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Effect of Zinc and Cobalt on Germination and Seedling Growth of *Pennisetum americanum* (L.) Schumann and *Parkinsonia aculeata* L.

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Abstract: The effects of heavy metals cobalt and zinc on germination, seedling growth, and dry biomass accumulation of two species viz., *Pennisetum americanum* and *Parkinsonia aculeata* were investigated. Cobalt and zinc were used as nitrate and chloride. Final germination was reduced greatly at higher concentrations. Germination reduction was markedly higher in *Pennisetum* compared to *Parkinsonia*. Reduction in root growth was relatively greater than the shoot growth. *Parkinsonia* exhibited some degree of tolerance against heavy metals in terms of germination, root and shoot growth and dry biomass accumulation. The mechanisms of tolerance and co-tolerance to heavy metals are discussed.

Key words: Heavy metals, zinc, cobalt, toxicity, tolerance, co-tolerance

Introduction

Heavy metals are generally considered toxic to cells. There are about 50 metals that are of special interest with respect to the toxicological importance, to human health, plants and animals. Use of pesticides and fertilizers, containing metals on agricultural land is a cause of heavy metal contamination (Vlamis *et al.*, 1985). Toxic metals first accumulate in soil to reach the plant through roots or are taken up by the leaves of the plants from atmosphere. High concentrations of zinc have been reported in some plant species growing along the Super Highway near Karachi (Iqbal *et al.*, 1999). Heavy metals can cause various toxic effects on plants such as inhibition of seed germination, plant growth and yield reduction (Shaukat *et al.*, 1999) and alternation of normal metabolic pathways including respiration and photosynthesis by disrupting cellular enzymes (Krupa *et al.*, 1993).

Zinc is an essential element in nutrition and its traces are present in many foods. Many workers have reported the toxic effects of zinc (Lavengood *et al.*, 1999).

Woolhouse (1983) reported the toxicity and tolerance in plants in response to heavy metals including cobalt and zinc. Rauser (1973) also reported the phytotoxic effects of cadmium, cobalt, nickel and zinc. The objectives of this study were to investigate: (1) the effect of cobalt and zinc on germination and early seedling growth of a crop plant *Pennisetum americanum* L. Schuman and a tree species *Parkinsonia aculeata* L., (2) the tolerance levels to heavy metals in the two test species.

Materials and Methods

The seeds of *Pennisetum americanum* were obtained from Cereal Disease Research Institute, Karachi University Campus, while that of *Parkinsonia aculeatea* were collected from the University Campus. Because of their hard seed coat, the seeds of *P. aculatea* were scarified using sand paper.

Different concentrations (25, 50, 100, 200 and 400ppm) of $CoCl_2$, $Co(NO_3)_2$, $ZnCl_2$, $Zn(NO_3)_2$ were prepared with distilled water. Germination was investigated in 9cm (diameter) sterilized Petri plates having Whatman No. 1 filter paper. Twenty surface sterilized seeds (with 0.3 percent calcium hypochlorite) of *Penisetum americanum* and 15 seeds of *Parkinsonia aculatea* were placed on filter paper in Petri plates containing 5 ml of aqueous solution of $CoCl_2$, $Co(NO_3)_2$, $ZnCl_2$ and $Zn(NO_3)_2$. All concentrations were based on the proportion of heavy metals in the salt. Distilled water was used for controls. The treatments were replicated four times each. 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 2Klux. 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, while small amount of distilled water was added in the controls.

The rate of germination was recorded daily and it was considered completed after 7 days when there was no chance for further germination. After the completion of germination, root and shoot lengths were measured and seedlings were placed in oven at 80°C for 24 h. and dry weights recorded. The collected data was subjected to appropriate statistical analysis following Zar (1994). A 50% tolerance level (TL_{50}) at which shoot growth was reduced to 50%, was computed using the 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% 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.

Results

Germination

Effect of cobalt nitrate and cobalt chloride on seed germination of *Pennisetum americanum*: Both cobalt nitrate and cobalt chloride significantly ($p < 0.05$) inhibited the final germination percentage at 200 and 400 ppm, but at lower concentrations germination remained unaffected (Fig.1a & 1b). The effect of cobalt chloride did not differ significantly from that of cobalt nitrate.

