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Evaluation of the effectiveness of *Azolla* fern in removing the toxicity of lead and cadmium in water

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Abstract

Background: Heavy metals are harmful to living organisms and are emitted into the environment from many different sources. Compared to alternative remediation technologies that are either too expensive or impractical, phytoremediation, in which plants are used to remove hazardous toxins from water and soil, is a viable option for use at extremely large field sites. Heavy metals may be removed from the environment by a wide variety of plants. *Azolla*, a kind of aquatic fern, is one example.

Methods: Different concentrations of heavy elements (5,10, and 25mg/ L) were prepared and treated with the plant in different masses (0.2,0.4, and 0.8 grams)and over time periods of one and two weeks. The concentration of each element was measured before and after the treatment periods using an atomic absorption device.

Results: As evidenced that spring *Azolla* (green type) was more effective at removing cadmium and lead elements than autumn *Azolla* (red type), where the spring *Azolla* recorded higher removal efficiency for Cd , where it was 1.80% under biomass 0.8g, contact time 10 days, and at metal concentration 10mg\L ,while the highest percentage removal for Pb , was 40.02% under biomass 0.4g, contact time 15 day and at metal concentration 5mg\L . *Azolla* has great potential for removing heavy metals from water resources and can be used in heavy metal phytoremediation initiatives in environmental improvement projects.

Conclusion: This research suggests using the aquatic macrophyte *Azolla*, especially spring fern, to treat sewage and household wastewater containing Cadmium and Lead.

Introduction

Heavy metal pollution is becoming an increasingly urgent environmental problem in emerging nations as a result of both natural and human-caused factors. These actions led to a rise in concentrations of these elements to dangerous levels that are bad for ecosystems and people [1].

As a result of the fact that heavy metals are known to have negative impacts on human health, heavy metal pollution of the food chain should get further focus. A great number of heavy metals and metalloids are poisonous, meaning that even at very low concentrations, they may induce unwanted consequences and serious issues [2]. In terms of their toxicity, the most troublesome heavy metals include mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), copper (Cu), zinc (Zn), and tin (Sn) [3]. Out of these, mercury, cadmium, lead, and arsenic are examples of non-essential heavy metals, which means that living organisms do not need them for any physiological or biochemical process [4,5]. On the other hand, copper and zinc are examples of essential heavy metals (trace elements) [6,7]. Heavy metals that are toxic to humans may cause a variety of health issues, depending on the specific heavy metal, its concentration, its oxidation state, and other factors [8].

One of the ways that the concentration of heavy metals in the environment may be reduced is via the use of phytoremediation. There are several types of plants that have the ability to take in quite large quantities of heavy metals. Hyperaccumulators are the term given to these organisms, and they are used for the purpose of cleaning up the environment [9]. It is an innovative solar-driven remediation technique that is cost-effective, efficient, environment- and eco-friendly, in-situ applicable, and environmentally benign [10-13].

The aquatic fern *Azolla* is a cosmopolitan genus that is found all over the world, native to Asia, Africa, and the Americas. In the natural surroundings, *Azolla* lives in lakes, swamps, rivers, and other waters, others have extended into different parts of the world through human or natural means. Some of them are purely marine, while others rise and grow in temperate or tropical regions [14]. It is a floating pteridophyte symbiotic of *Anabaena azollae* cyanobacterium [15]. It inhabits stagnant or slow-moving water and is observed to float as individual specimens on the water surface or, more commonly, as mats with a thickness of up to 20 cm [16]. For several years *Azolla* belonged to Azollaceae as a distinct family but now taxonomist has assigned *Azolla* to a monotypic family Salviniaceae as a synonym of Azollaceae according to genetic bases [17,18].

Azolla is an effective bioremediation plant for cleaning up a wide variety of wastewater types, from sewage to industrial effluents. It is also environmentally friendly and reliable [19,20], Also can uptake trace elements in water [21, 22, 23]. This research aimed to investigate whether or not the extract of *Azolla* can remove lead and cadmium from water and compare it with the effectiveness of fern detoxification if heavy metals are added directly to it.

