Bioaccumulation of (Ni, Cd, Pb, Cr, Hg, and Co) from Al-Rustomia wastewater using *A. filiculoids*

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**Abstract.** This study was conducted to apply phytoremediation technology in removing pollutants from wastewater in an attempt to use it as a tool in the subsequent reuse of the produced water. This technology considered one of the promising technologies, as it is environmentally friendly, cost-effective, and not selective of removing pollutants. The experiment was conducted in Al-Twitha location (IAEE). Six glass tubs with (30×30×30) cm dimension and 27 L capacity each of which were filled with sewage water that brought from Al-Rustomia sewage plant after primary treatment stage bowl. The other three (control) were filled with tap water, 100g of *Azolla* which brought from Al-Chibayish Marsh in the south of Iraq, was equally distributed into six tubs, after 28 days the plants were harvested. The results of accumulated minerals showed a high accumulation of (Ni, Cd, Pb, Cr, Hg, Co) and they were (60, 57, 24, 74, 9, 63) % respectively over the first couple of weeks. While in the next two weeks, minerals accumulation were (172, 73, 65, 126, 36, 97) % respectively. The results showed that the *A. filiculoids* can reduce the amounts of accumulated heavy metals from Al-Rustomya wastewater.

**Keywords:** Phytoremediation, *A. filiculoids*, Al-Rustomia, wastewater, Heavy metals

1. **Introduction**

The toxicity of heavy metals such as (Hg, Cd, As, Cr, Tl, and Pb) are back to the high atomic density and weight which is more than five times of water weight, that even at quite low density it may cause toxicity. The sources of heavy metals are either through the rock and soil leakage to water (nature reason) or by man activities. Recently, the latter become the prevalent due to the unprocessed wastewater discharging to rivers[1]. Heavy metals are the main focused among diverse water contaminants, due to their constant and its bioaccumulation normality [1-4]. The water is considered an essential section of human being maintenance, and due to growing realization about the ecosystem, particularly the water environment, research related to water resources around the globe, have dramatically increased, in terms of certain requirements of an affordable, and ecofriendly technology to remediate contaminants especially heavy metals, and ultimately improve the quality of water [5, 6]. In this research, *A. filiculoids* R. Br. (commonly known as water fern) was used as a plant remediator. This plant is considered as invasive or unwanted species for the use in phytoremediation such as rapid increase rate, nitrogen-fixing ability, superior biomass output, the root system expanded reasonably, harvest easily, and high allowance to a wide spectrum of heavy metals[7]. This plant exhibits different patterns of lead removal and can even accumulate a higher concentration of lead. It can remove 99.28% of lead as well as 65.89% of cadmium. It is considered as a hyperaccumulator for metals such as Zn, Ni, As, Cr, Ag, Cu, Hg, Fe, Mg, and Mn. Water lettuce is also efficient in the removal of nitrate. It is used in phytoremediation because compared to native plants it shows higher nutrient removal efficiency with their high nutrient uptake capacity [8]. *Azolla* is a fern frond...
consisting of the main stem growing at the surface of the water, with leaves and adventitious roots at regular intervals along the stem. Azolla is triangular or polygonal and floats on the water surface individually or in mats. It has several attributes that merit its consideration for widespread use as an amendment for bioaugmentation and biostimulation of contaminated sites. *A. filiculoids* remove Fe, Zn, Mn, Co, Cd, Hg, As, and Ni from the wastewater mixture. *Azolla caroliniana* based treatment has proved as a promising tool for the treatment of zinc at slightly higher concentrations. *Azolla pinnata* has been reported to accumulate a high level of arsenic from contaminated water [9, 10].

2. Material and methods

The aquatic fern *A. filiculoids* used in this experiment was collected from Al-Chibayish Marshes in the south of Iraq. The plants washed with raw water to remove the particles of adhering mud and was placed in a (30×30×30) cm, with 27 L capacity, glass tubs which filled with tap water for five days. The sewage water was brought from the Al-Rustomia sewage plant after the primary treatment stage bowl. The treatment was as follow:

Control (tap water)+ 100g *A. filiculoids* ×3 replicates.

