Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
5(1):38-43
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

In vitro Bioaccumulation of Heavy Metals by Water Lettuce *Pistia stratiotes Engl* (Araceae) and its Combustion Process as Manure Value by SEM-EDX Analysis

M.N.Abubacker[1]* and C.Sathya[2]

[1]PG & Research Department of Biotechnology, National College, Tiruchirappalli 620 001, Tamil Nadu, India
[2]Department of Botany, Seethalakshmi Ramaswami College, Tiruchirappalli 620 001, Tamil Nadu, India

Email: abubacker_nct@yahoo.com

ABSTRACT

In vitro experiments on chromium, copper, lead and zinc heavy metals bioaccumulation using water lettuce *Pistia stratiotes* Engl (Araceae) was conducted with 5, 10 and 20 mg/100 ml concentration for a period of 20 days. The SEM-EDX was used to characterize the interaction between the metal and plant. The results revealed the bioaccumulation of lead as high as 37.79% followed by chromium 8.63%, zinc 6.00% and copper 2.37%. The combustion process of bioaccumulated *P. stratiotes* biomass have shown the total reduction of lead 3.84%, copper 2.34%, zinc 1.26% and chromium 0.99%. The combusted biomass in the form of ash 10% and river sand passed through 1 mm sieve, sterilized and was amended to *Pennisetum typhoides* pot culture studies, revealed the healthy growth, ensure the manure value of metal-loaded biomass.

Keywords: Bioaccumulation, combustion process, heavy metal, manure value, *Pistia stratiotes*, SEM-EDX

INTRODUCTION

A number of chemical and pharmaceutical industries have been established since past three decades. Effluents from these industries are reportedly being directly discharged onto surrounding land and water bodies. Heavy metals from industrial and urban discharges are deposited in different components of the aquatic system such as water, sediments, soil and biota. Heavy metals like Cd, Cr, Ni, Cu, Pb and Zn may accumulate to a toxic concentration and cause ecological damage (Singh and Ghosh, 2005). The indiscriminate use of heavy metals containing fertilizers and pesticides in agriculture resulted in deterioration of water quality rendering serious environmental problems posing treat on human beings and sustaining aquatic biodiversity (Zhuang *et al*., 2007).

How to Site This Article:
M.N.Abubacker and C.Sathya (2017). *In vitro* Bioaccumulation of Heavy Metals by Water Lettuce *Pistia stratiotes Engl* (Araceae) and its Combustion Process as Manure Value by SEM-EDX Analysis. *Biolife*. 5(1), pp 38-43. doi:10.17812/blj.2017.5107

Published online: 24 January, 2017

Though some of the metals like Cu, Fe, Mn, Ni, Zn and Pb are essential as micronutrients for life processes in plants and microbes, while many other metals like Cd, Cr and Pb have no known physiological activity but they are proved detrimental beyond a certain limit (Subhashini and Swamy, 2013). Phytoremediation is the use of plants and to reduce the concentration or toxic effects of contaminants in the environment. Phytoremediation is the emerging technology, cost-effective, efficient, novel, ecofriendly with good public acceptance and long term applicability (Moosavi and Sehatoleslami, 2013; Vaziri *et al*.,2013).

In recent years, there has been an ever increasing interest in the study of metal accumulating plants for environmental remediation application, termed as phytoremediation. One method of phytoremediation is phytoextraction which uses metal accumulating plants to remove metal pollutants from contaminated sites by concentrating in the harvestable form from the plant (Zhuang *et al*., 2007). Phytoremediation of metal is a cost-effective 'green' technology based on the use of metal-accumulating plants to remove toxic metals from soil and water (Huang *et al*., 2011).

In the present study, the water lettuce *P. stratiotes* (Areaceae) was subjected to heavy metal concentrations in *in vitro* conditions to examine the bioaccumulation potential and its combustion process as manure value was carried out to highlight the possibility of using accumulated heavy metal from aquatic environment in the biomass and reused as manure for plant growth and development.

MATERIALS AND METHODS

Plant Sample Collection

Pistia stratiotes (Araceae), the water lettuce used in this study were collected from a polluted water body in Tiruchirappalli, Tamil Nadu, India. The plant is perennial monocotyleton with soft leaves that form a rosette. Its roots hanging submerged beneath floating leaves. The leaves green in colour, 14 cm long and 17 cm wide and fan shaped having parallel venation, covered in short hairs, wavy margins to form basket like structures. The plants were acclimatized for five days in tap water in tray and then subjected to *in vitro* studies.

