http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (4): 1307-1313, 1999 Research Article

Effect of Cadmium, Chromium and Lead on Seed Germination, Early Seedling Growth and Phenolic Contents of *Parkinsonia aculeata* L. and *Pennisetum americanum* (L.) Schumann

S. Shahid Shaukat, Mohammad Mushtaq and Zamin Shaheed Siddiqui*
Department of Botany, University of Karachi, Karachi-75270,
*Stress physiology and Environmental pollution lab., Karachi, Pakistan.

Abstract
The effect of heavy metals including lead, chromium and cadmium on germination, seedling growth, dry biomass accumulation and phenolic contents of two species viz. *Pennisetum americanum* and *Parkinsonia aculeata* were studied. Chromium and cadmium were applied as chloride while lead was used both as nitrate and chloride. Final germination percentage was greatly reduced by cadmium, chromium and lead salts in both the test species at concentrations of 50 ppm or more. Germination reduction was markedly higher in *Pennisetum americanum* compared to *Parkinsonia aculeata* treated with cadmium and chromium. Lead chloride inhibited germination more severely than did lead nitrate. Both root and shoot growth were also reduced significantly by Cd, Cr and $PbCl_2$. In both the species root growth was inhibited to a greater extent than shoot growth. Phenolic contents were substantially elevated in both the test species following treatment with heavy metals particularly at higher concentrations (200 and 400 ppm). *Parkinsonia aculeata* was less affected in terms of germination, root and shoot growth and dry matter accumulation as compared to *Pennisetum americanum*, exhibiting some degree of tolerance to heavy metals.

Introduction
The principal causes of elevated heavy metal concentration in soils include soil properties, agricultural, manufacturing, mining and waste disposal practices and the use of sewage sludge as fertilizer in agricultural fields (Mahar, 1986). Heavy metal contaminations also occur due to the application of metal containing pesticides and fertilizers on agricultural land (Bentine and Goldberg, 1977). Heavy metals like chromium, cadmium, lead and mercury are used extensively in industry (Raihan *et al.*, 1995). Chromium is used in chrome plating, steel and alloy manufacturing, leather tanning, film and photography equipment and metal cleaning.

Lead is released in the environment by mining, smelting, refining, lead based products, pesticides, vehicular exhaust and burning of coal and industrial rubbish (Dix, 1981). Roadside environment are grossly contaminated with lead as motor vehicles combusting gasoline containing lead alkyl release approximately 80 mg lead per km driven. Cadmium salts are commercially used as lubricant, as ice nucleating agents, in dry cell batteries, in photography, dyeing, calico printing, electroplating and engraving, and in the manufacture of special mirrors (Commission of the European Communities, 1978). Heavy metals are known to cause several toxic effects on plants such as inhibition of seed germination (Iqbal *et al.*, 991; Iqbal and Siddiqui, 1992; Al-Helal, 1995) reduction of plant growth and yield Iqbal *et al.*, 1991; Mushtaq, 1996) and altering normal metabolic pathway including respiration and photosynthesis by disrupting cellular enzymes (Tomasevic *et al.*, 1991; Krupa *et al.*, 1993).

Plant species, commercial varieties and cultivars of crop plants and ecotypes vary widely in their tolerance to different metals (Al-Helal, 1995). Though mechanism of heavy metal tolerance in plants has been studied in several species yet, no specific mechanism has so far been elucidated (Baker and Walker, 1990). Verkleij and Schatt (1990) in a review concluded that the present knowledge only permits comprehensive superficial and tentative mechanisms of heavy metal tolerance in plants. Apparently there is little information available pertaining to the effect of heavy metals on germination and growth of wild and crop plants in Pakistan (Iqbal *et al.*, 1991; Iqbal and Siddiqui, 1992).

The objective of the present study was to investigate the effect of cadmium, chromium and lead on germination and early seedling growth of a crop plant pearl millet (*Pennisetum americanum* (L.) Schuman) and a tree species *Parkinsonia aculeata* L., and to study the effect of heavy metal stress on the chosen plants in terms of production of total phenols, as it is known that toxic chemicals and various kinds of stresses lead to elevated levels of phenols in plants (Reid *et al.*, 1992).

