# CHAPTER 3

## 3 Lead Removal from Aqueous Solution in Batch Experiments by *Eichhornia crassipes*

### 3.1 Introduction

Lead (Pb) is non-essential heavy metal to biota, potentially toxic to living beings and the environment [1, 2]. As we known, plant photosynthesis is essential for the growth of plant. The photosynthetic processes of plants are affected by Pb through inhibition of enzyme activities [3] and in animals, Pb reacts with biomolecules and adversely affects the reproductive, nervous, immune, cardio-vascular, and other systems. Therefore, it is important to study the toxic effect of heavy metals on the plant photosynthesis. Recently, there has been a considerable interest in use of plant as a scavenger of heavy metals from aqueous solutions because, the methods are simple, and environment friendly, cost effective and both living and nonliving biomass are used [4,5,6,7,8]. But, most research work has been carried out by using terrestrial plants, grown hydroponically, and little is known on the process of heavy metals sorption by macrophytes [9]. Pb is often found in high concentrations in aquatic plants, particularly those growing in freshwaters and receiving mine or other industrial waste.

World's most troublesome aquatic weed, water hyacinth (*Eichhornia crassipes*), is known for its tendency to bio-accumulate and biomagnify the heavy metal contaminants present in water bodies. It is recognized as a very aggressive species of aquatic plant, which grows very fast and eliminates other aquatic species in its competition. We are interested in the capacity of water hyacinth to remediate aquatic environments that have been contaminated by the heavy metal Pb.

The water hyacinth which is an aquatic macrophyte has the ability to absorb and accumulate heavy metals from their environment and can withstand trace elements (such as Ag, Pb, Cd, Zn etc.) to a great extent [4]. Our present work was with the aim to examine its potential as a renewable resource to decontaminate polluted waste water, electron microscopy combined with X-ray microanalysis was used to investigate the localization of Pb in *E. crassipes* grown in hydroponic lead-rich solution.

The detoxification mechanisms of the plant have also been reported by various researchers [10, 11, 12]. The ability of water hyacinth to take up and translocate heavy metals was studied under controlled conditions but the number of such studies to date is still small as compared to field studies [13]. The metal uptake capacity of water hyacinth and other aquatic macrophytes are affected by some biological and non- biological factors via plant species and different organs, season, pH, metal concentration, and exposure time [12].

Scanning electron microscope (SEM) has often been used to localize metals in plants tissues, [14, 15, 16, 17] studied the different toxic effects of Mn and Hg on the cellular structure in leaves of aquatic plants, such as *P. vittata* and *N. exaltata* with the help of SEM micrograph. SEM is a powerful technique that can be used to investigate metal bindings to biomolecules. SEM allows us to evaluate morphological changes in the surface i.e. changes in cell wall composition after metal binding, but when combined with EDX techniques it can provide valuable inputs in determining the distribution of various elements over the biomolecules surface [18]. Raize et al. [19] used the SEM techniques to evaluate the surface of *Sargassum vulgaris* before and after Cd, Ni and Pb binding. After metal binding, minor morphological changes such as shrinking and layer sticking were seen in the cell wall matrix. Changes in surface morphology are usually related to disruption of the cross-linking between the metal ions and the negatively charged chemical groups e.g., carboxyl groups in the cell wall polymers. Plants can remove heavy metals by using their roots, stems or leaves for storage of these metals. The metals get converted within the plant into less harmful substances or gaseous form and are released into the air through transpiration activities [20]. *Hydrilla* sp. and *Chara* sp. can be used as bioabsorbant material for removal of chromium [21]. Activated carbon prepared from *Palmyra* palm nut has also been reported to be used for the removal of copper (II) from aqueous solution [22].

When a plant is exposed to a metal it was observed that in general metal concentration was significantly higher in roots than in other parts of the plants [23, 24]. The effects of Cr, Ni, Zn and P exposure on the chlorophyll a concentration of *Pistia stratiotes* L. showed that chlorophyll a was a more sensitive indicator of Zn and Cr toxicity than relative growth rate [25]. Most studies on pollutant bioaccumulation in macrophytes are aimed at assessing removal efficiency or toxic effects without taking into account the metal bioaccumulation process by

macrophytes, key knowledge not only to understand the behaviours of macrophytes but also to optimize effluent depuration by means of artificial wetlands. It has been proposed that the processes used by the plants are not necessarily the same for different species and for different metals. But, most research has been carried out on terrestrial plants grown hydroponically, and little is known on the process of heavy metal sorption by macrophytes [26].

SEM/EDX analysis is used in this present work to establish changes in morphology and elemental composition of the plants root, petiole surface with a view to establishing a mechanism of metal binding. The report of Pb accumulation by *E. crassipes* at different pH or at various initial concentration are available in the literature, however, in the present research work, the effect of these two factors on uptake capacity and their interplay have been studied. Moreover, a study on the effect of Pb on the photosynthetic rate and chlorophyll content was also done when the *E.crassipes* is exposed to $Pb^{2+}$ ion.

**3.1.1 Pb accumulation in plants**

The toxicity of Pb depends upon its absorption, transport and its cellular localization. Accumulation of Pb increases with the increase of metal in the environment as reported by Sinha, et al. [27] in maize and pea leaves. Aquatic and wetland plants usually accumulate high amounts of Pb usually grown in freshwater and receiving mine or other industrial wastes. Pb accumulation by most aquatic and wetland plant species such as *Potamogeton crispus, P. perfoliatus, Elodea canadensis* were very limited and did not exceed 40-fold the external Pb supply level.

**3.1.2 Effect of pH on accumulation of Pb**

Schneider et al. [28] studied the biosorption of metals on plant biomass extensively in order to determine whether it was exchange adsorption or surface precipitation. It was found that sorption of heavy metals on plant biomass is a function of pH, and was greatest at a pH value which was slightly more acidic than the pH at which there was a bulk precipitation of the metal hydroxide. The effect of pH on metal biosorption has been studied by many researchers, and the results indicated that pH of solution can significantly influence biosorption [29, 30, 31, 32].

## 3. 2 Materials and methods

### 3.2.1 Experimental setup and design

**Figure 3.1 *Eichhornia crassipes* (water hyacinth) plant**

*Eichhornia crassipes* (Mart.) Solm with approximately the same size and weight, 7-8 weeks old were collected from an uncontaminated pond. The plants were washed thoroughly with the tap water followed by de-ionized water prior to the experimentation. All the plants were grown hydrophonically for 10 days in modified Hoagland's nutrient solution. The modified Hoagland's solution contains (in M): $KNO_3$ $5X10^{-3}$, Ca $(NO_3).4H_2O$ $5X\ 10^{-3}$, $MgSO_4.7H_2O$ $2X10^{-3,}$ $KH_2PO_4$ $1x10^{-3}$, FeSO4.7H2O $0.02x10^{-3}$, $H_3BO_3$ $0.045x10^{-3}$, $MnCl_2.4H_2O$ $0.01x10^{-3,}$ CuSO4.5H2O $0.3x10^{-6}$, $Na_2MoO_4.2H_2O$ $0.1x10^{-6}$, and $ZnSO_4.7H_2O$ $0.8x10^{-6}$ (pH) [33, 34]. pH of the solutions was maintained at ~3, ~5 and ~7. Each set was examined for duration of ten days. Total chlorophyll content was estimated after 5-days interval using UV-Visible spectrophotometer. These were then put in polythene vessels (30× 9.5×9.5$cm^3$) for 7 days acclimatization. The *Eichhornia crassipes* was grown hydroponically without the addition of $Pb(NO_3)_2$ solution, considered as control.

