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Nitrate Assimilation in Intact and Excised Maize Leaves in the Presence of Lead

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Although Pb is a non-essential element, plants can absorb it from soil, water and air through their roots and leaves (Zimdahl and Koeppe 1977). It's growth inhibitory effects on plants have been reported in species such as maize, sunflower (Carlson et al. 1975), rice (Mukherji and Maitra 1977), oats (Fiussello and Molinari 1973). In tracing down the effects at physiological level, inhibition of photosynthesis in soybean (Bazzaz et al. 1974), sunflower and maize (Carlson et al. 1975) and of transpiration (Rolfe and Bazzaz 1975) have been reported, although the rate of respiration is increased at 100 µg/ml of Pb (Lee et al.1976). Several other physiological and metabolic aspects of plants are also known to be affected by Pb in the environment (Koeppe 1981). Although availability and assimilation of inorganic nitrogen is an important aspect of plant life, which often limits other metabolic functions and growth, the effect of Pb on the process has been rarely studied. The effects of Pb supply on nitrate reductase activity (NRA) and nitrate assimilation have been studied in the present investigation.

MATERIALS AND METHODS

Seeds of *Zea mays* L.Cv.GS-2, purchased from a local dealer were surface sterilized with 0.1%(W/V)$CaOCl_2$ for 5 min and then washed thoroughly with distilled water. Seedlings were grown in small plastic pots containing washed, sterilized sand for 14-d either in light (Ca 5 K lux, measured by using a lux meter Philips Model GLM 403 h), a mixture of cool white fluorescent tubes and incandescent bulbs or in the dark for chlorophyll biosynthesis investigation at 25 + 3°C. Seedlings were watered daily with modified 1/2 strength Hoagland's solution containing either KNO_3

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(10 mM) as sole nitrogen source or no nitrogen as per experimental design.

Lead acetate (0.01-100 mM) was included either in the nutrient medium or in the incubation medium as the case may be. The pH in each case was 6.0. Secondary leaves from uniformly grown seedlings were sampled and used for various studies. Pb was supplied to the excised secondary leaves in the incubation medium for 24 h at 25 $\pm$ 1°C in light of about 5 K lux in the growth chamber.

In vivo nitrate reductase activity was assayed by the method of Srivastava (1975) and *in vitro* by the method of Stevens and Oaks (1973). In intact seedlings NRA was measured only in the leaves, as they are believed to be major nitrate reducing organs in most plants. Total protein content in the leaf extract (in phosphate buffer, pH 7) was estimated as described by Lowry et al (1951) following precipitation with equal volume of 20% TCA, followed by alkali solublization. Total nitrogen content in the dried leaf samples was determined by a modified micro-kjeldahl method (Lang 1958), after digesting with concentrate H_2SO_4.

For the pigment content estimation fresh leaf samples were extracted with 80% acetone. Absorbance of clear supernatent was measured at 440, 645 and 663 nm. Total chlorophyll carotenoids were calculated by the method of Strain and Svec (1966) and Ikan (1969) respectively. The data presented are average $\pm$ S.D. of three duplicate experiments.

RESULTS AND DISCUSSION

The effect of Pb on NRA was dependent on the concentration of the metal, mode of its supply and whether the assay was done by *in vivo* or *in vitro* method. When Pb was supplied to the intact seedlings in the nutrient medium *in vivo* NRA in the leaves was stimulated slightly (11%) at 0.01 mM and inhibited (5-20%) at higher concentrations (Table 1). The *in vitro* activity however, was inhibited appreciably (13-64%) by each concentration of Pb. On the other hand, when the Pb was supplied in the incubation medium to the excised leaf segments both *in vivo* and *in vitro* NRA was drastically reduced; the inhibition increased with the increase in Pb concentration. This difference is possibly because of the reason that the uptake and mobilization of Pb to the actual site of action are different in intact seedlings and excised leaves. Perhaps in intact seedlings, it was

limited while in excised leaves it was easily accessible to the site of NR synthesis/activity. Inhibition of *in vivo* NRA by Pb salts has been observed in sunflower and sorghum leaves (Venkatramana et al.1978) and cucumber cotyledons (Burzynski and Grabowski 1984) also.

