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Phytoremediation of Heavy Metals by *Azolla microphylla* from Industrial Effluents

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Abstract
An aquatic fern, *Azolla microphylla* was tested to determine their growing capacity and heavy metal removal efficiency in different industrial effluents with different concentrations. Untreated industrial effluent samples were collected from three different sources (SE-1, SE-2 and SE-3) and poured in separate cement tanks and 5 grams of *A. microphylla* were introduced into each tank. After 30 days, the growth parameters such as fresh weight, doubling time and relative growth rate were assessed. The results revealed that the highest fresh weight, doubling time and relative growth rate of *Azolla* were observed in SE-3 than SE-1 and SE-2 samples. In SE-3, 50% concentration exhibited greater growth than other concentrations such as 25%, 75% and 100%. In general, the growth was greater in 50% concentration of effluent samples. At the concentrations of 25% and 100% the growth was found less and/or inhibited especially in the effluent samples of SE-1 and SE-2. *A. microphylla* expressed significant removal efficiency on all the four heavy metals tested and the removal efficiency was in the order of zinc > lead > cadmium > nickel. Based on these results, it was concluded that *A. microphylla* can be used in wastewater treatment.

Keywords
Azolla microphylla, Growth performance, Removal efficiency, Heavy metals, Industrial effluent.

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1. INTRODUCTION
The utilization of water has been replication on every twenty years. Fresh water temperament on earth is only one third of its province is well provide with water, and if sweeping efforts in water protection are not made by year 2025, 2.3 billion people will live in areas with persistent water shortage [1]. At present water is greatly polluted by different toxic metals, which create hazard troubles for all living organisms. Such metals are retarding farming efficiency and destructing the health of living things [2].

Quick urbanization, population explosion, industrialization, use of fertilizer and pesticide has resulted in heavy metal pollution of land and water resources. The rising consignment of heavy metals has source imbalance in aquatic ecosystems and the biota on the increase under such habitats accumulate huge amount of heavy metals especially zinc, copper, lead, chromium and cadmium etc, which in turn, are being assimilated and transferred to humans within food chain by the progression of biological magnification. Most of the heavy metals are highly toxic at exceeded deliberation and thus needs recent improving wastewater treatment techniques for their remediation [3].

Azolla microphylla Kaulf. is a local macrophyte growing in various water bodies and heterosporous free-floating freshwater fern that live symbiotically with *Anabaena azollae*, nitrogen fixing blue-green algae. *Azolla* is a genus of small aquatic fern belongs to the family Azollaceae with a world-wide distribution in temperate and tropical regions. Various research findings had proved its ability to absorb metal ions from the media, with single metal solution as well as multi-metal solutions and from wastewaters [4]. In this connection, the present study was carried out to assess the growth tolerance and heavy metal absorptions of *Azolla microphylla* in industrial effluents.

2. MATERIALS AND METHODS

2.1. Collection of industrial effluents

Untreated industrial effluents were collected from three different sources at the outlet point of an electroplating industry located in Madurai of Tamil Nadu, India, in plastic containers of about 10-liter capacities. The collected effluents were taken into the experimental site, poured in a separate cement tank and named as SE-1, SE-2 and SE-3.

2.2. Analysis of physico-chemical parameters of industrial effluents

The physico-chemical parameters of effluents such as pH, nitrogen, phosphorous, organic matter and heavy metals content present in the effluent samples were studied using standard methods outlined by APHA [5] before the introduction of the *A. microphylla* plants in the industrial effluents. Also, the heavy metal contents were evaluated after the completion of treatment.

2.3. Procurement of *Azolla microphylla*

Azolla microphylla was procured from the Agricultural College and Research Institute, Madurai. The plants were washed thoroughly and introduced into the cement tanks filled with water and maintained under natural conditions. Recommended dose of superphosphate and pesticide were added, in order to influence the better growth of *A. microphylla* and to avoid pest contamination. Well grown and healthy plants of *A. microphylla* were collected and used for further experiments.

