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EFFECTS OF CHROMIUM, CADMIUM, LEAD AND ZINC ON GERMINATION, SEEDLING GROWTH AND PHENOL CONTENTS OF *VIGNA UNGUICULATA* (L.) WALP.

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ABSTRACT

The effect of four heavy metals, including chromium, cadmium, lead and zinc were examined on germination, early seedling growth and soluble phenol content of cowpea *Vigna unguiculata*. The final seed germination percentage was significantly reduced by most of the heavy metals at 50ppm and 100ppm. Germination was suppressed by the metals in the order Cd> Cr> Pb> Zn. Both root and shoot growth of the seedlings were reduced by all the heavy metals tested. TL 50 values for Cd, Cr, Pb and Zn were 53,07, 55.86, 70.75 and 81.24, respectively. As a stress response, the soluble phenol content was remarkably elevated by the heavy metals at concentrations of 50ppm and 100ppm. Phenol accumulation due to heavy metals stress occurred in the order Cd> Cr> Pb> Zn. The mechanisms whereby heavy metals cause toxicity to plants are discussed.

Keywords: chromium, cadmium, lead, zinc, *vigna anguiculata*, germination, Phenol

INTRODUCTION

Increased industrial and agricultural activities have resulted in the problem of heavy metal contamination which poses problems to soil, biota and atmosphere (Friberg *et al.*, 1971; Raskin & Ensley, 2000; Violante *et al*., 2008). There are about 50 metals that are of special interest with respect to toxicological importance to human health, plants and animals (Brown and Welton, 2008; Brandl, 2008). The levels of heavy metals in soil may range widely from 1 mg Kg^{-1} to 100g kg^{-1} pertaining to their geological origin and human interference (Gardea-Torresey *et. al*., 1996; Blaylock and Huang, 2000; Brown and Welton, 2008).The extent of damage depends upon bioavailability and absorbability of metals (Hambridge, 1981; Misra and Mani, 2009).Heavy metal concentrations of 50 to 8000 µg g-1 are known to inhibit soil respiration when added as chloride salts even after 18 months (Doelman & Haanstra, 1984). It has been observed that certain plants have the ability to inhabit sites where soil contains high levels of heavy metals or other toxic compounds (Raskin and Ensley, 2000). Toxic metals first accumulate in soil to reach the plants through roots or are taken up by the leaves of the plants from the atmosphere such as lead (Misra and Mani, 2009). Heavy metals are highly toxic for plant growth affecting the enzyme systems and create ionic imbalances (Fargasava, 1994; Qureshi *et al.*, 2007). Plants respond to heavy metal toxicity by inducing different enzymes, creating ionic influx/efflux for ionic balance and synthesizing small peptides. These peptides bind metal ions and reduce toxicity (Reddy & Prasad, 1990, 1992). Some heavy metals in higher concentrations are therefore responsible for metabolic disorders and reduction in germination and growth for most of the plant species (Fernandez and Henriques, 1991; Krupa *et al*., 1993; Shaukat *et al* 1999; Duo *et al*., 2005).

Cadmium is closely related to zinc and will be found wherever zinc is found in nature. Cadmium is obtained as a by-product in refining zinc and some other heavy metals (Friberg *et. al.,* 1971). Cadmium is used mainly as an anticorrosive, electro-plating material on steel. Fertilizers produced from phosphate ores also constitute a major source of diffuse cadmium pollution. Cadmium concentrations of 10 ppm or more are shown to be inhibitory to germination (Hsu and Chou, 1992; Shaukat et al., 1999). Khan (2007) did not observe any inhibitory effect of cadmium on final germination of a hardy species *Prosopis juliflora*. However, root and shoot growth was suppressed at doses beyond 5 ppm *in vitro* (Shaukat *et al*., 1999, Peralta *et al*., 2001, Khan, 2007).

Chromium and its salts are used in leather tanning industry, the manufacture of catalysts, pigments and paints, fungicides, the ceramic and glass industry, and in photography. Chromium is also used in the manufacture of chrome alloy and in metallic form (Bradl, 2008; Misra and Mani, 2009).The suppressive effect of chromium on germination and seedling growth has been demonstrated by several workers (Shaukat *et al.*, 1999).

