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Studies on the effect of heavy metal stress on growth parameters of Soybean

N.C. Aery and Sunanda Sarkar

Department of Botany
College of Science
Sukhadia University, Udaipur - 313 001 (India)

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Abstract : The effect of various doses of zinc and cadmium on different growth parameters in soybean at different growth stages was studied. Lower doses of zinc (10-25 $\mu g\ g^{-1}$) as well as cadmium (5$\mu g\ g^{-1}$) were found to be promotory for all the growth parameters studied. The doses higher than the above resulted in a decrease. Negative correlations between different growth parameters and concentrations of zinc and cadmium with varying levels of significance were observed at all the stage of growth.

Key Words : Zinc - Cadmium - Metal stress.

INTRODUCTION

Today world is facing both an environmental and developmental crisis, and both these crisis seem to be intensifying and interacting to reinforce each others. The major environmental problems crop out from waste disposal either in form of air pollutants or water pollutants (Lagerwerff and Specht 1970, Friberg *et al.* 1971) having high concentration of heavy metals. Usually the pollutants are coming out through industrial, mining, milling and nuclear wastes (Francis and Rush 1973, Bachauer 1973). During the last decades, concentrated metal deposits, confined in the earth's crust and usually harmless to living beings, have been exploited on an increasing rate and discharged partly in the environment.

Among the heavy metals, some (for example zinc and copper) are essential for plant growth. But present at elevated levels in soils, they are generally toxic and can ultimately cause the death of plants. This toxicity is most conspicuously manifested in retardation or complete inhibition of root growth at higher metal levels (Rauser 1973, Lyon and Beeson 1948). Cadmium, though a 'ballast element' is accumulated in quite high amounts in different plant parts (Aery and Tiagi 1985 & 1988).

In this paper, studies made on different growth parameters of soybean as influenced

by heavy metal (zinc and cadmium) stress have been presented.

MATERIALS AND METHODS

The seeds of soybean (*Glycine max.* (L.) Merr. var. Gaurav) were obtained from the Rajasthan State Seed Corporation, Kota. Garden soil of average fertility was used for the study. Four kilogram of soil was filled in each earthen pot of 30 cm height and 25 cm diameter. Nine concentrations of zinc and seven concentrations of cadmium were prepared by taking corresponding amounts (calculated on the basis of their Atomic Weights) of $ZnSO_4 . 7H_2O$ (E. Merck, G.R.) and $CdCl_2 . 2H_2O$ (Analar BDH, England) per kilogram of air dried soil and mixed thoroughly in the soil. The zinc concentrations applied were in the range of 10, 25, 50, 100, 500, 1000, 2500, 5000 and 7500 $\mu g\ g^{-1}$ while for cadmium the range was 5, 10, 50, 100, 250, 500 and 1000 $\mu g\ g^{-1}$ of air dry soil. Pots without any added metal constituted the controls.

Ten duly inoculated seeds were sown equidistantly at 2 cm depth in each pot. Watering was done every day. After establishment, seedlings were thinned to 5 in number in each pot.

Table 1 : Showing seed weight (100 seeds^{-1}), seed number plant^{-1} and percentage decrease/increase (in parenthesis) over the controls in soybean under different treatments of cadmium.

S. No.	Cadmium concentrations ($ug\ g^{-1}$)	Seed weight (100 seeds)$^{-1}$	Seed No. plant^{-1}
1.	Control	8.4466 ± 0.35	47.61 ± 0.65
2.	5	8.4975 ± 3.3 (+ 0.60)	54.0 ± 2.8(+ 13.42)
3.	10	8.4329 ± 2.25 (–0.16)	16.12 ± 0.6 (+ 66.14)
4.	50	7.5937 ± 2.4 (–10.09)	14.4 ± 0.85 (–69.75)
5.	100	7.5000 ± 0.55 (–11.20)	12.5 ± 3.75 (–73.74)
6.	250	7.1078 ± 5.25 (–15.85)	10.1 ± 2.8 (–78.78)
7.	500	5.6593 ± 1.25 (–32.59)	7.5 ± 2.4 (–84.24)
8.	1000	—	—

Four sets in triplicate were prepared to take observations in all the four stages of life span i.e. seedling, vegetative, flowering and fruiting. Plant samples were taken out and observations were noted for shoot and root length and dry weight, nodule number and dry wight, leaf area and grain yield. The leaf area was measured by weight method (Ambasht 1971). Plants were dried in an oven at 80°C for 48 hours and weighed for the dry matter. The seeds were collected at maturity counted and weighed.