2.4. Screening of growth tolerance

In this phase, the predetermined effluents were filled as such in circular cement tanks (25 litre /tank) and introduced with *A. microphylla* (5g fresh weight/tank). For each set duplicates were maintained. One week after the introduction of *A. microphylla* was assessed by physical observation so as to determine the tolerance of *A. microphylla* strain (in terms of growth) in different effluents. When *A. microphylla* was unable to establish well is raw (100%) effluents, such effluents were diluted and used in different concentrations viz. 25%, 50%, 75%, and 100%. For further studies diluted effluents in different concentration were filled (10 litre/pot) in the earthen pots (5cm diameter) separately. Triplicates were maintained for each set. Pots filled with tap water, added with recommended dose of phosphorous served as control. In each set of pots filled with different concentrations of effluents, *A. microphylla* (3g fresh weight/pot) was introduced. The pots were maintained and incubated for about 30 days with occasional stirring. After the experimental period, the growth characteristics of *A. microphylla* were determined and the suitability of the effluent concentration for the growth of *A. microphylla* was evaluated.

2.5. Analysis of growth characteristics

At the end of the experimental period (after 30 days) the following growth characteristics were analyzed.

2.5.1. Biomass

The biomass was determined by weighing the fresh cultured mass and the change in biomass was evaluated by subtracting initial weight from final weight.

2.5.2. Doubling time

Doubling time usually referred as doubling day or the number of days that required for the biomass of the fronds of *A .microphylla* to double its weight under favorable conditions. From the observed rate of biomass (after 30 days) the doubling time was calculated and expressed in terms of number of days required to double the initial weight of *A. microphylla* introduced in each set of tanks.

2.5.3. Relative Growth Rate

Relative Growth Rate (RGR) was determined by utilizing the formula suggested by Williams [6] and expressed in g day^{-1}.

$$RGR = \frac{\log e^{w_2} - \log e^{w_1}}{t_2 - t_1}$$

Where, W_1 and W_2 are dry weights of whole plant at t_1 and t_2 times in days respectively.

2.6. Removal efficiency (%)

The heavy metal removal efficiency (%) of *A. microphylla* was calculated by using the following equation [7].

$$\text{Removal efficiency} = \frac{C_0 - C_1}{C_0} \times 100$$

Where C_1 is the concentration (ppm) after the treatment and C_0 is the initial concentration (ppm) of heavy metals.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical characteristics of industrial effluents

Industrial effluents from 3 different sources (SE-1, SE-2 and SE-3) showed difference in pH ranges from 5.0 – 7.5. In the industrial effluents, the organic content was less in SE-1 and SE-3, whereas it was high in SE-2 (13.0%). The level of phosphorus was high in SE-1 (17.0 mg/l), meanwhile it was less in SE-3 (8.0 mg/l) and SE-2 (6.0 mg/l). Nitrogen content was slightly high in S-2E and SE-3 (2.1 mg/1) than SE-1 (1.8 mg/1). Heavy metal analysis in the effluents showed that highest content of cadmium, zinc, nickel and lead were noted in SE-3 than other two effluents. They were recorded as 50.4, 105.0, 45.03 and 15.5 ppm (Table 1).

Table 1: Physico-chemical properties and heavy metal status of industrial effluents

Sample	pH	Organic matter (%)	Phosphorus (mg/l)	Nitrogen (mg/l)	Heavy metals (ppm)			
					Cadmium	Zinc	Nickel	Lead
SE-1	7.5 ± 1.21	4.29 ± 1.02	17.0 ± 2.21	1.8 ± 0.70	14.9 ± 1.7	11.71 ± 0.91	10.86 ± 0.78	12.8 ± 1.47
SE-2	6.5 ± 1.01	13.0 ± 1.47	6.0 ± 1.71	2.1 ± 0.91	30.4 ± 2.4	26.6 ± 2.00	54.00 ± 2.41	22.3 ± 2.05
SE-3	5.0 ± 0.76	2.92 ± 0.92	8.0 ± 1.83	2.1 ± 0.77	50.4 ± 3.1	105.0 ± 5.40	45.03 ± 2.2	15.5 ± 2.01

Values are mean of three replicates ± Standard Deviation

The analysis on physic-chemical characteristics of the effluents showed a significant difference in the selected chemical parameters like pH, organic matter, phosphorus, nitrogen and the heavy metals of different types. Such differences among the selected effluents might be due to usage of different consumables and variation in the means of processes in the selected respective industries. The differences in the physico-chemical status of several industrial effluents have been well-documented [4, 8-10].

