Tolerance to Heavy metals in the duckweed, *Lemna minor*

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1. Introduction

Duckweed is a variety of aquatic plant floating at the water surface. It is fast growing and adapts easily to various aquatic conditions. Wetlands and ponds are the most common sites to find duckweed. The macrophytes can tolerate a wide pH range but survive best between pH of 4.5 to 8.3 (Environnement Canada, 1999). The small size of *Lemna*, its simple structure and rapid growth make duckweed very suitable for toxicity tests (AFNOR, 1996; OECD, 2002). It is also used in wastewater treatment to remove mineral and organic chemicals and radionuclides (Susarla et al., 2002).

The purpose of this study was to investigate the tolerance to Cd, Cu, Ni and Zn in the duckweed *L. minor* as a first step to determine the use of this aquatic species to remove heavy metals from polluted water. The metal-related effects on the growth allowed to ascertain the concentration which caused the minimal growth and the inhibition parameters of *Lemna* growth (EC_{50}, LOEC and NOEC).

2. Experimental method

Duckweed species used in this study was *Lemna minor* native to a central region of France (Rhônes Alpes). The plants were exposed to different concentrations of Cd, Cu, Ni and Zn in a modified quarter coïc solution at pH = 6.1 (Remon et al., 2007) and under a daily regime of 16 h light (101 $\mu mol.m^{-2}.s^{-1}$). The test protocols (Fig. 1) were derived from the standard draft guideline 221 (OECD, 2002). Duckweed growth was measured after four days of exposure to different metal concentrations. 9 to 12 *Lemna* fronds (2 to 3 colonies) were gently placed in crystallising cups (7cm high, 5cm in diameter) containing 100 ml of metal solution diluted in the nutrient medium. *Lemna* fronds were observed daily for toxicity symptoms (chlorosis, necrosis and frond dislocation). The biomass based on the total frond area was evaluated by image analysis (scion image). Plants growth was calculated as follows: Growth = Biomass (t = 4 days)/ Biomass (t=0). The doubling time of frond number, T_d, was calculated according to the following equation: $T_d = \ln2/ \mu$, Where μ is the average growth rate in the control.