### 3.2.2 Heavy metal preparation

All experimental work was done using double distilled water, and all reagents were of analytical grade. Pb stock solution was prepared by dissolving 1.5984 mg of Pb $(NO_3)_2$ in 1000 ml of double distilled water which was later diluted as required. *Eichhornia crassipes*, which were acclimatized in the laboratory condition, applied to a solution of Pb of concentration of 15.0, 20.0 and 30.0 mg/L in nine plastic tubs of five litre capacity.

### 3.2.3 Heavy metal analysis in plants and water

Plant samples were harvested after 10 days and were washed in tap water, rinsed twice with distilled water and preceded for Pb analysis [35]. They were separated into roots, petioles and leaves dried at 60$^{o}$ C for 40 hours to a constant weight. Samples of dry plants were ground to a fine powder and digested in a mixture of concentrates $HNO_3$ and $HClO_4$ (4:1 V/V) and the extract was filtered through Whatman No. 42 ashless filter paper and then diluted to 25 ml with deionized water. The Pb analysis in water samples were carried by removing water samples from each tub after an interval of 24 hrs for a period of ten days. Pb present in different solutions was determined by Atomic Absorption Spectrophotometer (Perkin Elmer 2001).

The experiments were carried out in single concentration at each tub and no replica has been used for each concentration.

### 3.2.4 Scanning electron microscopy (SEM) and energy dispersion (EDS) studies

The SEM technique has been used in several studies to investigate the internal distribution of metals in plant tissues [36].

Root, petiole and leaf samples were washed in running tap water and carefully rinsed with distilled water before microscopy observations. For scanning electron microscopy analysis (SEM) with conventional preparation, small pieces of roots, petioles and leaves (3-4 mm) were immediately fixed in 3% glutaraldehyde in 0.05M phosphate buffer for 90 min, which was followed by secondary fixation in 2% osmium tetroxide in 0.01M sodium cacodylate buffer for 30 min [37]. The samples were dehydrated in an acetone series. SEM photograph were carried for the samples, using SEM model JEOL- JSM-6390 LV attach with energy dispersive X-ray unit, with an accelerating voltage of 20 kV. In order to confirm the internalization of Pb by the *E.crassipes* SEM studies in combination with EDX in root, petiole and leaf of the *E. crassipes* samples exposed to Pb $(NO_3)_2$ solution for $2^{nd}$, $4^{th}$ and $10^{th}$ days was studied.

### 3.2.5 Photosynthetic rate

The photosynthetic rate of *Eichhornia crassipes* was determined with the help of LI-6400 Portable Photosynthesis system.

### 3.2.6 BCF

Bioaccumulation factor (BCF) is the ratio of metal concentration in plant to metal concentration on the solution [38].

## 3.3 Results and discussion

### 3.3.1 Pb Removal

The present trend of bioaccumulation of Pb by *E. crassipes* shows that the rate of uptake of Pb by *E. crassipes* is found to be very high just up to 48 hours of exposure for 15mg/L, 20mg/L and 30mg/L concentration in low, medium and neutral pH (**Figure 3.2**). This show the plants have the maximum efficiency of accumulation upto 2$^{nd}$ day of its application and in some cases it almost reaches saturation. The efficiency of bioabsorption of Pb upto 48hrs was so high that it could uptake upto 85.05% at initial concentration of 20 mg/L with medium pH as compared to a total of 89.78% accumulation Pb after 240hrs. This show the plants have the maximum efficiency of accumulation up to 2nd of its application. It was found for 15mg/L initial solution concentration, maximum bioabsorption of Pb i.e 71.31% occurs at neutral pH while 53.45% and 64.23% of Pb were removed at low and medium pH just after two days exposure. After 10$^{th}$ day for 15mg/L initial concentration, the removal rate is only 68.90%, 69.09% and 84.54% at low, medium and neutral pH respectively, which shows the efficiency of uptake by *Eichhornia crassipes* get reduced after 2$^{nd}$ day.

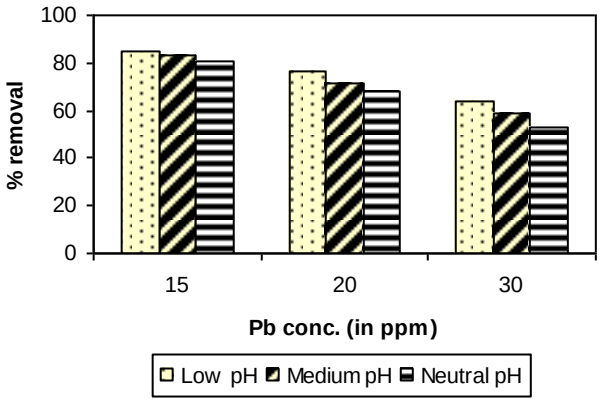



**Figure 3.2 Percent Pb removal by *E. crassipes* at 15, 20 and 30 ppm Pb at 2$^{nd}$ day**

The absorption of Pb by the root was found to be much higher at all initial concentration and pH. In the present study it was found that the roots absorb a high amount of Pb (11.04 mg/g after 10$^{th}$ day) as compared to petiole (1.129 mg/g) (**Table 3.1**) at initial concentration of 30 mg/L at low pH. This trend was noticed for all the concentrations and at different pH. This investigation showed that the

efficiency of absorption of Pb by root was found to be quite high as compared to the petiole.

**Table 3.1 Pb accumulation (mg/g) dry wt. and bioaccumulation factor (BCF) in plant segment after 10th day exposure**

| Initial external concentration (mg/L) | pH | Roots(mg/kg) | Accumulation after 5th day exposure(mg/g) dry wt Petiole(mg/kg) | | |
|---|---|---|---|---|---|
| | | | BCF | **Petiole** | BCF |
| 15.0 | Low | 7.995 | 533.0 | 0.15765 | 38.42 |
| | Medium | 5.248 | 349.8 | 0.17305 | 11.53 |
| | Neutral | 5.282 | 352.13 | 0.3084 | 20.55 |
| 20.0 | Low | 9.835 | 491.5 | 0.4873 | 24.36 |
| | Medium | 4.62 | 231 | 0.6725 | 33.625 |
| | Neutral | 4.98 | 249.15 | 0.4921 | 24.605 |
| 30.0 | Low | 11.04 | 368 | 1.129 | 37.629 |
| | Medium | 7.365 | 245.5 | 0.3354 | 11.1788 |
| | Neutral | 5.75 | 165.83 | 0.4888 | 16.2917 |
| Control | Neutral | 0.053 | - | 0.01 | - |

### 3.3.2 Photosynthetic Rate

**Table 3.2 Photosynthesis rate in carbon dioxide/meter-square/hr**

| Concentration of Pb ( mg/L) | 1st day | 3rd day | 10th day |
|---|---|---|---|
| Control | 11.06 | 10.76 | 10.97 |
| 15 | 10.97 | 8.44 | 5.07 |
| 20 | 9.25 | 7.8 | 4.13 |
| 30 | 6.18 | 5.42 | 3.26 |

