NITRATE ASSIMILATION AND BIOMASS PRODUCTION IN *SESAMUM INDICUM* L. SEEDLINGS IN A LEAD ENRICHED ENVIRONMENT

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Abstract. Seed germination was delayed and seedling growth inhibited by 0.04 to 1.9 mM Pb^{+2} in *Sesamum indicum* L. var HT-I. In root, shoot and leaf Pb^{+2} accumulation increased with increasing Pb^{+2} concentration in the nutrient solution. In root and leaf tissues *in vivo* and *in vitro* nitrate reductase activity was inhibited significantly which was well correlated with the concentration of Pb^{+2} supplied and its accumulation in the plant parts. The inhibition of the NR enzyme activity could be, however, reversed by simultaneous treatment of *Sesamum* seedlings with K_2HPO_4, $CaCl_2$ and KNO_3 dissolved in nutrient solution. Total organic N and soluble protein of roots and shoots/leaves, on the other hand, increased with increasing concentration of Pb^{+2} while the same treatment caused a decrease in the N content of cotyledons. It appears therefore, that the increase in N and protein in the roots, shoots/leaves may be a result of increased translocation of N from the cotyledons to the roots and shoots/leaves during early seedling growth in a Pb^{+2} enriched environment.

1. Introduction

Automobile exhaust, Pb^{+2}-containing paints and leaded organic chemicals used in agriculture and industry are causing consistent accumulation of Pb^{+2} in the biosphere (Thapa *et al.*, 1988; Assche and Clijsters, 1990). Lead absorption and accumulation in plant parts affects growth and productivity of plants on one hand, and human life on the other, via the food chain (Ward and Young, 1981). Inhibition of seed germination, seedling growth and productivity due to Pb^{+2} toxicity has been observed for certain plants (Miller *et al.*, 1977; Morzek and Funicelli, 1982; Gruenhage and Jaeger, 1985). Decreases in photosynthesis of soybean and maize leaves (Bazzaz *et al.*, 1974, 1975) and an increase in respiration (Lee *et al.*, 1976) in soybean have been noted. Nitrate reductase (E.C. 1.6.6.1), catalyzes reduction of nitrate to nitrite and is a rate limiting step in nitrate assimilation (Srivastava, 1980). Its activity is often correlated with the N-status of the plant (Mishra and Srivastava, 1983). Though the enzyme has been studied in certain species during Pb^{+2}-contamination (Burzynski and Grabowski, 1984; Sinha *et al.*, 1988a, b) it exhibits different behavior in different plant cultivars (Sinha *et al.*, 1988a, b). *Sesamum indicum*, an important oil seed crop of India, has been recommended for priority studies on physiological and genetic constraints on its production during stress (76th IS Congres Association, 1990†). The present study was planned with the following perspective; to find out

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the effect of Pb^{+2} on nitrate assimilation and biomass production in *Sesamum* seedlings during the early growth phase.

2. Materials and Methods

Sesamum indicum L. cv. HT-I seeds obtained from Haryana Agriculture University, Hisar, were surface sterilized with 0.1% (W/V) $CaOCl_2$ for 5 min and then washed thoroughly with distilled water before planting. Seedlings were raised within small perti-plates (4″) containing wet Whatman No. 1 filter paper for 5 d at 28 ± 2 °C in a prefabricated growth chamber (NSW model S.G., 1.92) under continuous light of approximately 5 klux. Seedlings were watered daily with modified 1/2 strength Hoagland's solution (Arditti and Dunn, 1969) containing KNO_3 (10 mM) as N source. pH of the nutrient solution was kept at 6.0. Desired concentrations of Pb^{+2} in the form of lead acetate were dissolved in distilled water and mixed with the nutrient solution to be supplied to the growing seedlings. As Pb^{+2} is sparingly soluble in nutrient solutions, the Pb^{+2} concentration in solution was elucidated with the help of an Atomic Absorption Spectrophotometer. The level of dissolved Pb^{+2} decreased in the nutrient solution with passage of time because of precipitation of Pb^{+2} as lead sulphate. Thus it is recommended to use only freshly prepared Pb^{+2} solution for such studies.

