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The Potential of *Lemna minor* to Uptake Iron in Water

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Abstract: Heavy metal, even necessary element (e.g. iron) may pose risks to human being and environment. This laboratory study investigated the potential of duckweed *Lemna minor* to uptake iron from solutions. The levels of iron in duckweed did not increase with the concentrations of iron in solutions. The amounts of iron in plants also did not enhance with the culture time. The results indicated that the capability of duckweed to uptake iron was limited as iron was easy to be precipitated and form metal plaque. The use of chelators to increase the bioavailability of iron is necessary to enhance the phytoremediation efficiency.

Key words: Duckweed, heavy metal, iron, phytoremediation.

1. Introduction

Various industrial practices such as wastewater treatment, metal processing and mining, contribute to a regular release of heavy metals, such as Fe, As, Cd, Cr and Ag. According to Niragu, et al. [1], 90% of the emissions from heavy metal have derived from anthropogenic sources since 1900 AD. Heavy metal can cause risks to environmental and human health [2]. Iron is utilized in many products such as, construction materials (particularly inter alia for drinking-water pipes), pigments in paints and plastics, and food colors for the treatment of iron deficiency in humans. Generally, mining sites used to extract these iron compounds for industrial use. These sites are contaminated due to the discharge of mine water which contains elevated levels of heavy metals from mining practices. These heavy metals (e.g. Fe, Al and Mn) can cause serious pollution of surface water, groundwater and soil upon its arrival to the surrounding environment [3]. Cost-effective phytoremediation techniques are being considered for use in these heavy metals contaminated medias.

Phytoremediation is to use green plants to extract contaminants from the environment [4]. Scientists are increasingly studying technological solutions for phytoremediation due to their potential to remediate large areas of land or water for a relatively inexpensive price. Extensive reports have indicated that plants (i.e. reeds and cattails) were able to sequester metals (e.g. Sr. Cr, Fe and Mn) from contaminated environment [5].

Lemna minor, otherwise known as duckweed, is a member of the family, *Lemaceae*. It is a simple, small flowering plant which flourishes in quiet, shallow water bodies. The individual green structures which can be seen above the water are known as fronds, and float freely with a shallow root system. Due to their ability to reproduce rapidly, they can blanket an entire surface of a pond or a slow moving stream within a short period of time. *Lemna minor* has been studied and accepted as a hyperaccumulator in the past for various heavy metals such as Pb, Cu, Cr, Zn and Cd. Some studies indicate that *L. minor* can accumulate high concentrations of several metals and metalloids, however, there are not many articles about using duckweed to uptake iron from an iron-rich effluent site [6]. This study aimed to determine whether or not this plant can successfully be used to remediate a media contaminated with iron.

2. Materials

2.1 Hydroponic Solutions

Duckweeds were collected from a natural pond in a

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residential area in Commerce, Texas. After acclimating in distilled water for 2 days, duckweeds were transferred to control and treatment solutions. The control group only contained nutrition which is in line with the modified Hutner Medium [7]. The treatment solution contained nutrients and different levels of iron (low 5 mg/L, middle 10 mg/L and high 15 mg/L Fe). Iron was added by using $FeCl_2$ and the pH of the solutions was adjusted to 7. Duckweeds were transferred into hydroponic solution immediately once it was prepared and cultured for 4 and 8 weeks. Triplicates were conducted for each experimental condition.

2.2 Plant Digestion

Upon harvesting, duckweeds were gently washed with deionized water, then air dried. One gram of dried tissue sections were digested as described by Cutright, et al. [8]. The solutions were analyzed by Shimadzu AA6300. All experiments were conducted in triplicate.

2.3 Statistical Analysis

Metal uptake in duckweeds was analyzed with one-way ANOVA using the Minitab statistical package (Minitab 16). Differences between specific CA levels were identified by the Tukey test at 5% probability.

3. Results and Discussions

3.1 Effect of Different Fe Levels

Fe was found in duckweed in all control and treatment groups. It was not surprising as Fe was a necessary element for the growth of plants. Duckweed cultured in control solutions could have already contained Fe in biomass when collected from the pond. The amounts of Fe in all treatment solutions were higher than those in control groups. However, Fe levels in *L. minor* treated with 5 mg/L Fe for 4 week (0.75 ± 0.03 mg/g) were not significantly different from Fe concentration in duckweeds cultured in 10 and 15 mg/L Fe (0.80 ± 0.07 mg/g, 0.89 ± 0.06 mg/g, respectively) (Fig. 1).

