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SCANNING ELECTRON MICROSCOPIC STUDY OF SUNFLOWER PLANT GROWN IN PRESENCE OF ARSENIC (SHORT COMMUNICATION)

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ABSTRACT
In West Bengal, during 1980 some cases of arsenical dermatosis in the districts of North 24 Paraganas, South24 Paraganas, Nadia, Murshidabad and Burdwan were reported. By the end of December 2001, this problem spreads from few villages to 2065 villages of 75 blocks in 8 districts. To overcome this situation phytoremediation may be a good remedy in west Bengal. Sunflower plant is capable to phytoremediate Uranium and this plant is easily cultivated in West Bengal. In the present communication sunflower plant was used to accumulate arsenic and the effects of arsenic on leaves and stem were studied by Scanning Electron Microscopic studies.

KEYWORDS: Arsenic, Phytoremediation, Sunflower plant, Scanning Electron Microscopy.

INTRODUCTION

Arsenic is the 20th most abundant element in the Earth`s crust 14th in the seawater and 12th in the human body (Woolson,1975) and is widely distributed throughout nature as a result of weathering, dissolution, fire, volcanic activity and anthropogenic input (Cullen and Reimer, 1989). The last includes the use of arsenic in pesticides, herbicides, wood preservatives and dye stuffs as well as production of arsenic-containing wastes during smelting and mining operations. In arsenic-enriched environments, a major concern is the potential for mobilization and transport of this toxic element to ground water and drinking water supplies. In Bangladesh, an estimated 57 million people have been exposed to arsenic through contaminated wells. This incident serves as an unfortunate reminder of the toxic consequences of arsenic mobilization and underscores the need to understand the factors controlling the mobility and solubility of arsenic in aquatic systems (Newman et al., 1997). The primary anthropogenic input derives from combustion of municipal solid waste, fossil fuels in coal and oil-fired power plants, release from metal smelters and direct use of arsenic-containing herbicides by industry and agriculture. There are a number of ways by which the human population can become exposed to arsenic.

The most important one is probably through ingestion of arsenic in drinking water or food. Toxicity and detoxification of heavy metal and transition metal oxyanions in living organisms are tightly bound to membrane transport systems of ions and oxyanions. Due to its un-ionized form at neutral pH, arsenite can passively move across the membrane bilayer or be transported by a carrier protein similar to those that transport un-ionized organic compounds. Arsenate poisoning generally results from the transport of this ion by the phosphate transport system thereby competitively inhibiting the oxidative phosphorylation pathway.

Arsenic and many of its compounds are especially potent poisons. Arsenic disrupts ATP production through several mechanisms. At the level of the citric acid cycle, arsenic inhibits pyruvate dehydrogenase and by competing with phosphate it uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD+, mitochondrial respiration, and ATP synthesis. Hydrogen peroxide production is also increased, which might form reactive oxygen species and oxidative stress. These metabolic interferences lead to death from multi-system organ failure probably from necrotic cell death, not apoptosis. A post mortem reveals brick red colored mucosa, due to severe hemorrhage. Although arsenic causes toxicity, it can also play a protective role. Some idea about arsenic uptake by Rice which is efficient at Arsenic accumulation owing to flooded paddy cultivation that leads to arsenite mobilization, and the inadvertent yet efficient uptake of arsenite through the silicon transport pathway. Iron, phosphorus, sulfur, and silicon interact strongly with As during its route from soil to plants. Plants take up arsenate through the phosphate transporters, and arsenite and undissociated methylated as species through the nodulin 26-like intrinsic (NIP) aquaporin channels.

Arsenate is readily reduced to arsenite in plants, which is detoxified by complexation with thiol-rich peptides such as phytochelatins and/or vacuolar sequestration. (Fang-JieZhao *et al.*, 2010). Sunflower plant is also able to remediate Arsenic (Lucia Cavalca *et al.*, 2013).

MATERIALS AND METHODS

Sunflower plant is capable of remediating arsenic (Shilev *et al.*, 2005). Our objective is to know whether arsenic remediation causes any morphological or micromorphological changes? (.For this purpose the micro morphology of root, stem and leaf of sunflower plant of both treated (soil mixed with 0.05mM of As2O3) and untreated sample has been studied under scanning electron microscope (HITACHI S-530) for comparative study of effect of arsenic on the plant. Two Plants was grown in presence of arsenite for 2 days (Figure 1 and 2), after that both the plants were removed from the soil (mixed with arsenite) and rinsed properly to remove the soil particles from the roots and cross section of root and stem (both treated and untreated) were prepared and leafs of both the plants were cut into pieces, then they were kept ready for SEM analysis.