Effect of zinc nitrate and zinc chloride on seed germination of *Pennisetum americanum*: Zinc nitrate significantly inhibited seed germination of *P. americanum* at all concentrations over the control (p at the most 0.05) (Fig. 2a). While zinc chloride also gave significant inhibition of germination at 50 ppm

Table 1: Effect of cobalt nitrate and chloride on dry biomass (g) of *Pennisetum americanum* and *Parkinsonia aculeata*

Conc. (ppm)	*Pennisetum americanum*		*Parkinsonia aculeata*	
	$Co(NO_3)_2$	$CoCl_2$	$Co(NO_3)_2$	$CoCl_2$
0	0.3457 ±0.0470	0.2487 ±0.0398	0.72852 ±0.0478	0.8952 ±0.01593
25	0.2547 ±0.0335	0.08907 ±0.0204	0.6932 ±0.0588	0.7448 ±0.0632
50	0.1855 ±0.0361	0.05983 ±0.0053	0.8233 ±0.01020	0.7693 ±0.0313
100	0.1538 ±0.0143	0.04465 ±0.0037	0.7692 ±0.05633	0.7424 ±0.01728
200	0.14795 ±0.0164	0.02737 ±0.0064	0.7407 ±0.035	0.7484 ±0.0277
400	0.08246 ±0.0178	0.0239 ±0.0049	0.6583 ±0.052	0.7278 ±0.032

Table 2: Effect of zinc nitrate and chloride on dry biomass (g) of *Pennisetum amerianum* and *Parkinsonia aculeata*

Conc. (ppm)	*Pennisetum americanum*		*Parkinsonia aculeata*	
	$Zn(NO_3)_2$	$ZnCl_2$	$Zn(NO_3)_2$	$ZnCl_2$
0	0.2986 ±0.022	0.3871 ±0.116	0.90713 ±0.0269	0.8046 ±0.0271
25	0.1096 ±0.011	0.0331 ±0.0016	0.8965 ±0.0387	0.801 ±0.032
50	0.0847 ±0.0035	0.03223 ±0.002	0.7476 ±0.0628	0.7362 ±0.0126
100	0.0768 ±0.0078	0.0358 ±0.0021	0.7450 ±0.05193	0.6959 ±0.026
200	0.115 ±0.0164	0.0391 ±0.0016	0.7097 ±0.1263	0.7680 ±0.0289
400	0.1197 ±0.0100	0.023 ±0.0016	0.6619 ±0.1209	0.5835 ±0.0718

Table 3: TL_{50} values of different compounds for *Pennisetum americanum* and *Parkinsonia aculeata*

Compound	*P. Americanum*	*P.aculeata*
Cobalt nitrate	25.88	350.00
Cobalt chloride	30.7	>400.00
Zinc nitrate	95.76	172.22
Zinc chloride	34.25	>400.00

onwards ($p < 0.05$ for 50, $p < 0.01$ for 100, 200 and 400 ppm) (Fig. 2b). Inhibitory effect of zinc nitrate was closely similar to that of zinc chloride.

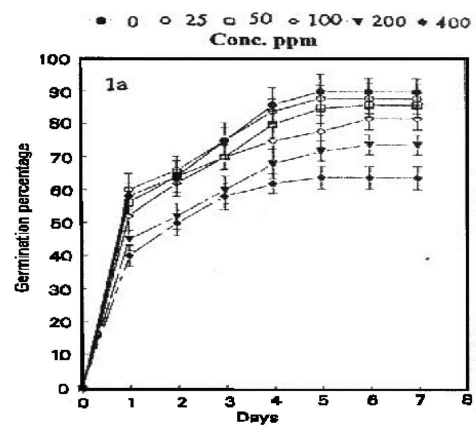
Effect of cobalt nitrate and cobalt chloride on seed germination of *Parkinsonia aculeata*: *P. aculeata* exhibited some degree of tolerance to cobalt nitrate and cobalt chloride compared to the *P. americanum* (Fig. 3a &3b).

Both cobalt nitrate and chloride significantly inhibited seed germination over the controls at 100 ppm onwards ($p < 0.05$). The effect of cobalt nitrate did not differ significantly from that of cobalt chloride.