It was not in agreement with previous research that accumulation of metals in plants was correlated with concentrations in substrate. For instance, metal (e.g. Pb, Cu) accumulation in *L. minor* increased with the levels of metals [7, 9]. Batty and Younger [10] indicated that the concentration of Fe in reeds collected from wetlands which contained high levels of metals was significantly higher than that grew in wetlands with low levels of metals.

It was not the case for Fe in this study, which may be due to that different metals possessing different toxicities to plants while plants have different tolerance abilities to various metals [11]. Further, the availability/mobility of metals also affects the amounts of metals sequestered in plants as decreased mobility of metals may reduce the amounts of metals accumulated in plants. It is reported that Fe^{2+} can form highly insoluble oxides and hydroxides (Fe^{3+}) in freshwater [12] which can decrease the availably of Fe. Fe oxides always exist as Fe plaque on the root system of plants which is reported to have certain effect on the elements uptake in plants. Greipsson and Crowder [13] indicated that iron plaque could act as a “barrier” for other elements uptake in plants. However, Zhang [14] pointed out Fe plaque can increase the accumulation of essential and nonessential metals. According to the study of Liu, et al. [15], metal plaque did not depict an apparent effect on metal accumulation in rice. Ye, et al. [16] also found that Cu accumulation and translocation in cattails were not impacted by Fe plaque. The overall effect of iron plaque on the uptake of elements in plants may be related to the amount of plaque, the type of metals and the species of plants [17]. Many other factors, such as the age of roots, concentration of metal and pH of medium can also influence the ability of metal plaque to inhibit or enhance uptake and translocation in plants [16]. High levels of metal plaque may inhibit the metal accumulation and translocation in plants while low