Lead was used in paints produced for use in homes and public buildings for many years until its toxicity became apparent. It was primarily used as a white or yellow pigment and also to reduce drying time, increased durability and moisture resistance (Bradl, 2008; Misra and Mani, 2009).Plants usually do not absorb lead from soil unless significant amount is present in the soil, for example, when crops are grown on contaminated grounds of old industrial areas. Lead pipes, galvanized pipes, bonfire ash and soils are the other common sources (Misra and Mani, 2009). Lead concentrations of 75 ppm or more are known to reduce the germination percentage as well as subsequent seedling growth (Hsu & Chou, 1992, Shaukat *et al.*, 1999, Shafiq *et al.*, 2008).

Zinc is an essential element in nutrition and its and its traces are present in many foods. Many workers have reported the toxic effects of zinc (Levengood *et al.*, 1999). The inhibitory effect of zinc on germination has been demonstrated by Burhan *et al*., (2001).

Plants have evolved various mechanisms to defend themselves from different types of biotic and abiotic stresses. There is evidence that secondary metabolism may modulate plant responses to various forms of stresses and thereby contribute to plant 'fitness for survival' (Altman & Colwell, 1998). By means of secondary metabolic pathways plants produce a wide range of compounds, including phenols, nitrogen-based compounds and terpenes, that play a protective role against a range of stresses (Seigler, 2001; Cseke *et al*., 2006; Harborne, 2007). In particular, phenols, phenolics are the major secondary metabolites that are involved in the protection of plants against a variety of biotic (Nicholson & Hammerschimidt, 1992, Dixon & Paiva, 1995; Harborne, 1999) and abiotic stress (Eliasova *et al*., 2004; Abreu & Mazzafera, 2005; Ganeva & Zozikova, 2007). Shaukat *et al*., (1999) demonstrated the accumulation of soluble phenols in response to exposure of plants to heavy metals.

Germination behaviour of seeds that are contaminated with heavy metals present in soils has not been fully understood. Therefore, this study has been designed to examine a) the inhibitory effect of heavy metals on germination and seedling growth, and b) to study the physiological stress response of cowpea [*Vigna unguiculata* (L.) Walp. Var. Elite] to heavy metals concentrations in terms of accumulation of soluble phenols.

MATERIALS AND METHODS

Germination and seedling growth:

The seeds of cowpea *Vigna unguiculata* var Elite were obtained from Cereal Disease Research Station, Karachi University Campus. Different concentrations (25,50,100 and 200 ppm) of chromium, cadmium, lead and zinc were prepared using their salts $CrCl_3$, $CdCl_2$, $PbCl_2$, and $ZnCl_2$ using deionized distilled water. Germination and early seedling growth were tested in 9 cm diameter sterilized plastic Petri plates in which two discs of Whatman No.1 filter paper were placed. Ten surface sterilized seeds (using 0.3 percent calcium hypochlorite) of cowpea *Vigna unguiculata* (L.) Walp. Var. Elite were place on filter paper in Petri plates 6 ml of aqueous solutions of the salts mentioned above. All concentrations were based on the proportion of heavy metals in the salts. Distilled water served as controls. The Petri plates were kept at 26±2°C day temperature and 18±2°C night temperature. Light intensity at the top of Petri plates was 2 Klux. The plates were kept wet throughout the experiment. Small amounts of respective solutions were added periodically when it was obvious that Petri plates were beginning to dry out . Small amount of distilled water was added in the controls

The germination percentage was recorded daily and it was considered as completed after 7 days when there was no chance for further germination. After completion of germination, root and shoot lengths were measured. The data were subjected to appropriate statistical analysis (Zar, 1999). A 50% tolerance level (TL_{50}) at which shoot growth reduced to 50% was computed following Davis *et al*., (1972) and Shaukat *et al*., (1999) as follows:

$$TL_{50} = C_1 + [(C_2 - C_1)(50 - P_1))/(P_2 - P_1)$$

Where, C_1=highest concentration giving less than 50% growth reduction, C_2 = lowest concentration giving more than 50% growth reduction, P_1 = percentage growth at C_1 and P_2= percentage growth at C_2. Since root growth was more sensitive to the phytotoxic action of heavy metals, it was used as the criterion of growth response.

