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Effect of lead on nitrate reductase activity and nitrate assimilation in pea leaves

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Abstract

The effect of Pb on nitrate reductase activity, protein, total organic nitrogen and on the chlorophyll content in excised and intact leaf tissues of *Pisum sativum* was examine. Enzyme activity assayed in vitro or in vivo in the excised leaves showed marked increase at lower concentrations of Pb while being inhibited at higher concentrations. In intact leaf tissues, the enzyme activity (in vivo or in vitro) was unaffected at lower concentrations but was inhibited at higher concentrations of Pb. Chlorophyll, carotenoids (non-nitrogenous pigments), soluble protein and organic nitrogen contents remained almost unaffected at all concentrations of Pb tested. It seems that nitrate reductase has a different response towards Pb pollution in this species, which is more tolerant to heavy metal pollution, especially Pb.

Key words: lead, nitrate reductase activity, pigments, protein, pea leaves

INTRODUCTION

Heavy metal pollution of the environment which is increasing day by day has exerted deleterious effects on plants and consequently to man and animals through the food chain (Petterson 1965, Jones et al. 1973). Mainly the monocotyledonous plants have been studied in detail for their responses to heavy metals (Broyer et al. 1972), although some physiological effects of Pb on dicotyledons have also been studied. Merakchiiska et al. (1976) demonstrated inhibition of growth in *P. vulgaris* seedlings by 10^{-5} to 10^{-3} M of $PbCl_2$.

Inhibitory effects of Pb on photosynthesis or photosynthetic components have been reported in soybean (Bazzaz et al. 1974), *Platanus occidentalis* L. (Carlson and Bazzaz 1977), corn (Bazzaz et al. 1975, Carlson et al.

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1975), loblolly pine, autumn olive (Rolfe and Bazzaz 1975) and in spruce (Keller and Zuber 1970). Inhibition of respiration in corn root tip (Koeppe 1977) or of protein and chlorophyll in submerged aquatic plants, *Potamogeton pectinatus* L., *Vallisneria spiralis* L. and *Hydrilla verticillata* L.F.J. (Jana and Choudhuri 1984) have also been reported. Since various species exhibited differential sensitivity to Pb toxicity (Fiussello and Molinari 1973), the responses recorded may not be generalised for all species. With this perspective, the effect of Pb on nitrate assimilation in pea, one of the most important leguminous crops, has been examined in the present investigation.

MATERIALS AND METHODS

Seeds of *Pisum sativum* L. cv. Archel purchased from a local deal were surface sterilized with a 0.1% $CaOCl_2$ solution for 5 min. and washed thoroughly with distilled water, then planted into plastic pots containing sterilized sand. Seedlings were raised for about 2 weeks in light (about 50 W m^{-2})/dark period of 10/14 h at $25 \pm 3°C$.

Excised fully developed leaves from uniformly minus nitrate grown seedlings were cut into small pieces of about 2 mm and floated on 1/10 strenght Hoagland's solution containing 10 mM KNO_3 as the sole nitrogen source and the desired concentration of Pb as lead acetate. This treatment was carried on for 24 h in light of about 60 W m^{-2} (measured by using a Philips Model GLM 403 H luxmeter) at $25 \pm 2°C$ in a growth chamber. Nitrate reductase activity (NRA), pigment and protein content in these leaf segments were measured.

In an alternate set (intact leaf tissue) the desired amount of Pb as lead acetate was supplied to the pea plants in 1/2 strength Hoagland's solution along with 10 mM KNO_3 as the nitrogen source in the plastic pots itself. The pH of the nutrient/incubation solution was 6.0. All of the results are presented with $\pm$ S.D. of at least three separate sets.

Nitrate reductase activity was assayed either by the in vivo (Srivastava 1975) or in vitro (Stevens and Oaks 1973) methods with slight modifications.

Chlorophyll and carotenoids were estimated in the clear supernatant obtained after extracting leaf segments with 80% acetone followed by centrifugation. We used the formulae for chlorophyll (Strain and Svec 1966) and carotenoids (Ikan 1969). The absorbance of the supernatant was read at 640, 645, and 663 nm.

Total soluble protein was estimated by the method of Lowry et al. (1951), after precipitating the extract in phosphate buffer (pH 7.0) with an equal volume of 20% TCA followed by solubilization with 1N NaOH solution. Total organic nitrogen was estimated by the micro-Kjeldahl method (Lang 1958) after digesting the dried leaf samples in concentrated H_2SO_4.