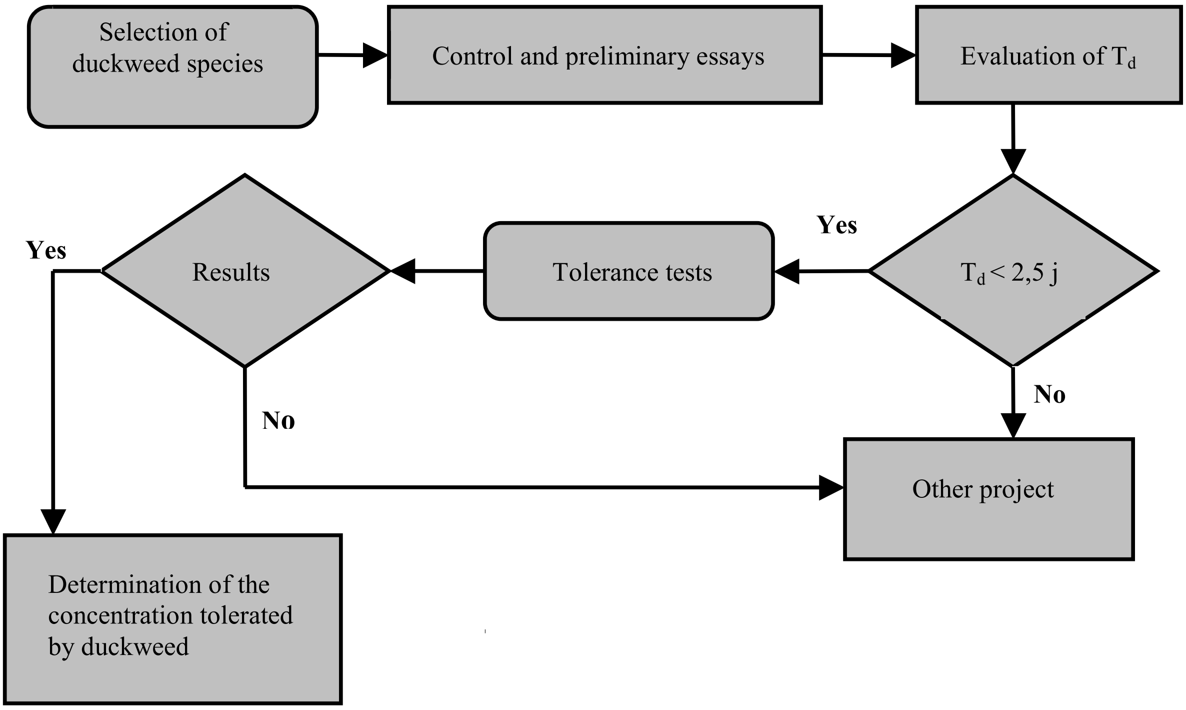

Fig. 1. Hierarchy of tolerance tests

3. Results

1) Morphological symptoms of toxicity

Cd, Cu, Ni and Zn caused visible damage to duckweed at concentrations of 0.5, 0.5, 4 and 18 mg/L respectively. Copper and cadmium were very toxic metals for *L. minor*. The plants exhibited chlorosis and/or necrosis when 0.5 mg/L of the element were added to the culture medium. Duckweed tolerated nickel and zinc up to 3 and 15 mg/L respectively. Zinc was thus the less toxic element for the aquatic plants since the toxicity symptoms (light decolourization and frond dislocation) were observed only at 18 mg Zn/L.

2) Concentration-growth relation (Fig. 2)

For the test to be valid, the doubling time of frond number in the control, Td, must be less then 2.5 days (OECD, 2002). According to the formula, the calculated Td was 1.9 day.
Copper and nickel had similar effects on *L .minor*. The two elements stimulated the growth for concentrations ranged between 0 and 0.2 mg/L for Cu and 0 and 0.5 mg/L for Ni. Over these values, the growth decreased until a minimal value (25% for Cu and 32% for Ni) corresponding to 0.5 mg Cu/L and 2 mg Ni/L. Cadmium and zinc were inhibitor elements for *Lemna* 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 52.5% and 65% respectively. The growth inhibition parameters (EC_{50}, LOEC and NOEC) are shown in Table 1.