Sewage water + 100g *A. filiculoids* ×3 replicates.

The experiment was conducted in the laboratories of the Iraqi Atomic Energy Agency (IAEA), in March of 2019, and have designed in Randomized Complete Design (RCD) with three replicates. The experiment took 28 days, have been started at the March the 1st, statistical analyses were performed using Gen Stat data analysis software and the significance level was set at p < 0.05. Plants and sewage water were analyzed before the start of the experiment, Table-1. The heavy metals (Ni, Cd, Pb, Cr, Hg, and Co) were analyzed every 14 days from water tubs, and the aquatic plants were harvested after 28 days. Table-2 shows the content of *A. filiculoides* prior the experiment.

- Analyses of heavy metals in plant tissues: this was done using X-ray Fluorecenc spectrometer (XRF), while Shimadzu was used to qualitatively and quantitatively determine the metals present in the tissue samples following the protocol of Klockenkamper[11].

- Rate of accumulation: The rate of accumulation was obtained by:

\[
Rate \ of \ Accumulation = \left( \frac{B - A}{A} \right) \times 100
\]

Whereas A= the initial concentration of heavy metal.

B= the final concentration of heavy metal.
Table 1. Illustrate the metals present in the wastewater from Al-Rustomia treatment plant

| Factor | Unit | Value |
|--------|------|-------|
| Ec     | μS/cm| 1344  |
| pH     | mg/L | 7.09  |
| TDS    | mg/L | 862   |
| BOD    | mg/L | 160   |
| DO     | mg/L | 1.7   |
| COD    | mg/L | 290   |
| NO3    | mg/L | 8.6   |
| PO4    | mg/L | 4.4   |
| Cl-    | mg/L | 320   |
| SO4    | mg/L | 550   |

Table 2. The content of studied heavy metals in *A. filiculoides* before carrying out the experiment.

| Factor | Unit | Value |
|--------|------|-------|
| Ni     | mg/kg| 15    |
| Cd     | mg/kg| 0.79  |
| Pb     | mg/kg| 7.2   |
| Cr     | mg/kg| 27.2  |
| Hg     | mg/kg| 1.1   |
| Co     | mg/kg| 10.5  |

3. Results and Discussion

Results presented in Table-3 shows a significant increase in bioaccumulation of all studied heavy metals indices (Ni, Cd, Pb, Cr, Hg, and Co) versus time for both specified periods (14, 28 day) along side the control (0 day), during the use of *A. filiculoides* for remediation of Al-Rustumiya plant wastewater. The indices heavy metals accumulation concentration was gradient concentration for the 1st fourteen days was:

Table 3. shows the impact of the phytoremediation by *A. filiculoides* on the accumulation efficiency of (Ni, Cd, Pb, Cr, Hg, and Co) in the wastewater of Al-Rustomia wastewater plant.
Nickle accumulatio efficiency: The Ni has accumulated in *A. filiculoides* significantly in a rate of (60, 172)% for both specified periods (14, 28 day), respectively, as shown in Figure-1, whereas the Ni initial concentration was 15 mg/kg and then became 41.5 mg/kg at the end of the experiment Table-3. These results were comparable with the previous studies regarding Azolla; however, the our results showed that the observed accumulation efficiency of *A. filiculoides* is lower than the one reported by Talebi [12] who obtained about 2900 mg/kg compared to the control (zero), yet higher than the efficiency reported by [13, 14] who recorded a removal efficiency of (100, and about 70)% respectively.

Cadmium (Cd) accumulation efficiency: The results in Table-3, showed a significant increase in Cd accumulation in *A. filiculoides* which treated Al-Rustomia plant wastewater by accumulation rate (57, 73)% for both specified periods (14 and 28 day) respectively (Figure-1). However, the Cd initial concentration was 0.79 mg/kg and became 3 mg/kg during the final experiment (Table-3). Regarding Azolla these results are comparable with the previous studies, as the current results were higher than Amare [15] who obtained 61%, Eribo [16] who obtained 44%, which was lower than Ugya [14] who obtained 90% removal efficiency [17] bioaccumulation increased more than 200%.

