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The uptake and accumulation of phosphorous and nitrates and the activity of nitrate reductase in cucumber seedlings treated with $PbCl_2$ or $CdCl_2$

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Abstract

Treatment of 4-day-old cucumber (*Cucumis sativus* L.) seedlings with $PbCl_2$ or $CdCl_2$ caused a significant increase in the accumulation of heavy metals by the plants, especially in the roots. The accumulated Pb initially enhanced the uptake of phosphorous after the plants had been transferred to a nutrient medium (6, 24 hrs), but after only 48 hrs the uptake had dropped to below control level. The plants treated with Cd exhibited a constant decreased phosphorous uptake level. The accumulated lead and cadmium also inhibited nitrate uptake and the activity of nitrate reductase. It is suggested that the reason for the decreased nitrate reductase activity lay rather in the lower nitrate uptake than in a direct effect of the heavy metals on the enzyme.

Key words: lead, cadmium, uptake and accumulation of phosphorous and nitrates, nitrate reductase

INTRODUCTION

The uptake by higher plants of macro- and microcomponents is dependent on heavy metals such as lead or cadmium. The presence of lead and cadmium in the medium or soil decreased the accumulation of K^+, Ca^{2+} and Mg^{2+} (Iwai et al. 1975, Walker et al. 1977, Burzyński 1987) and Fe^{3+}, Mn^{2+} and Zn^{2+} (Iwai et al. 1975, Burzyński 1987). It was found that $PbCl_2$ lowered the phosphorous level in lettuce leaves and oat seedlings (John and Laerhoven 1972) and in maize leaves (Walker et al. 1977). Cadmium reduced the level of phosphorous in maize leaves in a similar way (Walker et al. 1977). However, there is little data on the effect of heavy metals on the uptake of nitrates Hofslagare et al. (1985) observed a distinct decrease in the

uptake of nitrates by *Scenedesmus obliques*, and Burzyński and Grabowski (1984) showed the inhibition of nitrate uptake by roots of cucumbers growing in hydroponic cultures containing lead.

The disturbances in the uptake of macro- and microcomponents evoked by cadmium or lead may be the result of the effect of these metals on the structure and permeability of cell membranes (De Filippis 1979). Lindberg and Wingstrand (1985) showed that Cd^{2+} inhibited the activity of the K^+ and Mg^{+2}-dependent ATPase in the root cells of the sugar beet, while Keck (1978) showed the same in segments of oat roots. Zegers et al. (1976) suggested that lead acted on the ATPase located in the membranes of oat coleoptiles.

In this study, the uptake and accumulation of phosphorous and nitrates were examined in plants previously treated with $PbCl_2$ and $CdCl_2$. Also it was attempted to determine the relationship between the reduced uptake of nitrogen by plants treated with Pb or Cd and the activity of nitrate reductase (NR), a key enzyme in the synthesis of amino acids.

MATERIAL AND METHODS

Cucumber seeds (*Cucumis sativus* L. var. Wisconsin SMR 18) were germinated for 2 days in the dark at 27°C, after which the plants were grown in 250 cm^3 beakers (20 plants) on a diluted no-phosphorous medium of the following composition (in mM): KNO_3 – 0.5; $Ca(NO_3)_2 \cdot 4H_2O$ – 0.5; $MgSO_4 \cdot 7H_2O$ – 0.2; KCl – 0.1 and $FeC_6H_5O_7$ – 0.01. After 4 days of growth in a photothermostat (17 hrs of light at 15.83 W m^{-2}, 25°C and 7 hrs of darkness, 20°C) the plants were transferred for 24 hrs to solutions of $PbCl_2$ or $CdCl_2$ at the concentrations given in the tables and figures. The control plants were transferred to 30 μM solutions of NaCl. Next, the solution of metals was replaced with a nutrient medium of the following composition (in mM): KNO_3 – 1.7; $Ca(NO_3)_2 \cdot 4H_2O$ – 1.7; $MgCl_2$ – 0.33; KH_2PO_4 – 0.1; $FeC_6H_50_7$ – 0.1. The pH was brought to 5.8. After 6, 24 and 48 hrs of growth on this medium, the uptake of phosphorous and nitrates was determined, while the accumulation of phosphorous, nitrates, lead and cadmium was assayed only once after 48 hrs. The activity of nitrate reductase in leaves and coleoptiles was determined after 24 and 48 hrs.