Seeds were planted in each petri-plate, and germination of the seeds was counted after every 24 hr in the growth chamber continuously for 5 d. After 5 d plants were selected for uniformity, and root and shoot growth were measured. Length of the plant organs i.e. root and shoot was measured with the help of vernier callipers. They were then weighed for fresh weight. Roots and shoots were oven dried at 60 °C till the dry weight became constant.

Lead was estimated in the oven dried samples of roots, shoots and leaves by digesting the samples with concentrated HNO_3 + $HClO_4$ in 3:1 ratio. Lead content in the digested samples was estimated using a Shimdzu Double Beam Digital Atomic Absorption/Flame spectrophotometer AA-650.

Total double protein in roots and leaves was estimated by the method of Lowry *et al.* (1951), after precipitating the proteins with 20% tricarboxylic acid (TCA). Bovine serum albumin (BSA) was used as a standard.

Total organic N content of roots, shoots and cotyledons was estimated by microkjeldahl method of Lang (1958) after digesting with concentrated H_2SO_4. Ammonium sulphate was used as a standard.

In *vivo* NRA in the freshly harvested leaves was determined following the method of Srivastava (1975). The assay mixture contained 0.1 M sodium phosphate buffer (pH 7.4), 25% propanol, 2M KNO_3, 1% sulphanilamide in (1N HCl W/V) and 0.02% N(1-naphtyl) ethylene diamine dihydrochloride (NED).

In *vitro* enzyme activity was estimated by the method of Stevens and Oaks (1973). For *in vitro* assay the enzyme was extracted using 0.1 M sodium phosphate buffer (pH 7.4), 0.3 M ethylene diamine tetra acetic acid sodium salt (EDTA), 0.1% casein

and 0.01 M cysteine. The assay medium contained 2.0 mL of 0.1 M sodium phosphate buffer (pH 7.4), 0.5 mL of 2 M KNO_3, 0.3 mL of nicotinamide adenine dinucleotide, reduced form (NADH) (1 mg mL^{-1}) and 0.2 mL of enzyme preparation.

The data presented in this paper are averages of at least three experiments each with duplicate determinations. Paired '*t*' tests were applied to test the significance of differences. Variance and critical differences of the effects observed were calculated using an '*F*' test.

3. Results and Discussion

3.1. Seed germination and seedling growth as affected by Pb^{+2}

A concentration dependent inhibition of seed germination was observed in *Sesamum indicum* L. var HT-I treated with 0.04 to 1.9 mM Pb^{+2} on the first day after planting. However, this effect was reduced as a function of time and on the 4th day after planting all seeds germinated even at 1.9 mM Pb^{+2} (Figure 1). Length, fresh weight and dry weight of roots and shoots of 5 d old seedlings were, however, reduced considerably due to Pb^{+2} supply (Table I). Further, number and size of root hairs, total leaf area and greening of the leaves also reduced during Pb^{+2} toxicity (data not shown). The effect of Pb^{+2} was, however, more pronounced at higher concentrations of the metal and the root was more affected than the shoot as far as the growth parameters were concerned which is well correlated with Pb^{+2}