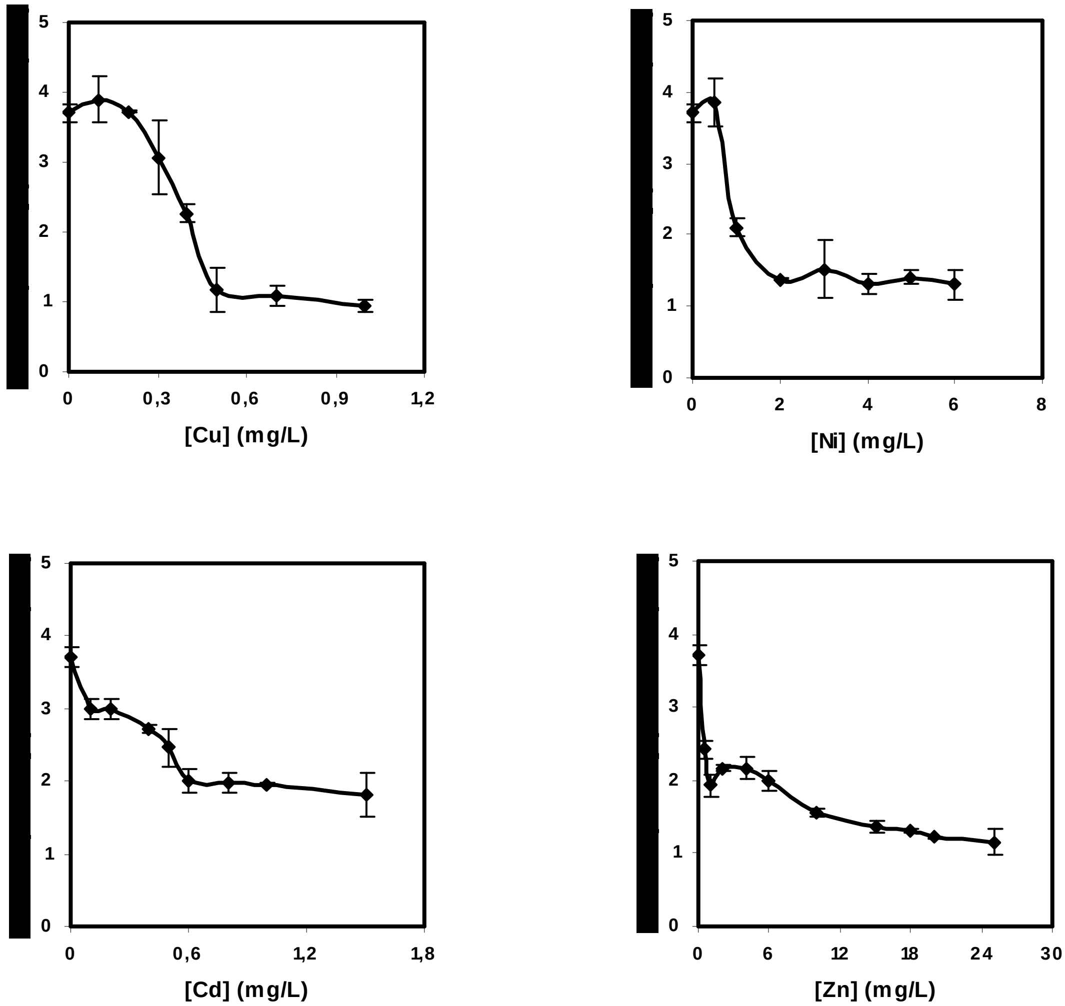

Fig. 2. The effect of metal ions on the growth of *L. minor*
Cu, (b) Ni, (c) Cd, (d) Zn

Table1. Inhibition parameters of *Lemna* growth in the presence of Cd, Cu, Ni and Zn

Metal	EC_{50} (mg/L)	R^2	LOEC (mg/L)	NOEC (mg/L)
Cd	0.64	0.96	0.1	*
Cu	0.45	0.95	0.3	0.2
Ni	1.90	0.95	1.0	0.5
Zn	5.50	0.92	0.5	*

4. Discussion

In this work, the tolerance tests to the metals Cd, Cu Ni and Zn in *L. minor* allowed to evaluate the metal concentration tolerated by the plants. The tolerance is defined as the ability of the plants to survive to concentrations of metals in their environment that are toxic to other plants (Kamal et al., 2004). Cadmium inhibited duckweed growth even at low concentrations. The inhibition consisted on the reduction of the biomass which might be explained by an excessive absorption of the metal. Copper when present in the nutrient solution at concentrations ≤ 0.2 mg. L-1 was an essential element for the development of *Lemna* fronds because of its important role in cellular metabolism. At a concentration higher than 0.4 mg/L, Cu caused the photosystem alteration by reducing electron transport. This effect was explained by a rapid development of chlorosis.

L. minor tolerated nickel up to 3 mg/L with an optimal development at 0.5 mg/L. Concentrations higher than 3 mg/L of Ni were toxic for the macrophytes and decreased considerably the growth rate. Axtell et al. (2003) reported in a comparative study of the absorption of Pb and Ni by *L. minor* that this vegetable species showed a preference to remove Ni. This aquatic macrophyte could be effective to remove nickel from polluted water with low concentrations (3 mg Ni/L). Zinc was more tolerated by duckweed which exhibited no toxicity visible symptoms, but showed a reduction of biomass for concentrations ranged between 0.5 and 15 mg. L-1. The toxicity metal in *Lemna* tissues was in decreasing order of damage: Cu > Cd > Ni > Zn. We concluded that *L. minor*, could be a good candidate for the phytoremediation of water polluted with Zinc or Nickel.

Conclusion

The metals Cd, Cu, Ni and Zn were tolerated by *L. minor* at 0.4, 0.4, 3 and 15 mg/L respectively. At these concentrations, biomass and growth rate were affected without toxicity signs (chlorosis, necrosis and frond dislocation). Thus, the aquatic macrophytes could survive in a medium containing 3 mg Ni/L or 15 mg Zn/L. Although growth was reduced at these concentrations, the aquatic species could be a good candidate for cleaning up wastewater polluted with Zinc and Nickel (industrial and residential effluents). This result will be discussed in forthcoming study. Copper and cadmium are considered as very toxic for the macrophytes. Thus, *Lemna minor* is not suitable for treating water containing Cu and Cd. To remove copper and cadmium from wastewater, other plants more tolerant than *L. minor* must be tested.

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