Phytoextraction of al and fe using *Eichhornia crassipes* in contaminated water from the madín dam

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ABSTRACT
The phytoextraction potential of water hyacinth (*Eichhornia crassipes*) in the treatment of a representative sample of water of Madín Dam, contaminated with 24.45 mg/L of aluminum (Al) and 5.10 mg/L iron (Fe) was evaluated. Two 60 days experiments were performed, one using young and adult plants and the other with young ones, extracted and substituted every 15 days. The percentage of maximum removal observed in the hyacinth exposed for 60 continuous days was the one of young hyacinths, namely 72%. Besides, those hyacinths extracted every 15 days extracted about 86% of the metals in contaminated water. *Eichhornia crassipes* turned to be a Al and Fe tolerant species, but not capable of transporting those metals from the roots to the leaves. However, its harvest implies the removal of the whole specimen and therefore the complete extraction of the plant. This indicates that a post-treatment of the extracted hyacinth as a hazardous material would be required, due to its high content of heavy metals.

Keywords: phytoextraction, water hyacinth, eichhornia crassipes, heavy metals, aluminum, iron.

1 INTRODUCTION

Metal pollution is a latent problem that represents a serious threat due to its persistence, bioaccumulation, non-biodegradability, and toxicity, even at low concentrations [1]. Metals are present in aquatic ecosystems due to discharge of wastewaters without treatment, which pollute rivers, lakes, lagoons and dams, as well as to coastal and marine environments [2].

1.1 MADÍN DAM

Madín Reservoir is a water body located in the Valley of Mexico that covers part of the municipalities of Naucalpan and Atizapán in the Mexico State [3]. With a total area of 100 km^2, it is supplies drinking water to the nearby communities; it hosts species such as the common carp and is a space for recreational activities such as fishing, navigation and canoeing [4]. The Madín Water Treatment Plant distributes water through the municipal supply to about 280,000 habitants of the communities of Atizapán and Naucalpan [5].

The water treatment process consists of coagulation-flocculation, sedimentation, sand filtration and final disinfection mainly by chlorine [3]. Usually the sludge generated during the process are not properly disposed of, according to NOM-004-SEMARNAT-2002 [6], at the time of filter cleaning, decanter purging and general maintenance. They are directly disposed in the water body or in unconditioned land, contributing significantly to the pollution of the Madín Dam, surrounding soils, and affecting the ecosystems of the area [7].

Unfortunately, in recent years, the reservoir has experienced significant deterioration due to population growth and development, leading to negative socio-environmental impacts such as displacement of native species, introduction of invasive ones, water scarcity, soil pollution, and irregular discharges, being the nearby settlements the main the main source of pollution [8].

According to different studies, among other pollutants and nutrients, high concentrations of metals, especially iron (Fe) and aluminum (Al), have been found, which exceed permissible levels [9][10].

The water hyacinth or *Eichhornia crassipes* is a freshwater perennial plant that is listed as one of the 100 most damaging invasive exotic species in the world [11]. Due to the elevated concentrations of nutrients, specially nitrogen and phosphorus from wastewatersdam has repeatedly been affected by this plague. One of the most significant crises occurred in 2019, when for more than 160 days, members of the Mexico State Water Commission (CAEM), the National Water Commission, the residents, and various social organizations managed to remove approximately 3,516 tons of water hyacinth from Madín Dam [12].

1.2 PHYTOREMEDIATION

Phytoremediation, specifically phytoextraction, is a technology that uses plants that can absorb, translocate, and accumulate heavy metals from contaminated soil or water [13]. These plants have a high capacity to transport and concentrate metals in their roots, stems and leaves; they can also develop a significant amount of biomass, which helps to accumulate a high concentration of the contaminant [14].

One family of plants that has shown great potential in the field of phytoextraction of metals are aquatic macrophytes [15]. Their species *Eichhornia crassipes*, known as water hyacinth, has shown to be a potential metal phytoremediator in water bodies [16] [17].

