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Growth and Chlorophyll Biosynthesis of *Vigna angularis* under Lead Stress

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The effect of various supplies of lead singly and in combination with aluminium on growth and chlorophyll biosynthesis was investigated in 7-day-old *Vigna angularis* seedlings. Expose to 50 μM Pb or more drastically reduced root elongation rate. Significant depressions in root growth was observed within 1 day and no recovery of growth was seen over the duration of treatment period. Root elongation decreased depending on the Pb concentrations. Root growth inhibition was stronger than shoot growth inhibition. The initiation of lateral roots appeared to be more sensitive to Pb than the growth of main roots. Inhibition of root and shoot elongation by Pb was lessened by combined exposure of Pb and Al, suggesting that the presence of Al reverse the inhibitory effect of Pb alone. With the histochemical sodium rhodizonate method the rate of Pb uptake was dependent on the Pb concentration and exposure time of the roots to Pb salts. Pb was first deposited on the root surface and then translocated radially in the root cap cells. During a longer Pb administration (up to 72 h) Pb penetration was nonuniform, with accumulation within the cortex or endodermis. There was drastic reduction in chlorophyll content by Pb. The Pb inhibition of chlorophyll synthesis was concentration dependent. δ-Aminolevulinic acid dehydratase (ALAD) activity exhibited distinct inhibition from control. Reduction in chlorophyll content was accompanied by proportional changes in ALAD activity. Chlorophyll content and ALAD activity were less affected by combined exposure of Pb and Al, suggesting that Al has a protective effect against the inhibiting action of Pb on photosynthetic activity.

Key words : root elongation, lateral root initials, chlorophyll content, ALAD activity, *Vigna angularis*, lead, aluminium

1. Introduction

Lead acts as an environment pollutant and contaminates soils and plants when it is given off from car exhaust, dusts and gases from various industrial sources. Lead contamination of soils can cause a variety of environmental problems, including loss of vegetation, ground water contamination and Pb toxicity in plants, animals and humans (Body, 1991). Although lead is considered to be a non-essential element, plants can absorb it from soil, water and air through their roots and leaves.

Lead can affect the plant growth and also become accumulated in plant tissue and then enter the food chain of man, and at high levels has a wide range of negative effects on plants (Pahlsson, 1989). Toxic effect of lead on most plant life processes have been shown repeatedly. In particular, lead strongly inhibit plant growth (Salim, 1992), root elongation (Godbold and Kettner, 1991), cell divisions (Mukherji and Maitra, 1976), photosynthesis (Moustakas, 1994; Stoyanova and Tchakalova, 1993), transpiration

(Rolfe and Bazzaz, 1975) and nitrate assimilation (Bharti and Singh, 1993). Several other physiological and metabolic aspects of plants are also known to be affected by Pb in the environment.

Root growth is highly sensitive to metals ; it has been used for a number of years as a parameter for assessing metal tolerance of plants (Godbold and Kettner, 1991a), and has also commonly been used to assess the toxicity of Pb to seedlings. In a recent view of the response of seedlings to Pb, the visual non-specific symptoms of Pb toxicity are stunted growth, chlorosis and blackening of root systems. In nutrient solutions, Pb reduced root elongation of primary roots and uptake of mineral nutrients in *Picea abies* (Godbold and Kettner, 1991b). Garland and Wilkins(1981) showed that increased levels of Ca reduced the effect of Pb on root elongation.

Photosynthetic processes are very sensitive to heavy metals such as cadmium and lead and many data have been reported on chlorophyll content and activities of a number of enzymes essential for various photosynthetic functions (Lidon and Henriques, 1993 ; Stoyanova and Tchakalova, 1993). δ-Aminolevulinic acid dehydratase(ALAD) is necessary enzyme for chlorophyll synthesis and photosynthesis and locates in proplastids or chloroplasts (Le Pabic, 1987). Yet little information is presently available on heavy metal effect on the activities of key enzymes in the chloroplast development involved in chloroplast synthesis and enzyme activity during greening process.