Soluble phenols

Soluble phenol contents were ascertained at 7 days after treatments. Levels of soluble phenols in seedlings were determined in accordance with Dihazi *et al*., (2003). Seedlings (500 mg) were taken from each treatment and control plant and homogenized in an ice bath with 2ml 80% ethanol v/v. The homogenate was centrifuged three times at 6000 g for 3 min. One hundred µl of the supernatant was added to Folin-Ciocalteau reagent (0.5 ml) and 1 ml saturated sodium carbonate. The mixture was incubated at 40° C for 30 min. and the absorbance of the developed blue colour was read at 725 nm. Catechol was used as standard. The amount of soluble phenols was expressed as µg g^{-1} fresh weight. All analyses were performed using samples from five replicates.

Statistical analysis

Data were subjected to statistical analysis following Zar (1999). One-way analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) was performed appropriate to the experimental design used. Post-hoc test was conducted using multiple comparisons. However, instead of the original probabilities, Bonforoni modified probabilities were used for the tests.

RESULTS

1) *Effect on germination*

The final germination percentage was significantly reduced (P at the most 0.050) by most of the heavy metals at 50 and 100ppm (Fig.1). Drastic reduction in the final percentage germination occurred at 100ppm. Germination percentage was suppressed by different metals in the order Cd > Cr > Pb > Zn.

2) *Effect on seedling growth*.

Root growth was significantly (P at the most 0.01) suppressed by all the heavy metal at all the concentrations (Table 1). The degree of root growth reduction increased with increasing concentration. Root growth was inhibited by the metals in the order Cd > Cr > Pb > Zn. Shoot growth of the seedling was also significantly (P at the most 0.05) retarded by the heavy metals at all the concentrations except 25 ppm. Shoot growth was inhibited in the heavy meats in the order Cd > Cr > Pb >Zn. The suppressive effect was greater on root growth compared to shoot growth. TL_{50} values for Cd, Cr, Pb and Zn, on the basis of seedling growth were 53.07, 55.86, 70.75 and 81.24 ppm, respectively.

3) *Effect on soluble phenols*.

Soluble phenol content of the seedlings was remarkably elevated by the metal salts at 50 and 100 ppm concentrations ($P < 0.001$) (Fig. 2). However, at 25 ppm the soluble phenol content remained unaltered by the heavy metals. Soluble phenol content of seedling increased with increasing concentrations of heavy metals. Phenol accumulation by the metals occurred in the order: Cd > Cr > Pb >Zn.

Table 1. Effect of heavy metals on root and shoot growth of *Vigna unguiculata* seedlings.

Treatments	**Root length (cm)**	**Shoot length (cm)**
Cadmium		
Control	8.7 ± 1.2	5.6 ± 0.5
25 ppm	5.2 ± 0.7	4.9 ± 0.4
50 ppm	4.5 ± 0.6	4.2 ± 0.4
100 ppm	2.1 ± 0.3	2.5 ± 0.2
Chromium		
Control	8.9 ± 1.8	6.0 ± 0.4
25 ppm	5.8 ± 0.6	5.3 ± 0.5
50 ppm	4.7 ± 0.4	4.4 ± 0.3
100 ppm	2.0 ± 0.4	2.8 ± 0.5
Lead		
Control	8.5 ± 1.5	5.8 ± 0.4
25 ppm	6.1 ± 8.0	5.2 ± 0.3
50 ppm	5.0 ± 0.7	4.6 ± 0.45
100 ppm	3.2 ± 0.6	2.9 ± 0.5
Zinc		
Control	8.8 ± 1.2	5.6 ± 0.55
25 ppm	6.5 ± 0.8	5.4 ± 0.45
50 ppm	5.4 ± 0.6	4.7 ± 0.4
100 ppm	3.8 ± 0.5	3.2 ± 0.5