1.3 WATER HYACINTH (EICHHORNIA CRASSIPES)

The water hyacinth is a floating plant belonging to the plant kingdom and the *Pontederiaceae* family. Its structures called petioles have large air-filled intercellular spaces that allows it to stay afloat on water [18]. It has a short stem, circular bright green leaves and purple flowers (figure 1). Its roots are purple due to the presence of anthocyanin, a plant pigment that acts as a defense mechanism against ultraviolet light [19].

Due to its reproductive capacity and resistance, *Eichhornia crassipes* is considered one of the most invasive and damaging plagues in the current world. It is found in almost all aquatic habitats and represents an ecological, social, economic and political threat. The proliferation of the water hyacinth creates dense floating mats that impede water bodies flow, reduce the amount of light and directly affect aquatic habitats. In addition, it has an inverse relationship with dissolved oxygen concentration in water, which can cause a reduction in fish growth, failed eggs and larvae, immunological effects and death, turning the water body unable to support aquatic life [20][21].

Figure 1. Morphology of *Eichhornia crassipes*

The objective of this research was to evaluate the water hyacinth living biomass performance the removal of aluminum and iron from a representative sample of Madín Dam water and determine the tolerance of the species *Eichhornia crassipes* to the presence of high concentrations of these metals.

2 MATERIALS AND METHOD

Experiments were carried out in a sample of water collected from the Madín Dam. The highest values of heavy metals reported in literature were used in designing our experiments. Al and Fe initial concentrations used in spiking the sample to cover the ranges of reported concentrations [9] are summarized in Table 1.

Table 1. Maximum metals concentrations reported in the Madín Dam.

Metal	**Maximum concentration found (mg/L) [9]**
Aluminum	24.45
Iron	5.10

Aluminum chloride ($AlCl_3$) and ferric chloride ($FeCl_3$) were added (Table 2). The solubility of these salts was considered relevant as it determines the bioavailability of metals for plants.

Table 2. Reagents used to prepare water at maximum conditions reported for aluminum and iron.

Metal	**Salt**	**mg/L added**
Aluminum	$AlCl_3$	218.7
Iron	$FeCl_3$	24.7

Al and Fe extraction capacity of *Eichhornia crassipes* was studied in two experimental blocks. Block A contained young and adult plants cultivated in water for 60 days. Block B only contained young plants, which were extracted and substituted every 15 days of exposure to the same sample of water.

2.1 PLANTS USED IN EXPERIMENTS

All plants used in the current study were acquired in the Cuemanco Flower and Plant Market, Xochimilco Mexico. In addition, all experiments were conducted in a greenhouse environment in plastic reactors.

2.2 EXPERIMENTAL SETUP

In Block A, four duplicate experiments were set with the young species *Eichhornia crassipes* and its corresponding control, measuring Al and Fe concentration every 15 days. Additionally, a duplicate experiment with adult hyacinths and its control was run over an 8-week period.

Block B contained only young plants in two experiments with the same metal concentrations as Block A. Samples of water and the two plants were extracted every 15 days, and two clean young specimens were substituted in weeks 2, 4, and 6.

Due to the high evapotranspiration rate of the water hyacinths, distilled water was added to compensate the loss and maintain metal concentration balance.

2.3 WATER SAMPLES ANALYSIS

Contaminated water sample was treated with HNO_3 and digested in a microwave, using EPA-3015A method for 20 minutes. The solution was filtered for analysis in an atomic absorption spectrophotometer.

2.4 PLANT TISSUE ANALYSIS

Plant tissue was sampled by separating the root from the aerial part and drying it in an oven at 60°C until crispy texture, and each part was weighted. The sample was digested in a microwave with nitric acid, filtered, analyzed by atomic absorption spectrometry.

2.5 MICROBIOLOGICAL ANALYSIS

The presence of total and fecal coliforms in Madín Dam water was determined using the most probable number (MPN) method.

2.6 BIOCONCENTRATION AND TRANSLOCATION FACTORS

Heavy metals accumulation and adsorption effect in plants were studied using the bioconcentration factor (BCF) and translocation factor (TF).

