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EFFECT OF LEAD ON GERMINATION, EARLY SEEDLING GROWTH, SOLUBLE PROTEIN AND ACID PHOSPHATASES CONTENT IN *ZEA MAYS* L.

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Maize seeds were grown in 0.0 to 100 mM of lead nitrate to evaluate the toxic effects of lead on seedling growth and metabolism. Lead treatment, in general, was inhibitory for seed germination and seedling growth, as reductions in percentage germination and root-shoot lengths were observed at 5-100 mM. Root-shoot dry weight/seedling registered an increase at 5-100 mM. Moreover, the lead content in roots and shoots also registered an increase with increasing concentrations of lead over the control. Relative increase in lead content was more in roots at any given concentration of lead in comparison with the shoots. The above data of growth analysis are based on 7-day old seedlings.

Soluble protein content declined at all lead treatments in shoots of 1, 4 and 7-day old seedlings. The acid phosphatase activity registered an increase in 1-day (30-100 mM) and 4-day (5-100 mM) and a decrease (5-100 mM) in 7-day old seedlings.

The possible morpho-physiological causes underlying the deleterious effects of lead on growth are discussed.

Key words: Lead, *Zea mays*, Proteins.

INTRODUCTION

The effects of heavy metals within the biosphere has become a subject of great importance in the past few years [1]. Lead is of particular interest because of its toxic nature, wide spread occurrence, long half-life in biological systems and because plants facilitate its entry into food chains which eventually enter man's food and livestock [2]. Major sources of lead toxicity to plants are the parent materials from lead mines, smelting operations, use of lead arsenate as an insecticide, fungicide and pesticide in agriculture, from fertiliser impurities and from automobile exhausts (which is a major hazard to vegetation adjacent to highways [3].

Lead toxicity is deleterious to plant growth. Inhibition to germination and retardation of growth are the commonly reported effects [4,5,6]. There are few data available on the toxicity of lead at physiological and biochemical levels [7-10] rendering lead toxicity to be poorly understood.

The present study reports the effect of lead on seed germination, early seedling growth, soluble protein and acid phosphatases content in maize. This may help in elucidation of the mechanism of the action of lead toxicity on growth.

MATERIAL AND METHODS

Maize (*Zea mays* L. cv. Akbar) seeds were obtained from the Punjab Seed Corporation, Lahore. Seeds were sterilised with 1 % Chloramine for 15 min, washed thoroughly in distilled water and sown in single salt lead nitrate solution of 0, 5, 10, 30, 50, 75 and 100 mM conc. Sowing and growth conditions were the same as reported earlier [11]. Data on germination, root-shoot length and dry weight were collected as outlined in a previous study [12]. For the estimation of lead content in root and shoot parts of 7-day old seedlings, the method of Garcia *et al.* [13] was followed. The oven dried plant material was ground in a mill to less than 20 mesh. One g of material was prepared for lead analysis using HNO_3 and $HClO_4$ acid digestion with heating; the diluted acid solution was filtered (<1 μm) and then evaporated to 10 ml. Flame atomic absorption spectrophotometry was used to determine the lead concentration from the sample.

The extraction of soluble protein and estimation was according to the procedure of an earlier study [14]. The assay of acid-phosphatases was based on enzyme hydrolysis of *p*-nitrophenyl phosphate at 30° and pH 5, as reported before [11].

Three independent experiments were carried out and the data presented are the mean of these experiments. The data on growth was analysed by analysis of variance and significance at P=0.05 were calculated by Duncan's multiple range test of composite means, while for lead content the Dunnett and S-N-K tests were carried as reported in Steel and Torrie [15].

RESULTS

(a) *Effects on germination and early seedling growth.* The effects of lead on germination and growth of maize seedlings are given in Table 1. As evident from the Table, lead is injurious to growth. Reduction in percentage germination was observed at all lead treatments (5–100 mM) and decreases at 10–100 mM were significant. Likewise a decline in shoot and root length was observed at all lead treatments. Reductions in root and shoot length at all treatments were significant. Percent reduction at 5 mM was 7.77 and 23.29 and at 100 mM 18.41 and 30.82 in shoot and root lengths, respectively. Inhibition in root length was higher than in the shoot at all lead treatments. Contrary to the effects on germination and root-shoot length, an increase in dry weights of roots and shoots was observed. Relatively more increase in dry weights of roots than shoots was observed at all lead treatments. All increases in root-shoot dry weights at all treatments (5–100 mM) were significant.

