



Assessment of Selected Heavy Metals in Concentrations in Water and Azolla Samples Collected from Different Regions in Iraq

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ABSTRACT

This study was performed on the aquatic plant Azolla, an aquatic fern. A measure of the concentrations of the heavy metals Cadmium, Copper, Iron, Nickel, and Lead in the plants and water samples, which were chosen from three different regions: Baghdad, Al-Ghalibiya, and Khan-Bani-Saad. The results showed higher measurements in the plant compared to the water. The highest value in the plant was for Cadmium in the Al-Khan region at 0.653 ppm, while the highest result for Copper was in the Azolla plant from the Baghdad region, reaching 0.800 ppm. On the contrary, the highest value for Iron in the Azolla plant was in the Al-Khan region, reaching 2.283 ppm. In the Al-Khan region, the highest value for Nickel was 0.316 ppm for the Azolla plant. The measurement of Lead showed the highest percentage present in the plant from the Al-Ghalibiya region at 1.100 ppm. The results for DO, Tw, TDS and pH showed their highest values of 10.033 mg/l-1, 20.233 °C, 643.333 ppm, and 8.080, in the same order, in the Al-Ghalibiya region. It is obvious from the heavy metal results that there is an increase in the density of these elements in the plants compared to the water, referring to the plant's ability to absorb these elements from its environment.

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INTRODUCTION

The term “heavy metals” points to any metal or metalloid element with a relatively high density ranging from 3.5-7 gm/cm³, which can be toxic at high concentrations, with somehow being toxic even at low concentrations. However, these include Cadmium (Cd), Arsenic (As), Chromium (Cr), Zinc (Zn), Nickel (Ni), Copper (Cu), and Lead (Pb). Even though this is a general term, it is widely used and often applied to pollutants widespread in soil and water bodies (Sharma, 2016). The common dispersion of heavy metals in the environment due to human activities, such as industry, agriculture, mining, and incorrect waste disposal, has led to the accumulation of harmful heavy metals in ecosystems, posing serious risks to human health as well as the environment (Singh et al., 2022). Heavy metal pollution in water, especially in areas heavily reliant on groundwater for drinking purposes, where these metals leach into groundwater due to soil and rock weathering or through improper disposal of industrial and liquid waste, is a critical environmental problem (Karim et al., 2024). Therefore, we resort to specific solutions, including bioremediation, defined as the technological process that harnesses biological systems, plants, animals, including microorganisms, to clean pollutants from the

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aquatic environment to treat wastewater contaminated with heavy metals and metalloids, and how to mitigate them using plants from different environmental conditions via cost-effective traditional techniques (Wei Xu et al., 2024). Stemming from the interest in environmental sustainability, the aquatic plant Azolla is a well-established product in the field of biotechnology (Nasir et al., 2022). Azolla has multiple positive effects on production, including improving soil fertility, reducing harmful weeds, increasing soil organic carbon, improving microbial biomass, and thus nutrient cycling, and enhancing rice growth and productivity (Marzouk et al., 2023). Azolla production primarily depends on the chemical and biological characteristics of the water, and regardless of its quality, the continuous availability of water throughout the year is crucial for regular Azolla production (Sadashiv et al., 2021). The nutritional value of Azolla as a good source of protein has been recognized, and it has been found to contain essential minerals like Iron, Calcium, Magnesium, Potassium, and significant amounts of Vitamins A and B-12 (Rehman et al., 2022). Some study results have also indicated that Azolla is a rich source of protein and has a clear benefit in meeting part of the nutritional needs for livestock production, effectively serving as a low-cost dietary supplement, a biological control for mosquito repellent, and a biological environmental treatment (Abdullah and Al-wahab, 2025). Azolla plants are known for their benefits in greener and more sustainable lifestyles, in addition to improving quality and the bio-based economy compared to traditional methods. Therefore, this plant was chosen as a suitable and environmentally friendly solution for a long and healthy lifestyle (Korsa et al., 2024).

