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A Study on the Phytoremediation Potential of *Azolla pinnata* under Laboratory Conditions

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Abstract

Heavy metal contamination in aquatic environments has become one of the major environmental problems all over the world. Phytoremediation is a plant based technology that utilises special plants known as hyperaccumulators to purify heavy metal contaminated sites. Hyperaccumulators are capable of absorbing heavy metals in greater concentrations. *Azolla pinnata* is an aquatic macrophyte that has been earmarked for its hyperaccumulation ability. This study was conducted under laboratory conditions to assess the ability of *A. pinnata* for the removal of Cr, Ni, Cd and Pb through rhizofiltration. Under three main experiments, phytoremediation ability of this species was investigated. In the first experiment, *A. pinnata* was exposed to prepared solutions of Cr, Ni and Pb of 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm and of Cd solutions of 0.5 ppm, 1.0 ppm, 1.5 ppm, 2.0 ppm, 2.5 ppm and 3.0 ppm respectively. Experiments were carried out separately for Cr, Ni, Cd and Pb concentrations for 7 days.

Presence of Cr, Ni, Cd and Pb caused a maximum inhibition of *A. pinnata* growth by 47%, 54%, 52% and 45% respectively while the highest removal percentages of Cr-98%, Ni-57%, Cd-88% and Pb-86% were recorded in 2 ppm, 2 ppm, 0.5 ppm and 8 ppm treatments respectively. The highest Bio-concentration Factor (BCF) for Cr was 1,376.67 when treated with 6 ppm, 684.95 at 4 ppm for Ni, 1,120.06 at 0.5 ppm for Cd and 1,332.53 at 8 ppm for Pb respectively. At the end of the experiments toxic symptoms were observed in plants exposed to Cd and Ni. The findings of this experiment revealed that *A. pinnata* is an excellent candidate for the removal of Pb and Cr even at higher concentrations and for Cd at lower concentrations while it is only partially efficient for Ni removal. The ability of *A. pinnata* to remove Cr, Ni, Cd and Pb from open dump site leachate was investigated in the experiment three. *A. pinnata* was exposed to a leachate dilution series of 5%, 15%, 25%, 50%, 75% and 100%. For all four metals, the highest removal percentages as well as the highest BCFs were given by the plants exposed to 5% leachate concentration. Influence of interactive effects of Cr, Cd, Ni and Pb on their removal capacities of *A. pinnata* and the metal selectivities were determined by the experiment two. The metal selectivity of *A. pinnata* was Pb> Cd> Cr> Ni. *A. pinnata* can be designated as a good phytoremediation tool for the mitigation of heavy metal pollution due to its high BCFs (over 1,000) in relation to Pb (2-10 ppm), Cr (2-10 ppm) and Cd (0.5-1 ppm).

Keywords: Phytoremediation, Azolla pinnata, Heavy metals, Bio Concentration Factor, Metal accumulation

1. Introduction

Many aquatic environments are polluted by heavy metals due to a myriad of reasons including industrial effluents, leaching of heavy metals from soil or bedrock and agrochemical usage in contemporary agriculture. Heavy metals are metallic chemical elements with a high atomic weight and densities five times greater than water (Sood et al., 2011). Many heavy metals are essential to the life cycles of both flora and fauna but are capable of reaching toxic levels when the supply of heavy metals through natural and anthropogenic sources exceeds their demand by inherent biological systems.

Heavy metals are considered a dangerous threat to the environment due to three key criteria, i.e. persistence, bioaccumulation and toxicity. Heavy metals are non-degradable chemical residues which possess long persistence times in the environment and gradually enter food chains and accumulate within higher trophic levels, endangering both animal and human life. Therefore the mitigation or attenuation of heavy metals from polluted aquatic environments is of great importance in protecting both the environment and human health.