The BCF is related to the plants capacity to absorb metals during the phytoextraction process [22]. It is calculated as a ratio of metal concentration in the plant tissue (C_T), both roots and aerial part; and the metal concentration in water (C_W), as follows:

$$BCF = \frac{C_T}{C_W}$$

The TF indicates the plants ability to translocate heavy metals from roots to stems and leaves. It is calculated as the ratio of metal concentration in the aerial (C_L) and root (C_R) parts, as:

$$TF = \frac{C_L}{C_R}$$

BCF and TF values above 1.0 indicate potential hyperaccumulation, values between 0.1 and 1.0 indicate tolerance, and values lower than 0.1 indicate low extraction and translocation capacity.

3 RESULTS AND DISCUSSION

3.1 WATER INITIAL CHARACTERIZATION

The results of the characterization of the Madín Dam water are shown in Table 3.

Table 3. Water initial characterization

Parameter	Result	Maximum allowable limit / Optimum range
Al	0.72 mg/L	0.2 mg/L [1]
Fe	0.45 mg/L	0.3 mg/L [1]
pH	8.84	4-10 [2]
Temperature	18 °C	28-30 °C [2]
Total coliforms	920 MPN/100 mL	---
Fecal coliforms	140 MPN/100 mL	240 MPN/100 mL [3]

Note:
NOM-127-SSAI-1994. Mexican Official Standard that establishes the maximum permissible limits of contaminants in wastewater discharges in waters and national assets
Optimal growth conditions for the species *Eichhornia crassipes* [18].
NOM-003-SEMARNAT-1997. Mexican Official Standard that establishes maximum permissible limits of contaminants for treated wastewater reused in public services.

Al and Fe concentrations in the initial water sample from Madín Dam were 0.72 mg/L and 0.45 mg/L respectively, both above the maximum permissible limits but below the maximum concentrations found in 2014 [9]. The water temperature was below the optimal range for the growth of the *Eichhornia crassipes* species while pH value was within the appropriate range.

The results of the water characterization prepared with aluminum and iron are shown in Table 4.

Table 4. Al and Fe contents of trial water

Metal	Result (mg/L)
Al	20.37
Fe	5.13

3.2 REMOVAL OF METALS IN WATER

3.2.1 Block A: removal after 60 days

The results of metal removal with *Eichhornia crassipes* are shown in Table 5.

Both young and adult specimens were able to remove at least 50% of the initial metal concentration in water. The adult specimen showed similar behavior to the young one. Additionally, both specimens showed a higher capacity for absorbing Fe than Al, at 72% and 68%, respectively.

Table 5. Removal results in water after 60 days and comparison of young and adult hiacynths before and after treatment.

Specimen	Metal	Result (mg/L)		Removal percentage
		Initial	Final	
Young	Al	20.368	9.171 ± 1.390	55%
	Fe	5.128	1.438 ± 0.610	72%
Adult	Al	20.368	8.540 ± 0.485	58%
	Fe	5.128	1.644 ± 0.042	68%

3.2.2 Block B: removal during the first 15 days of exposure

The purpose of Block B was to determine the Al and Fe removal potential of young *E. crassipes* during the first 15 days of exposure. The final concentration of contaminated water was calculated after 60 days and the results in Table 5 show a highly significant decrease in Al and Fe concentration with removal percentages of 86% and 84%, respectively.

Table 6. Removal results in water after 60 days of placing clean hiacynths every 15 days, before and after treatment.

Metal	Result (mg/L)		Removal percentage
	Initial	Final	
Al	20.368	2.935 ± 0.030	86%
Fe	5.128	0.800 ± 0.140	84%

3.2.3 Block A *vs* block B

The exposure time of water hyacinths to contaminated water has a direct influence on the metals removal. A removal percentage of 55% for Al was observed in experimental Block A, while in Block B it was 86%. A similar trend was observed with Fe with a removal of 72% in Block A and 84% in Block B.