Methods

The preparation of heavy metals stock solutions

Cd(II) and Pb(II) metals stock solutions with an initial concentration of 1000 mg/L were generated by dissolving a suitable quantity of nitrate salts of these metals (i.e., $Cd(NO_3)_2$ _$4H_2O$ and $Pb(NO_3)_2$, respectively) in an adequate volume of double-distilled water. Three dilutions were prepared from each heavy-metal stock solution (5, 10, and 25 mg/L) using double-distilled water

Collection of plant samples

Fresh plant samples were collected several times during field trips from different locations along the Euphrates River, which passes through the Al-Musayib region in Babylon to the Al-Jimijma region during two periods, the spring period from February to April 2022 and the autumn period from September to December 2022, by placing them in containers containing an amount of water and placed in the refrigerator at temperature 4°C until use. The fresh leaves collected during field trips were cleaned and washed with running water several times to clean them from dirt and other plants, the parts exposed to fungal infections were removed, then left at room temperature to dry, and after making sure that they were completely and well dried, they were ground in an electric grinder and kept in bags.

Preparation of experimental samples

After that, the samples were weighed in (0.2,0.4, and 0.8 grams), placed in cylindrical plastic dishes with 9 cm depth and roughly 200 cm^3 volumes, and filled with 100 ml of test solutions containing Cd (II), Pb (II), and deionized water as a reference Inoculum solution. The dishes were then weighed in again. Up to the 15th day of the trial, samples from each concentration were taken weighed, and digested once every five days on average. Heavy metal concentrations were measured using an atomic absorption spectrophotometer, and water samples were kept in polyethylene containers for storage [25].

Results

In this study, two types of *Azolla* were used, the red type which spreads on the surfaces of ponds and rivers in the autumn season, while the other type (with green color) spreads in the spring season. As can be seen from Fig. 1, when using the autumnal *Azolla* fern, the highest removal efficiency of cadmium was recorded at 1.52% under biomass of 0.8 g, contact time 15 days of treatment, and at a metal concentration of 5 mg/L, while the lowest removal efficiency was 0.15% under biomass 0.2g, contact time 5 day of treatment and a metal concentration of 5 mg/L. Whereas Fig. *2* shows the highest removal efficiency of lead was recorded at 15.27% under biomass of 0.8 g, contact time of 5 days of treatment and at a metal concentration of 5 mg/L, and the lowest percentage of removal was recorded at 0.027% under biomass 0.2 g, contact time 15 day and at a metal concentration of 25mg\L.

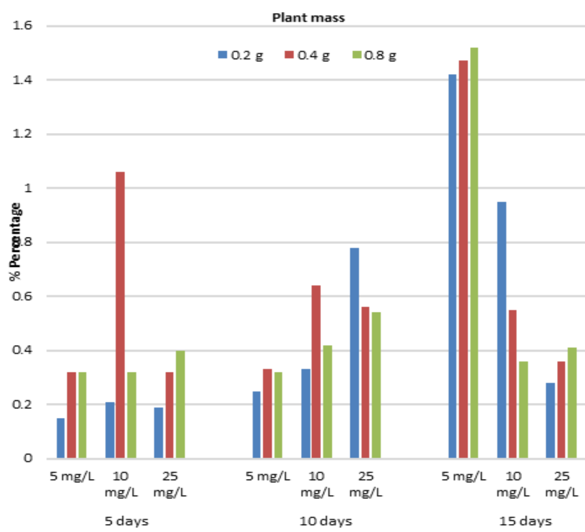

Figure 1: Shows the removal efficiency of Cadmium by red type of *A. fern* under different conditions of biomass, contact time, and metal concentration.

