

GROWTH RESPONSE OF THE DUCKWEED *LEMNA MINOR* TO HEAVY METAL POLLUTION

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Received 20 March 2009; revised 18 July 2009; accepted 2 August 2009

ABSTRACT

To assess the tolerance and effect of heavy metals pollution on the duckweed *Lemna minor*, the aquatic plants were exposed to different concentrations of copper (Cu), nickel (Ni), cadmium (Cd) and zinc (Zn) in a quarter Coïc and Lessaint solution at pH = 6.1 (± 0.1) and under a daily regime of 16 h light ($101 \mu\text{mol}/\text{m}^2.\text{s}^1$). Copper at 0.2 mg/L and nickel at 0.5 mg/L promoted the growth of *Lemna* fronds. At higher concentrations, Cu and Ni inhibited the growth of duckweed; the EC_{50} (concentration causing 50% inhibition) were 0.47 mg/L for Cu and 1.29 mg/L for Ni. Cadmium and zinc decreased by 50% the growth of fronds when the medium contained respectively 0.64 and 5.64 mg/L (EC_{50}). Duckweed tolerated Cu, Ni, Cd and Zn at concentrations of 0.4, 3.0, 0.4 and 15.0 mg/L respectively without showing any visible signs of toxicity (chlorosis, frond disconnection and necrosis). On the basis of visible symptoms and the EC_{50} values, the toxicity of the metals on *Lemna minor* was in decreasing order of damage: Cu > Cd > Ni > Zn. It was concluded that the duckweed *Lemna minor* is very sensitive to copper and cadmium pollution.

Key words: Growth, *Lemna minor*, Heavy metal, Tolerance, Toxicity

INTRODUCTION

Heavy metal pollution is an important environmental problem in the world. In contrast with most organic materials, metals cannot be transformed by microorganisms and therefore accumulate in water, soil, bottom sediments and living organisms (Miretzky *et al.*, 2004). These pollutants are present in the environment as natural components or as a result of anthropogenic activities (agricultural and industrial activities). Industries such as smelters, metal refineries and mining operations have been indicated as major sources of metal release into the environment (Gardea-Torresdey *et al.*, 1997; Srivastava *et al.*, 2007). Most of the heavy metals are toxic or carcinogenic in nature and pose a threat to human health and the environment (Shakibaie *et al.*, 2008; Vinodhini and Narayanan, 2009). Copper (Cu), nickel (Ni), cadmium (Cd) and zinc (Zn) are considered as toxic since they cause deleterious effect in plants, animals and humans. The metals are responsible for many alterations of the plant cell (photosynthesis, chlorophyll production,

pigment synthesis and enzyme activity) (Teisseire and Vernet, 2000; Prasad *et al.*, 2001; Vaillant *et al.*, 2005; Kanoun-Boulé *et al.*, 2008; Zhou *et al.*, 2009).

Duckweeds are aquatic plants which often form dense floating mats in eutrophic ditches and ponds (Driever *et al.*, 2005). The macrophytes are fast growing, adapt easily to various conditions and can tolerate a wide pH range (4.5-8.3) (Environnement Canada, 1999). The small size, simple structure and rapid growth make duckweed very suitable for toxicity tests (OECD, 2002). It is also used in wastewater treatment to remove mineral and organic contamination and radionuclides (Davis *et al.*, 2002; Susarla *et al.*, 2002).

The present study investigates the effect of Cu, Ni, Cd and Zn on the duckweed (*Lemna Minor*) to assess tolerance of this aquatic plant to metal pollution. This effect is determined from the concentration that results in a 50% reduction in the growth of duckweed (EC_{50}), the lowest observed effect concentration (LOEC) and the no-observed effect concentration (NOEC).

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MATERIALS AND METHODS

Plant material and culture medium

Lemna minor native to the Rhône Alpes region of France was collected from a natural pond and placed in plastic aquaria containing quarter Coïc and Lessaint solution at pH = 6.1 ± 0.1 (Khellaf and Zerdaoui, 2009). A continuous aeration system provides oxygen for the *Lemna* fronds and prevents root fungal diseases (Kamal *et al.*, 2004). Aquatic macrophytes were cultured at 22 °C with a 16 h photoperiod (101 µmol of photons/m².s).