Unlike NRA, total protein and total nitrogen content in the leaves from intact seedlings increased slightly (1 to 14%) with the Pb supply in the nutrient solution but for that at 1mM Pb when there was 5% inhibition in protein content (Table 2). The protein and organic nitrogen in the excised leaves on the other hand were inhibited (2 to 20%) by Pb in the incubation medium. Inhibition increased with increase in Pb concentration. Nitrate reductase is a rate limiting enzyme in overall assimilation of nitrate (Srivastava, 1980). Its activity may determine the nitrate assimilation status of the plant. Consistent with this, protein and organic nitrogen, the eventual products of nitrate assimilation also declined during Pb supply specially in the excised leaf segments.

However, such an inhibitory effect of Pb in intact seedlings was not seen. Obviously, the enzyme activity is much more susceptible to inhibition than other metabolic steps in intact seedlings. Experiments with other species are in progress, to test whether this enzyme can be taken as a marker of Pb toxicity. Again the insensitivity of protein or organic nitrogen content to Pb inhibition in intact seedlings may be due to restricted presence of the metal at the actual site of action in the leaf.

Although several reports indicate that plants can absorb lead from the environment (Keoppe 1981) and accumulate preferentially in the roots (Atkins et al. 1982). Alternatively, the intact plants may possess some kind of detoxification mechanism which apparently lacks in excised leaves. A slight increase in protein and organic nitrogen contents as in *in vivo* NRA at 0.01 mM Pb in intact leaves may also be either because of very small quantity of metal available at the site of action or of some detoxifying mechanism at this concentration.

The nitrogenous pigment chlorophyll and non-nitrogenous carotenoid in the leaves from intact seedlings increased slightly with Pb supply, although there was no correlation between Pb concentration and the increase (Table 3). However, in the excised tissues,

Table: 1. Effect of Lead on nitrate reductase activity.

Pb^{2+} (mM)	Nitrate reductase activity (μ mole $NO_2 h^{-1} g^{-1}$ fresh weight)			
	Leaves from intact seedlings		Excised Leaves	
	In vivo NRA			
		%		%
0.0	1.31 ± 0.17	(100)	10.6 ± 1.90	(100)
0.01	1.45 ± 0.21	(111)	4.83± 0.79	(46)
0.1	1.24 ± 0.14	(95)	4.43± 0.54	(42)
0.5	1.20 ± 0.16	(92)	1.72± 0.43	(16)
1.0	1.05 ± 0.12	(80)	0.44± 0.10	(4)
	In vitro NRA			
0.0	2.75 ± 0.58	(100)	2.92± 0.72	(100)
0.01	2.41 ± 0.36	(87)	1.69± 0.10	(58)
0.1	1.55 ± 0.33	(56)	0.76± 0.10	(26)
0.5	1.98 ± 0.40	(72)	0.35± 0.0	(12)
1.0	0.99 ± 0.20	(36)	0.35± 0.0	(12)

For intact seedlings treatment the desired concentration of lead was included in the nutrient medium (1/2 strength Hoagland's solution containing 10 mM KNO_3) supplied to the plants in the pots. For excised leaf treatment plants were raised with nutrient solution containing no nitrogen and then excised leaf segments were incubated in nutrient solution containing the desired concentration of Pb and 10 mM KNO_3. Details of incubation conditions are described in materials and methods. Values related to control as a percent are given in parentheses.

Pb inhibited the two pigment levels, the inhibition of chlorophyll being more pronounced than that of carotenoids. Inhibition of chlorophyll content by Pb has also been reported in oat (Fiussello and Molinari 1973) and some hydrophytes (Jana and Choudhuri 1984).