In forest sites the highest concentration of Pb and Al are spacially separated down in soil profile (Godbold and Kettner, 1991b). Pb are found in the uppermost mineral soil, whereas Al are found in the deeper mineral soil layers. Exposure to more than one metal may have antagonistic or synergistic effects on root growth. A synergistic effect of Pb and Cd on the root growth was observed in *Platanus occidentalis* (Carlson and Bazzaz, 1977). Alternatively, tolerance to one metal may protect against the action of another.

In the earlier study, growth and morphology in azuki bean seedlings have been found to be inhibited by exogenously supplied aluminium (Koo and Hong, 1996). In this present work, we planned to test *Vigna angularis* to draw a more conclusion on the response of the crop under Pb toxicity. The present work has been taken to characterize the effect of deleterious concentration of Pb on growth and chlorophyll biosynthesis of *Vigna angularis*. The protection of aluminium against the inhibitory action of lead was also taken into consideration with regard to growth and chlorophyll biosynthesis.

2. Materials and Methods

2.1. Growth conditions and treatments

Seeds of azuki bean(*Vigna angularis*) were sterilized in 1% sodium hypochlorite solution for 20 min, rinsed five times in sterile distilled water, and soaked in running tap water for 12 h. The seeds were germinated in the dark at 25±1 ℃ in Petri dishes lined with filter paper moistened with distilled water. 7-day-old seedlings were placed in 10 ㎝ Petri dishes lined with filter paper containing 10 ㎖ of 50, 100, 200 and 500 μM Pb singly or in combination with Al and grown for 7 days. Pb and Al were supplied as $Pb(NO_3)_2$ or $AlK(SO_4)_2 \cdot 12H_2O$, respectively. Control seed received no Pb. To prevent depletion Pb solutions were renewed every 2nd day. Growth conditions were 25±1℃ with a 16-h light/ 8-h dark regime.

2.2. Measurements of root and shoot length

Root and shoot growth measurements were determined every 24 h. Root and shoot elongation were determined using a binocular microscope with an eyepiece micrometer. In another experiment, the whole root and shoot length were measured with a ruler. When possible, measurements were made along the middle of the root to avoid errors associated with root curvature. Lateral root initials were also counted using a dissecting microscope. Twenty seeds were used and three replicates carried out per test. Data represent the means and SD from three replicate experiments.

2.3. Lead localization in whole roots by the rhodizonate method

The analyses of lead distribution in whole roots of 7-day-old seedlings were performed macroscopically using sodium rhodizonate (Glateer and Hernandez, 1972) after treating the roots with Pb at all concentrations described above for 24, 48 and 72 h. Following the respective incubation periods, roots were excised and submerged for 30 min in 0.2 % sodium rhodizonate solution (pH 2.8). The presence of lead was confirmed by pink or red root staining. Cross sections of fresh roots were made. The sections were analyzed in the light microscope in a drop of sodium rhodizonate solution.

2.4. Determination of leaf chlorophyll

To determine chlorophyll content the first leaves of the seedlings were collected. Each preweighed leaves were homogenized with a mortar and pestle in 80% (v/v) acetone and centrifuged at 10,000 x g for 15 min at 0℃ to 4 ℃. The supernatant was brought up to 10 ㎖ volume and the absorbance of the acetone extract was measured at 663 and 645 nm with UV-visible spectrophotometer (UV-260, Shimadzu).

Chlorophyll content was calculated according to the method of Arnon (1949) and expressed in mg chlorophyll per gram fresh weight.

2.5. Estimation of δ-aminolevulinic acid dehydratase

The first leaves were homogenized with a prechilled mortar and pestle in 5 ㎖ of 50 mM Tris-HCl buffer (pH 8.2) containing 0.1 mM dithiothreitol (Naito, 1980). The homogenate was centrifuged at 27,000 x g for 20 min at 0℃. The supernatant was assayed for enzyme activity.

