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Evaluation of localization of lead and nickel in plant cells of *Amaranthus* sp. and *Brassica* sp. absorbed from mine spoil waste

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Abstract: A detailed survey was undertaken in the sewage water contaminated areas of Coimbatore to select the natural hyper accumulators to rehabilitate the contaminated mine spoils. From this experiment the Pb and Ni accumulators, *Amaranthus* sp. and *Brassica* sp. were selected for further studies towards remediating the metal contaminated mine spoils. Microtomy of root, stem and leaf of *Amaranthus* sp. and *Brassica* sp. showed that the colour development in the plant species is evidence for accumulation of metals in different parts of plants and also tolerance mechanism employed by plant species under metal stress condition. The accumulation of heavy metals from soil to plant did not follow any particular pattern and varied with respect to metals, species and plant parts. However, the maximum Pb localization took place in root portion than in aerial parts. But the Ni accumulation was almost equal or higher in aerial parts (leaf and stem) compared to roots. This study revealed that the *Amaranthus* sp and *Brassica* sp stored lead and nickel in roots, leaves and stems. The roots showed more localization of metals followed by leaves and stems.

Keywords: *Amaranthus* sp.,*Brassica* sp., Lead ,Mine spoil, Microtomy, Nickel

INTRODUCTION

Mining is one of the necessary evils of the modern world. It provides the materials required to sustain quality of life, but does immense, sometime irrepairable, damage to the environment. In India, 80% of mining is in coal and the balance 20% is in various metals and other raw materials such as Gold, Copper, Iron, Lead, Bauxite, Zinc and Uranium. India with diverse and significant mineral resources is the leading producer of some of the minerals. India is not endowed with all the requisite minerals resources. Of the 89 minerals produced in India, 4 are fuel minerals, 11 metallic, 52 non-metallic and 22 minor minerals (RMMII- Report on mining and mineral industry in India, 2015). Magnesite and Coal are the most abundant mineral resources in India. It is the largest contributor to the industrial growth of the country. It is a crucial and enduring element in a modern, balanced energy portfolio, providing very good economical status to India (Ministry of Mines, Annual report 2014-2015). To develop a solution for removing contaminants especially heavy metals from mine spoil areas as well as to reduce the ecological stress, phytoremediation technology is used. Phytoremediation has been tested by various green house and pilot scale field experiments in the USA and Europe. By adoption of this remediation method the biological properties and physical structure of the soil is maintained and the technique is environment friendly, potentially cheap, and offer the possibility of bio-recovery of heavy metals (Jeba sweetly *et al.*, 2014). In this regard, the present study was undertaken to develop an economically feasible and environmentally sound phytoremediation technology for remediating Pb and Ni containing mine spoils.

MATERIALS AND METHODS

Representative magnesite and coal mine spoil samples were collected from the mining areas and were characterized. The sub cellular distribution of Pb and Ni in plant cells was investigated by conventional microtomy technique to completely understand the complex tolerance mechanisms adopted by plant species. The microtomy technique is a well established method which permits the analysis of very small areas (10-15 nm) in thin sections. The selected hyperaccumulators (*Amaranthus* sp. and *Brassica* sp.) were grown in a soil artificially contaminated with Pb and Ni separately at a rate of 1000 mg kg^{-1}. The treated plants were har-

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vested on 30 days after the addition of metal solution, separated into root, shoot and leaves and rinsed with distilled water. The microtomy technique of Pb and Ni in plant tissues followed was the protocol as described by Jensen (1962). The plant tissues were cut into a small pieces measuring 4-5 mm length and fixed in a mixture of 5 parts of 35 per cent formalin, 5 parts of glacial acetic acid and 90 parts of ethyl alcohol fixative for 24 hours. The tissues were then dehydrated using a series of baths consisting of water, ethyl alcohol and tertiary butyl alcohol and embedded in wax. The thin sectioning was done using rotary microtome and the sections were then placed on slides previously coated with Haupt's adhesive. The wax was removed by passing the slides gently through xylol for 10 minutes and rehydrated in a series of baths consisting of xylol –100 per cent (2 changes), xylol + ethanol – 50 : 50 (1 change) and ethanol –100 per cent (2 changes). The slides were kept in each bath for 10 minutes and then stained with dithizone reagent for Lead and dimethyl glyoxime reagent for Nickel (USEPA, 1979) and viewed under Nikon light microscope (20 X).

RESULTS AND DISCUSSION

During the present study intracellular distribution of Lead and Nickel in plant cells was investigated by conventional microtomy method, to understand the metal tolerance mechanisms adopted by plant species. The thin section of root, stem and leaf of both *Amaranthus* sp. and *Brassica* sp. grown in soils artificially contaminated with 1000 mg kg^{-1} of Lead and Nickel were examined.