In another experiment, when leaf segments from dark grown, seedlings (raised with nutrient solution containing no nitrogen) were incubated in nutrient solution containing nitrogen and different concent-

Table: 2. Effect of Lead on protein and total organic nitrogen contents in the leaves.

Pb^{2+} (mM)	mg protein or Nitrogen (g^{-1} fresh weight ± S.D.)			
	Leaves from intact seedlings		Excised Leaves	
		Total soluble protein		
		%		%
0.0	12.80 ± 2.02	(100)	13.52 ± 2.96	(100)
0.01	14.55 ± 1.22	(114)	13.25 ± 1.95	(98)
0.1	13.32 ± 1.63	(104)	11.34 ± 2.28	(84)
0.5	13.55 ± 1.75	(106)	10.93 ± 1.65	(81)
1.0	12.15 ± 1.17	(95)	10.80 ± 1.42	(80)
		Total Nitrogen		
0.0	2.01 ± 0.30	(100)	2.17 ± 0.35	(100)
0.01	2.20 ± 0.23	(109)	1.97 ± 0.19	(91)
0.1	2.04 ± 0.31	(102)	2.00 ± 0.38	(92)
0.5	2.03 ± 0.10	(101)	1.97 ± 0.19	(91)
1.0	2.18 ± 0.20	(109)	1.83 ± 0.36	(84)

Details as in Table 1.

rations of lead, the synthesis of chlorophyll was appreciably inhibited (Table 4). The inhibition generally increased with the increase in the concentration of Pb. Chlorophyll biosynthesis may be inhibited due to inhibition of δ-aminolevulinic acid dehydratase, an important enzyme in the biosynthesis of heme compounds including chlorophyll, as has been observed in animal tissues (Sordo et al. 1982). Besides, Pb may also affect the general organization and biogenesis of chloroplasts, as carotenoid synthesis is also inhibited. Lead induced structural changes in the chloroplasts of *Ceratophyllum demersum* (Rebechini and Hanzely 1974).

Table: 3. Effect of Lead on total chlorophyll and carotenoid levels in the leaves.

Pb^{2+} (mM)	Total chlorophyll or carotenoid ($mg\ g^{-1}$ fresh weight ± S.D.)			
	Leaves from intact seedlings		Excised Leaves	
		%		%
	Chlorophyll			
0.0	0.892 ± 0.16	(100)	0.508 ± 0.07	(100)
0.01	0.928 ± 0.12	(104)	0.454 ± 0.09	(89)
0.1	0.965 ± 0.10	(108)	0.460 ± 0.10	(91)
0.5	1.03 ± 0.19	(115)	0.404 ± 0.07	(80)
1.0	0.901 ± 0.08	(101)	0.359 ± 0.07	(71)
	Carotenoid			
0.0	0.875 ± 0.18	(100)	0.679 ± 0.11	(100)
0.01	0.923 ± 0.14	(105)	0.668 ± 0.09	(98)
0.1	0.958 ± 0.10	(109)	0.672 ± 0.06	(99)
0.5	1.04 ± 0.23	(119)	0.639 ± 0.10	(94)
1.0	0.918 ± 0.15	(105)	0.569 ± 0.11	(84)

Details as in Table 1.

Table: 4. Effect of Lead on Chlorophyll and carotenoid synthesis in greening leaf segments.

Pb^{2+} (mM)	Total Chlorophyll or Carotenoids ($\mu g\ g^{-1}$ fresh weight ± S.D.)			
	Chlorophyll	%	Carotenoid	%
0.0	107 ± 20	(100)	91 ± 8	(100)
0.01	80 ± 10	(75)	74 ± 10	(81)
0.1	64 ± 0	(60)	64 ± 6	(70)
0.5	30 ± 4	(28)	53 ± 3	(58)
1.0	20 ± 3	(19)	37 ± 1	(41)

Seedlings were raised for 14 days in dark with 1/2 strength Hoagland's solution containing no nitrogen. Excised leaf segments were incubated in 1/10 strength nutrient solution containing 10 mM KNO_3 and desired concentration of Pb. The incubation was carried on for 24 h in light.

