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# *In vitro* bioaccumulation metabolic studies of heavy metals by water lettuce *Pistia stratiotes Engl.* (Araceae)

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## ABSTRACT

In vitro experiments on bioaccumulation of heavy metals, chromium, copper, lead and zinc was conducted using water lettuce Pistia stratiotes Engl. (Araceae) with 5, 10 and 20 mg/ 100 ml concentration for each metals for a period of 20 days. The Scanning Electron Microscopy equipped with Energy Dispersive X-ray (SEM-EDX) results revealed the bioaccumulation of lead as high as 37.79% followed by chromium 8.63%, zinc 6.00% and copper 2.37%. There was a change in the pH of the medium, the bioaccumulation of metals were confirmed by FTIR analysis and a unique metabolic studies of heavy metal sorption have been predicted by GC-MS analysis.

**Keywords:** Bioaccumulation, FT-IR, GC-MS, Heavy metals, Pistia stratiotes Engl., SEM-EDX

## INTRODUCTION

Increase in accumulation of heavy metals in land and aquatic ecosystem caused due to anthropogenic impacts resulting in nutrient imbalance and productivity loss. These heavy metals such as Cd, Cr, Cu, Ni and Zn are assimilated and transferred within the food chains by biomagnifications process[1]. Related researches have been done on bioaccumulation of essential and non-essential metals by aquatic macrophytes[2,3]. This property of bioaccumulation was found useful in monitoring and ameliorating the water bodies[4,5]. Usually the plants have the ability to accumulate heavy metal such as Cr, Cu, Fe, Mn, Ni, Pb and Zn which are incorporated in their system for their growth and development. Certain aquatic plants also have the tendency to absorb and accumulate heavy metals with no known specific biological function. However, excessive accumulation of heavy metals will be toxic to plants. The ability to tolerate elevated levels of heavy metals and accumulation in high concentration has evolved independently or in combination has happened in different plant species[6,7]. The emphasis of most studies gradually shifted towards the use of aquatic plants as monitors for heavy metal water pollution.

Soil and water contaminated with metals pose a major environmental and human health hazard that needs an effective and affordable technological solution. Microbial bioremediation has been successful in degradation of specific organic contaminants, but is ineffective at addressing the challenge of certain toxic heavy metal contamination[8]. In recent years, there has been a lot of interest in the study of heavy metal accumulating plants which are used for environmental remediation as well as for application, termed as phytoremediation. Phytoextraction is one method of phytoremedition in which the metal accumulating plants are used to remove pollutants from contaminated sites by concentrating in the harvestable form from the plant[9,10]. This is a cost effective 'green' technology which can be employed to remove toxic metals from soil and water[11,12].

In the present study, the water lettuce *Pistia stratiotes* Engl. (Araceae) plant was subjected to heavy metal concentrations in *in vitro* conditions to examine the bioaccumulation potential of metal, bioaccumulation metabolic studies was carried out through SEM-EDX, FTIR and GC-MS analyses.

## MATERIALS AND METHODS

### Plant sample collection:

*P. stratiotes* Engl. (Araceae) the water lettuce weed used in this study were collected from a polluted water

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body in Tiruchirappalli, Tamil Nadu, India. The plant is perennial monocotyledon with soft leaves arranged in rosette manner. Roots found hanging submerged below the floating leaves[13]. The plants were acclimatized for five days in tap water in plastic tray under normal sunlight exposure, than 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), copper (Copper-II - Sulphate, Himedia), lead (lead acetate, Merck), and zinc (zinc sulphate, Himedia) at 5, 10 and 20 mg/100 ml respectively for 20 days as experimental time. Triplicate batch tests were conducted in Petridishes. Desired heavy metal concentration were added in each Petridish from prepared stock solution. All the Petridishes were exposed to normal sunlight for experimental time of 20 days. The Petridishes were shaken gently at regular interval for uniform distribution of metals in aqueous medium. The bioaccumulation of Cr, Cu, Pb and Zn were tested for pH, SEM-EDX, FTIR, GC-MS studies for metal accumulation and metabolic studies.

