsaturated fatty acids of biological membranes, and its increase indicates that plants are in a state of oxidative stress (Wenhua *et al*., 2007). Cell membrane stability has been widely used to distinguish stress-tolerant from susceptible cultivars of many crops (Premachandra *et al*., 1991) and, in some cases, higher membrane stability could be correlated with better performance (Sudhakar *et al*.,

2001). The oxidative stress imposed to the plants after Cd and Cu exposure was evident from the significant increase in MDA and EC at higher concentrations. Fronds treated with Cd were experienced higher oxidative stress than those treated with Cu. Because Cd had a stronger effect on growth rates than Cu and plants exposed to the highest Cd concentrations developed more chlorosis and necrosis than Cu. Contrary to our results, Razinger *et al*. (2007) and Razinger *et al*. (2008) found that Cu elicits a stronger oxidative stress as compared to Cd and is therefore more toxic to the test plants. In accordance with our observations, similar results were obtained after Cu treatment in *Ceratophyllum demersum* L. (Devi and Prasad, 1998; Mishra *et al*., 2006) and *Pistia stratiotes* (Sinha *et al*., 2003).

5. CONCLUSIONS

We have investigated the toxic effects of cadmium and copper on *L. gibba*, and showed that nutrient enrichment increased the tolerance of *L. gibba* to metal contamination. This effect has important implications in the use of constructed wetlands for industrial wastewater treatment. Many metallurgic industrial processes produce wastewater containing high concentrations of metal ions. Increased tolerance may be useful for wastewater treatment by allowing macrophyte growth at metal concentrations that would otherwise impair their development. Nutrient addition will thus aid metal removal by increasing macrophyte production, leading to a higher metal uptake by the macrophyte biomass, and also by enhancing overall biological activity, reaching a higher metal retention in the detrital fractions.

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