In vitro Experimental Design

After acclimatization, the plants were tested in *in vitro* condition for three different concentration of chromium (Potassium dichromate, Merck), and copper (Copper-II sulphate, Himedia), lead (lead acetate, Merck) and zinc (zinc sulphate, Hemedia) at 5, 10 and 20 mg/100 ml respectively for 20 days as experimental time. Selected heavy metal concentrations were added in each Petridish from prepared stock solution. All the Petridishes were exposed to normal sun-light for detention time of 20 days. The Petridishes were shaken gently at regular interval for uniform distribution of metals in aqueous medium.

Anatomical Studies

Control plants and bioaccumulated chromium, copper, lead and zinc leaves of *P. stratiotes* were washed with deionised water, and were subjected to anatomical studies. The cross sections was taken with a thickness of 200-300 μm using a clear stainless steel razor blade, the unstain sections were mounted in microscopic slides using a drop of glycerine covered with a coverslip and photographed with the help of light microscope with 100X magnification (Olympus CH20i).

SEM-EDX Elemental Analysis

SEM-EDX analysis were carried out for bioaccumulation of Cr, Cu, Pb and Za. The combustion process for manure value of all the metals was also evaluated in this study. To study the nature of *P. stratiotes* after bioaccumulation, its leaves were collected on the 20th day after exposure to respective heavy metals. They were initially dried in shade, followed by hot air oven (at 50°C for one hour). Using mortar and pestle, the dried materials were powdered and placed in steel stub with carbon tape and sputter coated with fold particle for 50 second in high vacuum conditions for SEM-EDX analysis. The images of bioaccumulated *P. stratiotes* biomass was captured using scanning electron microscope coupled with energy dispersive X-ray consisting 3.5 nm and 2.5 nm resolution for tungsten filament (LaBb) and EDX detector resolution 133 eV (TESCON, Czechoslovakia) (Jamari *et al*., 2014).

Pot Culture Studies

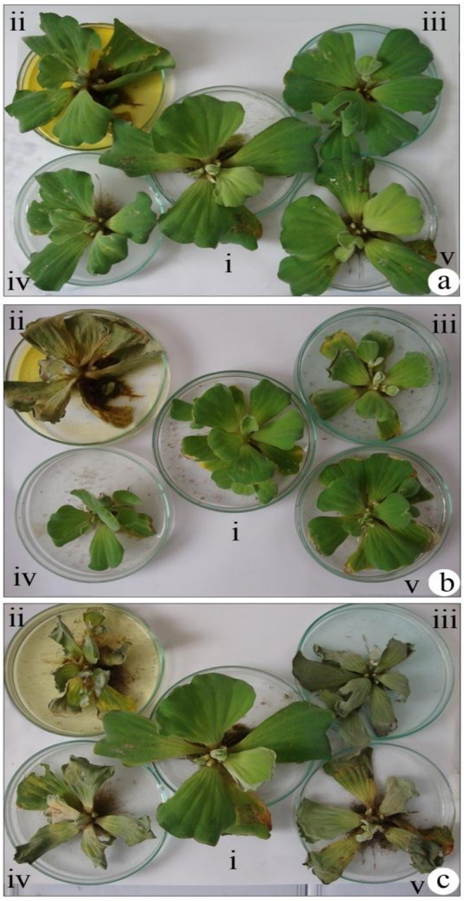
Pot culture studies were conducted using *Pennisetum typhoides* at three different formulations to analyze the growth conditions. Each pot incorporated with 20 mg/100 ml: (i) heavy metals (Cr, Cu, Pb, and Zn), (ii) dried *P. stratiotes* biomass after phytoremediation, and (iii) combusted metal-loaded biomass as manure. The controls were maintained with tap water and were carried out for a period of 20 days.

RESULTS

Bioaccumulation analysis of heavy metals

Studies on bioaccumulation of heavy metals such as Cr, Cu, Pb and Zn were conducted in *P. stratiotes* (water lettuce - aquatic weed) at 5, 10 and 20 mg/10 ml concentrations for a period of 20 days (Figure-1).

Figure-1. Bioaccumulation of heavy metals by Pistia stratiotes engl. (Araeae).
a) Initial stage of bioaccumulation-Day1; b) Second stage of bioaccumulation-Day10; Third stage of bioaccumulation-Day20; i) Control (water); ii) Chromium; iii) Copper; iv) Lead; v) Zinc (20mg/100ml)



The results indicated that *P. stratiotes* was able to absorb the heavy metals and there were no morphological changes observed and remain healthy till 7th day of experimental condition. Subsequently, observation on the 8th day indicated that the plant morphology has changed due to the accumulation of heavy metals upto 20th day of observation (Figure-1).