For the determination of ALAD activity, 1 ㎖ of extract was incubated with 0.3 ㎖ of 1 $mg \cdot m\ell^{-1}$ ALA, 1.3 ㎖ of 50 mM Tris-HCl buffer (pH 8.2) containing 0.1 mM dithiothreitol and 0.8 ㎖ of 0.2 mM $MgCl_2$ for 2.5 h at 37℃. The enzymatic reaction was stopped with 0.3 ㎖ of 3 M trichloroacetic acid containing 0.1 M $MgCl_2$. Samples were then centrifuged at 400 x g for 10 min. PBG formed in the supernatant was measured according to Mauzerall and Granick (1956) : one ㎖ of supernatant was mixed with an equal volume of a modified Ehrlich's reagent. The absorbance at 555 nm was determined after 15 min and the concentration of PBG was calculated using an extinction coefficient of 61 $mM^{-1} \cdot cm^{-1}$. ALAD activity was expressed in nmole PBG formed per h and per leaf.

3. Results

3.1. Lead effects on root and shoot growth

Effects of various concentrations of Pb on the root growth of *Vigna angularis* seedlings esti-

mated as the daily elongation rate are shown in Figs. 1 and 2. Exposure to 50, 100, 200 or 500 μM Pb decreased the rate of root elongation within 1 day, and root elongation rate was significantly reduced throughout the treatment period, the strongest inhibition occurring at treatment longer than 7 days. Significant depressions were also observed in shoot growth from 50 to 500 μM Pb treatment (Figs. 3 and 4). At 50 μM, shoot inhibition was rather small (about 46% from the control), whereas root inhibition amounted to 63% after 7 days. Rate of inhibition increased with increasing concentrations and root growth inhibition was stronger than shoot growth inhibition. After 7 days of 50 μM Pb exposure, total length in shoot was twofold higher than that in root. No significant recovery of growth was observed in the Pb treated seedlings over the duration of the treatment period. This result is different from the previous observation obtained for Al where the same treatment showed no further significant decrease (Koo and Hong, 1996).

To investigate the effects of a combined exposure to Pb and Al, the seedlings were grown at same levels of Pb and Al singly and in combination (Fig. 2 and 4). An exposure to 50 μM Pb or Al produced similar results to those found in previous experiments. The main root length decreased by 22% and 63% in the 50 μM Al and 50 μM Pb treatment after 7 days exposure. At 50 μM Al, after an initial inhibition of root elongation rate, root growth recovered after 7 days supply. At lower levels of both Pb and Al exposure, the combined exposure to 50 μM Pb and Al inhibited the rate of root elongation compared with the control over the duration of the treatment period. However, the degree of inhibition was less than that produced by exposure to 50 μM Pb alone.

Exposure to 50 μM Pb and 50 μM Al caused a progressive increase in shoot growth throughout the treatment period, reaching the rate of control plants after 7 days, after which no further significant decrease was observed (Fig. 4). In the combined Al and Pb, main root and shoot length decreased by 56% and 8%, respectively. The presence of Al lessened the inhibitory effect of 50 μM Pb alone.

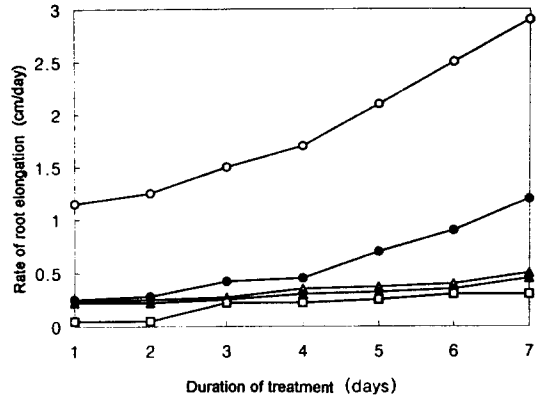

Fig. 1. Rate of root elongation of *Vigna angularis* seedlings exposed to 50, 100, 200 and 500 μM Pb. ○, control ; ●, 50 μM Pb ; △, 100 μM Pb ; ▲, 200 μM Pb ; □, 500 μM Pb.