(b) *Lead content in roots and shoots.* The data on lead content of root and shoot at different lead concentrations are given in Table 2. These results fully substantiate the dry weight data of these two organs. Firstly, the lead content increased both in roots and shoots with increasing lead concentration. Secondly, the relative increase in the lead content was relatively larger in roots. For example, lead content in control shoot and root were 0.286 and 0.288 (ppm, dry basis), which increased to 0.358 and 0.403 at 100 mM of lead treatment. Furthermore, the relative percent increase was 25.17 for shoots and 39.93 for roots.

(c) *Effects on soluble protein content.* The data on the effects of lead on soluble protein content of shoots are given in Fig. 1. For control seedlings (0.0 mM lead) the protein content of shoots showed an increase with age (19.76, 24.01 and 31.59 mg/seedling at 1, 4 and 7 days, respectively). A decrease in protein content at all lead treatments was observed in shoots of one and seven day old seedlings, while only at 50–100 mM, for 4-day old seedlings. Of the decreases the significant were at 5–100 mM, i.e. all treatments in 7 day, 75 and 100 mM in 4 day and 30–100 mM in 1 day old seedlings.

(d) *Effects on acid phosphatases activity.* The data on the effects of lead on total activity of acid phosphatases in shoots of 1, 4 and 7 day old seedlings are given in Fig. 2. In control seedlings the maximum activity of acid phosphatases was observed in 1 day (69.72 μmole/15 min/mg fresh wt) and minimum in 7 day (48.75 μmole/15 min/mg fresh wt) old seedlings. With lead grown seedlings a decline in total activity at 5 and 10 mM (non-significant) and a rise at 30–100 mM (from 7.32 % to 28.18 %) was observed in

Table 1. Effect of lead on germination and early seedling growth of *Zea mays* L.

		7-day old seedlings			
Lead Conc. (mM)	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot dry wt. mg/ seedling	Root dry wt mg/ seedling
0	98	11.84	17.13	49.22	29.86
5	94	10.92–	13.14–	52.91+	32.49+
10	90–	10.11–	12.97–	54.09+	33.54+
30	83–	10.01–	12.79–	56.70+	35.78+
50	78–	9.92–	12.63–	58.69+	36.81+
75	71–	9.88–	12.55–	59.95+	38.92+
100	67–	9.66–	11.85–	61.28+	41.79+

–/+ = Decreases/increases significant at P= 0.05 (Duncan's multiple range test of composite means).

Table 2. Comparison of lead concentration values (ppm, dry basis) for root and shoot parts of 7-day old *Zea mays* seedlings.

	Lead content (ppm, dry basis)			
Lead Conc. (mM)	Shoot	Increase (%)	Root	Increase (%)
0	0.286 a		0.288 a	
5	0.309+ bc	8.04	0.314+ bc	9.02
10	0.315+ bcd	10.13	0.323+ c	12.15
30	0.330+ cde	15.38	0.346+ de	20.13
50	0.341+ def	19.23	0.354+ e	22.91
75	0.345+ g	20.62	0.374+ f	29.86
100	0.358+ h	25.17	0.403+ g	39.93

Values are means of six different root and shoot samples.
+ = Treatment means significant from control for a joint confidence coefficient of P = 0.95 (Dunnett 1955).
a,b – – = Values followed by different letters in the same column differ significantly at α = 0.05 (S-N-K test, 1952).

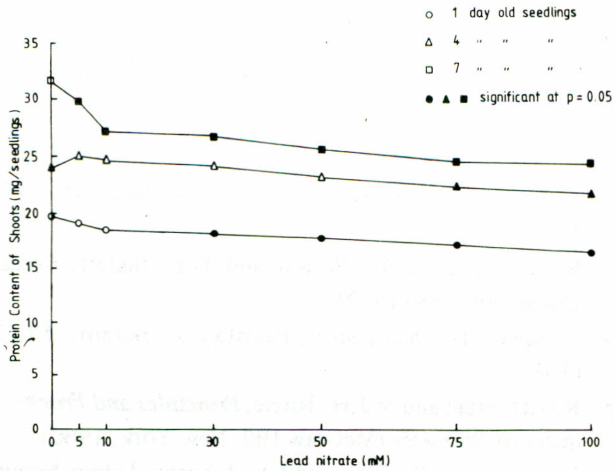

Fig. 1. Effect of lead nitrate on the soluble protein content in shoots of *Zea mays* L. cv. Akbar.

