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Alleviation of Cadmium-Induced Decrease in Biomass of *Pisum* and *Sesamum* by Inorganic Nutrients

N. Bharti,[1] S. Srivastava,[1] P. S. Srivastava[2]

[1] Department of Genetics, University of Delhi, South Campus, New Delhi 110021, India
[2] Centre for Biotechnology, Faculty of Science, Hamdard University, Hamdard Nagar, New Delhi 110062, India

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Although heavy metals like Cd^{2+} and Pb^{2+} are not essential for plant growth and metabolism, they are often readily taken up and accumulated by plants. These metals not only reduce the qualitative and quantitative productivity of the species but also cause a serious health hazard as they travel through the food chain unaltered. These metals have been reported to affect seed germination (Mukherji and Maitra 1976), seedling growth (Kumar *et al.* 1992), photosynthesis (Muthuchelian *et al.* 1988), nitrate assimilation (Chugh *et al.* 1992) and other metabolic processes (Stiborova *et al.* 1988) of plants. However, the nature and magnitude of sensitivity varies from species to species and varieties to varieties.

Pea (*Pisum sativum* L.) is one of the important leguminous crops grown extensively for its varied uses as vegetable, pulse, feed and fodder. Pea can contribute very effectively in augmenting the total production of pulses thus meeting the ever increasing demands of leguminous protein. Sesame (*Sesamum indicum* L.) is the third largest oil seed crop, extensively grown in India, and is utilized for edible purposes. In respect of aiding calcium and phosphorus retention, the oil is reported to be superior to groundnut oil.

The present study was planned to assess the comparative effects of Cd^{2+} and Pb^{2+} on generation of biomass of a legume, *Pisum sativum* L. and an oil crop, *Sesamum indicum* L. As our results have shown that Cd^{2+} is more toxic to these plants than Pb^{2+}, we have also looked at its interaction with some inorganic nutrient ions, such as KH_2PO_4, K_2SO_4, KNO_3 and $(NH_4)_2SO_4$ (K^+, NH_4^+, PO_4^{2-}, NO_3^-, and SO_4^{2-}).

MATERIALS AND METHODS

Seeds of two varieties of *Pisum sativum* L. (cv *P.arvense* and P 1563) and one of *Sesamum indicum* L. (cv. PB-1) procured from National Seed Corporation, New Delhi, India were surface sterilized with 0.1% $HgCl_2$ for 5 min and then washed thoroughly with distilled water. The seeds were germinated for 10 and 5 days at 20 ± 2°C and 30 ± 2°C, respectively, in petridishes (4" diameter) lined with two layers of moist filter paper and maintained in the growth chamber under 9 hr light (of 100 $\mu mol\ m^{-2}s^{-1}$) / 15 hr dark cycle. Filter papers were kept moist with different concentrations of acetate salt solutions of Pb^{2+} and Cd^{2+} in the absence as well as in the presence of KNO_3, K_2SO_4, KH_2PO_4 and $(NH_4)_2SO_4$ (5-30mM). Different concentrations of lead acetate (0.01 to 16.0mM) and cadmium acetate (0.01 to 9.0 mM) were dissolved in distilled water and equal volumes were used for watering 100 seeds of *Pisum* and 200 seeds of *Sesamum*. The germinated seeds were scored every 24 hrs. To assess growth, the FW of root, stem and leaves and the length of root and shoot of

Correspondence to: P. S. Srivastava

10-day-old seedlings of *Pisum* and 5-day-old *Sesamum* were measured. The tissues were then dried at 80°C until the dry weight became constant. The data presented are an average of atleast 3 independent replicate experiments conducted in duplicate. Student 't' test was applied to test the significance of difference, if any from the mean value.