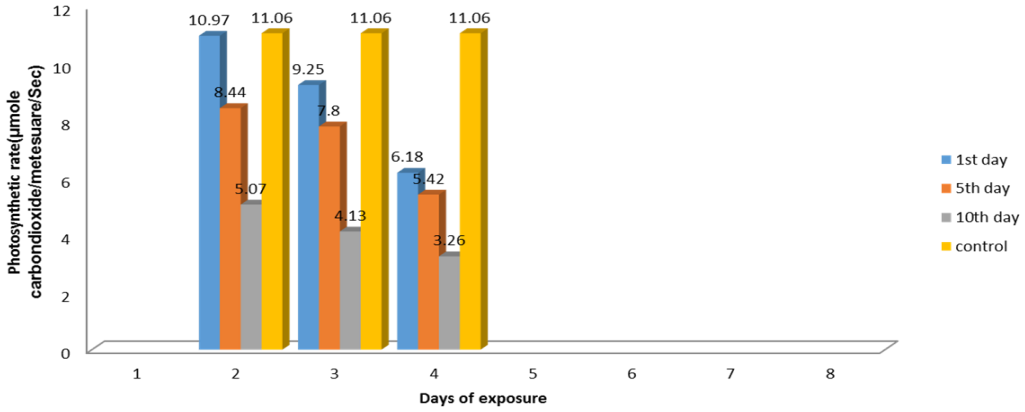



**Figure 3.3 Photosynthetic rate in *E. crassipes* at different concentrations of Pb for 10 days of exposure**

From the above table (**Table 3.2**) it is shown that the photosynthesis rate was reduced as the concentration of Pb increases and also the day's increases (**Figure 3.3**). The reduction in photosynthesis rate is due to Pb induced changes in

several gas exchange and water relations. Sharma and Dubey [39] reported that plants exposed to Pb ions showed a decline in the photosynthesis rate as a result of distorted chloroplast, restrained systhesis of chlorophyll, obstructed electron transport, inhibited of Calvin cycle enzymes as well as deficiency of $CO_2$ as a result of stomatal closing. The main site of heavy metal attack was water splitting system at the oxidixing site photo system II and add additional site of inhibition at the plastoquinone level. It was observed that the photosynthetic rate and the toxic effect depend on the applied concentration and the species of the plants [40, 41, 42].

### 3.3.3 Total Chlorophyll estimation

The total chlorophyll content of *E. crassipes* significantly decreased when the exposure time and Pb concentration were increased. The reduction was highest at 30.0 mg/L concentration with only 0.353 mg/g fresh weight at low pH on 10$^{th}$ day (**Figure 3.4**). Maximum decrease in chlorophyll content occurred at low pH and highest solution concentration (30.0mg/L). The higher reduction of chlorophyll content at higher initial concentration of the solution and low pH is obvious because of higher accumulation of Pb that takes place under these two factors.

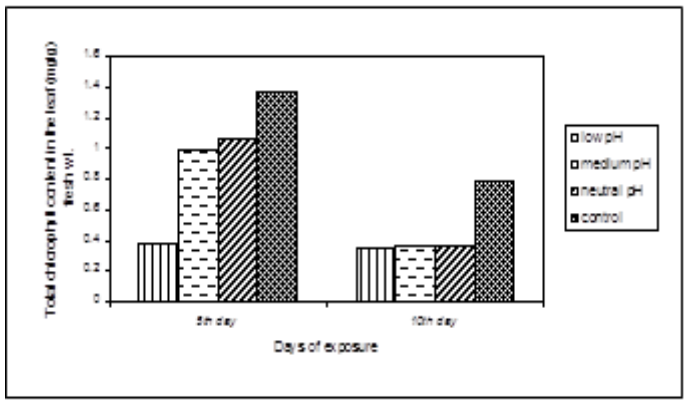



**Figure 3.4 Total chlorophyll content of *E. crassipes* at different pH with 30.0 mg/L initial conc. as observed after 5$^{th}$ and 10$^{th}$ day of exposure**

### 3.3.4 SEM- EDX analysis

SEM-EDX analysis was performed to localise Pb at tissue level in *E. crassipes* grown in $Pb(NO_3)_2$ solution in a hydroponic culture.

In this study, SEM allows examination of the topography of the root surface and internal accumulation of Pb and identification of any morphological

changes which might take place in root, shoot and leaf of the plant after metal exposure.

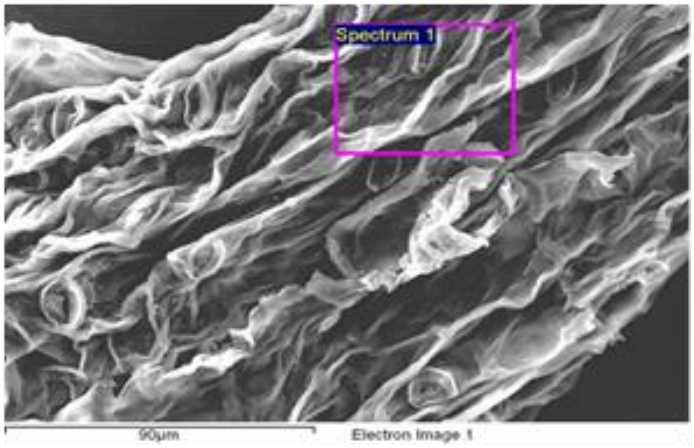
An EDX instrument attached to an SEM provided supplementary information. As the SEM electron beam strikes the sample surface X-rays are produced. An X-ray photon impinging on the surface of the EDX detector produces electron hole pairs which are detected as a single pulse by the liquid nitrogen cooled preamplifier. The pulse energy is determined by the X-ray energy which in turn is determined by the element being determined. The EDX analyser produces a spectrum of the elements present in targeted areas of the samples allowing detectable elements to be quantified or mapped.

SEM can be used to visualise the surface morphology of the plant root before and after metal binding, allowing for direct observation of any change. Raize et al. [19] analysed the effect of metal binding to *Sargassum vulgaris* using combined SEM-with EDX. SEM analysis revealed that there were significant morphological changes, including shrinking and layer sticking in the sea weed after metal binding. SEM-EDX analysis was performed to localise Pb at tissue level in plants grown in hydroponic culture plus $Pb(NO_3)_2$ 30mg/L.

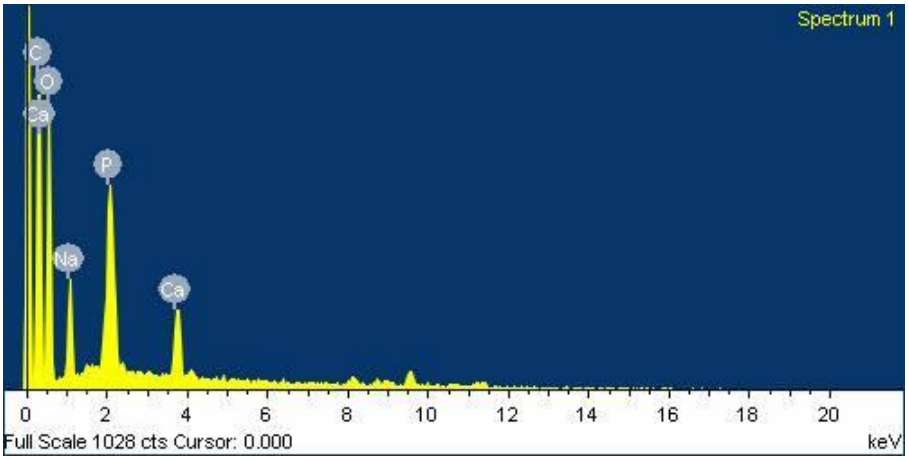
**Table 3.3 Percentage of elements in *E. crassipes* roots determined by EDX (wt %)**

| **Elements** | **Root part (Control)** | | **Root part (Pb treated)** | |
|---|---|---|---|---|
| Tissue | Cortex (External) | Vascular tissue (Internal) | (External) Cortex | Vascular (Internal) |
| C | 41.25 | 32.48 | 53.36 | 37.22 |
| O | 42.38 | 50.8 | 56.86 | 36.99 |
| Na | 5.62 | 7.83 | 2.77 | 13.21 |
| Mg | 1.56 | 2.43 | 1.27 | 4.49 |
| K | 0.04 | 0.14 | 0.04 | -- |
| Ca | 0.43 | 0.17 | 0.43 | 0.33 |
| Zn | 0.31 | 0.09 | 2.68 | 0.62 |
| Pb | -- | -- | 2.47 | 0.76 |