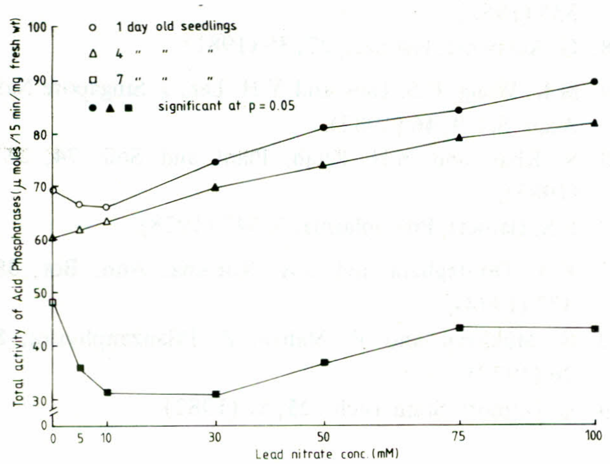

Fig. 2. Effect of lead nitrate on the total activity of acid phosphatases in shoots of *Zea mays* L. cv. Akbar.

shoots of 1 day old seedlings. All observed increases were significant. In shoots of 4-day old seedlings, at all lead treatments enhancement in acid phosphatases activity was observed. Of the increase that at 30 to 100 mM of lead were significant. Contrary to 1 and 4 day old seedlings, in shoots of 7-day old seedlings, a decline in all lead treatments was observed. All decreases in enzyme activity were significant.

DISCUSSIONS

Environmental contamination by lead has been a major concern in biological studies as it is toxic to plants, man and animals. That lead is injurious to plant growth has been also manifested by the present investigation. Reduced germination and retarded shoot-root growth are among the common effects.

Reduction in the rate of germination in *Spartina* [5] and in several other crops have also been reported [4]. In *Spartina* reduction in seed germination was attributed to alterations of selective permeability properties of the seed cell membrane. Retardation in root-shoot length under lead stress is also well documented for different crops, e.g. *Bispinosa rapa* [16], *Sorghum vulgare* [9], *Pisum sativum* [7], soybean [8], radish [17] and many other crops [4]. Increase in cell number and cell enlargement are important contributory factors in growth. Both these phenomena are adversely affected by lead treatment. In *Secale cereale* cellular divisions were hindered and the formation of a large number of binucleate cells indicated that cytokinesis was hampered by lead [18]. Lead has been shown to antagonise the effects of auxin in cell elongation in *Avena* coleoptiles, where it reduces the cell wall plasticity [10].

Several reports are available confirming our observations on the increases in dry weights under lead stress [6,18,19,20]. An increase in dry weight appears to be a contradictory observation to reduced shoot and root elongation under the influence of lead. The explanation to this phenomenon lies in the fact that relatively less amount of lead is transported to the shoot in comparison with the amount absorbed by the root. Hence roots accumulate lead, the amount of which passively increases with the concentration of lead. This is also shown from the present investigation. The lead content for any given treatment of lead was greater in roots than the shoots. This phenomenon is also reported for other plants [18,19,20]. Moreover, within the plant the order of accumulation is roots > stems > leaves [6]. In the present study also a greater accumulation of lead was observed in roots in comparison with the shoot.

Lead mediated reduction in soluble protein content was observed in the present study. In *Pisum* a decrease in total N content with increasing concentrations of lead has been reported [7]. Hamett [21] suggested a denaturation of protein by lead treatment. It appears, therefore, that a reduction in soluble protein content may be due to the non-utilisation of elemental nitrogen and/or by denaturation of the protein themselves.

Acid phosphatases content in shoots of control seedlings were maximum in 1-day and minimum in 7-day old seedlings. The greater activity of acid phosphatases in early stages of growth in the present study is in conformity with the postulated role of acid phosphatases in those physiological processes as are involved in the early growth and development of plant [22]. Under lead stress the acid phosphatases activity registered an increase in 1 and 4-day and a decrease in 7-day old seedlings. Both activation [23] and inactivation [24] of the enzyme, as symptom of lead toxicity has been reported. A critical analysis of the present data on growth and acid phosphatases activity on *a priori* basis suggest that an initial increase in phosphatases content may be symptomatic of lead toxicity. An increase denotes non-utilisation of the enzyme in various metabolic processes. A decrease, on the other hand, denotes an extremely toxic response, where growth altogether ceases and biosynthetic processes are inactivated.

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