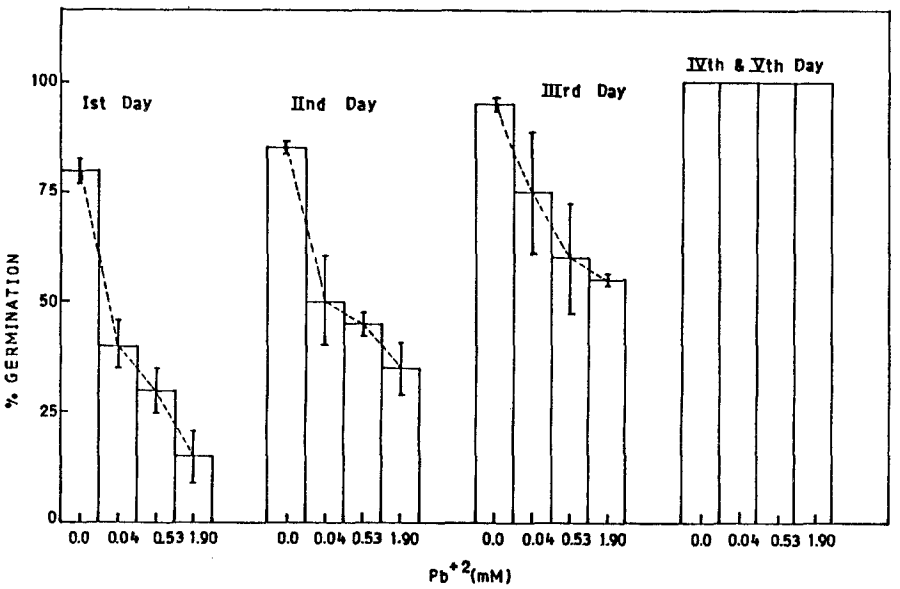

Fig. 1. Seed germination pattern in Pb^{+2} enriched environment. *Sesamum* seedlings were raised for 5 d with ½ strength Hoagland's solution containing 10 mM KNO_3 as a N source and desired concentration of lead acetate within a growth chamber at 28 ± 2 °C under continuous light of 5 klux.

TABLE I

Growth parameters of *Sesamum* seedlings 5 d after planting, as affected by Pb^{+2} supply

Pb^{+2} (mM)	Seedling length[a] (cm±S.D.)	mg[b]	
		F.Wt[b1] ± S.D.	D.Wt[b2] ± S.D.
Root			
0.0	4.6 ± 0.33	2.25 ± 0.16	0.75 ± 0.12
0.04	2.3 ± 0.08	1.62 ± 0.10	0.47 ± 0.12
0.53	1.5 ± 0.29	1.07 ± 0.16	0.35 ± 0.08
1.90	0.7 ± 0.09	0.72 ± 0.18	0.22 ± 0.04
Shoot			
0.0	4.2 ± 0.23	10.7 ± 0.67	2.5 ± 0.20
0.04	2.3 ± 0.18	5.9 ± 0.66	1.7 ± 0.24
0.53	2.1 ± 0.28	5.1 ± 0.31	1.2 ± 0.18
1.90	1.7 ± 0.36	3.8 ± 0.71	0.95 ± 0.19

Sesamum seedlings were raised for 5 d with ½ strength Hoagland's solution containing 10 mm KNO_3 as N source and desired concentration of lead acetate within a growth chamber at 28 ± 2 °C under continuous light of 5 klux. Uniformly grown seedlings of each treatment were sampled and measured for growth parameters. Data are significant (P = 0.01). Critical difference at 1% level of significance; Root-a, 0.58; b_1, 0.33; b_2, 0.14; shoot-a, 0.4; b_1, 0.74; b_2, 0.37.

[The concentration of Pb^{+2} dissolved in the nutrient solution was 0.0, 0.1, 1.0, 2.0 mM, however, as the Pb^{+2} is partially soluble in the complete nutrient solution, the effective Pb^{+2} concentration (to which roots were exposed) in this solution was estimated. The effective concentrations of Pb^{+2} for these solutions were 0.0, 0.04, 0.53, and 1.90 mM respectively].

[a] = Seedling length.
[b] = mg Weight.
b_1 = F.Wt.
b_2 = D.Wt.

accumulation in roots, shoots and leaves of *Sesamum* seedlings (Table II). Reduction in root size and number and size of root hairs may cause restriction in absorption of water and minerals from the nutrient solution. Inhibition of seed germination by Pb^{+2} has been reported in *Spartiana alterniflora* (Morzek and Funicelli, 1982), *Pinus halepensis* (Nakos, 1979) and *Lupinus* (Wozny *et al.*, 1982). Inhibition/delaying of germination may be due to interference with some important enzymes involved in the process, as Mukherji and Maitra (1976), observed inhibition in protease and amylase by about 50% in rice endosperm, though at a very high (60 mM) Pb^{+2} concentration. Further in rice seedlings Pb^{+2} causes deleterious effects on growth and metabolism only at higher concentrations (Mukherji and Maitra, 1976). Inhibition in pod F.Wt. in *Glycine max* at 300 μM Pb^{+2} (Huang *et al.*, 1974), yield of tomato and egg plant (Khan and Khan, 1983), barley (Keaton, 1937), D.Wt. of *Pisum sativum* (Huang *et al.*, 1974), nodulation in soybean (Paivoke, 1983), root