Accumulation of heavy metal in plant causes negative growth effects and also reduces their photosynthetic process (Sandelio *et al.*¸ 2001). The metal sorption in *P. stratiotes* and its subsequent anatomical studies have shown that the heavy metal were accumulated in the mesophyll tissue in accordance with the study of *Thlaspi caerulescens* as reported by Wojcik *et al*. (2005). The bioaccumulated leaf of *P. stratiotes* was sectioned, examined and microphotographed. The microphotography of unstained leaf anatomy revealed the bioaccumulation of Cr and Cu in the mesophyll tissue (Fig. 2). Since Pb and Zn are colourless heavy metals, leaf anatomy appears similar to control.

SEM-EDX elemental analysis

Scanning Electron Microscopy equipped with Energy Dispersive X-ray (SEM-EDX) analysis was conducted to detect the bioaccumulation of heavy metal at cellular and sub-cellular levels in *P. stratiotes* biomass, which revealed 8.63% for chromium, 2.37% for copper, 37.79% for lead and 6.0% for zinc. In control sample, these metals were not detected (Table-1, Fig.3).

SEM analysis of *P. stratiotes* biomass samples clearly reveals the surface texture and pores in the materials along with the morphological changes with respect to shape and size of the materials after accumulation of heavy metal ions. It is found that there is a difference in the shape of control and metal-loaded samples ensure the metal sorption as reported by Giri and Patel (2012).

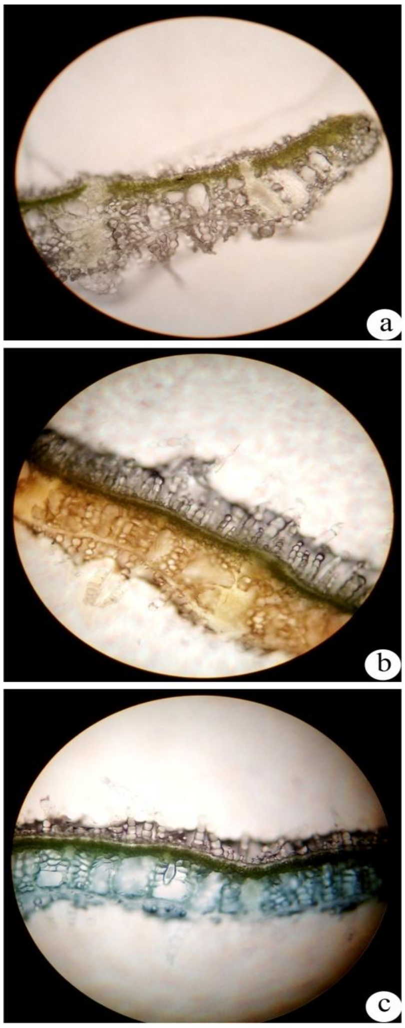
Pot Culture Studies

In final stage of experiments, the metal-loaded biomass was subjected to combustion process showed remarkable reduction in the percentage of heavy metals of 0.99% for Cr, 3.84% for lead, 1.26 for Zn and there is little reduction 2.34% for copper (Fig.3) than that of metal-loaded biomass before combustion process.

Table-1. SEM-DEX analysis of bioaccumulation of metals and its combustion process in *P. stratiotes* biomass as manure value

Metals	Bioaccumulation 20 mg/100 ml					
	Control %	Cr	Cu	Pb	Zn	Combustion Process (manure)
C	56.54	–	43.06	31.69	43.91	49.07
F	0.77	3.24	–	–	–	–
O	25.74	58.06	39.50	25.41	41.37	30.19
Na	1.30	1.20	–	0.21	–	1.45
Mg	0.37	0.98	–	0.33	0.38	0.78
Si	9.07	9.57	8.20	0.89	3.22	1.39
P	0.29	2.53	0.26	0.76	0.61	0.82
S	0.22	0.91	0.35	–	1.03	0.60
Cl	0.13	1.04	0.10	0.65	1.00	2.07
K	2.04	3.60	2.81	0.24	0.40	1.85
Ca	1.14	4.18	–	2.02	0.91	2.56
Fe	0.12	1.66	0.29	–	0.43	0.38
Al	0.41	3.47	2.49	–	0.74	0.42
Cr	–	8.63	–	–	–	0.99
Cu	–	–	2.37	–	–	2.34
Pb	–	–	–	37.79	–	3.84
Zn	1.34	0.94	0.43	–	6.00	1.26

Figure-2. Leaf anatomy (100x) of *Pistia stratiotes* *Engl.* Showing bioaccumulation of chromium and copper. a) Control; b) Bioaccumulation of chromium in mesophyll tissue; c) Bioaccumulation of copper in mesophyll tissue.

