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Phytoaccumulation of zinc using the duckweed *Lemna gibba* L.: effect of temperature, pH and metal source

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ABSTRACT

The use of aquatic plants in the removal of pollutants is an alternative technique for the remediation of contaminated waters. In this work, the capacity of accumulation of soluble Zn by the duckweed *Lemna gibba* L. native to a Mediterranean area (North-east Algeria) was studied under controlled conditions. The effect of temperature (17, 21, 25 and 29°C), medium pH (3, 4, 5 and 6) and Zn source ($ZnSO_4$, $Zn(NO_3)_2$ and $ZnCl_2$) was investigated in order to assess the ability of the plants to remove metal pollutant under various conditions. The results revealed that, at 21, 25 and 29°C, the plants reduced initial Zn concentration ($\sim 18\,mg\,L^{-1}$) to 4.64, 1.69 and $1.92\,mg\,L^{-1}$, respectively, with 9.1–22.1 $mg\,g^{-1}$ DW of Zn accumulated in the biomass. Low Zn accumulation in *L. gibba* biomass (7.5 $mg\,g^{-1}$ DW) corresponding to a low plant growth was observed at pH 3 and 4 indicating that this species is not tolerant to water acidity. A very low Zn concentration in *Lemna* biomass (1.04 $mg\,g^{-1}$ DW) was observed when $Zn(NO_3)_2$ was used as Zn source. The results obtained in this study demonstrated that temperature $\geqslant 21$°C and pH values of 5 and 6 are favourable factors for the phytoaccumulation of Zn by *L. gibba* L.

Keywords: Abiotic factor; Duckweed; Metal removal; Zn accumulation

1. Introduction

More recently, the use of aquatic plants in the removal of toxic elements has been an alternative technique for the remediation of contaminated waters. This technology, commonly called constructed wetland, is more and more widely studied and employed throughout the world [1–4]. The selection of species to be used in a wetland system is largely governed by location and site conditions since metal phytoaccumulation depends upon numerous biotic and abiotic factors such as features of plant species, temperature, pH, salinity and dissolved ions in water [5–7]. The successful application in the field of metal phytoremediation is largely dependent on the effect of the environmental conditions. Many studies have demonstrated that floating macrophytes were able to accumulate metal ions in their tissues [8–13]. Increasingly, attention is more and more focused on the knowledge of the ability of different plant species to absorb metal ions under different physical and chemical conditions.

Temperature and pH are the most important environmental characteristics affecting chemical uptake and distribution within living plants [14,15]. Temperature is one of the key factors governing the physiological processes of all organisms. It maintains a direct

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relationship with growth rate and other functions involved in the energy metabolism [16]. Medium pH could be a parameter that affects the uptake of metal ions [17]. pH affects the solubility of metals and the ionization state of some functional groups of the cell wall [18]. An increase of pH is generally accompanied by a decrease of the solubility of many heavy metals; thus, total concentrations of most metals decrease in the water body because of the sedimental process [16].

Earlier experiments in our laboratory demonstrated the capacity of the duckweed *Lemna gibba*, originated from North-east Algeria, in Zn removal. The macrophytes were effective in removing metal ions from the nutrient medium (61–71%). However, there was no detailed study on the effect of abiotic factors on the remedial process. In this work, it was aimed to investigate the effect of some physicochemical parameters namely, temperature, medium pH and Zn source in order to evaluate which of the factors led to an optimal Zn phytoaccumulation by *L. gibba*. For this purpose, we had chosen Zn concentration of approximately 18 $mg\,L^{-1}$ to conduct the different experiments. This concentration has been chosen as a test concentration based on our previous study that showed this as the highest metal concentration tolerated by *L. gibba* [19].

2. Materials and methods

2.1. Experimental protocol

The plants used in this study were selected from a natural pond located in the city of Annaba (North-east Algeria). The climate of this region is typically Mediterranean, characterized by wet winters and hot dry summers with a mean temperature of 27°C. The plants were cultured in laboratory at 21 ± 1°C in quarter Coïc and Lesaint solution at $pH = 6.0 \pm 0.1$. Illumination with a 12-h photoperiod was supplied by two lamps (each having 40 W white light). To evaluate the effect of temperature, pH and Zn source on Zn phytoaccumulation by *L. gibba*, three sets of experiments (in triplicate) were conducted in 1.2 L capacity vessels. About 1.00 ± 0.05 g (FW) of *Lemna* fronds were added to the Coïc solution containing approximately 18 $mg\,L^{-1}$ of Zn. Control treatments were necessary to compare differences in plant growth. The treatment duration was seven days and the change in volume due to evaporation and sampling was compensated with distilled water.