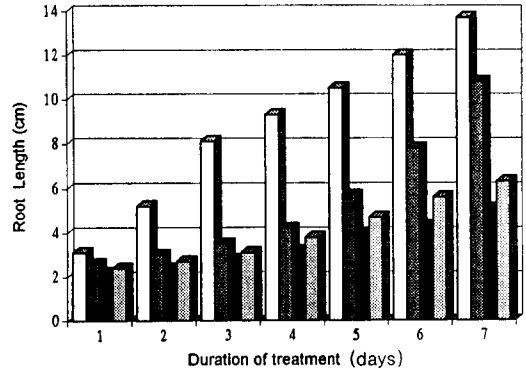

Fig. 2. Root length of seedlings of *Vigna angularis* exposed to 50 μM Pb and 50 μM Al singly and combined. □, control ; ▒, 50 μM Al ; ■, 50 μM Pb ; ▒, 50 μM Pb + 50 μM Al.

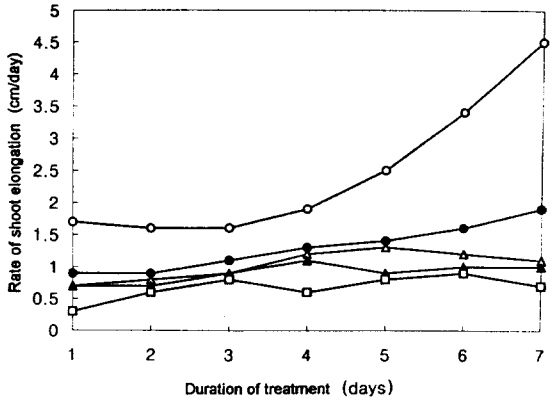

Fig. 3. Rate of shoot elongation of *Vigna angularis* seedlings exposed to 50, 100, 200 and 500 μM Pb. ○, control ; ●, 50 μM Pb ; △, 100 μM Pb ; ▲, 200 μM Pb ; □, 500 μM Pb.

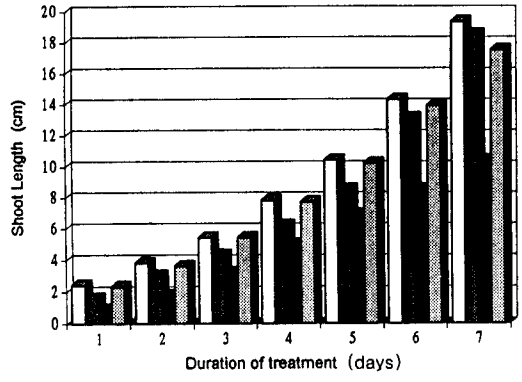

Fig. 4. Shoot length of seedlings of *Vigna angularis* seedlings exposed to 50 μM Pb and 50 μM Al singly and combined. □, control ; ▒, 50 μM Al ; ■, 50 μM Pb ; ▒, 50 μM Pb + 50 μM Al.

3.2. Effects of Pb on lateral root initiation

Lead treatments that affected root elongation also influenced lateral root growth and development. The initiation of lateral roots by the approximately 10 cm of root tissue, which grew after the initiation of Pb treatment, was significantly reduced at concentrations as high as 50 μM Pb after a 7-day treatment period (Table 1). The number of lateral root initials decreased by 71% and 21% in the 50 μM Pb and combined Pb and Al treatment. A drastic decrease of lateral root initials with increasing Pb supply was observed at higher Pb concentrations. However, the combined exposure to 50 μM Pb and Al caused a significant increase in the initiation of lateral roots, although not up to the levels of the control plants.

Table 1. The number of lateral root initials at a distance of 10 cm from the root tip of *Vigna angularis* seedlings exposed to Pb singly or Al and Pb combined for 7 days. Each value denotes mean ± SD (n=3).