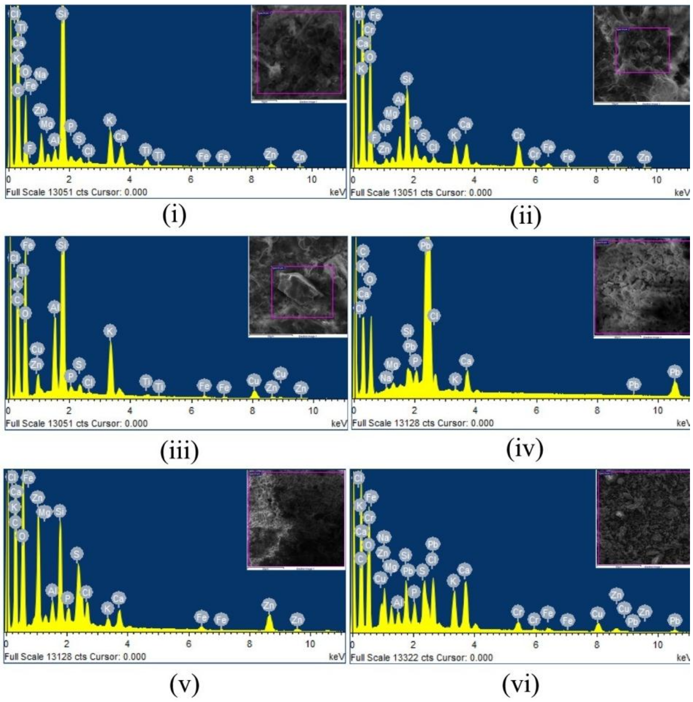


The product after combustion (ash) were supplemented to *Pennisetum typhoides* pot culture experiment and found healthy growth of seedlings (Fig.4), which ensure the manure value of metal-loaded *P. stratiotes* biomass. This result reveals the possible mechanisms of use of metal-loaded products in detoxification of heavy metals as reported by Lassat (2002).

DISCUSSION

Contamination of the aquatic bodies by various pollutants like heavy metal and polyaromatic hydrocarbons have caused imbalance in the natural functioning of the aquatic ecosystem. Phytoremediation works best at sites by reducing the pollutant concentration through bioaccumulation on biomass. SEM-EDX analysis confirms the bioaccumulation of heavy metals by *P. stratiotes* biomass. Due to this special characteristic feature, this aquatic plant can be employed easily for cost effective and ecofriendly green technology for heavy metal reduction from the polluted aquatic ecosystem and also recycle this heavy metal pollutant is manure through combustion process.

Figure-3. Elemental analysis of *Pistia stratiotes* *Engl.* Using SEM-EDX. i) Control; ii) Bioaccumulation of chromium; iii) Copper; iv) Lead; v) Zinc; vi) Bioaccumulation of metals and its product after combustion



Acknowledgements

Author (MNA) wish to thank DST-FIST, Government of India, New Delhi for providing the infrastructure facilities to the Department of Botany, National College, Tiruchirappalli, Tamil Nadu. Authors also expresses thanks to Padmavibhushan, Dr. V. Krishnamurthy, President, Sri. K. Ragunathan, Secretary and Dr. K. Anburasu, Principal, National College, Tiruchirappalli for all the supports and encouragement given to PG and Research Department of Biotechnology to carry over the research work.