Experience 1: Plants were placed at 17, 21, 25 and 29°C (±1°C) with 1 treatment control (without Zn supplementation) for each temperature ($pH = 6.0 \pm 0.1$, Zn added as $ZnSO_4$).

Experience 2: Plants were exposed to Zn solutions at pH = 3.0, 4.0, 5.0 and 6.0 (±0.1) with 1 treatment control for each pH ($T = 21 \pm 1$°C, Zn added as $ZnSO_4$). Medium pH was adjusted using pH buffers.

Experience 3: Plants were exposed to Zn solutions in which Zn was added as $ZnSO_4$, $Zn(NO_3)_2$ and $ZnCl_2$ ($T = 21 \pm 1$°C, $pH = 6.0 \pm 0.1$). One treatment control was used in each case.

2.2. Data analysis

Plant biomass (mg DW) was determined at the beginning and the end of experiments and used to calculate the percentage of *L. gibba* growth:

$$\%\,\text{Growth} = \frac{\text{Final biomass} - \text{Initial biomass}}{\text{Final biomass}} \times 100$$

Water samples (2 mL) were drawn at the start and the end of the treatment in order to measure Zn concentration removed from the nutrient medium. Zinc amount accumulated in *L. gibba* biomass was measured at the end of metal exposure; plants were dried at 70°C until constant weight and digested in 69% HNO_3. The percentage metal removal (% removal), the metal amount accumulated in plant biomass (mg) and the metal accumulation capacity of *L. gibba* ($mg\,g^{-1}$ DW) served as parameters to evaluate the efficacy of treatments [20]. The metal concentration in water and in duckweed biomass was quantified with an atomic absorption spectrophotometer (Shimadzu AA 6601 F).

The results were analysed by one-way ANOVA and differences in Zn concentration were considered significant for $p < 0.05$.

3. Results

3.1. Effect of temperature

The effect of temperature on Zn phytoaccumulation by *L. gibba* was investigated in a temperature range from 17 to 29°C, representing the temperature change (on average) throughout spring, summer and autumn in North-east Algeria. The effect of temperature on the removal and accumulation of Zn is given in Fig. 1. The lowest metal removal (~62%) was obtained at 17°C. At this temperature, 0.56 mg of Zn was accumulated in *L. gibba* biomass. At elevated temperatures, approximately 74–90% of Zn was removed from the solution. Metal amount accumulated in duckweed biomass at 21, 25 and 29°C were 2.03, 1.14 and 0.77 mg, respectively.

Temperature not only affects Zn removal but also influences the plant growth. Fig. 2 shows the growth

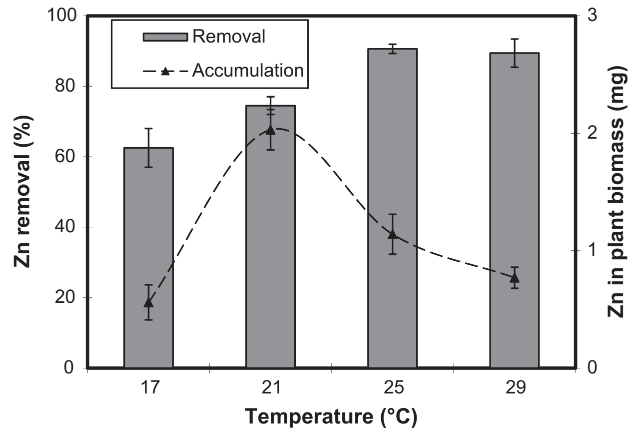

Fig. 1. Effect of temperature on the removal and accumulation of Zn by *L. gibba*. Initial Zn concentration was $18.12 \pm 0.09\,mg\,L^{-1}$.