### 3.3.4.1 Distribution of Pb in leaves

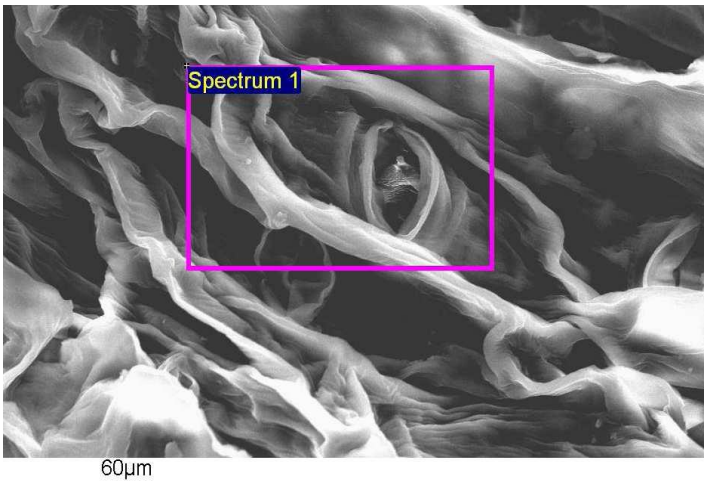



**(a)**

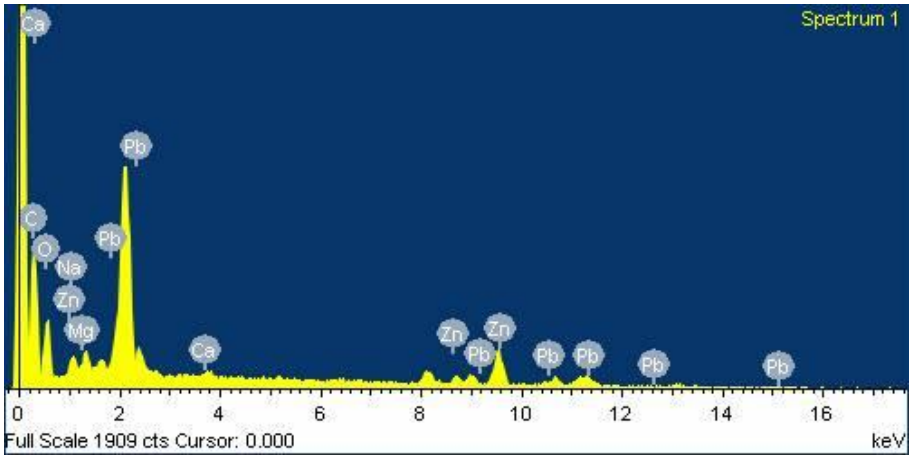



**(b)**

**Figure 3.5 (a) SEM image of *E.crassipes* leaves after 2nd days in control; (b) The EDX spectrum of cross-section of leaf of control of *E.crassipes***




**(c)**



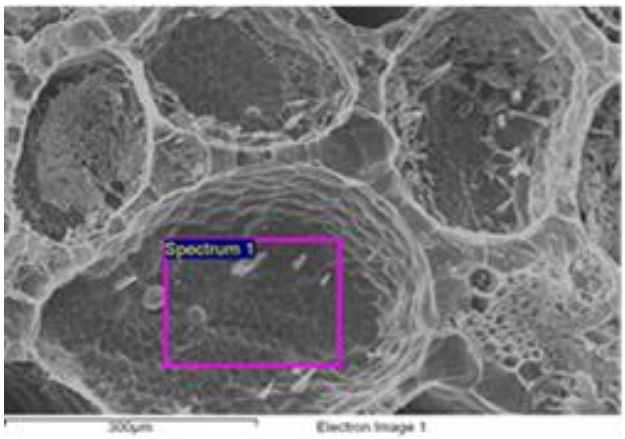

**(d)**

**Figure 3.5 (c) SEM images of longitudinal sections of *E. crassipes* leaf treated with Pb (30 mg/L); (d) EDX taken from the leaf of Pb exposed plants**

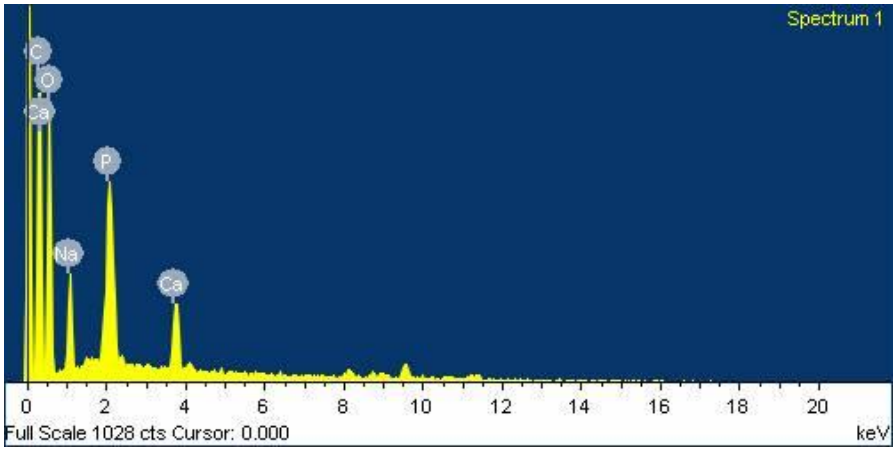
It reveals from the SEM-EDX micrograph of control plants that it did not show the presence of Pb (**Figure 3.5a, b**). However, in the treated plant, at an intensity of 20 kV, the characteristic peak of Pb was seen inside the leaf (**Figure 3.5c**, **d**). SEM observations found abundant inclusions in epidermal cells of leaves treated with Pb ions. Analyses by EDX confirmed that these inclusions contained Pb (**Figure 3.5 d**).

### 3.3.4.2 Characterizations of *Eichhornia crassipes* shoot biomass (before and after absorption of $Pb^{2+}$ ions) using SEM-EDX

The surface morphology of *E. crassipes* shoot biomass without and with removal of $Pb^{2+}$ ions during absorption process was observed with the help of SEM-EDX (JOEL model JSM-6480LV, Japan) and presented in **Figure 3.6**. It shows the morphological changes with respect to shape and size of the materials after absorption of $Pb^{2+}$ ions.