## RESULTS

Studies on bioaccumulation of heavy metals such as Cr, Cu, Pb and Zn was conducted for a period of 20 days at 5, 10 and 20 mg/100 ml concentrations using *P. stratiotes,* water lettuce (Figure-1).

### *pH*:

The *pH* of each sample was measured by using *pH* meter, *pH* reduction was noticed in all the samples (Cr, Cu, Pb and Zn) from the initial stage to the 20th day of experimental time (Table-1). The maximum *pH* 7.80 was noticed in 20 mg/100 ml concentration of chromium at the initial stage was reduced to 7.30 on the 20th day of bioaccumulation as against 6.90 for control. For copper, the maximum *pH* 7.60 was recorded at the initial stage was reduced to 7.20 on the 20th day. For lead the maximum *pH* 7.70 was reduced to 7.40, and for zinc the maximum *pH* 7.90 was reduced to 7.20 on the 20th day of bioaccumulation.

***Table-1. pH reduction during bioaccumulation process by Pistia stratiotes***

| Heavy metals (20 mg/100 ml) | No. of days treated | | |
|---|---|---|---|
| | 1 | 10 | 20 |
| Chromium | 7.80 | 7.40 | 7.30 |
| Copper | 7.60 | 7.30 | 7.20 |
| Lead | 7.70 | 7.50 | 7.40 |
| Zinc | 7.90 | 7.40 | 7.20 |
| Water (Control) | 6.90 | 6.90 | 6.90 |

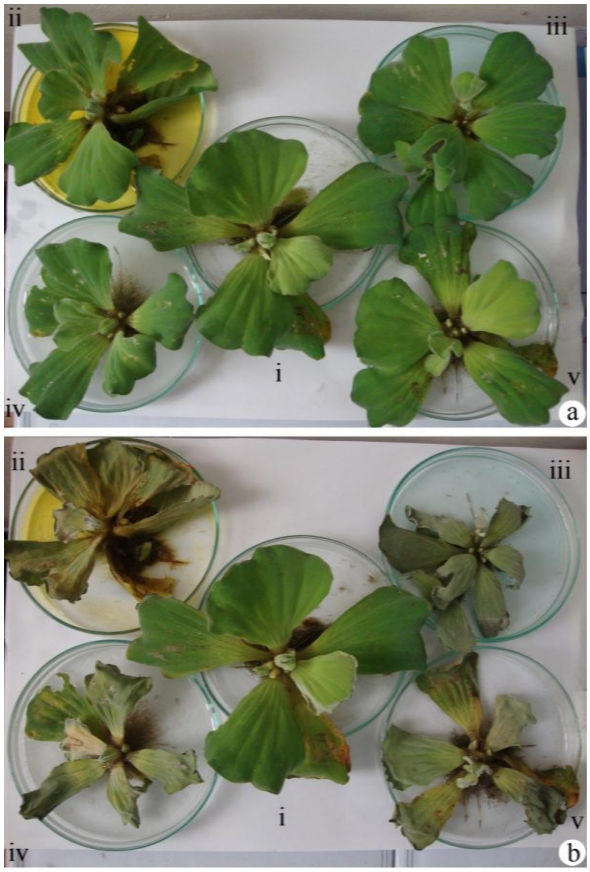
### SEM-EDX analysis:

Scanning Electron Microscopy equipped with Energy Dispersive X-ray (SEM-EDX) analysis was performed to determine the cellular and sub-cellular bioaccumulation of heavy metals in *P. stratiotes* biomass revealed 37.79% for Pb, 8.63% for Cr, 6.00% for Zn and 2.37% for Cu. In control sample only zinc is detected (1.34 and 0.94%) and the other metals copper and lead are not detected (Table-2).

