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Uptake and localization of Lead in *Eichhornia crassipes* grown within a hydroponic system

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ABSTRACT

Lead (Pb) uptake, localization within and around root, petiole and leaf of Eichhornia crassipes grown hydroponically in a Pb $(NO_3)_2$ solutions were studied by Scanning electron microscopy coupled with elemental X-ray analysis (SEM-EDX) at different increasing concentrations of Pb. Analysis by scanning electron microscopy coupled with the elemental X-ray analysis (SEM-EDX) showed upward Pb transport by root vascular tissues to leaf. Important changes in surface morphology of root and metal binding mechanism were also studied by using SEM/EDX. The photosynthetic rate of Eichhornia crassipes sharply decreased when grown in aquatic medium containing Pb.

Keywords: *Eichhornia crassipes*, photosynthetic rate, SEM-EDX.

INTRODUCTION

Lead (Pb) is non-essential heavy metal to biota, potentially toxic to living beings and the environment [1]. 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 [2] 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, environment friendly, cost effective and both living and nonliving biomass are used [3-5]. 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 [6]. Lead is often found in high concentrations in aquatic plants, particularly those growing in freshwaters and receiving mine or other industrial waste. 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 [7]. *Hydrilla sp*. and *Chara sp*. can be used as bioabsorbant material for removal of chromium [8]. Activated carbon prepared from Palmyra palm nut has also been reported to be used for the removal of copper (II) from aqueous solution [9].

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 [2]. 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 lead (Pb) in *E. crassipes* grown in hydroponic lead-rich solution.

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 seaweed surface [10]. Raize et al., [11] also 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.

SEM/EDX analysis is used in this present work to establish changes in morphology and elemental composition of the plants root surface with a view to establishing a mechanism of metal binding.

MATERIALS AND METHODS

Experimental setup

Eichhornia crassipes 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 hydroponically for 10 days in modified Hoagland's nutrient solution.

Heavy metal preparation

All experimental work was done using deionised 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 deionised 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 30.0mg/L in nine plastic tubs of five litre capacity. A plant control, i.e. plant grown in tap water and metal control, metal solution without any plants were also established.

Photosynthetic rate

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

Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) studies

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 [12]. 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.

RESULTS AND DISCUSSION

Effect on Photosynthetic rate

Eichhornia crassipes grown in aquatic medium containing Pb $(NO_3)_2$ adversely affect the process and showed a decline in photosynthetic rate. The photosynthetic rate of *Eichhornia crassipes* sharply declined when grown in aquatic medium containing Pb (Fig1).

Analysis of SEM with an EDX

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 can be attached to an SEM to provide 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. [11] 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.