Figure 1. plant treated with arsenite (0.05mM) (48hrs)

Figure 2. Plant Untreated(48 hrs)

Method for sample preparation for Scanning Electron Microscope

The micro morphology of leaves, stem and root was studied under scanning electron microscope (HITACHI S-530) for the comparative study of the effect of arsenic on the sunflower plant. Arsenic treated and untreated plant samples were prepared for studying under SEM was as follows-

The leaf stem and root of two plants species (i.e. treated and untreated) were cut into small pieces of different shapes. Then the small pieces of leaves stem and roots were immersed in 2.5% glutaraldehyde in six different beakers. Each containing different sections (leaf, stem and root) and Plant specimens treated with arsenic and the untreated one also prepared separately and incubates for 4hours and rinsed with buffer solution after incubation. Then they were passed through serial gradations of alcohol for fixation. At first sample pieces were immersed in 50% alcohol for 5mins and then in 70% alcohol for 30minutes, thereafter in 90% and in absolute alcohol respectively for 30mins.Then they were immersed in the mixture of different ratios of absolute alcohol and amyl acetate [3:1, 2:2, 1:2 respectively].In each solution the sample pieces were immersed for 30minutes. Next, they were dipped into amyl acetate for 30mins and then in CPD [Critical Point Dryer], where after the sample pieces were dried at 31ºC and at high pressure. The samples were removed and gold coating was done by IB2 ion coater machine. Now the samples were ready for scanning electron microscopic study. Under scanning electron microscope the features were observed are given below:

RESULTS AND DISCUSSION

SEM observation-

No such significant changes were found in the arsenic treated plant cells of roots, stems and leaves in comparison to the untreated plant. It might be the reason that as sunflower plant can remediate arsenic; it can survive easily in the presence of arsenite (0.05mM). It has been found that the cells were not so much affected by arsenic treatment (Figure 3 to 8).

It might be due to short term exposure with arsenite (0.05mM).Some metal deposition was found in the cell surface of leaf of the treated plant (Figure 8).