Heavy metals from aquatic environments can be removed by a variety of techniques which include chemical, physical and biological techniques such as precipitation, ion exchange, chemical reduction and oxidation, membrane filtration, solvent extraction, reverse osmosis and activated carbon adsorption. However, the applications of these advanced remediation strategies are limited especially in developing countries as they are not economically feasible and require high sophisticated equipment which can be expensive to acquire. Generation of secondary wastes, metal specificity and suitability only in a narrow range of concentrations, are the other major limitations of the above methods.

One technology that is widely utilised for remediation of polluted environment is phytoremediation which is based on exploiting a plant's intrinsic mechanisms to accumulate or detoxify heavy metals from the soil or from aquatic environments. Phytoremediation is a low cost green technology and has been shown to be effective in diverse aquatic systems including reservoirs and ponds. Certain plants known as hyperaccumulators are capable of absorbing heavy metals which have no importance for plant metabolic processes. These hyperaccumulating plants which possess the capacity to absorb non-essential heavy metals are suitable candidates for efficient phytoremediation. In hyperaccumulation, heavy metal pollutants are absorbed by the roots of the plants and are concentrated in the plant tissues or decomposed to less harmful forms. Plants that can absorb and tolerate high levels of heavy metals are considered as potent candidates of phytoremediation.

Floating aquatic macrophytes are defined as plants that float on the water surface with submerged roots. Many aquatic macrophytes are potential candidates of phytoremediation as they demonstrate strong capacities to absorb non-essential heavy metals and concentrate them in tissues (Salt et al, 1995). In addition, the rapid proliferation and direct contact of aquatic macrophytes with the contaminated environment facilitates the purification process and ensures the sanitation of polluted water bodies.

Azolla pinnata is a small free-floating aquatic fern that lives in a mutual symbiosis with a cyanobacterium (*Nostoc azollae*), which resides within dorsal cavities of the fronds. *Nostoc azollae* can fix atmospheric nitrogen into ammonium ions, which are a source of nitrogen to the water fern. Subsequent decomposition of the water fern enriches the soil of paddy fields with nitrogen and due to its benefit as a nitrogen biofertilizer, *A. pinnata* is cultivated widely among rice cultivars to ensure that there is a constant supply of nitrogen to the soil.

In this study, the phytoremediation potential of *A, pinnata* was assessed in an *ex-situ* tank-based experimental system, where its phytoaccumulation capacity for four heavy metals, namely chromium (Cr), cadmium (Cd), nickel (Ni) and lead (Pb). The data generated in this study showed that *A. pinnata* is a good phytoremediation candidate for the sequestration of Cr and Pb and possesses limited capacity to be an efficient phytoremediation agent for Cd.

2. Materials and Methods
2.1 Plants acquisition and acclimatization
A. pinnata was obtained from paddy fields at the Rice Research and Development Institute Labuduwa, Galle. Healthy mature plants were selected for the experiment and rinsed with tap water in order to remove adhering mud particles and epiphytes. In order to adapt to the experimental conditions and to obtain substantial biomass, those plants were grown in a plastic trays containing distilled water with Albert solution for 10 days in the greenhouse of Department of Forestry and Environmental Science, University of Sri Jayewardenepura.

2.2 Preparations of stock solutions and treatment/experiment series
Experiment 1
Concentrations of heavy metals used in the experiments largely agreed with the environmentally measured values, although in certain experiments, higher treatments exceeded the environmental pollution levels.

Treatment solutions of Cr, Cd, Ni and Pb were prepared by diluting the respective stock solutions of 1,000 ppm concentration. A treatment series of 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm was performed for Cr, Pb and Ni, while a series of 0.5 ppm, 1.0 ppm, 1.5 ppm, 2.0 ppm, 2.5 ppm and 3.0 ppm was used for Cd, on par with the environmental levels of the respective heavy metals.

Experiments for all four metal ions were carried out separately in the greenhouse at an ambient temperature of 25-35 ℃. Rectangular glass aquariums with the dimensions of 28×15×10 cm were used to perform the experiment. Aquariums were filled with 2 L of each treatment solution in triplicates and distilled water in the absence of the metal was used as the control. One gram of Albert solution was added into each aquarium to supply the nutrient requirements and pH of the solutions were maintained between 6.5-7.5 throughout the experiment by titration with HCl and NaOH.