For study of localization of Lead and Nickel in plant cells a wide distribution of pale-pink coloured complex, formed between Lead and dithizone and dark purple-violet coloured complex formed between Nickel and dimethyl glyoxime, were seen in intracellular spaces of plant cells (Plate 1 and 2). The colour development observed in the plant species (*Amaranthus* sp. and *Brassica* sp.) is evidence for accumulation of metals (Lead and Nickel) in roots, stems and leafs of *Amaranthus* sp. and roots and stems of *Brassica* sp. and also tolerance mechanism employed by plant species under metal stress condition. The stems and roots of *Amaranthus* sp. and *Brassica* sp. showed both Pb and Ni accumulation. In case of leaf, Pb accumulation was seen in *Amaranthus* sp. only. This is in confirmation with the findings of Skaar *et al.* (1973) who found that the Lead was deposited near the rhizome cell wall of *Typha angustifolia*. Autoradiography of roots showed ^{51}Cr was present mostly in soluble non-particulate form in the vacuoles of barley seedlings (Shewry and Peterson, 1974).

Malone *et al.* (1974) used the electron microscope to study localization of Lead accumulated by corn plants

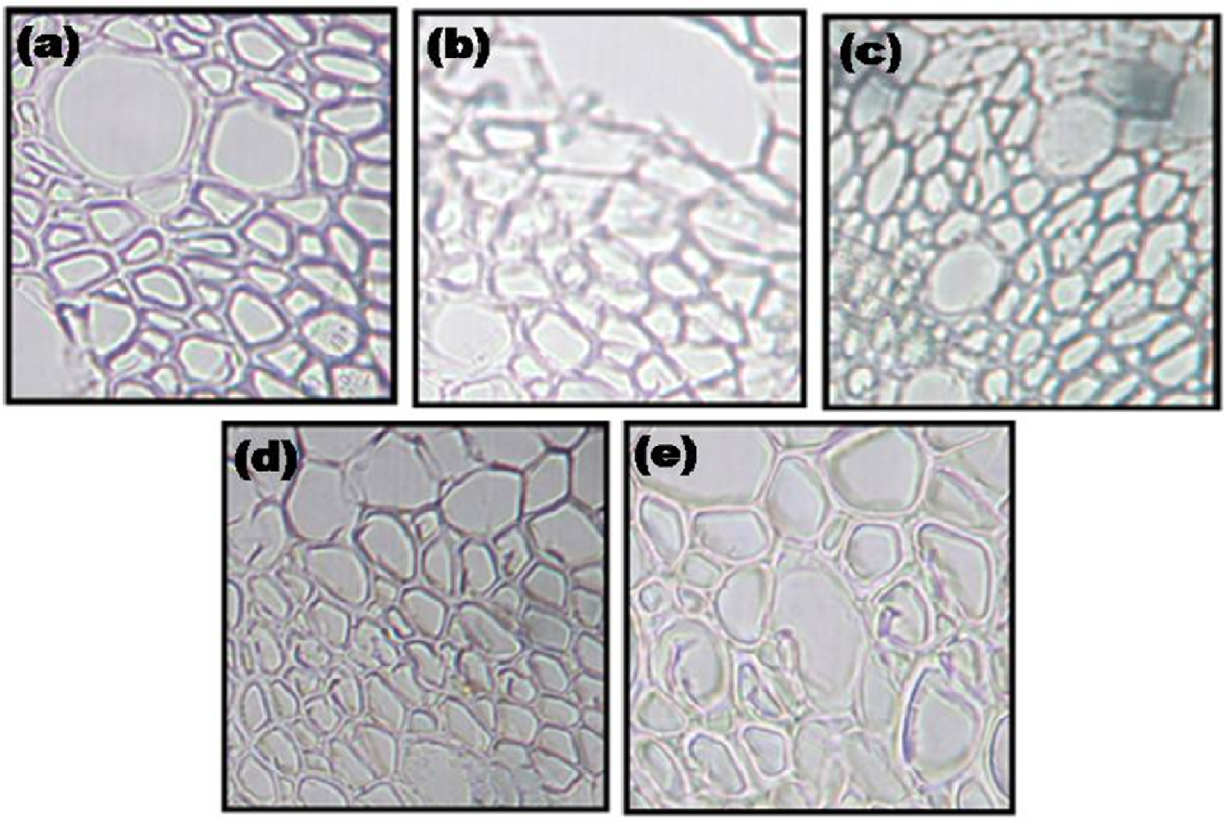

Fig. 1. *Localization of lead in (a) root (b) stem (c)leaf and nickel in (d) root; (e) stem cells of Amaranthus sp.*