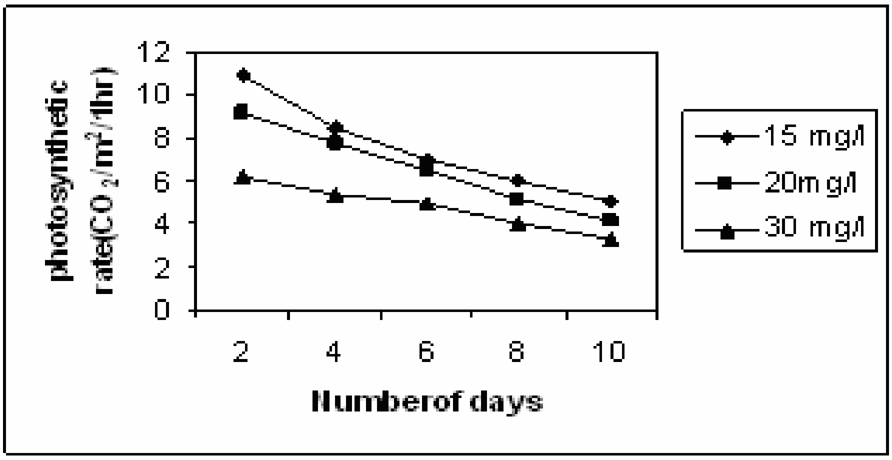
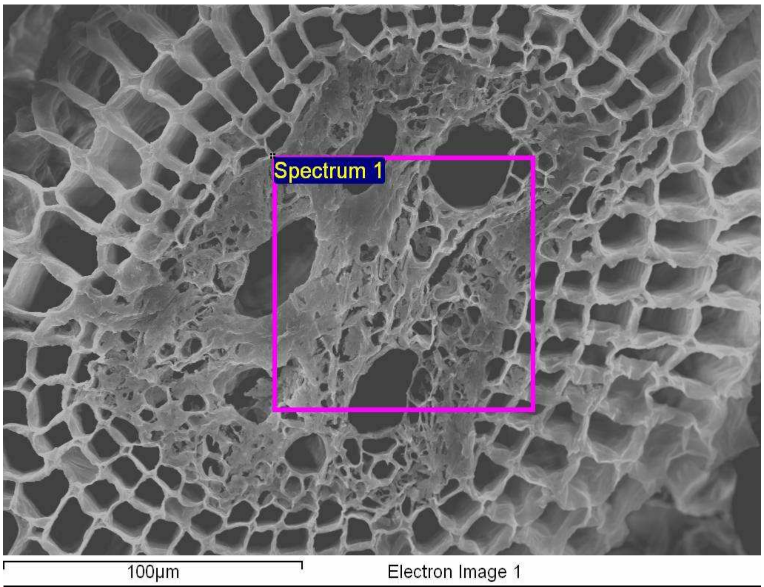


Fig 1:Photosynthetic rate in *E. crassipes* at different concentrations of Pb for 10 days of exposure.



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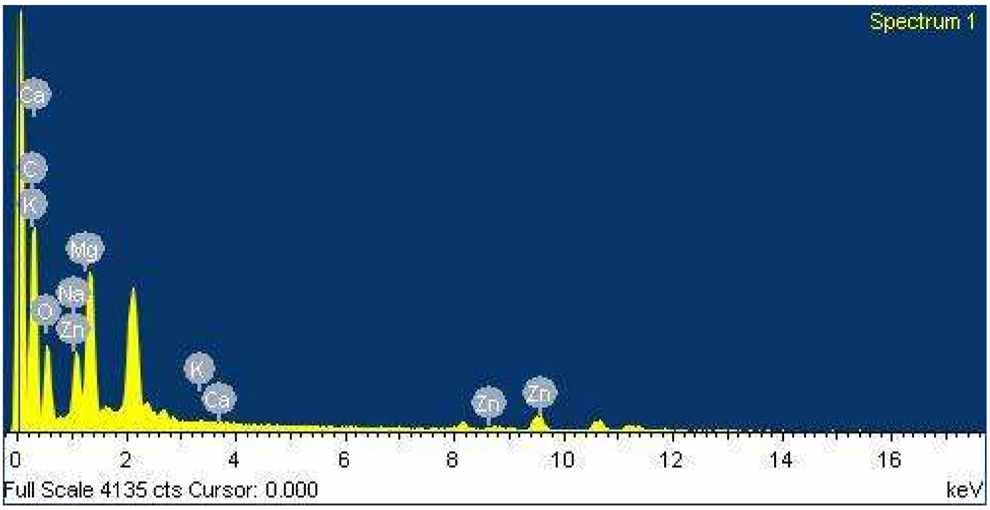
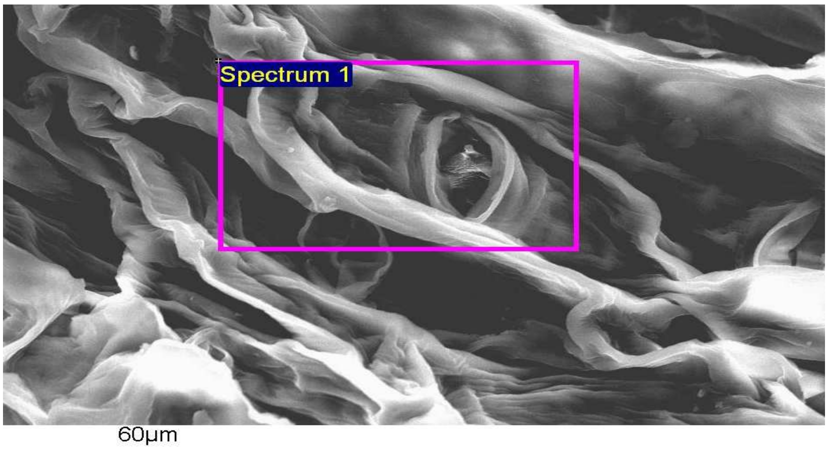