TABLE II

Lead (Pb^{+2}) content in 5 d old *Sesamum* seedlings supplied with lead acetate

Pb^{+2} (mM)	Lead content in *Sesamum* seedlings ($\mu g\ g^{-1}$D.Wt)±S.D.		
	Roots	Shoots	Leaves
0.0	24 ± 2 (100)	1.3± 0.5(100)	17 ± 1 (100)
0.04	592.3 ± 2.0(2468)	83 ± 2.0(6384)	44.3± 2.0(260)
0.53	6577 ± 75 (27404)	723.3± 7.5(55638)	1452.7±18.5(8545)
1.90	5840 ± 36 (24333)	44633 ±152 (3433307)	1764.7±25.0(10380)

Details as in Table I. Data are significant (P=0.01). Critical difference at 1% level of significance; leaves, 40.8; shoots, 228; roots, 53. Percentages relative to control are given in brackets.

and stem elongation and leaf expansion in *Raphanus* (Lane and Martin, 1980) have also been observed. These inhibitory effects of Pb^{+2} on growth and biomass production are possibly a consequence of its effect on metabolic processes of the plant (Assche and Clijsters, 1990). It may also arise from interference by Pb^{+2} with auxin metabolism (Lane *et al.*, 1978, Burzynski and Jakob, 1983).

3.2. Lead content of 5 d old *Sesamum* seedlings in roots, shoots and leaves, supplied with lead acetate

Supply of various concentrations of Pb^{+2} to intact *Sesamum* seedlings increased Pb^{+2} concentration in roots, shoots and leaves. In each tissue, the increase in internal Pb^{+2} concentration was well correlated with external Pb^{+2} supply, although the magnitude of increase varied with the plant part and concentration supplied (Table II). At 0.04 mM Pb^{+2} concentration the order of Pb^{+2} accumulation was roots > shoots > leaves and at 0.53 mM, roots > leaves > shoots. However, at 1.9 mM the order was shoots > roots > leaves. In general roots accumulate more Pb^{+2} than shoots and leaves. Lead toxicity, to some extent depends upon its transport and accumulation. Lead absorbed mostly through the root hair zones reaches the vascular system of the root via cortex and endodermis, (Tanton and Crowdy, 1971). Its accumulation in the cell wall of xylem has been demonstrated by using histochemical techniques (Glater and Hernandez, 1972; Lane and Martin, 1977; Sieghardt, 1984).

3.3. Nitrate reductase activity in roots and leaves under influence of lead environment

Lead acetate (0.04 to 1.9 mM) supplied to the intact *Sesamum* seedlings decreased *in vivo* nitrate reductase activity drastically (Table III). At 0.04 mM Pb^{+2} inhibition in the enzyme activity was 42 and 25% in roots and leaves, respectively, which was well correlated with the Pb^{+2} accumulation in these tissues (Table II). A similar trend in NRA was observed when the enzyme was assayed by an *in vitro* method (Table III).

TABLE III

In vivo and *in vitro* nitrate reductase activity in roots and leaves of *Sesamum* seedlings during lead supply

Pb^{+2}(mM)	Nitrate reductase activity (μ Mole NO_2 h^{-1} g^{-1} F. Wt ± S.D.)	
	Roots[1]	Leaves[2]
In vivo Assay[a]		
0.0	2.4±0.11(100)	2.66±0.1(100)
0.04	1.4±0.10(58)	2.0 ±0.1(75)
0.53	0.7±0.06(29)	1.66±0.17(61)
1.90	0.4±0.02(17)	0.17±0.05(6)
In vitro Assay[b]		
0.0	4.1±0.3 (100)	5.28±0.13(100)
0.04	2.6±0.35(63)	3.80±0.21(72)
0.53	1.0±0.3 (25)	2.64±0.08(50)
1.90	0.5±0.2 (12)	0.33±0.05(6)

Details as in Table I. The enzyme activity was assayed by *in vivo* and *in vitro* methods. Percentages relative to control are given in brackets. Data are significant (P=0.01). Critical difference at 1% level of significance; 1a, 0.23; 1b, 0.18; 2a, 0.30; 2b, 0.20.