MATERIALS AND METHODS

Samples were collected from three different regions, including Baghdad, Al-Ghalibiya, and Khan Bani Saad, in narrow-necked, tightly sealed glass bottles with a capacity of (250-300) milliliters. These bottles were filled quietly and flowingly. Sample Drying and Measurement by Atomic Absorption spectrophotometer (AAS). The plant samples were dried in an oven at 150°C for two days. 1 gram of the sample was taken, placed in the digestion bottle of the instrument, and 9 ml of concentrated nitric acid was added, followed by 1 ml of 35% hydrogen peroxide solution. The instrument's container was tightly closed and placed inside the instrument under high pressure with a gradual increase from 10 to 100 bar and a temperature starting from 25°C to 200°C with a gradual increase over 15 minutes, then maintained at 200°C for 15 minutes, making the total digestion period 30 minutes. After half an hour, the instrument was turned off, the sample container was taken, and the contents were filtered into a 50 ml volumetric flask. The sample was washed, and then the volumetric flask was filled to the mark with water. Field Measurement of TDS and pH, then taking the samples in tightly sealed bottles with a capacity of (250-300) milliliters, bringing them to the laboratory, and measuring DO using a dissolved oxygen meter, which relies on the method of oxygen diffusion rate through the electrode membrane.

Experimental Design and Statistical Analysis

Three replicates were taken for the mentioned regions (Baghdad, Al-Ghalibiya, Khan Bani Saad) within an area not exceeding approximately 50 m² per site. TDS and pH were measured in the field. A Completely Randomized Design (C.R.D.) was used in conducting the simple experiments using the SPSS program for data analysis, and the means were compared according to Duncan's multiple range test at a probability level of 0.05.

RESULTS & DISCUSSION

- Estimation of Cadmium in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya,

Table 1. Estimation of Cadmium in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions

Samples From	Regions			Estimation
	Baghdad	Al-Ghalibiya	Al-Khan	
Water	0.033b	0.023b	0.023b	0.026b
Plant	0.350ab	0.633a	0.653a	0.545a
	0.191a	0.328a	0.338a	

Table 2. Estimation of Copper in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions

Samples	Regions			Estimation of the simple
	Baghdad	Al-Ghalibiya	Al-Khan	
Water	0.013c	0.070c	0.023c	0.035b
Plant	0.800a	0.403b	0.336b	0.513a
	0.406a	0.236b	0.180b	

Table 3. Estimation of Iron in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions

Samples	Regions			Estimation of the simple
	Baghdad	Al-Ghalibiya	Al-Khan	
Water	0.063d	0.153d	0.380cd	0.198b
Plant	1.400ab	1.210bc	2.283a	1.631a
	0.731a	0.681a	1.331a	

and Al-Khan Regions. Table 1 shows the estimation of Cadmium in water and plants from three different regions. The results indicate no significant differences between the regions. However, the results of the comparison between water and plant showed significant differences, reaching 0.545 mg/L for the plant compared to water samples, which reached 0.026 mg/L. The results of the interactions between water, plant, and regions showed the highest result was in the Al-Khan region at 0.653 mg/L, while the lowest values were in the water sample within the Al-Ghalibiya and Al-Khan regions at 0.023 mg/L.

- Estimation of Copper in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions. Table 2 shows the estimation of Copper (Cu) in water and plants from three different regions. It is clear from the results that there are significant differences among the regions, with the highest value in the Al-Ghalibiya region, at 0.236 mg/L. The results of the comparison between water and plant samples showed the highest value, with a significant difference, in the Azolla plant sample, at 0.513 mg.L⁻¹. The interaction results showed that the highest values for the plant among the regions were the highest value in the plant sample from the Baghdad region, which reached 0.800 mg/L, while the lowest values were in the water sample from the Baghdad region, which reached 0.013 mg/L.

- Estimation of Iron in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions. Table 3 shows the estimation of Iron (Fe) in water and plants from three different regions. The results show no significant differences between the regions, but the highest value was in the Al-Khan region, which reached 1.331 mg/L. The results of the comparison between water and plant showed significant differences, with the highest value for the plant reaching 1.631 mg/L, significantly different from the water sample, which reached 0.198 mg/L. The interaction results showed the highest value was for the plant sample in the Al-Khan region, which reached 2.283 mg/L, while the lowest value was in the water sample from the Baghdad region, which reached 0.063 mg/L.