(b)

Fig 2: (a) SEM image of *E.crassipes* root after 2nd days in control. (b) EDX spectra of cross-section of root of control of *E.crassipes*.



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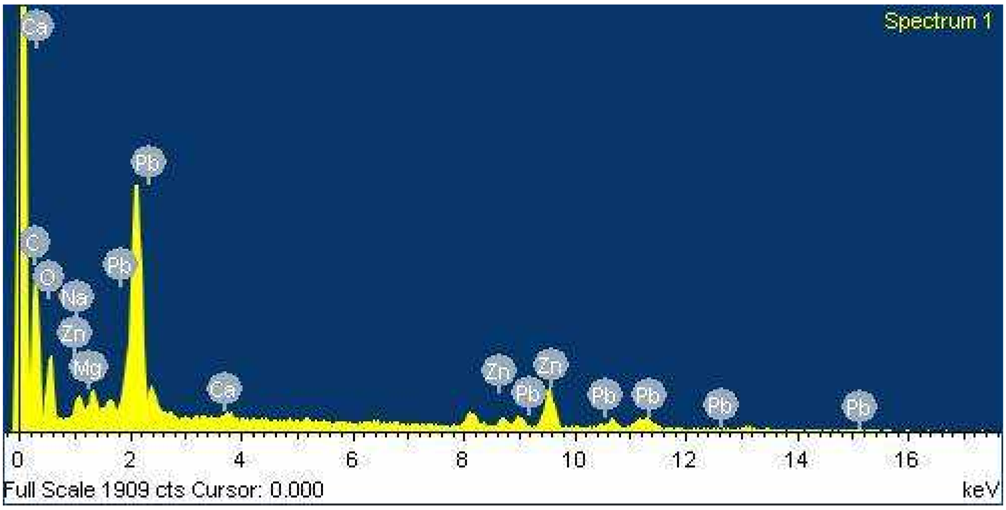
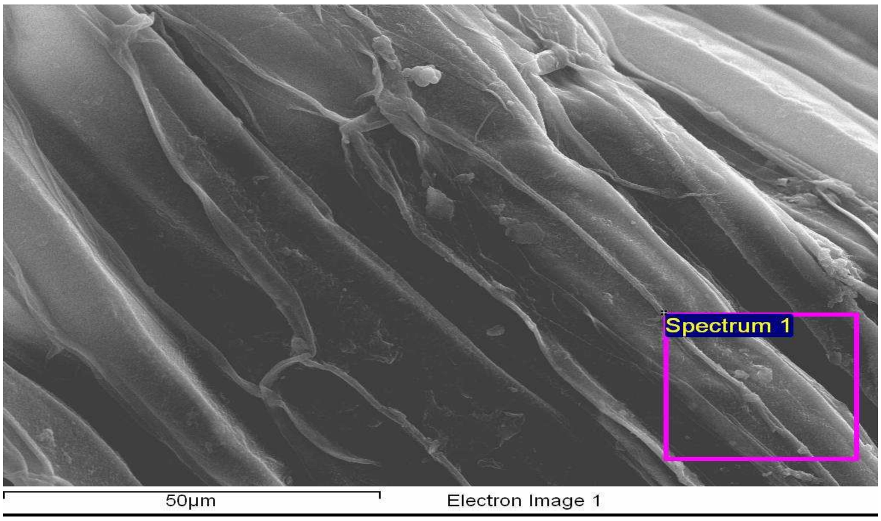


(b)

Fig 3: (a) SEM images of longitudinal sections of *E. crassipies* leaf treated with Pb (30 mg/l).(b) EDX taken from the leaf of Pb exposed plants.