**Table-2. Bioaccumulation of heavy metals by *Pistia stratiotes* by SEM-EDX analysis**

| Metal accumulation | Control (%) | Cr % | Cu % | Pb % | Zn % |
|---|---|---|---|---|---|
| Chromium | – | 8.63 | – | – | – |
| Copper | – | – | 2.37 | – | – |
| Lead | – | – | – | 37.79 | – |
| Zinc | 1.34 | 0.94 | – | – | 6.0 |

**Figure-1. Bioaccumulation of heavy metals by *Pistia stratiotes Engl.* (Araceae)**




a) Initial stage of bioaccumulation (Day 1)
b) Final stage of bioaccumulation (Day 20)
(i) Control (water) (ii) Chromium (iii) Copper
(iv) Lead (v) Zinc concentration 20mg/100mL

### Fourier transform infrared (FTIR) spectroscopy analysis:

FTIR spectroscopy was used to detect vibration frequency change in *P. stratiotes* biomass before and after the heavy metal bioaccumulation. The spectra were collected by Perkin Elmer spectrometer with the range 4000-400 $cm^{-1}$ using ethanol as mulling agent. The background obtained from the scan of ethanol was automatically subtracted from the sample spectra (Figure-2). The FTIR spectrum of *P. stratiotes* control plant showed the wavelength at 3403.43 $cm^{-1}$ for $NH_2$ aromatic amines and amides followed by these peak 2980.17 $cm^{-1}$ peak revealed the C–H stretch. *P. stratiotes* Cr accumulated plant sample showed 3400.22 $cm^{-1}$ that is –OH in alcohol and phenols and three peaks deviated showed at 2980.01, 2903.89 and 2834.97 $cm^{-1}$. Cu accumulated plant sample showed the peak at 3433.67 which is $–NH_2$ in aromatic amines followed by two peaks at 2981.45 $cm^{-1}$ and 2834.81 for aliphatic compounds.

*P. stratiotes* Zn accumulated plant sample showed two deviated peaks at 3466.87, 3434.02 $cm^{-1}$ are –CH stretch. Pb accumulated plant sample had the peak at 3433.8 $cm^{-1}$ which is –OH stretch and two deveated peaks are at 2906.16, 2838.41 $cm^{-1}$ revealed the $–CH_3$ and $CH_2$ in aliphatic compounds (Table-3, Figure-2).

### GC-MS analysis of metabolic pathway of heavy metal sorption in *P. stratiotes* biomass:

The GC-MS analysis of *P. stratiotes* control plant have certain specific compounds like 3-Ethoxy-1, 5-Hexamethyl, bis-ether, 3-Butoxy-1, 2,5-Dihydroxyacetophenone, whereas chromium bioaccumulated plants have shown propanedioic acid, ethnol, methanol, propanoic acid, 2-Butanol, 2-propanol, 2-fluoropropene, 2-pentanol. Copper bioaccumulated plants have propanedioic acid, Ethanol, 2-propanol, 2-fluropropene, 2-Butanol, oxoethyl methyl ester. The lead bioaccumulated plants have tetrahydrofuran-2-one, 2-hydroxypropyl 1-oxacyclopentadecan-2-one, 9-oximino-2, methyl ester, benzoic acid, 14-octadecental and the zinc bioaccumulated plant have shown 3-buten-2-ol, Dodecanoic acid, 2-Furanone, d-Mannose, L-Glucose, 1-pantoyl lactone, pantolactone, dodecanoic acid, d-Glycero-d-galacts-hetose (Figure-3).