Al and Fe removal trend by young *E. crassipes* in both Blocks over 60 days of exposure is shown in figures 2 and 3, respectively.

A significant decrease in both metals concentration was observed during the first 15 days. However, at day 30 an important increase in metal concentration was noted due to the shedding of plant tissue, which remains as sediment. From 45 days on, the removal trend stabilized. The reduction of metal concentration generally followed two patterns: a rapid initial decrease followed by a slower constant decrease and stabilization.

Therefore, *Eichhornia crassipes* resulted effective in removing metals from water, especially when clean hyacinths were added every 15 days. According to Haddad *et al.*, [23], rapid absorption and accumulation of metals through the roots is due to adsorption on the cell walls and chemical processes like chelation and ionic exchange. The slower process of metal removal is due to precipitation in the roots and biological processes like intracellular uptake.

Figure 2. Trend of aluminum removal in phytoextraction treatment after 60 days using *Eichhornia crassipes*.

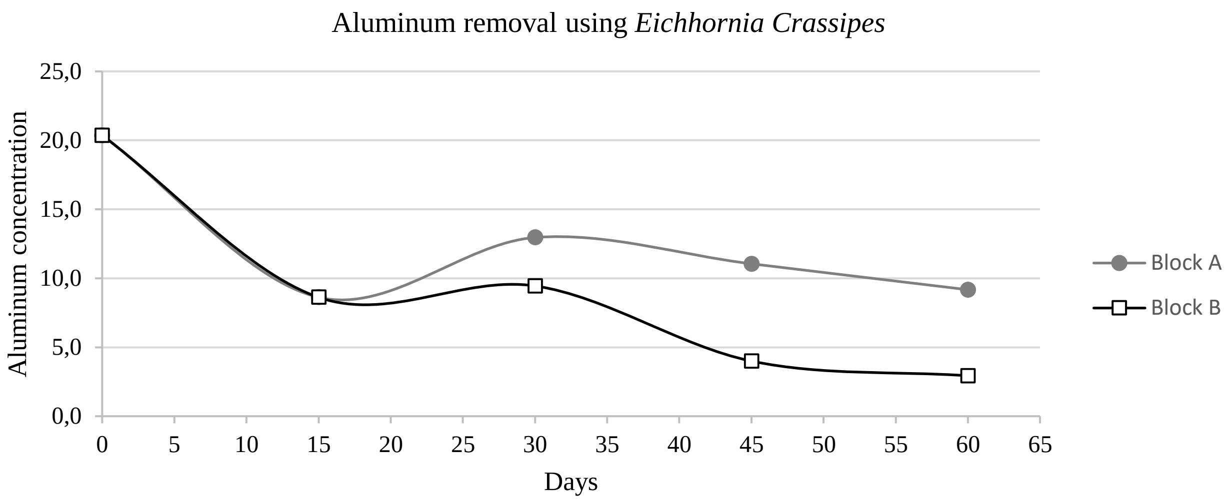
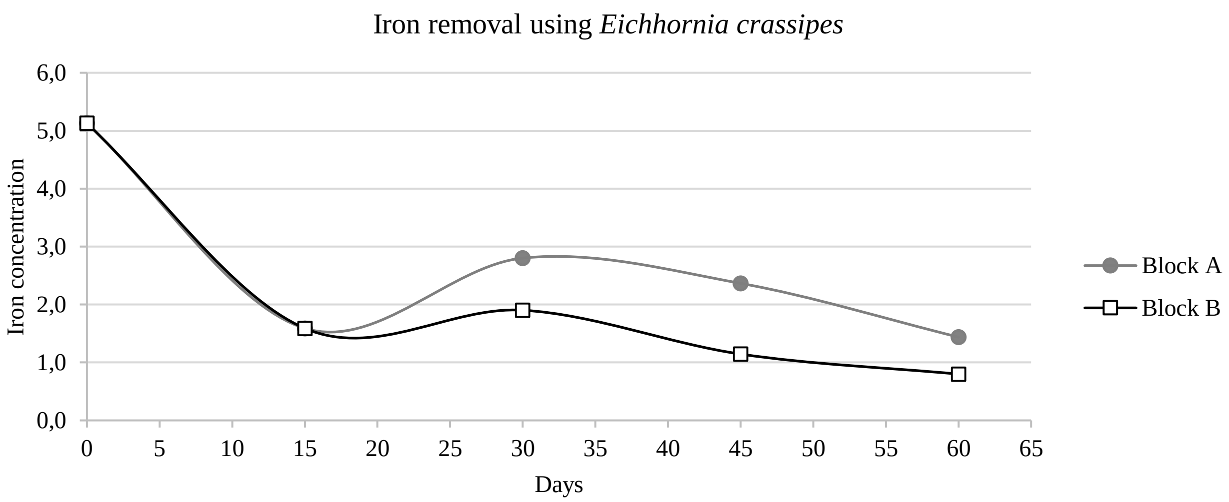