The uptake of phosphorous and nitrates was determined on the basis of the depletion of the medium of these anions. Phosphorous was determined according to Fiske and Subbarow (1925), nitrates according to Cataldo et al. (1975). The decrement of phosphates and nitrates was determined in 5 beakers for each combination, the experiment was repeated 4 times.

Total phosphorous was determined by the Fiske and Subbarow (1925) method separately for roots, hypocotyls and cotyledons, by incinerating the appropriate samples of fresh plant material in concentrated sulfuric and nitric acids.

The nitrates content in the individual plant parts was determined in dried material (the plant parts were dried to a constant weight at 70°C). Nitrates

were extracted from 20 mg of dry material by heating the samples in distilled water for 2 hrs at 80°C and then for 10 min in a boiling water bath. After filtration, the nitrates were assayed in the filtrate according to Cataldo et al. (1975). The P and NO_3^- contents in seedlings are the averages of 5 determinations.

Cadmium and lead were determined using an atomic absorption spectrophotometer (AAS 1N Zeiss) after incineration of dry samples of the appropriate plant parts in a 4:1 mixture of 65% HNO_3 and 60% $HClO_4$.

The activity of nitrate reductase was assayed in crude extracts from cotyledons and roots. The tissue was ground in a mortar at a ratio of 4:1 with 0.05 M K-phosphate buffer (pH 7.4) containing 1 mM cysteine hydrochloride, 3 mM EDTA and 1.5% polyvinylpyrrolidone. The homogenate was centrifuged for 15 min at 15 000 × g and the supernatant (crude extract) was used to determine NR activity. The described procedure was carried out at a temperature of 0-4°C. NR (EC. 1.6.6.1) activity was determined according to Hageman and Flesher (1960) by measuring the NADH-dependent nitrite production. NR activity was expressed in μmoles NO_2^- per g fresh weight per hour.

The effect of cadmium on NR activity *in vitro* was determined by adding increasing concentrations of Cd^{2+} and crude extracts of control plants to the incubation medium.

RESULTS

The accumulation of heavy metals in the particular plant parts is given in Tables 1 and 2. These results indicate that Pb^{2+} and Cd^{2+} accumulated mainly in roots, although the distribution of both metals in the plant clearly differed. Ninety-six to 99% of the absorbed lead accumulated in the roots, depending on the concentration of the solution with which the plant had been treated. This was the reason that the concentration of this metal in the roots, as expressed per unit of dry weight (1-9 mg per 100 mg), was very high. In the cotyledons, the lead concentration did not exceed 12 μg per 100 mg dry weight. On the other hand, Cd^{2+} was taken up in much smaller quantities (Table 2) and its highest concentration in the roots, at 50 μM Cd^{2+} in the external solution, did not exceed 0.45 mg per 100 mg dry weight. Moreover, it was found that Cd^{2+} was transported more easily to the cotyledons than Pb^{2+}, which is indicated by the ratio of Cd accumulated in the roots to that in the cotyledons (Tables 1 and 2). These results, as well as the distribution of Pb^{2+} and Cd^{2+} in the individual plant organs, indicate that the translocation of heavy metals to leaves is dependent on the concentration of these metals in the medium. The lower the concentration of Pb or Cd in the medium, the greater the amount of the absorbed metals, especially cadmium, which was transported to the cotyledons.

Previous treatment of plants with Pb or Cd clearly influenced the uptake of phosphorous from the medium, although the effect of both metals differed. For

Table 1

The concentration and content of lead in cucumber organs related to the Pb^{2+} concentration in the pretreatment solution

Organ	μM Pb^{2+} in solution		
	25	50	100
	μg Pb^{2+} per 100 mg of dry weight		
Roots	1333.3±48.73	3124.2±143.71	9268.2±370.73
Hypocotyls	11.1±0.42	11.1±0.47	27.5±1.07
Cotyledons	7.6±0.30	11.4±0.43	10.8±0.38
Roots / Cotyledons	1:0.0057	1:0.0036	1:0.0012
	μg Pb^{2+} per organ		
Whole plant	42.37±1.63 (100)	105.08±4.20 (100)	317.6±12.70 (100)
Root	40.80±1.71 (96)	103.10±4.23 (98)	315.12±13.55 (99)
Hypocotyl	0.41±0.02 (1)	0.40±0.02 (0.4)	1.12±0.04 (0.4)
Cotyledon	1.17±0.05 (3)	1.68±0.07 (1.6)	1.55±0.06 (0.6)
Root / Cotyledon	1:0.029	1:0.016	1:0.005

The plants were grown for 4 days in ten-strength Hoagland solution without P, next for 1 day in aqueous solutions of $PbCl_2$ or $CdCl_2$, then in fresh nutrient solution without Pb or Cd (for composition see in Material and Methods). After 48 h, the concentration of Pb was determined. Values represent the mean ±SE of four replications of five independent series. Relative values in brackets.