RESULTS AND DISCUSSION

Exogenous supply of cadmium acetate (up to 2.0 mM) and lead acetate (up to 4.0 mM) to *Pisum* cultivar *P.arvense* and up to 3.0 mM of Cd^{2+} and 6.0 mM of Pb^{2+} to cultivar P 1563 showed no adverse effect on germination for 10 days. With an increase in Cd^{2+} concentration to 9.0 mM and Pb^{2+} to 11.0 mM, seed germination was significantly inhibited ($P< 0.05$-0.001) in both the varieties.

Likewise, lower concentrations of Cd^{2+} (0.1mM) and Pb^{2+} (6.0 mM) did not produce any drastic effect on germination in *Sesamum* for five days. Any further increase in their concentration, however, inhibited the germination significantly ($P < 0.05$-0.001).

Cadmium, proved more toxic than Pb^{2+} for both *Pisum* and *Sesamum*. Mathur *et al.* (1987) reported that higher concentrations of Cd^{2+} and Cr^{2+} were fatal to seed germination in Allium cepa. Differential inhibition of seed germination in maize and wheat cultivars treated with Cd^{2+} has also been reported by Singh *et al.* (1991). Inhibition of seed germination in Spartiana alterniflora was explained on the basis of interference of Pb^{2+} with some important enzymes (Mukherji and Maitra 1976). Inhibitory effects of Cd^{2+} and Pb^{2+} and other metals on seed germination of Helianthus annuus were reported by Chakravarty and Srivastava (1992). These authors also showed that Pb^{2+} is more toxic than Cd^{2+}.

Growth of 10-day-old seedlings of both the cultivars of *Pisum* declined at all the concentrations of Cd^{2+} (1.0-7.0 mM), but the effect was less pronounced in P 1563 than in *P.arvense*. The percentage decrease in FW effected by Cd^{2+} (7.0mM) was significant at $p < 0.001$. In *P.arvense* this decrease in root, stem and leaves was 98, 95 and 96%, respectively, and in root and shoot length 84 and 80%, respectively. In P 1563, the same was 79, 72 and 80%, respectively in FW of root, stem and leaves and in length of root and shoot 81 and 76%, respectively (significant $P < 0.05$-0.001) (Fig. 1A). The decrease in FW of root, stem, and leaves of *P.arvense* by Pb^{2+} (7.0 mM) was only 68, 59 and 59% and in length of root and shoot 54 and 64%, respectively. Reduction in FW of root, stem and leaves in P 1563 was 68, 48 and 52% and decrease in root and shoot length was 52 and 58%, respectively. The differences in both the cases were significant at $P<0.001$ (Fig. 1B). Similar response was recorded for dry weight of both the cultivars (see Fig 1A,B).

Even at lower concentrations (0.01-1.0 mM) of Cd^{2+}, S. indicum cv. PB-1 showed a loss in FW of root (76-84%), stem (47-76%), and leaves (28-51%) and reduction in root and shoot length by 48-73 and 20-57%, respectively significant at $P < 0.001$ (Fig.1C). However, the reduction in FW of root, stem and leaves due to Pb^{2+} (1.0 mM) was only 6, 30 and 19% and decrease in length of root and shoot was 63 and 23%, respectively. With increase in concentration of Pb^{2+} (1.0-3.0 mM), toxicity became more pronounced causing further reduction in FW, DW and length of root and shoot (Fig. 1D). A similar decrease in DW of various parts was noticed when concentration of Cd or Pb was increased (see Fig. 1C,D). The reduction in seed germination and subsequent growth of seedlings in the above cultivars relate to Cd^{2+} induced suppression of various physiological and metabolic processes.Cadmium indeed has been shown to interfere with seed germination and seedling growth in a number of crop plants (Gruenhage and Jaeger 1985; Sheoran *et al.* 1990; Chakravarty and Srivastava 1992).

Deleterious effects of higher concentrations of Pb^{2+} has been reported on growth and metabolism of rice seedlings by Mukherji and Maitra (1976). Earlier reports on reduction in fresh weight of pod of *Glycine max*, yield of tomato and egg plant (Khan and Khan 1983), dry weight of pea (Huang *et al.* 1974) and root and stem elongation in *Raphanus* (Lane and Martin 1980) corroborate our observations. Inhibition in root and shoot elongation may arise from interference of lead with auxin-regulated cell elongation (Burzynski and Jakob 1983) or decrease in the cell wall extensibility (Barcelo *et al.* 1986).

