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Influence of lead on NO_3^- uptake and reduction in cucumber seedlings

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Abstract

The influence of $PbCl_2$ on NO_3^- uptake and activity level of nitrate (NR) and nitrite (NiR) reductases in cotyledons and roots of cucumber seedlings was studied. $PbCl_2$ in a 10^{-5} M concentration decreased by 50 per cent NO_3^- uptake and reduced NR and NiR activity *in vivo*. In *in vitro* experiments only high lead concentrations (10^{-3} M) almost completely inhibited NR activity and reduced by 14-28 per cent that of NiR. Lower lead concentrations which inhibited *in vivo* NR activity did not affect enzyme induction, but depressed tissue hydration. These results indicate that lead in low concentrations (10^{-5}, 10^{-4} M) indirectly affected NR and NiR activity by producing water stress and reducing NO_3^- uptake, whereas the direct influence of lead on the proteins of the tested enzymes was noticeable at high concentrations.

Key words: lead, nitrate reductase, nitrite reductase

INTRODUCTION

Plants are known to take up and accumulate heavy metals the presence of which in the cells frequently has a negative effect on plant metabolism and development. To such metals belongs lead. A number of enzymes functioning in the cell are exposed to the action of Pb^{2+} ions, owing to their reaction with SH groups. Thus, lead reacts with proteins containing cystein or histidin, purin proteins or those bound with porphyrins (Vallee and Ulmer 1972), this leading to cell metabolism disturbance. The influence of Pb^{2+} on the activity of acid phosphatases in maize is known (Maier 1978), as well as on the activity and quantity of malate dehydrogenase isoenzymes in alfalfa (Maier 1977), nitrogenase activity in soybean (Huang et al. 1974) or enzymes of the pentose phosphate cycle (Hampp et al. 1973). The influence has also

been demonstrated of Pb^{2+} on the enzymes of IAA metabolism (Mukherji and Maitra 1977) and the activity of enzymes participating in cell wall synthesis and in ATPase connected with plasmalemma (Zegers et al. 1976). It has, moreover, been demonstrated that lead accumulated in the tissues influences plant transpiration. Bazzaz and Windle (1974) and Carlson et al. (1975) noted a reduction of transpiration intensity under the influence of lead, owing to its action on the stomates. Burzyński and Jakób (1983) demonstrated, however, that lead diminished water uptake by sunflower hypocotyl tissues, thus depressing their hydration. Since the activity of many enzymes, particularly inductive ones depends on the water potential and is depressed with the diminution of the latter (Bradzik et al. 1971, Shaner and Boyer 1976a, b), the indirect influence of lead on the activity of numerous enzymes cannot be ruled out.

The present study was undertaken to investigate the direct, or indirect influence of Pb^{2+} on nitrate reductase (inductive enzyme) and nitrite reductase (constitutive enzyme) activity in cucumber seedlings.

MATERIAL AND METHODS

Two-day-old cucumber (*Cucumis sativus* L. var. Wisconsin SMR 18) seedlings were placed in 250-cm^3 beakers filled with —N solution of the following composition: 3 mM K_2SO_4, 1 mM $Ca(H_2PO_4)_2$, 2 mM $CaSO_4$, 1 mM $MgSO_4$ with basic microelements added (Buczek 1979). The solution pH was adjusted to 5.5. After 24 h the plants were transferred for 18 h to a $PbCl_2$ solution (concentrations shown in Tables). Both seed germination and plant growth in both solutions were run in darkness at 27°C. The plants were then transferred to a light thermostat (5.83 $W \cdot m^{-2}$) where the seedlings were placed in lead solutions containing 5.5 mM KNO_3 and 1 mM $Ca(NO_3)_2$, with pH adjusted to 5.5. The control solution contained, instead of $PbCl_2$, NaCl in an amount equivalent to the Cl^- introduced with $PbCl_2$. After 4, 24, and 48 h the plants were taken for determinations. In one part NR and NiR activity was determined in the cotyledons and roots, and in the remaining one fresh and dry weight.