Toxicity test

The test protocols were derived from the standard draft guideline 221 (OECD, 2002). Duckweed growth was measured after four days of exposure to different concentrations of metals. The metals used for this study were supplied as CuSO₄.5H₂O,

NiCl₂.6H₂O, CdCl₂ and ZnSO₄.7H₂O.

Nine to twelve *Lemna* fronds were gently placed in crystallising cups (7 cm high, 5 cm I.D.) containing 100 mL of metal solution diluted in the nutrient medium. The dose- response tests were performed in conditions similar to those of the plant cultures but without aeration (the treatment duration was four days). Preliminary assays defined the variation of concentrations for each metal. The nominal concentrations selected were based on the response of the fronds in the presence of the metal ions and thus, the upper limit of the concentration ranges were defined when the necrosis was observed. The nominal and the interpolated concentrations of the tested elements are shown in Table 1. Control treatment (without metal) was necessary to compare the results. For the four metals, each bioassay was performed in triplicate.

Table 1: Nominal and interpolated concentration ranges of metal toxicity

Metal	Range of the nominal concentration (mg/L)	concentration in the medium (µg/L)	Range of the interpolated concentration (mg/L)
Copper	0.1 to 1.0	9.98	0.11 to 1.01
Nickel	0.5 to 6.0	4.34	0.50 to 6.00
Cadmium	0.1 to 1.5	0	0.1 to 1.5
Zinc	0.5 to 25.0	16.34	0.51 to 25.01

Data analysis

Lemna fronds were observed daily for toxicity symptoms (chlorosis, necrosis and frond disconnection). Total frond area was determined on days 0 and 4. The biomass based on the total frond area was determined by image analysis. A video camera captured each cup and then the image was analysed by the software scion image (Scion image, 2004. www.scioncorp.com) to determine the total frond area. A plant growth index was calculated as follows:

$$\text{Growth index} = \frac{\text{Biomass}(t = 4 \text{ days})}{\text{Biomass}(t = 0)} \quad (1)$$

The doubling time of frond number, T_d , was calculated according to the following equation:

$$T_d = \frac{\ln 2}{\mu} \quad (2)$$

Where $\mu[\text{day}^{-1}]$ is the average growth rate in the control,

$$\mu_{ij} = \frac{\ln N_j - \ln N_i}{t_j - t_i} \quad (3)$$

Where: $i = 0$, $j = 4$, N is frond number and t is the time.

Comparison between control and treatments was statistically analyzed by one-way ANOVA; the validity of investigation was expressed as probability value of $p < 0.05$.

RESULTS

Symptoms of toxicity

The metals Cu, Ni, Cd and Zn caused visible damage to duckweed at concentrations of 0.5, 4, 0.5 and 18 mg/L, respectively. Chlorosis (a progression of green to yellow colour on the frond) and frond disconnection (detachment

of fronds from colonies) were toxicity signs observed at the start of exposing *Lemna* fronds to the metal elements. These signs progressed to necrosis at the end of the treatment with Cu, Ni and Cd (the necrosis was not observed in the case of Zn for the concentrations selected in this work). Copper was a very toxic metal for *L. minor*. At low concentrations of Cu (≥ 0.5 mg/L), fronds were chlorotic and some fronds separated from the others (necrosis was observed after 24 h of exposure of plants to 0.5 mg/L of Cu). Cadmium was also very toxic for the plants; 0.5 mg Cd/L in the culture medium, caused visible damage one day after the treatment. Duckweed, as the visible responses, tolerated Ni and Zn up to 3 and 15 mg/L, respectively. Zinc was thus visibly the less toxic element for the duckweed since the toxicity symptoms (light decolorization and separation of the fronds) were observed only at 18 mg Zn/L and at higher concentrations.

Concentration-growth relation

The concentration-growth index curves are presented in Fig. 1. The standard deviation was 0.21, 0.19, 0.14 and 0.099 on average, respectively for Cu, Ni, Cd and Zn. Copper and nickel had similar effects on *L. minor*. The two elements stimulated the growth for concentrations between 0 and 0.2 mg/L for Cu and 0 and 0.5 mg/L for Ni. Over these values, the growth index decreased until a minimal value (indicated by broken line on Fig. 1) corresponding to 0.5 mg Cu/L and 2 mg Ni/L. For concentrations ≥ 0.5 mg Cu/L and ≥ 2 mg Ni/L, treatments were not statistically different from each other ($p > 0.05$). Cadmium and zinc were inhibitory for *L. minor* for all the selected concentrations. The growth depended on the initial concentration metal in the solution and showed a monotonic decline with the concentration. At 0.6 mg Cd/L and 15 mg Zn/L, *Lemna* growth decreased by 46% and 65%, respectively.

