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Morphoanatomical Response of Two Varieties of *Brassica juncea* (L.) Czern. Grown on Tannery Sludge Amended Soil

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Since the dawn of civilization, metals have been a part of human history, however, toxic metal pollution of the biosphere has intensified rapidly since the onset of industrial revolution. Among industries, tannery industry occupies a place of prominence in the Indian economy in view of its massive potential for employment, growth and exports. In rural and peri-urban areas of most developing countries, the use of sewage and industrial wastewater and sludge for growing the crops is a common practice (Nan and Cheng 2001; Wong et al. 2001). The presence of high concentration of toxic metals in the tannery waste pose a constraint on its application on agricultural land, which cumulatively elevate the heavy metal loading in soil and plants growing therein. Chromium is one of the major elements, which is being released from tannery industry, used in chrome tanning of raw leather. Cr (VI) acts principally on plant roots, resulting in an intense growth inhibition. The initial symptoms of Cr toxicity appear as severe wilting and chlorosis of the plants. The primary toxic effect seemed to be membrane damage due to high oxidative potential of Cr (VI) and its compounds, which easily enter the cells and produce cytotoxic and mutagenic effects. Besides Cr, iron is an another major element present in the tannery sludge and comes from animal's hides and ferrous aluminum sulphate, which is used for the precipitation of suspended solids during the wastewater treatment. The deficiency of Fe results in chlorosis, however, excessive concentration of Fe is believed to generate oxidative stress (Halliwell and Gutteridge 1993). Visible symptoms of metal toxicity in plants are an expression of metal induced alterations at the structural and ultrastructural levels. These changes at the cell, tissue and organ levels, in turn, are the result of a direct interaction of the toxic metals with structural components at these sites.

Brassica juncea (L.) Czern. is an important oil bearing crop of India. The present experiment was planned to assess the growth performance of two varieties of *B. juncea* (*rohini* and *vaibhav*) and the Scanning Electron Microscopic studies of their leaf surface grown on tannery sludge amended soil.

MATERIALS AND METHODS

Dried tannery sludge cakes were collected randomly from the surface of sludge beds of UASB (Upflow Anaerobic Sludge Blanket) Treatment Plant, Jajmau, Kanpur (Uttar Pradesh, India) in large plastic bags and brought to the field laboratory. The uncontaminated garden sub soil collected from National Botanical Research Institute, Lucknow was used to make the various amendments for experimental studies. The

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physico-chemical parameters of the tannery sludge and garden soil were estimated by the method of Kalra and Maynard (1991). The tannery sludge and soil were air dried, finely powdered and sieved to 2 mm mesh size before use. The various amendments of tannery sludge (10, 25, 35, 50, 75 and 100%) were prepared using garden soil, however only garden soil served as control. The seeds of *Brassica juncea* (var. *rohini* and *vaibhav*) were obtained from Chandra Shekhar Ajad Agriculture University, Kanpur. All the seeds were sterilized in 3% formalin for 5 min to avoid fungal contamination, washed with redistilled water for three changes and soaked in water overnight. The soaked seeds were evenly sown in pots of 14 inches in diameter, which were filled with different amendments (10, 25, 35, 50, 75 and 100%) of tannery sludge (10 kg dw) along with one set of control in soil, each in three replicates. A total of 21 pots were randomly divided into 7 groups (amendments), each group with 3 pots (replicates). Ten seeds were sown in each pot to a depth of 0.5 cm. The pots were watered daily till seed germination. When the seedlings were developing 5 or 6 leaves, they were thinned out to retain 3 uniform ones per pot and allowed to grow. Pots were placed in a green house in a randomized block design at an average diurnal temperature of 25-45°C. The tap water was provided to the pots to keep the top soil moist and avoid leaking from the pots. The plants (one plant from each pot) were harvested after 30, 60 and 90 days after sowing. All the plants were free from any disease in whole of the experiment duration. All the experiments were repeated twice. At each harvesting, the length of roots and shoots of all the plants was measured by Vernier calipers, photosynthetic area by a delta T device, while the number of leaves was recorded manually. The experiment was performed in a completely randomized block design. To confirm the variability of data and validity of results, all the data were subjected to an analysis of variance (ANOVA) and to determine the significant difference between treatments, least significant difference (LSD) was performed (Gomez and Gomez 1984).