Healthy and matured *A. pinnata* plants were selected, rinsed with distilled water and blotted on filter papers to remove adherent water and 10 g of the water fern were laid on the surface of each aquarium. All the experiments were run for a 7 day period. Twenty milliliters water samples were

withdrawn from each individual replicate at 12 hour intervals. Distilled water was added based on necessity, to compensate for the water loss through evaporation and transpiration.

Sample analysis

Water samples were filtered with Whatmann No.1 filter papers and analysed by Flame Atomic Absorption Spectrometer (FAAS, GBC 932 plus) for Cr, Ni, Cd and Pb.

On the 7th day of the experiment *A. pinnata* plants in each individual replicate were harvested, rinsed with distilled water to remove any ions adhere to plant's surface and dried out by blotting on filter papers separately. Then the fresh biomass was weighed, dried for 48 hours in an oven at 80℃ and the dry weights were subsequently measured. Dried plant biomass were digested by wet digestion method according to Kalra, (1998) and analyzed by FAAS (GBC 932 plus) to determine the metal concentrations in *A. pinnata* tissues.

Experiment 2

The objective of this experiment was to determine the influence of the interactive/competitive effects of Cr, Cd, Ni and Pb on their removal capacities of each metal by *A. pinnata*.

Experimental design

The treatment solution was prepared as a mixture of all four metals. Thus, it comprised of Cr, Cd, Ni and Pb concentrations which gave the highest Bio Concentration Factor (BCF) values in Experiment 1.

Table 1: Initial concentrations of Cr, Ni, Cd and Pb in the treatment solution.

Metal	Treatment Concentrations that gave the highest BCFs in Experiment 1 (ppm)
Cr	6.0
Ni	4.0
Cd	0.5
Pb	8.0

Same experimental conditions were maintained as in experiment 1. Fifty millilitres of water samples were withdrawn from each individual replicate at 24 hours intervals and on the last day of the experiment *A. pinnata* plants were harvested, weighed after rinsing with distilled water and the adherent water removed using filter papers. Sample analysis was also done in the same manner as experiment 1.

Experiment 3
Sample collection

Leachate samples were collected from Karadiyana solid waste dumpsite which is located adjacent to the Bolgoda river. All samples were collected at the inlets of leachate collection drains around the dumpsite in order to obtain leachate with maximum contamination of heavy metals. Leachate samples were collected in polypropylene bottles and immediately transported to the greenhouse.

Experimental design

All collected leachate samples were mixed in order to get a homogenous mixture. A concentration series of 5%, 15%, 25%, 50%, 75% and 100% were prepared by diluting the leachate and 2 L of each was filled into 28×15×10 cm sized rectangular glass containers in the form of triplicates. Tap water was used as the control treatment. Healthy mature *A. pinnata* plants were selected, rinsed with distilled water and the water removed by blotting on filter paper. Ten grams of *A. pinnata* was introduced to each aquarium. The experiment was run for seven days at an ambient temperature of 25-35℃. of leachate samples were withdrawn from each individual replicate at 24 hour intervals. *A. pinnata* plants were harvested at the end of the experiment and fresh weights determined by removing adherent water using filter papers, following rinsing with distilled water.

2.3 Atomic absorption spectrometry analysis

Water samples were filtered with Whatmann No.1 filter papers and analysed by Flame Atomic Absorption Spectrometer (FAAS, GBC 932 plus) for Cr, Ni, Cd and Pb. Dried plant biomass digested by wet digestion method according to Kalra (1998), were also analysed by FAAS (GBC 932 plus) to determine the metal concentrations in *A. pinnata* tissues.

