

PHYTOREMEDIATION OF HEAVY METALS USING MACROPHYTE CULTURE

Sufia Irfan

Biology Department, University of Tabuk, Tabuk-71491, Saudi Arabia

Abstract

Aquatic macrophytes *Pistia stratiotes* was grown in various concentrations of Cr (VI) and Zn solution for 15 days. The trend of bioaccumulation was reported high in *Pistia* in Zn culture. Metal toxicity in the floating plants showed significant effect on phytomass along with the inhibition in chlorophyll, protein, sugar, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ uptake inhibition. The uptake and accumulation of Cr in the root was always higher than that of shoot except between 4 h to 48 h duration at initial concentration of test metal.

Keywords: Cr, Heavy metals, *Pistia*, Phytoremediation, Zn

1. INTRODUCTION

Heavy metal removal from the wastewater through bio-absorption has benefits of releasing negligible secondary pollutants over conventional physicochemical water treatments plants. Development technologies and industrial progress has widely disrupted the aquatic ecosystems by various pollutants damaging the ecosystem balance and water quality. (Chakraborty et al. 2003). Heavy metals generally filter from aquatic medium into human system through water–plant–human or water–plant–animal–human biological chains (Tangahu 2011). Therefore finding solution to the problem of toxin tolerance in an aquatic medium is essential for an ecosystem and its components. There are reports with a variety of data detailing the effects of heavy metals on water and water plant structure and their properties, their enzymatic activity and nutrition pattern (Wittman 1979). A negative correlation was revealed between heavy metal content in growth medium and plants' submerged organs and green biomass (Zayed et al. 1998). Metallothionein and phytochelatins (low molecular weight protein) appears to work against metal toxification and other functions to protect plant cells from environmental strain (Memon & Schroder 2009).

The potentiality of aquatic macrophytes for heavy metal pollution has been investigated and reviewed extensively (Cheng 2003; Rai 2009). Heavy metals are among major water pollutants because of their persistent and bioaccumulative nature (Chang et al. 2009; Yadav et al. 2009). Implementing phytoremediation technology in environmental remediation is a budget friendly green technology. The plants applied in the phytoremediation technique are categorized as metal hyperaccumulator.

Since, macrophytes represents the base of aquatic food chain they consume metals from water and sediment and release them on senescence and decomposition (Brix & Schierup 1989; Shardendu et al. 2003; Azaizeh et al. 2006). Metal suspension in soil and water are major environmental and human threats. Living plants works as a machine under solar energy that can consume certain elements from the environment. Phytofiltration of heavy metals and their storage by aquatic plants represents the green pathway where plants extensive root structure consumes the toxic metals from polluted water via evapotranspiration over an extended period of time.

Pistia stratiotes is considered as the best option for phytoremediation with the established rhizofiltration mechanism in the aquatic ecosystems for wastewater treatment than terrestrial plants as their rapid growth and large biomass production supports higher pollution uptake and better purification mechanism due to direct contact with water column. Phytofiltration mechanisms of aquatic plants have often been treated in various water purification systems.

The aim of this study was to assess the total heavy metal removal capacity of *Pistia stratiotes*, role of root and leaves in metal uptake, preferential uptake of metals by macrophyte.

The specific objectives of the study are:

1. Uptake of Zn and Cr at various concentrations for 15 days by *Pistia stratiotes*
2. Preferential uptake of metals by *Pistia stratiotes*.
3. Effect of metals on chlorophyll.
4. Effect of metals on plant biomass.
5. Effect of metals on proteins
6. Effect of metals on Nitrate & Phosphate uptake

2. MATERIALS AND METHODS

Pistia stratiotes was isolated from local pond and was grown in garden pond water in the laboratory. The test metals (Cr and Zn) were introduced into the experimental vessels at various concentrations (Cr: 0.3, 0.6, 1.2 and 3.0 mg l⁻¹, Zn: 0.6, 1.2, 2.4 and 6.0 mg l⁻¹). Growth was measured in terms of dry weight (dw) after 15 days of treatment. Chlorophyll a and Protein estimation was performed by Broody & Broody (1961) and Lowry et al (1951) methods respectively. NO₃ – N uptake was estimated by phenoldisulphonic acid method (Nicholas & Nason 1957) and PO₄ – P uptake was estimated by phospho-molybdenum blue color method (APHA, 1993). Metal uptake was measured by the method of Martin (1979) with the help of a Perkin–Elmer 2380 Atomic Absorption Spectrophotometer. A control experiment without plant was performed simultaneously with the experimental batch.