In order to confirm the internalization of Pb by the *E.crassipes* X-ray microanalysis, roots of *E.crasspes* plant not exposed to Pb $(NO_3)_2$ solutions did not show the presence of Pb (Fig2). It reveals from the SEM-EDX micrograph of control plants that it did not show the presence of Pb. SEM-EDX analysis was performed to localise Pb at tissue level in plants grown in hydroponic culture plus $Pb(NO_3)_2$ 30mg/l. However, in the treated plant, at an intensity of 20 kV, the characteristic peak of lead was seen inside the leaf (Fig 3) and root (Fig 4) indicating that this macrophyte can absorb and transport heavy metals inside the root. 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 (Fig 5).



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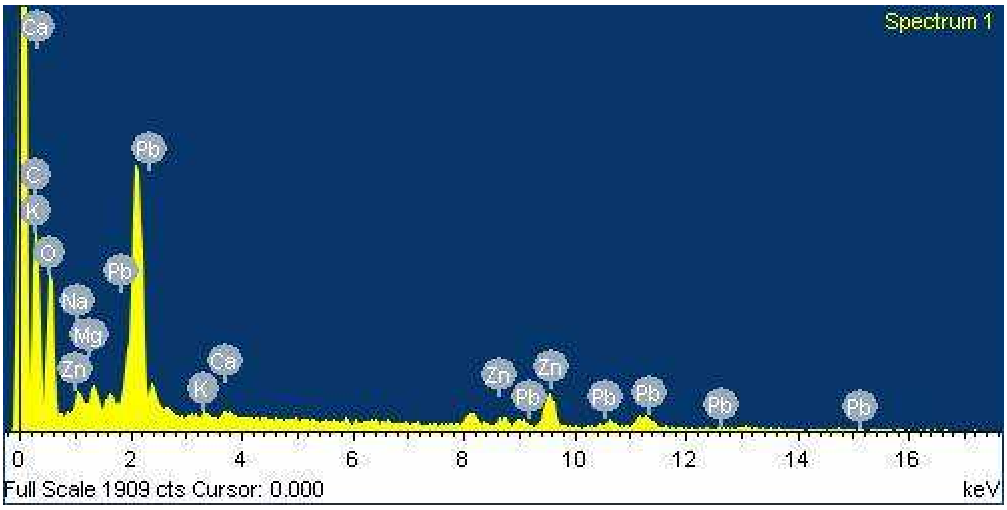
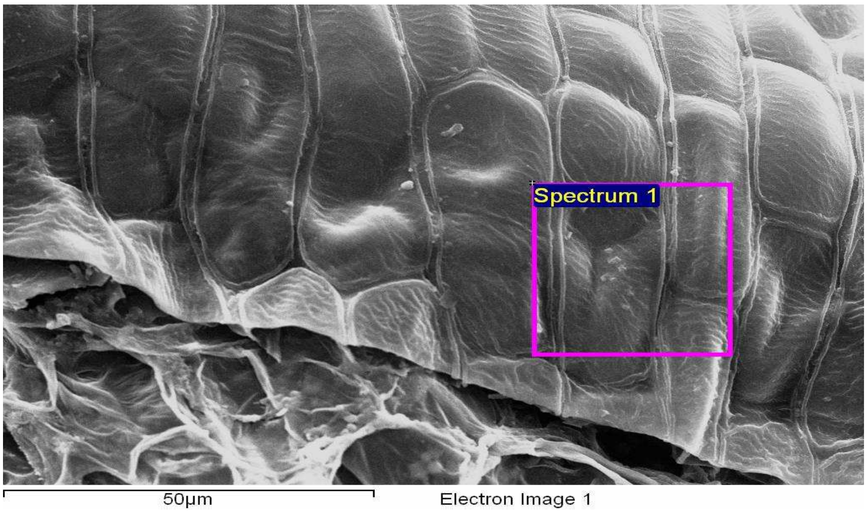


(b)

Fig 4: 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).