[1] is root nitrate reductase activity.

[2] is leaves nitrate reductase activity.

[a] is *in vivo* nitrate reductase activity.

[b] is *in vitro* nitrate reductase activity.

Inhibition of NRA by Pb^{+2} has also been observed in *Helianthus* and *Sorghum* leaves (Venketramana *et al.*, 1978), cucumber cotyledons and roots (Burzynski and Grabowski, 1984), soybean (Huang *et al.*, 1974), *Zostera marina* roots (Brackup and Capone, 1985) and *Zea* and *Pisum* leaves (Sinha *et al.*, 1988a, b). The cause of inhibition of NRA due to Pb^{+2} supply may be multifacial. It may be due to (i) reduced supply of NADH; NADH may be oxidized by Pb^{+2} or its production may be restricted due to swelling of mitochondria in a Pb^{+2} environment (Gengenbach *et al.*, 1973), (ii) disorganization of chloroplasts (Rebechini and Hanzely, 1974 and our own unpublished data), (iii) a smaller NO_3 supply to the site of the enzyme synthesis because Pb^{+2} treatment can create water stress in the plants (Shaner and Boyer, 1976; Burzynski and Jakob, 1983; Burzynski and Grabowski, 1984) and (iv) a direct effect of Pb^{+2} on the enzymatic protein synthesis/activity as Pb^{+2} has a strong affinity for the functional-SH group of the enzyme (Prasad and Prasad, 1987; Sinha *et al.*, 1988b). Since a supply of NADH in the assay system of *in vitro* NRA did not prevent the decline of the enzyme activity in *Sesamum*, it appears that NADH was not a limiting factor in our case. However, the absorption of nutrient and water by the seedling may be restricted in the presence of Pb^{+2} as it caused a reduction in the number and size of roots as well as the number of root hairs (data not shown).

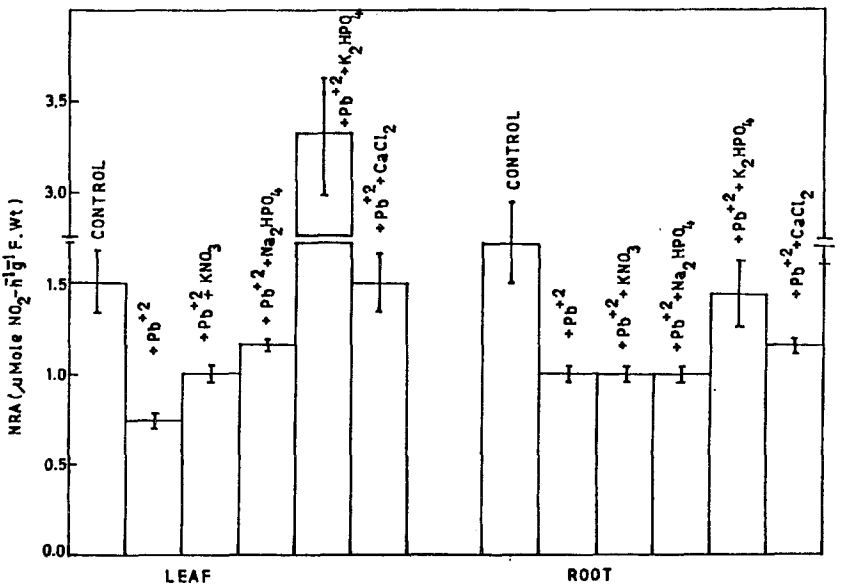

Fig. 2. Effect of Pb^{+2} supply on NRA in *Sesamum* seedlings in the presence of additional levels of some nutritive salts. Growth conditions as in Figure 1.

