Pak. K. Bot., 39(4): 1089-1096, 2007.

BIOSORPTION OF Hg: I. SIGNIFICANT IMPROVEMENT WITH MARINE GREEN ALGAE IN THE ANATOMY OF HYPOCOTYL OF *TRIGONELLA FOENUMGRAECUM* UNDER Hg STRESS

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Abstract

Biosorption of mercury (Hg) on marine green algae, *Codium iyengrii* was observed in relation with seed germination, morphology of plant and anatomy of hypocotyls of *Trigonella foenumgraecum*. Results were compared with plants grown in garden soil as a control plant to that of Hg and seaweeds treated plants. Adverse affect of Hg was observed on root hairs, which was retained in presence of seaweeds too. However a significant improvement in tissue structure of vascular bundles was pragmatic and anatomy of hypocotyls reached to normal plant structure at 10ppm, showing the removal of Hg through biosorption on *Codium iyengrii.* This indicates that seaweeds correct the soil condition by supplying macro and micronutrients which develop resistance in plants in stress of heavy metal by adsorbing mercury on surface of seaweeds due to metal and ligand interaction.

Introduction

Biosorption used for removing heavy metals and other pollutants is a newly developed environmental protection technique (Azmat *et al*., 2006a& b). Extensive studies on marine resources decontamination revealed that seaweeds can efficiently accumulate heavy metal, due to presence of polysaccharides, proteins which provides wide range of ligands for interaction with heavy metals ions and other macro and micronutrients (Same *et al.*, 2002; Stirk & Staden, 2002; Schiewer & Wong, 2000; Norama, 1972). Seaweeds extracts are used at the recommended times and rates for increasing growth of plants (Azmat *et al*., 2007). These extracts supply the amounts of iron, zinc, copper, molybdenum, cobalt, boron, manganese and magnesium that most crops require. They form complex with metal ions by changing the oxidation state of metal consequently detoxification is occurred (el- Sheekh & el Saied, 2000; Azmat *et al.,* 2006b). Seaweed can be used directly on the plant in the form of a spray, which may directly absorb through skin of leaves or absorbed by the root. Seaweeds is a potential candidate algae for biosorption of a number of heavy metals, but little is known about the phytotoxicity of mercury (Hg) in different plant species, like distribution and phytotoxicity in the whole plant and at cellular level. Bio availability of Hg in soil, uptake of Hg at phytotoxic level, growth retardation, affects on palisade and spongy parenchyma cells in leaves (Ahmed, 2003, Ladygein, 2004), collated deposition in the vascular bundles and change in vacuoles with electron dense material along the walls of xylem and phloem vessel (Ladygein & Semenova**,** 2003; Boulia *et al.*, 2006)**.** Shaw & Rout (1998**)** observed significant inhibition of root elongation, which was more prominent with Hg than Cd leading to increase in the cell size grown in aquatic medium with Hg. Mor *et al.,* (2002) reported growth inhibitory effect of Mercuric chloride in cucumber leading to the disorientation of root and shoot, while hypocotyle elongation, growth and cell wall loosing in young *Phaseolus vulgaris* was observed due to inhibition of cell wall division in apical meristem region.

Hg which is common in irrigation water and soil sediments, causes irreversible damages in tissue structure of plants leading to reduction in the productivity of crops. Therefore this research has been designed to remove the toxic metal Hg from soil by biosorption on green seaweeds as a very low cost effective technique. Experiment were carried out to study the growth rate and anatomy of *Trigonella foenumgraecum* with removal of Hg in relation with the biosorption of toxic metal on green seaweed (*Codium iyengrii*).

Material and Methods

The green seaweed *Codium iyengrii w*ere harvested in the morning from Buleji coastal beach of Arabian Sea, Karachi, in the months of February and March 2005 when it is abundant at the beach. The seaweed were dried at room temperature and then finally ground by rotary mill to dry seaweed powder (Azmat *et al*., 2007).

Seeds of *Trigonella foenumgraecum* were surface sterilized with Tween 20 solution then soaked in distilled water for two hours. Thirty seeds were kept in each polythene bag containing one kg sterilized garden soil. The soil was supplied with different concentrations of Hoagland based $HgCl_2$ solution (i.e. 5, 10, 15, 20 & 25 ppm Hg) and termed as experimental bags or plants. Half among them were also provided with two gm green seaweed per polythene bag and considered as treated plants. First seed germination was recorded on 4^{th} day. Plants were harvested after two weeks. Morphology of hypocotyls was recorded and transverse section of hypocotyls were observed under Camera Lucida microscope and figures were drawn by pencil on white paper.

Results and Discussion

The present work aimed at studying the germination rate and anatomy of *Trigonella foenumgraecum* after its interaction with mercury and seaweeds. So far no detailed work on anatomy and germination with Hg and green algae is available. The anatomical features of roots were studied in comparison with control plant and treated plants.

