ROLE OF PH IN THE ACCUMULATION OF LEAD AND NICKEL BY COMMON DUCKWEED (LEMNA MINOR)

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Abstract: Heavy metals are the most common non-biodegradable pollutants. Various remediation methods have been used for the treatment of heavy metal contaminated water. However, phytoremediation technology is currently being used due to its economically feasible and ecologically viable advantages. The aim of this study was to examine the role of pH in the accumulation capacity of Lemma minor in Pb and Ni contaminated water. The removal rate of Pb and Ni from L. minor was 99.99% and 99.30% respectively, after 28 days of exposure to the 10 mg/l Pb and Ni contaminated water at pH 5.6 and pH 6 respectively.

Keywords: Lead, Lemma minor, nickel, pH, Phytoremediation

INTRODUCTION

Presence of heavy metals in aquatic environment is detrimental as they are harmful to human health. Unlike organic pollutants, heavy metals are non-biodegradable and therefore, the removal of them is extremely important in terms of health of living specimens [1, 2]. Lead (Pb) is extensively used in many industrial applications, such as storage battery, manufacturing, printing pigments, fuels, photographic materials and explosive manufacturing [3]. The Pb, one of the most toxic heavy metals may cause health problems such as behavioral anomaly, learning disabilities and seizures [4]. Nickel (Ni) is widely used in plating plants, steel factories, Ni batteries and in the production of some alloys [5]. The Ni intake over the permissible levels results in different types of disease such as acute and chronic disorder in man, pulmonary fibrosis and renal edema, and skin dermatitis such as severe damage to lungs and kidney, and gastrointestinal distress (e.g. nausea, vomiting, diarrhea) [6].

Various technologies such as ion exchange, reverse osmosis, membrane filtration, and precipitation have been employed for heavy metal removal from aqueous solutions and effluents. However, these technologies are not economically feasible and also generate toxic sludge [7-9]. Phytoremediation is one of the most promising techniques for removal of heavy metal pollution from aquatic ecosystems [10, 11]. Different aquatic plants have been used for the removal of heavy metals [12-16]. Common duckweed (Lemma minor) plant was chosen for the current investigation because aquatic plants are the first link in relation to metal contents of aquatic environments [17, 18]; it is a bioindicator of the ecological relevance for the detection and monitoring of metal pollution [19], and has a known ability to accumulate heavy metals [20-23]. Most members of the Lemna genus are used as model plants for phytoremediation, nutrient and metal uptake studies, and bioassays [24]. The aim was to determine the accumulation capacity of lead and nickel in L. minor under the influence of pH in the contaminated water. The ultimate goal was to assess the possibility to use L. minor for bioindication and phytoremediation of lead and nickel contaminated water. The maximum waste water discharge limits of lead and nickel in small scale industries allowed by the Central Pollution Control Board (CPCB), New Delhi are 0.1 mg/l and 3.0 mg/l [25]. In this study, concentrations significantly higher than the maximum amount allowed were selected (10 mg/l), in order to examine the phytoremediation potential of L. minor when exposed to extremely elevated levels of lead and nickel in the water.

MATERIALS AND METHODS

Experimental setup:
Lesser duckweed plants (Lemma minor) were collected from a waste stream in Indian Institute of Technology Delhi campus. They were cultured in a water tank. All experiments were carried out in six litre plastic containers with triplicates. The initial concentration of lead and nickel were kept at 10 mg/l in all the experimental sets. The pH of the experiments was set as pH 4, 5, 6, 7, 8, 9 and 10. The pH of the medium was adjusted by using 0.1 N HCl or 0.1 N NaOH. The water level was maintained constant up to six litres.

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throughout the experiment. Plants were harvested after 7, 14, 21 and 28 days. Water samples were also collected at the same intervals. The fresh weight of biomass was determined after removing excess liquid over a filter paper (Whatman No. 1) for 15 minutes and was dried in an oven at 25-35°C for 24 hours to get constant weight. The effect on plant growth was measured by calculating relative growth (RG) to the method of Lu et al. [26], following the formula $\text{RG} = \frac{\text{Final fresh weight}}{\text{Initial fresh weight}}$.

**Sampling and analyses:**

Stock solution of lead (Pb) and nickel (Ni): The solutions were prepared by dissolving 159.845 mg of Pb(NO$_3$)$_2$ and 495.332 mg Ni(NO$_3$)$_2$.6H$_2$O in 1000 ml double-distilled water to prepare Pb$^{2+}$ and Ni$^{2+}$ solution of strength 1 mg/l respectively. The various standards were prepared by using the stock solution for obtaining the calibration curves of different ranges for subsequent analyses of lead and nickel.

The concentrations of Pb and Ni remaining in the plastic containers were measured at an interval of a week up to 28 days. The treated plants were analyzed for Pb and Ni content. The dried plant samples were heated in a muffle furnace at 500°C for 6 hours. The ash of each sample was dissolved in 5 ml of 20% HCl to dissolve the residue. Samples were heated on a hot plate to boiling. Required amount of HCl (20%) was added to avoid sample drying. The resulting solutions were filtered and diluted to 50 ml with deionized water in volumetric flasks. Pb and Ni content of plant samples and water samples were determined by flame atomic absorption spectrophotometer of Electronics Corporation of India Limited (ECIL model no. AAS4129) with the following settings: For Pb - wavelength 217 nm, lamp current 5 mA, slit 1 nm, fuel – acetylene and oxidant air; and for Ni - wavelength 232 nm, lamp current 3.5 mA, slit 0.2 nm, fuel – acetylene and oxidant air. Pb and Ni estimation were made against standard calibration curves prepared with known concentrations of Pb and Ni and expressed in mg per kg of dry weight of the plant.