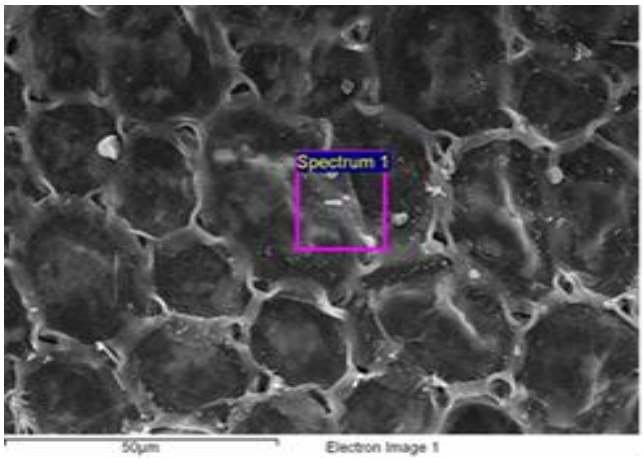



**(a)**

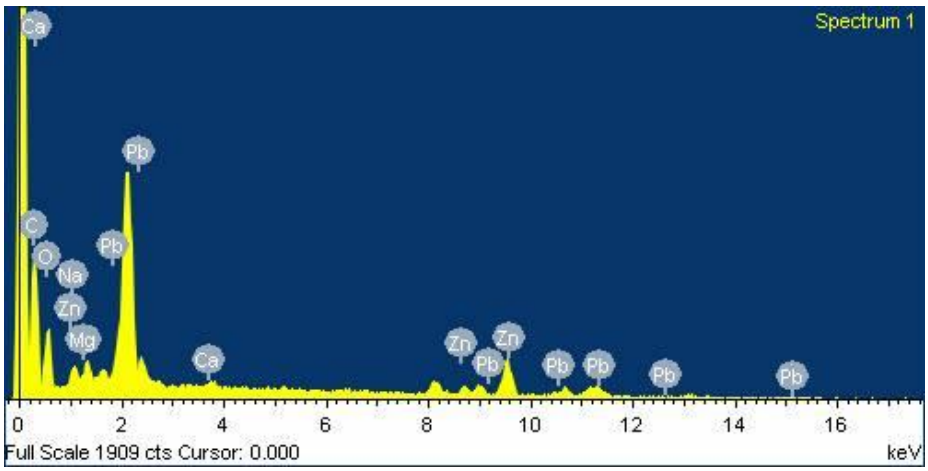



**(b)**

**Figure 3.6 (a, b) SEM-EDX images of *Eichhornia crassipes* shoot (petiole) biomass of control plant**




**(c)**



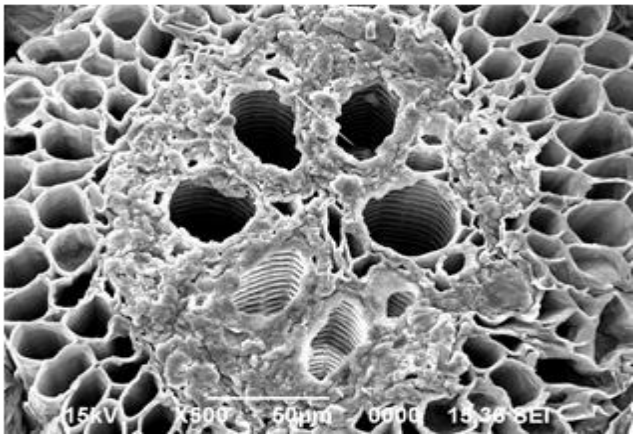

**(d)**

**Figure 3.6 (c, d) SEM-EDX images of *Eichhornia crassipes* petiole biomass treated with Pb ions**

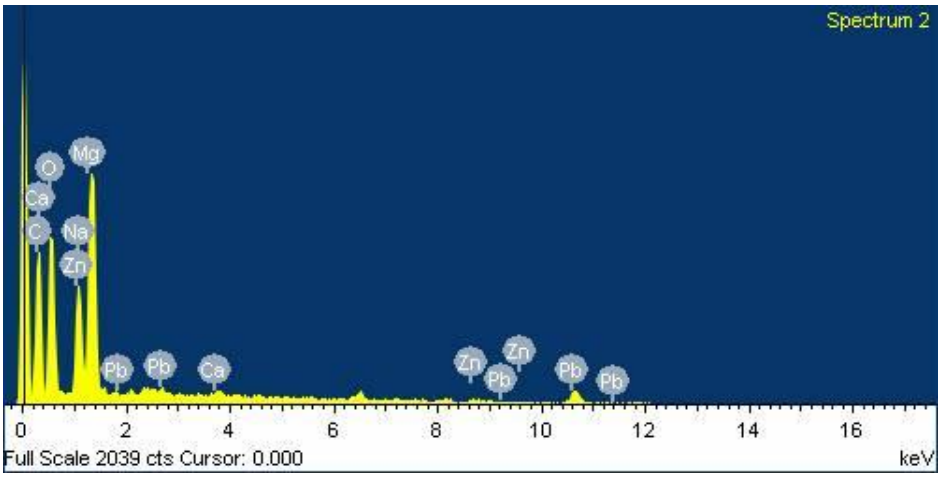
The SEM micrographs showed changes of the cells of the shoot samples (**Figure 3.6c, d**). Exposure to 30mg/L Pb for 10 days resulted in a loss of cell shape, decrease in the intercellular spaces, and shrinkage of vascular bundle in *E. crassipes*) (**Figure 3.6d**).

### 3.3.4.3 Characterization of *E. crassipes* root biomass (before and after absorption of Pb ions) using SEM-EDX

Exposure to 30mg/L Pb for 10 days resulted in a loss of cell shape, decreases in the intercellular spaces, and shrinkage of root vascular bundle in *E. crassipes* (**Figure 3.7a**, **b**) compared to the control (**Figure 3.7c**,**d**).

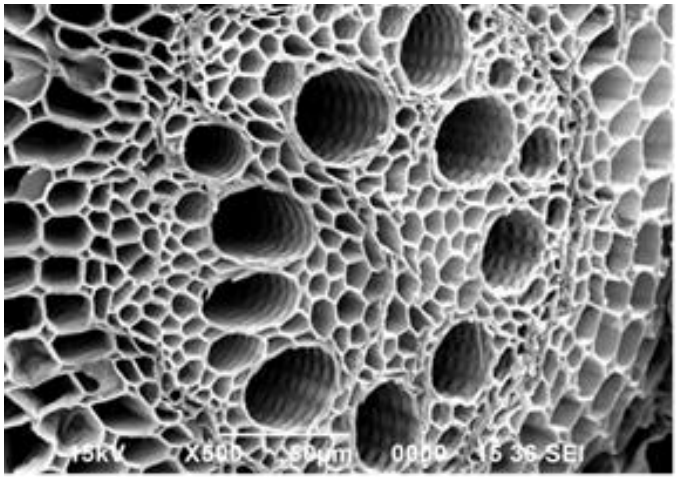


**(a)**

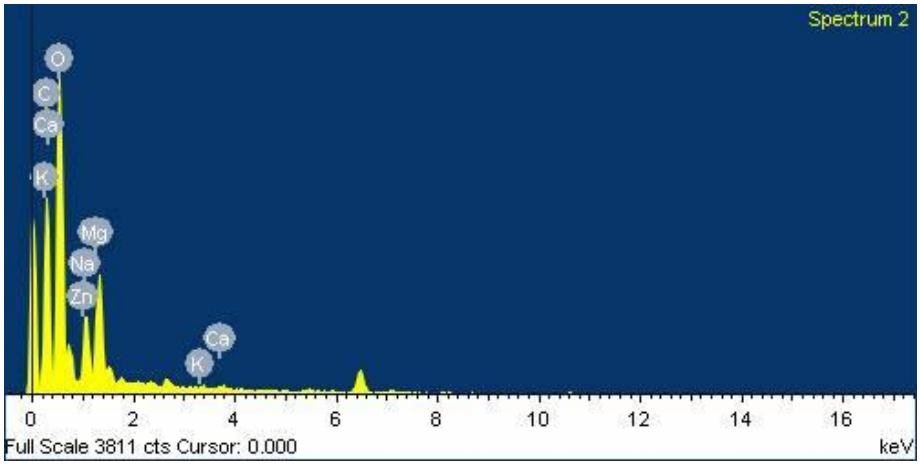



**(b)**

**Figure 3.7(a, b) The SEM micrographs showed changes of the vascular cells of the root samples exposed to 30mg/L Pb treatment; (b). EDX confirmed the presence of Pb**