Table 2

The concentration and content of cadmium in cucumber organs related to the Cd^{2+} concentration in the pretreatment solution

Organ	μM Cd^{2+} in pretreatment solution			
	5	10	25	50
	μg Cd^{2+} per 100 mg of dry matter			
Roots	33.43±1.40	122.50±5.14	230.00±9.66	450.00±18.00
Hypocotyls	4.74±0.17	7.95±0.32	1.50±1.62	78.75±3.15
Cotyledons	2.00±0.09	3.75±0.15	6.06±0.27	6.09±0.23
Roots / Cotyledons	1:0.06	1:0.03	1:0.026	1:0.13
	μg Cd^{2+} per organ			
Whole plant	1.63±0.08 (100)	4.28±0.17 (100)	8.67±037 (100)	17.46±0.71 (100)
Root	1.17±0.06 (72)	3.43±0.14 (80)	6.21±0.25 (72)	13.50±0.61 (77)
Hypocotyl	0.18±0.01 (1)	0.31±0.02 (7)	1.66±0.08 (19)	3.15±0.12 (18)
Cotyledon	0.28±0.01 (17)	0.54±0.03 (13)	0.80±0.03 (9)	0.81±0.04 (5)
Root / Cotyledon	1:0.24	1:0.16	1:0.13	1:0.06

Explantations as in Table 1.

6 hours immediately after treatment with $PbCl_2$, the treated plants took up more phosphorous from the medium containing 0.1 mM P than did control plants (Table 3), moreover, the higher the concentration of Pb with they had been treated, the more phosphorous they took up. After 24 hrs, the P uptake of these plants was only slightly higher than that of control plants, while after 48 hrs, their take up of phosphorous was inhibited. The degree this inhibition increased with the rising concentration of the Pb^{2+} treatment. Cadmium, however, caused decreased phosphorous uptake after only 6 hrs of growth on the nutrient medium (Table 3). The degree of inhibition of uptake after 48 hrs of growth on the nutrient medium was independent of the Cd^{2+} concentration used, and equalled 40-59% of the phosphorous taken up by the control plants.

The determination of total P contents in plants after treatment with Pb and Cd (Table 4) showed that lead and higher Cd concentrations (10 and 25 μM)

Table 3

The effect of lead and cadmium concentrations in the pretreatment solution on the phosphorus uptake (μmoles of Pi per plant) after 6, 24 and 48 hrs of growth of seedlings in nutrient solution

Concentration of Pb or Cd in pretreatment solution, μM	Hours of growth of seedlings in nutrient solution		
	6	24	48
Control	0.103±0.005 (100)	0.423±0.022 (100)	0.986±0.021 (100)
Pb 25	0.145±0.004 (140)	0.542±0.025 (128)	0.875±0.018 (93)
50	0.197±0.005 (190)	0.458±0.028 (108)	0.749±0.020 (80)
100	0.207±0.006 (200)	0.458±0.016 (108)	0.684±0.027 (73)
Cd 5	0.084±0.004 (81)	0.355±0.022 (84)	0.487±0.019 (52)
10	0.077±0.003 (75)	0.339±0.019 (80)	0.470±0.021 (50)
25	0.013±0.001 (19)	0.201±0.012 (49)	0.371±0.017 (40)

Explanations as in Table 1.

Table 4

Increment of phosphorus content (μmoles P per 100 mg of dry matter) in organs of cucumber seedlings treated with different Pb or Cd concentrations after 48 hrs of growth in nutrient solution

Concentration of Pb or Cd in pretreatment solution, μM	Roots	Hypocotyls	Cotyledons
Control	0.1±0.02	6.0±0.21	6.7±0.24
Pb 25	11.8±0.61	7.7±0.23	2.9±0.10
50	15.9±0.63	9.7±0.31	3.7±0.12
100	12.0±0.55	5.7±0.20	2.6±0.11
Cd 5	0.0	7.6±0.21	5.4±0.20
10	9.8±0.31	10.6±0.31	0.0
25	9.8±0.37	9.1±0.33	0.0

Explanations as in Table 1.

elevated, as compared with control plants, the total P content in roots, whereas Pb and Cd at all of the concentrations used decreased the increment of total P in the cotyledons. In the plants treated with cadmium at 10 and 25 μM, no increase in the total phosphorous content in the studied organs was noted.