Correlation coefficients (r) between zinc and cadmium concentration and different parameters studied were calculated as per the methods of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The results are presented in Tables 1 to 5; and Figs. 1 to 5. It is evident that the lower concentrations (10-25 μg^{-1}) of zinc as well as cadmium (5$\mu g\ g^{-1}$) proved to be beneficial to the crop with respect to all the parameters studied (Tables 1-4; Figs. 1-3). The essentiality of zinc to the growth of plants was established long back (Maze 1919, Sommer 1928). Yield increase by the application of low amounts (5–10 $\mu g\ g^{-1}$) of zinc had also been reported by Kanwar (1964) for wheat, Patel *et al.* (1976) for *Chrysanthemum* and Saxena and Singh (1970) for a number of pulses.

Table 2 : Showing seed weight (100 seeds^{-1}), seed number plant^{-1} and percentage decrease/increase (in parenthesis) over the controls in soybean under different treatments of zinc.

S. No.	Zinc concentrations (ug g^{-1})	Seed weight (100 seeds)$^{-1}$	Seed No. plant^{-1}
1.	Control	8.4465 ± 0.35	47.61 ± 0.65
2.	10	8.9100 ± [illegible].25 (+ 5.48)	49.1 ± 0.5 (+ 3.12)
3.	25	8.7392 ± 2.0 (+ 3.4641)	34.5 ± 2.25 (–27.53)
4.	50	8.2195 ± 1.13 (–2.6886)	25.71 ± 4.35 (–45.90)
5.	100	8.3920 ± 2.26 (–0.6464)	22.2 ± 6.89 (–53.37)
6.	500	7.5786 ± [illegible].83 (–10.27)	21.0 ± 2.73 (–55.89)
7.	1000	7.4140 ± [illegible] (–12.22)	17.25 ± 0.65 (–63.76)
8.	2500	7.1706 ± 1.55 (–15.10)	13.86 ± 3.25 (–70.88)
9.	5000	6.6821 ± 3.80 (–20.89)	9.0 ± 4.5 (81.09)
10.	7500	5.1807 ± 3.43 (–38.66)	7.98 ± 1.25 (–83.23)

Table 3 : Showing nodule dry weight (mg plant^{-1}), nodule number plant^{-1} and precentage decrease/increase (in parenthesis) over the controls in soybean under different treatment of zinc.

Zinc concentrations ($\mu g\ g^{-1}$)	Vegetative		Flowering	
	Weight	No.	Weight	No.
Control	27.0	31.05	72.0	54.0
10	50.0 (+85.18)	50.25	78.8 (+9.44)	88.50
25	47.0 (+74.07)	60.08	76.8 (+6.66)	68.25
50	20.0 (–25.92)	36.11	70.0 (–2.77)	50.18
100	21.8 (–19.25)	30.15	64.0 (–11.11)	44.62
500	17.5 (–35.18)	24.56	42.4 (–41.11)	36.15
1000	10.0 (–62.96)	20.0	14.0 (–80.55)	11.05
2500	—	—	12.0 (–83.33)	8.75
5000	—	—	—	—
7500	—	—	—	—

In the present study 10$\mu g\ g^{-1}$ added zinc was found to be the most suitable for plant