**(c)**




**(d)**

**Figure 3.7(c, d) SEM-EDX micrograph of control root cross section of *E. crassipes***

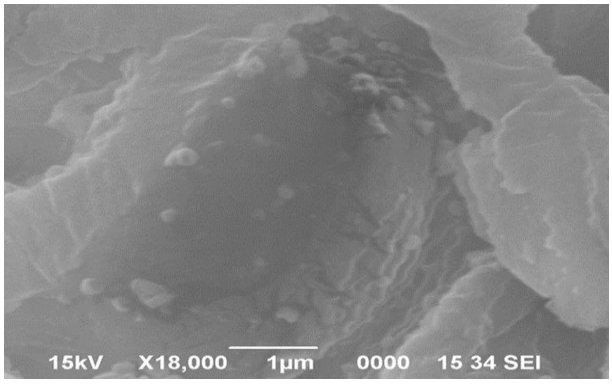
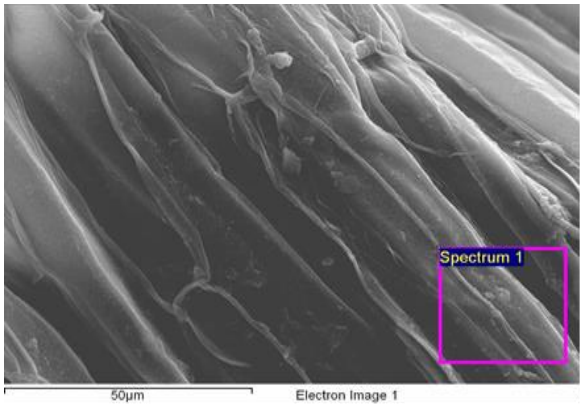


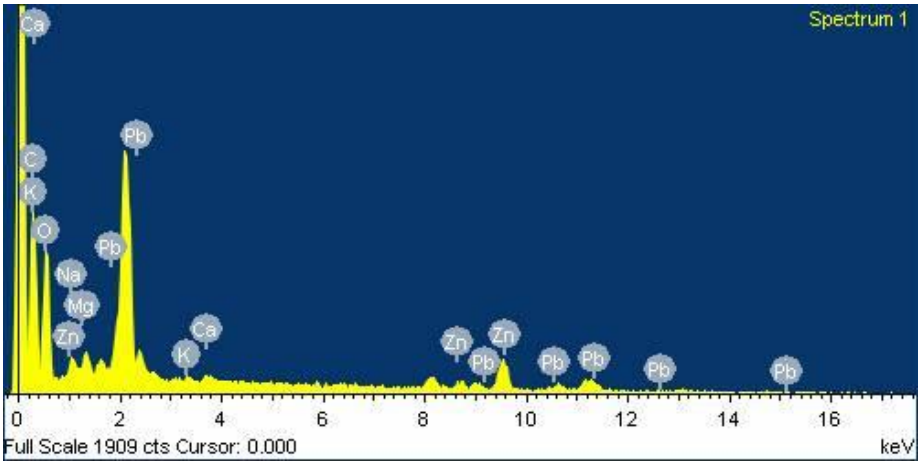

**Figure 3.8 Pb accumulation in vascular bundles of root of *E. crassipes***

The results of the study reveal that *E. crassipes* absorbed Pb from the aqueous solutions through the epidermal regions of the roots. Then the Pb are translocated from the epidermis into the vascular bundles and conducted their movement upwards to the plants. In shoot samples some inclusions were seen. In

these inclusions, a distinct signal and high atomic values for Pb were also noticed in Energy dispersive X-ray (EDX) analysis (**Figure 3.6 c, d**).

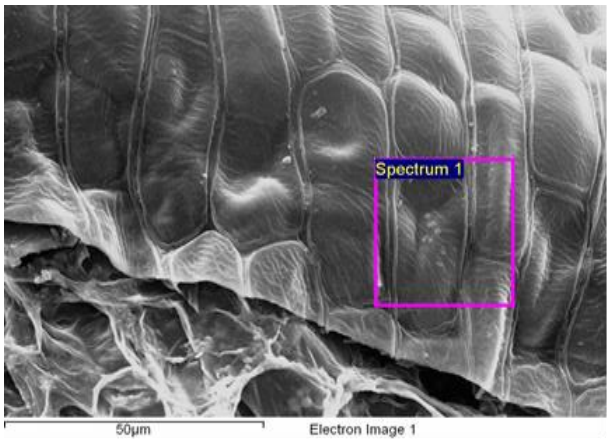



(**a**)

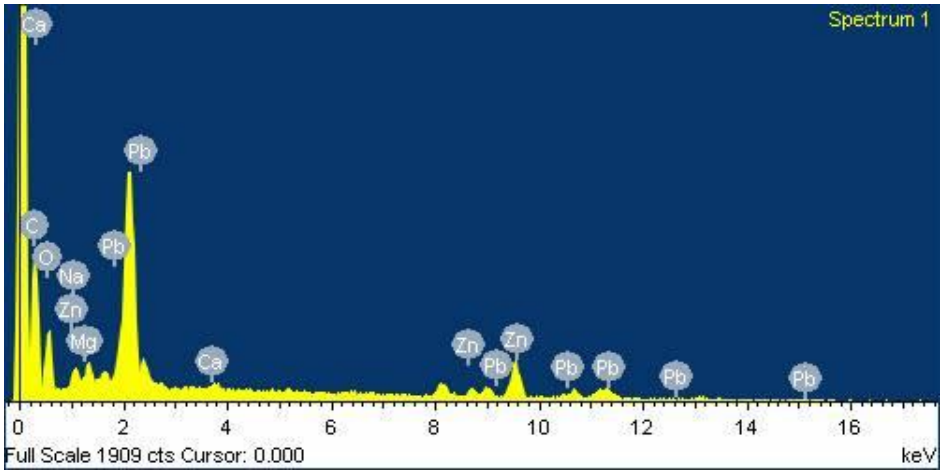



**(b)**

**Figure 3.9 Scanning electron micrograph and EDX spectra of Pb distribution in root of *E.crassipes*. Pb internalization was confirmed by SEM (a); studies in combination with EDX (b)**



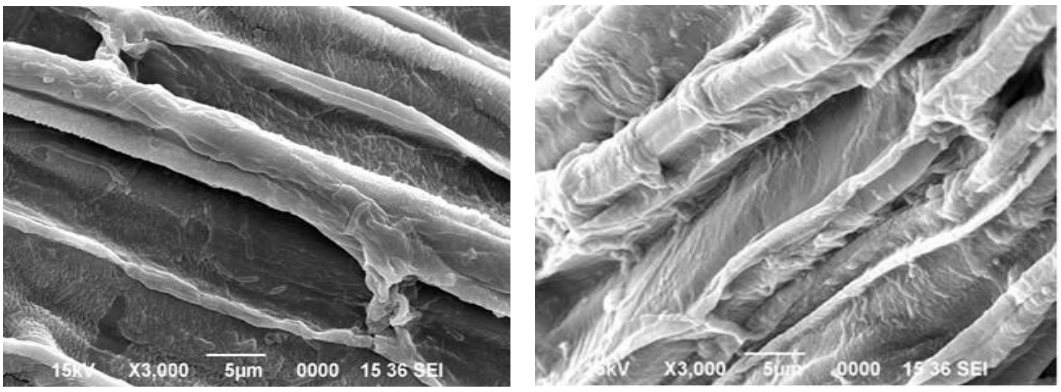

(**a**)




**(b)**

**Figure 3.10 SEM-EDX spectra of Pb distribution in epidermis of root of *E. crassipes***

Transversal analysis of root of *E. crassipes* demonstrated that Pb accumulated in a higher proportion on the root surface (epidermis), decreasing in concentration towards the centre (**Figure 3.10**).