The leaves of *B. juncea* var. *rohini* and *vaibhav* grown on 50 and 100% tannery sludge for 90 d were examined by Scanning Electron Microscope. The leaves of the plants grown on soil served as control. The plant samples were kept in 2.5% gluteraldehyde for fixation and left overnight. The plant material was passed through alcohol series (30, 50, 70, 90 and 100%) for dehydration and drying was done in BAL-TEC CPD-030 critical point drier using liquid CO_2 as carrier gas. The mounted specimens (six samples from each category) were coated with 15 nm thin gold and studied with Philips XL-20 Scanning Electron Microscope.

RESULTS AND DISCUSSION

Table 1 showed the physico-chemical properties of the tannery sludge and garden soil used in the present experiment. The analysis of the data showed that the tannery sludge contains high concentrations of metals. The data presented in Fig. 1A showed that the root length of *B. juncea* var. *rohini* increased up to 25% tannery sludge after 30 and 60 d and up to 35% tannery sludge after 90 d of exposure followed by significant ($p<0.01$) decrease, compared to their respective controls. The shoot length of the plant (Fig. 1A) increased with increase in sludge amendments at all the exposure periods as compared to control except at 100% tannery sludge after 90 d. In case of *B. juncea* var. *vaibhav*, the root length of the plant increased significantly ($p<0.01$) up to 35% tannery sludge followed by decrease at higher amendments of tannery sludge at all the exposure periods, compared to their respective controls (Fig. 1C). The shoot length of the plant increased significantly with increase in sludge amendment except at 100%

Table 1. Physico-chemical properties of tannery sludge and garden soil.

Parameters	Tannery sludge	**Garden soil**
pH	8.31±0.34	7.8±0.39
Electrical conductivity (mS m^{-1})	7.08±0.83	1.32±0.06
Cation exchange capacity (me L^{-1})	224±15	1.10±0.05
Na (mg L^{-1})	268.6±8.11	71.93±4.29
K (mg L^{-1})	32.1±2.87	10.93±1.82
Organic matter (%)	1.48±0.87	0.82±0.26
Cr (μg g^{-1} dw)	12,500±18.61	1.83±0.09
Fe (μg g^{-1} dw)	842.25±18.35	207.82±15.22

All the values are mean of three replicates ± SD.

tannery sludge after 30 d, compared to their respective controls. The number of leaves in *B. juncea* var. *rohini* (Fig. 2A) of the exposed plant increased up to 25 and 50% tannery sludge after 30 and 90 d, respectively, compared to their respective controls. However, at 60 d, the number of leaves increased with increase in sludge amendment ratio as compared to control. Leaf area of the plant, *B. juncea* var. *rohini* grown on different amendments of tannery sludge increased significantly ($p<0.01$) up to 75% sludge at 30 and 60 d and up to 50% sludge at 90 d followed by decrease, compared to their respective controls. The number of leaves in *B. juncea* var. *vaibhav* (Fig. 2C) increased significantly ($p<0.01$) up to 75% tannery sludge followed by no change at 30 and 90 d of exposures, compared to their respective controls. At 60 d, it increased significantly ($p<0.01$) at all the amendments of tannery sludge as compared to control. The leaf area of the plant increased at all the sludge amendments at 30 and 60 d of exposures, compared to their respective controls. At 90 d, it increased up to 50% tannery sludge followed by decrease as compared to control.