Lead (Pb) accumulation efficiency: The results confirmed that Pb accumulation increased by (24, 65)% using the *A. filiculoides* for both specified periods (14 and 28 day) respectively (Figure-1), as the Pb initial concentration was 7.2 mg/kg and then reached 11.9 mg/kg in the final experiment (Table-3). The results of Pb accumulation were comparable with previous studies regarding Azolla with rates of removal efficiency slightly lower than those of [16], 66% with[14] and about 80% with[17] bioaccumulation increased more than 200%.

Chromium accumulation efficiency: The Cr has accumulated in *A. filiculoides* significantly in a rate of (74, 126)% for both specified periods (14 and 28 day), respectively, as shown in Figure-1, whereas the Cr initial concentration was 27.2 mg/kg and became 61.4 mg/kg during the final experiment (Table-3). These results were comparable with the previous studies regarding Azolla; however, the current results showed that accumulation efficiency is lower than [18] who recorded 1095 mg/kg of Cr(III) accumulation compared to the used control (3 mg/kg), Arora [5] who recorded 9125 mg/kg of Cr(VI) accumulation compared to the control 20 mg/kg.

Mercury accumulation efficiency: The results in Table-3 showed a significant increase of Hg accumulation in *A. filiculoides* which treated Al-Rustomia plant wastewater by accumulation rate (9, 36)% for both specified periods (14 and 28 day) respectively (Figure-1). However, the Hg initial concentration was 1.1 mg/kg and then became 6.3 mg/kg during the final experiment (Table-3). These results were comparable with the previous studies regarding Azolla; however, the recorded efficiency is lower than [19] who recorded 1095 mg/kg of Cr(III) accumulation compared to the control 3 mg/kg, Arora [5] who recorded 9125 mg/kg of Cr(VI) accumulation compared to the control 20 mg/kg.

**Table 3.** Nickel accumulation efficiency: The Ni has accumulated in *A. filiculoides* significantly in a rate of (60, 172)% for both specified periods (14, 28 day), respectively, as shown in Figure-1, whereas the Ni initial concentration was 15 mg/kg and then became 41.5 mg/kg at the end of the experiment Table-3. These results were comparable with the previous studies regarding Azolla; however, the our results showed that the observed accumulation efficiency of *A. filiculoides* is lower than the one reported by Talebi [12] who obtained about 2900 mg/kg compared to the control (zero), yet higher than the efficiency reported by [13, 14] who recorded a removal efficiency of (100, and about 70)% respectively.

Cadmium (Cd) accumulation efficiency: The results in Table-3, showed a significant increase in Cd accumulation in *A. filiculoides* which treated Al-Rustomia plant wastewater by accumulation rate (57, 73)% for both specified periods (14 and 28 day) respectively (Figure-1). However, the Cd initial concentration was 0.79 mg/kg and became 3 mg/kg during the final experiment(Table-3). Regarding Azolla these results are comparable with the previous studies, as the current results were higher than Amare [15] who obtained 61%, Eribo [16] who obtained 44%, which was lower than Ugya [14] who obtained 90% removal efficiency [17] bioaccumulation increased more than 200%.

Lead (Pb) accumulation efficiency: The results confirmed that Pb accumulation increased by (24, 65)% using the *A. filiculoides* for both specified periods (14 and 28 day) respectively (Figure-1), as the Pb initial concentration was 7.2 mg/kg and then reached 11.9 mg/kg in the final experiment (Table-3). The results of Pb accumulation were comparable with previous studies regarding Azolla with rates of removal efficiency slightly lower than those of [16], 66% with[14] and about 80% with[17] bioaccumulation increased more than 200%.

