Potential of *Azolla pinnata* for removal of cadmium from wastewater by phytoremediation

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Abstract

In this study, *Azolla pinnata* is a free- floating plant was obtained from Agric. Microbial Dept., Soils, Water and Environment Research Institute (SWERI), Agric. Res. Center (ARC), Giza, Egypt and used to investigate its bioindicative value by evaluating its ability to accumulate different concentrations of Cd^{+2} (0, 0.1, 0.2, 0.3, 0.4 and 0.5 ppm) in the form of Cd $(NO_3)_2.4H_2O$. The effect of different concentrations of Cd^{+2} on biomass production of this plant, doubling time and metal accumulation was studied. In such concern, growth of *A. pinnata* was determined during 25 days of incubation under greenhouse conditions. From the results of this study, it can be concluded that *A. pinnata* could be used as a reliable way for biomonitoring of cadmium and in pollution assessment. Overall, *A. pinnata* is an effective, eco-friendly and low-cost treatment technology.

Keywords: *Azolla pinnata*, Phytoremediation, Wastewater, Cadmium

I. Introduction

One of the most important environmental problems related to water pollution throughout the world is the contamination of water bodies by heavy metal ions because of their toxic effects on the environment and human health **(Akpor and Muchie, 2010)**. Heavy metals are chemical elements with a specific gravity that is at least five times the specific gravity of water **(Charan *et al.*, 2014)**. Cadmium may be released to water by natural weathering processes, by discharge from industrial facilities or sewage treatment plants, atmospheric deposition, by leaching from landfills or soil, or phosphate fertilizers **(Morrow, 2001)**. Cadmium accumulates in the human body affecting negatively several organs: liver, kidney, lung, bones, placenta, brain and the central nervous system **(Castro and Méndez, 2008)**.

Phytoremediation can be prepared from the naturally abundant plants which are very economical **(Rai, 2011)**. Aquatic macrophytes are known as good indicators of heavy metal contamination in aquatic ecosystems and they act as biological filters by accumulating heavy metals from the surrounding environments **(Alaa and Elsayed, 2015)**. The application of *Azolla* is a very common practice in phytoremediation, because it has very good potential for hyperaccumulation of different pollutants, minerals and heavy metals, restoring polluted aquatic resources **(Hossein *et al.*, 2014)**. *Azolla* spp. is heterosporous free-floating freshwater ferns that live symbiotically with *Anabaena azollae*, a nitrogen-fixing blue-green algae **(Punita and Soma, 2015)**. *Azolla* is a better macrophyte for aquatic phytoremediation because of its short doubling time (2-3 d), easy harvest, nitrogen fixation ability and tolerance to and accumulation of a wide range of heavy metals **(Sood *et al.*, 2012)**.

Therefore, the aim of this investigation is to evaluate the role of *A. pinnata* and *L. gibba* in accumulation of heavy metals such as cadmium existing in wastewater. *A. pinnata* and *L. gibba* were compared for their growth, fresh, dry weights, doubling time and Cd^{+2}accumulation.

II. Materials and Methods

2.1. Propagation of *A. pinnata*

A. pinnata was cultivated on modified Yoshida medium **(Yoshida *et al.*, 1976)**. Then it was collected and washed gently in running deionized water for several times by using 0.2 meshes screen and then the plants were air dried for 30 min.

2.2. Experimental procedure

This experiment was carried out in the greenhouse of Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt during July 2014. Cultivation of *A. pinnata* was carried out in plastic pots (10.0 cm diameter and 7.0 cm in depth). Pots were filled with 1000 ml of Yoshida medium and supplemented with different concentrations of Cd^{+2}. Wastewater samples were prepared by dissolving their corresponding analytical grade salts of $Cd(NO_3)_2.4H_2O$ in deionized water at nominal concentrations of 0, 0.1, 0.2, 0.3, 0.4 and 0.5 ppm. The pots were inoculated with 1 g fresh of *A. pinnata*, which was used as a standard inoculum **(El- Berashi, 2008)**. The inoculated pots were incubated at 35°C ± 2, 14 hr light and 10 hr dark for 25 days under greenhouse conditions. Samples of the treatments were taken after zero time, 5, 10, 15, 20 and 25 days of incubation.