Our findings that tannery sludge favours the growth of the plants up to certain percentage are also strengthened by Chandra et al. (1997). They have reported an increase in the biomass of plants, *Phragmites karka* (633%), *Scirpus lacustris* (620%) and *Bacopa monnieri* (433%) grown on tannery sludge for 12 weeks. Om et al. (1994) also recorded an increase in root length, shoot length and biomass of Okra seedlings treated with 25% effluent (Distillery and sugar mill) concentration. Roots are the first target sites for the metal stress, thus, root growth inhibition is considered as first symptom of metal toxicity. (Ebbs and Kochian 1997; Fargasova 1998). At low Zn concentrations, the growth of *Brassica juncea* and *Cajanus cajan* (Alia et al. 1995) and *Brassica juncea* seedlings (Prasad et al. 1999) was promoted followed by suppression at higher metal concentrations.

Scanning electron micrographs of the leaf surface of *B. juncea* var. *rohini* (control plant) showed that the stomata are anisocytic, surrounded by three subsidiary cells, two equal sized and one smaller than that (Fig. 3A,B). In the leaves of plants grown on 50% tannery sludge (Fig. 3C,D), few stomata were found open and few towards closure, compared to control. The size of stomata was found increased in the plants grown on 50 (0.0215 mm ±0.0012, n = 5) and 100% (0.020 mm ±0.0015, n = 5) tannery sludge amendments (Fig. 3C,E) than their control (0.018 mm ±0.0010, n = 5). Stomatal frequency in the leaves of the plant growing in 50 (0.242 stomata/ mm^2) and 100% (0.253 stomata/ mm^2) tannery sludge (Fig. 3C,E) seems to be increased than that of control (0.143 stomata/mm^2) (Fig. 3 A). In the leaves of the plant grown on 100%