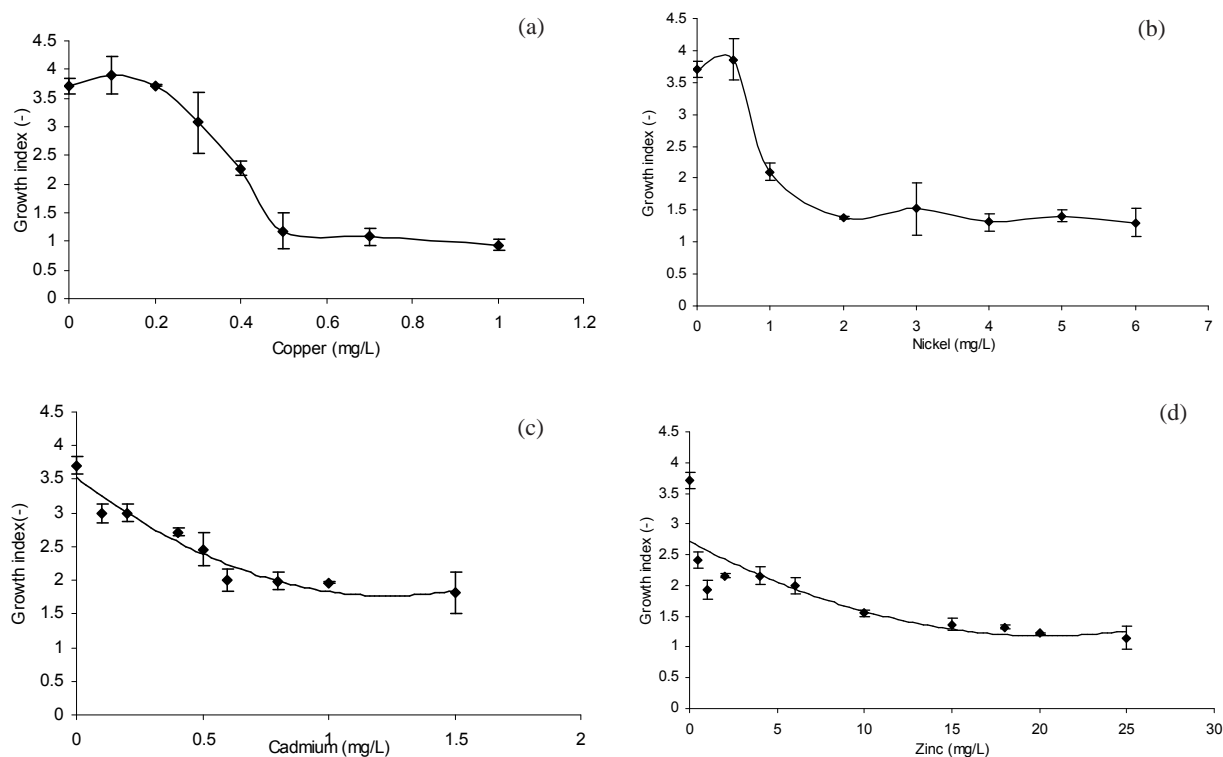


Fig. 1: The effect of various metal concentrations on the growth of *L. minor*.
(a) Copper, (b) Nickel, (c) Cadmium, (d) Zinc. Vertical bars indicate standard deviation, $n = 3$.

Growth inhibition parameters

For metal toxicity testing to be valid, the doubling time of the frond number in the control, T_d , must be less than 2.5 days (OECD, 2002). The observed T_d was 1.9 days. The calculated concentration that results in a 50 % reduction in the growth of *Lemna* (EC_{50}) in the presence of Cu, Ni, Cd and Zn were interpolated from linear regression

of growth index as a function of concentration. The metals Cu, Ni, Cd and Zn decreased the fronds growth index by 50 % when the medium contained respectively 0.47, 1.29, 0.91 and 5.64 mg/L ($R^2 = 0.89 - 0.95$). The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) are also estimated. The parameters values are shown in Table 2.