Materials and Methods
Seeds of *Pennisetum americanum* var. White hegari were obtained from Cereal Disease Research Institute, Karachi University Campus, while that of *Parkinsonia aculeata* were collected from an old-field at Karachi University Campus. The seeds of *Parkinsonia aculeata* were scarified using sand paper because of their hard coat.

Different concentrations of lead chloride, cadmium chloride, chromium chloride and lead nitrate including 25, 50, 100, 200 and 400 ppm were prepared in deionized distilled water. Germination was performed in 9 cm diameter sterilized petri plates having Whatman No. 1. filter paper. Twenty surface sterilized seeds with 0.1 per cent sodium hypochloride of *Pennisetum americanum* and fifteen seeds of *Parkinsonia aculeatai* were placed on filter paper in sterilized petri plates containing 5 ml of an aqueous solution $PbCl_2$, $CdCl_2$, $CrCl_3$, and Pb $(NO_3)_2$, 25, 50, 100, 200 and 400 ppm. All concentrations were based on the proportion

of heavy metals in the salts. Deionized distilled water was used for control. The treatments were replicated thrice. The petri plates were kept in the laboratory at 26±2°C day temperature and 18±2°C night temperature light intensity at the top of petri plates was 2000 lux (14 day length). Small amounts of solutions were added when it was obvious that petri dishes were beginning to dry out.
The rate of germination was recorded daily and it was considered completed after 8 days when, there was no chance for further changes. A seed was considered germinated when the radicle had attained a length of not less than 1.5 mm (Taylor, 1942). After the completion of seed germination, root and shoot of all germinated seedlings were measured, placed in oven at 80°C for 24 hours and dry weights recorded.
A 50 per cent tolerance level (TL_{50}) the concentration at which shoot growth was reduced to 50 per cent was computed using formula adduced by Davis *et al.* (1972) 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 per cent growth reduction, C_2 = lowest concentration giving more than 50 per cent growth reduction, P_1 = percentage growth at C_1 and P_2 = percentage growth at C_2.
The phenolic contents of entire seedlings were measured using Folin-Ciocalteu reagent (1:1) by the method of Swain and Hills (1959) after preparing the extract in 1N HCl. The data sets were subjected to factorial analysis of variance. All percentage data were transformed using an arcsine function before analysis (Steel and Torrie, 1980). Programs FANOVA (factorial analysis of variance), TRANS (data transformation) and TOLR (TL_{50}) were written in GWBASIC for microcomputers by the first author and are available on request.

Results

Germination:

Effect of lead chloride and lead nitrate on seed germination of *Pennisetum americanum*: Lead chloride caused greater reduction in germination of *Pennisetum* than did lead nitrate (Fig. 1a,b). Germination was inhibited ($p<0.05$) significantly by lead chloride at 100 ppm onwards and there was no significant inhibition at lower concentration i.e., at 25 ppm and 50 ppm. On the other hand, lead nitrate significantly affected seed germination only at 200 and 40 ppm ($p<0.01$).

Effect of chromium chloride on seed germination of *Pennisetum americanum*: Chromium chloride showed marked toxicity and significantly inhibited seed germination of *Pennisetum* at all concentrations over the control ($p<0.001$). Germination was completely inhibited at 400 ppm (Fig. 1c). The inhibitory effect of chromium chloride on germination was significantly greater than that of lead chloride and lead nitrate ($p<0.001$).

Effect of cadmium chloride on seed germination of *Pennisetum americanum*: Cadmium chloride had more drastic effect on seed germination of *Pennisetum* than chromium and lead. There was significant inhibition at 25 pm onwards, while at 200 ppm and 400 ppm no germination was observed (Fig. 1d).

Effect of lead chloride and lead nitrate on seed germination of *Parkinsonia aculeata*: *Parkinsonia aculeata* exhibited some degree of tolerance to lead nitrate and chloride compared to *Pennisetum americanum* (Fig. 2a, b). Lead chloride delayed and inhibited seed germination significantly ($p<0.05$) at 50 ppm onwards over the control, while lead nitrate affected the rate of germination though the final percentages remained unaffected.