Chromium accumulation efficiency: The Cr has accumulated in *A. filiculoides* significantly in a rate of (74, 126)% for both specified periods (14 and 28 day), respectively, as shown in Figure-1, whereas the Cr initial concentration was 27.2 mg/kg and became 61.4 mg/kg during the final experiment (Table-3). These results were comparable with the previous studies regarding Azolla; however, the current results showed that accumulation efficiency is lower than [18] who recorded 1095 mg/kg of Cr(III) accumulation compared to the used control (3 mg/kg), Arora [5] who recorded 9125 mg/kg of Cr(VI) accumulation compared to the control 20 mg/kg.

Mercury accumulation efficiency: The results in Table-3 showed a significant increase of Hg accumulation in *A. filiculoides* which treated Al-Rustomia plant wastewater by accumulation rate (9, 36)% for both specified periods (14 and 28 day) respectively (Figure-1). However, the Hg initial concentration was 1.1 mg/kg and then became 6.3 mg/kg during the final experiment (Table-3). These results were comparable with the previous studies regarding Azolla; however, the recorded efficiency is lower than [19] who recorded 1095 mg/kg of Cr(III) accumulation compared to the control 3 mg/kg, Arora [5] who recorded 9125 mg/kg of Cr(VI) accumulation compared to the control 20 mg/kg.

**Table 3.**

| factors | Control mg/kg | 14 days contact time | 28 days contact time | LSD |
|---------|---------------|----------------------|----------------------|-----|
|         | conc. mg/kg   | Accumulation efficiency % | conc. mg/kg   | Accumulation efficiency % |       |
| Ni      | 15            | 24.1                 | 60                   | 41.5 | 172   | 4     |
| Cd      | 0.79          | 1.7                  | 57                   | 3    | 73    | 0.7   |
| Pb      | 7.2           | 8.9                  | 24                   | 11.9 | 65    | 0.6   |
| Cr      | 27.2          | 47.2                 | 74                   | 61.4 | 126   | 4     |
| Hg      | 1.1           | 1.2                  | 9                    | 1.5  | 36    | 0.1   |
| Co      | 10.5          | 17.1                 | 63                   | 20.7 | 97    | 2     |
mg/kg and became 1.5 mg/kg during the final experiment (Table-3). Regarding Azolla these results are comparable with the previous studies however, the current results showed that accumulation efficiency is lower than [19] who recorded 767 mg/kg of Hg accumulation compared to the control (3 mg/kg)[20] who recorded 450 mg/kg of Hg accumulation compared to the control (10 mg/kg).

Cobalt accumulation efficiency: The results confirmed that Co accumulation significantly increased by (63, 97)% using the A. filiculoides for both specified periods (14 and 28 day) respectively (Figure-1), as the Co initial concentration was 10.5 mg/kg and has reached 20.7 mg/kg in the final experiment (Table-3). These results were comparable with the previous studies regarding Azolla; however, the current results showed that accumulation efficiency is lower than [21] which recorded 115% accumulation of Co in plant tissues.

Figure 1. Impact percentage of the heavy metals accumulation efficiency (Ni, Cd, Pb, Cr, Hg, and Co) with the time.

4. Conclusion

The conclusion remarks of the present application of Azolla in phytoremediation; is that the plant can be adopted and maintained growth in harsh environment despite its original habitat. The plant Azolla could accumulate many harmful heavy metals from sewage water collected from Al- Rustamia; heavy metals accumulated with time to reach its final concentration in Azolla biomass after 28 days. The most powerful application is to develop a biofilter based on Azolla befits in sewage treatment to produce high quality water besees different purposes. For future perspective, heavy metals accumulated in the biomass can be mineralized again by chemical methods to repossesses the metals.

5. Reference:

[1] Rai, L., J. Gaur, and H. Kumar, Phycology and heavy-metal pollution. Biological Reviews, 1981. 56(2): p. 99-151.
[2] Lokeshwari, H. and G. Chandrappa, *Effects of heavy metal contamination from anthropogenic sources on Dasarahalli tank, India*. Lakes & Reservoirs: Research & Management, 2007. **12**(3): p. 121-128.

[3] Chang, J.-S., I.-H. Yoon, and K.-W. Kim, *Heavy metal and arsenic accumulating fern species as potential ecological indicators in As-contaminated abandoned mines*. Ecological Indicators, 2009. **9**(6): p. 1275-1279.