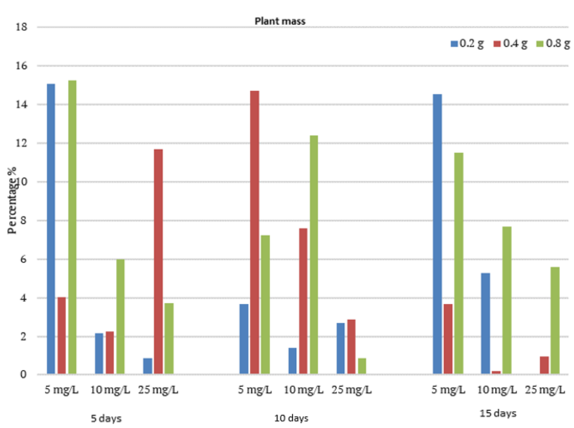

Figure 2: Shows the removal efficiency of Lead by red type of *A. fern* under different conditions of biomass, contact time, and metal concentration.

The spring *Azolla* recorded higher removal efficiency for Cadmium, where it was 1.80% under biomass 0.8g, contact time 10 days, and at metal concentration 10mg\ L as in Fig. 3, while the lowest removal efficiency was 0.04% under biomass 0.8g, contact time 10 day of treatment and a metal concentration of 25 mg/L .However the highest percentage removal for the lead as in Fig. 4, was 40.02% under biomass 0.4g, contact time 15 day and at metal concentration 5mg\L .On the other hand, the lowest removal rate of Lead was 0.89% under biomass 0.2g, contact time 10 day and at metal concentration 25mg\L. Moreover, it was determined that more biomass growth nearly led to more removal.

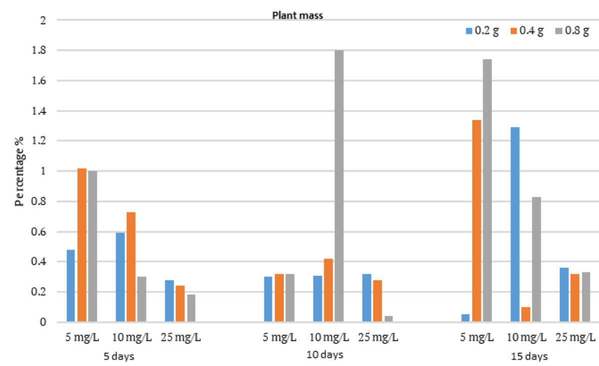

Figure 3: Shows the removal efficiency of cadmium by green type of *A. fern* under different conditions of biomass, contact time, and metal concentration.

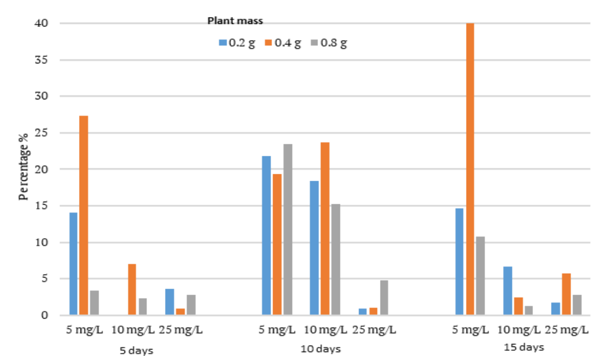

Figure 4: Shows the removal efficiency of Lead by green type of *A. fern* under different conditions of biomass, contact time, and metal concentration.

In table 1, the effect of removing cadmium element on the two types of *Azolla* fern used in this study is shown, as it was found that the autumn fern with a red color had a significant effect in reducing the toxicity of the cadmium element after the tenth day at a concentration of 10 mg/L at a P-value equal to 0.001 and became equal to 0.004 after 15 days and at concentrations of 5 and 10 mg/L, respectively. While the green spring fern showed a P-value equal to zero since the first five days at concentrations 5, 10, and 25 mg/L and also after 15 days at a concentration of 0 mg/L only, while the rest of the concentrations did not show any significant effect according to P-values.