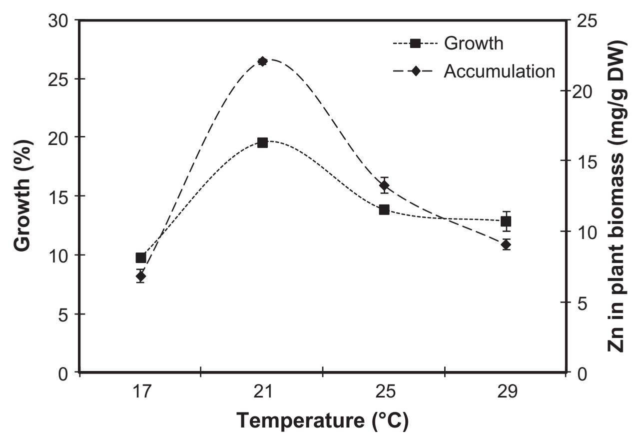

Fig. 2. Effect of temperature on the growth and Zn accumulation capacity of *L. gibba*. Initial Zn concentration was $18.12 \pm 0.09\,mg\,L^{-1}$.

and Zn amount ($mg\,g^{-1}$ DW) accumulated in *L. gibba* biomass as a function of temperature at 17, 21, 25 and 29℃. The curves show that optimal growth and the greatest accumulation of Zn ($22.1\,mg\,g^{-1}$ DW) were determined at 21℃. Over a temperature of 25℃, the growth of the aquatic plants reached a constant value while the accumulation decreased slightly. The optimal temperature of 21℃ was chosen for the next study on the influence of pH and Zn source.

3.2. Effect of pH value of the medium

Based on the distribution diagram of the possible hydroxo-soluble species of Zn, the effect of pH medium on the Zn phytoaccumulation by *L. gibba* was investigated in a pH range of 3–6 (the increase in pH does not produce soluble species different from the aquo-ions until precipitation, pH ~ 7).

There was an increase of Zn phytoaccumulation with increasing pH from 3 to 6. The maximum removal and accumulation of Zn was observed at pH 5 and 6 (Fig. 3). The amount of Zn accumulated in plant biomass at these pH values was $19.4\,mg\,g^{-1}$ DW on average, where the growth was approximately 20% (Fig. 4). At pH $\leqslant 4$, the metal amount accumulated in duckweed biomass was less than $7.5\,mg\,g^{-1}$ DW and the removal percentage was about 50%. At these pH values, growth (either in control than in Zn treatment) was very low and plants developed visible signs of toxicity. The accumulation of Zn was not significantly different ($p > 0.05$) at pH 5 and 6, so pH value of 6 was chosen for the study on the effect of Zn source on the phytoaccumulation process.

3.3. Effect of Zn source

In natural waters, Zn can be found in several chemical forms. Inorganic Zn species which have high solubility are mainly sulphates ($2.2 \times 10^5\,mg\,L^{-1}$ at 20℃), nitrates ($1.8 \times 10^6\,mg\,L^{-1}$ at 25℃) and chlorides ($4.32 \times 10^6\,mg\,L^{-1}$ at 25℃). The other inorganic species have very low solubility in water [21]. We used zinc sulphate, zinc nitrate and zinc chloride as Zn source to assess the effect of Zn salt in the phytoaccumulation process. Fig. 5 shows greater removal and accumulation ($p < 0.05$) when Zn ions were added as sulphates and chlorides. However, a very low Zn concentration ($1.04\,mg\,g^{-1}$ DW) corresponding to a low growth of *L. gibba* (<6%) was observed when $Zn(NO_3)_2$ was used as Zn source (Fig. 6).

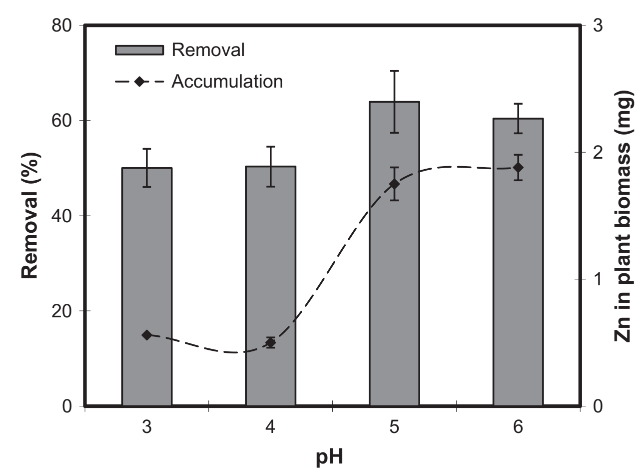

Fig. 3. Effect of pH values on the removal and accumulation of Zn by *L. gibba*. Initial Zn concentration was $17.15 \pm 0.14\,mg\,L^{-1}$.