Effect of chromium chloride on seed germination of Parkinsoni *aculeata*: Chromium chloride inhibited seed germination more adversely than lead chloride or nitrate. Germination was significantly inhibited by chromium chloride at concentrations of 50 ppm and more ($p<0.01$) (Fig. 2c).

Effect of cadmium chloride on seed germination of Parinsonia *aculeata*: Cadmium significantly inhibited seed germination of parkinsonia at concentration of 200 ppm and 400 ppm ($p<0.01$). *Parkinsonia* exhibited greater tolerance to cadmium chloride compared to chromium chloride (Fig. 2d). *Parkinsonia* exhibited marked tolerance to cadmium with regard to germination, compared to *Pennisetum*.

Seedling growth

Effect of lead chloride and nitrate on seedling growth of parkinsonia and *Pennisetum*: Lead chloride adversely affected seedling growth of both *Pennisetum* and *Parkinsonia* but leads nitrate had no significant effect on root or shoot growth (Fig. 3a,b,c,d). Greater effect of lead chloride was observed on *Pennisetum* as compared to *Parkinsonia* ($p<0.05$). *Pennisetum* root growth was significantly ($p<0.001$) inhibited at 200 and 400 ppm of lead chloride, whereas shoot growth was inhibited at all concentrations ($p<0.05$). Root growth of *Parkinsonia* was significantly reduced by lead chloride at 100 ppm onwards while shoot growth was not significantly retarded at any of the concentrations.

Effect of cadmium chloride on seedling growth of *Pennisetum* and *Parkinsonia*: Cadmium chloride showed marked inhibitory effect on the seedling growth of both the species. *Pennisetum* was found more susceptible to cadmium toxicity than *Parkinsonia*. In *Pennisetum* both shoot and root growth were significantly reduced at all concentrations ($p<0.001$) and there was no growth at 200 ppm and 400 ppm whereas in *Parkinsonia* root cadmium was inhibited at all concentrations, but shoot growth was reduced at 50 ppm onwards (Fig. 4a, b).

Effect of chromium chloride on seedling growth of *Pennisetum* and *Parkinsonia*: Chromium chloride caused greater inhibition of seedling growth in *Pennisetum* as compared to *Parkinsonia* (Fig. 5a,b). In *Pennisetum* root length was significantly retarded at all concentrations ($p<0.001$) over the controls while shoot growth decreased significantly at 50 ppm and 200 ppm ($p<0.05$). There was no growth at 400 ppm. *Parkinsonia* exhibnited some tolerance to chromium chloride; both root and shoot growth were significantly inhibited only at 400 ppm.

Seedling dry weight

Effect of lead chloride and nitrate on the dry weight accumulation of *Pennisetum* and *Parkinsonia*: Lead chloride significantly decreased the dry weight accumulation in *Pennisetum* at all concentrations over the control, but the reduction was more pronounced at 200 ppm and 400 ppm. In *Parkinsonia* significant reduction was observed at all concentration, but there was equal reduction at 200 ppm and 400 ppm (Table 1). Lead nitrate reduced significantly the dry biomass of both species at all concentrations but the adverse effect was more pronounced in case of *Pennisetum* (Table 2).

Effect of chromium chloride on the dry biomass accumulation of *Parkinsonia* and *Pennisetum*: Chromium chloride showed much more drastic effect on dry biomass accumulation of pennisetum compared to *Parkinsonia* (Table 3). In *Pennisetum* the dry biomass accumulation was significantly decreased ($p<0.01$) at all concentration over controls while at 200 ppm the reduction was greater than at other concentrations and no germination occurred at 400 ppm. *Parkinsonia* exhibited some tolerance to chromium chloride and dry weight reduction occurred at 100 ppm onwards over the control.

Effect of cadmium chloride on the dry biomass accumulation of *Pennisetum* and *Parkinsonia*: *Pennisetum* was highly susceptible to cadmium chloride toxicity and significant reduction occurred in dry weight (p at the most 0.001). *Pennisetum* was much more sensitive to cadmium chloride as compared to chromium chloride. *Parkinsonia* showed remarkable tolerance to cadmium and dry biomass accumulation was reduced at 200 and 400 ppm only (Table 4).

