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Effect of Pb and Zn stress on growth parameters of *Spinach oleracea*

Shivom Singh[1], Neetu Saxena[2], Kajal Srivastava[1] and D. K. Saxena[3]

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Abstract

The influence of metals (Pb and Zn) was investigated using plant growth as stress indicators. Spinach plant was treated with various concentrations of Pb and Zn (2, 5, 10 50, 100 and 200 mM). Lead has a stimulating effect upto 5mM concentrations whereas, zinc was find to promote the growth upto 10mM level. Overall, a negative correlation was found between metal concentration and chlorophyll, carbohydrate, nitrogen and protein content of the plant.

Keywords: C*orrelation, Growth response, Metal, Stress, Spinach*

Introduction

Present scenario of industrialization and development gives clear picture of environmental pollution of gaseous as well as metal precipitation. Metal toxicity (especially by pollutants) is an increasing problem in agriculture due to urbanization and industrialization. The uptake of metal by plants is due to the availability of these elements in the soil as a result of agricultural, manufacturing, mining and waste disposal practices (Veerappa and Samy, 1999).

The toxic metal Pb is well known to react with protein, enzymes and amino acids by binding forming tetraethyl lead and gives reduction in photosynthetic rate, resulting to disrupt many metabolic pathways. Zinc is an essential element for both plants and animals. It plays an important role in several plant metabolic processes. However, like other heavy metals (Doncheva *et al*., 1996, Doncheva, 1997, 1998) when Zn is accumulated in excess in plant tissues, it causes alterations in vital growth processes such as photosynthesis and chlorophyll biosynthesis (Doncheva *et al*., 2001) and membrane integrity (De Vos *et al*., 1991). The present study had been taken with the aim of examining the effect of increased lead and zinc concentrations on growth and productivity aspects of spinach plant.

Materials and Method

Seeds of *Spinach oleracea* were sown in circular plastic pots (20 seeds/pot) containing loamy clay soil. The pots were kept in controlled lab conditions, irrigated with water and left until emergence of seedlings. Thereafter, the pots were divided into various lots and were sprayed with different concentrations of lead (Lead acetate) and zinc (Zinc sulphate): control, 2 mM, 5 mM, 10 mM, 50 mM, 100 mM and 200 mM for 7 days. The concentrations were calculated on the basis of metal ion portion. The control sets of pots were irrigated with tap water only.

The data on germination was taken at 6, 8 and 10 days after sowing (DAS) and the germination relative index (GRI) was calculated as per the following equation (Garg and Jasleen, 2004)

$$\mathbf{GRI = (S)\ X_n\ (k\text{-}n)}$$

Where, X_n= number of seeds germinated on n^{th} day

k= number of counts, n = number of days

After 20 DAS, seedlings from each treatment were selected and analysed for the following parameters. Photosynthetic pigments (chlorophyll-

Author's Address

[1] Biological Science Department, C.B.S.H, G.B. Pant University of Agriculture and Technology, Pantnagar, US Nagar, U.K.(India)
Email - shivom101@rediffmail.com
[2] Department of Zoology and Environmental Science, Gurukul Kangri University, Haridwar, U.K.(India)
[3] PGD Environmental Management, Department of Botany, Bareilly College, Bareilly, (U.P.), India

a, chlorophyll-b, total chlorophyll and carotenoids) were analysed according to the method of Tuba (1987). Carbohydrate content was estimated by anthrone method of Hedge and Horfreiter (1962). Percent nitrogen and crude protein were determined by Micro-Kjeldhal method (Jackson, 1962).

Results and Discussion

GRI of *Spinach oleracea* differed in response to lead and zinc metal stress (Fig.1). GRI of zinc treated samples was higher than that of lead treatment up to 5mM metal concentration while at further higher concentrations, GRI of zinc treated samples had decreased in comparison to lead treated samples which indicates that higher concentrations of zinc causes more toxic effect than lead. The lower level of lead metal upto 5mM induced significant increase in chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids (59.8%, 35.65%, 21.22% and 27.64% increase respectively) when compared with control untreated plants (Table 1). Further increasing the metal stress, there is decline in all the photosynthetic pigments which decreased significantly at higher levels. However, overall a negative correlation was found between lead toxicity and chlorophyll content having correlation coefficient 'r' –0.901. Same trend was observed in case of zinc toxicity having correlation coefficient of –0.794 (Fig. 2). However, in case of higher metal toxicity, the decrease in total chlorophyll is more pronounced in Zn toxicity as compared to Pb metal. An enhancement of chlorophyll degradation occurs due to increased chlorophyllase activity (Drazkiewicz, 1994).