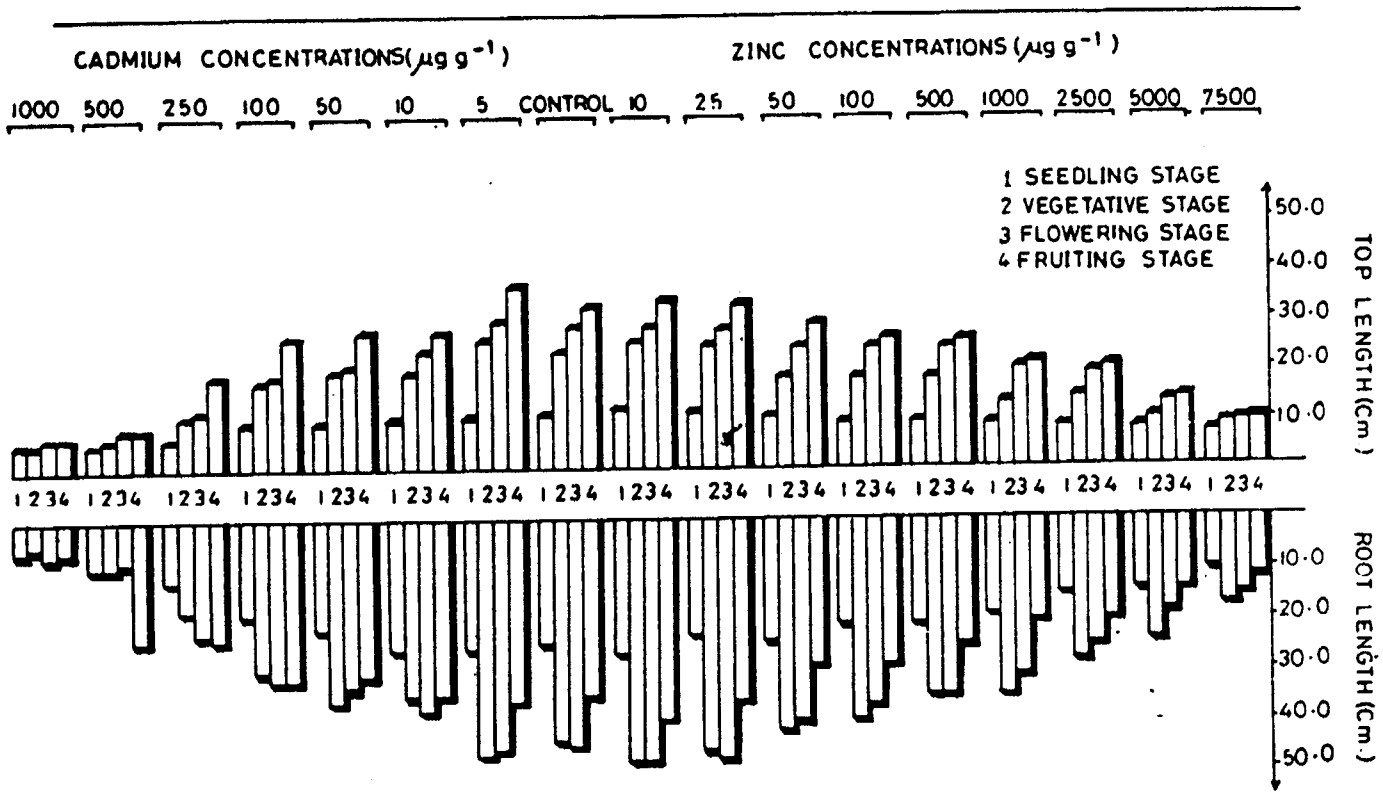

Fig. 1 : The effect of different zinc and cadmium concentrations on root-shoot length (cm) of soybean in different stages of growth.