Control treatment (plant without metal) which contained only a nutrient medium, was used to compare it with the effects Cd^{+2} concentrations on fresh, dry weight **(El-Shahat, 1997)**, doubling time of *A. pinnata* growth and

the accumulation of Cd^{+2} by this plant were determined on dry weight basis by using Inductively Coupled Plasma Spectrometry (**ICP**) according to **Chapman and Pratt (1961)**. *Azolla* culture was kept at a constant volume throughout the experimental periods by frequent changing of culture medium every 5 days to compensate water loss by evaporation when it is necessary **(El- Berashi, 2008)**.

2.3. The measured parameters

2.3.1. Fresh and Dry Weight

Samples of *A. pinnata* fronds were harvested, washed by deionized water and placed under shade between two thick layers of blotting tissue papers for approximately 1 hr. before determining fresh weight. The dry weight of *A. pinnata* was determined by drying fronds in an oven at 70°C to constant weight. Fresh and dry weights of *A. pinnata* were expressed as g/m^2 (**El- Berashi, 2008**).

2.3.2. Doubling time calculation

Growth rate of *A. pinnata* in terms of doubling time (D.T.) was calculated by using the following equation according to **Aziz and Watanabe (1983)**:

Doubling time = t/r, whereas:

t = the duration of *Azolla* growth, r = [log (wt/wo) / 0.301], wt = weight of *Azolla* at time t,

wo = weight of *Azolla* at zero time i.e. weight of inoculum.

2.4. Determination of Cd^{+2} accumulation by *A. pinnata*

Before digestion to analyze Cd^{+2}, harvested plant was washed with deionized water, air dried, dried at 70°C until constant weight and weighted for the dry weight. The digestion method was applied involving sulfuric acid and perchloric acid as wet digestion procedure according to **Chapman and Pratt (1961)**. 0.1 g dry weight of *A. pinnata* was used for digestion for each sample. Concentrations of Cd^{+2} were determined by using **ICP** (**Chapman and Pratt, 1961)**. Read of the instrument (mg L^{-1}) multiplied by an inverted extraction ratio (total volume for sample (cm) / sample weight (g)) = mg kg^{-1}.

2.5. Statistical analysis

The data were presented by mean ± standard deviation (n=3). Statistical analysis was carried out as a randomized complete design **(Snedecor and Cochran, 1980)** using LSD test to compare means of treatments in investigation. Statistical significance was defined as $p < 0.05$.

III. Results

3.1. Effect of different concentrations of Cadmium (Cd^{+2}) on fresh, dry weight (g/m^2) and doubling time (days) of *A. pinnata*

Fresh and dry weights gradually increased with increasing the incubation period from zero time up to 25 days. Fresh and dry weights also gradually increased with increasing the concentrations of Cd^{+2} from 0.1 to 0.2 ppm and then decreased from 0.3 up to 0.5 ppm during all the tested incubation periods from zero time up to 25 days as illustrated in **Table (1)**. It was obvious from the results that, maximum growth density were observed for *A. pinnata* at 0.2 ppm (1145.29 ± 37.12 and 85.90 ± 2.78 g/m^2) for fresh and dry weight, respectively after 25 days of incubation. These parameters were compared with the control (1135.07 ± 31.76 and 85.13 ± 2.38 g/m^2) for fresh and dry weight, respectively. There was non significant difference between the values of fresh, dry weights at 0.2 ppm and control after 25 days of incubation.

Doubling time generally decreased with increasing the concentrations of Cd^{+2} from 0.1 to 0.2 ppm and then increased with increasing the concentrations from 0.3 up to 0.5 ppm during all the tested incubation periods from zero time up to 25 days **(Table (1))**. The lowest value of doubling time was recorded at 0.2 ppm (7.91 ± 0.12 days) and this value decreased than that of the control (7.94 ± 0.10 days) after 25 days of incubation. The values of doubling time were significantly different at concentrations (0.3, 0.4 and 0.5 ppm) compared to the control after 25 days of incubation; whereas the values of doubling time was non significantly different at concentrations (0.1 and 0.2 ppm) after the same incubation period.