Table 2: Inhibition parameters of *L. minor* growth in the presence of copper, nickel, cadmium and zinc

Metal	EC_{50} (mg/L)	R^2	LOEC (mg/L)	NOEC (mg/L)
Copper	0.47	0.953	0.3	0.2
Nickel	1.29	0.951	1	0.5
Cadmium	0.91	0.892	0.1	*
Zinc	5.64	0.929	0.5	*

*Not observed

DISCUSSION

In this work, it was aimed to compare the growth response to four heavy metals (Cu, Ni, Cd and Zn) of the hydrophyte *L. minor*. Metal phytotoxicity was assessed through the visible symptoms of toxicity and determination of the concentration that results in a 50% reduction in the growth of duckweed (EC_{50}). On the basis of these two parameters, the toxicity of metal in *Lemna* biomass was in decreasing order of damage: Cu > Cd > Ni > Zn.

Copper when present in the nutrient solution at concentrations ≤ 0.2 mg/L (NOEC) was essential to develop the fronds of *L. minor*. At a concentration higher than 0.4 mg/L, Cu caused the photosystem alteration by reducing electron transport. This effect was manifest by a rapid development of chlorosis; after exposing *Lemna* to the cupric ions (2 or 3 hours), the fronds colour changed from green to yellow and some fronds were separated from the colonies. According to Teisseire and Vernet (2000), $CuSO_4$ at 10 μ M was inhibitory for *L. minor*; at this concentration, activities of glutathione S-transferase and glutathione

reductase were inhibited. However, Zayed *et al.* (1998) used *L. minor* for the phytoaccumulation of copper in quarter-strength Hoagland's solution at pH 6; the lowest Cu concentration causing > 10% growth reduction was 5 mg/L.

L. minor tolerated Ni up to 3 mg/L but had an optimal growth at 0.5 mg/L (NOEC). Concentrations higher than 3 mg/L were toxic for the plants and decreased considerably the growth. Zayed *et al.* (1998) demonstrated that *L. minor* fronds accumulated low amounts of Ni in their tissues (1.79 g/kg in a medium containing 10 mg Ni/L). However, Axtell *et al.* (2003) reported the absorption of lead (Pb) and Ni by *L. minor*; duckweed showed a preference to remove Ni (the removal rate was equivalent to 82% in an aqueous solution containing 5 mg Ni/L and 10 mg Pb/L). Authors did not report the effect of Ni on *L. minor* tissues.

Cadmium inhibited duckweed growth at all concentrations selected for this investigation. The inhibition consisted of the reduction of the biomass; at concentrations up to 0.4 mg/L, Cd

caused reduced growth rate, separation of the colonies and changes in fronds colour. Zayed *et al.* (1998) reported that Cd was toxic for *L. minor* at 5 mg/L in a Hoagland solution at pH = 6. At 10 mg Cd/L, frond growth decreased by 25% when compared to the control. These results were different from those of the present study. We found that a concentration of 0.91 mg Cd/L in the growth medium reduced the growth index by 50%. According to Prasad *et al.* (2001), the toxic effect of Cd on *Lemna trisulca* (a duckweed species) was explained by a reduction of the respiration rate due to the alteration of cytoplasm and mitochondrial structures.

Zinc was more tolerated by duckweed which exhibited no visible toxicity, but showed a reduction of biomass and growth rate, for concentrations between 0.5 and 15 mg/L. The growth index and frond number of producing were more sensitive than visible signs of toxicity. The inhibitor effect of Zn was investigated by several studies. Vaillant *et al.* (2005) demonstrated that Zn induced an important reduction in growth, photosynthesis and chlorophyll production. The activity of the nitrogenase enzyme in *Azolla filiculoides* (water fern) was entirely inhibited in the presence of Zn (Sela *et al.*, 1989).

To conclude, the metals Cu, Ni, Cd and Zn were tolerated by *L. minor* at 0.4, 3, 0.4 and 15 mg/L, respectively. At these concentrations, biomass and growth rate were affected without visible toxicity signs (chlorosis, frond disconnection and necrosis). Thus, these aquatic macrophytes could survive in a medium containing elevated concentrations of Ni and Zn (3 mg Ni/L or 15 mg Zn/L). Copper and cadmium are considered as toxic for *L. minor*. At low concentration (0.5 mg/L) the plants exhibited chlorosis and frond disconnection which progressed to necrosis (dead fronds).

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Prof J.C. Leclerc and Doctor O. Faure (LEPA, Saint-étienne, France) for their help and assistance.

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