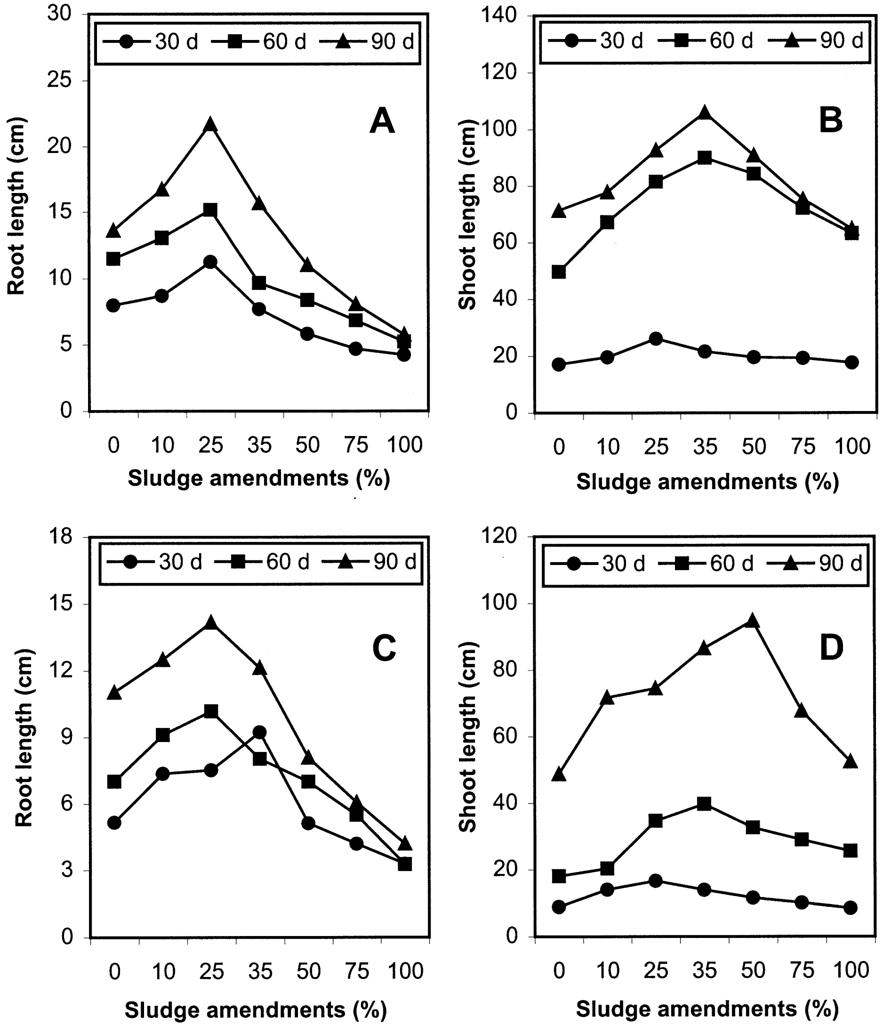

Figure 1. The effect of tannery sludge amendments on (A, B) root length (cm) and shoot length (cm) of B. juncea var. rohini, (C, D) root length (cm and shoot length (cm) of B. juncea var. vaibhav, n=3. LSD ($p<0.01$): var. rohini; amendments; root length = 1.26, shoot length = 5.88, exposures; root length = 0.82, shoot length = 3.79. var. vaibhav, amendments; root length = 0.41, shoot length = 2.12, exposures; root length = 0.27, shoot length = 1.39.

tannery sludge, most of the stomata were found closed (Fig. 3 E,F). The plants of *B. juncea* var. *vaibhav* grown on different amendments of tannery sludge (50 and 100%) showed considerable changes in the surface structures of the leaves after 90 d of exposure along with control (Fig. 4). In the leaves of the plant grown on 50 and 100% tannery sludge (Fig. 4C,E), few stomata were found closed and stomatal frequency was increased than the control (Fig. 4A). The size of stomata increased in the plants grown on 50 (0.0197 mm ±0.0011, n = 5) and 100% (0.020 mm ±0.0012, n = 5) tannery sludge amendments (Fig. 4C,E) than their control (0.017 mm ±0.0010, n = 5). Slight