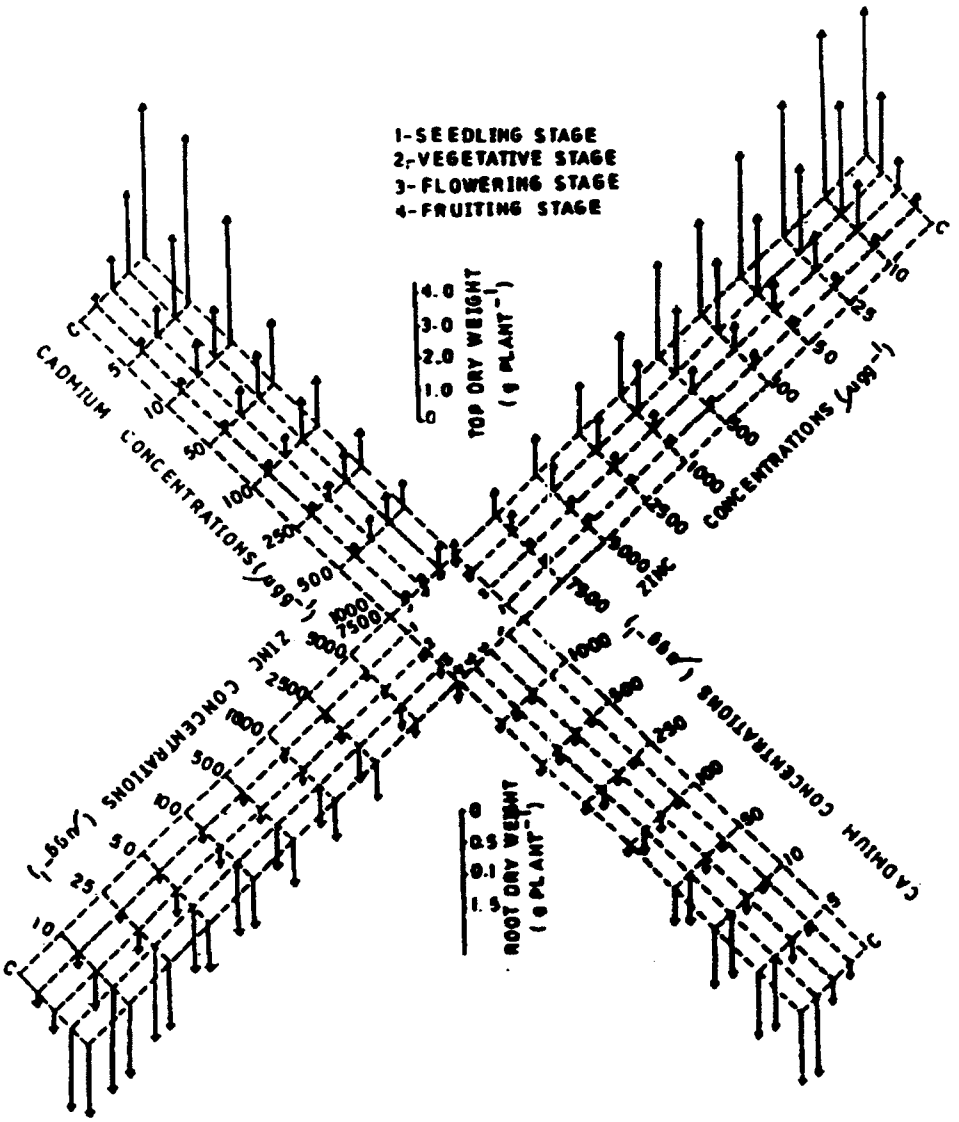

Fig. 2 : The effect of different zinc and cadmium concentrations on below and above ground dry weights (g plant^{-1}) of soybean at different stages of growth.

growth and resulted in a substantial percent increase over the controls in all the parameters studied. On shoot-root length the maximum effect was observed during the seedling stage (Fig.1). On dry matter accumulation the effect was maximum during the vegetative stage (Fig.2). As the plant developed, root growth continued until about the time of seed development, after which total root weight decreased. Even before the

Fig. 1 : The effect of different zinc and cadmium concentrations on root-shoot length (cm) of soybean at different stages of growth.