Figure 3. Trend of iron removal in phytoextraction treatment after 60 days using *Eichhornia crassipes*.



3.3 PLANT TISSUE INITIAL CHARACTERIZATION

Al and Fe concentrations in water hyacinth was found to be very low in both roots and leaves. The adult specimen had a higher concentration due to its longer exposure to metals in water.

The results of the analyses performed during the characterization of the water hyacinths initial samples are shown in Table 7.

Table 7. Al and Fe concentrations present in the initial sample of water hyacinths

Specimen	Metal	Dry mass concentration (mg/L)	
		Roots	Leaves
Young	Al	0.054 ± 0.029	0.016 ± 0.010
	Fe	0.051 ± 0.029	0.022 ± 0.012
Adult	Al	0.190 ± 0.003	0.024 ± 0.002
	Fe	0.250 ± 0.036	0.041 ± 0.004

3.4 METAL PHYTOEXTRACTION IN PLANT TISSUE

3.4.1 Block A: removal for 60 days

The results of Al and Fe final accumulation in the plant tissue of young and adult specimens of the *E. crassipes* in experimental Block A, in which they were exposed to contaminated water for 60 days, are shown in Table 8.

Water hyacinth has higher capacity for metal absorption and retention in its roots, with higher concentrations of Al and Fe found in the submerged parts of the roots in both young and adult specimens. There was no significant translocation of metals to the aerial part of the plant during the experiment.

3.4.2 Block B: removal during the first 15 days of exposure

Al and Fe accumulation in plant tissue of the species *E. crassipes* for experimental Block B, where a destructive sample was taken every 15 days and a clean hyacinth was added to the contaminated water are shown in Table 9.

Table 8. Accumulation of Al and Fe in the aerial (leaves) and submerged (roots) part of *E. crassipes* after 60 days of treatment.

Specimen	Metal	Dry mass concentration (mg/L)	
		Roots	Leaves
Young	Al	2.289 ± 0.345	0.727 ± 0.309
	Fe	1.568 ± 0.123	0.211 ± 0.050
Adult	Al	2.823 ± 0.111	0.800 ± 0.089
	Fe	2.059 ± 0.877	0.255 ± 0.012

Table 9. Accumulation of aluminum and iron in the aerial (leaves) and submerged (roots) parts of *E. crassipes* during block B removal.

Day	Metal	Dry mass concentration (mg/L)			
		Roots		Leaves	
		Al	Fe	Al	Fe
15	1	4.150 ± 0.102	2.375 ± 0.168	0.102 ± 0.031	0.041 ± 0.001
30	2	1.262 ± 0.192	0.376 ± 0.107	1.108 ± 0.016	0.267 ± 0.042
45	3	0.735 ± 0.254	0.353 ± 0.139	0.149 ± 0.064	0.097 ± 0.057
60	4	0.632 ± 0.136	0.248 ± 0.045	0.302 ± 0.111	0.86 0.024

3.4.3 Block A vs Block B

Al and Fe absorption and accumulation trends in the plant tissue, roots and leaves, of *E. crassipes* after 60 days of both blocks treatment are presented in figures 4, 5, 6 and 7, respectively.