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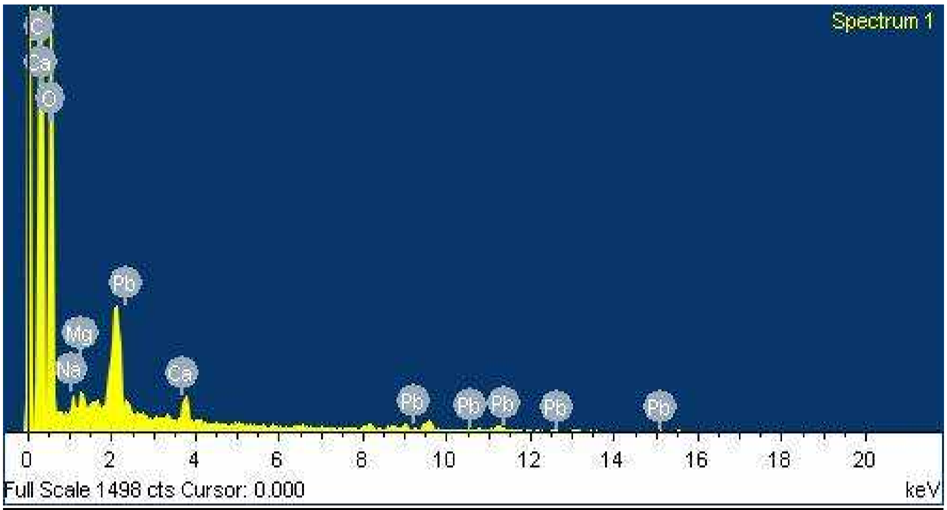
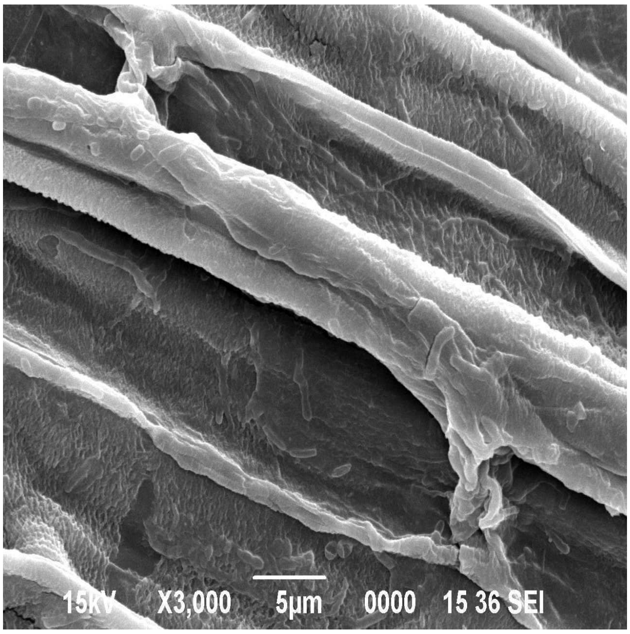
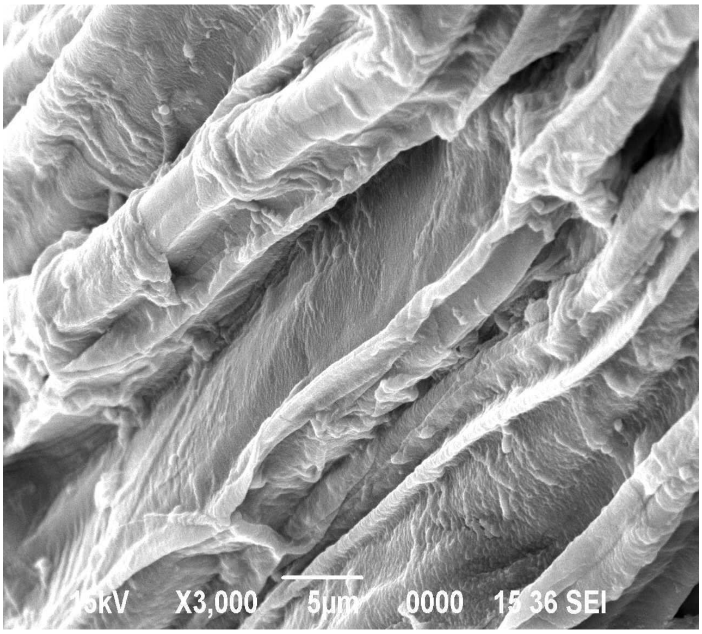