Treatment	Number of lateral root initials
Control	14 ± 2.17
50 μM Pb	4 ± 0.82
100 μM Pb	1 ± 0.82
200 μM Pb	0
500 μM Pb	0
50 μM Pb + 50 μM Al	11 ± 2.45

3.3. Localization of Pb in the root tissues

With the histochemical sodium rhodizonate method Pb was detected along the entire root length of *Vigna angularis* within 24 h incubation. All concentrations of $Pb(NO_3)_2$ resulted in roots that were slightly pink. The colour intensity was higher with increasing Pb dose (Table 2). No root zone takes up more Pb than others. The amount of Pb that is taken up depends on its concentration in the incubation solution. Pb was first deposited on the root surface, and then was translocated radially through the middle lamella of root cap cells. Within the root tip stained most intensely, significant alterations in the Pb

distribution sequence in particular tissues and their layers were observed. During a longer Pb administration (up to 72 h) nonuniform distribution of Pb and proportional increase of root staining were observed.

Cross sections showed that Pb penetration throughout the root is generally nonuniform, with accumulation mainly within the cortex (Fig. 5). A supply of Pb during the 24 h of incubation stained the root by pink or red in epidermis and subepidermal cell. During the 48 h, Pb deposition reached outer cortex at 50 μM Pb and inner cortex at 100 μM Pb. After 72 h exposure Pb distribution appeared in the innermost cortex and endodermis at all Pb levels tested.

Table 2. Lead binding pattern in the roots of *Vigna angularis* seedlings detected by the sodium rhodizonate method.