Phenolic contents: In general heavy metals elevated the phenolic contents over the controls in the seedlings of both *Pennisetum* and *Parkinsonia* and greater increase occurred at high concentrations (6a, b). Both species showed more or less same trend with respect to phenolic contents. Lead chloride caused greater increase of phenolic content than lead nitrate. Chromium and cadmium usually induced greater levels of phenols compared to lead.

TL_{50} Values: For *Pennisetum americanum* TL_{50} values ranged from 13.18 to >400 ppm for different compounds (Table 5). For *Pennisetum* it was in the order.

$$CdCl_2 < CrCl_3 < PbCl_2 < Pb(NO_3)_2$$

In case of *Parkinsonia* TL_{50} ranged between 38.88 to >400 ppm. The order of TL_{50} for *Parkinsonia* was

$$CdCl_2 < PbCl_2 < CrCl_3 < Pb(NO_3)_2$$

The results show that $CdCl_2$ had lowest TL_{50} value and $Pb(NO_3)_2$ had the highest TL_{50} value in both the test species.

Discussion

The salts of all three heavy metals lead, chromium and cadmium had an inhibitory effect on germination as well as early seedling growth of the test species. Inhibition of seed germination by the heavy metals has often been reported (Iqbal *et al.*, 1991; Al-Helal, 1995). The inhibitory effect of salts on seed germination could be the result of ionic toxicity (Redman, 1974) or due to an osmotic effect (Micheal *et al.*, 1972) or it could be due to decreased levels of auxin resulting from enhance destruction of auxin by the metal ions (Mukherji and Das Gutpa, 1972).

Plant species vary widely in their tolerance to different metals. In addition, cultivars and populations within the same species differ widely in tolerance to excess heavy metals (Ryan *et al.*, 1992; Joyo and Kazi, 1993). Varying results were obtained for the different concentrations of lead chloride, lead nitrate, chromium chloride and cadmium chloride on the percentage of germination, seedling growth, dry biomass and phenolic content of *Parkinsonia aculeata* and *Pennisetum americanum.* It was obvious that cadmium chloride and chromium chloride were much more phytotoxic to *Pennisetum* as compared to *Parkinsonia* Lead chloride affected germination and seedling growth more strongly than did lead nitrate. *Parkinsonia* exhibited lesser reduction in all growth parameters relative to *Pennisetum*. Thus *Parkinsonia* seems to exhibit tolerance and co-tolerance to heavy metals. It grows mostly in the polluted areas, such as sewage farm, industrial areas, etc. In Karachi it grows usually on roadsides and highly polluted areas. Therefore, over the years it has developed resistance to heavy metals. The environmental pollution problem specifically land pollution due to sewage sludge in industrial areas where the sludge often contains large amounts of heavy metals like Cd, Cr, Pb, Zn, Cu and Ni is well known and documented (Iqbal *et al.*, 1991; Area *et al.*, 1996).

Pennisetum is a species grown in fields that are less polluted particularly with respect to heavy metals. It has no ability to tolerate the heavy metals. Therefore, it was highly susceptible to heavy metals. Germination and seedling growth of *Pennisetum* were greatly reduced by the application of lead chloride, chromium chloride and cadmium chloride, whereas lead nitrate caused lesser toxicity. Cadmium chloride toxicity was considerably more significant in both *Pennisetum* and *Parkinsonia* as compared to other heavy metals. Chromium chloride was also highly toxic and inhibitory to germination and growth in both the species, though more so in *Pennisetum*. Mechanisms of metal tolerance have been studied in several species yet, no clear mechanism of tolerance has been elucidated so far (Baker and Walker, 1990; Verkleji and Schat, 1990). though some mechanism have been postulated. The most

Table 1: Effect of lead chloride, lead nitrate, chromium chloride and cadmium chloride on seedling biomass of *Pennisetum americanum* and *Parkinsonia aculeata*