For both metals, a similar behavior was observed: the first 15 days, the roots showed the maximum metals extraction, the following 15 days, part of the metal is released back to water, while another part is translocated to the leaves. After 45 days, the roots extract again a portion of metal and stabilize.

In general, metal absorption followed two different patterns: a rapid initial absorption followed by a slower and constant absorption, a stabilization.

3.5 BCF AND TF DETERMINATION

3.5.1 Block A: removal for 60 days

In Table 10, the bioconcentration factor (BCF) and the translocation factor (TF) during the 60-day duration of experiment block A are shown.

3.5.2 Block B: removal during the first 15 days of exposure

The results of the calculation of the bioconcentration factor (BCF) and the translocation factor (TF) during the 60-day duration of experiment block B are shown in Table 11.

Table 10. BCF and TF factors calculated during block A

Specimen	Metal	BCF	TF
Young	Al	0.33	0.32
	Fe	1.23	0.13
Adult	Al	0.42	0.28
	Fe	1.41	0.12

Table 4. BCF and TF factors calculated during block B

Days	Hyacinth	BCF		TF	
		Al	Fe	Al	Fe
15	1	0.49	1.52	0.02	0.02
30	2	0.25	0.34	0.88	0.71
45	3	0.22	0.39	0.20	0.28
60	4	0.32	0.42	0.48	0.35

The water hyacinths absorbed the most metals in their roots in the first 15 days, as seen by the highest BAC observed. At day 30, a decrease in BAC and an increase in TF was noticed, indicating that the plant achieved its maximum translocation capacity from day 15 to 30. After day 30, the trend of metal absorption stabilized.

Thus, *E. crassipes* was observed as potentially hyperaccumulator of Fe and could be considered Al tolerant, as seen by BCF values greater than 1.0 for iron and between 0.1 and 1.0 for aluminum for both specimens and blocks. Nevertheless, the capacity of the plant to transport absorbed Al and Fe to the aerial part is limited, as indicated by FT values being less than 1.0.

For being an aquatic species, its harvest involves the total removal of the specimen and, therefore, the complete extraction of the plant. This implies considering the post-treatment of the harvested water hyacinth as hazardous material, due to its high levels of heavy metals [23].

As mentioned earlier, *Eichhornia crassipes* is considered an invasive plague [11] and therefore, its application as metal phytoextractor must be considered in confined environments, such as plant "farms", which should be thoroughly monitored and frequently removed from the water. Exposed plants, with high concentrations of heavy metals, should be correctly managed and disposed off as hazardous residuals.

Figure 4. Comparison of block A and B trends in aluminum accumulation young *Eichhornia crassipes* roots after 60 days of treatment