Enzyme activity *in vitro* was estimated in fresh material. NR and NiR extracts were prepared from leaves and roots at a temperature around 0°C, the tissues were homogenised in medium containing 0.05 M Tris-HCl (pH 7.8), 1 mM cystein and 0.3 mM EDTA (Buczek 1976). The homogenate was centrifuged for 20 min at 20 000 × g and enzyme activity was determined in the supernatant. Nitrate reductase (EC 1.6.6.1) was determined after Hageman and Flescher (1960) by measuring NADH-dependent NO_2^- production. Nitrite reductase (EC 1.6.6.4) activity was measured according to Hucklesby et al. (1972) with the

use of methylviologen reduced with dithionite as reductive agent. Enzyme activity was expressed as μmols or nmols NO_2^- produced (NR) or utilised (NiR) per 1 g of fresh weight within one hour. The direct influence of Pb on NR and NiR activity (Table 1) was tested in extracts prepared from plants growing 20 h on full Hoagland medium (KNO_3 — 5 mM, $Ca(NO_3)_2$ — 5 mM, $MgSO_4$ — 1 mM, KH_2PO_4 — 1 mM) in light. In this case phosphate buffer was not added to the incubation medium (Pb precipitates phosphates).

Table 1

Influence of lead *in vitro* on nitrate and nitrite reductase activity (μmole $NO_2^- \cdot gram^{-1}$ fresh weight $\cdot hour^{-1}$)

Lead concentration, M	Nitrate reductase activity		Nitrite reductase activity	
	cotyledons	roots	cotyledons	roots
Control	2.7	5.5	35.6	37.3
10^{-7}	2.1 (76)*	5.0 (90)	32.5 (91)	35.7 (96)
10^{-5}	2.2 (82)	5.4 (97)	32.5 (91)	33.3 (89)
10^{-3}	0.4 (14)	0.0	30.6 (86)	26.9 (72)

* — per cent of control. Each value represents mean of 5 replications.

Nitrate reductase *in vivo* was determined according to the modified method of Jaworski (1971). The cut off cotyledons and roots (about 250-300 mg) were infiltrated under reduced pressure with medium containing 0.1 M KNO_3, the tested $PbCl_2$ concentration and propanol in a final concentration of 1 per cent. Phosphate buffer was not used. After infiltration the tissues were placed in vessels containing 5 cm^3 medium and incubated in a water bath in darkness at 30°C. After 2 and 4 h an adequate amount of the solution was taken for nitrite determination. To the sample taken 1 cm^3 of 1 per cent (w/v) sulphanilamide was added in 1 N HCl and 1 cm^3 of N-(1-naphthyl)ethylendiamine dihydrochloride. The whole was diluted to 5 cm^3 with distilled water. After 15 min extinction was read at 540 nm. The influence of Pb on NR activity *in vivo* (Table 3) was tested on roots and cotyledons of 2-day-old cucumber seedlings growing afterwards for 24 h under light on Hoagland medium (composition given above). The influence of Pb on the appearance of active NR (induction) (Table 2) was studied on cotyledons and roots cut off from plants growing for 24 h in a —N solution (composition given above) and incubated after filtration for 4 h.

Nitrate uptake was measured in terms of the loss of NO_3^- in the medium after a definite time, nitrates were determined after Cataldo et al. (1975).

Table 2

Influence of various lead concentrations on nitrate reductase induction

Lead concentration, M	Nitrate reductase activity, nmoles $NO_2^- \cdot g^{-1}$ fresh weight	
	cotyledons	roots
Control	442±62* a	111±21 a
10^{-5}	387±79 a (87)**	114±15 a (102)
10^{-4}	375±51 a (84)	88±22 a (79)
10^{-3}	281±21 b (63)	13±3 b (12)

* — mean ±SE. ** — per cent of control. Amount of NO_2^- formed, measured after 4 h of incubation. Means denoted by different letters within one column differ significantly at 5 per cent level. Experiment was repeated three times with five replications for each combination.

RESULTS

The results concerning the influence of lead on NR and NiR activity *in vitro* are listed in Table 1. Lead chloride in a 10^{-7} and 10^{-5} M concentration only slightly inhibited NR activity in extracts from cotyledons and had no effect on the NR activity in roots or on NiR activity in preparations from both types of tissues. NR activity was, however, almost reduced to zero in the presence of 10^{-3} M Pb, whereas NiR activity at the same Pb concentration was diminished by about 14-28 per cent as compared with that in the control.