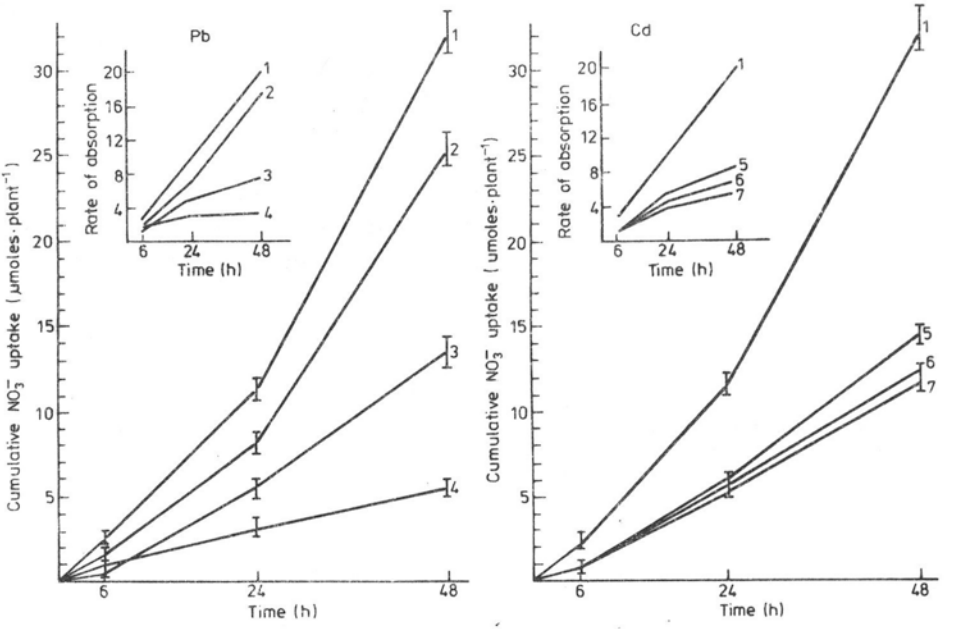

Fig. 1. Cumulative uptake and rate of NO_3^- absorption (μmoles NO_3^- per plant) by cucumber seedlings treated with different concentrations of Pb^{2+} or Cd^{2+}: 1 — control, 2 — 25 μM Pb, 3 — 50 μM Pb, 4 — 100 μM Pb, 5 — 5 μM Cd, 6 — 10 μM Cd, 7 — 25 μM Cd. Values represent the mean ± SE of 5 replications of four independent series. Four-day-old plants were treated with Pb or Cd and transferred to the nutrient solution. After 6, 24 and 48 hrs of growth in the nutrient solution, NO_3^- absorption was determined

Although the accumulated lead and cadmium highly inhibited the uptake of NO_3^-, the effect of Pb^{2+} differed from that of Cd^{2+} (Fig. 1). Inhibition was already noticeable 6 hrs after transfer of plants from the metal-containing solutions to the nutrient medium, and was maintained for 24 and 48 hrs of growth in the medium. The degree to which the uptake of NO_3^- by the plants was reduced was directly dependent on the concentration of the metal in the external solution. However, in the case of the plants treated with cadmium, the degree of inhibition of NO_3^- uptake was the same, irrespective of the concentration of the metal in the external solution. The rate of nitrate uptake by the control plants and those treated with 25 μM Pb increased with time as the plants grew in the nutrient medium, while in plants previously treated with 50 or 100 μM Pb^{2+} or cadmium at all of the used concentrations, this parameter increased much more slowly and after 24 hrs, was inhibited. Table 5 presents an analysis of the uptake, accumulation and reduction of NO_3^- after 48 hrs of growth on the mineral nutrient medium after previous treatment with

Table 5

Absorption, accumulation and reduction of NO_3^- (μmoles per seedling) by cucumber seedlings as affected by different Pb^{2+} or Cd^{2+} concentrations in the pretreatment solution