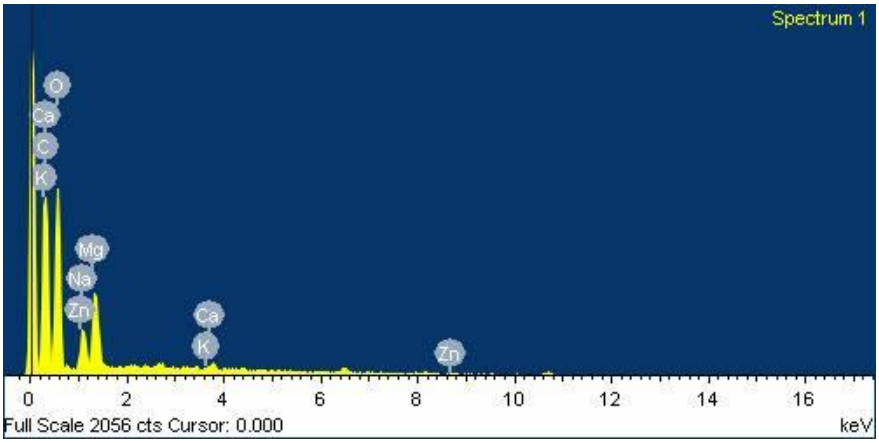

(**a**) (**b**)

**Figure 3.11 SEM micrograph of *E. crassipes* root (a) control; (b) Pb (II) loaded Magnification: 3000X**

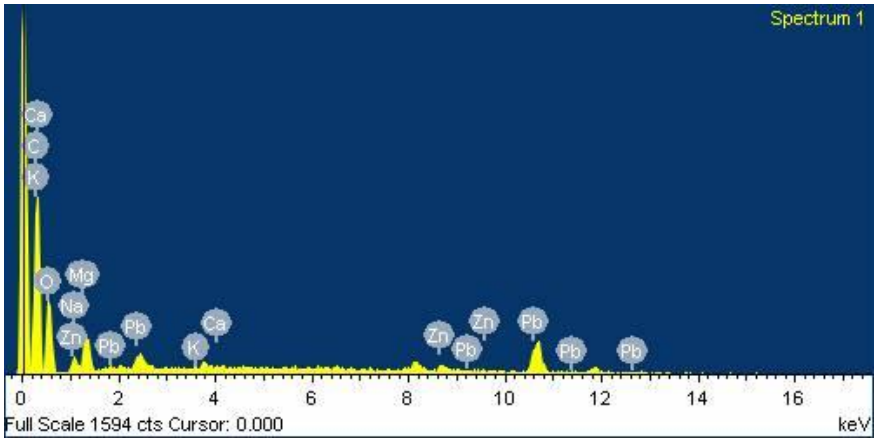
From the above figures it was seen that some morphological differences found between the control and metal loaded root surface. In both the cases, the control surface appears smoother than that the metal-loaded samples with some surface shrinking after metal binding also apparent.

The elemental composition of the root surfaces were simultaneously measured by EDX. The EDX spectra of the control (**c**) and metal-loaded root (**d**) surfaces are shown in the **Figure 3.11**.

Zhu et al. [43] also reported that the main route of heavy metal uptake in wetland plants was through the roots in case of emergent and surface-floating plants like water hyacinth and that much of the accumulation into the plant tissue is by absorption to the anionic sites in the cell walls. This explains why wetland plants can have very high magnitude of heavy metal concentration in their tissues compared to their surrounding environment.




**(c)**




**(d)**

**Figure 3.11 EDX micrograph of *E. crassipes* root (c) control; (d) Pb (II) loaded Magnification: 3000X**

SEM and EDX are useful tools for evaluating the elemental characteristics of the adsorbents and these two techniques have been widely used in heavy metals adsorption studies especially in determining the adsorption mechanism [44, 45, 46, 47]. The SEM images and EDX spectra for control and after lead adsorption are shown in **Figure 3.11(a, b, c, d).** The SEM images (at 500× magnification) show the rough surface and porosity of control, conditions which might favour the

adsorption of Pb (II) ions. The EDX spectrum for before adsorption (**Figure 3.3.11c, d**) showed some peaks for inorganic (Na, K, Ca, Mg and Mn) species. The presence of K and Mn on control plant surface could originate from $KMnO_4$ solution used during treatment.

## 3.4 Conclusion

The present study shows the *E. crassipes* have the maximum efficiency of accumulation of accumulation up to 2$^{nd}$ day of its application for all initial concentration and pH. *E. crassipes* can accumulate high concentrations of toxic metals and has a great potential of removing pollutants, including toxic metals, from wastewater. We are interested in the capacity of the water hyacinth to remediate aquatic environments that have been contaminated by the heavy metal Pb as they grow easily, propagate readily and their large biomass facilitates handling and tissue manipulations.

The declined rate of photosynthesis in *E. crassipes* with increased Pb concentrations suggests the accumulation of Pb within the leaves. Histochemical studies using scanning electron microscopy (SEM) reveals the ultimate adsorption nature of Pb. The higher concentration of metal in the aquatic weed signifies the biomagnification that lead to filtration of metallic ions from polluted water. The impact of bio-filtration of metals by using a weed is not only a sustainable technique but it is also cost effective with no maintenance.

The EDX spectra showed that Pb was detected inside the roots, petioles and leaves of *E.crassipes*, indicating that this macrophyte can absorb and transport Pb inside the roots, which represent an important mechanism of *E. crassipes* in the accumulation of the metal. The examination of root cross-section pointed out the upper epidermis is the main site for lead accumulation. *E. crassipes* can be used as low cost treatment material for the removal of Pb.

The metals accumulation in *Eichhornia crassipes* increases linearly with the solution concentration in the order of leaves < stems < roots [48, 49, 50]. It can be proposed that the roots reached saturation during the period and there exists some mechanism in roots that could detoxify heavy metals or transfer them to aerial parts.

## References


1. Ferner, D.J., et al. Toxicity, heavy metala, *eMed. J*. **2**(5), 1, 2001.
2. Sharma, P., & Dubey, R.S. Lead toxicity in plants, *Braz.J.Plant Physiol*. **17**, 35--52, 2005.
3. Mishra, S., et al. Lead detoxification by coontail (*Ceratophylum demersum* L.) involves indication of phytochelatins and antioxidant system in response to its accumulation, *Chemosphere* **65**(6), 1027--1039, 2006.
4. Mishra, V.K., & Tripath, B.D. Accumulation of chromium and zinc from aqueous solutions using water hyacinth (*Eichhornia crassipes*), *J.Hazard.Mater*. **164**, 1059--1063, 2009.
5. Dhir, B., et al. Physiological and antioxidant responses of *Salvinia natans* exposed to chromium-rich wastewater, *Ecotoxicol. Environ. Saf*. **72**, 1790--1797, 2009.
6. Zhang, S., et al. A newly found cadmium accumulator - *Malva sinensis* Cavan, **173,** 705--709, 2010.
7. Zhu, Y.Q., Li, Y., & Chen, J.J. Accumulation of Pb, Cd, Cu and Zn in plants and hyperaccmulator choice in lanping lead-zinc mine area, China, *Environ. Int*. **30**, 567--576, 2004.
8. McGrath, S.P., & Zhao, F.J. Phytoremediation of metals and metalloids from contaminated soils, *Curr. Opin. Biotechnol*. **14**, 277--282, 2003.
9. Maine, M.A., et al. Removal efficiency of a constructed wetland for wastewater treatment according to vegetation dominance, *Chemosphere* **68**, 105--111, 2007.
10. Gupta, M., & Chandra, P. Bioaccumulation and toxicity of mercury in rooted submerged macrophyte *Vallisneria spiralis*, *Environ Pollut.* **103**, 327--32, 1998.
11. Tokunaga, T., Furuta, N., & Morimoto, M. Accumulation of cadmium in *Eichhornia crassipes*, *J. Hyg. Chem*. **22**, 234--239, 1976.
12. Xiaomel, L., et al. Removal of Cadmium and Zinc by Water hyacinth, *Eichhornia crassipes*, *Science Asia* **30**, 93--103, 2004.
13. Jana, S. Accumulation of Hg and Cr by the three aquatic species and subsequent changes in several physiological and biochemical plant parameters, *Water Air Soil pollut* **38**, 105--109, 1988.
14. Fernando, D.R., et al. Manganese accumulation in the leaf mesophyll of four tree species: a PIXE/ EDXA localization study, *New Phytol*. **171**, 751--758, 2006.