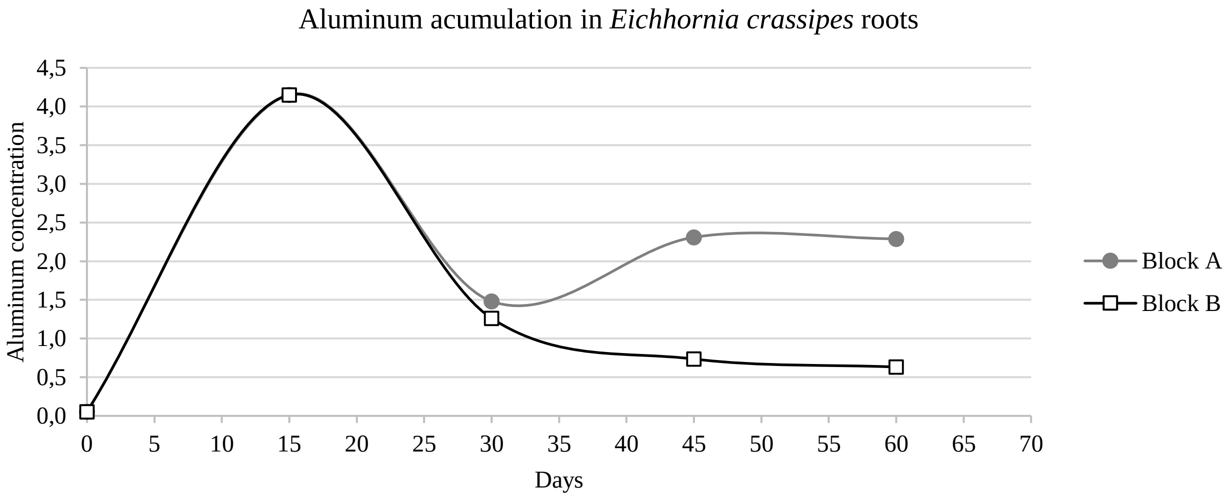


Figure 5. Comparison of block A and B trends in irion accumulation young *Eichhornia crassipes* roots after 60 days of treatment

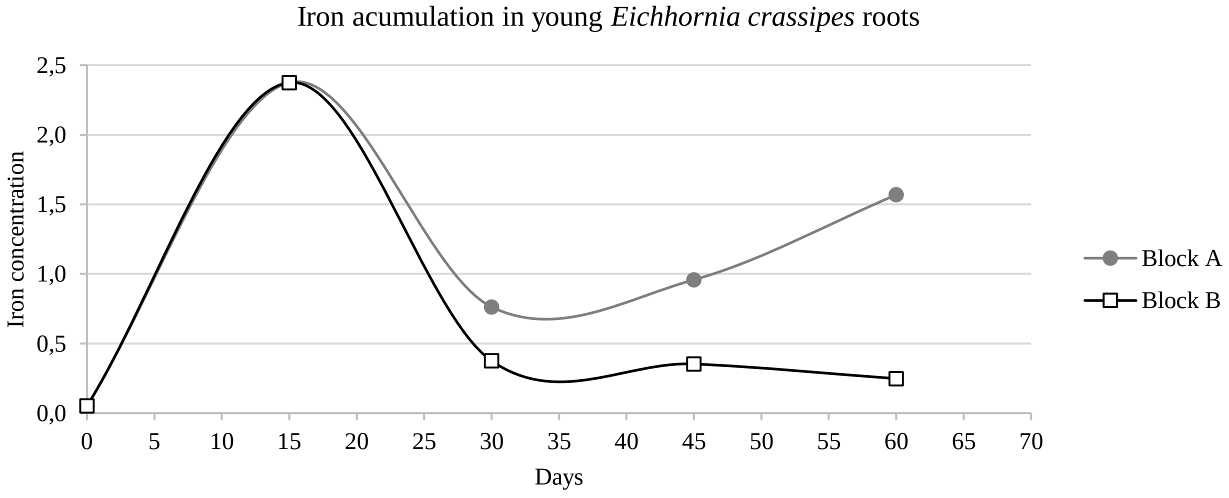


Figure 6. Comparison of block A and B trends in aluminum accumulation young *Eichhornia crassipes* leaves after 60 days of treatment