For experiment 3, a portion of original leachate was analysed for Cr, Cd, Ni and Pb in order to determine their initial concentrations. Leachate samples withdrawn at 24 hours intervals were digested according to APHA, (1999) and analyzed by FAAS (GBC 932 plus) to determine the remaining Cr, Cd, Ni and Pb concentrations in leachate treatments. Plant materials were digested and analyzed in the same manner as described above.

2.4 Measurement of fresh and dried biomass

On the 7th day of the experiment *A. pinnata* plants in each individual replicate were harvested, rinsed with distilled water to remove any ions adhering to plant's surface and dried out by blotting on filter papers separately. Then the fresh biomass was weighed, dried for 48 hours in an oven at 80℃ and the dry weights subsequently measured.

2.5 Calculations

The following parameters were calculated using the mean values of the data obtained from the experiments.

Relative growth

The relative growth of the plants exposed to the treatment solutions were calculated using the initial fresh weights and final fresh weights of *A. pinnata* biomass as follows (Xiaomei et al., 2004).

$$\text{Relative growth} = \frac{\text{Final fresh weight}}{\text{Initial fresh weight}}$$

(1)

Removal efficiency

The removal percentage of metal ions by *A. pinnata* was determined by using the initial metal concentrations of the treatment and the final concentrations at the end of the experiment (Bakar et al., 2013).

$$Removal\ efficiency = \frac{(Initial\ metal\ concentration - Final\ metal\ concentration) \times 100}{Initial\ metal\ concentration} \quad (2)$$

Metal uptake capacity

The accumulation of metal ions in *A. pinnata* tissues was calculated by using the dried weights. The metal concentrations of digested biomass were calculated as follows (Bediako, 2013).

$$Metal\ uptake\ (mg/\ kg) = \frac{Metal\ concentration\ of\ the\ dried\ biomass \times Total\ diluted\ volume}{Dry\ weight} \quad (3)$$

Bio Concentration Factor (Thayapara et al., 2013)

$$Bio\ Concentration\ factor = \frac{Metal\ concentration\ of\ the\ dried\ biomass\ (mg/\ l)}{Initial\ concentration\ of\ the\ metal\ in\ the\ external\ solution\ (mg/l)} \quad (4)$$

3. Results

3.1 Phytoremediation of chromium by Azolla pinnata

High removal efficiencies were observed in all treatments, with 98% (2 ppm) to 77% (10 ppm) removal efficiencies detected in the tested range (2 to 10 ppm) of Cr concentrations. In all treatments, high BCFs of over 1000 were observed with the highest BCF (1376.67) recorded in the 6 ppm Cr treatment. The relative growth of all Cr treatments were comparatively reduced to the negative control with a relative growth value of 1.14 observed in *A. pinnata* plants treated with the most concentrated treatment of Cr (10 ppm) (Table 2).

Table 2: Mean values of removal efficiency, relative growth, uptake and bio concentration factor (BCF) of chromium by *A. pinnata*.

Parameter	Control	2 ppm	4 ppm	6 ppm	8 ppm	10 ppm
Removal efficiency (%)	0	98	93	96	80	77
Relative growth	2.14	1.94	1.50	1.53	1.45	1.14
Cr uptake (mg/kg)	13.63	2,158.60	5,498.70	8,260.01	10,887.66	11,229.30
BCF	-	1,078.81	1,374.67	1,376.67	1,360.96	1,122.93

3.2 Phytoremediation of cadmium by A. pinnata

The highest Cd removal efficiencies were observed in the 0.5 ppm and 1 ppm treatments, where 88% and 76% removal efficiencies were recorded in the respective treatments. The calculated BCFs of the 0.5 ppm and 1 ppm treatments were above 1,000. In contrast, at concentrations above 1 ppm, the calculated BCFs were well below 1,000. In all treatments, there was a significant reduction in growth with only scarce growth observed after 7 days in all treatments above 0.5 ppm Cd.