Germination of seeds: Almost all seeds germinated in the control plant (Table1) which got reduced (Neelima & Reddy, 2003) after the addition of Mercuric chloride and at 25ppm of Hg concentration only few seeds germinated which were very weak and it was difficult to make the T.S. of hypocotyls that indicate the toxic effects of Hg on roots system where transportation of water was inhibited due to the accumulation of Hg (Fig1). The roots of control plant were healthy and pale to light brown whereas different treatment of Hg showed dark brown to black color, while plant with seaweeds showed healthy growth in different concentration of Hg (Table 2).

Morphology of plant: Control plant was erect with green leaves while plant grown in Hg showed bend over position with very small size of leaf and weak hypocotyls whereas treatment with seaweeds showed overall healthy growth of hypocotyls with broader structure of leaves. Accumulated mercury inhibited the growth of *Trigonella foenumgraecum* and induced disorientation in roots and shoots while disorientation effects enhanced with concentration of Hg (Table 2).

Table 1. Percentage of seed germination in experimental and in treated plants of *Trigonella foenumgraecum*.

Hg (ppm)	Control (%)	Experimental (%)	Treated (%)
0	87.0 ± 20.0	-	-
5	-	77.0 ± 14.5	83.3 ± 18.2
10	-	47.0 ± 15.2	67.0 ± 16.2
15	-	57.0 ± 12.2	63.3 ± 13.3
20	-	37.0 ± 11.3	40.0 ± 11.9
25	-	27.0 ± 9.6	40.0 ± 10.2

Table 2. Morphology of roots of *Trigonella foenumgraecum*.

	Hg (ppm)					
	0	**5**	**10**	**15**	**20**	**25**
Control	Healthy, yellow/ Light brown	-	-	-	-	-
Experimental plants	-	Yellow or pale	Weak yellow	Weak brown	Damaged dark brown	Damaged dark brown
Treated plants	-	Healthy yellow	Healthy Yellow	Healthy Yellow	-Do-	-Do-

Anatomical features of cross section of hypocotyls: Cross section of hypocotyls of control plant (Table 3 and 4) showed the epidermal hairs with single layer, compactly arranged rectangular cells of epidermis (Fig. 1), which were found to be absent at 10, 15, 20 and 25 ppm of Hg while root hairs were not observed in seaweeds treated plants which indicated the adverse affects of Hg on hairs. Root hairs are responsible for the conduction of water from soil into roots of plant that ultimately affect the conduction of water into xylem tissue cells and nutrients enter directly from water into root but compact root structures indicated inhibition of nutrients and water into plant. This results in weakness of seedlings. Epidermis in roots was characteristically constant feature of this species (Figs. 1-11). The cortex consists of eight layered parenchymatous cells which get reduced into 6 layers with longer size (Shaw & Rout 1998) of the cell and indistinguishable at 20 ppm of Hg with ground tissue (Figs. 2-6). The reduced cell size were observed after the addition of seaweed showing the cell division which were retarded due to the accumulation of mercury in cells at high concentration of Hg (Fig. 10). Endodermis in control plant and at 5ppm of Hg and seaweed was single layered (Figs. 1 and 2), comparatively small barrel shaped which was absent at 15, 20 & 25 ppm of Hg whereas at 20ppm of Hg with seaweeds it appeared in the T.S. of hypocotyls while again indistinguishable at higher concentration of Hg with seaweeds (Figs. 4- 6).

The most significant feature of this investigation was the change in structure of vascular system and development of xylem tissues in the given changed structure. In control plants vascular bundles consists of triarch shaped cells, occupying central region and phloem cell consists of three prominent patches, which were alternative with xylem cells (Fig. 1) whereas at 5ppm of Hg concentration, xylem cells were split into tetraarch and phloem cells were not identified in cross section. It appears that mercury affected the cell division in the apical meristem, thus affecting directly the auxin synthesis, which in turn affects the xylem cells and they become large in size (Shaw & Rout 1998) and prominent due to accumulation of mercury but in seaweeds treated plant it becomes in patches and phloem cells were also observed due to which healthy growth of plant is observed because phloem translocate the prepared food material from leaves to all body

Table 3. Histomorphology of transverse section of hypocotyls of *Trigonella foenumgraecum* with Hg.