Concentration of Pb or Cd in pretreatment solution, μM	Absorption	Accumulation	Reduction	Absorbed / Reduced
Control	32.22±1.72	18.82±0.94	13.46	2.4
Pb 25	25.66±1.07	15.51±0.78	10.15	2.5
50	13.74±1.00	8.24±0.61	5.50	2.5
100	6.45±0.72	3.87±0.09	2.58	2.5
Cd 5	14.35±0.87	7.95±0.55	6.40	2.2
10	12.64±0.89	5.67±0.23	6.97	1.8
25	12.01±0.83	5.88±0.24	6.13	2.0

Explanations as in Table 1.

Pb^{2+} or Cd^{2+}. The results show that treatment with Pb^{2+} or Cd^{2+} decreased both the uptake and reduction of NO_3^-, although the ratio of absorbed to reduced NO_3^- ions was equal in Pb-treated and control plants, while in Cd-treated plants it was slightly lower. These results suggest that the lead or cadmium which accumulated in the tissues inhibited the uptake of NO_3^- rather than its reduction.

Decreased activity of nitrate reductase both in cotyledons and roots was found in plants treated with lead or cadmium. Only the lowest used concentration of cadmium (5 μM) had no effect on the activity of this enzyme in the roots (Tables 6, 7). A greater decrease in the activity of NR due to the accumulated heavy metals was observed in the cotyledons than in the roots.

Table 6

The effect of Pb and Cd pretreatment of cucumber seedlings on nitrate reductase activity in roots and cotyledons after 24 and 48 hrs of growth of plants in nutrient solution

Concentration of Pb or Cd in pretreatment solution, μM	Nitrate reductase activity (μmoles $NO_2^- \cdot g^{-1}$fr.wt. h^{-1})			
	cotyledons		roots	
	24 h	48 h	24 h	48 h
Control	3.76 (100)	3.60 (100)	1.00 (100)	0.77 (100)
Pb 25	3.02 (80)	3.00 (85)	0.70 (83)	0.67 (90)
50	1.38 (37)	2.19 (61)	0.40 (61)	0.51 (68)
Cd 5	2.04 (54)	2.83 (79)	1.02 (102)	0.79 (103)
10	1.38 (37)	1.50 (41)	0.54 (41)	0.58 (74)
25	0.88 (23)	1.35 (37)	0.27 (37)	0.45 (56)

For explanation see Table 1.

The reduction in the activity of the enzyme decreased with time after the transfer of the plant from the solution of metals to the nutrient medium. The *in vitro* effect of cadmium on NR activity similarly as that of lead (Burzyński and Grabowski 1984), became visible only after the addition of large concentrations of the metal to the incubation mixture.

Table 7

The influence of cadmium *in vitro* on nitrate reductase activity

Cd concentrations in incubation media, μM	Nitrate reductase activity (μmol. $NO_2^- \cdot g^{-1}$fr.wt. h^{-1}	
	cotyledons	roots
Control	9.75 (100)	1.86 (100)
1	9.90 (101)	1.80 (97)
10	10.20 (105)	1.76 (95)
50	9.30 (95)	1.77 (89)
100	6.90 (71)	1.65 (89)

Enzyme extracts were prepared from the tissues of plant grown for 24 h on three-strenght Hoagland solutions. Each value represents the mean of determinations. Relative values in brackets.

DISCUSSION

A high Pb^{2+} or Cd^{2+} concentration in the tissues of individual plant organs was correlated with an increased total phosphorous content. This relationship was seen mainly in the roots where over 90% of the absorbed Pb^{2+} and 70-80% of the Cd^{2+} were accumulated; a similar relationship was noted in the hypocotyls. In the cotyledons, which accumulated only trace amounts of heavy metals, the total phosphorous level was always lower than in the controls. According to the studies by De Filippis (1979), in plant tissues Pb^{2+} cations and, to a much lesser degree, Cd^{2+}, exhibit an ability to permanently bind phosphates. This ability in roots, which usually accumulate Pb^{2+} in the greatest amounts, may be a specific defense mechanism which inactivates excess amounts of this heavy metal in plants. This is confirmed by the studies of Koeppe and Miller (1970) who observed that the toxic effect of lead is decreased in plants intensely fed with phosphorous. The intense phosphorous uptake by seedlings treated with $PbCl_2$ seen during the initial phase of the experiment (up to 24 hrs) was probably the result of a constant phosphorous insufficiency in the roots brought about by the binding of Pb^{2+} with phosphates. A similar relationships was not found in the case of Cd^{2+}, which is understandable in light of the experiments by Wagner and Trotter (1982). These authors showed that Cd^{2+} induces the formation of low molecular weight protein complexes, called Cd-thioneins, in root tissues, which was later confirmed by Rauser (1984). In respect to lead, however, the formation of such complexes has not to date been found. However, from recent

works (Grill et al. 1986) it seems that lead, similarly as cadmium, induces the synthesis of so-called phytochelatins or homo-phytochelatins.