Table 1: Effect of Pb and Zn metal toxicity on chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids (mg g^{-1} FW) of *Spinach oleracea*.

Treatment		Chlorophyll-a	Chlorophyll-b	Total chlorophyll	Carotenoids
Pb	Control	0.122 ± 0.019	0.359 ± 0.085	0.589 ± 0.125	0.123 ± 0.006
	2mM	0.124 ± 0.010 (1.64% I)	0.437 ± 0.029 (21.73%I)	0.655 ± 0.040 (11.20%I)	0.147 ± 0.001 (19.51%I)
	5mM	0.195 ± 0.004 (59.84% I)	0.487 ± 0.005 (35.65%I)	0.714 ± 0.061 (21.22%I)	0.157 ± 0.001 (27.64%I)
	10mM	0.163 ± 0.007 (33.60%I)	0.321 ± 0.004 (10.58%D)	0.554 ± 0.016 (5.94%D)	0.169 ± 0.001 (37.39%I)
	50mM	0.105 ± 0.007 (13.93%D)	0.258 ± 0.010 (38.13%D)	0.335 ± 0.010 (43.12%D)	0.093 ± 0.002 (24.39%D)
	100mM	0.053 ± 0.002 (56.55%D)	0.149 ± 0.013 (58.50%D)	0.173 ± 0.020 (70.27%D)	0.091 ± 0.002 (26.01%D)
	200mM	0.045 ± 0.001 (63.11%D)	0.074 ± 0.008 (79.39%D)	0.102 ± 0.015 (82.68%D)	0.090 ± 0.002 (26.82%D)
Zn	Control	0.141 ± 0.008	0.389 ± 0.062	0.604 ± 0.125	0.171 ± 0.022
	2mM	0.167 ± 0.003 (18.44%I)	0.481 ± 0.036 (23.65%I)	0.686 ± 0.061 (13.57%I)	0.179 ± 0.012 (4.68%I)
	5mM	0.255 ± 0.005 (80.85%I)	0.540± 0.002 (38.82%I)	0.743 ± 0.005 (23.01%I)	0.188 ± 0.013 (9.94%I)
	10mM	0.078 ± 0.004 (44.68%D)	0.257 ± 0.011 (33.93%D)	0.332 ± 0.016 (45.03%D)	0.046 ± 0.018 (73.09%D)
	50mM	0.057 ± 0.004 (59.57%D)	0.148 ± 0.007 (61.95%D)	0.204 ± 0.004 (66.23%D)	0.024 ± 0.001 (85.96%D)
	100mM	0.030 ± 0.001 (78.72%D)	0.063 ± 0.005 (83.80%D)	0.127 ± 0.005 (78.97%D)	0.018 ± 0.002 (89.47%D)
	200mM	0.021 ± 0.003 (85.10%D)	0.055 ± 0.010 (85.86%D)	0.079 ± 0.004 (86.92%D)	0.015 ± 0.001 (91.22%D)

Values represent the mean ± SE (% Increase (I)/ Decrease (D) w.r.t. control)