[4] Yadav, S.K., et al., *Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by Jatropha curcas L.: impact of dairy sludge and biofertilizer*. Bioresource Technology, 2009. **100**(20): p. 4616-4622.

[5] Arora, A., S. Saxena, and D.K. Sharma, *Tolerance and phytoaccumulation of chromium by three Azolla species*. World Journal of Microbiology and Biotechnology, 2006. **22**(2): p. 97-100.

[6] Umali, L., J. Duncan, and J. Burgess, *Performance of dead Azolla filiculoides biomass in biosorption of Au from wastewater*. Biotechnology letters, 2006. **28**(1): p. 45-50.

[7] Sood, A., et al., *Phytoremediation potential of aquatic macrophyte, Azolla*. Ambio, 2012. **41**(2): p. 122-137.

[8] Gupta, P., S. Roy, and A.B. Mahindrakar, *Treatment of water using water hyacinth, water lettuce and vetiver grass—a review* system, 2012. **49**: p. 50.

[9] Deval, C., et al., *Phytoremediation potential of aquatic macrophyte Azolla caroliniana with references to zinc plating effluent*. Emirates Journal of Food & Agriculture (EJFA), 2012. **24**(3).

[10] Al-Baldawi, I.A., et al., *Phytotransformation of methylene blue from water using aquatic plant (Azolla pinnata)*. Environmental Technology & Innovation, 2018. **11**: p. 15-22.

[11] Mouhamad, R., et al., *Determination of heavy metal uptake in transgenic plants harbouring the rabbit CYP450 2E1 using X-ray fluorescence analysis*. International journal of environmental studies, 2014. **71**(3): p. 292-300.

[12] Talebi, M., B.E.S. Tabatabaei, and H. Akbarzadeh, *Hyperaccumulation of Cu, Zn, Ni, and Cd in Azolla species inducing expression of metallothionein and phytochelatin synthase genes*. Chemosphere, 2019. **230**: p. 488-497.

[13] Ugya, A., T. Imam, and A. Hassan, *Phytoremediation of Textile Waste Water Using Azolla pinnata; A Case Study*. World Journal of Pharmaceutical Research, 2017. **6**(2).

[14] Ugya, A., X. Hua, and J. Ma, *Phytoremediation as a tool for the remediation of wastewater resulting from dyeing activities*. APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH, 2019. **17**(2): p. 3732-3735.

[15] Amare, E., F. Kebede, and W. Mulat, *Wastewater treatment by Lemma minor and Azolla filiculoides in tropical semi-arid regions of Ethiopia*. Ecological Engineering, 2018. **120**: p. 464-473.

[16] Eribo, O. and M. Kadiri, *Growth performance and phytoremediation ability of Azolla pinnata in produced water*. Journal of Applied Sciences and Environmental Management, 2016. **20**(4): p. 1053-1057.

[17] El-Shahat R. Mohamed, M.S.A., Ahmed A. Tantawy, Nasr H. Gomaa and Hassan A. Mahmoud, *Phytoremediation of Pb+2, Cd+2 and Cu+2 by an Aquatic Macrophyte Azolla pinnata from Industrial Wastewater in Egypt*. Middle East J. Appl. Sci, 2016. **6**(1): p. 13.

[18] Rai, P.K., *Microcosm investigation on phytoremediation of Cr using Azolla pinnata*. International journal of phytoremediation, 2009. **12**(1): p. 96-104.

[19] Rai, P.K., *Phytoremediation of Hg and Cd from industrial effluents using an aquatic free floating macrophyte Azolla pinnata*. International journal of phytoremediation, 2008. **10**(5): p. 430-439.

[20] Mishra, V.K., B. Tripathi, and K.-H. Kim, *Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent*. Journal of Hazardous Materials, 2009. **172**(2-3): p. 749-754.
[21] Amare, E., et al., *Field-based investigation on phytoremediation potentials of Lemma minor and Azolla filiculoides of Ethiopia*. International journal of phytoremediation, 2018. 20(10): p. 965-972.