Plant type	Days	motive con. mg/L	Cadmium effect/ppm Mean ± Std. D.	P *value* (P ≤ 0.05)	Lead effect / ppm Mean ± Std. D.	P *value* (P ≤ 0.05)
Red type	5	5	.02356 ± .008853	.182 NS	1.52111 ± .852658	.572 NS
		10	.07244 ± .062095		1.34333 ± .843096	
		25	.09278 ± .032077		2.86778 ± 2.959734	
		Control	19.25333 ± 11.748218		34.23333 ± 19.421981	
		Total	4.86053 ± 10.021206		9.99139 ± 16.867610	
	10	5	.02656 ± .003405	.001 *	1.13333 ± .748539	.305 NS
		10	.06311 ± .021818 *		2.75889 ± 2.132232	
		25	.18889 ± .039182		1.13333 ± .579233	
		Control	17.14667 ± 10.511828		37.50000 ± 21.656408	
		Total	4.35631 ± 8.920956		10.63139 ± 18.688495	
	15	5	.12867 ± .004177	.004*	1.31678 ± .743463	.879 NS
		10	.08500 ± .041030		1.69389 ± 1.474361	
		25	.10544 ± .020156		1.15811 ± 1.568900	
		Control	15.78667 ± 11.051246		32.91333 ± 18.969727	
		Total	4.02644 ± 8.514578		9.27053 ± 16.421882	
Green type	5	5	.07322 ± .026769	.000 **	2.42444 ± 1.042355	.366 NS
		10	.07389 ± .029321		1.20878 ± 1.387329	
		25	.06511 ± .009311		1.27333 ± .715914	
		Control	19.25333 ± 11.748218		34.23333 ± 19.421981	
		Total	4.86639 ± 10.018081		9.78497 ± 16.936165	
	10	5	.02767 ± .000882	.370 NS	2.85667 ± .274246*	.002 *
		10	.11522 ± .112609		7.38222 ± 1.663485*	
		25	.06589 ± .045272		1.17778 ± 1.185722*	
		Control	17.14667 ± 10.511828		37.50000 ± 21.656408	
		Total	4.33886 ± 8.930019		12.22917 ± 17.996846	
	15	5	.09144 ± .076919	.000**	2.89578 ± 2.106230	.478 NS
		10	.10067 ± .081206		1.33778 ± 1.101223	
		25	.10200 ± .006984		1.81556 ± 1.098273	
		Control	15.78667 ± 11.051246		32.91333 ± 18.969727	
		Total	4.02019 ± 8.517815		9.74061 ± 16.195213	

* mean significant difference (P ≤ 0.05) ** mean highly significant difference (P ≤ 0.05) Std .D: stander deviation
NS: Non-significant difference (P ≤ 0.05) by ANOVA

Table 1: Effect of Cadmium and Lead on different plant types (based on growth season) according to motive concentration.

On the other hand, the effect of removing the lead element on the two types of *Azolla* fern displayed that the autumn fern with a red color did not show any significant effect according to P-values at all concentration during the treatment period. While the green spring fern showed a P-value equal to 0.002 after 10 days at a concentration of 25mg/ L only. In comparison between the two types, the current results showed that the spring *Azolla* is better by removing the cadmium and lead elements than the autumn *Azolla*.

Discussion

The mechanism that is responsible for metal accumulation may be detoxicated through the confiscation of heavy metal ions in vacuoles. In vacuoles, heavy metal ions bind with organic acids, proteins, and individual peptides with the help of enzymes, the selective transport and uptake of ions, osmotic adaptation, and salt [26,27]. Once they have been absorbed, the necessary surplus as well as non-essential metals are stored in the vacuoles of the leaf cells. It has been hypothesized that vacuolar uptake is involved in the process of differential metal accumulation and storage, despite the fact that the precise molecular mechanisms that drive these processes are not understood [28].