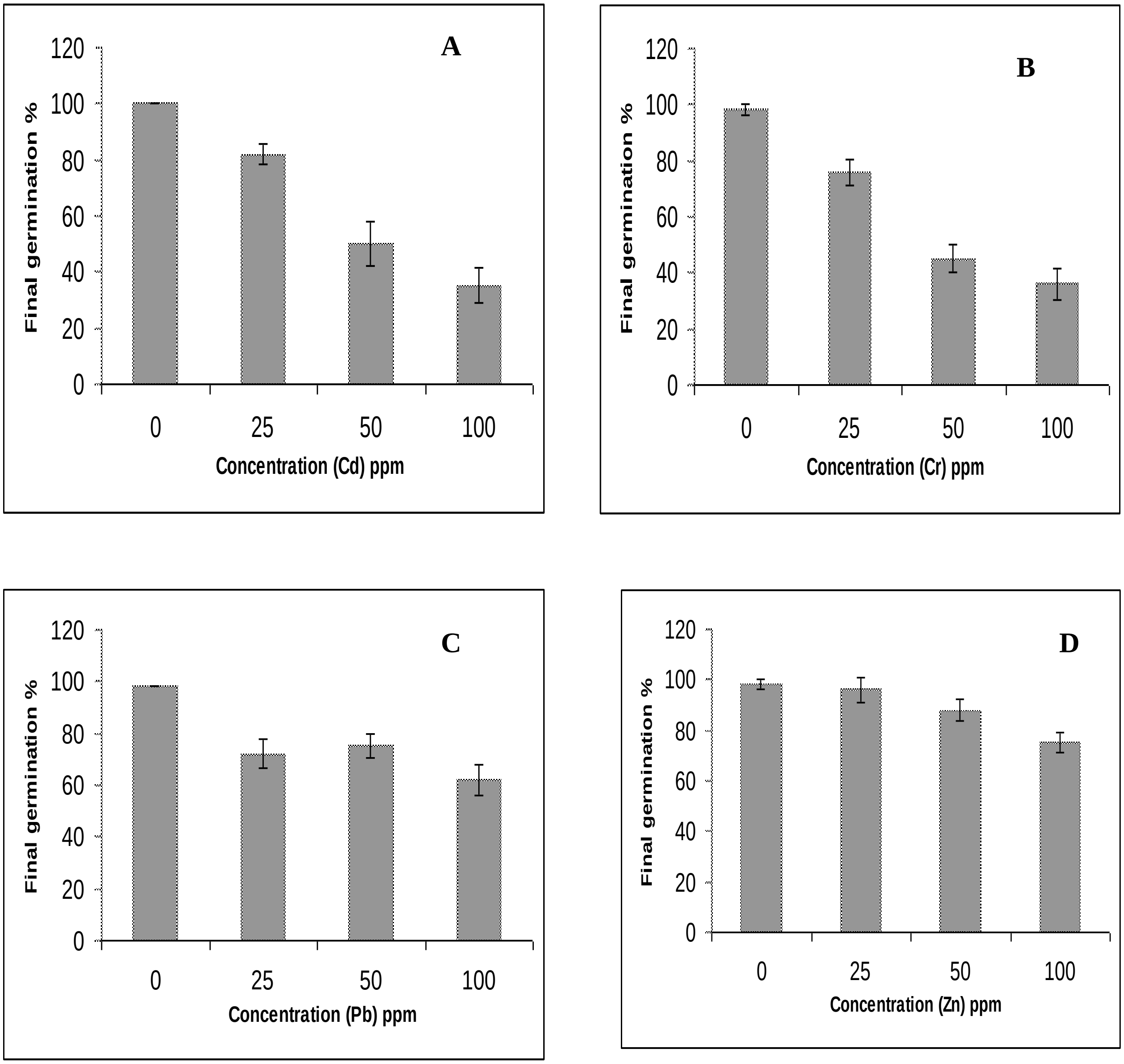

Fig. 1. Effect of heavy metals on the germination of *Vigna anguiculata* . A (Cd), B (Cr), C (Pb), D (Zn).