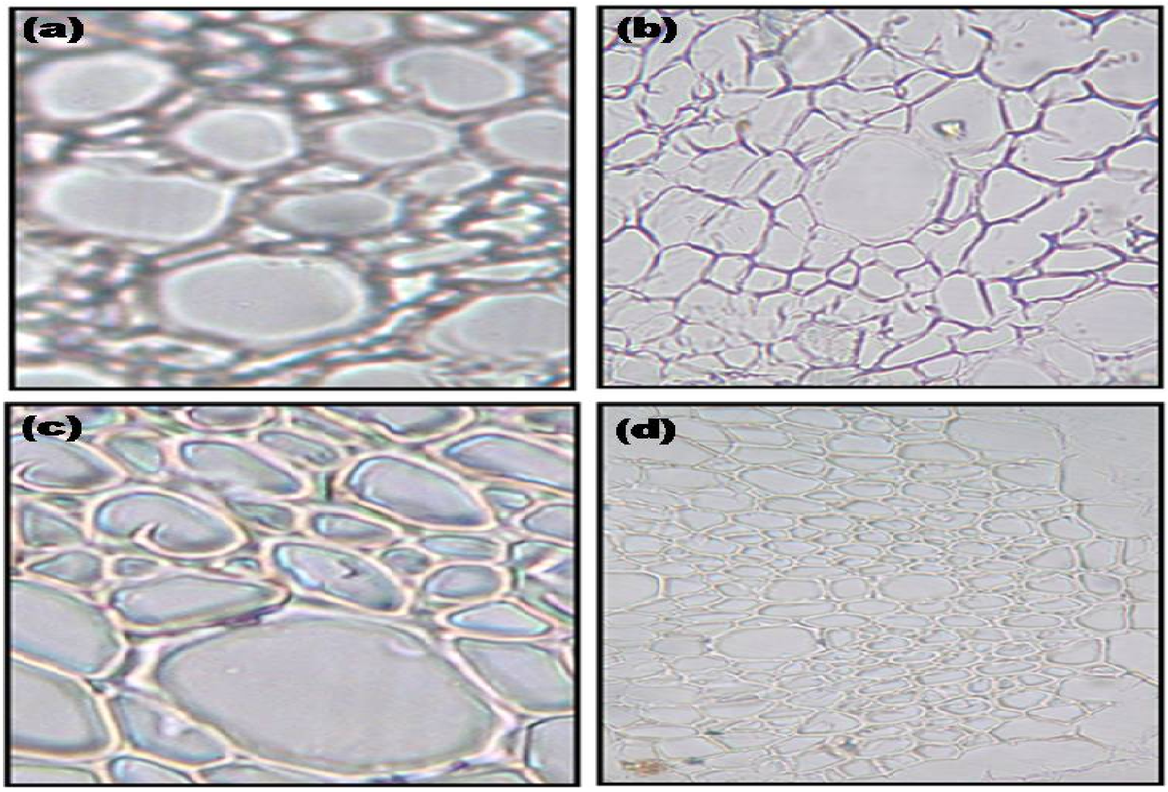

Fig. 2. *Localization of lead in (a) root (b) stem (c)leaf and nickel in (d) root; (e) stem cells of Brassica sp.*

(*Zea mays* L.) and found Lead deposition in the root system. Neumann et al. (1997) reported that the cellular and intracellular distributions of heavy metals were investigated by conventional electron microscopy, EDX, ESI and EELS. Considerable amounts of Fe, Cu, Zn and Al found on the leaf surface are excreted by hydrathodes. Intracellular spaces and cell walls of the leaf parenchyma contain Fe, Cu, Zn and Pb, whereas no metals could be detected in the cytoplasma, vacuole or cellular organelles. Sumathi (2003) reported that the microtomy technique on localization of Cr showed a wide distribution of Cr (VI) in the cells of root, stem and leaf of *Arundo* sp. and *Typha* sp. Vijayakumar and Udayasoorian (2007) reported the effect of increasing concentration of the ions in the effluent let out from the paper mill on the root anatomy of the *Cenchrus celiaris* treated grass in comparison with their control. Anatomical changes were observed in root tissues under effluent irrigation including greater number of cortical layer and thick epidermis, compared to that in well water irrigation. Raw effluent irrigation had damaged the phloem and the total vascular region was seemed to be reduced.

Conclusion

Microtomy technique was used for visualizing of Pb and Ni accumulation and distribution pattern in the cells of root, stem and leaf of *Amaranthus* sp., and *Brassica* sp. The wide distribution of colour complex formed between metals and dye were seen in intracellular spaces of parenchyma cells of *Amaranthus* sp. and *Brassica* sp. The colour development observed in the *Amaranthus* sp. and *Brassica* sp. is a evidence for accumulation of metals (Lead and Nickel) in different parts of plants viz. roots, stems and leaves and also tolerance mechanism includes the induction of proteins, activation of anti-oxidant enzymes and organic acids production for chelation tolerance mechanism employed by plant species under metal stress condition.

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