3.2. Growth tolerance of *A. microphylla* to industrial effluents

The tolerance of *Azolla* was analyzed by its growth characteristics and its responses in various concentrations of each effluent. The growth of *A. microphylla* in different concentrations of industrial effluents exhibited that the growth (assessed in terms of biomass, doubling time and relative growth rate) was influenced much in 75% concentration of SE-1. In SE-3 50% concentration exhibited greater growth than other concentrations. In SE-2 the growth was much reduced in all concentration than that of other effluents. In general, the growth was greater in 50% concentration of effluent samples ES-3. At the concentrations of 25% and 100% the growth was found less and/or inhibited especially in the effluent samples of SE-1, SE-2 and SE-3 (Table 2).

Table 2: Biomass, doubling time and Relative Growth Rate of *A. microphylla* treated with different concentrations of effluents

Sample	Biomass (g fresh weight)				Doubling time (Days)				Relative Growth Rate (g/day)			
	25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%
Control	16.8±2.1	19.6±2.2	20.1±2.1	19.1±1.4	8.4±1.7	7.8±2.1	7.7±1.9	7.0±1.8	0.27±0.03	0.31±0.06	0.32±0.07	0.30±0.07
SE-1	1.9 ± 0.7	14.4±1.7	18.3±1.9	4.5 ± 0.7	–	9.4±1.4	8.1±1.0	–	0.03±0.01	0.18±0.01	0.29±0.04	0.35±0.01
SE-2	3.1 ± 1.1	3.3 ± 0.9	2.0 ± 0.7	2.1 ± 0.6	–	–	–	–	0.05±0.01	0.05±0.01	0.04±0.01	0.30±0.07
SE-3	4.5 ± 2.1	18.9±1.2	17.1±1.1	3.0 ± 0.7	9.2±1.0	7.9±1.0	8.4±1.0	7.5±1.0	0.23±0.04	0.30±0.05	0.27±0.05	0.45±0.07

Values are mean of three replicates ± Standard Deviation

From the findings of present study, it was cleared that *A. microphylla* grown in 100% concentration (raw effluent) of effluents showed the state of senescence in all the three effluents. It indicates the existence of undesirable physico-chemical status and heavy metals in the effluents. It has been reported that the growth of aquatic plants is attributed to the inhibitory effect of toxic chemicals on chlorophyll, protein, RNA, dry weight and activities of several key enzymes [4].

The results of present study also revealed that 50% and 75% concentration of the various effluents had lesser effect on the growth in terms of biomass, doubling time and relative growth rate in *A. microphylla*, whereas concentrations at 25% and 100% inhibited the growth and /or exhibited lesser rate of growth. It evidences that the dilution of the concentration of effluents to 50% and 75% could provide preferential and also adequate level of pH, minerals, organic matter and heavy metal content for the growth of *A. microphylla*. The reduction in the extreme concentrations (25% and 100%) might be due to the inadequate (25%) and undesirable (100%) physical and chemical status and heavy metals in the effluents.

In the earlier studies, reduction in the growth of freshwater green algae by heavy metals like cadmium, lead and nickel [11], *Anacystis nidulans* by copper [12] and *Azolla*, *Lemna* and *Salvinia* by selenium [13] were reported. Similar result was also observed by Kannaiyan [14] in *A. pinnata* and *A. filiculoides*. It was reported that in general the growth of several plants was inhibited by industrial effluents due to presence of toxic chemicals [15].