3.2. Effect of different concentrations of Cadmium (Cd^{+2}) on accumulation of this metal (g/m^2) by *A. pinnata*

Results of Cd^{+2}accumulation by *A. pinnata* in **Table (2)** particularly increased with increasing the concentrations of Cd^{+2} from 0.1 to 0.2 ppm and then decreased from 0.3 to 0.5 ppm at all incubation periods from zero time up to 25 days. Higher Cd^{+2}accumulation obtained in **Table (2)** as compared with the control at 0.2 ppm (130.57 ± 7.85 g/m^2) after 25 days of incubation. The results also showed that, Cd^{+2}accumulation generally increased with increasing the incubation period. The values of Cd^{+2}accumulation were highly significantly different compared to the control at all concentrations during all the tested incubation periods from zero time up to 25 days.

Table (1): Effect of different concentrations of Cadmium (Cd^{+2}) on fresh, dry weight (g/m^2) and doubling time (days) of *A. pinnata* (Data expressed as mean ± SD).

Period (days) C (ppm)	F.wt. (g/m^2)						D.wt. (g/m^2)						D.t. (days)					
	Zero-time	5	10	15	20	25	Zero-time	5	10	15	20	25	Zero-time	5	10	15	20	25
Control	128.21	333.33 ± 11.05	393.20 ± 9.26	543.60 ± 17.34	733.33 ± 24.61	1135.07 ± 31.76	9.62	25.00 ± 0.83	29.49 ± 0.69	40.77 ± 1.30	55.00 ± 1.85	85.13 ± 2.38	0.00	3.62 ± 0.12	6.17 ± 0.12	7.21 ± 0.14	7.94 ± 0.16	7.94 ± 0.10
0.1	128.21	353.87 ± 11.03	422.22 ± 9.84	565.82 ± 18.53	760.67 ± 25.17	1140.18 ± 29.83	9.62	26.54 ± 0.83	31.67 ± 0.74	42.44 ± 1.39	57.05 ± 1.89	85.51 ± 2.24	0.00	3.42 ± 0.11	5.81 ± 0.11	7.01 ± 0.17	7.78 ± 0.16	7.94 ± 0.10
0.2	128.21	360.67 ± 14.20	446.18 ± 13.46	600.00 ± 22.31	789.73 ± 26.82	1145.29 ± 37.12	9.62	27.05 ± 1.07	33.46 ± 1.01	45.00 ± 1.68	59.23 ± 2.01	85.90 ± 2.78	0.00	3.36 ± 0.12	5.56 ± 0.13	6.73 ± 0.15	7.63 ± 0.15	7.91 ± 0.12
0.3	128.21	345.29 ± 13.42	408.53 ± 8.28	557.29 ± 15.18	741.86 ± 23.93	998.31 ± 27.95	9.62	25.90 ± 1.01	30.64 ± 0.62	41.80 ± 1.14	55.64 ± 1.80	74.87 ± 2.10	0.00	3.50 ± 0.14	5.99 ± 0.11	7.08 ± 0.14	7.91 ± 0.14	8.45 ± 0.12
0.4	128.21	319.69 ± 7.48	382.89 ± 9.92	546.98 ± 17.91	735.02 ± 20.25	938.44 ± 26.20	9.62	23.98 ± 0.56	28.72 ± 0.75	41.02 ± 1.35	55.13 ± 1.52	70.38 ± 1.97	0.00	3.79 ± 0.09	6.33 ± 0.16	7.18 ± 0.17	7.94 ± 0.13	8.71 ± 0.12
0.5	128.21	278.62 ± 5.63	357.25 ± 8.67	531.65 ± 14.09	709.38 ± 19.92	882.09 ± 24.15	9.62	20.90 ± 0.43	26.79 ± 0.65	39.87 ± 1.06	53.20 ± 1.50	66.15 ± 1.81	0.00	4.46 ± 0.11	6.76 ± 0.16	7.32 ± 0.14	8.10 ± 0.13	8.99 ± 0.13
LSD at 0.05	-	19.394	17.876	31.588	41.958	53.010	-	1.455	1.339	2.370	3.148	3.974	-	0.184	0.235	0.269	0.255	0.203

F.wt*: Fresh weight;* ***D.wt****: Dry weight;* ***D.t****: Doubling time;* ***C:*** *Concentration****; L.S.D****: Least Significant Differences*

Table (2): Effect of different concentrations of Cadmium (Cd^{+2}) on accumulation of this metal (g/m^2) by *A. pinnata* (Data expressed as mean ± SD).