**RESULTS**

**Plant growth test:**

Relative growth of *L. minor* plants is reported in Figure.1 as weekly measured fresh weight. The biomass of both control and treated plants was 6.0 g at the start of the experiment. Relative growth of pH 6 treated plants showed the highest value of 1.66 ± 0.32 after 7 days. The lowest relative growth was measured in pH 10 treated plants after 14 days (1.28 ± 0.25).

**Lead and nickel accumulation:**

The total amount of Pb and Ni accumulated in plant biomass is reported in Figure.2 and Figure.3. Accumulation of Pb and Ni by *L. minor* increased with time. The highest accumulation was determined as 327 ± 24 mg/kg of pH 5 treated plants after 28 days exposure for Pb and 395 ± 29 mg/kg in pH 6 treated plants after 28 days exposure for Ni, respectively. However, the lowest Pb accumulation was found in pH 10 treated plants after 7 days exposure (21 ± 5 mg/kg). The lowest accumulation of Ni were found in pH 10 treated plants after 7 days exposure (181 ± 5 mg/kg) respectively. Figure.4 represents the effect of pH on lead and nickel accumulation by *L. minor* after 28 days.
Lead and Nickel removal:

The effect of pH on the removal of Pb and Ni in solution was studied by changing pH values in the range of 4-10 and the results were presented in Figure 5 and Figure 6 respectively. The highest concentrations of Pb and Ni remained in the media were 0.40 ± 0.05 mg/l and 2.62 ± 0.28 mg/l in pH 10 for 7 days treatments. The lowest Pb remained in water samples was found in pH 5-6. However, the lowest Ni concentration was 0.07 ± 0.02 mg/l in pH 6 for 28 days treatments. The maximum removal was found to be 99.99% for Pb at pH 5-6 and 99.3% for Ni at pH 6 after 28 days exposure. The lowest Pb removal was 95.94% in pH 10 after 7 days exposure. Otherwise, the pH did not have a significant influence on the removal capacity of Lema for Pb being in the range of 95% to 99.99% for a pH of 4 to 10. The order of Pb removal in ascending order is as follows: pH 5>pH 6>pH 8>pH 9>pH 10. The lowest Ni removal was achieved at pH 10 for 7 days treatments (73.78%). The ascending order of Ni removal is as follows: pH 6>Control>pH 8>pH 5>pH 4>pH 9>pH 10.

DISCUSSION

Alterations of the physiochemical conditions of the environment (pH, redox potential, organic and inorganic ligands, temperature, etc.) can strongly influence the relative proportions of the metal ions that can be taken up. Evidently, the implementation of bioindicator studies to assess the accumulation of metals and other pollutants in biota must be evaluated under well-defined physiochemical environmental conditions [27].

The pH of solution is the most important variable affecting metal ions biosorption [28, 29]. This is partly because hydrogen ions themselves are strongly competing with metal ions. At higher pH values, the accumulation for Ni was dramatically decreased. At pH values lower than 5, the accumulation was 78% for Ni. These results can be explained by the pH effect on both metal binding sites on the biomass surface and metal chemistry in aqueous solutions. Increased positive charge (protons) density on the sites of biomass surface at low pH value (pH 4) restricted the approach of metal cations as a result of repulsive force. At lower pH more protons will be available to protonate active groups of biomass surface, such as chitin, acidic polysaccharides, lipids, amino acids and other cellular components of the plant and metal ions are competing with the H⁺ in the solution at low pH values for adsorption [30]. Similar results have been reported by other researchers for the uptake of heavy metal by activated sludge and aquatic moss Fontinalis antipyretica [31, 32]. However, Salvinia minima BAKER removed 97.44% Pb at pH 4 at 3 mg/l-1 of Pb treatment under outdoor conditions [33].

In contrast, when the pH values increased, adsorbent surfaces were more negatively charged and the biosorption of the metal ions with positive charge (Pb²⁺ and Ni²⁺) process was reached maximum around pH 5-6. Decrease in biosorption at higher pH (>8) is due to the formation of soluble hydroxylated complexes of the metal ions and their competition with the active
sites, and as a consequence, the retention would decrease again. A similar theory was proposed by several earlier workers for the biosorption of same metal ions on different biomasses [34, 35]. Furthermore, it has been suggested that there are two mechanisms taking place in the sorption of heavy metal ions onto plant biomass surfaces: adsorption through a specific ion exchange mechanism and surface precipitation onto solid surfaces [36].

**CONCLUSION**

The results obtained from the present study confirm the capacities of accumulation of lead and nickel by *Lemna* and its great potential for phytoremediation. The removal from the medium was attributed to precipitation of metal salts, adsorption on the plants and absorption and sequestration inside the plant. This study demonstrated that *L. minor* could be used as an effective bio accumulator for the treatment of Pb and Ni containing wastewater. It is also an environmentally acceptable plant for phyto accumulation of heavy metals.

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**Conflict of interest:** None Declared