Treatments	Epidermis	Cortex	Endodermis	Phloem	Xylem	Pith
Control	Numerous hairs's single layered	Parenchymatous, 7-8 layered	Single layered, barrel shaped	3 prominent patches, alternating to xylem	Triarch, occupying central region	Absent
5ppm Hg	Few hairs, single layered	-Do-	-Do-	Not distinct	Tetrarch	Large
10ppm Hg	Hairs, absent single layered	Parenchymatous more pronounced, 6-7 layered	-Do-	A small patches, alternating to xylem	Tetrarch	Absent
15ppm Hg	-Do-	Not distinct	Absent	A small patches, at the apex of each xylem strand	-Do-	Small pith is present
20ppm Hg	-Do-	Not distinct,ground tissues are a little larger	-Do-	Scanty phloem present at each end of xylem strand	Triarch,one strand is large and like a wave and other two are same all, like a triangle	Absent
25ppm Hg	-Do-	-Do-	-Do-	Not distinct	Two strands, one is wavy and other is like an inverted bowl	-D-

Table 4. Histomorphology of transverse section of hypocotyls of *Trigonella foenumgraecum* with seaweeds and Hg.

	Epidermis	Cortex	Endodermis	Phloem	Xylem	Pith
Control						
5ppm Hg + green seaweed	Hairs absent, single layered	Eight layered small parenchymatous	Single layered	Four patches, alternating to xylem	Four strands	Small
10 ppm Hg + green seaweed	-Do-	-Do-	-Do-	Not distinct	4 strands, extending from centre to periphery	Absent
15ppm Hg + green seaweed	-Do-	-Do-	Not distinct	Not distinct	Tetrarch	Small
20ppm Hg + green seaweed	-Do-	8-10 layered small cells	Single layered	4 patches, alternating to xylem	Tetrarch, small cells	-Do-
25ppm Hg + green seaweed	-Do-	-Do-	Not distinct	-Do-	-Do-	-Do-

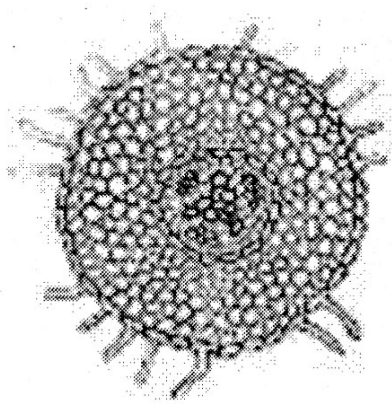

Fig. 1. T.S. of control plant.

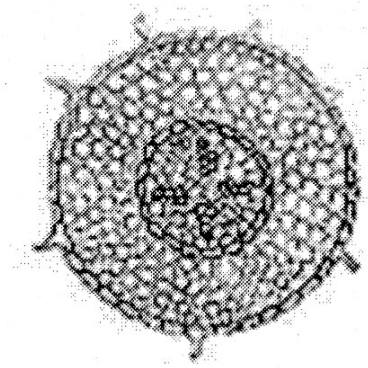

Fig. 2. T.S. at 5 ppm of Hg.

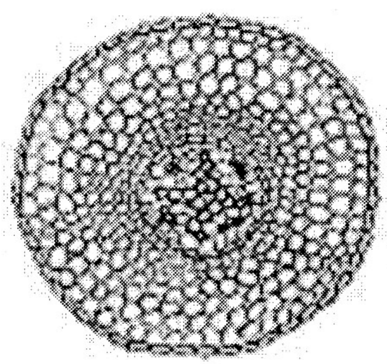

Fig. 3. T.S. at 10 ppm of Hg.

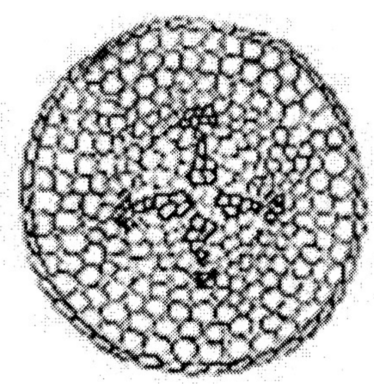

Fig. 4. T.S. at 15 ppm of Hg.

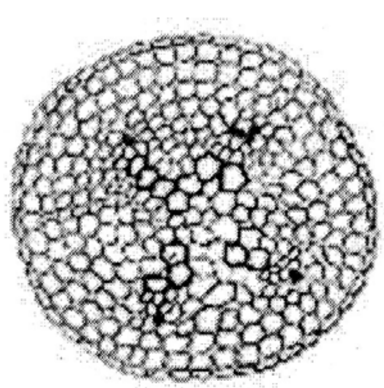

Fig. 5. T.S. at 20 ppm of Hg.

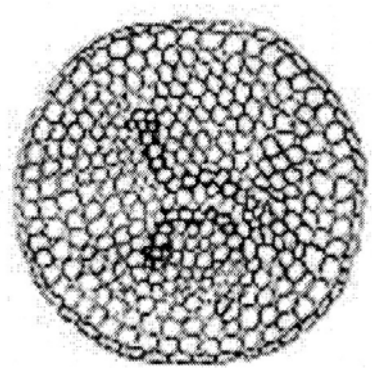

Fig. 5. T.S. at 25 ppm of Hg.