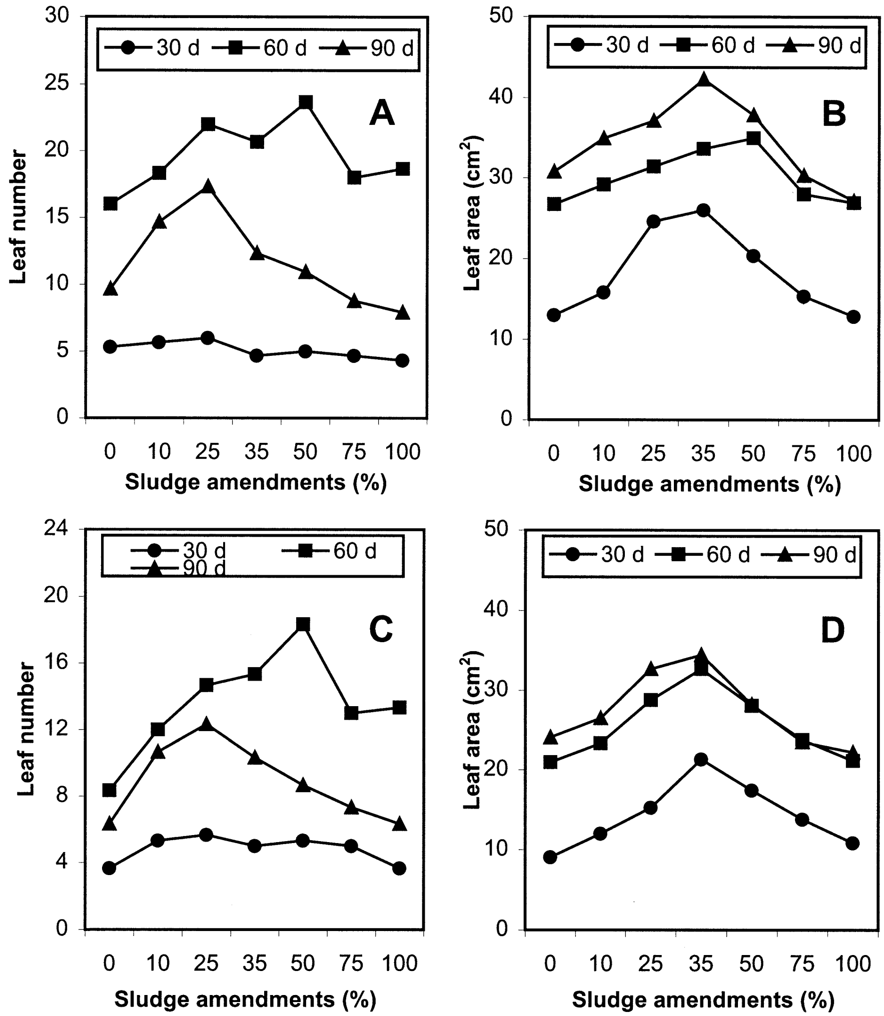

Figure 2. The effect of tannery sludge amendments on (A, B) leaf number and leaf area (cm2) of B. juncea var. rohini, (C, D) leaf number and leaf area (cm2) of B. juncea var. vaibhav, n=3. LSD ($p<0.01$): var. rohini; amendments; leaf number = 2.09, leaf area = 1.46, exposures; leaf number = 1.37, leaf area = 0.95, var. vaibhav, amendments; leaf number = 1.08, leaf area = 1.70, exposures; leaf number = 0.71, leaf area = 1.11.

elevation in the stomatal complex is also observed in the plants growing in 100% tannery sludge (Fig. 4F) as compared to control (Fig. 4B). Linear striations emerging from stomata present in control (Fig. 4A,B) is completely insignificant in 100% tannery sludge (Fig. 4E,F). These results are in agreement with the findings of several authors (Bazzaz et al. 1974; Barcelo et al. 1988) showing closure of stomata in the leaves of metal treated plants which may be a strategy to prevent the water loss through transpiration as the translocation of water and solutes get disturbed in the presence of metals. Bondada and Oosterhuis (2000) reported that closed stomata of the leaf result in a slower rate of diffusion due to greater diffusion gradient of water vapour.