Effect of zinc nitrate and zinc chloride on seed germination of *Parkinsonia aculeata*: Zinc nitrate showed significant inhibition of final germination at 200 and 400 ppm only ($p < 0.05$) (Fig. 4a). On the other hand zinc chloride showed some fluctuations and the final germination was significantly inhibited only at 400 ppm (Fig. 4b). *P. aculeata* exhibited tolerance to zinc, with regard to germination, as compared to *P. americanum*.

Seedling growth

Effect of cobalt nitrate and chloride on seedling growth of *Pennisetum* and *Parkinsonia:* Cobalt nitrate significantly inhibited the root and shoot length of *P. americanum* ($p < 0.01$) at all concentrations except that shoot growth was not significantly influenced at 25 ppm (Fig. 5a). Whereas cobalt chloride inhibited the root length at all concentrations ($p < 0.001$), while shoot growth was inhibited over the controls at 50 ppm onwards (p at the most 0.05) (Fig. 5b). Root growth was affected to a greater extent in comparison



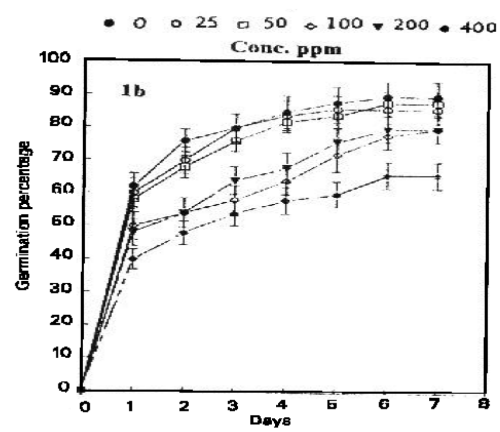


Fig. 1: Effect of cobalt on seed germination percentage of *Pennisetum americanum* a) cobalt nitrate b) cobalt chloride

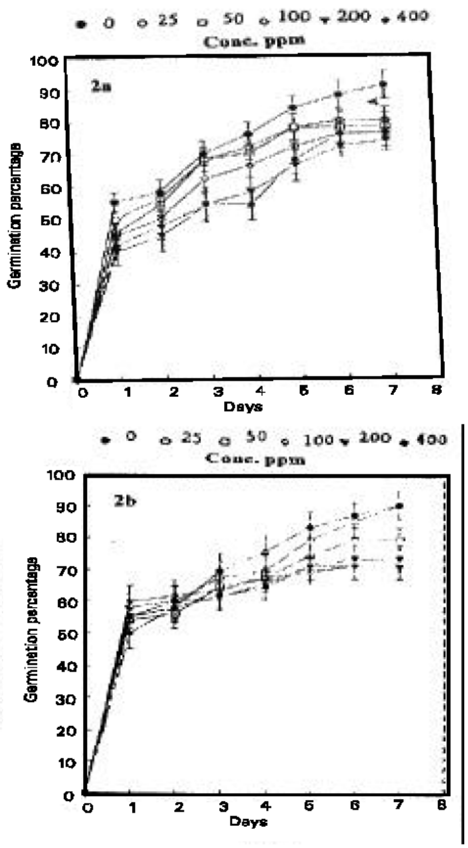


Fig. 2: Effect of zinc on seed germination percentage of *Pennisetum americanum* a) zinc nitrate b) zinc chloride