3. RESULTS

Plants are vulnerable to heavy metal toxicity and behave in multiple ways to avoid detrimental effects. Toxicity concentrations depend on the type of ion, ion concentration, plant species and growth phases of the plant. Time and concentration dependent uptake of Zn and Cr by *Pistia stratiotes* was measured for 15 days culture. The uptake and accumulation of metals were estimated in roots and shoots separately and were added to get total uptake by the species. Uptake rates varied greatly according to relative concentration to test metals, growth form of the plant, type of absorption mechanism, affinity of metals for the adsorption sites and metal speciation. In general, at the beginning of the growth period there was rapid rate of uptake of all the metals considered and there was gradual reduction in uptake and rate with increasing time and concentration.

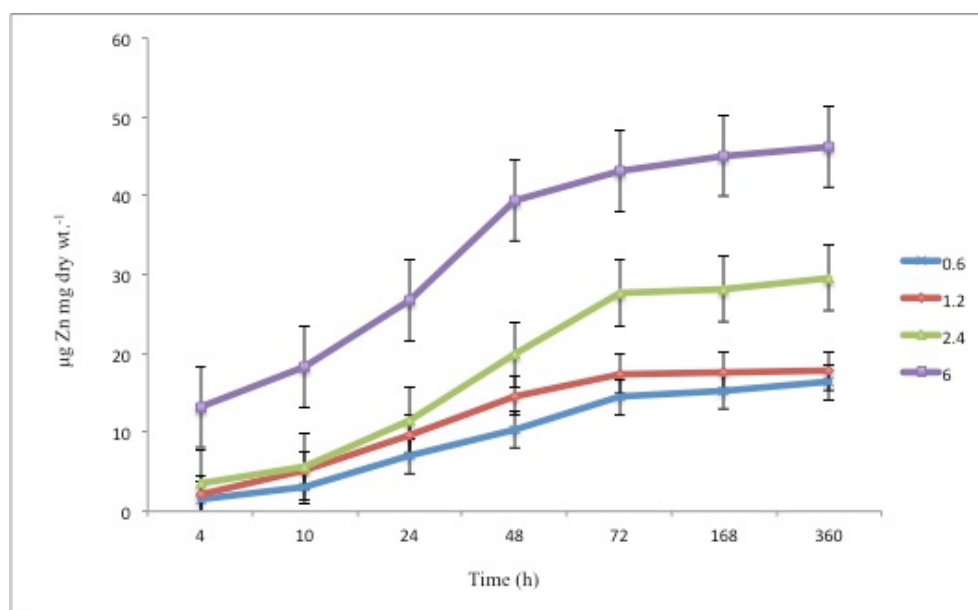


Fig. 1 Uptake and accumulation of Zn metal by *P. stratiotes*

The minimum uptake of Zn was $1.1 \mu\text{g mg}^{-1}\text{dw}$ of *Pistia* at 6 mg l^{-1} at 2h time interval where 41 % was absorbed by the shoot and 59 % by the root; the uptake amount increased about five fold after 15 days at the above concentration of test metal. The percentage of shoot uptake was less as compared to root uptake at lower time duration but after 7 and 15 days, the shoot uptake was about 57 % at initial concentration of test metal. The initial rate of uptake was quite high and it gradually declined. The amount and rate of uptake increased with increasing test metal concentration and maximum amount of Zn uptake was $18.5 \mu\text{g mg}^{-1}\text{dw}$ at 6 mg l^{-1} metal concentration after 15 days of treatment (Fig- 1). In most of the cases, percentage ratio of shoot uptake of Zn at longer time duration was higher than the root.

The rate and amount of Cr uptake was minimum as compared to Zn (Fig-2). The rate of uptake increased with concentration and decreased with increasing time duration. At the interval of 2 hour, the rate of uptake changed between $0.022 \mu\text{g Cr mg}^{-1}\text{dw h}^{-1}$ at initial concentration to $0.069 \mu\text{g Cr mg}^{-1}\text{dw h}^{-1}$ at final concentration of the test metal. After 15 days of experiment, the rate of uptake varied from $0.0016 \mu\text{g Cr mg}^{-1}\text{dw h}^{-1}$ to $0.0024 \mu\text{g Cr mg}^{-1}\text{dw h}^{-1}$ at initial and final concentration of test metal respectively. The uptake and accumulation of Cr in the root was always higher than that of shoot except between 4 h to 48 h duration at initial concentration of test metal. After 2 hours, the root did 54 % to 59 % Cr uptake and after 15 days the shoot did 49 to 60 % uptake of Cr.