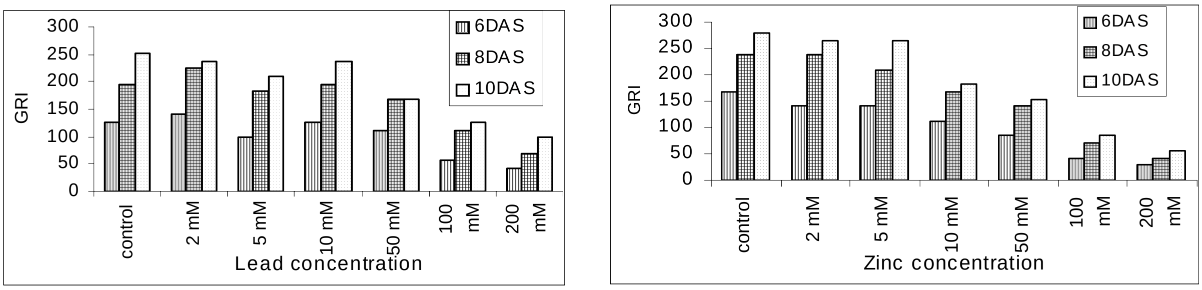

Fig. 1: Effect of different levels of metal toxicity (Pb and Zn) on the germination relative index (GRI)

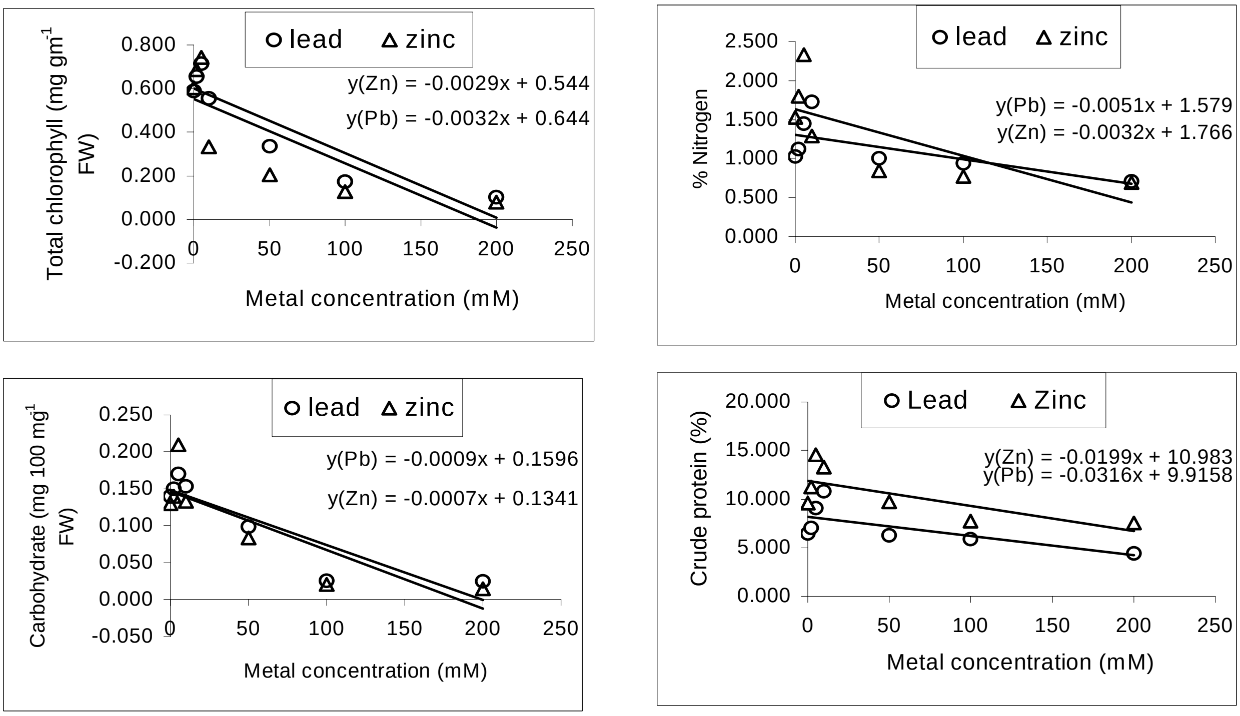

Fig. 2 : Relationship between metal toxicity (Pb and Zn) and productivity parameters of *Spinach oleracea*