The inhibition in the growth of 5- to 10-day-old seedlings as well as at the later stages is correlative of inhibition of some primary physiological processes such as photosynthesis (Singh and Singh 1987) and nitrogen assimilation (Chugh *et al.* 1992). As proposed by several other investigators, Cd^{2+} and perhaps other heavy metals (Pb^{2+} and Cu^{2+}) inhibit growth by affecting uptake of water, activity of hydrolytic enzymes and hydrolysis of storage macromolecules in endosperms or cotyledons, transfer of hydrolytic products to the embryonic axis, root and shoot, and resynthesis of essential macromolecules such as nucleic acids, proteins etc.

In the present study, var P 1563 appeared more tolerant to Cd^{2+}. The order of Cd^{2+} tolerance was P 1563 > *P. arvense* > *S. indicum* cv. PB-1. The degree of effect of both the metals on seedling growth was $Pb^{2+} < Cd^{2+}$. Roots proved more sensitive than the aerial parts, perhaps because of direct exposure to metals while translocation to the aerial parts is gradual. Besides, metal-induced phytochelatins in aerial parts are more effective to combat metal toxicity than in the roots.

As Cd^{2+} turned out to be more toxic, we looked at the possibility of overcoming its negative effect by exogenous supply of different concentrations (5-30 mM) of nutrient salts such as KH_2PO_4, K_2SO_4, KNO_3 or $(NH_4)_2SO_4$ alongwith Cd^{2+} (1.0 mM). We found that inhibition of growth can be reversed to variable extent by these nutrients. The maximum recovery in *P. arvense* was obtained with 20.0 mM KH_2PO_4 or with 15.0 mM of both K_2SO_4 and $(NH_4)_2SO_4$. In cv. P 1563, 15.0 mM of all the three salts provided maximum reversal. The increasing order of recovery was $KH_2PO_4 < (NH_4)_2SO_4 < K_2SO_4$ in *P. arvense* and $KH_2PO_4 < K_2SO_4 < (NH_4)_2SO_4$ in P 1563 (Fig. 2A,B). As described earlier *S. indicum* cv. PB-1 was most sensitive to Cd^{2+}. This effect could be reversed by the combination of different concentrations of KH_2PO_4, KNO_3 and K_2SO_4, with the order of recovery as $KH_2PO_4 < KNO_3 < K_2SO_4$ (Fig. 2C).

Recovery in seedling growth due to supply of these salts alongwith Cd^{2+} may be attributed to the increased activity of auxin and other substances that help overcome the negative effect of metal on one hand, and less absorption and accumulation of the metal in the presence of the inorganic salts on the other. Further, exogenous addition of sulphur in the form of SO_4^{2-} is known to trigger enhanced synthesis of metal-binding peptides rich in sulphur-containing amino acids (Popovik *et al.* 1996). One of the sulphur containing compounds, especially GSH (reduced glutathione) is reported to induce the synthesis of phytochelatins (Pc) against the toxic Cd^{2+} (Gupta and Goldsbrough 1991). Zhao *et al.* (1996) observed that the ratio of glutathione to cysteine increased with exogenous supply of sulphur(S). Glutathione is the major form of organic S in the soluble fraction of plants, and the predominant form of reduced