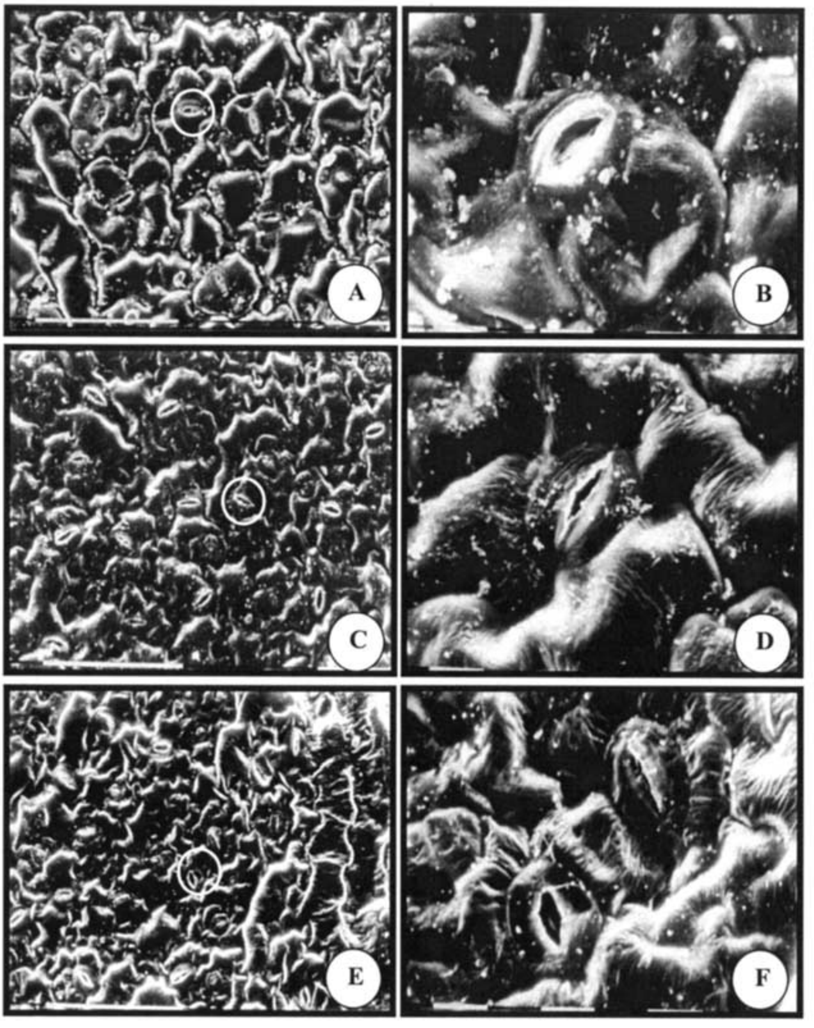

Figure 3. SEM micrographs of leaf surface of *B. juncea* var. *rohini* (A-F). **A.** Leaf epidermis of control plant (400x); **B.** Magnified view of plate A (1550x); **C.** Leaf epidermis of plants growing in 50% tannery sludge (400x); **D.** Magnified view of Plate C (1550x); **E.** Leaf epidermis of plants growing in 100% tannery sludge (400x); **F.** Magnified view of Plate E (1550x).

The decrease in the size of stomatal aperture in the leaves is in line with the hypothesis that metals induce water stress (Barcelo et al. 1988). The SEM study suggested that the shape of some stomatal complexes is disrupted in the plants exposed to tannery sludge amendments. Nishizono et al. (1987) reported that the metal ions seem to attack various cellular components including cell wall and membranes resulting in differential alterations that ultimately lead to their disorganization and mechanical injury i.e., necrosis. In conclusion, the morphological parameters of both the varieties of *B. juncea* increased up to 50% tannery sludge showing that the tannery sludge supports the growth of the plant at lower amendments. Further, SEM micrographs revealed that increased