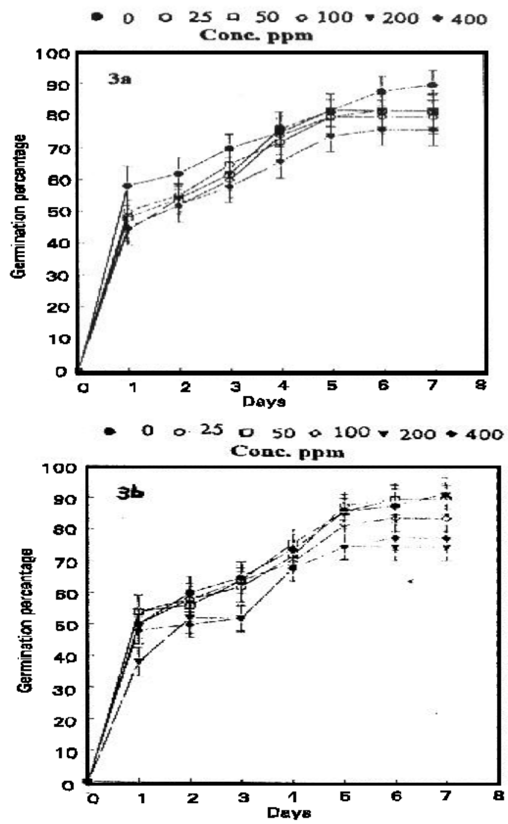


Fig. 3: Effect of cobalt on seed germination percentage of *Parkinsonia aculeata* a) cobalt nitrate b) cobalt chloride

with shoot growth.

In case of *P. aculeata,* cobalt nitrate significantly retarded the root length at 200 and 400 ppm, while the shoot growth was significantly reduced at 400 ppm only ($p < 0.01$) (Fig. 6a). Both root and shoot growth were reduced by cobalt chloride at 50 ppm onwards (p at the most 0.05) (Fig. 6b).

Cobalt nitrate showed greater inhibitory effect on the seedling growth of both the species compared to cobalt chloride. *P. americanum* was found more susceptible to cobalt toxicity, than *P. aculeata.*

Effect of zinc nitrate and zinc chloride on seedling growth of *Pennisetum* and *Parkinsonia:* Zinc nitrate showed inhibitory effect on seedling growth of *P. americanum* and both root and shoot growth were significantly reduced at all concentrations (p at the most 0.01) (Fig. 7a). Similarly, zinc chloride also significantly inhibited root and shoot growth at all concentrations ($p < 0.001$) (Fig. 7b).

Root growth of *P. aculeata* was significantly reduced by zinc nitrate at 100 ppm and above ($p < 0.05$), whereas shoot growth was significantly reduced at all concentrations (p at the most 0.05) (Fig. 8a). Zinc chloride inhibited the root growth of *P. aculeata* at 100 ppm and above ($p < 0.05$), while shoot growth was reduced at 400 ppm only ($p < 0.05$)(Fig. 8b).

Seedling dry biomass

Effect of cobalt nitrate and chloride on the dry biomass accumulation of *Pennisetum* and *Parkinsonia:* Cobalt nitrate significantly reduced the dry biomass in *P. americanum* at all concentrations over the control (p at the most 0.01). Greater reduction occurred at higher concentrations. *P. aculeata* exhibited some tolerance to cobalt nitrate since dry weight reduction occurred at only 400 ppm (Table 1).

Cobalt chloride drastically reduced the dry weight accumulation in *P. americanum* at all concentrations over the control ($p < 0.001$). Although in *P. aculeata* significant reduction in dry biomass was observed at all concentrations but the reduction was of less order as compared to that in *P. americanum* (p at the most 0.05) (Table 1).

Effect of zinc nitrate and chloride on biomass of *Pennisetum* and *Parkinsonia:* Dry biomass of *P. americanum* was significantly reduced at all concentrations over the control by zinc nitrate ($p < 0.001$). In case of *P. aculeata* significant decrease in biomass occurred at all concentrations (p at the

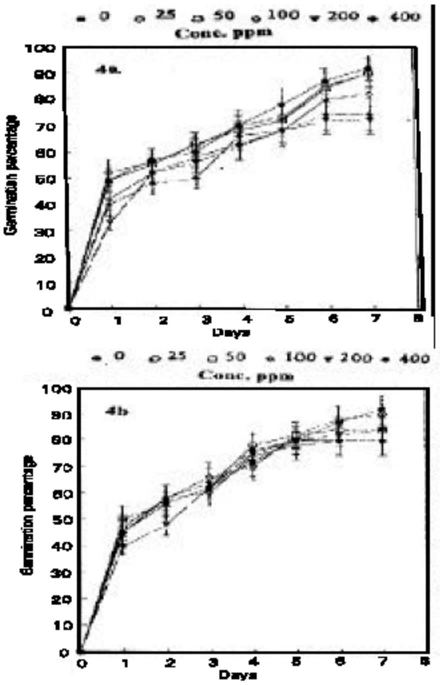


Fig. 4: Effect of zinc on seed germination percentage of *Parkinsonia aculeata* a) zinc nitrate b) zinc chloride