The cell walls and vacuoles of *Azolla* leaves are the sites of lead accumulation in the plant. Lead accumulated in greater aggregates in the older leaves of the *Azolla* than it did in the younger leaves. Through their H+-ATPase activity, the tonoplasts may play a role in the buildup of lead in the vacuoles, which are secondary ion transporters. [29]. According to Thayaparan [30]. The capacity of *Azolla* sp. to absorb lead and its bioconcentration factor both increased as the concentration of lead in the growing medium rose. The most that *Azolla* is capable of absorbing is 4%. However, its ability to absorb grew significantly with the length of time it was exposed to the substance

In addition, Melo [31] found that there were size differences in epidermal tissues as a result of the circumstances of water pollution. Increased thickness of the abaxial and adaxial may be associated with the adsorption of metals in the cell walls, which constitutes an alternate channel for the allocation of these ions and prevents their translocation to photosynthetic tissues when triggered by heavy metals [32]. According to Sridhar, [33] who cited that being subjected to heavy metals causes a decrease in the thickness of mesophyll cells. This might explain the thinner leaf blades that were seen in the treatments that were exposed to pollution. Some species, depending on the degree of morphological plasticity they possess, generate changed leaf tissues that enable improved adaptation to a variety of environmental stresses [31].

The toxicity of heavy metals has an adverse effect on photosynthesis because it causes a distortion of chloroplast ultrastructure, which in turn inhibits the production of photosynthetic pigment in chlorophyll content as well as enzymes involved in the Calvin cycle [34]. Under the influence of heavy metals, a number of studies have seen a reduction in the amount of chlorophyll present in a variety of plant species. Changes in pigment content are connected to visual signs of plant sickness and the productivity of photosynthetic processes, hence it is common practice to test the chlorophyll content of plants when attempting to determine the influence of environmental stress [35]. In a study with *Salix viminalis* L. cultivated in the presence of Cd observed increased thickened walls of the collenchyma and pericycle, with higher concentrations of metal than the other tissues [36]. This suggests that a plant's strategy for tolerating toxic levels of heavy metals may involve directing the deposition of heavy metals to non-photosynthetic tissues. Metals have a tendency to be distributed throughout the leaf tissues in order to minimize their concentration in the chlorophyll parenchyma, thus protecting the photosynthesis process from being disrupted [36].

According to the current results, *Azolla* has a significant amount of potential for eliminating heavy metals from water resources. Additionally, it is suitable for use in heavy metal phytoremediation efforts within environmental improvement projects. Heavy metals are among the most concerning of the many contaminants that may be found in water because of the nature of their ability to remain in the environment, bioaccumulate, and biomagnify [37]. A significant quantity of metals may be absorbed by *Azolla* fern, where they are then stored [38]. The ability of several free-floating aquatic plants, such as *Salvinia molesta*, *Pistia stratiotes*, *Echhornia*, *Spirodela* and *Bacopa monnieri*, to remove heavy metals from the sewage of varying quantities was tested, and the results showed that these plants were effective [39,40].

Phytoremediation is an option that may be used at extremely large field sites, in situations when other remediation strategies are either not cost-effective or cannot be implemented [41]. When compared the results reached the results of Naghipour [25]. It is found that the fern is more effective in removing the toxicity of Cd and Pb when added directly to the fern without the extract, as well as phytoremediation has relatively modest costs for both installation and ongoing maintenance in contrast to other cleanup methods [42].

Toxic metal contamination is a big issue in most large cities. Geo-accumulation, bioaccumulation, and biomagnification may result from harmful metals entering the environment. Because heavy metals are indestructible and poisonous at high concentrations, they pollute the environment worldwide. According to this study, the aquatic macrophyte *Azolla*, particularly spring type, may be employed as a phytotool to remediate sewage and residential wastewater containing heavy metals like Cadmium and Lead.

Author Contributions

Wurood H. Muttaleb: Study conception and design the experiment and make the part related to heavy metals, data collection and analysis and interpretation of results.

Huda J. Altameme: Study conception and design on the part related to the plant, checking the data, writing and manuscript preparation.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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