DISCUSSION

All the metals tested including cadmium, chromium, lead and zinc clearly have inhibitory effect on germination and early seedling growth of the test species, *i.e.*, cowpea (*Vigna unguiculata*). Suppression of germination due to heavy metals has been reported by many workers (Al-Helal, 1995; Shaukat *et al*, 1999; Burhan *et al.*, 2001).

Cadmium is shown to be most inhibitory to germination of many test plant species (Fargasova, 1994; Shaukat *et al*., 1999; Peralta *et al*, 2001; Shafiq *et al*., 2008). However, working with *Prosopis juliflora*, Khan (2007) found no detrimental effect of cadmium up to concentration of 400 ppm on the basis of overall germination. It appears that cadmium toxicity at germination level is a species-specific phenomenon (Khan, 2007)

Chromium is also shown to be highly toxic to the process of seed germination (Shaukat *et al*., 1999; Peralta *et al*., 2001). Generally, lead, as compared to chromium, has been shown to be less inhibitory to germination (Shaukat *et al.*, 1999; Burhan *et al*., 2001; Shafiq and Iqbal 2006). Zinc was found to be least inhibitory to germination compared to other heavy metals tested. Zinc has been shown to be less inhibitory to germination compared to lead, cadmium and chromium (Burhan *et al*., 2001). Anwar *et al*., (2000) observed zinc tolerance in pasture and mine populations of *Anthoxanthum odoratum.* Several workers have demonstrated the presence of heavy metals like Cd, Cr, Pb, Zn, Cu and Ni in sewage and industrial effluents (Smith, 1997). The germination of seeds in soils receiving such effluents is likely to be inhibited by the metal ions. Reduction of seed germination can be caused by ionic toxicity of heavy metals or it could be due to osmotic pressure of the salt solution (Shaukat *et al*., 1999). Decreased levels of auxins due to their destruction by the toxic metal ions could also be responsible for reduced germination and/or early seedling growth (Mukharji & Das, 1972). Reduction in germination as well as early seedling growth may also be attributed to changes in permeability and selectivity properties or the impairment of the cell membrane system (Fargasava, 1998). Heavy metals in higher concentrations are also responsible for metabolic disorders and therefore reduction in germination and growth for most of the plant species (Fernandez and Henroques, 1991; Krupa *et al*., 1993; Shaukat *et al* 1999; Duo *et al*., 2005).