(b)

Fig 5: SEM-EDX spectra of Pb distribution in epidermis of root of *E. crassipes*.

The SEM micrograph obtained for control (without metal) (a) and metal loaded *E. crassipes* root (b) are shown in the Fig 6.

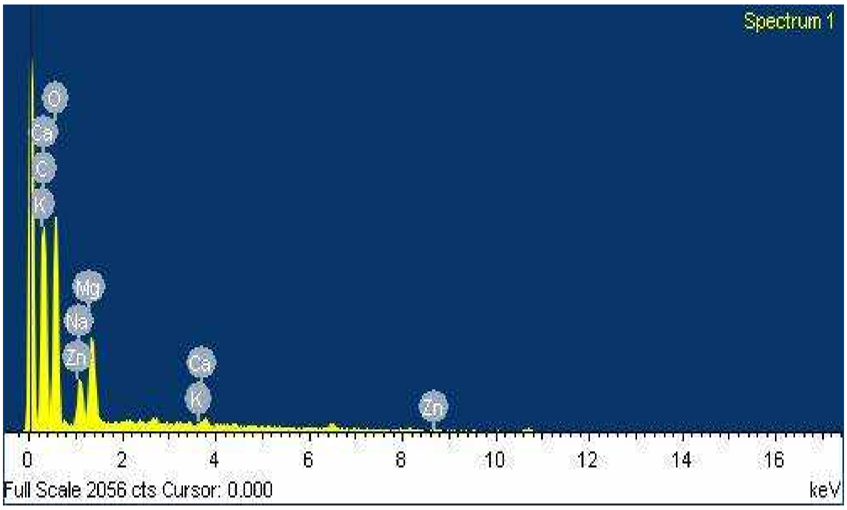




(a) **(b)**

Fig 6: SEM micrograph of *E. crassipes* root (a) control, (b) Pb (II) loaded. Magnification: 3000X.

From Fig 6 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 (a) and metal-loaded root(b) surfaces are shown in the Fig 7. Exposure to 30mg/l lead for 10 days resulted in a loss of cell shape, decreases in the intercellular spaces, and shrinkage of root vascular bundle in *E. crassipes* (Fig 9) compared to the control (Fig 8).