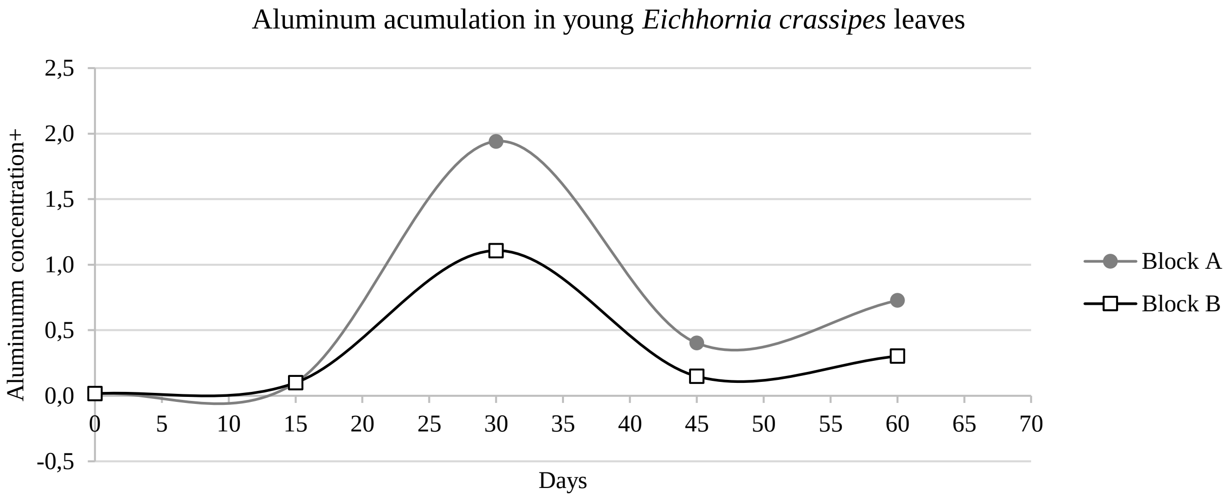
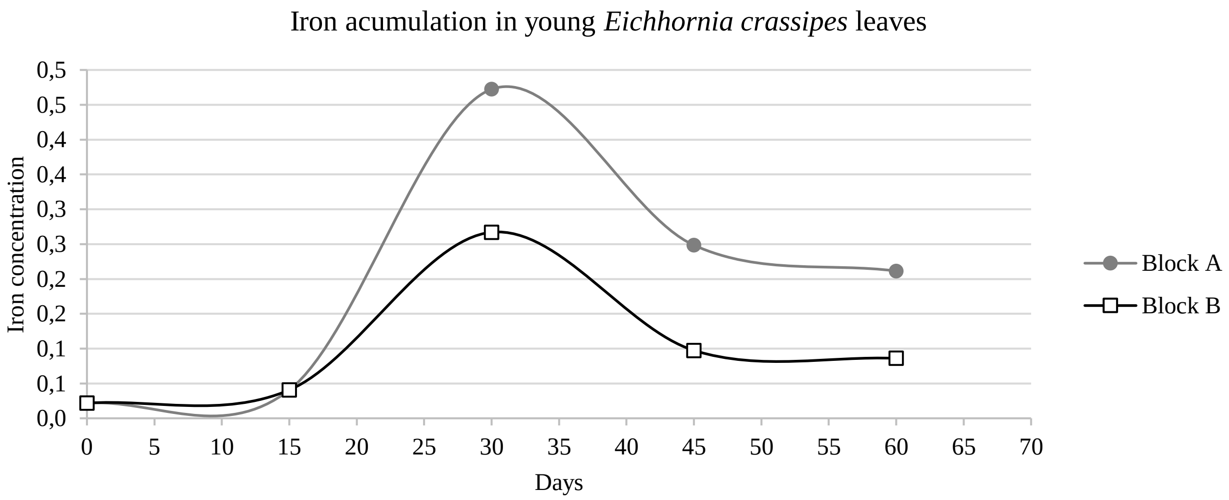


Figure 7. Comparison of block A and B trends in iron accumulation young *Eichhornia crassipes* leaves after 60 days of treatment



4 CONCLUSION

Phytoextraction employing water hyacinth is a promising technology to separate Al and Fe from contaminated water, this vegetable species has the capacity to bioaccumulate metals especially in its roots. Furthermore, harvesting these aquatic plants every 15 days and replacing them with clean hyacinths enhances the bioaccumulation factor. However, as an aquatic species, the complete removal of the exposed plant implies that it must be considered as hazardous material due to its high content of heavy metals.

Eichhornia crassipes is a viable solution for cleaning contaminated water in the Madin Reservoir or any other water body high in Al and Fe. To maximize cleaning efficiency, a 20-day exposure system and subsequent removal is recommended. Nevertheless, due to the high ability of reproduction of this species, it is common to observe it as aquatic plague, contributing to the eutrophication of dams and lakes. Therefore, the plant system must be carefully confined and monitored to prevent any negative environmental, social, or economic impact.

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