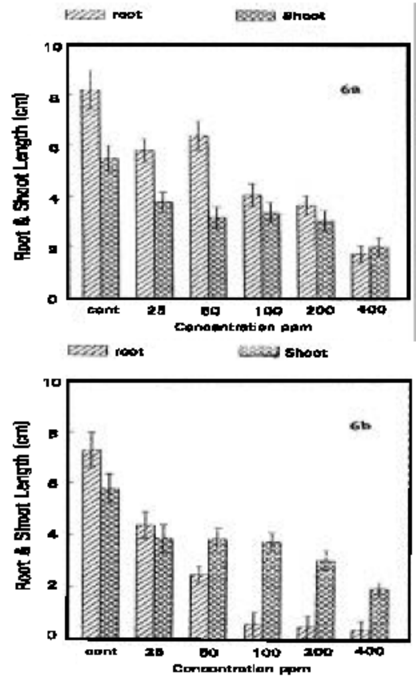


Fig. 6: Effect of cobalt on seedling growth (root and shoot length) of *Parkinsonia aculeata* a) cobalt nitrate b) cobalt chloride

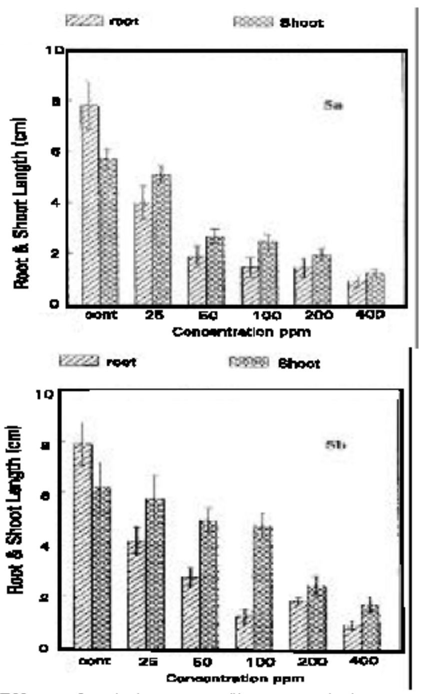


Fig. 5: Effect of cobalt on seedling growth (root and shoot length) of *Pennisetum americanum* a) cobalt nitrate b) cobalt chloride

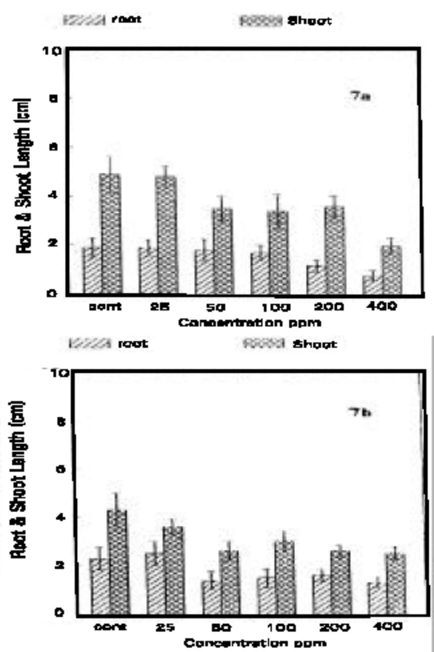


Fig. 7: Effect of zinc on seedling growth (root and shoot length) of *Pennisetum americanum* a) zinc nitrate b) zinc chloride

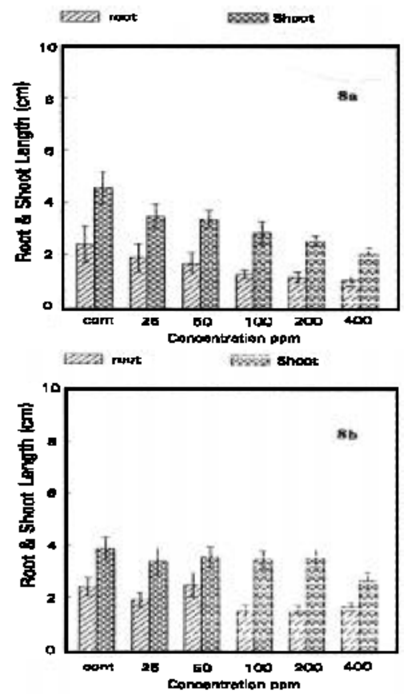


Fig. 8: Effect of zinc on seedling growth (root and shoot length) of *Parkinsonia aculeata* a) zinc nitrate b) zinc chloride

most 0.05), though the reduction was not as high as in *P. americanum* (Table 2).