Lead and cadmium inhibited the uptake of nitrates and the activity of nitrate reductase. From our results it seems that the limiting factor in the reduction of nitrates was the low NO_3^- supply. As shown by Beevers and Hageman (1969), nitrates are not only the substrate for nitrate reductase, but also a factor inducing the activity of the enzyme. The fact that the level of nitrate reductase activity is regulated by the constant supply of NO_3^- to the cell from the outside, is well documented, while the NO_3^- accumulated in the tissue is of little effect (Heimer and Filner 1971, Shaner and Boyer 1976, Buczek 1985). One can then suppose that the inhibition of NO_3^- uptake was the reason behind the decrease in NR activity. This is indicated by the experiments using various lead and cadmium concentrations. Namely, all of the lead and cadmium concentrations which inhibited NO_3^- uptake also inhibited NR activity, whereas the very slight decrease in NO_3^- uptake induced by treatment of the seedlings with 25 μM lead caused only an insignificant decrease in the enzyme's activity. In addition, in *in vitro* experiments both Pb (Burzyński and Grabowski 1984) and Cd^{2+} inhibited NR activity only at very high, unphysiological concentrations, which was not observed in *in vivo* experiments. Our results indicate, however, that NR activity increased as time passed from the moment the plants had been transferred from the metal solutions to the nutrient medium. This fact can suggest a certain direct effect of Pb^{2+} and Cd^{2+} on NR, which is constantly renewed in plant tissues (Huffaker and Peterson 1974). Namely, as the accumulated Pb^{2+} or Cd^{2+} ions are inactivated due to the formation of complexes with phosphates or proteins, these metals have a decreasing effect on the proteins of nitrate reductase.

The results of our study do not explain the mechanism of the effect of Pb or Cd on the uptake of nitrates or phosphates. The "incrustation" of cell walls by lead (Lane and Martin 1977), may create a barrier in the uptake and transportation of all mineral components since, as it seems, it creates such a barrier in the uptake and transportation of water (Burzyński 1987). The decreased hydration of tissues treated with heavy metals may also be the reason for the worse uptake of ions, as was shown to be the case with nitrates by Moiilla et al. (1973), or Shaner and Boyer (1976). It is also known that both lead and cadmium cause disturbances in the uptake of cations such as K^+, Ca^{2+} or Fe^{3+} (Burzyński – in press), which adds to the cation-anion imbalance. Phosphates and nitrates are actively absorbed, while Cd, as well as Pb, act on plasma membrane ATPase and disturb the cell's energy metabolism (Zegers et al. 1976, Keck 1978, Lindberg and Wingstrand 1985).

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Pobieranie i akumulacja fosforu i azotanów oraz aktywność reduktazy azotanowej w siewkach ogórka traktowanych $PbCl_2$ lub $CdCl_2$

Streszczenie

24-godzinne traktowanie 4-dniowych siewek ogórka (*Cucumis sativus* L.) $PbCl_2$ lub $CdCl_2$ wpływało na znaczną akumulację metali ciężkich w tkanakch rośliny, głównie w korzeniach. Zakumulowany Pb zwiększał pobieranie fosforu w początkowym okresie po przeniesieniu roślin do pożywki (6, 24 godz.), ale już po 48 godzinach pobieranie fosforu było mniejsze niż w roślinach kontrolnych. Rośliny traktowane Cd wykazywały stale zmniejszone pobieranie fosforu. Zakumulowany ołów i kadm hamowały również pobieranie azotanów oraz zmniejszały aktywność reduktazy azotanowej. Sugeruje się, że przyczyną zmniejszenia aktywności reduktazy azotanowej było raczej hamowanie pobierania NO_3^-, niż bezpośredni wpływ metali ciężkich na aktywność enzymu.