RESULTS

EXPERIMENT 1

When excised leaf segments from minus nitrate grown seedlings were incubated in 1/10 strength Hoagland's solution containing nitrate and varying concentration of lead, the in vitro and in vivo NRA increased substantially at 0.01 and 0.1 mM Pb (Table 1). However, when lead was supplied to the seedlings in the nutrient solution, the in vivo enzyme activity in the leaves (intact leaves) was inhibited consistently. The inhibition increased with the increase in Pb concentration. However, the in vitro activity of the enzyme either increased or was unaffected at low concentrations of lead while inhibited at higher concentrations.

Table 1

The effect of lead on in vivo and in vitro nitrate reductase activity in pea leaves

	Pb^{2+}(mM)	Nitrate reductase activity, μmole $NO_2^- h^{-1} g^{-1}$ f. w.	
		excised leaves	leaves from intact seedlings
In vivo	0.0	2.54 ± 0.17 (100)	1.18 ± 0.20 (100)
	0.01	4.73 ± 0.88 (186)	1.14 ± 0.35 (97)
	0.1	7.74 ± 0.35 (303)	0.71 ± 0.09 (60)
	0.5	1.84 ± 0.18 (72)	0.67 ± 0.06 (56)
	1.0	0.87 ± 0.20 (34)	0.65 ± 0.07 (55)
In vitro	0.0	0.695 ± 0.15 (100)	0.165 ± 0.01 (100)
	0.01	0.867 ± 0.17 (125)	0.182 ± 0.02 (110)
	0.1	1.210 ± 0.08 (174)	0.172 ± 0.02 (104)
	0.5	0.502 ± 0.002 (72)	0.133 ± 0.02 (81)
	1.0	0.343 ± 0.02 (49)	0.133 ± 0.02 (81)

The desired concentration of lead was included either in the incubation medium, 1/10 strength Hoagland's solution (excised leaves) or in the nutrient solution, 1/2 strength Hoagland's solution (intact leaves). In either case the medium/solution contained 10 mM KNO_3 as the sole nitrogen source.

EXPERIMENT 2

Subjection of excised leaf segments to varying concentrations of Pb for 24 h had very little effect on total chlorophyll and carotenoid contents (Table 2). However, when the Pb was supplied to the seedlings, the chlorophyll content decreased, the reduction generally was correlated with the Pb concentration. Although the total carotenoid content was also slightly reduced by Pb, there was no correlation between Pb concentration and the fall in the carotenoid content.

Table 2

The effect of Pb on the pigment content in pea leaves

	Pb^{2+} (mM)	Excised leaves	Leaves from intact seedlings
Chlorophyll content (mg g^{-1} f. w.)	0.0	1.022 ± 0.12 (100)	1.596 ± 0.02 (100)
	0.01	1.01 ± 0.24 (99)	1.227 ± 0.07 (76)
	0.1	1.047 ± 0.21 (102)	1.153 ± 0.12 (72)
	0.5	1.086 ± 0.20 (106)	1.09 ± 0.03 (69)
	1.0	1.056 ± 0.20 (103)	1.255 ± 0.13 (79)
Carotenoid content (mg g^{-1} f. w.)	0.0	0.527 ± 0.06 (100)	0.708 ± 0.09 (100)
	0.01	0.505 ± 0.13 (96)	0.689 ± 0.07 (97)
	0.1	0.526 ± 0.10 (100)	0.574 ± 0.05 (81)
	0.5	0.543 ± 0.11 (103)	0.631 ± 0.08 (89)
	1.0	0.507 ± 0.07 (96)	0.658 ± 0.14 (92)

Details as in Table 1.

EXPERIMENT 3

Exposure of excised leaf segments to varying concentrations of Pb had a small negative effect on the amount of total soluble protein, although there was no correlation between Pb concentration and the decrease (Table 3). On the other hand, total organic nitrogen in the leaf segments increased with Pb supply except for 0.01 and 1.0 mM, where there was no effect. Supply of Pb to intact seedlings had a small promotory effect on leaf protein with the exception of 0.5 mM where there was a small decline. The total organic nitrogen was generally unaffected except for 1.0 mM where a slight drop was noted.