- Estimation of Nickel in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions. Table (4) shows the estimation of Nickel in water and plant samples from three different regions. It is clear from the results that there are no significant differences

Table 4. Estimation of Nickel in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions

Samples	Regions			Estimation of the simple
	Baghdad	Al-Ghalibiya	Al-Khan	
Water	0.186a	0.027a	0.015a	0.076b
Plant	0.280a	0.213a	0.316a	0.269a
	0.233a	0.120a	0.165a	

Table 5. Estimation of Lead in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions

Samples	Regions			Estimation of the simple
	Baghdad	Al-Ghalibiya	Al-Khan	
Water	0.063ab	0.016b	0.006b	0.028b
Plant	0.346ab	1.100a	0.733ab	0.726a
	0.205a	0.558a	0.369a	

Table 6. Measurement of DO in Water Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions

Regions	Do estimation mg.l ⁻¹
Baghdad	9.373b
Al-Ghalibiya	10.033a
Al-Khan	9.406b

between the regions. However, the results of the comparison between water and plant samples showed significant differences, with the highest value in the plant sample reaching 0.269 mg/L, compared to the lowest value of 0.076 mg/L in the water sample. The interaction results showed the highest result in the plant sample from the Al-Khan region at 0.316 mg/L, while the lowest value was in the water sample from the Al-Khan region at 0.015 mg/L.

- Estimation of Lead in Water and Azolla Plant Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions. Table (5) shows the estimation of the element Lead (Pb) in water and plants from three different regions. It is clear from the results that there are no significant differences between the regions, but the highest value was 0.558 mg/L in the Al-Ghalibiya region. Meanwhile, the results of the comparison between water and plant showed significant differences between them, with the highest value of 0.726 mg/L for the plant sample. The interaction results showed the highest value in the plant sample from the Al-Ghalibiya region, which reached 1.100 mg/L, while the lowest value was in the water sample from the Al-Khan region, which reached 0.006 mg/L.

- Estimation of DO Value in Water Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions. Table (6) shows the estimation of the percentage of dissolved oxygen in water from three different regions. It is clear from the results that there are no significant differences between the two regions of Baghdad and Al-Khan, only with the Al-Ghalibiya region. The highest value was 10.033 mg/L in the Al-Ghalibiya region, while the lowest value of 9.373 mg/L was in the Baghdad region.

- Estimation of Tw Value in Water Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions. Table 7 shows the estimation of water temperature for three different regions. It is clear from the results that there are no significant differences between the two regions of Baghdad and Al-Khan, only with the Al-Ghalibiya region. The highest value was 20.233 °C in the Al-Ghalibiya region, while the lowest value of 18.900 °C was in the Baghdad region.

- Estimation of TDS Value in Water Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions. Table 8 shows the estimation of dissolved solids in water for three different regions. The results indicate the presence of significant differences between the regions. The highest value was 643.333 ppm in the Al-Ghalibiya region, while the lowest value of 269.333 ppm was

Table 7. Measurement of Tw in Water Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions

Estimation °C Tw	Regions
18.900b	Baghdad,
20.233a	Al-Ghalibiya
19.266b	Al-Khan

Table 8. Measurement of T.D.S in Water Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions

estimation ppm T.D.S	Regions
394.000b	Baghdad
643.333a	Al-Ghalibiya
269.333c	Al-Khan

Table 9. Measurement of pH in Water Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions

pH estimation	Regions
7.733a	Baghdad
8.080a	Al-Ghalibiya
8.080a	Al-Khan

in the Al-Khan region.

• Estimation of pH Value in Water Samples from Baghdad, Al-Ghalibiya, and Al-Khan Regions Table 9 shows the estimation of the pH percentage in water for three different regions. The results indicate no significant differences between the three regions. The highest value was 8.080 in the Al-Ghalibiya and Al-Khan regions, while the lowest value was 7.733 in the Baghdad region.

The results showed an increase in the concentration of heavy metals in the Azolla plant. These results can be explained by the expansion of industrial and tourist activities, consequently raising the concentration of heavy metals to dangerous levels (Hassan zadeh et al., 2021). The Azolla plant can hyper accumulate heavy metals, which makes it a reliable phytoremediation agent for industrial wastewater and sewage polluted with metals, together with being a prospect for its use with irrigation water polluted with mixed heavy metals) (Zeid et al. 2024). Researchers have discovered that Azolla can reduce Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and the concentrations of nitrogen, phosphorus, and heavy metals in wastewater, and thus can be used in wastewater treatment (Jayasundar, 2022). Moreover, Azolla can eliminate heavy metal pollutants thanks to its ability to hyper accumulate heavy metals, allowing it to purify industrial waste, water reservoirs, and any water bodies (Lestari et al., 2024).