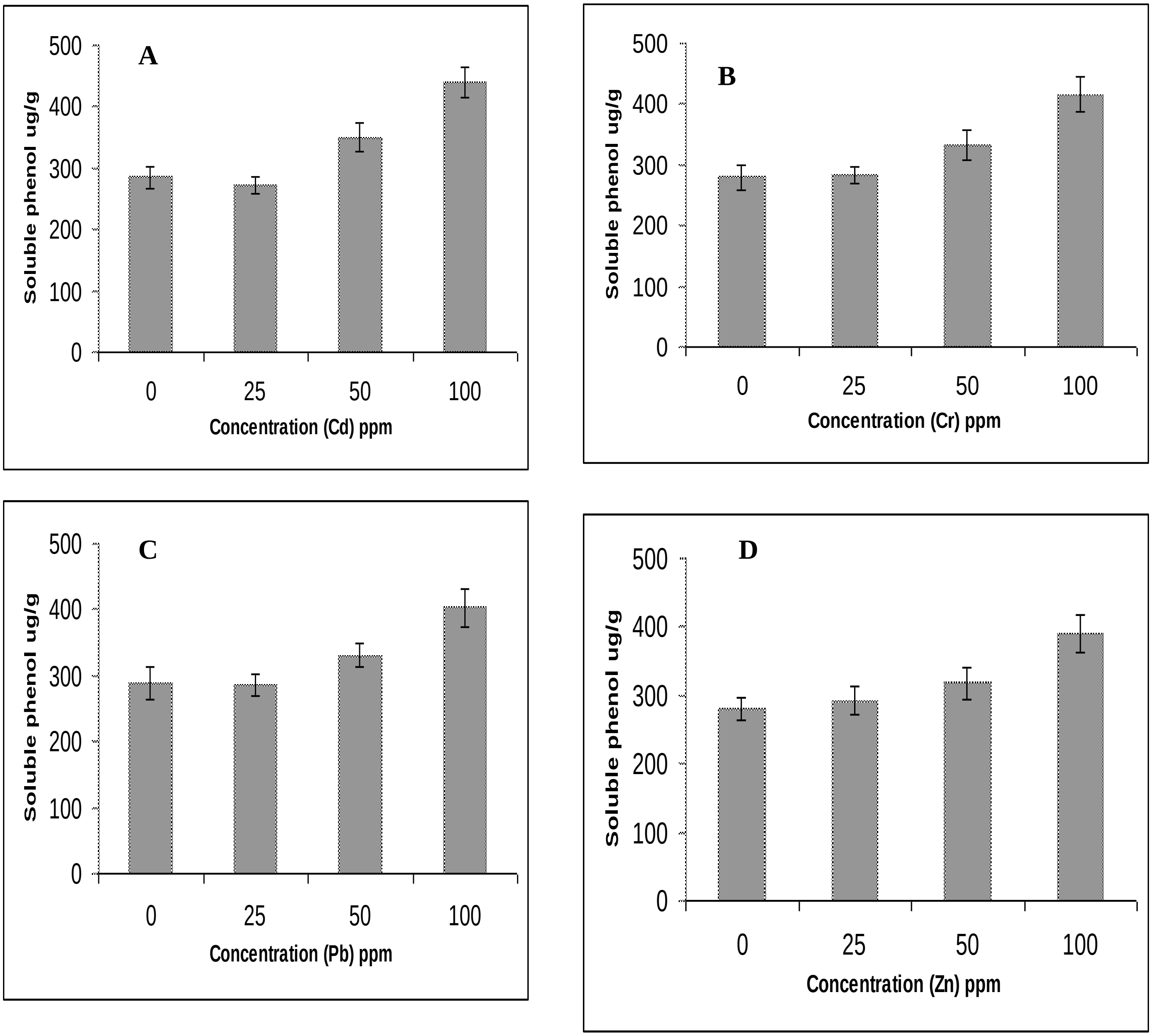

Fig. 2. Effect of heavy metals on the phenolic content of *Vigna anguiculata.* A (Cd), B (Cr), C, (Pb), D (Zn).

Root growth was suppressed to a greater extent than the shoot growth by all the metals tested. The differential response of roots and shoots to heavy metals might be due to greater accumulation of the toxic metal ions in the roots than in the shoots and/or rapid detoxification in the shoots compared to roots (Al-Helal, 1995).

Exposure of cowpea seedlings to heavy metal salt solutions resulted in accumulation of soluble phenols. It has been established that phenol metabolism is activated in plants as a reaction to abiotic stress (Abreu & Mazzafera, 2005; Olenchenko & Zagoskina, 2005; Ganeva & Zozikova, 2007). Shaukat *et al.* (1999) demonstrated that the exposure of plants to heavy metals such as Cd, Cr and Pb results in the accumulation of soluble phenols. Plant phenolics have been regarded as defences against pathogens and herbivores (Dixon & Paiva, 1995) and provide protective mechanism against a variety of abiotic stresses including stress due to heavy metals. Our results provide additional support to this conjecture. Secondary metabolic pathway is physiologically important as it provides the means of channelling and storing carbon compounds, accumulated from photosynthesis during periods when nitrogen is limiting and whenever leaf growth is curtailed. In this connexion, it is noteworthy that the cotyledon and first leaf growth was suppressed by the metal salts as has also been reported in *Brassica rapa* (Ghos *et .al*., 2004) *and Prosopis juliflora* (Khan, 2007). The protective role of phenolics may be due to structural stabilization of cell wall through condensation-polymerization of phenols and quinines. Secondly, they can provide photo-protective mechanism *i.e*., by altering the absorbance of visible and UV-radiation. Thirdly, they act as powerful antioxidant and antiradical agents (Harborne, 1999; Edreva *et al*., 2008)

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