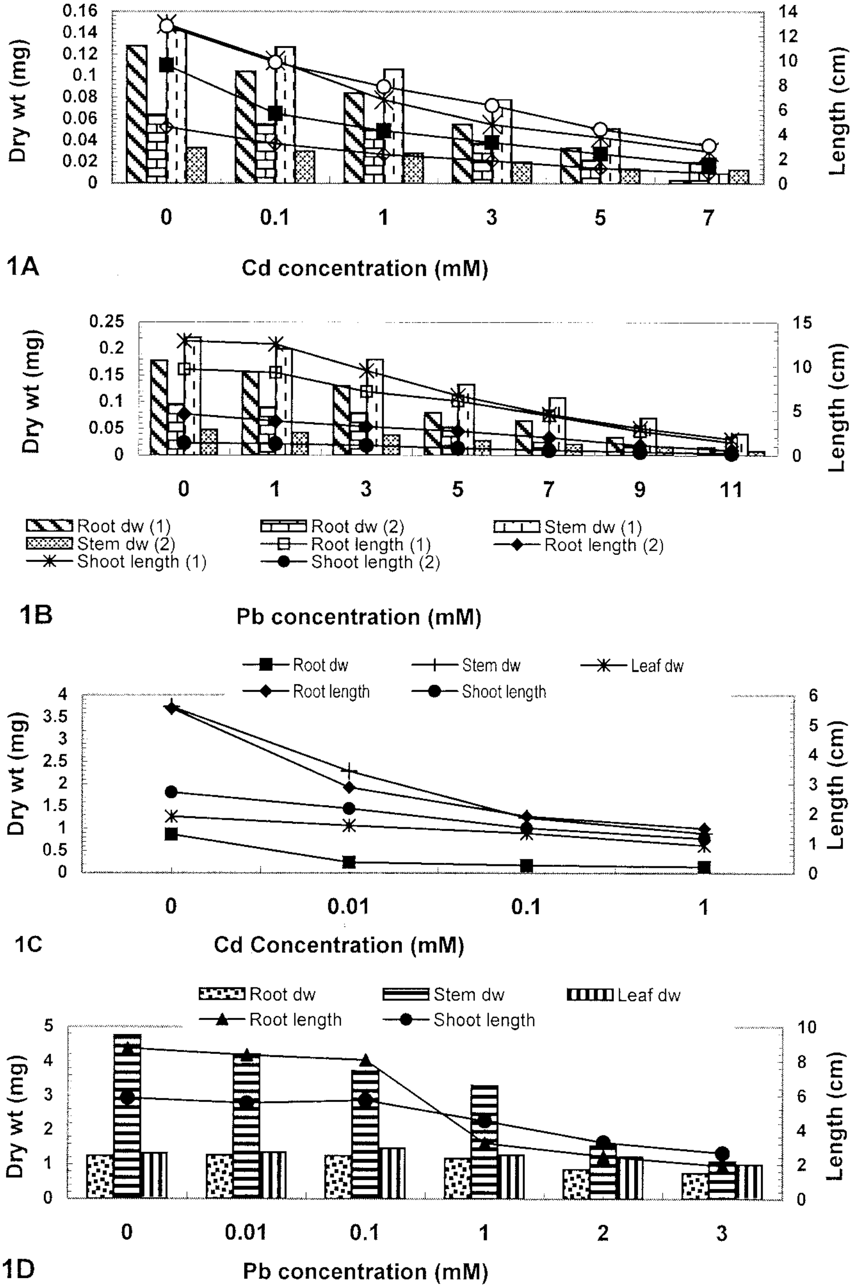

Figure 1A-D. Length and DW of 10-day-old seedlings of *Pisum - Parvense* = (1), and P1563 = (2) treated with Cd^{2+} (A), or Pb^{2+} (B), C, D. Length and DW of 5-day-old seedlings of *S.indicum* cv. PB-1 treated with Cd^{2+}(C) or Pb^{2+}(D)