Table 3

The effect of Pb on total soluble protein and total organic nitrogen in pea leaves

	Pb^{2+} (mM)	Excised leaves	Leaves from intact seedlings
Total soluble protein (mg g^{-1} f. w.)	0.0	7.917 ± 1.04 (100)	9.74 ± 0.44 (100)
	0.01	7.519 ± 1.03 (95)	11.235 ± 0.97 (115)
	0.1	7.309 ± 0.879 (92)	11.164 ± 1.10 (114)
	0.5	7.889 ± 0.736 (99)	9.296 ± 0.74 (95)
	1.0	7.376 ± 0.752 (93)	10.253 ± 0.9 (105)
Total organic nitrogen (mg g^{-1} f. w.)	0.0	6.65 ± 0.31 (100)	5.18 ± 0.79 (100)
	0.01	6.80 ± 1.01 (102)	5.32 ± 1.07 (103)
	0.1	8.06 ± 0.52 (121)	5.29 ± 0.80 (102)
	0.5	7.60 ± 0.68 (114)	5.18 ± 0.79 (100)
	1.0	6.53 ± 0.17 (98)	4.64 ± 0.99 (89)

Details as in Table 1.

DISCUSSION

Increasing concentrations of Pb had an inhibitory effect on nitrate reductase activity assayed either in vivo or in vitro in intact pea leaves (Table 1). Inhibition in nitrate reductase activity has been reported in cucumber cotyledons and roots (Burzyński and Grabowski 1984) in *Helianthus annuus* and *Pennisetum typhoides* leaves as well (Venketramana et al. 1978). The cause of inhibition might be the reduced supply of NADH due to its oxidation by Pb or reduced production due to swelling of mitochondria (Gengenbach et al. 1973) or due to disturbances in the structure of chloroplasts (Rebechini and Hanzely 1974). Burzyński and Grabowski (1984) had suggested that the inhibitory action of Pb was due to lesser NO_3^- supply to the site of enzyme synthesis because of water stress (Morilla et al. 1973, Shaner and Boyer 1976) created by lead treatment (Burzyński and Jakób 1983).

In the present study, intact and excised leaves of seedlings treated with Pb showed a markedly different pattern in enzyme activity both in vivo as well as in vitro. The lower concentration of lead considerably increased NRA in vivo as well as in vitro in excised tissue of pea leaves (Table 1). The possibility of direct influence of Pb on enzyme synthesis can not be ruled out. From in vitro studies it appeared that cofactor NADH may not be limiting for NR in the presence of Pb, because exogenous NADH is supplied during in vitro assay. Still, inhibition at higher concentrations of Pb was recorded in intact as well as in excised tissues. Therefore, it may be inferred that Pb has a direct effect on enzyme synthesis.

Since the total protein level was not much altered by Pb (Table 3), it is possible that Pb affects a specific protein only. Chlorophyll content is not much effected in either case of treatment of intact or excised leaf tissues. This may be through non-translocation of Pb at the active site of chlorophyll metabolism, although structural changes in chloroplast have been reported in *Ceratophyllum demersum* (Rebechini and Hanzely 1974). But at the same time the insensitivity of mature tissue towards heavy metals has been also demonstrated (Koeppe 1981).

Since protein and organic nitrogen content also remained unchanged, the experiments described demonstrate that the enzyme, nitrate reductase, is much more sensitive to Pb pollution than other components of nitrate assimilation, such as protein organic nitrogen and chlorophylls. It also appears that pea is more tolerant to Pb pollution in respect to these parameters than are other species such as the maize where similar treatment with Pb caused greater effects on the examined parameters (Sinha et al. 1988).

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Wpływ ołowiu na aktywność reduktazy azotanowej i na przyswajanie azotanu w liściach grochu

Streszczenie

Badano wpływ ołowiu na aktywność reduktazy azotanowej, na poziom białka, na zawartość azotu organicznego i chlorofilu w tkankach uciętych i nienaruszonych liści *Pisum sativum*. Aktywność reduktazy azotanowej, badana in vitro i in vivo w uciętych liściach wyraźnie była większa przy stosowaniu mniejszych stężeń ołowiu, natomiast w większych stężeniach była inhibowana. W tkankach liści nienaruszonych aktywność enzymu (in vivo i in vitro) była nie zmieniona przy małych stężeniach ołowiu, a w większych stężeniach – inhibowana. Zawartość chlorofilu, karotenoidów (pigmenty bezazotowe), białka rozpuszczalnego i azotu organicznego pozostała prawie nie zmieniona w przypadku wszystkich stosowanych stężeń ołowiu. Wydaje się, że reduktaza azotanowa grochu reaguje inaczej na zanieczyszczenie ołowiem niż innych gatunków, ponieważ groch jest stosunkowo tolerancyjny na zanieczyszczenie matalami ciężkimi, szczególnie ołowiem.