The influence of lead *in vivo* on NR and NiR activity was much more pronouced (Fig. 1A, B) as compared with its influence on the enzymes *in vitro*. Three-day seedlings treated preliminarily for 18 h with 10^{-5} M $PbCl_2$ and then transferred to a nitrate solution with the same Pb^{2+} concentration and exposed to light showed a distinct decrease of NR activity in the roots (Fig. 1B) after 4 h and in the cotyledons (Fig. 1A) after 24 h of growth. Higher Pb concentration (10^{-3} M) caused a decrease almost to zero of NR activity both in roots and cotyledons of the plants. Nitrite reductase was less resistant to the influence of lead introduced into the medium. Lead chloride in 10^{-5} and 10^{-3} concentrations depressed the enzyme activity in the cotyledons, however, the reduction of NiR activity was only 30-40 per cent as compared with the control (Fig. 2A). Similarly, 10^{-5} M lead reduced slightly but significantly NiR in the roots, whereas at a 10^{-3} concentration it depressed this activity more distinctly.

The presence of 10^{-5} M lead in the nitrate solution diminished twofold NO_3^- uptake after 24 h as compared with that in control plants. At the same time 10^{-3} M Pb inhibited completely nitrate uptake, but after 48 h their traces could be detected (Fig. 3).

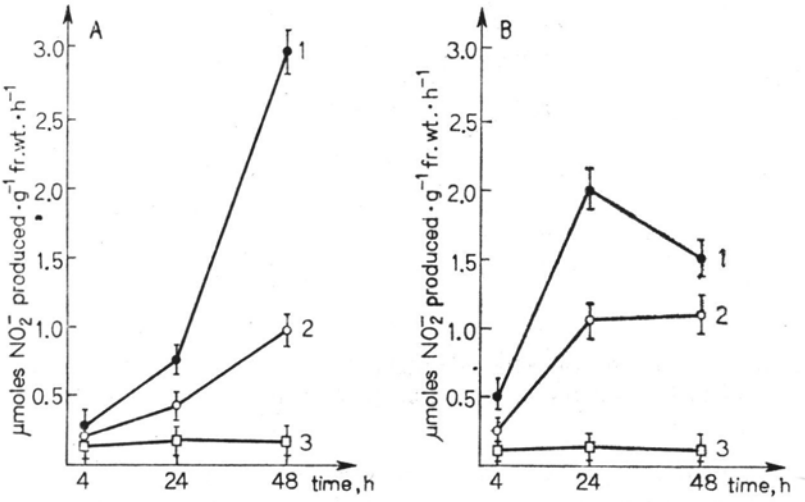

Fig. 1. Changes in nitrate reductase activity under the influence of lead in the cotyledons (A) and roots (B). 1 — control, 2 — 10^{-5} M Pb. 3 — 10^{-3} M Pb. Vertical lines denote 2 SE value

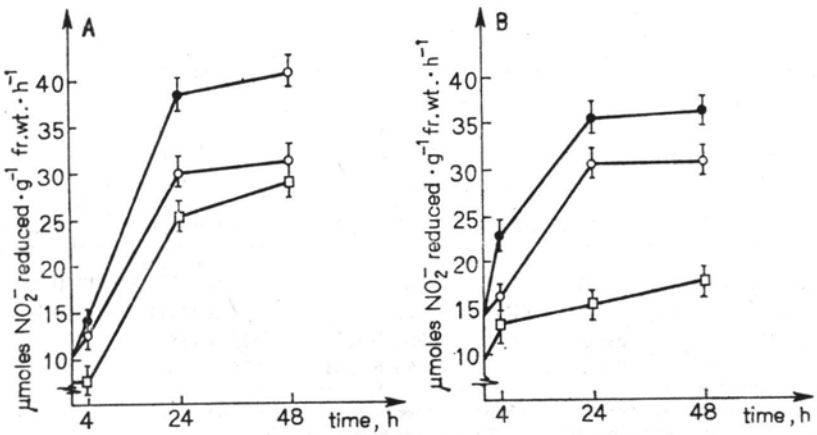

Fig. 2. Changes in nitrite reductase activity under the influence of lead in the cotyledons (A) and roots (B). 1 — control, 2 — 10^{-5} M Pb, 3 — 10^{-3} M Pb. Vertical lines denote 2 SE value