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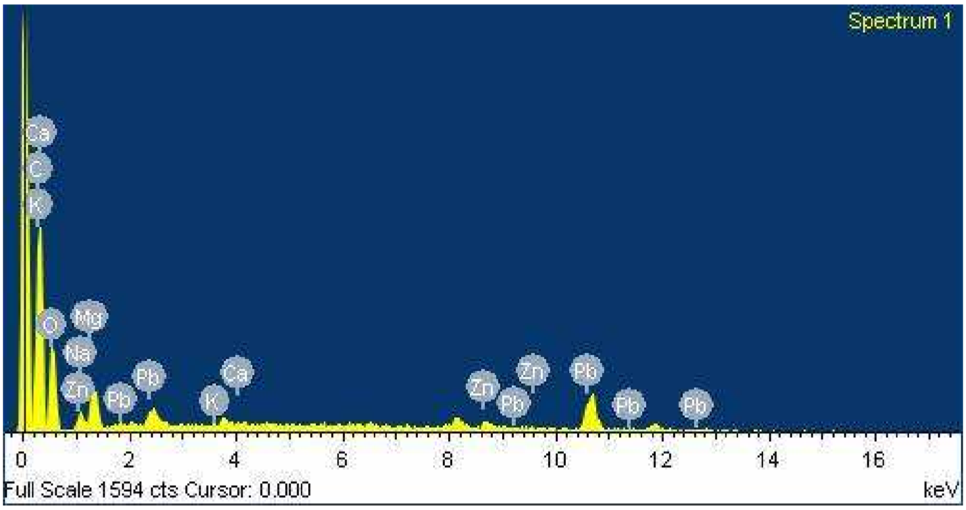
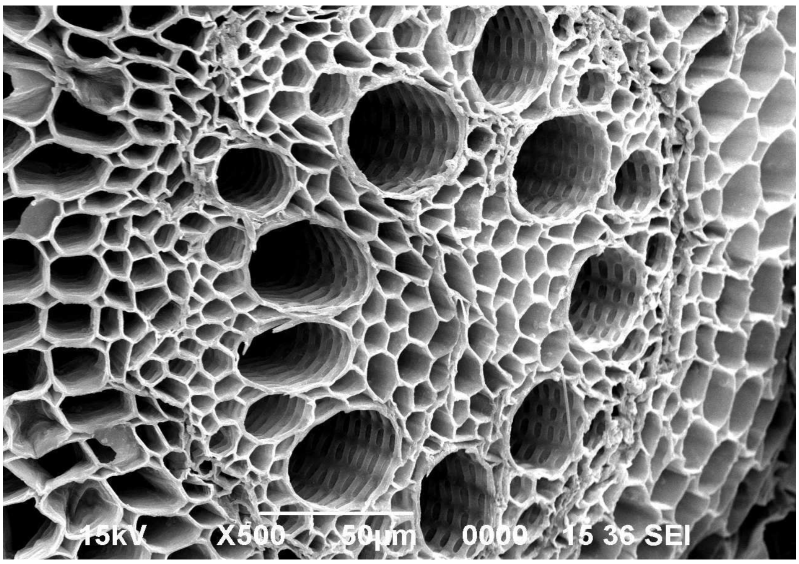


(b)

Fig 7: EDX spectra of (a) control and (b) metal loaded root surface of *E. crassipes*. Magnification: 3000X.



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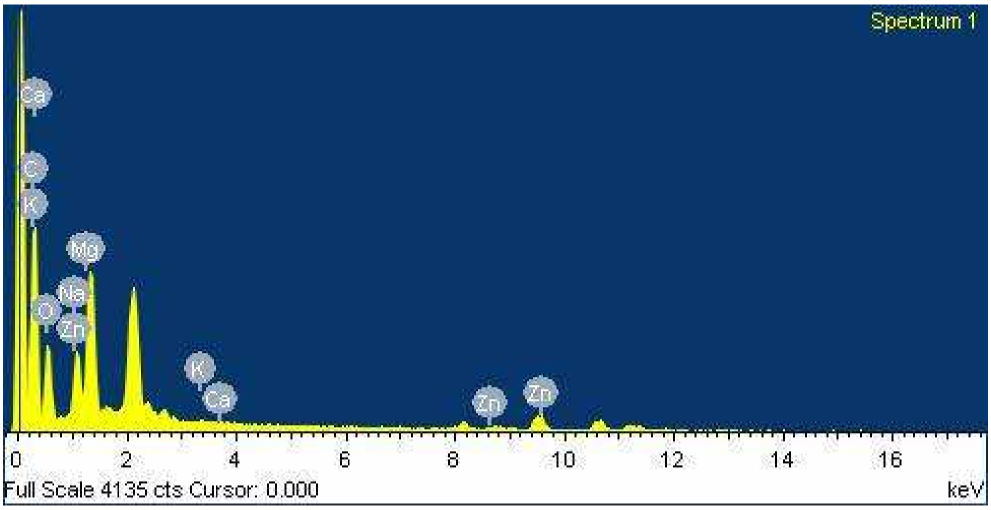