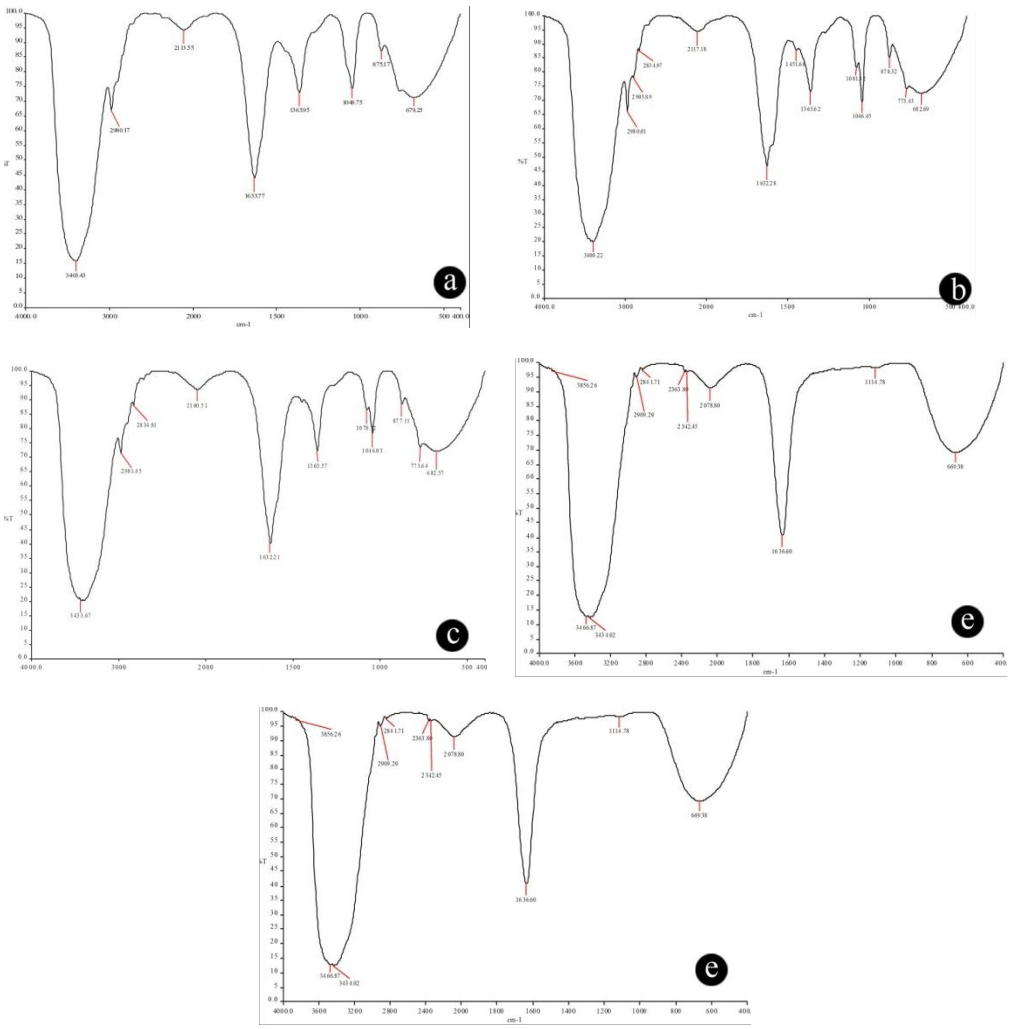
## DISCUSSION

SEM-EDX analysis of *P. stratiotes* biomass clearly reveals the surface texture and pores in the materials shows the morphological changes with respect to shape and size of materials after absorption of heavy metal

**Table-3. Bioaccumulation of metals by *P. stratiotes* by FTIR peaks wave length**

| Peaks | Bioaccumulated Plant | | | | |
|---|---|---|---|---|---|
| | Control | Cr | Cu | Pb | Zn |
| Peak-I | 3403.43 | 3400.22 | 3433.67 | 3433.84 | 3466.87 |
| | | | | | 3434.02 |
| Peak-II | 2980.17 | 2980.01 | 2981.45 | 2906.16 | 2841.71 |
| | | 2903.89 | 2834.81 | 2834.41 | 2909.20 |
| | | 2834.97 | | | |
| Peak-III | 2113.55 | 2117.18 | 2100.51 | 2340.95 | 2363.80 |
| | | | | 2370.05 | 2342.45 |
| | | | | 2083.12 | 2078.80 |
| Peak-IV | 1633.77 | 1632.28 | 1632.21 | 1637.97 | 1636.60 |
| Peak-V | 1365.95 | 1363.62 | 1363.57 | – | – |
| | | 1451.66 | | | |
| Peak-VI | 1048.75 | 1081.32 | 1078.72 | – | 1114.78 |
| | | 1046.45 | 1046.03 | | |
| Peak-VII | 875.17 | 878.32 | 877.11 | – | – |
| Peak-VIII | 679.25 | 773.43 | 773.64 | 687.10 | 6669.38 |
| | | 682.69 | 682.57 | | |