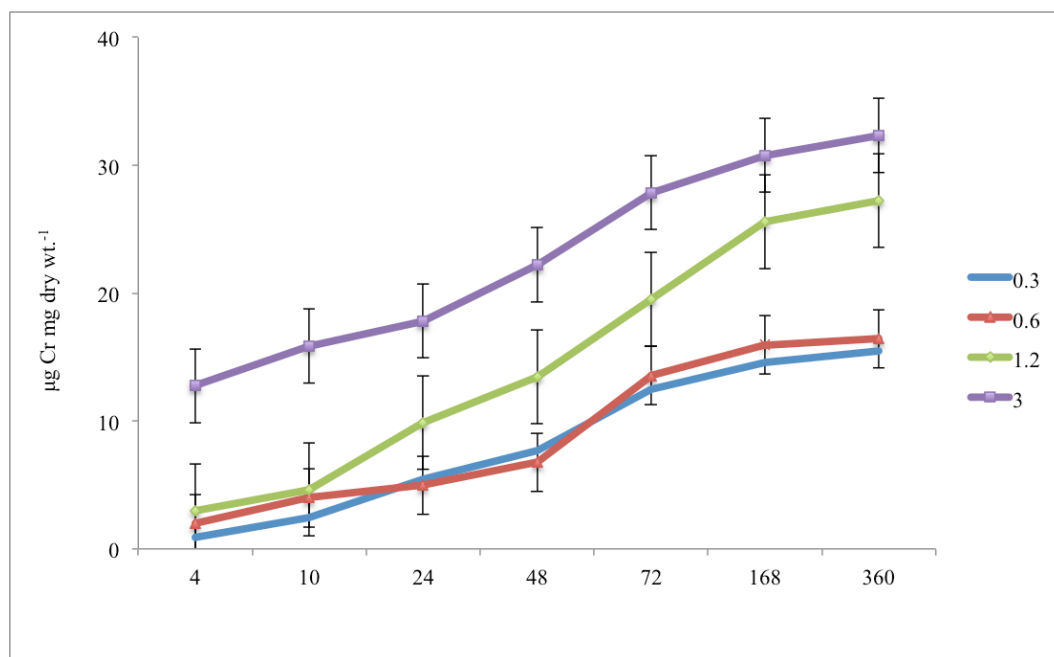


Fig. 2 uptake and accumulation of Cr metal by *P. stratiotes*

3.1 Root versus shoot uptake of Zn and Cr in *Pistia stratiotes* L.

The floating aquatic macrophytes designed to have a higher metal accumulation capacity in shoots than terrestrial plants. The reason could be the capacity of aquatic plants to take metals up by shoot floating on the surface water. During active growth submersed and free-floating plants accumulate metals mostly from the water and they function as an effective filter machine in the polluted water system. The uptake of heavy metal through root systems of aquatic macrophytes and subsequent release of metal during decomposition of plant material represent a pathway, in the study of biology of metals in aquatic ecosystems. Such a mechanism could have an important effect on levels of toxic metals in lake waters and therefore could exert some control on the toxicity of these metals to sensitive organisms. Both the living and dead biomass of aquatic macrophytes can be applied for removal of heavy metal contaminants from the aquatic systems. (Rai 2009; Mishra et al. 2009).

There are studies on estimation of metal concentration of macrophytes growing in natural and metal enriched water bodies (Adams et al. 1973; Welsh & Denny 1979, 1980; Pip 1990). There are reports on metal uptake and their toxicity to macrophytes (Sutton et al. 1971; Stanley 1974; Beham et al. 1979; Peter et al. 1979; Kay et al. 1984). There are reports of shoot versus root phosphorus storage in *Pistia stratiotes* (Agami & Reddy 1990) and in *Eichhornia crassipes* (Tucker & DeBusk, 1983, Agami & Reddy 1990).

The uptake of P by leaves or roots of aquatic plants was investigated by several authors (McRoy & Barsdate 1970, Bristow & Whitecomb 1971; McRoy et al. 1972; Demaetre & Hartman 1974). A study has confirmed roots of aquatic plants may absorb that nitrogen nutrient and translocate them to stems and leaves (Toetz 1974). Sutton et al. (1971) observed that when plants that had accumulated high levels of copper were placed into water containing no copper, concentration of metal in the plants decreased; this apparent release indicates the potential role of hydrophytes in metal cycling.