Dry weight of *P. americanum* was remarkably reduced by zinc chloride at all concentrations over the control ($p < 0.001$). In *P. aculeata* significant decrease by zinc chloride occurred at 100 and 400 ppm ($p < 0.01$) (Table 2).

TL_{50} Values: For *P. americanum* TL_{50} values ranged from 25.88 to 34.25 ppm for different compounds (Table 3) in following order:

$Co(NO_3)_2 < CoCl_2 < ZnCl_2 < Zn(NO_3)_2$

In case of *P. aculeata* TL_{50} ranged from 350 to >400 ppm. The order of TL_{50} for *P. aculeata* was:

$Zn(NO_3)_2 < Co(NO_3)_2 < CoCl_2 = ZnCl_2$

The results showed that cobalt nitrate had lowest TL_{50} value in *P. americanum* and zinc nitrate had lowest value in *P. aculeata*.

Discussion

Cobalt and zinc clearly have inhibitory effect on the germination and seedling growth of the test species. Germination inhibition due to heavy metals has been reported by many workers (Al-Helal, 1995; Shaukat *et al.*, 1999). Ionic toxicity might be the cause of drastic effects of salts on seed germination (Shaukat *et al.*, 1999), or it could be due to osmotic effect (Shaukat *et al.*, 1999). Different species show different levels of tolerance to heavy metals (Anwer *et al.*, 2000).

It is clear from the results that *Pennisetum* is more susceptible to the salts of cobalt and zinc than *Parkinsonia*. Zinc nitrate had greater inhibitory effect on germination and seedling growth than zinc chloride. Similarly, cobalt nitrate had greater effect than cobalt chloride. *Parkinsonia* was found less liable to heavy metal toxicity with respect to all parameters of growth compared to *Pennisetum*. Thus, it exhibits certain degree of tolerance and co-tolerance to heavy metals. It is observed that *Parkinsonia* usually grows in polluted areas and therefore, it is plausible that it has developed resistance against heavy metals. Several workers have reported the presence of heavy metals like Cd, Cr, Pb, Zn, Cu and Ni in industrial and sewage effluents (Smith, 1997). Earlier studies of Shaukat *et al.* (1999) also suggested certain degree of heavy metal tolerance for *Parkinsonia aculeata*. *Pennisetum americanum* which is cultivated in the agricultural fields, grows in comparably less polluted conditions. Evidently, it exhibits much less tolerance to heavy metals as compared to *Parkinsonia*. This result paralleled the findings of Von Frenckell-Insam and Hutchinson (1993). Since salts of both zinc and cobalt had less inhibitory effect on germination and seedling growth of *Parkinsonia*, it exhibits the phenomenon of co-tolerance. It is also evident that the root growth of both the test species was significantly reduced at higher concentrations of salts. These results accord with those of Anwer *et al.* (2000) who observed zinc tolerance in pasture and mine populations of *Anthoxanthum odoratum*. Likewise, Schat and ten Bookum (1992) found reduction in root growth of *Silene vulgaris* while studying copper tolerance in this specie. The results also showed that root growth of the test species was affected to a greater degree than shoot growth, by heavy metals. Similar results have also been reported by other investigators (Shaukat *et al.*, 1999; Anwer *et al.*, 2000). The differential response of root and shoot to heavy metals might be due to more swift accumulation of heavy metals in roots than in shoots, or rapid detoxification in the shoot than in roots (Al-Helal, 1995).

From the above study it is concluded that seed germination, seedling growth and dry weights of both the test species were reduced by different concentrations of cobalt chloride, cobalt nitrate, zinc chloride and zinc nitrate. Furthermore, *Pennisetum* was found more susceptible to heavy metals than *Parkinsonia* and root and shoot growth responded differentially to the heavy metal stress.

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