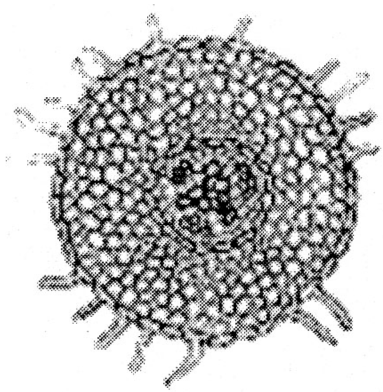

Fig. 1. T.S. of control plant.

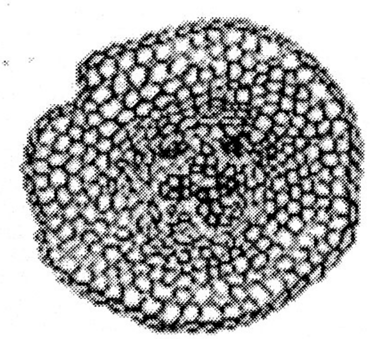

Fig. 7. T.S. at 5 ppm with sea weed.

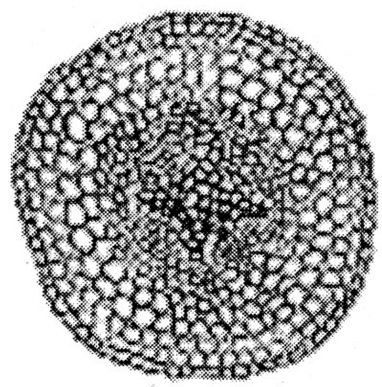

Fig. 8. T.S. at 10 ppm with sea weed.

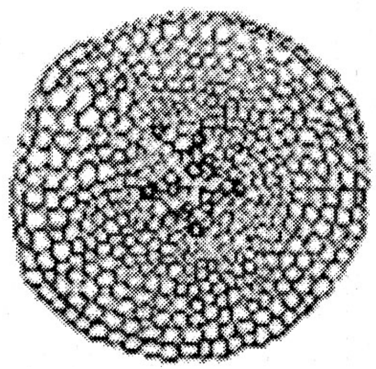

Fig. 8. T.S. at 15 ppm with sea weed.

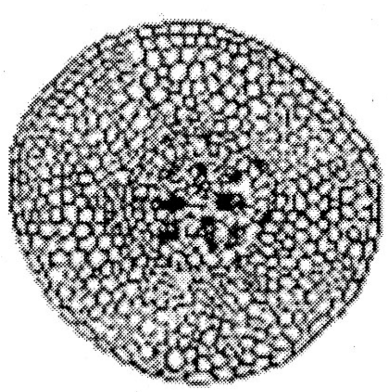

Fig. 9. T.S. at 20 ppm with sea weed.

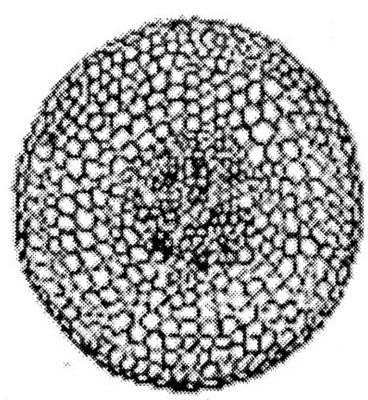

Fig. 9. T.S. at 25 ppm with sea weed.

parts. Spray of green seaweeds in mercury contaminated soil showed significant improvement in the structure of root and approximately reach to the structure of normal plant at 10ppm of Hg, whereas at all given treatment of Hg with seaweed showed improvement in the structure of root.

A large pith was observed at 5, 15, 20, 25 ppm of Hg due to split of xylem tissues, indicated the destructive effect of Hg in the plants. Pith was absent at 10 ppm of Hg.

Histological study at the hypocotyls level revealed that mercury induced a restriction of the tissues territories as well as meristem formation differentiating in a root structure where pith is formed due to the distorted structure of xylem indicated that vascular menistematic cells are affected by Hg (Figs. 3-6) while Figs. 7-11 characterized by a positive effects of seaweed with well developed structure with reduced sized of cells in which phloem tissues are also present (Sela *et al*., 1998; Tung *et al*., 1988; Azmat *et al*., c & d 2006). This is related with the healthy growth of the plant even in high concentration of Hg showing the biosorption of Hg by marine green algae.

It would suggest that Hg can be removed from soil contaminated with toxic metals through biosorption by green seaweeds, *Codium iyengri*i which is abundant at our coastal areas which proved to be a good biomass biosorbent for remediation of the soil.

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(Received for publication 30 April 2007)