Metal concentration mg l ⁻¹	DW mg g ⁻¹	Chlorophyll µg mg ⁻¹ dw	Protein µg mg ⁻¹ dw
Shoot			
Control	228 ±13	4.2 ±.11	.48 ±.02
Zn			
0.6	163 ±9	0.91± 0.03	.37 ±0.02
1.2	137±8	.55±0.05	.24±.01
2.4	96±8	.46±.03	.15±.01
6.0	62±5	.32±.06	.08±.01
Cr			
0.3	183±10	0.22±.02	0.34±.02
0.6	146±14	0.15±.01	0.25±.02
1.2	83±6	0.11±.01	0.14±.01
3.0	28±2	0.07±.01	0.04±.01
Root			
Control	126 ± 10	-	0.34 ±.02
Zn			
0.6	95±4	-	0.22±.02
1.2	72±3		0.16±.01
2.4	46±3		0.09±.01
6.0	32±2		0.04±.01

Cr			
0.3	97±7		0.05±.01
0.6	66±2		0.18±.01
1.2	35±3		0.14±.01
3.0	18±1		0.03±.005

Table 1 Effect of Zn and Cr on dry weight (dw), chlorophyll a and protein content of shoot and root of *Pistia stratiotes*

Supply mg l ⁻¹	NO ₃ -N uptake Shoot µg NO ₃ -N mg ⁻¹ dw	PO ₄ -P uptake Root µg PO ₄ -P mg ⁻¹ dw	NO ₃ -N uptake Shoot µg NO ₃ -N mg ⁻¹ dw	PO ₄ -P uptake Root µg PO ₄ -P mg ⁻¹ dw
Control	120±4	123±6	80±6	86±4
Zn				
0.6	72±3	65±6	46±2	47±3
1.2	63±4	50±3	36±2	36±3
2.4	38±3	30±2	22±2	22±2
6.0	30±2	16±2	14±1	16±2
Cr				
0.3	64±5	60±6	41±5	4341±53
0.6	32±2	47±7	21±1	24±2
1.2	18±1	21±3	12±1	20±2
3.0	11±1	11±1	7±1	9±1

Table 2 Metal induced inhibition of NO₃-N and PO₄-P uptake by *Pistia stratiotes* after 15 days.

Metal	Shoot (µg metal mg ⁻¹ dw)		Root (µg metal mg ⁻¹ dw)	
	0.3	0.9	0.3	0.9
Zn	1.7±.14	2.4±.14	2.2 ±.08	2.8±.14
Cr	0.14±.01	0.26±.01	0.20±.01	0.39±.01

Table 3 Preferential uptake of metals (µg metal mg⁻¹dw) by shoot and root of *Pistia stratiotes*.

Shoot	Time	Concentration	Time* Concentration
Zn			
F	541	690	30

P<	.001	.001	.001
d.f	15	3	45
Cr			
F	519	94	8
P<	.001	.001	.001
d.f	15	3	45
Root			
Zn			
F	523	902	47
P<	.001	.001	.001
d.f	15	3	45
Cr			
F	1328	176	18
P<	.001	.001	.001
d.f	15	3	45

Table 4 Two-way ANOVA to test for variation of metals in shoot and root of the species between time (hours), concentration and interaction between hours and concentration

Shoot			
F- values			
Metals	Time	Concentration	Time* Concentration
Zn	541*	690*	30*
Cr	519*	94*	8*
Root			
Zn	523*	902*	47*
Cr	1328*	176*	18*

Table 5 Two-way ANOVA to test for variation of metals in shoot and root of the species between time (hours), concentration and interaction between hours and concentration

* All the F values are significant at $p < 0.001$, at time 15, concentration 3 and time and concentration 45

4. DISCUSSION

All plants have ability to accumulate essential elements from the abiotic surrounding of the ecosystem. Metals are required by the plants in different phases of their growth and development. These metals do not degradelike organic pollutants (Schroder 1973). When the metal deposition in plant tissues reach above optimal levels they start damaging cell structre by phytotoxicity. *Pistia stratiotes* in the aquatic system is an efficient cosmopolitan, metal hyperaccumulator because itsexensive root system that favours the selective metal uptake. A linear uptake trend was observed in the metal bioabsorption by *Pistia*. *P.stratiotes* indicates more uptake of Zn in comparison to Cr (Fig-1). The root zone technology in floating macrophytes have significant performance in phytoremediation. Submerged extensive root