3.3. Heavy metal removal efficiency (%) of *A. microphylla*

The data pertained in Table 3 showed the removal efficiency of the *A. microphylla* biomass from the industrial effluents for cadmium, zinc, nickel and lead ions. The results revealed that *A. microphylla* expressed significant removal efficiency on all the four heavy metals tested and the removal efficiency was in the order of zinc > lead > cadmium > nickel. The highest removal efficiency was found for zinc in all the three effluents (SE-1, SE-2 and SE-3) as 27.41, 19.92 and 13.60% respectively. *A. microphylla* showed least removal efficiency (0.35%) for nickel in both SE-2 and SE-3 effluents. These results were corroborated with the findings of Naghipour *et al*. [16] and this study revealed that the highest removal efficiency of *A. filiculoides* was recorded for lead (97.12), followed by cadmium (92.84%) and nickel (76.82%) after 15 days of treatment. Other studies showed that the contents of metals determined in the solution medium decreased whereas accumulation of the metals inside *Azolla* tissues was increased [17]. Taghi-Ganji *et al*. [18] reported that, the removal of heavy metal ions throughout *Azolla* fronds depends on the treatment conditions of biomass and the biosorption process.

Table 3: Changes in heavy metal content (ppm) in industrial effluents after 30 days of treatment with *A. microphylla*

Samples	Cadmium		Zinc		Nickel		Lead	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
SE-1	14.9 ± 1.7	13.92 ± 1.9	11.71 ± 0.91	8.5 ± 0.97	10.86±0.78	10.63±0.61	12.8 ± 1.47	11.76± 1.7
SE-2	30.4 ± 2.4	28.95 ± 2.8	26.6 ± 2.00	21.3 ± 1.9	54.00±2.41	53.81±2.41	22.3 ± 2.05	20.54± 1.2
SE-3	50.4 ± 3.1	49.8 ± 3.0	105.0 ± 5.40	90.71 ± 5.01	45.03 ± 2.2	44.87 ± 3.1	15.5 ± 2.01	14.0± 2.21

Values are mean of three replicates ± Standard Deviation

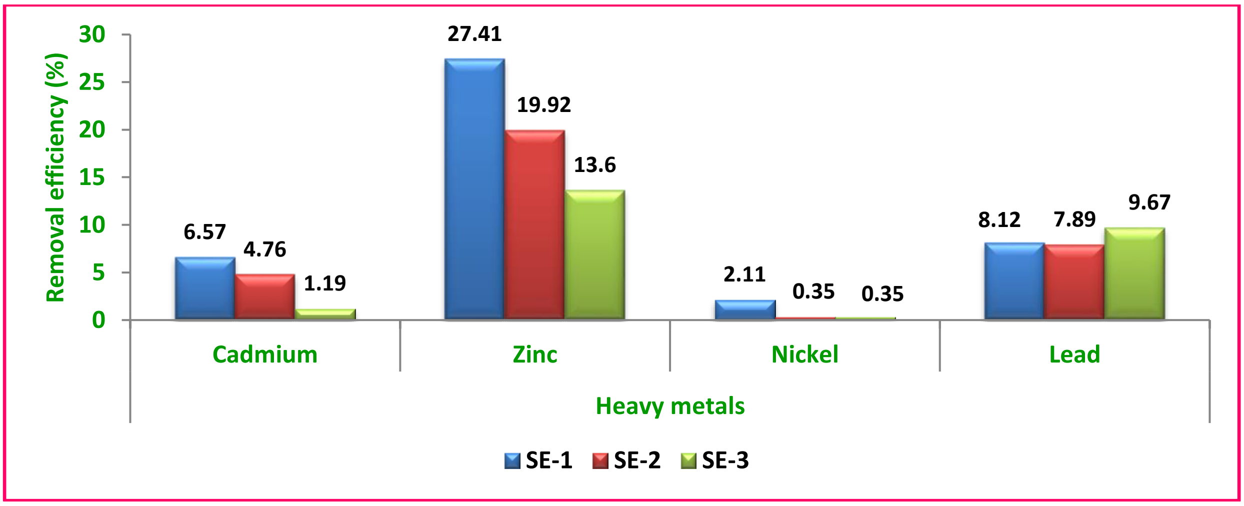

Figure 1: Removal efficiency of heavy metals by *A. microphylla*

4. CONCLUSION

The results of present study highlighted that *Azolla microphylla* had significant potential for the removal of heavy metals from industrial effluents and it can be used in phytoremediation of heavy metals for large scale wastewater treatment plants in environmental refinement projects. The exact mechanism of absorption of heavy metals from wastewater should also be studied and explained.