Lead in a 10^{-5} concentration which *in vivo* reduced NR activity had no significant influence on nitrate reductase induction (Table 2) either in roots or in cotyledons. A similar effect was achieved by applying a ten times higher Pb concentration (10^{-4} M). Only at 10^{-3} M concentration did lead decrease by 37 per cent NR induction in cotyledons and almost completely inhibit it in roots.

It would seem, therefore, that the influence of lead on NR consists rather in the action of this metal on the enzyme activity level than on

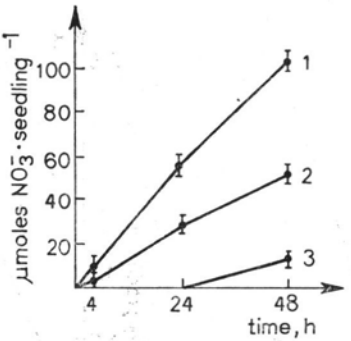

Fig. 3. Influence of Pb on nitrate uptake by cucumber seedlings. 1 — control, 2 — 10^{-5} M Pb, 3 — 10^{-3} M Pb. Vertical lines denote 2 SE value

enzymatic protein synthesis, as indicated by the experiments shown in Table 3. Infiltration of Pb^{2+} into the cut cotyledons, namely, containing like the roots active nitrate reductase and their placement in the nitrate solution with Pb^{2+} added, gradually reduced the NR activity, the effect being dependent on the lead concentration and the time of exposure to it.

Table 3

Influence of various lead concentrations on nitrate reductase activity measured *in vivo*

Lead concentration, M	Nitrate reductase activity, nmoles $NO_2^- \cdot g^{-1}$ fresh weight			
	cotyledons		roots	
	after 2 h	after 4 h	after 2 h	after 4 h
Control	6180 a	12000 a	625 a	1330 a
10^{-5}	5800 a (94)*	12200 a (102)	695 a (111)	880 b (65)
10^{-4}	3500 b (57)	8300 b (69)	612 a (88)	790 b (60)
10^{-3}	3500 b (57)	7150 b (59)	225 b (36)	260 c (20)

* — per cent of control. Experiment was repeated three times with five replications for each combination. Means denoted by various letters within one column differ significantly at 5 per cent level.

The results shown in Table 4 indicate that lead rather rapidly caused a decrease in fresh weight increment of the plants and did not influence the dry weight increment in the course of the 48 h of the experiment. Hydration of the plants expressed as percentage of that of the controls indicates a distinct water stress in plants growing in the presence of lead. Detailed analyses of the degree of hydration of the cotyledons, roots and hypocotyls (data no included) indicate that the water content in the roots and cotyledons decreases equally in a degree close to the mean for the whole plant. Least pronounced was the influence of lead on the hypocotyls water content.

Table 4

Influence of lead on fresh and dry weight of cucumber seedlings

Duration of growth, h	Pb concentration, M	Fresh weight, mg · plant^{-1}	Dry weight, mg · plant^{-1}	Hydration as per cent of control
4	control	194±5.1* a	20.1±1.1 a	100
	10^{-5}	169±5.3 b	19.9±1.2 a	86
	10^{-4}	140±4.1 c	18.2±2.1 a	70
	10^{-3}	124±3.8 d	16.7±1.5 a	61
24	control	224±4.8 a	21.3±0.8 a	100
	10^{-5}	166±4.2 b	20.6±1.1 a	71
	10^{-4}	178±5.7 b	20.5±0.7 a	71
	10^{-3}	163±3.3 b	20.5±1.5 a	71
48	control	237±5.8 a	20.9±1.2 a	100
	10^{-5}	208±2.4 a	21.6±0.4 a	85
	10^{-4}	161±3.2 b	20.3±0.8 a	65
	10^{-3}	145±2.7 c	21.4±1.2 a	57

* — mean±SE. The values are means from 20 seedlings. Means denoted by various letters within one column in each time of measurement differ significantly at 5 per cent level.