Concentration (ppm)	Dry biomass root and shoot			
	Lead chloride	Lead nitrate	Chromium chloride	Cadmium chloride
	Pennisetum americanum			
0	0.04605±0.002	0.16910±0.013	0.0392±0.005	0.0652±0.0083
25	0.03293±0.001	0.11400±0.018	0.0328±0.002	0.0220±0.0040
50	0.03066±0.001	0.09337±0.007	0.0299±0.003	0.0159±0.0020
100	0.02766±0.003	0.08780±0.003	0.0263±0.004	0.0152±0.0040
200	0.02960±0.001	0.06920±0.001	0.0148±0.008	0.0000±0.0000
400	0.01556±0.002	0.06100±0.002	0.0000±0.000	0.0000±0.0000
	Parkinsonia aculeata			
0	0.7853±0.052	0.6920±0.032	0.6290±0.052	0.86110±0.073
25	0.6615±0.043	0.6135±0.046	0.5832±0.061	0.77739±0.094
50	0.6420±0.056	0.5952±0.038	0.5280±0.093	0.78540±0.134
100	0.5992±0.048	0.5623±0.053	0.5245±0.135	0.78280±0.058
200	0.5615±0.037	0.5550±0.091	0.5100±0.086	0.72460±0.072
400	0.5600±0.054	0.4820±0.042	0.4820±0.148	0.69630±0.110

Table 2: TL_{50} values (ppm) of different heavy metal salts in *Pennisetum americanum* and *Parkinsonia aculeata*

Compounds	*Pennisetum americanum*	*Parkinsonia aculeata*
Lead chloride	297.2	95.12
Lead nitrate	>400	>40
Chromium chloride	16.23	374.6
Cadmium chloride	13.38	38.88

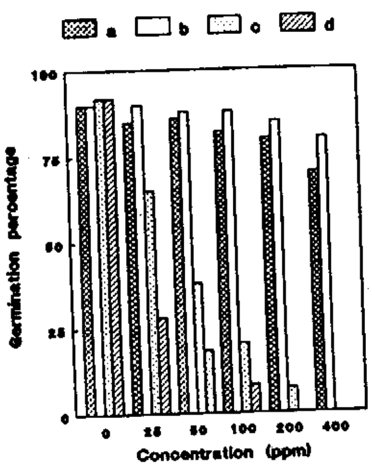

Fig. 1: Effect of heavy metals on germination of *Pennisetum americanum*; (1a) lead chloride, (1b) lead nitrate, (1c) chromium chloride, (1d) cadmium chloride.

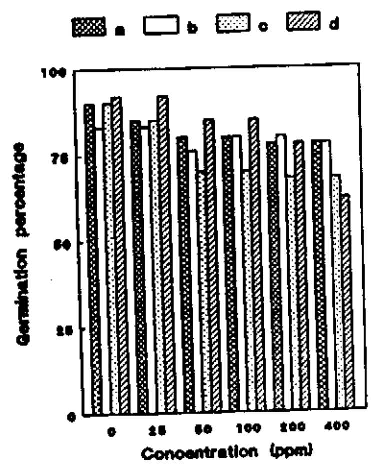

Fig. 2. Effect of heavy metals on germination *Parkinsonia aculeata*; (2a) lead chloride, (2b) nitrate, (2c) chromium chloride, (2d) cadm chloride.

widely proposed mechanism according to Mehrag (19 are: (1) biochemical detoxification, (2) compartmentaliza of the metal within the cell, (3) limited uptake of ions; heavy metal are taken up by tolerant than non-tol plants, (4) restricted transport from the root to the sho (5) formation of metal binding polypeptides, (6) chealtin organic.acids and (7) the plasmalemma may have a ro metal tolerance.

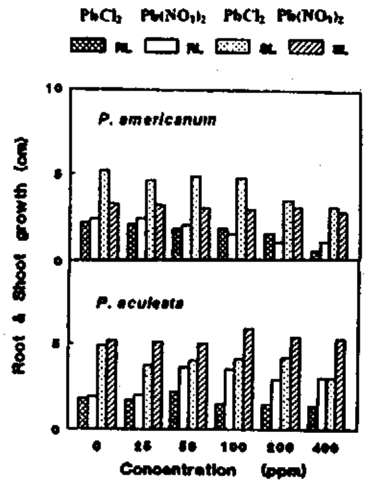

Fig. 3. Effect of lead chloride and lead nitrate on seedling growth; Pennisetum *americanum*, *Parkinsonia aculeata*.