Dissolved Oxygen (DO)

The results of Dissolved Oxygen (DO) indicate a significant difference between the sample collection sites, for example, the Al-Ghalibiya region recorded the highest value (10.033 mg/L) as compared to other regions. The reason for this can be due to the environmental conditions of the region, and this increase may be attributed to the possibility of water movement or low organic pollution, moreover, to the possibility of relatively lower temperatures enhancing oxygen solubility in water (Wetzel, 2001). In contrast, the Khan and Baghdad regions showed lower values (9.406 and 9.373 mg/L, respectively), and no notable difference was observed between the two regions, due to similar environmental conditions, which include increasing decomposing organic matter or reduced water movement due to pollutants such as sewage and wastewater (Omar et al., 2022).

Temperature water (Tw)

The temperature results specified that the statistical analysis showed significant differences between the three study sites, where the Al-Ghalibiya region recorded the highest values, reaching 20.233 °C. This increase can be explained based on the known hydrological attributes of Iraqi waters in some areas, which are often characterized by better water flow and a relative decrease in the concentrations of organic and industrial pollutants. As for the Khan and Baghdad regions, the values were (19.266 and 18.900 °C), respectively, indicating no significant difference between them. The reason is the slight difference in air temperatures between the study sites due to different sampling times, as temperatures are low in the early morning and then rise as we approach midday (AL-Dulaimi, 2013).

TDS Measurement

The results of the TDS measurement comparison indicated the presence of significant real differences between the regions, confirming that these are actual differences in water characteristics. Compared with international standards, all values fall within the acceptable limits set by the World Health Organization (WHO, 2022). The highest values were recorded in the Al-Ghalibiya region at (643.333 ppm), while the lowest value was in the Al-Khan region at (269.333 ppm). The results are very similar to those obtained by Omar et al. 2022.

pH Level

The measurement of the pH level for water samples taken from the Baghdad, Al-Ghalibiya, and Khan regions showed no significant differences between the three regions, as all recorded similar values. The pH value in Baghdad was 7.733, while the Al-Ghalibiya and Khan regions recorded approximately the same value (8.080). These values indicate that the water in all regions falls within the natural alkaline range, a range that conforms to the standard specifications of the World Health Organization (WHO, 2022), which set the acceptable limit for pH in drinking water between 6.5 and 8.5. These results indicate that the water in the three regions is chemically stable and in a state of equilibrium, because pH values have a strong relationship with carbon dioxide concentrations in water (Wetzel, 2001). Algae and aquatic plants consume carbon dioxide to perform photosynthesis, leading to an increase in pH value in some areas (Weiner, 2000).

CONCLUSION

This study demonstrates that *Azolla* possesses a remarkable capacity for heavy metal bioaccumulation, consistently showing significantly higher concentrations of Cadmium, Copper, Iron, Nickel, and Lead in plant tissues compared to surrounding water across all three study regions. The highest accumulation values were observed for Iron (2.283 ppm) in the Al-Khan region and Lead (1.100 ppm) in the Al-Ghalibiya region, confirming *Azolla*'s efficacy as a hyperaccumulator and its potential utility in phytoremediation strategies for contaminated aquatic ecosystems. The physicochemical parameters, including Dissolved Oxygen, Temperature, Total Dissolved Solids, and pH, remained within acceptable ranges according to WHO standards, indicating that the aquatic environments across the three regions are chemically stable, with variations largely attributable to local environmental conditions and pollution loads. The findings underscore *Azolla*'s dual role as both an effective bioindicator for monitoring heavy metal pollution and a sustainable, cost-effective bioremediation agent for wastewater treatment. Given the increasing anthropogenic pressures on water resources through industrial, agricultural, and domestic activities, the integration of *Azolla*-based phytoremediation into environmental management practices offers a promising, eco-friendly approach to mitigating heavy metal contamination and safeguarding aquatic ecosystem health.

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