(b)

Fig 8: (a-b) SEM micrograph of control root cross section of *E. crassipes*

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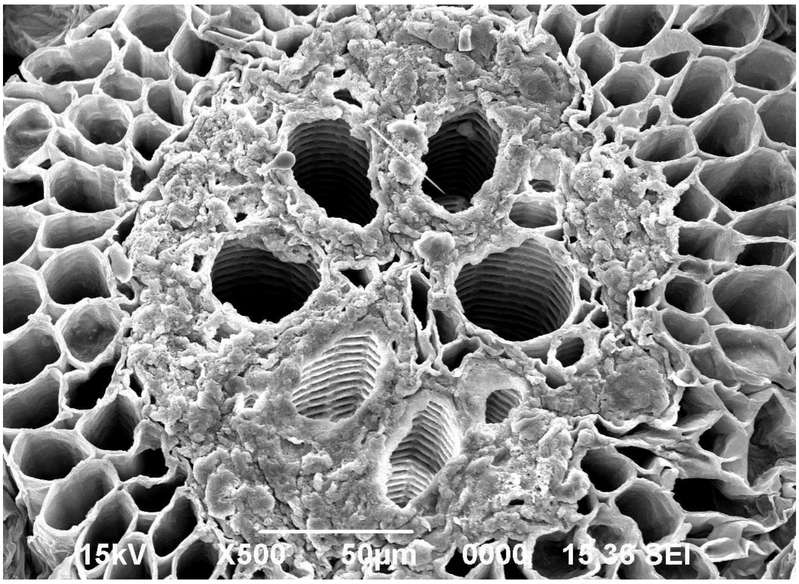

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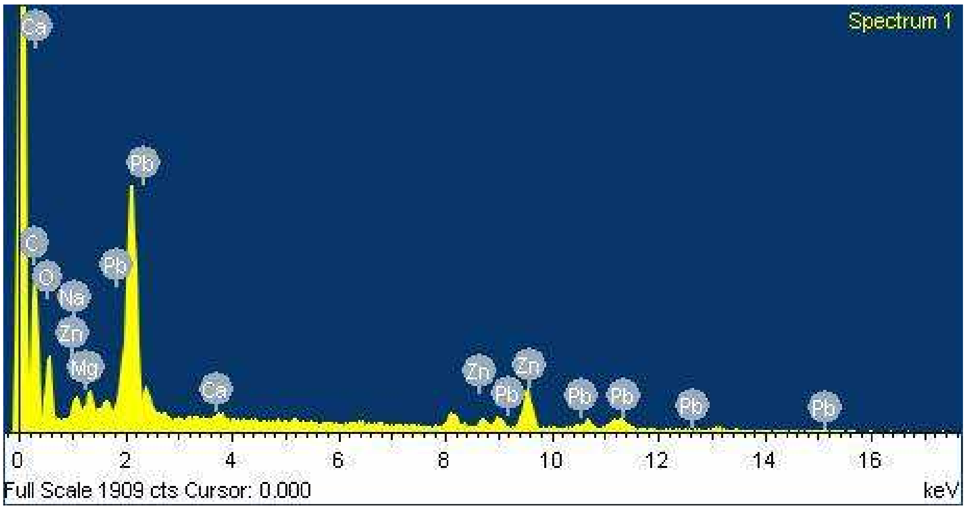

(b)

Fig 9: The SEM micrographs showed changes of the vascular cells of the root samples exposed to 30mg/l Pb treatment (a). (b) EDX confirmed the presence of Pb .

CONCLUSION

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. *Eichhornia crassipes* can be used as low cost treatment material for the removal of Pb.

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