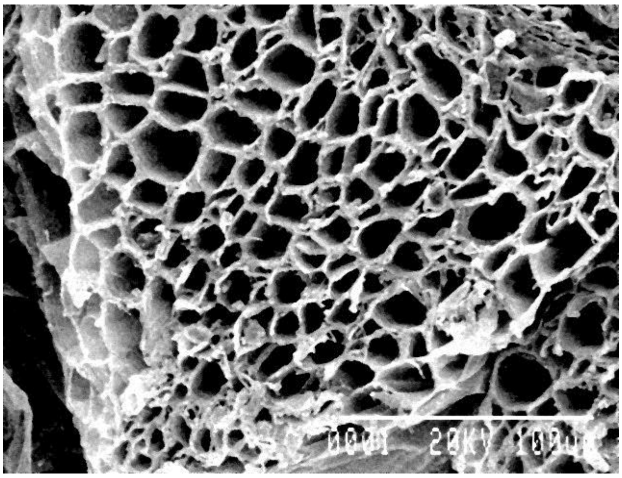


Figure 3. Untreated root at 500X

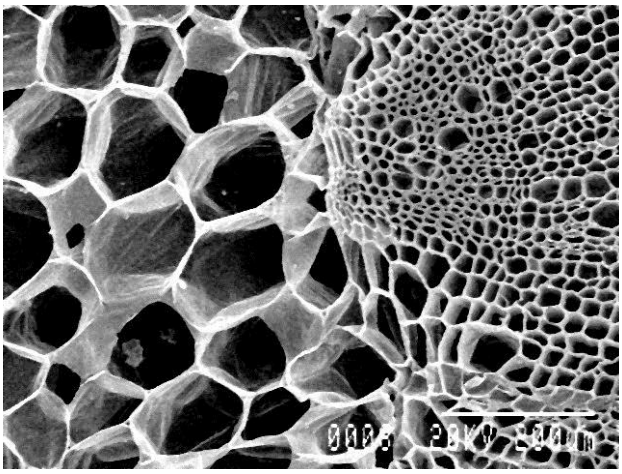


Figure 4. Arsenic treated root at 150X

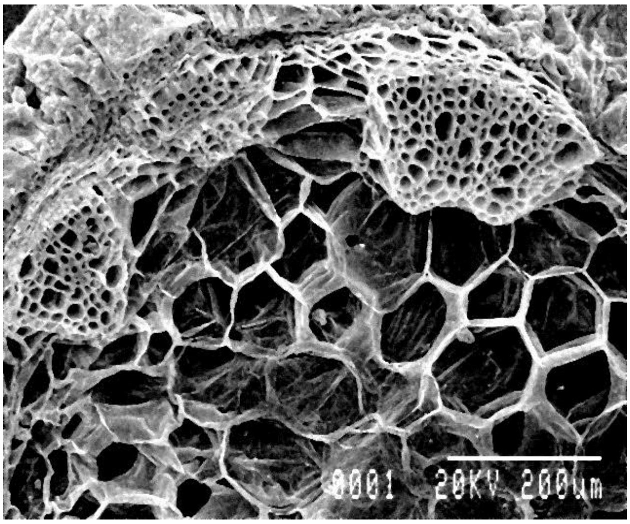


Figure 5. Untreated stem at 150X

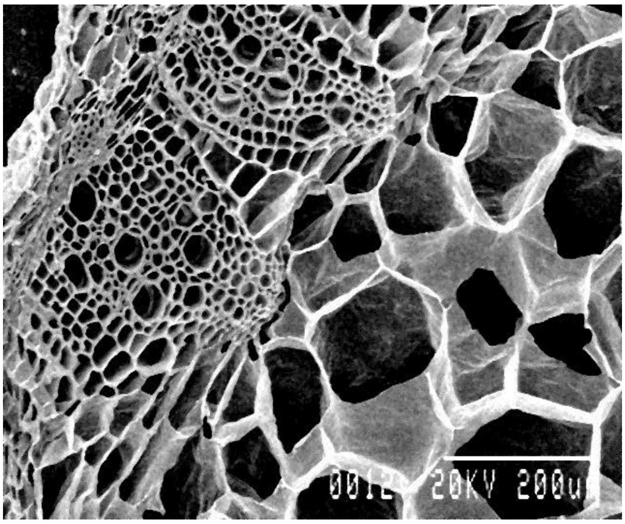
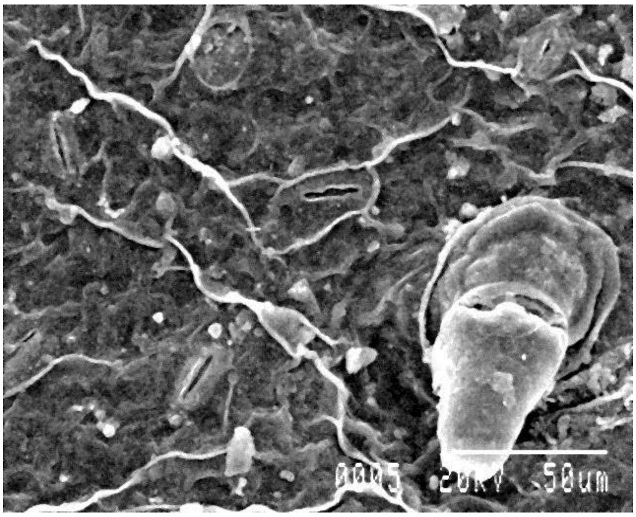


Figure 6. Arsenic treated stem at 150 X



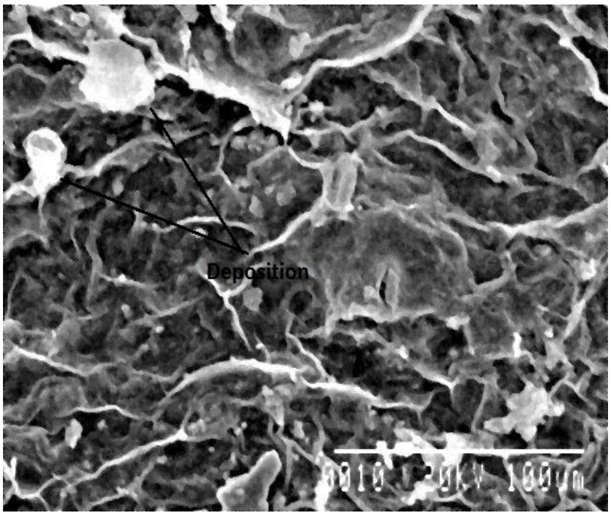


Figure 7. Arsenic untreated leaf at 600X **Figure 8. Arsenic treated leaf at 800X**

CONCLUSION

We can use this plant to phytoremediate arsenic in arsenic prone area which would be a easy solution of arsenic pollution as this plant easily cultivated in West Bengal for oil. The qualitative and quantitative estimation of Sunflower oil in presence of arsenic is our future area of research.

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