fraction of the *Pistia* can absorb greatest concentrations of heavy metals this could be explained by the greater surface area (Smith et al. 1979) and thereby greater heavy metal adsorption capacity of the roots compared to the shoots. Studies revealed the role of total ecosystem effects, involving not only macrophytes but also sediments and other biota necessary to provide a complete picture of the effects of heavy metals on aquatic ecosystems. Metal uptake comprises various factors surrounding the ecosystem such as the availability and amount of contaminants, plants ability to interrupt the process of metal uptake, bioabsorption of metals in the shoot system via root zone, interaction between metals in the aqueous medium and sediment and biota. Many researchers have reported the application of FAM design grown with *Pistia* (Qin Lu 2011), *Eichhornia* (Maine et al. 2004) and *Lemna* (Jhon et al. 2008) as a suitable heavy metal accumulator. It has been reported that metal accumulating plant species can concentrate heavy metals in proportion to 100 or 1000 times compared to non accumulator plants. Microorganisms bacteria and fungi associated with the root zone of the plants helps mobilization of metal ions and enhances the bioavailability of the same (Tangahu 2011).

Function of heavy metal in the plant results in the physiological disorders of the system. A reduction in plant biomass was observed in metal treated plants especially at high dose of Zn and Cr (Table-1). Heavy metal translocation was negligible in the old age plants in the leaves of eelgrass while pectic compounds from the cell wall material thought to play an important role in the absorption of ions. (Maeda et al. 1966). *Pistia* population showed a high reduction in biomass when compared to control at a concentration of (6 mg l⁻¹) of Zn and (3mg l⁻¹) of Cr solution. The reason behind reduction in biomass could be root deformity causing poor transport of nutrients and water to the shoots. The results obtained clearly demonstrate that the ion exchange is the mechanism solely responsible for the ions uptake. The carboxyl groups in the ion exchange reactions are found related to the protein content in the *P. lucens* with high protein content (Schneider & Rubio 1999). Photosynthetic pigment is one of the most studied biological characteristics used to determine the physiological disorders as a result of metal phytotoxicity. It is reported that some metals decreased chlorophyll content in many plant species (Fargosova 2000). *Pistia* plant exposed in to metal culture of Zn and Cr for long duration affected the chlorophyll biosynthesis and thereby resulted in visible symptoms of chlorosis, petiolar chlorosis and necrosis leading to plant decay in the culture flask (Table-1). Metal toxicity degrades the chlorophyll synthesising substance and hinders the chlorophyllase activity by Cr metal (Jana & Bhattacharjee 1988).

The progressive decrease of proteins from leaves and roots of *P. stratiotes* may be caused by lower NR activity because they acts as limiting factor for protein content of the plants (Solomonson & Barber 1990) essential for growth and development of the plant. The loss in protein content may also be ascribed to leakage and diffusion of soluble protein out of the plant or catabolic enzymes induction destroyed the protein (Jana & Bhattacharjee 1988). The two forms of nitrogen used up by plants are NO₃⁻N and NH₄⁺ with different utilization by plants (Solomonson & Barber 1990). The inhibition in NO₃⁻N uptake by leaf and root tissues was clearly reflected by the increase metal concentration. Nitrate is an essential component for chlorophyll molecule (Sela et al. 1989) gradual decrease in N uptake may be due to binding of metals with enzyme, which finally resulted in damage of catalytic functions (Nichols et al. 2000).

In the metal mixture solution *Pistia* showed higher Zn uptake compared to Cr (Table 3). Metal chemistry in water attracts the opposite ends of water molecules. The cations with smaller atomic radius create a more intense electric field and thus penetrate more water ions (Levine, 1988). A study with metal mixture was shown in which bioabsorption of Cr⁶⁺ (atomic radius= 0.52 Å) was low in comparison to Zn (atomic radius= 0.74 Å). The slow uptake of Cr in metal combination solution might be because of large hydrated radius and hexavalent valency of the metal (Mallick et al. 1996). Different metals have different metal affinity based on cell wall adhesion, active influx of ions in to the vacuole, and development of complex compounds with organic acids or peptides (Memon & Schroder 2009).

The decaying organic matter of the aquatic macrophytes enters detritus food chains (Fenchel 1977) and the heavy metals bound in the biomass may thereby be released to higher trophic levels. The paper describes the mechanisms of heavy metal accumulation and tolerance in *Pistia stratiotes*. The

phytotoxicity of *Pistia* was visible in the presence of metals (Zn & Cr) Culture and can be applied in the contaminant removal system as heavy metal pollutant monitoring agent.

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