15. Turnau, K., et al. Metal uptake and detoxification mechanisms in *Erica andevalensis* growing in a pyrite mine tailing, *Environ*. *Exp*. *Bot*. **61**, 17--23, 2007.
16. Solis-Domingues, F.A., et al. Accumulation and localization of cadmium in *Eichnochola polystachya* grown within a hydroponic system, *J*. *Hazard*. *Mater*. **141**, 630--636. 2007.
17. Chen, J., et al. Bioaccumulation and physiological effect of mercury in *Pteris vittata* and *Nephrolepis exaltata, Ecotoxicology* **18**, 110--121, 2009.
18. Figueira, M.M., Volesky, B., & Mathieu, H. J. Instrumental Analysis Study of Iron Species Biosorption by *Sargassum* Biomass, *Environ. Sci. Technol*. **33**, 1840--1846, 1999.
19. Raize, O., Argaman, Y. & Yannai, S. Mechanisms of biosorption of different heavy metals by brown marine macroalgae, *Biotechnol. Bioeng*. **87**, 451-- 458, 2004.
20. Ahalya, N., Ramachandra, T.V. & Kanamadi, R.D. Biosorption of Heavy Metals, *Res. J. Chem. Environ*. **7**, 4, 2003.
21. Shaikh, P. R., & Bhosle, A. B. Bioaccumulation of Chromium by Aquatic Macrophytes *Hydrilla sp. & Chara sp, Adv. Appl. Sci. Res.* **2**(1), 214--220, 2011.
22. Nwabanne, T. J., & Igbokwe, K. P. Copper (II) Uptake by Adsorption Using Palmyra Palm Nut, *Adv. Appl. Sci. Res.* **2** (6), 166--175, 2011.
23. Paris, C., et al. Efficiencia de dos macrofitas flotantes libres en la absoreion de metales pesados, *Limnetica* **23**(3-4), 237--244, 2005.
24. Hadad, H., Maine, M.A., & Bonetto, C., Macrophyte growth in a pilot-scale constructed wetland for industrial wastewater treatment, *Chemosphere* **63**(10), 1744--1753, 2006.
25. Mufarrege, M.M., Hadad, H.R., & Maine, M.A. Response of *Pistia stratiotes* to Heavy Metals (Cr, Ni and Zn) and Phosphorous, *Arch*. *Environ*. *Contam*. *Toxicol*. DOI 10.1007/s00244-009-9350-7.
26. Maine, M.A., et al. Cadmium and chromium removal kinetics from solution by two aquatic macrophytes, *J. Environ. Poll.* **145**, 467--473, 2007.
27. Sinha, S.K., Srivastava, H.S., & Tripathi, T.D. Influence of some growth regulators and divalent cations on the inhibition of nitrate reductase activity by lead in maize leaves, *Chemosphere*, **29**, 1775--1782, 1994.
28. Schneider, I.A.H., et al. Biosorption of heavy metals onto plant biomass: exchange adsorption or surface precipitation? *Int. J. Mineral Process*, **62**, 111—120, 2001.

29. Salinas, E., et al. Removal of cadmium and lead from dilute aqueous solutions by *Rhodotorula rubra*, *Bioresour. Technol*, **72**(2), 107--112, 2000.
30. Sheng, P. X., et al. Sorption of lead, copper, cadmium, zinc and nickel by marine algal biomass: Characterization of biosorptive capacity and investigations of mechanisms, *J. Colloid. Interface Sci*, **275**(1), 131--141, 2004b.
31. Mohanty, K., et al. Biosorption of Cr(VI) from aqueous solutions by *Eichhornia crassipes*, *Chem. Eng J*, 117(1), 71--77, 2006.
32. Luo, F., et al. Biosorption of lead ion by chemically- modified biomass of marine brown algae *Laminaria japonica*, *Chemosphere*, **64**(7), 1122--1127, 2006.
33. Hoagland, D. R., & Snyder, W.C. Nutrition of strawberry plant under controlled conditions: (a) effects of deficiencies of boron and certain other elements: (b) susceptibility to injury from sodium salts. Proceedings of the American Society for Horticultural Sciene 30, 288–294, 1993.
34. Xiong, Z.T., et al. Nutrition influence on copper accumulation by *Brassica pekinensis* Rupr, *Ecotoxicol. Environ. Saf*. **53**, 200--205, 2002.
35. Sasmaz, A., et al. The accumulation of heavy metals in *Typha latifolia* L. grown in a stream carrying secondary effluent, *Ecol. Eng*. **33**, 278--284, 2008.
36. MacFarlane, G. R., & Burchett, M. D. Cellular distribution of Cu, Pb and Zn in the Grey Mangrove *Avicennia marina* (Forsk.) Vierh, Aquat. Bot. **68**, 45--59, 2000.
37. Sandalio, L. M., et al. Cadmium-induced changes in the growth and oxidative metabolism of pea plants, *J. Exp. Bot.* **52**, 2115--2126, 2001.
38. Kumar, P., et al. Phytoextraction: The use of plants to remove heavy metals from soil, *Environ. Sci. Technol*. **29**, 1232--1238, 1995.
39. Sharma, P., & Dubey, R. S. Lead toxicity in plants, *Braz. J. Plant Physiol*. **17**, 35--52, 2005.
40. Costa, G., & Morel, J. L. Water relations gas exchange and amino acid content in Cd treated lettuca, *Plant Physiol. Biochem*. **32**, 561--570, 1994.
41. Vassilev, A., et al. Effect of cadmium stress on growth and photosynthesis of young barley (*H. vulgare* L.) plants. 2. Structural and functional changes in photosynthetic apparatus, *Bulg. J. Plant Physiol*. **21**(4), 12--21, 1995.
42. Lang, F., Sarvari, E., Szigeti, Z., Fodor, F., Cseh., E. Effects of heavy metals on the photosynthetic apparatus in cucumber. In: Photosynthesis: from Light to Biosphere, IV, Kluwer Acad. Publishers, 1995, 533–536.

43. Zhu, Y. L, et al. Phytoremediation of trace elements by wetland plants: II. Water hyacinth, *J. Environ. Qual.* **28**, 339--344, 1999.
44. Akar, T., & Tunali, S. Biosorption performance of *Botrytis cinerea* fungal by-products for removal of Cd(II) and Cu(II) ions from aqueous solutions, *Miner. Eng*. **18**, 1099--1109, 2005.
45. Tunali, S. Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil, *Chem. Eng. J*. **115**, 203--211, 2006.
46. Panda, G. C., et al. Adsorption of nickel on husk of *Lathyrus sativus*: Behavior and binding mechanism, *Colloids Surf. B Biointerfaces* **57**, 135--142, 2007.
47. Pino, G. H., et al. Biosorption of cadmium by green coconut shell powder, *Miner. Eng.* **19**, 380--387, 2006.
48. Maine, M. A., et al. Chromium bioaccumulation: comparison of the capacity of two floating aquatic macrophytes, *Water Res*. **38**, 1494--1501, 2004.
49. Qian, J. H., et al. Phytoaccumulation of trace elements by wetland plants: III. Uptake and accumulation of ten trace elements by twelve plant species, *J. Environ. Qual.* **28**, 1448--1455, 1999.
50. Keith, C., Borazjani, H. S., Diehl, V., Su2, Y., & Baldwin, B.S. Removal of Copper, Chromium, and Arsenic by Water Hyacinths, Forest Products Department, Forest and Wildlife Research Center, Mississippi State University, MS., 2006.