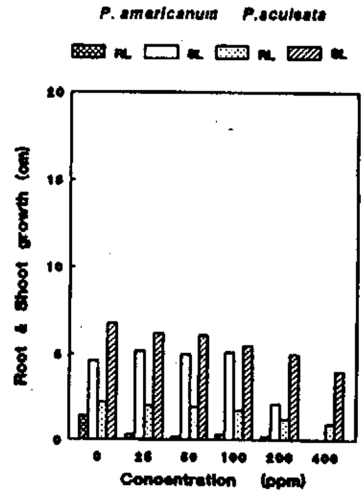

Fig. 5: Effect of chromium chloride and lead nitrate on seedling growth; *Pennisetum americanum*, *Parkinsonia aculeata*.

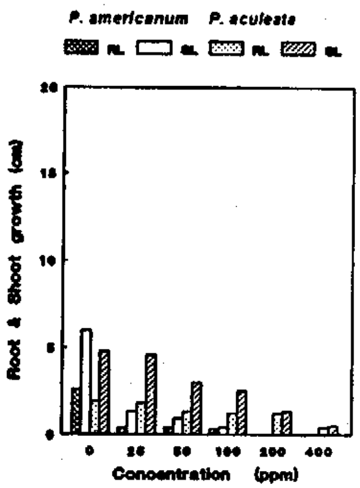

Fig. 4. Effect of cadmium chloride and lead nitrate on seedling growth; *Pennisetum americanum*, *Parkinsonia aculeata*.

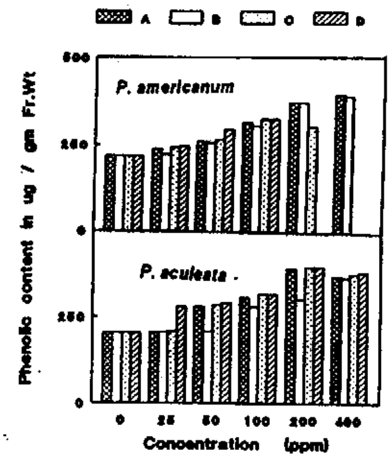

Fig. 6: Effect of heavy metals on total phenolic content; *Pennisetum americanum*, *Parkinsonia aculeata*. Symbol stand for A = Lead nitrate, B = Chromium chloride, C = Lead chloride, D = Cadmium chloride

The results also suggest that radicle elongation of *Pennisetum* and *Parkinsonia* was effected more adversely by the heavy metals than shoot growth. Similar results have been reported by some other workers (Brown and Wilkins, 1986). The reason for the differential response of root and shoot to heavymetals is not known but it might be due, in part, to more rapid accumulation of heavy metals in root than in shoot, or to a faster rate of detoxification in the shoot compared to root (Al-Helal, 1995). Dayton and Lewin (1975) reported that lead affects the metabolism indirectly by causing phosphate deficiency. Shulz-Baldes and Lewin (1976) suggested that lead arrests cell division and uptake of essential elements as it deposits on the cell membrane. Tolerance levels of Solanum nigrum to heavy metal have been analyzed by Joyo and Kazi (1993). They found that Solanum nigrum was tolerant to heavy metals like chromium, nickel, lead, copper, cadmium as well as some trace elements such as iron, sodium, potassium, calcium and magnesium. Due to the presence of these toxic metals in Solanum nigrum it becomes toxic to other living organisms. However, no particular mechanism was suggested.

It is known that stress conditions elevate the phenolic contents of plants (Reid *et al.*, 1992; Siddiqui and Ahmed, 1996). The enhanced phenolic content in the test species could be such a response. Thus high levels of phenolic compounds, particularly at higher concentrations of the heavy metal could be at least in part responsible for germination and growth inhibition. Phenolic acids have been shown to exert dramatic effect on membrane permeability and membrane electrical potentials (Glass, 1974; Glass and Dunlop, 1974) which are remarkably similar to the effects of uncoupling agent such as dinitrophenol (DNP). It is likely that phenolic compound caused enhanced rates of oxygen consumption by uncoupling electron transfer. Whether this is the primary effect of phenolic compound or simply a general manifestation of impaired membrane function has to been ascertained. However, it is known that membrane system plays a role in the metal tolerance mechanism presumably by the extent to which it is damaged or its function is altered.