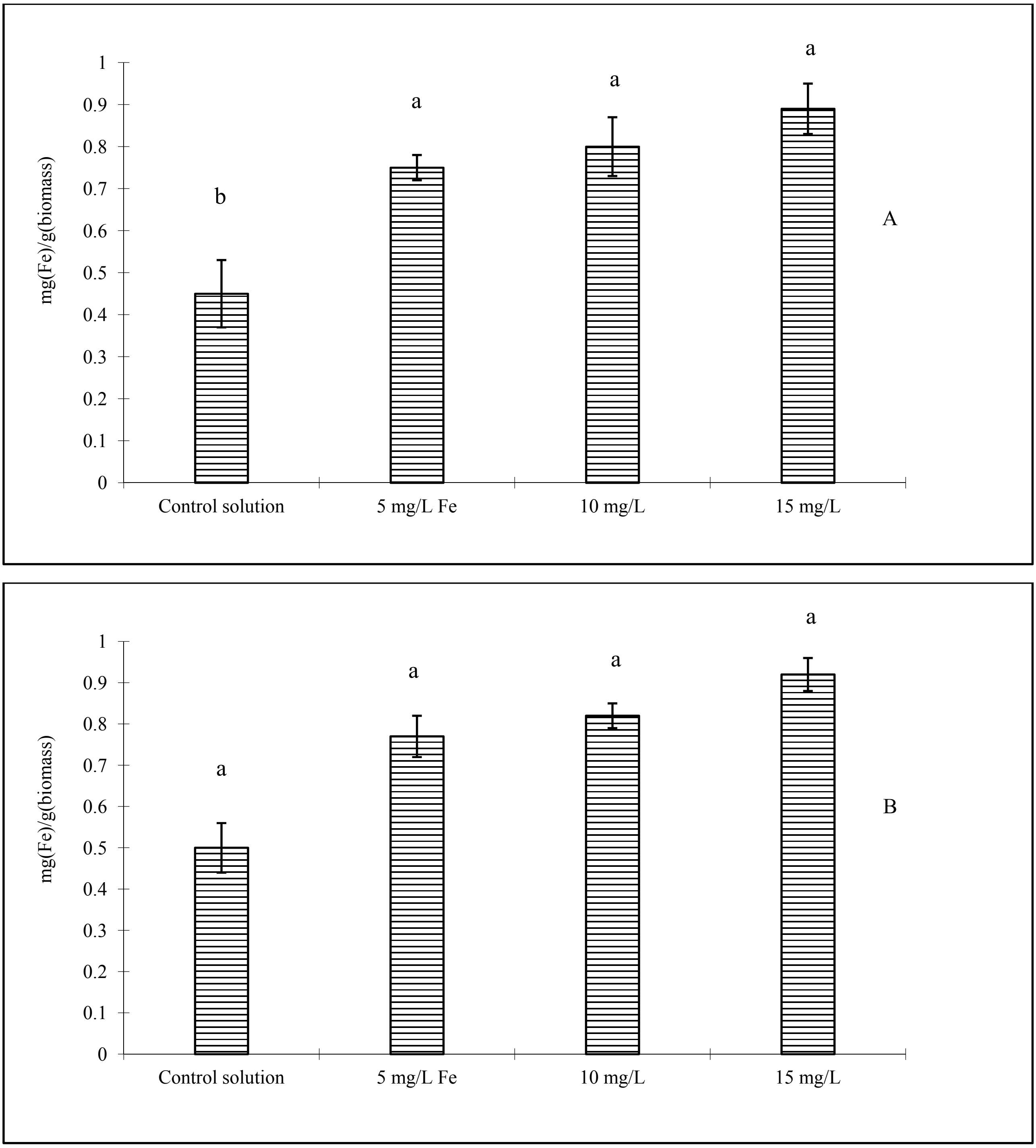

Fig. 1 Fe (mg/g) in duckweeds under different treatment for 4 (A) and (B) 8 weeks.

amounts of metal plaque may not influence the metal uptake in plants. In our study, orange iron plaque was observed on the roots of duckweed and it may reduce the availability of iron and inhibit iron uptake in biomass. Thus, it is not surprising that Fe accumulation by *L. minor* did not increase with Fe supplies.

3.2 Effect of Culture Time

According to previous report, the mechanism of metals uptake in plants from solutions was due to the metal diffuse through a membrane towards the bulk phase of the plant [18]. Thus with the time passing by and the growth of plants, more metals can enter the biomass of reeds. Taylor and Crowder [19] also indicated the growth of plants may increase the metal and nutrient transport. However, in authors' study, the iron uptake in duckweed did not increase with time. Duckweed accumulated (0.82 ± 0.03 mg Fe/g) from 10 mg/L Fe solutions after 4 weeks, which was not significantly different ($p < 0.05$) from Fe concentration in duckweed cultured for 8 week (0.80 ± 0.07 mg Fe/g) (Fig. 1).

Heavy metal transportation mechanisms in plants differ with the type of metals [20]. As mentioned earlier, the tendency of ferrous iron (Fe^{2+}) to quickly and easily oxidize into ferric iron (Fe^{3+}) as orange tinted precipitants when in a still water environment, may inhibit its translocation in plants with time. According to Stumm and Lee [21], factors including pH, temperature, and concentration of dissolved oxygen may increase the rate of oxidation. In our study, pH of all treatment groups was adjusted to 7 prior to the addition of *L. minor*. The oxidation rate of Fe^{2+} was slow at a low pH, but when pH is above 5, the oxidation rate increases greatly [21, 22]. In addition, as the containers were exposed to air; the dissolved oxygen levels could have increased. As dissolved oxygen levels increased, Fe^{2+} was oxidized into Fe^{3+} which was not readily absorbed by *L. minor* [21].

It is also reported that the oxidation of Fe^{2+} was first-order [22], thus the Fe^{2+} concentration decreased sharply in the first several hrs. The oxidation of Fe^{2+} was described in the following equation $\mathrm{In}[Fe(II)t] = -kt + \mathrm{In}[Fe(II)initial]$ [22]. The oxidation of dissolved Fe(II) and hydrolysis of Fe(III) can produce H^+ and decrease the pH of water [23]. In this study, pH in solutions was tested (Table 1). Generally, pH decreased with time. It is further proved that Fe^{2+} was precipitated and H^+ was produced; the precipitation of Fe^{2+} makes it unavailable for plants.

Results were reported as average ± standard deviation, $n = 3$. Different letters in the same column indicated a significant difference at $p < 0.05$.(it should be under the table)

3.3 Effect of Fe Speciation

In order to further test whether Fe precipitation would affect Fe uptake in duckweeds, iron solution was prepared and settled for 24 hrs, then duckweed was transferred into the solution. The amounts of Fe in duckweed were compared with those in duckweeds transferred to Fe solutions immediately. As expected, Fe levels in *L. minor* treated with15 mg/L Fe for 4 weeks (0.89 ± 0.06 mg/g) were significantly different from Fe concentration in duckweeds cultured in 15 mg/L pre-precipitated Fe solutions for the same time period (0.59 ± 0.05 mg/g) (Fig. 2). The amounts of Fe in plants cultured in solutions for 8 weeks were not significantly different from those in solutions for 4 weeks. It is further proved that the Fe^{2+} was oxidized into insoluble Fe^{3+} precipitates and inhibited the metal accumulation in plants.

Most of the iron (II) sulfate heptahydrate used in the treatments was precipitated within 24 hours of mixing. This was in accordance with previous findings that oxidation of Fe^{2+} in higher pH waters (> 5) oxidized in the first several hours [21, 22], especially sharply in the first 6 hrs [22]. It further proved that iron was predominantly in the form of ferric iron which is not readily bioavailable to plants due to Fe^{3+} insolubility. It is reported that the precipitated Fe^{3+} which is also called "yellow boy"

Table 1 pH of solutions cultured with duckweeds for 4 and 8 weeks.