3.3 Phytoremediation of nickel

The highest Ni removal efficiency of 57.05% was observed in the 2 ppm treatment. All BCFs of *A. pinnata* exposed to Ni treatments were well below 1,000. In all treatments, there was a strong growth retardation observed in *A. pinnata* plants, even though the Ni removal efficiencies were below 57% for all concentrations (2 to 10 ppm) of Ni tested (Table 3).

Table 3: Mean values of removal efficiency, relative growth, uptake and bio concentration factor of nickel by *A. pinnata*.

Parameter	Control	2 ppm	4 ppm	6 ppm	8 ppm	10 ppm
Removal efficiency (%)	0	57	50	42	40	25
Relative growth	2.26	1.64	1.26	1.14	1.13	1.05
Ni uptake (mg/kg)	0	1,095.84	2,739.79	3,173.48	3,567.91	4,366.93
BCF	-	547.92	684.95	528.91	445.99	436.69

3.4 Phytoremediation of lead by A. pinnata

The highest removal efficiency of Pb (86%) was observed in the 8 ppm treatments. The highest Pb accumulation of 10,660.22 mg/ kg of dry weights of plants was shown in *A. pinnata* plants exposed to 8 ppm treatment. Bio concentration factors of *A. pinnata* exposed to Pb treatments of 4, 6 and 8 ppm, exceeded 1000, where respective BCFs of 1,158.51, 1,113.29 and 1,332.53 were calculated (Table 4).

Table 4: Mean values of removal efficiency, relative growth, uptake and bio concentration factor (BCF) of lead by *A. pinnata*.

Parameter	Control	2 ppm	4 ppm	6 ppm	8 ppm	10 ppm
Removal efficiency (%)	_	79	81	85	86	76
Relative growth	2.81	2.40	2.37	2.13	2.02	1.54
Pb uptake (mg/kg)	6.29	1,875.31	4,634.03	6,679.72	10,660.22	9,824.71
BCF	_	937.65	1,158.51	1,113.29	1,332.53	982.47

3.5 Phytoremediation of each heavy metal ion in a collective pool of each other

A treatment of all four heavy metal ions was formulated by collectively pooling each heavy metal ion at the concentration at which the highest BCF was reported in each of our individual experiments. The concentration of each heavy metal ion that was used for this collective treatment is as follows; Cr–6 ppm, Cd–0.5 ppm, Ni–4 ppm and Pb–8 ppm.

Table 5: Relative growth, removal efficiency, metal uptake and BCFs of *A. pinnata* for Cr, Ni, Cd and Pb in a collective pool of each other. The initial concentrations of each metal in the collective treatment are provided in the table below.

Removal percentage (%)			
Heavy metal	Initial con.	Final con.	Removal %
Cr	6	1.15	81
Ni	4	1.78	56
Cd	0.5	0.09	83
Pb	8	1.20	85
Relative growth			1.39
Uptake capacity (mg/kg)			
Cr			6142.64
Ni			2719.87
Cd			524.19
Pb			7518.18
BCF			
Cr			1023.77
Ni			679.97
Cd			1048.39
Pb			939.77

Our results demonstrate that in the collective pool of heavy metal ions, Cr (6 ppm) and Cd (0.5 ppm) were sequestered efficiently as reported by their high BCF factors. However, *A. pinnata* showed a significant decline in the phytoremediation potential of Ni in the collective presence of the four heavy metal ions and is likely weakened by competitive ions and polluters which act to perturb the growth and physiology of the *A. pinnata* plant. Furthermore, consistent with what was reported earlier in this study, *A. pinnata* was a poor candidate in the removal of Ni from heavy-metal infested reservoirs.

3.6 Phytoremediation of industrial effluents containing heavy metals

Since data are strongly favorable for the use of *A. pinnata* in the phytoremediation of Cr, Cd and Pb, it was important to extend the *ex-situ* experiments to assess the sanitisation of industrial effluents by the *A. pinnata* water fern. The collected leachate were serially diluted to a form a series of dilutions (5%, 15%, 25%, 50%, 75%, 100%) and the dilutions were phytoremediated in triplicate using *A. pinnata*. Our data demonstrate that up to a dilution strength of 15%, Cr, Cd and Pb are

efficiently sanitized by *A. pinnata,* as indicated by the high BCF factors calculated for *A. pinnata* in the 5% and 15% leachates.