**Figure-2. FTIR analysis: Bioaccumulation of heavy metal by *Pistia stratiotes Engl.***



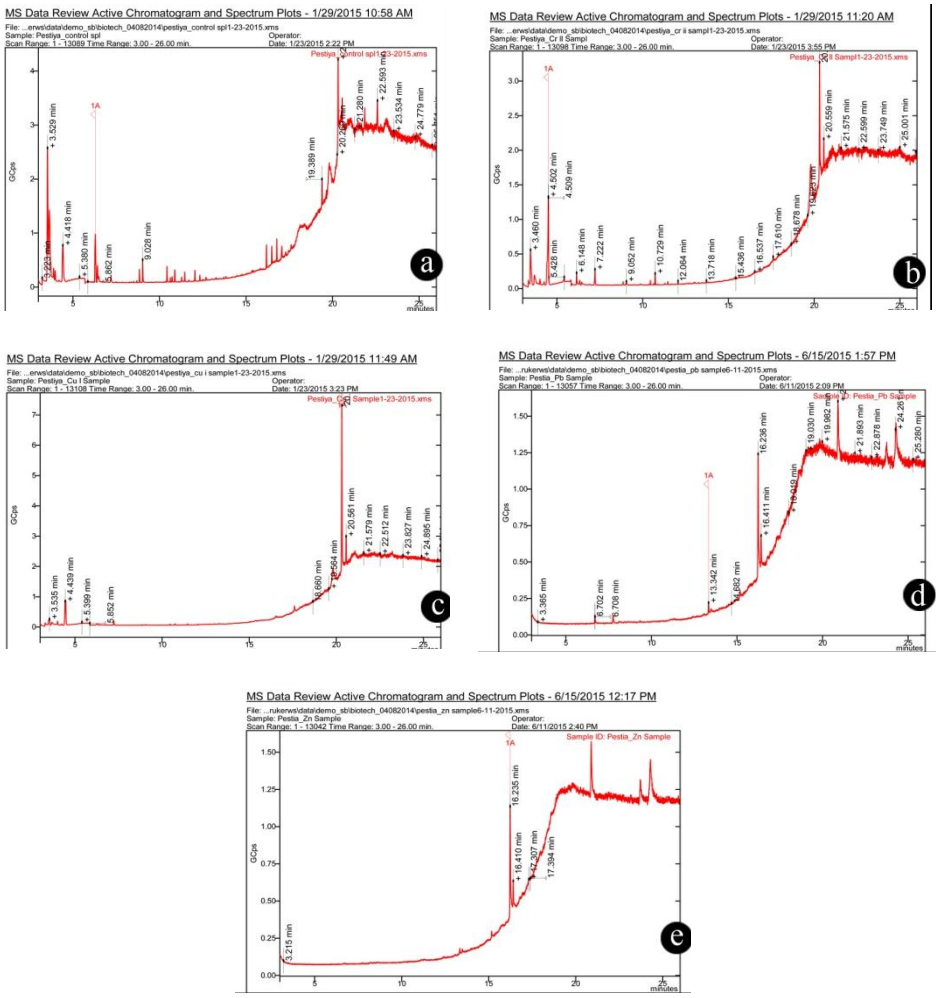

***a) Control b) Chromium c) Copper d) Lead e) Zinc***

ions. It is clearly observed that the surface of materials shape has changed into new particles ensure the metal sorption as reported by Giri and Petel (2012)[14] and Jamari *et al*. (2014)[15]. Certain plants have the ability to accumulate heavy metals such as Pb, Cr, Cd, and Zn[16]. The plants like *Avicennia marina* and Rhizophora accumulate Al, Cu, Cr, Cu, Fe, Mg, Mn and Zn[17]. *Canna indica* accumulate Pb, Ni, Zn, Cd and Cr[18], such bioaccumulation efficiency is noticed in *P. stratiotes*, able to accumulate 37.79% of Pb, 8.63% of Cr, 6.0% of Zn and 2.37% of Cu.