DISCUSSION

The above described results indicate that only high lead concentrations (10^{-3} M) decrease directly *(in vitro)* the activity of nitrate reductase isolated from roots and cotyledons of cucumber and that they inhibit biosynthesis of active enzyme (induction) in the roots. As regards cotyledons, however, lead affected less NR biosynthesis. Even high Pb concentrations exerted but a slight influence on NiR *in vitro*. It is, therefore, doubtless that Pb in high concentrations affected directly the NR protein, probably by way of interaction of with the SH groups of nitrate reductase (Beevers and Hageman 1969, Vallee and Ulmer 1972). The fact that lead in a lower concentration than 10^{-3} had no influence on NR and NiR *in vitro*, but inhibited this activity *in vivo* in cotyledons and roots points rather to an indirect action of Pb^{2+} on nitrate or nitrite reductase.

The indirect influence of Pb^{2+} ions on the activity level of both reductases may be interpreted on the basis of the present results either as a strong influence of Pb^{2+} on NO_3^- uptake or its depressing action on tissue hydration causing water stress.

One of the factors influencing the NR activity in plant tissues is the continuous supply of NO_3^- from the external environment both to roots and from roots to leaves. The investigations of Heimer and Filner (1971), Jackson et al. (1973), Neyera and Hageman (1975) and Shaner and Boyer (1976a, b) demonstrated that NR activity

in plant leaves depends not so much on the amount of nitrates accumulated, but rather on continuous NO_3^- transport from roots to leaves. A similar dependence was demonstrated by Buczek (personal communication) as regards leaves and roots of cucumber and maize. The results of the present investigations indicate that the presence of Pb^{2+} in the external solution depresses twofold the amount of NO_3^- ions taken up by cucumber roots as compared with that in control plants and this may be one of the causes of the reduction in NR activity under the influence of low Pb^{2+} concentrations in the medium.

The water stress in plants taking up Pb^{2+} which was manifested in our experiments by a reduced water content in the plant tissues (mainly in cotyledons and roots) amounting to 30-40 per cent as compared with the control may suggest an indirect influence of Pb^{2+} on the activity level of nitrate reductase. This decrease due to water stress has been demonstrated in the experiments of Morilla et al. (1973) and Shaner and Boyer (1976b). The latter authors demonstrated also that water stress depresses nitrate uptake and suggested that this may be the main cause of enzyme activity inhibition, whereas it affects less its bioinduction. The present experiments seem to confirm this supposition. Water stress, namely, caused by lead uptake distinctly reduces the uptake of NO_3^- and this led as consequence to NR activity inhibition. It seems, however, that the specific influence of Pb as metal on enzymic protein in plant tissues should not be disregarded. The present experiments suggest that the direct influence of lead on NR and NiR proteins was mainly dependent on the concentration of lead introduced into the medium. Evidence of this would be the influence of only high lead concentrations on NR and NiR activity *in vitro*.

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Wpływ ołowiu na pobieranie i redukcję NO_3^- w siewkach ogórków

Streszczenie

Badano wpływ $PbCl_2$ na pobieranie NO_3^- oraz na aktywność reduktazy azotanowej (NR) i azotynowej (NiR) w liścieniach i korzeniach siewek ogórków. $PbCl_2$ w stężeniu 10^{-5} M zmniejszał o 50 procent pobieranie NO_3^- i wpływał na zmniejszenie aktywności NR i NiR *in vivo*. W doświadczeniach *in vitro* jedynie ołów w dużym stężeniu (10^{-3} M) prawie całkowicie zahamował aktywność NR i zmniejszył o 14-28 procent aktywność NiR. Ołów w mniejszym stężeniu, który *in vivo* hamował aktywność NR nie miał wpływu na indukcję enzymu, zmniejszał natomiast uwodnienie tkanek. Wyniki wskazują, że ołów w małych stężeniach (10^{-5}, 10^{-4} M) wpływał pośrednio na aktywność NR i NiR wywołując stres wodny i zmniejszając pobieranie NO_3^-, natomiast bezpośredni wpływ ołowiu na białko badanych enzymów zaznaczył się przy dużych jego stężeniach.