Table 6**:** Bio concentration factor of *A. pinnata* for Cr, Cd, Ni and Pb in different leachate concentrations.

Metal ion	5%	15%	25%	50%	75%	100%
Cr	1248.51	1185.60	609.11	721.98	449.32	473.97
Cd	1199.19	1040.90	882.02	442.50	341.64	328.62
Ni	867.33	746.78	570.71	272.87	165.97	114.85
Pb	1355.43	999.19	993.24	535.38	336.45	227.62

4. Discussion

A. pinnata possesses a remarkable capacity to hyperaccumulate heavy metals from polluted water bodies (Wagnar, 1997). *Ex situ* research carried out by Salt et al*.,* (1995), Bennicelli et al., (2004), Jangwattana*.,* (2010), Sood et al., (2011), Deval et al., (2012), Moradi et al*.,* (2013), Sufian et al*.,* (2013), Thayapara et al., (2013) have shown the uptake and retention capacities of *A. pinnata* species to different heavy metal ions. These findings suggest at the potential and the applicability of *A. pinnata* species to phytoremediate heavy metal polluted water reservoirs.

The primary characteristics of macrophytes that possess strong phytoremediation capacities are their faster growth rates, higher biomass and greater adaptability to wide range of environmental conditions. The ability of the endosymbiont in *A. pinnata* (*Nostoc azollae*) to assimilate atmospheric nitrogen and to enhance the overall growth of the plant is an important attribute that is utilised to engender profuse propagation of the water fern in irrigated environments. Moreover, the free floating nature of *A. pinnata* and other macrophytes facilitates harvesting and their higher water content in fresh biomass (90- 94%), which drastically reduces in volume after drying, solves to a great degree the disposal dilemma of harvested material. The dry *A. pinnata* biomass can easily be transported to recycling sites for the recovery of the heavy metals (Sood, 2011). Furthermore, *A. pinnata* possesses a remarkable ability to survive in highly polluted waters with wide spectra of pH, temperature and salinity, reflecting its suitability for phytoremediation applications.

4.1 Phytoremediation potential of A. pinnata in environmentally-relevant ranges of heavy metals

There are many sources of heavy metal ions to the wider environment including waterways. Cr is primarily generated through disposed batteries, anodizing and other metal finishing operations, tannery, paint and textile industries while Pb is released from disposable batteries, paints, via fossil fuel combustion and through runoff from road sediments of Pb which pollute urban waterways. Cd though is largely produced by the agrochemical Triple Super Phosphate (TSP) which contains between 23.5 to 71.7 Cd/kg (Noble et al*.,* 2014) and such release of Cd is thought to be the main contributory factor for the presence of the often fatal illness, CKDu, in the dry zone of Sri Lanka.

There are environmental standards determined by international bodies and local regulatory organisations that ensure that heavy metals are kept below a threshold level of contamination of

public waterways/catchments as well as public water supply. For example, the maximum concentration of Cd in drinking water as enforced by WHO stands at 0.003 ppm. However, it is of strong concern that in a study undertaken by Bandara, (2003), Cd levels of 0.03-0.06 ppm were reported in water samples from several reservoirs especially in North Central Province. Therefore, although in this study we have strived to treat *ex-situ* reservoirs with environmentally-relevant level of the tested heavy metals, it should be noted that higher contamination levels are often encountered in pollution sites.

Our results demonstrate that environmentally-relevant Cr and Pb, are easily phytoremediated up to a concentration of 10 ppm of the treated heavy metal. The BCFs for Cr at all treated concentrations were above a value of 1000 and for Pb, only the BCF values at 2 ppm and 10 ppm were marginally lower to the threshold level (937 and 982 ppm respectively), suggesting that for both Cr and Pb, there is strong phytoremediation of the respective heavy metal ions from the treated water aquariums. In the case of Cr, the removal percentages dropped with increased treatment concentrations which were analogous to the data reported on *A. pinnata* (Bennicelli*et* al., 2004).