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REFERENCES

Atkins DP, Truemam IC, Clarke CB, Bradshaw AD (1982) The evolution of lead tolerance by *Festuca rubra* on a motorway verge, Environ Pollut Sev 27: 233-241.

Bazzaz FA, Rolfe GL, Windle P (1974) Differing sensitivity of corn and Soybean photosynthesis to lead contamination. J Environ Qual 2: 156-158.

Burzynski M, Grabowski A (1984) Influence of lead on NO_3 uptake and reduction in cucumber seedlings, Acta Soc Bot Pol 53: 77-86.

Carlson RW, Bazzaz FA, Rolfe GL (1975) The effects of heavy metals on plants II. Net photosynthesis and transpiration of whole corn and sunflower plants treated with Pb, Cd, Ni, Tl, Environ Res 10: 113-120.

Fiussello N, Molinari MT (1973) Effect of lead on plant growth. Allionia 19: 89-96.

Ikan R (1969) Natural products: A laboratory guide, Academic Press, New York, p.101.

Jana S, Choudhuri MA (1984) Synergistic effects of heavy metal pollutants on senescence in submerged aquatic plants. Water, Air and Soil pollution 21: 351-357.

Koeppe D (1981) Lead: Understanding the minimal toxicity of lead in plants. In effects of heavy metal on plant function, (Lepp. NW ed) Applied Science Publisher, London p.55-76.

Lang CA (1958) Simple micro determination of Kjeldahl nitrogen in biological materials. Ann Chem 30; 196,1692-1694.

Lee KC, Cunningham BA, Chung KH, Paulsen GM, Liang GH (1976) Lead effects on several enzymes and nitrogenous compounds in soybean leaf. J Environ Qual 5: 357-359.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin-phenol reagent. J. Boil Chem 193:265-275.

Mukherji S, Maitra P (1977) Growth and metabolism of germinating rice (*Oryza sativa.*) seeds as influenced by toxic concentration of lead.Z. Pflazenphysiol 81: 26-33.

Rebechini HM, Hanzely L (1974) Lead induced ultrastructural changes in chloroplast of the hydrophytes *Ceratophyllum demersum* Z.Pflanzenphysiol 73: 377-386.

Rolfe GL, Bazzaz FA (1975) Effect of lead contamination on transpiration and photosynthesis of loblolly pine and autumn olive. For Sci 21:33-35.

Sordo JC, Arroyo M, Macarulle JM, Fedriani JR, Marino A (1982). Neveles de plomo Y activated delta-amino-levulinic dehydratase senquinaos an unapoblaction espenola. Effecto de la exprescion professional Y de los habitos sociales. Rev Expan Fisiol 38: 427-432.

Srivastava HS (1975) Distribution of nitrate reductase in ageing bean seedlings. Plant cell Physiol 16: 995-999.

Srivastava HS (1980) Regulation of nitrate reductase activity in higher plants. Phytochem 19: 725-733.

Stevens DL, Oaks A (1973). The influence of nitrate on the induction of nitrate reductase in maize roots. Can J Bot 51: 1255-1258.

Strain HH, Svec WA (1966) Extraction, separation, estimation and isolation of chlorophylls. In the chlorophylls (Vernon L P.Seely GR ed) Academic Press, New York p. 21-66.

Venkatramana S, Veeranjaneyulu K, Rmadas VS (1978). Heavy metal inhibition of nitrate reductase. J Exp Biol 16: 615-616.

Zimdahl RL, Koeppe DE (1977). Uptake by plants. In lead in the environment, (Boggess WR ed) National Science foundation, Washington D.C. p.99-104.

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