FTIR analysis indicates shift in wavelength in all bioaccumulated samples. The *P. stratiotes* control plant showed the wavelength at 3403.43 $cm^{-1}$. The NH stretch is typically not as broad as strong as the OH amine or amide N–H is *P. stratiotes* Cr bioaccumulated sample shows 3400.22 $cm^{-1}$, Cu bioaccumulated sample deviated into two peaks at 3433.67 $cm^{-1}$ $NH_2$ in aromatics amine and amides. Zn bioaccumulated plant sample shows that same to place deviated into two peaks at different wavelength of 3466.87, 3434.02 $cm^{-1}$ are –CH stretch alkyne. Alcohol O–H amine or amide N–H, *Pistia* Pb bioaccumulated sample have the peak at 3433.8 $cm^{-1}$ –OH stretch. Control sample shows the alkyl C–H at 2980.17 $cm^{-1}$ this peak show changes in Cr, Cu, Pb and Zn. In Cr sample deviated into three different wavelength at 2980.01, 2903.89 and 2834.97 $cm^{-1}$ alkyl group, in Cu bioaccumulated sample this peak deviated into two different wavelength at 298145 $cm^{-1}$ and 2834.81 $cm^{-1}$ aliphatic compounds, the Zn bioaccumulated sample 2909.20, 2841.71 $cm^{-1}$. This is normally a very broad signal centered near 3000 $cm^{-1}$ which is O–H stretch. Finally in the Pb bioaccumulated sample shows this peak at 2906.6, 2833.41 $cm^{-1}$ revealed the $–CH_3$ and $CH_2$ in aliphatic compounds. All these results are comparable with the reports of Phugare *et al.* (2011)[19] and Harshad Lade *et al.* (2012, 2015)[20,21].

The GC-MS studies of *P. stratiotes* control plant biomass showed specific aliphatic alcohol, aromatic compound, Cr bioaccumulated plant shows aliphatic alcohol, ethyl group shown the propanoic acid, 2-butanol, 2-propanol, 2-fluoropropene, 2-pentanol, copper bioaccumulated plant have alcohol, ester

**Figure-3. GC-MS analysis: Bioaccumulation of heavy metal by *Pistia stratiotes Engl.***




***a) Control b) Chromium c) Copper d) Lead e) Zinc***

compound, acids-propanedioc acid, ethanol, e-propanol, 2-fluoro propene, 2-butanol, oxyethyl ester, the lead bioaccumulated plants have tetrahydrofuran, 2-hydroxypropyl-1, oxacyclopentadecan-2-one, methyl ester – so it have the ester compounds, alicylic, aliphatic alcohol and zinc bioaccumulated plant shows 3 buten 2-ol-dodecanoic acid, 1-pantoyl lactone, dodecanoic acid with ol-alcohol, al-aldehydes. The biological mechanism of biosorption of metals by metal chelating proteins related to metallothioneins as stated by Lassat (2002)[22] and Kidd *et al*. (2009)[23]. From these report it is to ascertain that the acidifying process was resulted in the bioaccumulation of heavy metals in *P. stratiotes*.

## CONCLUSION

Contaminations of the aquatic bodies by various pollutants like heavy metal, poly-aromatic hydrocarbons have caused imbalanced in the natural functioning of the aquatic ecosystem. The mechanism of metal uptake, accumulation, exclusion and translocation are varying with each plant and the specific role in phytoremediation. SEM-EDX and FTIR analyses confirm the bioaccumulation of heavy metals in the *P. stratiotes* biomass. GC-MS analysis revealed the bioconversion pathway of metals in the plant biomass. This plant can be easily utilized for cost effective and eco-friendly green technology in pollutant reduction from the polluted aquatic ecosystem. This technology is a commercial reality in the near future.

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## Conflict of Interests

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

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