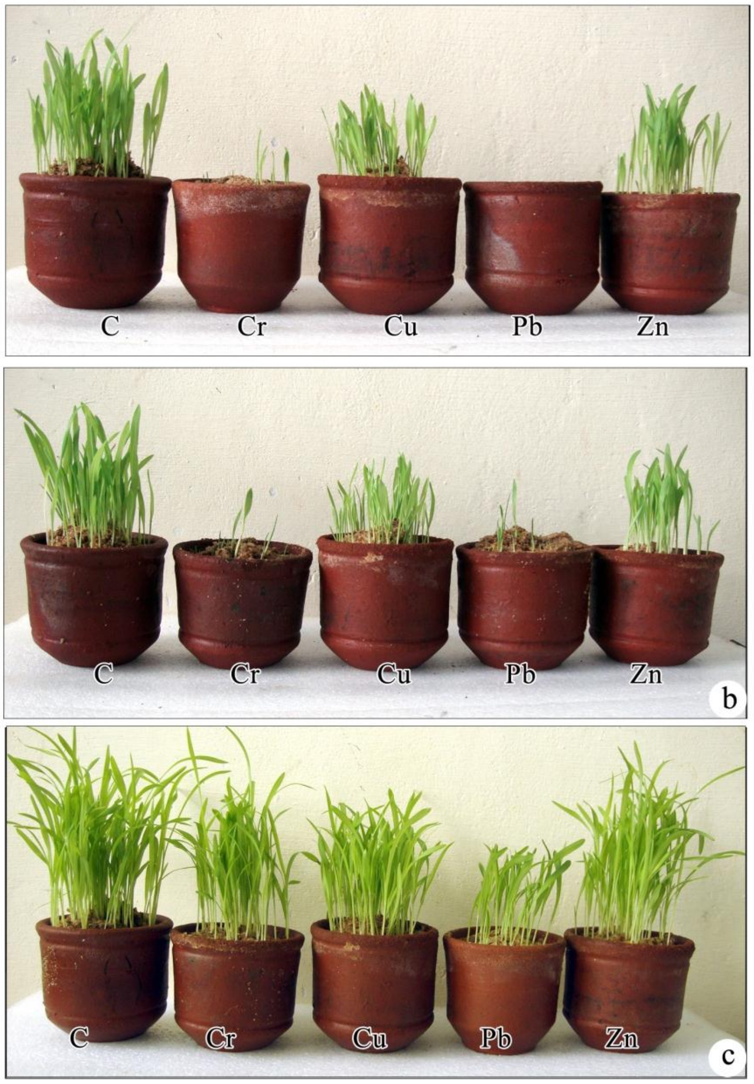
Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

Figure-4. Pot culture studies of *Pennisetum typhoides*

C-Control; Cr-chromium; Cu-Copper; Pb-Lead; Zn-Zinc

a) Pot culture supplemented with 20 mg/100 ml of heavy metals (Cr, Cu, Pb, Zn)
b) Pot culture supplemented with 20 mg/ml of respective heavy metals bioaccumulated *Pistia stratioles.*
c) Pot culture supplemented with 20 mg/100 ml of respective heavy metals bioaccumulated *P. stratioles* combusted biomass



References

[1]. Giri, A. K. and Patel, R. K. (2012). Phytoaccumulation potential and toxicity of arsenic ions by *Eichhornia crassipes* in hydroponic system. *J. Bioremed. Biodegrad.*, 3: 137.

[2]. Huang, H., Yu, N., Wang, L. and Yang, X. (2011). The phytoremediation potential of bioenergy crop *Ricinus communis* for DDTs and Cadmium cocontamination soil. *Bioresour. Technol.* 102: 11034-11038.

[3]. Jamari, S., Embong, Z. and Bakar, I. (2014). Elemental composition study of heavy metal (Ni, Cu, Zn) in riverbank soil by electrokinetic assisted phytoremediation using XRF and SEM-EDX. *AIP Conference Proceedings*, 1584: 221-227.

[4]. Lassat, M. (2002). Phytoextraction of toxic metals: A review of biological mechanisms. *J. Environ. Qual.*, 31: 109-120.

[5]. Moosavi, S. G. and Seghataleslami, M. J. (2013). Phytoremediation: A review. *Advance in Agriculture and Biology*, 1: 5-11.

[6]. Sandalio, L. M., Dalurzo, H. C., Gomes, M., Romero-Puertas, M. C. and Del Rio, L.A. (2001). Cadmium induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.*, 52: 2115-2126.

[7]. Singh, S. P. and Ghosh, M. (2005). A review on phytoremediation of heavy metals and utilization of its by-products. *Applied Ecology and Environmental Research,* 3: 1-18.

[8]. Subhashini, V. and Swamy, A. V. V. S. (2013). Phytoremediation of Pb and Ni contaminated soils using *Catharanthus rosens* L., *Universal Journal of Environmental Research and Technology*, 3: 465-472.

[9]. Vaziri, A., Panchpour, E. and Mirzaea Beni, M. H. (2013). Phytoremediation: A method for treatment of Petroleum Hydrocarbon Contaminated Soils. *International Journal of Farming and Allied Sciences*, 2: 909-913.

[10]. Wojcik, M., Vangronsveld, J., Dl Haen, J. and Yukiendort, A. (2005). Cadmium tolerance in *Thlaspi caerulescens. Environ. Exp. Bot*., 53: 163-171.

[11]. Zhuang, P., Yang, Q. W., Wang, H. B. and Shu, W. S. (2007). Phytoextraction of heavy metals by eight plant species in the field. *Water Air Soil Poll.*, 184: 235-242.