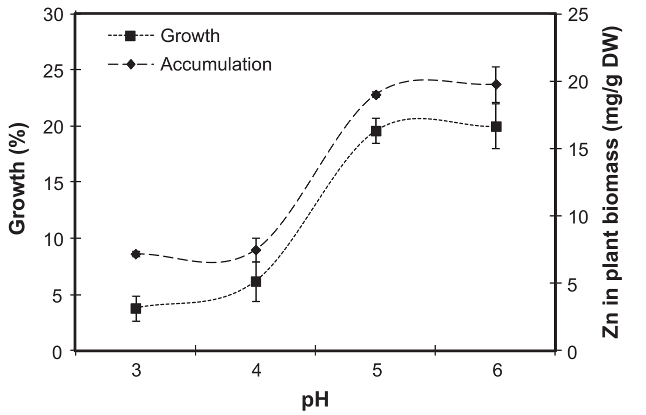

Fig. 4. Effect of pH values on the growth and Zn accumulation capacity of *L. gibba*. Initial Zn concentration was $17.15 \pm 0.14\,mg\,L^{-1}$.

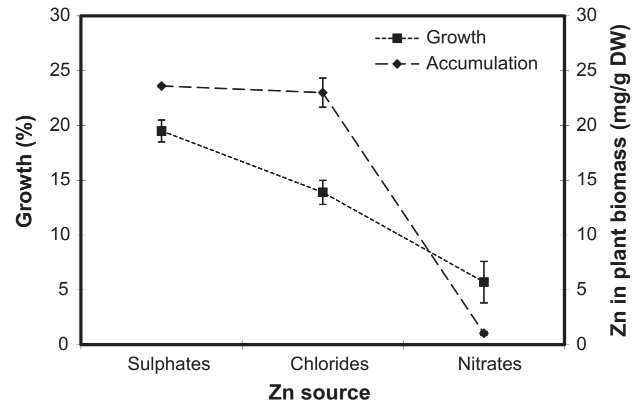

Fig. 6. Effect of Zn source on the growth and Zn accumulation capacity of Zn by *L. gibba*. Initial Zn concentration was 17.15 ± 0.14, 16.83 ± 0.09 and $17.15 \pm 0.10\,mg\,L^{-1}$ in the case of $ZnSO_4$, $ZnCl_2$ and $Zn(NO_3)_2$, respectively.

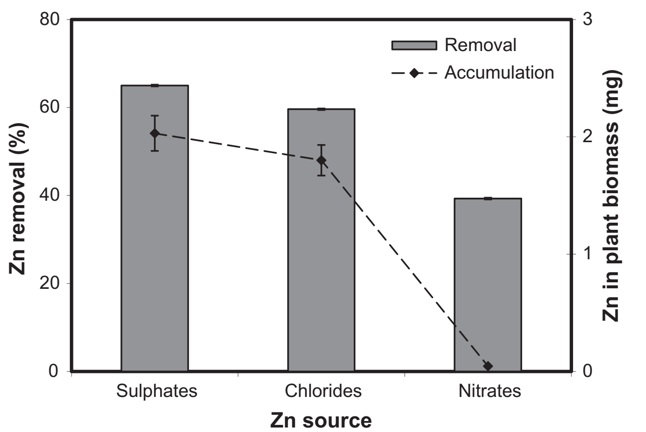

Fig. 5. Effect of Zn source on the removal and accumulation of Zn by *L. gibba*. Initial Zn concentration was 17.15 ± 0.14, 16.83 ± 0.09 and $17.15 \pm 0.10\,mg\,L^{-1}$ in the case of $ZnSO_4$, $ZnCl_2$ and $Zn(NO_3)_2$, respectively.

4. Discussion

Due to the toxicity of zinc, the American Environmental Protection Agency (US EPA) has set the maximum acceptable concentration in contaminated water supply to $5\,mg\,L^{-1}$ and in drinking water to $3\,mg\,L^{-1}$ [22]. The duckweed *L. gibba* can be used as biological filters for Zn-contaminated waters. It is effective in the removal and accumulation of Zn from a nutrient medium containing approximately $18\,mg\,L^{-1}$ of Zn [20].