5. REFERENCES

[1] WHO. (2004). Meeting the MDG drinking water and sanitation target: A mid-term assessment of progress. WHO, Geneva, Switzerland/UNICEF, New York. 33 p.

[2] Varsha, M., Madaan, N. and Mudgal, A. (2010). Phytoremediation: Plants used to remediate heavy

metal pollution. Agriculture and Biology Journal of North America, 5(6): 112-115.

[3] He, Z.L., Zhang, M.K., Calvert, D.V., Stoffella, P.J., Yang, X.E. and Yu, S. (2004). Transport of heavy metals in surface runoff from vegetable and citrus fields. Soil Science Society of America Journal, 68: 1662-1669.

[4] Rathinavel, S., Mohan, E. and Sundararajan., K.S. (1999). Bioremoval of heavy metals by *Azolla*. Recovery, Recycling, Re-integration World Congress Proceedings, Geneva, Switzerland. Volume IV, pp. 245-250.

[5] APHA. (1995). Standard methods for the examination of water and waste water. 14th edition, American Public Health Association, Washington, D.C.

[6] Williams, R.F. (1996). The physiology of plant growth with special reference to the concept of net assimilation rate. Annals of Botany, 10: 41-72.

[7] Ali, H., Khan, E., and Sajad, M.A. (2013). Phytoremediation of heavy metals-concepts and applications. Chemosphere, 91(7): 869–881.

[8] Haniffa, M.A. and Arul Selvan, S. (1991). Relative toxicity of bleaching drying and mixed textile mill effluents to the freshwater fish *Oreochromis mossambicus* Trewaves. Journal of Environmental Biology, 12(4): 359-362.

[9] Moitra, J.K. and Pandey, G.S. (1991). Toxicity of integrated steel plant wastewater to true mirrows *Barbus dorsalis*. Journal of Environmental Biology, 12 (4): 329-333.

[10] Swaminathan, K. and Vaidheeswaran, P. (1991). Effect of dyeing factory effluents on seed germination and seedling development of ground nut *Arachis hypogea*. Journal of Environmental Biology, 12(4): 353-358.

[11] Deviprasad, P.V. and Deviprasad, P.S. (1992). Effect of cadmium, lead and nickel on three freshwater green algae. Water, Air and Soil Pollution, 17: 263-268.

[12] Lee, L.H., Lustigman, B. and Maccarn, J. (1993). Effects of copper on the growth of *Anacystis nidulans*. Bulletin of Environmental Contamination and Toxicology, 50: 600-607.

[13] Ornes, W.H., Sajwar, K.S., Dosskey, M.G. and Adriano, D.C. (1991). Bioaccumulation of selenium by floating aquatic plants. Water, Air and Soil Pollution, 32: 53-57.

[14] Kannaiyan, S. (1990). Effect of sodium chloride on the growth, nitrogenase and photosynthesis of *Azolla*. Indian Journal of Plant Physiology, 33(2) : 125-129.

[15] Taghavi, S.M. and Vora, A.B. (1994). Effect of industrial effluent on germination, growth and development of guar seed (var. PNB). Journal of Environmental Biology, 15(3): 209-212.

[16] Naghipour D., Ashrafi, S.D., Gholamzadeh M., Taghavi, K. and Naimi-Joubani M. (2018). Phytoremediation of heavy metals (Ni, Cd, Pb) by *Azolla filiculoides* from aqueous solution: A dataset. Data in Brief, 21: 1409-1414.

[17] Stepniewska, Z., Bennicell, R.P. and Balakhae, T.I. (2005). Potential of *Azolla carolinana* for the removal Pb and Cd from wastewater. International Agrophysics, 19: 251-255.

[18] Taghi-Ganji, M., Khosravi, M. and Rakhshaee, R. (2005). Biosorption of Pb^{+2}, Cd^{+2}, Cu^{+2} and Zn^{+2} from the wastewater by treated *Azolla filiculoides* with $H_2O_2/MgCl_2$. International Journal of Environmental Science and Technology, 1(4): 265-271.