The carbohydrate content at low and moderate metal treatment showed significant differences between control and metal stress (Table 2). It has increased with the increasing metal concentration upto 5 mM. Thereafter, there was marked decline as compared to control. The percent increase in carbohydrate content was higher in Zn metal in comparison to Pb metal at 5 mM. The values of correlation coefficient was measured –0.907 and -0.847 with Pb and Zn metal toxicity respectively (Fig. 2). The pattern of changes in carbohydrates was similar to that of chlorophyll which gives a reason to believe that low chlorophyll content causes a relevant reduction of light absorption by leaves (Evass, 1996) and consequently reduces the biosynthesis of carbohydrates. Study of growth in *Spinach oleracea* with respect to % nitrogen and crude protein revealed that over 5 mM, treatment of lead crosses the tolerance limit showing decreasing trend of % nitrogen with further increasing metal levels (Table 2), depicting the correlation coefficient as –0.690. In case of zinc metal treatment, the % nitrogen had increased upto 10mM level after which it had declined with the increasing metal level and having the correlation coefficient as –0.733 (Fig. 2). The crude protein content of Pb and Zn treated samples showed same trend as found in case of % nitrogen. The results of nitrogen and crude protein suggest that plant metabolism and their growth are interrelated parameters. It is thus evident that Pb treatment promotes the growth at lower level of 5 mM in the

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Spinach oleracea. Dabas *et al*. (1995) has reported that lower level of Pb increased the fresh weight and dry weight of roots and shoots of Mungbean. Zn is less toxic than Pb stimulating the growth upto 5mM concentration, whereas, at higher metal concentrations, Zn produces more toxic effects than Pb which shows less % decrease in different productivity parameters as compared to control. Veerappa and Samy (1999) have also reported the promontory role of Zn in *Zea mays* L. at lower levels. Thereafter, both the metals become toxic and the plant suffered metal stress which is significant at higher levels of 100 and 200 mM inhibiting the productivity in plant.

Table 2: Effect of Pb and Zn metal toxicity on carbohydrate content (mg 100 mg^{-1} FW), % nitrogen and crude protein(%) of *Spinach oleracea*

Treatment		Carbohydrate (mg100mg^{-1} FW)	% Nitrogen	% Crude protein
Pb	Control	0.140 ± 0.007	1.030 ± 0.027	6.438 ± 0.167
	2mM	0.150 ± 0.015 (7.14%I)	1.120 ± 0.047 (8.73%I)	7.000 ± 0.294 (8.73%I)
	5mM	0.170 ± 0.071 (21.42%I)	1.450 ± 0.082 (40.77%I)	9.063 ± 0.512 (40.77%I)
	10mM	0.153 ± 0.006 (9.28%I)	1.730 ± 0.117 (67.96%I)	10.812 ± 0.731 (67.96%I)
	50mM	0.099 ± 0.009 (29.29%D)	1.000 ± 0.020 (2.91%D)	6.250 ± 0.127 (2.91%D)
	100mM	0.026 ± 0.007 (81.43%D)	0.940 ± 0.119 (8.73%D)	5.875 ± 0.741 (8.73%D)
	200mM	0.025 ± 0.001 (82.14%D)	0.710 ± 0.035 (31.06%D)	4.437 ± 0.220 (31.06%D)
Zn	Control	0.130 ± 0.015	1.530 ± 0.133	9.563 ± 0.833
	2mM	0.190 ± 0.042 (46.15%I)	1.800 ± 0.231 (17.64%I)	11.250 ± 1.443 (17.64%I)
	5mM	0.200 ± 0.035 (53.84%I)	2.330 ± 0.067 (52.28%I)	14.563 ± 0.417 (52.28%I)
	10mM	0.123 ± 0.049 (5.38%D)	1.290 ± 0.133 (15.68%D)	8.065 ± 0.833 (15.68%D)
	50mM	0.084 ± 0.008 (35.38%D)	0.840 ± 0.023 (45.09%D)	5.250 ± 0.146 (45.09%D)
	100mM	0.021 ± 0.007 (83.84%D)	0.770 ± 0.023 (49.67%D)	4.813 ± 0.144 (49.67%D)
	200mM	0.015 ± 0.003 (88.46%D)	0.690 ± 0.058 (54.90%D)	4.313 ± 0.363 (54.90%D)

Values represent the mean ± SE (% Increase (I)/ Decrease (D) w.r.t. control)

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