Period (days) / Concentrations (ppm)	Cd^{+2} accumulation (g/m^2)					
	Zero-time	5	10	15	20	25
Control	0.00	0.00	0.00	0.00	0.00	0.00
0.1	0.00	8.17 ± 0.30	20.43 ± 1.04	35.65 ± 1.98	65.32 ± 2.95	103.89 ± 5.70
0.2	0.00	10.48 ± 0.38	22.33 ± 1.37	42.41 ± 2.23	83.07 ± 4.81	130.57 ± 7.85
0.3	0.00	7.94 ± 0.23	19.40 ± 0.91	30.54 ± 1.51	56.35 ± 3.29	93.51 ± 4.58
0.4	0.00	6.41 ± 0.19	13.27 ± 0.61	26.23 ± 1.43	49.89 ± 2.45	90.20 ± 4.49
0.5	0.00	3.55 ± 0.12	10.29 ± 0.37	23.46 ± 1.40	45.76 ± 2.09	87.78 ± 4.47
LSD at 0.05	-	0.422	1.506	2.831	5.289	9.046

IV. Discussion

In this study, *A. pinnata* was grown in Yoshida medium **(Yoshida *et al.*, 1976)**. This medium was the most suitable environment where it had the essential nutrients needed for *Azolla* growth. Fresh and dry weights of *A. pinnata* gradually increased with increasing the incubation period from zero time up to 25 days. The recorded results are in the same line with those of **El- Araby *et al.* (1999)**; they found that *A. pinnata* recorded its maximum growth with increasing the incubation period up to 25 days. *Azolla* is a better macrophyte for aquatic phytoremediation because of its short doubling time (2-3 d), easy harvest, nitrogen fixation ability and tolerance to and accumulation of a wide range of heavy metals **(Sood *et al.*, 2012)**.

Higher Cd^{+2}accumulation was recorded at 0.2 ppm after 25 days of incubation. These results were harmony with **Calabrese and Baldwin (1999)**, who reported that at low concentrations of cadmium in the medium (0.01, 0.1 mg/L), *A. filiculoides* was a valid tool for water phytoremediation use; however, at the highest concentrations of cadmium in the medium (0.4, 0.8 and 1 mg/L), the accumulation was less than in the other treatments. This phenomenon is known as hormesis, where the small concentrations increase a response while greater concentrations diminish a response.

Similar trends were found for **Sheo and Anita (2011)**, a greater accumulation of Cd in plants led to a decrease in growth and disturbance in the metabolic activities. Therefore, *Azolla* can be used for the remediation of heavy metal to certain extent and as a sustainable technique to remove the heavy metal from contaminated fields. In addition, **Dai *et al.* (2006)** reported that, Cd accumulation and its effects on the fronds (*Azolla*) were closely related with Cd concentration in the growth medium. Aquatic macrophytes can accumulate significant quantity of heavy metals in their tissues ($10\text{-}10^6$) times greater concentration than in the water **(Snežana *et al.*, 2005)**. According to **Nuzhat *et***

***al*. (2015)** has revealed the role of free floating macrophyte (*A. pinnata*) in phytoremediation technology has an excellent performance in removing the metals and was able to remove huge amount of heavy metals in 10 days of the experimentation period.

Conclusion

A. pinnata is a potential candidate for the removal of Cd^{+2} from wastewater, therefore *A. pinnata* can play an important role in the remediation of aquatic ecosystems and wastewater treatment which are under heavy stress of anthropogenic pressure.

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