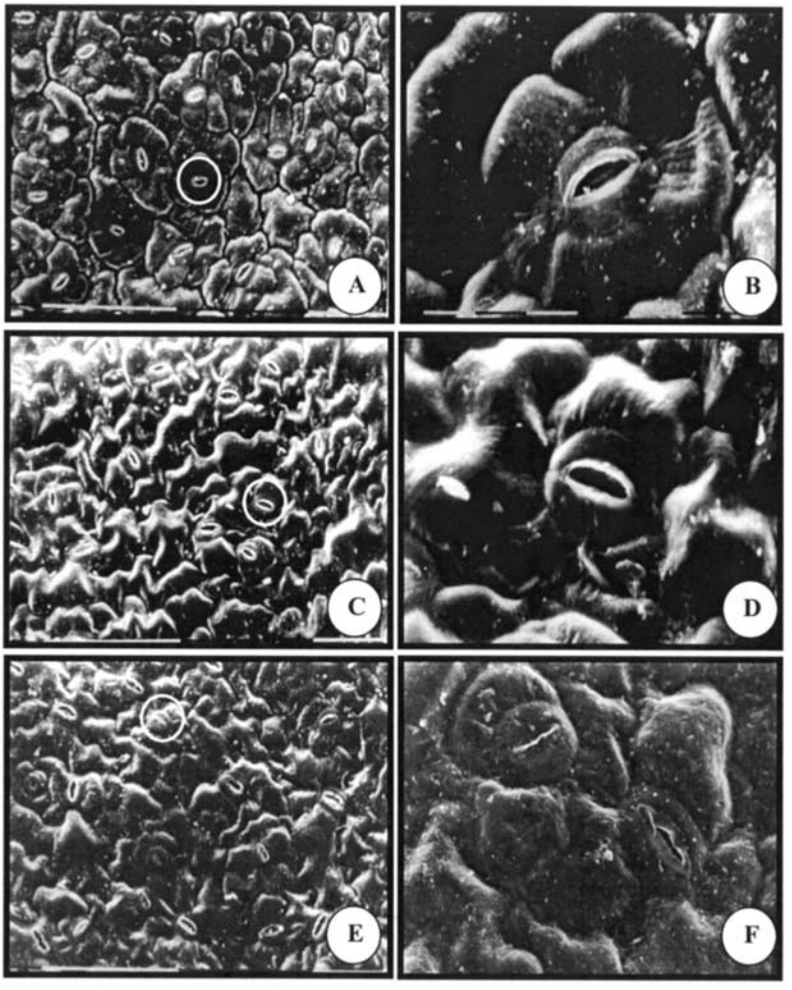

Figure 4. SEM micrographs of leaf surface of *B. juncea* var. *vaibhav* (A-F). **A.** Leaf epidermis of control plant (400x); **B.** Magnified view of plate A (1550x); **C.** Leaf epidermis of plants growing in 50% tannery sludge (400x); **D.** Magnified view of Plate C (1550x); **E.** Leaf epidermis of plants growing in 100% tannery sludge (400x); **F.** Magnified view of Plate E (1550x).

amendments of tannery sludge led to closure of stomata, increase in their frequency and degeneration of certain cells.

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REFERENCES

Alia KV, Prasad SK, Saradhi PP (1995) Effect of zinc on free radicals and proline in

Brassica and *Cajanus*. Phytochem 39: 45-47.

Barcelo J, Vazquez MD, Poschenrieder C (1988) Structural and ultrastructural disorders in cadmium treated bush bean plants (*Phaseolus vulgaris* L.) New Phytol 108: 37-49.

Bazzaz FA, Carlson RW, Rolfe GL (1974) The effect of heavy metals on plants. I. Inhibition of gas exchange in sunflower by Pb, Cd, and Tl. Environ Pollut 7: 241-246.

Bondada BR, Oosterhuis DM (2000) Comparative epidermal ultrastructure of cotton (*Gossypium hirsutum* L.) leaf, bract and capsule wall. Ann Bot 86: 1143-1152.

Chandra P, Sinha S, Rai UN (1997) Bioremediation of chromium from water and soil by vascular aquatic plants. In: Kruger EL, Anderson TA & Coats JR (ed) Phytoremediation of soil and water contaminants, American Chem Society, Washington DC, p 274-282.

Ebbs SD, Kochian LV (1997) Toxicity of zinc and copper to Brassica species: Implications for phytoremediation. J Environ Qual 26: 776-781.

Fargasova A (1998) Root growth inhibition, photosynthetic pigments production and metal accumulation in *Sinapois alba* as the parameters for trace metals effect determination. Bull Environ Contam Toxicol 61: 762-769.

Gomez KA, Gomez AA (1984) Statistical procedure for agricultural research. John Willey & Sons, New York.

Kalra YP, Maynard DG (1991) Methods manual for forest soil and plant analysis.. Forestry Canada, Northwest Region, Northern forest Centre, Edmonton, Alberta. Information Report NOR-X-319.

Nan Z, Cheng G (2001) Accumulation of Cd and Pb in spring wheat (*Triticum aestivum* L.) grown in calcareous soil irrigated with wastewater. Bull Environ Contam Toxicol 66: 748-754.

Nishizono H, Ichikawa H, Suziki S, Ishi F (1987) The role of root cell wall in the heavy metal tolerance of *Athyrium yokoscense*. Plant Soil 101: 15-20.

Om H, Singh N, Arya MS (1994) Combined effect of wastes of distillery and sugar mill on seed germination, seedling growth and biomass of okra [*Abelmoschus esculentus* (L) Moench]. J Environ Biol 15: 171-175.

Prasad KVSK, Saradhi PP, Sharmila P (1999) Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. Environ Exp Bot 42: 1-10.

Wong JWC, Lal KM, Su DS, Fang M (2001) Availability of heavy metals for *Brassica chinensis* grown in an acidic loamy soil amended with a domestic and an industrial sewage sludge. Water Air Soil Pollut 128: 339-353.