Supplying K_2HPO_4, $CaCl_2$ and KNO_3 (10 mM each) in the nutrient media increased *in vivo* NRA in *Sesamum* leaves considerably by 177, 67 and 22%, respectively (Figure 2). Although, inhibition of root NRA caused by Pb^{+2} toxicity (0.53 mM) could not be counteracted completely by the addition of these salts, K_2HPO_4 and $CaCl_2$ partially prevented this decline in NRA. On the other hand, Na_2HPO_4 and KNO_3 reduced the decrease of leaf NRA by 22 and 12% (P=0,01), respectively, and $CaCl_2$ could prevent the Pb^{+2} effect on leaf NRA completely. Addition of K_2HPO_4 to the nutrient solution, however, increased leaf NRA by 122% even in the presence of 0.53 mM Pb^{+2}. Thus it appears that the inhibition of NRA caused by Pb^{+2} was not an irreversible phenomenon and the addition of certain inorganic salts, especially K_2HPO_4 and $CaCl_2$ could prevent this loss in the enzyme activity if they were provided along with the nutrient solution in a Pb^{+2} enriched environment. The mechanism of action of these salts on NRA in the presence of Pb^{+2} is yet to be elucidated. Among various possibilities, the ion may increase the membrane permeability and the availability of NO_3 and other enzyme activators. A direct effect of cations and anions on *de novo* synthesis/activity of the enzyme protein is also possible.

3.4. Protein and total organic nitrogen of various organs of the seedlings

Supplying Pb^{+2} (0.04 to 1.9 mM) to *Sesamum* seedlings had a positive effect on protein and total organic N contents in some cases (Tables IV and V). Organic N of the cotyledons (2 d old), however, decreased with increased Pb^{+2} concentration (Table V). Further, the effect of Pb^{+2} on protein and N content was concentration

TABLE IV

Effect of lead supply on total soluble protein in roots and leaves of *Sesamum* seedlings

Pb^{+2} (mM)	mg protein g^{-1} F.Wt. ± S.D.	
	Roots	Leaves
0.0	1.73±0.04(100)	4.79±0.13(100)
0.04	2.19±0.07(126)	6.66±0.20(133)
0.53	2.53±0.06(146)	8.26±0.26(166)
1.90	2.99±0.10(172)	9.06±0.28(189)

Details as in Table I. Data are significant (P=0.01). Critical difference at 1% level of significance; roots, 0.21; leaves 1.18. Percentages relative to control are given in brackets.

TABLE V

Effect of Pb^{+2} on total organic N content of *Sesamum* seedlings

Pb^{+2}(mM)	mg nitrogen g^{-1} F.Wt.± S.D.		
	Roots[a]	Shoots[b]	Cotyledons[c]
0.0	1.37±0.20(100)	4.12±0.20(100)	6.79±0.46(100)
0.04	1.92±0.08(150)	4.73±0.12(115)	6.48±0.46(95)
0.53	2.2 ±0.07(160)	5.35±0.20(129)	5.87±0.31(86)
1.90	2.6 ±0.14(202)	7.00±0.31(170)	5.25±0.31(77)

Details as in Table I. Data are significant (P=0.01). Critical difference at 1% level of significance; a, 0.03; b, 0.7; c, 1.22. Percentages relative to control are given in brackets.

[a] Roots.

[b] Shoots.

[c] Cotyledons.

dependent and the 1.9 mM Pb^{+2}-level increased soluble protein of roots and leaves by 72 and 89% and total organic N of the root and shoot by 90 and 70%, respectively.

A slight increase in protein and N of *Zea* leaves due to Pb^{+2} supply has been reported (Sinha *et al.*, 1988a). As inhibition of NRA should be correlated with decreased organic N (Srivastava, 1980; Mishra and Srivastava, 1983), increase in root and shoot N and decreased NRA in the present case is rather unexpected. It is likely, however, that the metal translocates N from the cotyledons for transport to the roots and shoots. Thus the increase in root-shoot N was correlated with the decrease in total organic N of cotyledons on the 2nd day after planting (Table V).

Sesamum indicum thus seems to be responding significantly to the presence of Pb^{+2} in the environment as far as processes such as seed germination, seedling growth, NRA and total organic N are concerned. However, the toxic effect of Pb^{+2} on nitrate assimilation can be ameliorated by supplying K_2HPO_4 and $CaCl_2$ in the nutrient solution.

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