Temperature is one of the key factors governing the physiological processes of the plants. The results obtained in this work revealed a maximal biomass growth of 20% and a maximal phytoaccumulation capacity ($22.1\,mg\,g^{-1}$ DW) at an optimal temperature of 21˚C. In addition, the study demonstrated that Zn removal by *L. gibba* L. was more effective at elevated temperature (21, 25 and 29˚C); about 74–90% of Zn was removed from water, reducing Zn concentration to a value which is below the safety limit set by the Environmental Protection Agencies [21]. At 17˚C, either removal or accumulation was much lower than those obtained at 21, 25 and 29˚C. Frond chlorosis was observed at this temperature (in treatment and control) and the reasons were that low temperature probably inhibited nutrient assimilation [16]. For the four temperatures tested in this work, amount of Zn eliminated from water was higher than that absorbed by duckweed. Zinc may have been removed through a chemical precipitation of zinc phosphate (See our previous study [20]).

The growth of *L. gibba* was influenced either by temperature or by medium pH. The effect of pH on the growth and Zn phytoaccumulation by *L. gibba* was tested across the pH range of 3–6. Extremely low pH values are toxic for the growth of aquatic floating plants because an increased influx of H^+ would decrease the electrochemical gradient across the plasma membrane and thus the uptake of cations [17]. The treatment was found to be dependent on hydrogen ion concentration present in solution. The results showed that lower pH had adverse effect on the growth either in control (data not shown) or in treatment. When the pH value was $\leqslant 4$, the growth was very low (~6%) and the metal amount accumulated in duckweed biomass was less than $7.5\,mg\,g^{-1}$ DW with a removal percentage of about 50%. The decrease in plant growth and metal accumulation efficiency at pH 3 and 4 may be due to the tolerance of the plants towards acidity. According to Saygideger et al. [23], at lower pH, more protons will be available to protonate active groups of biomass surface, such as lipids, amino acids and other cellular components of the organism and metal ions are competing with the H^+ in the solution. The maximum removal and accumulation of Zn was observed at pH 5 and 6 (Fig. 3).

When Zn was added as zinc sulphate or zinc chloride, the metal removal percentage was > 65% corresponding to a final Zn concentration of $6\,mg\,L^{-1}$. When zinc nitrate was used in experiments, large number of small fronds were produced. The presence of high concentration of nitrate in the medium was probably responsible for the significant decrease in *L. gibba* growth, the low Zn accumulation ($1.04\,mg\,g^{-1}$ DW) in plant biomass and the low metal removal from water [24]. In our experiments, *L. gibba* grown on NO_3^--rich media has probably developed nitrate toxicity symptoms. Several studies have showed that for most freshwater aquatic macrophytes, the preferred form of inorganic nitrogen is ammonium (assimilation of NH_4^+ is less complex compared to NO_3^-). Jampeetong and Brix [24] examined the effect of inorganic nitrogen on the growth of *Salvinia natans* (water fern). Their results showed that plants supplied with only NO_3^- had lower growth ($0.17 \pm 0.01\,g\,g^{-1}\,d^{-1}$) rates than plants supplied with NH_4^+ alone or in combination with NO_3^- ($0.28 \pm 0.01\,g\,g^{-1}\,d^{-1}$). The results obtained from the present study indicate that Zn phytoaccumulation is efficient only when the metal was used as $ZnSO_4$ or $ZnCl_2$. On the other hand, Leblebici and Aksoy [25], investigating the impact of nutrient enrichment (P, $NO_3^- - N$ and SO_4^{2-}) on growth and lead accumulation capacity of *L. minor* demonstrated that nutrient enrichment reduced the accumulation of Pb in plant biomass and enabled growth of the duckweed species at Pb concentration that impaired growth in plants without nutrient addition. To confirm these observations with *L. gibba*, more work is needed to show the effect of different concentrations of $NO_3^- - N$ on the growth of these aquatic species.

5. Conclusion

The duckweed *L. gibba* is a viable species in an artificial wetland of water effluent treatment plant. It proved to be a potent tool for the removal of Zn from contaminated water, mainly at temperatures $\geqslant 21\,^{\circ}C$ and pH of 5 and 6. The lower growth at low temperature, water acidity and high nitrates concentration are some limitations of the use of duckweed as phytoremediation agent. More work may be developing to ameliorate these limitations.

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