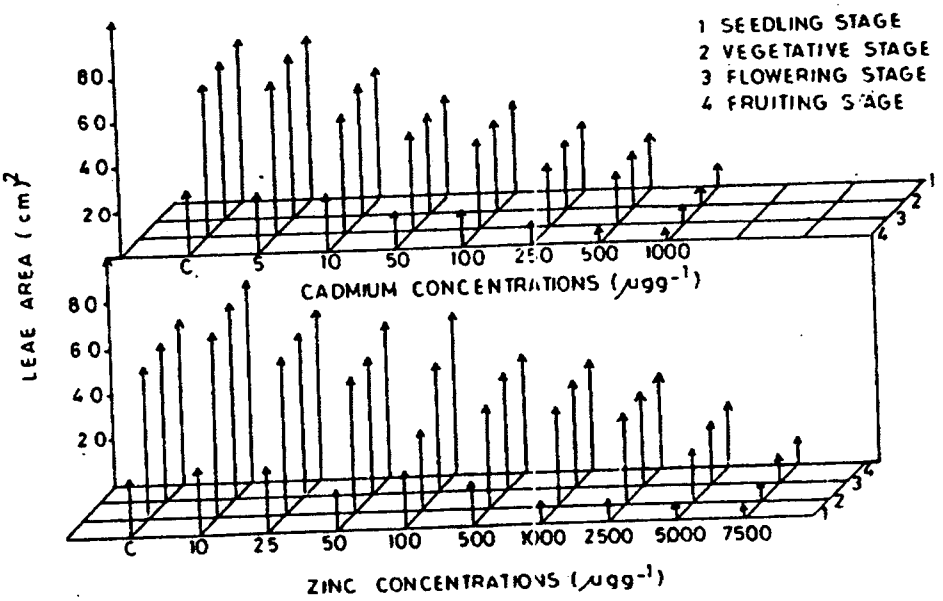

Fig. 3 : The effect of different zinc and cadmium concentrations on the leaf area (cm^2) of soybean in different stages of growth.

cessation of root growth, aboveground organs grew more rapidly than the root so that the shoot-root ratio increased steadily throughout the life of the plant (Fig.2). The leaf area of the plant also experienced the maximum percentage increase (2-9%) over the controls at 10µg g^{-1} added zinc. A steady increase in the leaf area was observed even upto the fruiting stage (Fig.3). The total weight of nodules/plant, as well as nodule number/plant increased throughout the life of the plant. The nodule weight and number were observed to be much higher during the flowering than in the vegetative stage. It was maximum at 10µg g^{1} added zinc level (Table 3). The same was the case for seed number and weight.

Table 4 : Showing nodule dry weight (mg $plant^{-1}$), nodule number $plant^{-1}$ and precentage decrease/increase (in parenthesis) over the controls in soybean under different treatment of cadmium.

Cadmium concentrations	Vegetative		Flowering	
	Weight	No.	Weight	No.
Control	27.0	31.0	72.0	54.0
5	13.6 (–49.62)	9.67	65.6 (–8.88)	86.67
10	8.5 (–68.51)	11.16	35.6 (–50.55)	26.53
50	2.4 (–91.11)	6.50	30.0 (–58.33)	30.17
100	1.6 (–94.07)	5.13	26.0 (–63.88)	24.08
250	0.5 (–98.14)	7.06	9.0 (–87.49)	6.05
500	—	—	9.0 (–87.49)	6.05
1000	—	—	—	—

Zinc concentrations upto 25µg g^{-1} proved to be beneficial in respect of the above parameters after which a decrease occurred, (Figs. 1-3; Tables 1-4). The positive role of the lower concentrations 10-25 µg g^{-1} of zinc in promoting the general growth could be explained on the basis that the soils of Udaipur in general and experimental soils in particular were zinc poor, the ammonium acetate exchangeable zinc contents being 0.7 µg g^{-1} only. But the lower doses 5 µg g^{-1} of cadmium, a 'ballast element' (Aery and Tiagi 1988), also invariably induced increment in all the parameters studied. No adequate explanation for cadmium stimulation of yield could be thought off.

As the zinc concentrations in the soils were increased beyond 25 µg g^{-1} a steady drop up to 90% over the controls in different parameters could be observed. The percent decrease in dry weight over the control was more in roots than in the shoots. The maximum percent decrease (88.1%) over the control was observed in the roots at fruiting stage at a zinc concentration of 7500 µg g^{-1} (Fig.2). Yield reduction due to higher concentrations of zinc have also been reported by Gupta and Singh (1972), Patel *et al.* (1976) and Okhi (1975, 1976 & 1978).

Table 5 : Showing correlation coefficients and level of significance in zinc and cadmium concentrations and parameters studied in soybean.