In the case of Cd, there was sound phytoremediation at 0.5 and 1 ppm, where BCF values were over 1,000, but beyond this value Cd sequestration was hindered in particular by toxicity effects of Cd. Cd, which is both a residue of agrochemicals and industrial effluents has been pointed as one of the primary candidates for the etiology of CKDu and the transmission of Cd up the food chain is of grave danger to human health. Therefore it appears that *A. pinnata* is an efficient tool for the removal of low levels of Cd (below 1 ppm) from polluted water reservoirs. A separate study has also demonstrated that *A. pinnata* is a sound phytoremediation tool and a useful bio fertilizer up to a concentration of 1ppm Cd, beyond which both growth and photosynthesis was handicapped due to oxidative damage induced by superoxide radicals and hydrogen peroxide (Prasad and Freitas, 2003).

It is of significance that Ni was not efficiently remediated by the *A. pinnata* as indicated by the low bio-concentration factors for all treatments tested. Still, there was some removal of Ni from waterways suggesting that even Ni can by accumulated in tissues of *A. pinnata*.

4.2 Phytoremediation by A. pinnata in collective enrichments of heavy metals

Past studies have reported that in the presence of an array of contaminants, there can be varying consequences on the individual uptake of a heavy metal ion, due to complex formation, reactivities, decreased bioavailability, competition and inhibitory effects. It is due to such phenomena that we tested the sequestration of individual heavy metal ions, in aquariums treated with all heavy metal ions used in this study. It has been reported that one of the most critical influences on individual heavy metal uptake is the supplementary metal concentrations in the medium (Marbaniangand Chaturvedi*.,* 2014). For example, in a study by Rofkar et al., (2014), it was demonstrated that the presence of silicon (Si) and copper (Cu), decreased the accumulation of arsenic (As) by *Azolla caroliniana*.

This experiment was designed to entertain collectively the concentrations with the highest BCFs for individual ions, as determined by the BCFs calculated for *A. pinnata* in a series of individual treatments for each heavy metal ion. The BCF values obtained from collective treatment

of Cr, Cd, Pb and Ni, demonstrated that high BCF values were retained for Cr (6 ppm) and for Cd (0.5 ppm) even in the presence of competing ions, suggesting that there was minimal displacement of uptake of Cr and Cd by the presence of auxiliary heavy metals. On the other hand, the phytoremediation of Pb was marginally decreased in the presence of three other co-polluting heavy metal ions, suggesting that there may have been interactive or competitive effects that could have hindered the accumulation of lead by the *A. pinnata*.

4.3 Applications of A. pinnata to industrial effluents/treated samples

Numerous studies have demonstrated that industrial effluents can be sanitised by using plants of high phytoremediation potential. For example, in a study by Rizwana et al., (2014), the phytoremediation potential of *Eicchornia crassipes, Lemna trisulca, Oenathe javanica* and *Lepronia articulate* were identified, due to their efficient removal of Cd and Pb from textile industry effluents. However growth retardations, such as in *Vetivaria zizanioides,* due to the presence of Mn, Fe and Cu, as reported by Roontanakiat et al*.,* 2007, are a common characteristic due to the toxic nature of heavy metal ions.

In this experiments with industrial effluents, it was observed that Cr and Cd was amenable for phytoremediation at 5% and 15% dilutions of leachate as indicated by BCFs over 1000 for both. Pb too showed a high BCF values nearing or above 1000 for the 5% and 15% leachates demonstrating that at lower dilutions of effluents (such as following chemical or physical treatment), there is good potential for phytoremediation by *A. pinnata*.