On the basis of this study it could be concluded that seed germination, seedling growth and dry biomass were reduced while phenolic compounds elevated of both the test species by different concentrations of lead chloride, chromium chloride, cadmium chloride and lead nitrate and that *Parkinsonia* was found more tolerant species than *Pennisetum*. The experiments suggest much greater toxicity for cadmium and chromium compared to lead.

References

Al-Helal, A. L., 1995. Effect of cadmium and mercury on seed germination and early seedling growth of rice and alfalfa. J. Univ. Kuwait. (Sci.), 22: 76-82.

Ara, F., M. Z. Iqbal and M. S. Qureshi, 1996. Determination of heavy metals contamination of trees and soils due to vehicular emission in Karachi city. Kar. Univ. J. Sci., 24: 80-84.

Bache, C. A., D. J. Lisk and G. T. Doss, 1985. Cadmium and nickel in main stream particulates of cigarettes containing tobacco grown on low cadmium soil sludge mixture. Cornell Univ. J. Toxicol. Environ. Health, 16: 547-552.

Baker, A. J. M. and P. L. Walker, 1990. Ecophysiology of heavy metal uptake by tolerant plants. In: Shaweid, A. J. (Ed.). Heavy Metal Tolerance in Plant. Evolutionary Aspect. CRC Press, Boca Raton, pp: 155-177.

Baura, I., M. Basalle, H. J. Sasadhar and K. Gupta, 1986. Effect of heavy metals on germination and seedling growth of gram (Cicer aurietinum). Enivron. Ecol., 4: 300-303.

Commission of the European Communities, 1978. Criteria (dose/effect relationship) for cadmium. Report of a working group of experts prepared for the Commission of European Communities, pp: 15-23.

Davis, F. S., A. Villarreal, J. R. Baur and I. S. Goldstein, 1972. Herbicidal concentration of picloram in cell culture and leaf bud. weed Sci., 20: 185-188.

Dayton, L. and R. A. Lewin, 1975. The effect of lead on algae. III. Effect of Pb on population growth curves in two-membered cultures of phytoplankton. Arch. Hydrobiol. (Suppl.), 49: 25-36.

Dix, H. M., 1981. Environmental Pollution. Wiley, New York.

Foy, C. D., R. L. Chaney and M. C. White, 1978. The physiology of metal toxicity in plants. Ann. Rev. Pl. Physiol., 29: 511-566.

Glass, A. D. M., 1974. Influence of phenolic acids upon ion uptake. III. Inhibition of potassium absorption. J. Exp. Bot., 89: 1104-1113.

Glass, A. D. M. and J. Dunlop, 1974. Influence of phenolic acids on ion uptake. IV. Depolarization of membrane potentials. Plant Physiol., 54: 855-858.

Goldblod, D. L. and A. Huttermann, 1985. Effect of zinc, cadmium and mercury on root elongation of Picea abies Karst, seedling and the significance of these metals to forest dieback. Environ. Pollution (Series A), 38: 375-385.

Iqbal, M. Z., M. T. Mahmood and F. Ahmed, 1991. Influence of cadmium toxicity on germination and growth of some common tress. Pak. J. Sci. Ind. Res., 34(4): 140-142.

Iqbal, M. Z. and A. D. Siddiqui, 1992. Effects of lead toxicity on seed germination and seedling growth of some tree-species. Pak. J. Sci. Ind. Res., 35 (4): 149-150.

Joye, M. and T. G. Kazi, 1993. Determination of trac elements in Solanum nigrum by atomic absorptio spectroscopy. Sind Univ. Res. J. (Sci. Sr.), 25 (1 & 2 29-33.

Krupa, Z., O. Gunnar and N. P. A. Humer, 1993. Th effects of cadmium on photosynthesis of Phaseolu vulgris: a fluorescence analysis. Physiol. Plant, 3 626-630.