Parameter	Seedling	Vegetative	Flowering	Fruiting
		ZINC		
Shoot length	–0.7915 S*	–0.8387 S*	–0.9509 S**	–0.9145 S**
Root length	–0.9139 S**	–0.9243 S**	–0.8977 S**	–0.8641 S*
Shoot dry wt.	–0.8907 S**	–0.7745 S*	–0.8399 S*	–0.8116 S*
Root dry wt.	–0.7785 S*	–0.7361 S	–0.7961 S*	–0.8849 S**
Leaf area	–0.8155 S*	–0.9334 S**	–0.9315 S**	–0.9358 S**
Seed weight	—	—	—	–0.9400 S**
Seed number	—	—	—	–0.7126 S*
Nodule weight	—	0.7018 PS	—0.7790 PS	—
Nodule number	—	–0.7471 PS	–0.8664 S	—
		CADMIUM		
Shoot length	–0.8709 S*	–0.8566 S*	–0.8265 S*	–0.8865 S*
Root length	—0.9348 S**	–0.8708 S*	–0.8926 S*	–0.9881 S**
Shoot dry weight	–0.8306 S	–0.8461 S*	–0.7088 S	–0.7075 PS
Root dry weight	–0.8538 S*	0.9622 S**	–0.7081 S	–0.6485 PS
Leaf area	0.8208 S	–0.8782 S*	–0.8660 S*	–0.8641 S*
Seed weight	—	—	—	–0.9660 S**
Seed number	—	—	—	–0.5262 NS
Nodule weight	—	–0.7330 S	–0.7526 S	—
Nodule number	—	–0.522 NS	–0.6374 NS	—

S** – Very highly significant (< 0.001) ; S* – Highly significant (0.001 < P < 0.01) ; S – Significant (0.01 < P < 0.05) ; PS - Possibly significant (0.05 < P < 0.10) ; NS - Not significant (P > 0.10).

Cadmium concentrations above 5 $\mu g\ g^{-1}$ resulted in a decrease in all the growth parameters. This decrease in root–shoot length was found to be maximum at 1000 $\mu g\ g^{-1}$ cadmium concentration and ranged from 77 to 86% and 56 to 80%, respectively at different growth stages (Fig.1). Roots got more severely affected than shoots. The maximum decrease in shoot and root dry weight (92.9% and 96.4%, respectively) over the controls was also observed at a cadmium level of 1000 $\mu g\ g^{-1}$ (Fig.2). Cadmium concentrations responsible for a 50% decrease in different growth parameters at different growth stages ranged from 10 $\mu g\ g^{-1}$ to 1000 $\mu g\ g^{-1}$. Similar decreases at higher cadmium levels in growth parameter have also been observed in different crops by a number of workers (Turner 1973, Lagerwerff and Biersdorf 1972, Williams and David 1977, John and Laerhoven 1976). Higher concentrations of cadmium decreased seed output also. At 500 $\mu g\ g^{-1}$ of cadmium the decrease was 32.99% in seed weight and 84.24% in seed number (Table 1). Beyond this level plants could not survive.

Nodule number and weight, a measure of the nitrogen fixation ability of the plants was also affected due to higher levels of zinc and cadmium. The effect was so severe that no nodules could be observed beyond 2500 $\mu g\ g^{-1}$ zinc level and 500 $\mu g\ g^{-1}$ cadmium level (Table 3-4). Huang *et al* (1974) have also observed a decrease in the nodule dry weight and nitrogenase activity in soybean plants stressed under high doses of cadmium and lead.

Quite significant negative correlations exist between zinc and cadmium concentrations and different growth parameters studied (Table 5) indicating the toxic effect of the metals studied on these parameters at all the stages of growth.

The steady decrease in leaf area at different applied zinc and cadmium doses was observed to be in the range of 4-6% to 86.9% and 14.7% to 87.4% respectively (Fig.3). The decrease in leaf area at higher concentrations of zinc and cadmium can be attributed either to a reduction in the number of cells as judged by Nieman (1965) in the leaves of *Phaseolus vulgaris* stunted by a salination of 72 meq litre^{-1} or due to reduction in cell size (Meiri and Poljakoff-Mayber 1967). The metals might be inhibiting mitotic activity and therefore, cell division as also observed in *Crepis capillaris* under the influence of salt (Kulieva *et al.* 1975). Cytological abnormalities (Ruposhev and Garima 1976) mutagenic activities and degradation of DNA (Rohr and Baughinger 1976) have been observed under the effects of toxic levels of zinc and cadmium.

The overall decrease in different growth parameters due to the toxic effects of higher concentrations of cadmium and zinc might be due to the reason that the stressed plants have to expend more energy for their survival in the hostile environment, which otherwise would be available for their other growth processes. This may lead to a

decrease in the overall growth of the stressed plants. This expected growth inhibition by the metal stress has been confirmed experimentally in the present studies.

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