Treatment	pH after 4 weeks	pH after 8 weeks
Control	6.98 ± 0.04^{a}	7.00 ± 0.03^{a}
5 mg/L	7.00 ± 0.02^{a}	6.81 ± 0.04^{a}
10 mg/L	7.00 ± 0.04^{a}	6.62 ± 0.06^{ab}
15 mg/L	7.00 ± 0.03^{a}	6.43 ± 0.05^{b}

Results were reported as average ± standard deviation, n = 3. Different letters in the same column indicated a significant difference at $p < 0.05$.

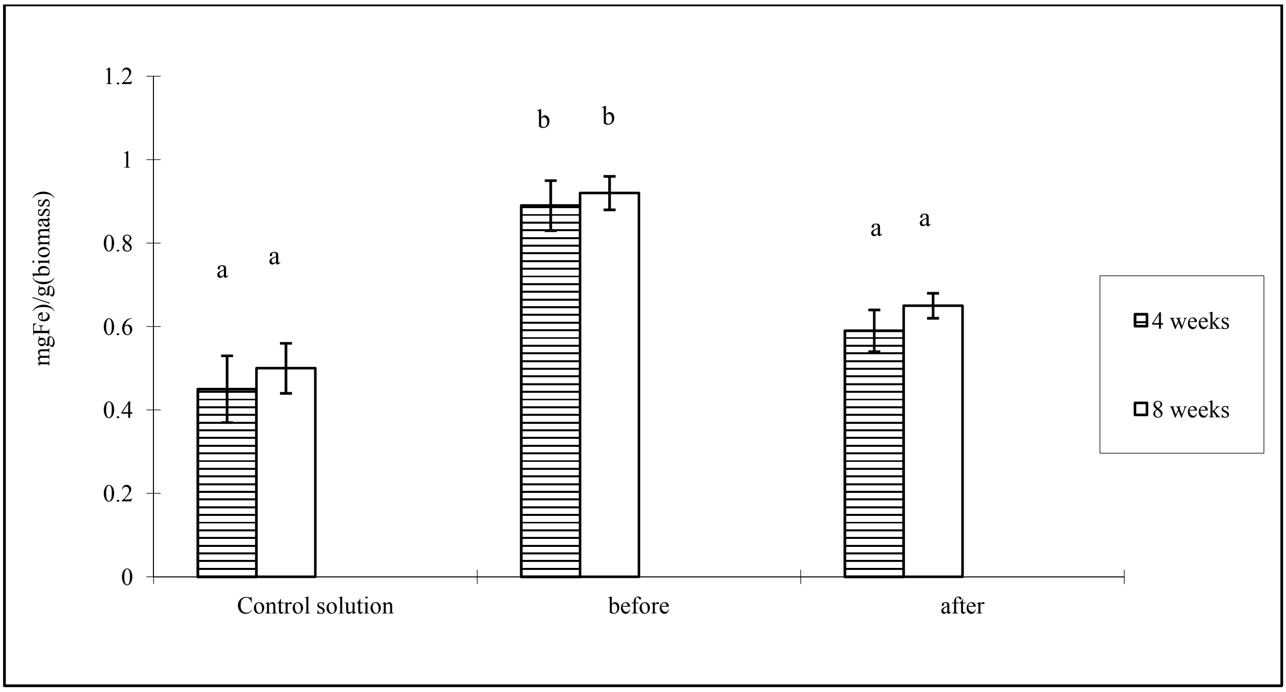

Fig. 2 The amounts of Fe (mg/g) in duckweed transferred to solutions before iron was precipitated or after iron was precipitated

can decrease water quality and affect the growth of aquatic organisms [22]. Thus it is significant to control the formation of Fe^{3+} and maintain the solubility of Fe^{2+} for phytoremediation. Previous research indicated that chelators can be applied to increase the mobility of metal and the accumulation of metals in plants. Future research is needed to study the application of chelator to enhance the phytoremediation efficiency for iron contaminated media.

4. Conclusions

This study aimed to determine whether duckweed can successfully be used to remediate iron contaminated solutions. The levels of iron in the duckweed did not increase with the concentrations of iron in solutions while it also did not increase with the culture time. The results indicated that the capability of duckweed to uptake iron was limited as iron was easy to be precipitated and form metal plaque. Under natural conditions, especially when pH of solution is more than 5, Fe^{2+} was easy to be oxidized to Fe^{3+} which was precipitated and unavailable for plant to uptake. The use of chelators to increase the bioavailability of iron is necessary to enhance the phytoremediation efficiency in future.

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