Mehrag, A. A., 1993. The role of the plasmalemma in met tolerance in angiosperms. Physiologia Pl., 88: 19 198.

Micheal, D., D. Williams and L. A. Unagar, 1972. The effe of environmental parameters on the germination development of Suaeda depressa (pursch) Wats. Ame J. Bot., 59: 912-918.

Mukherji, S. and B. Das Gupta, 1972. Characterization copper toxicity in lettuce seedlings. Physiol. Pl., 2 126-129.

Mukherji, S. and P. Maitrá, 1977. Growth and metabolis of germinating rice (*Oryza sativa* L.) seeds influenced by toxic concentration of lead. Z. P Physiol., 81: 26-33.

Nasu, Y., M. Kugmoto, O. Tanaka and A. Takimoto, 19 Comparative studies on the absorption of cadmium copper in Lemma paucicostata. Environ. Pollut. (S A), 32: 201-209.

Pollard, A. J., 1980. Diversity of metal tolerances in Plantago lanceolata L. from the southestern United States. New Phytol., 8: 109-117.

Prasad, D. D. K. and A. R. K. Prasad, 1987. Effects of lead and mercury on chlorophyll synthesis in mung bean seedling. Phytochemistry, 26: 881-884.

Rauser, W. E., 1973. Early effects of phytotoxic burdens of cadmium, cobalt, nickel and zinc in white bean. Can. J. Bot., 56: 1744-1749.

Raihan, S., F. Sarwar, M. Azim, O. Y. Khan and N. Ahmed, 1995. Isolation, characterization and assessment of nickel and cadmium accumulation of bacterial isolates from industrial waste. In: Ahmed, N., M. Ishaq, O. Y. Khan and F. Sarwar (Eds.). Biotechnology for Environmental and Agriculture. Centre for Molecular Genetics, University of Karachi.

Redmann, R. E., 1974. Osmotic and specific ion effects on the germination of alfalfa. Can. J. Bot., 52: 803-808.

Reid, L. M., D. E. Mather, J. Arnason, T. Hamilton and R. J. Bolton, 1992. Changes in phenolic constituent of maize silk infected with Fusarium graminerum. Can. J. Bot. 90: 1697-1670.

Ryan, P. R., J. E. Shaff and L. V. Kochain, 1992. Aluminum toxicity in roots. Correlation among ionic currents, ion fluxes and root elongation in aluminum-sensitive and aluminum-tolerant wheat cultivars. Plant Physiol., 99: 1190-1200.

Schulz-Baldes, M. and R. A. Lewin, 1976. Lead uptake in two marine phytoplankton organism. Biol. Bull., 150; 118-127.

Siddiqui. Z. S. and S. Ahmed, 1996. Effect of systemic fungicide Topsin-M (methyl thiophenate) and Bayleton (Triedimefon) on germination, seedling growth and phenolic contents of Vigna radiata. Pak. J. Bot., 28: (2): 191-193.

Steel, G. D. and J. T. Torrie, 1980. Principles and Procedures of Statistics, 2nd ed. McGraw-Hill, New York.

Swain, T. and W. E. Hills, 1959. The phenolic constituents of Prunus domestic. J. Sci. Food Agric., 10: 63-68.

Taylor, I., 1942. Influence of oxygen tension on respiration, fermentation and cadmium kin wheat and rice. Am. J. Bot., 29: 721-738.

Tomasevic, M., M. Bogdanovic and D. Stojanovic, 1991. Influence of lead on some physiological characteristic of bean and barley. Periodium Biologurum, 93: 337-338.

Verkleij, J. A. and H. Shcat, 1990. Mechanisms of heavy metal tolerance in plants. In: Shaw, A. J. (Ed.). Heavy metal tolerance in plants. Evolutionary Aspects. CRC Press, Boca Raton, pp: 179-193.

Wu, L. and J. Antonovics, 1976. Experimental ecological genetics in Plantago. II. Lead tolerance in Plantago lanceolata and Cyndon dactylon from a roadside. Ecology, 57: 205-208.