It is suggested that industrial leachates which are treated initially with chemicals or filtered through membranes/adsorption surfaces, and thus diluted, are sound contenders to be phytoremediated by macrophytes such as *A. pinnata.* Therefore a shallow to medium-depth pond-based system where *A. pinnata* is cultivated as a surface lining can be used successfully to cleanse industrial effluents such as Cr, Cd and Pb and such a system is worth an effort to exploit the potential of biological heavy metal quenchers such as *A. pinnata* to sanitize industrial residue, prior to their contamination of public waterways.

4.4 Growth responses of A. pinnata to selective and collective heavy metals

Growth changes are often the first and most obvious indicators of plants under heavy metal stress (Deval et al., 2012). In this study, plant growth was measured in terms of relative growth of the treated plants as compared to the corresponding negative control (in the absence of heavy metal treatment).

In the presence of Cr and Pb (between a range of 2 and 10 ppm), the growth of *A. pinnata* was inhibited by 9-47% and 14%-45% respectively compared to the corresponding controls and there was a dose-dependency of the retardation of growth to the level of treatment of the selected heavy metal. The presence of Ni (2 to 10 ppm) caused a direct reduction of *A. pinnata* growth by 27-54% relative to the negative control even though the removal efficiencies of Ni were low at all concentrations tested.

A growth reduction of 12-52% was observed in the presence of Cd (0.5–3 ppm) relative to the negative control. *A. pinnata* exposed to 0.5 ppm treatment showed the highest relative growth of

1.95 and in all other treatments (1-3 ppm), there was no significant growth recorded, while the plants appeared to undergo chlorophyll degradation due to the decoloration of fronds. Similar observations were made by Lu et al., (2004) and Hasan et al., (2006) where the relative growth of water hyacinth plants were significantly decreased with moderately high Cd concentrations (2 and 4 ppm). Uysal and Taner (2009) also reported that high Cd concentrations created various toxicity symptoms on *Lemna minor* such as yellow coloration of plant leaves and decomposition of plants. In another study by Delgado et al., (1993) concerning the phytotoxic effect stemming from the uptake of Cd, Cr and Zn by water hyacinths, Cd was identified as most toxic to the water hyacinth plants. Therefore, our results agree with past findings on the formidable toxicity of Cd to macrophytes and other water plants.

5. Conclusion

We conclude by nominating *A. pinnata* as a phytoremediation agent that can be used for the sanitization of selective heavy metals, namely Cr, Cd and Pb and this technology offers efficient, easy, cost-effective, environmentally-sustainable solution to the problem of heavy metal accumulation in public reservoirs and waterways. It is of significance that in Sri Lanka, a fatal and debilitating condition designated as CKDu is fast reaching epidemic status and Cd which has been shown to be relevant to the etiology of this illness, requires to be extracted from contaminating sites such as irrigated rice fields, waterways and reservoirs. *A. pinnata,* which is abundantly used as a nitrogen-infuser for rice cultivation provides a vehicle for purifying water bodies infested with Cd, and thus allows for the arrest of transport of Cd up the food chains and through water consumption and consequently will contribute towards the management of the disease and the diseased in relation to CKDu.

It is also suggested that artificial pond-based systems cultivated with *A. pinnata* can easily be employed as a biological filter to quench heavy metal ions from industrial effluents and to let the resultant flow-through exit from the drainage system. Using such a filter-system will ensure that industrial pollutants are trapped, removed and destroyed prior to their contamination of public water bodies. The easily harvestable nature of *A. pinnata* is of significant benefit both in industrial ponds and in rice fields where pollution from effluent discharge and agrochemical usage is of major concern. The use of mechanical or manual harvesting can easily be performed and the heavy-metal enriched water ferns can be used for the chemical extraction of heavy metals such as Cr and Pb, which have numerous applications once recycled. Therefore, *A. pinnata,* although not a panacea for heavy metal contamination, offers a feasible solution to remediate polluted water bodies by the entrapment of selective heavy metals within the biological tissues of this miniature green wonder.

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