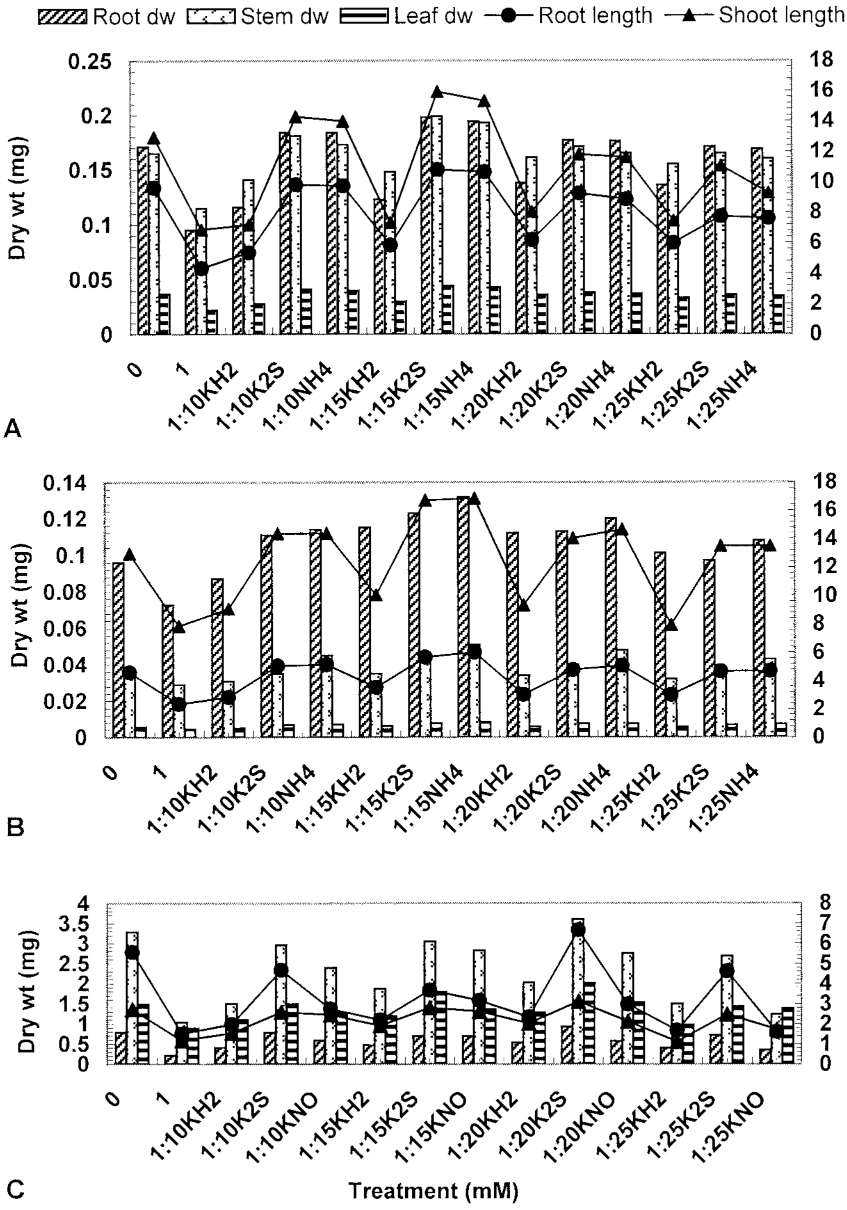

Figure 2A-C. Length and DW of 10-day-old seedlings of *Pisum* treated with Cd^{2+} in the presence of KH_2PO_4 = (KH2), K_2SO_4 = (K2S) or $(NH_4)_2SO_4$ = (NH4). A. *Parvense*, B. P1563, C. Five-day-old seedlings of *S. indicum* cv. PB-1 treated with Cd^{2+} in the presence of KH2, K2S or KNO_3 = (KNO)

S in long distance transport (Renenberg 1995). Phytochelatins (PCs) synthesized in response to Cd^{2+} cause decline in the concentration of GSH in a concentration dependent manner (Tukendorf and Rauser 1990).

Our data indicated that Cd^{2+} toxicity in *Pisum* and *Sesamum* during early growth can be overcome to a great extent by adding nutrient salts such as K_2SO_4, $(NH_4)_2SO_4$, KNO_3 or KH_2PO_4. Thus, metal toxicity may be reduced in nutritionally-rich soils.

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