Concentration (μM)	24 h	48 h	72 h
50	+	+	++
100	+	++	+++
500	++	+++	++++

Degree of staining : +, weak ; ++, moderate ; +++, intense ; ++++, very intense

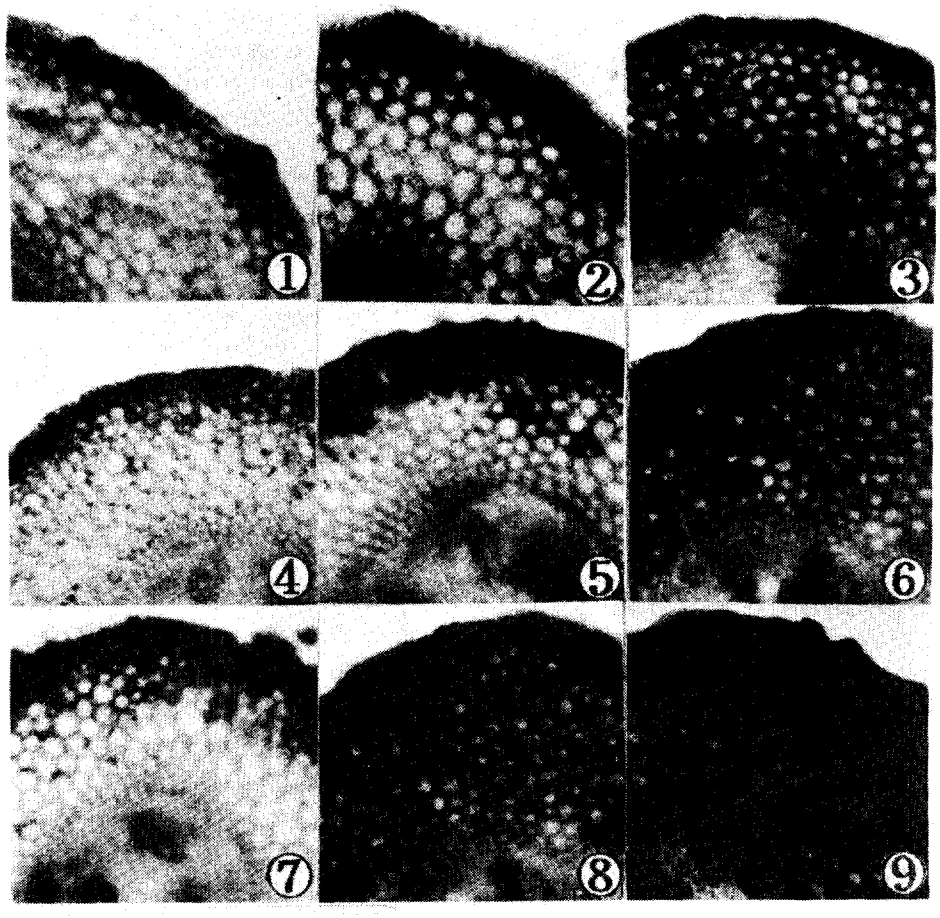

Fig. 5. Cross sections showing lead distribution in root of Vigna angularis seedlings by the sodium rhodizonate method. x 40. 1, 50 μM Pb treatment for 24 h ; 2, 50 μM Pb treatment for 48 h ; 3, 50 μM Pb treatment for 72 h ; 4, 100 μM Pb treatment for 24 h ; 5, 100 μM Pb treatment for 48 h ; 6, 100 μM Pb treatment for 72 h ; 7, 500 μM Pb treatment for 24 h ; 8, 500 μM Pb treatment for 48 h ; 9, 500 μM Pb treatment for 72 h.

3.4. Chlorophyll determination and ALAD activity in leaf segments treated with Pb

Leaf segments prepared from the primary leaves of 7-day-old seedlings were subjected to various concentrations of Pb. After 7-day exposure to Pb the chlorophyll content and ALAD activity in leaves were determined. There was drastic reduction in total chlorophyll content of *Vigna angularis* leaves following Pb treatment (Table 3). A concentration-dependent decrease in chlorophyll level was well correlated with exogenous supply of the metal. At 50 μM, there was about 33 % reduction from the control and the rate of chlorophyll destruction increased at higher Pb doses amounting to about 82 % loss at 500 μM. Combination of 50 μM Pb and Al caused an increase in the chlorophyll content, although not up to the levels of the control plants. Al reversed the inhibitory effect of Pb in chlorophyll synthesis.

The amount of ALA in leaves is related to the quantity of the enzyme ALAD in such tissue. Since Pb has been shown to lower the amount of chlorophyll it was considered worthwhile to measure the effect of Pb on the ALAD activity in leaves. Supply of Pb to the intact seedlings caused a large decrease in ALAD activity (Table 3). Decrease in the enzyme activity was 90 % in the leaf tissue at 50 μM Pb. The degree of inhibition was dependent upon the Pb supply. The activity, however, was significantly increased in the combined 50 μM Pb and Al treatment.

4. Discussion

In the soil, heavy metals such as lead, cadmium and copper operate stress factors causing physiological constraints after their absorption by the root system which result in a decreased vigour of the plants and retardation of plant growth (Pahlsson, 1989 ; Van Assche and Clijsters, 1990 ; Ouzounidou, 1993). Root growth is highly sensitive to metals and has been used to assess the toxicity of heavy metals to plants. Inhibition of root elongation by Pb has been reported for many plants. The results presented show that a supply of 50μM Pb or more drastically depressed root growth of *Vigna angularis* seedlings estimated as the daily elongation rate (Figs. 1 and 2). The results are consistent with the findings (Godbold and Kettner, 1991a) that supply of Pb inhibited root elongation of *Picea abies* seedlings within 1 day.

Pb supply that strongly affected primary root growth also influenced lateral root growth and development. Both the total length and number of lateral root initiation per seedling were signifi-

Table 3. Effect of Pb singly or Pb and Al combined on the chlorophyll content and ALAD activity in leaves of *Vigna angularis* seedlings. Values are mean ± SD (n=3).

Treatment	Total chlorophyll (mg/g fr. wt.)	ALAD activity (nmole PBG · h^{-1} · $leaf^{-1}$)
Control	12.03 ± 0.75	2.83 ± 0.07
50 μM Pb	8.10 ± 0.21	0.25 ± 0.09
100 μM Pb	4.36 ± 0.19	0.18 ± 0.10
200 μM Pb	3.17 ± 0.34	0.16 ± 0.05
500 μM Pb	2.18 ± 0.30	0.07 ± 0.03
50 μM Pb + 50 μM Al	10.78 ± 0.28	2.03 ± 0.23

cantly reduced after exposure to Pb (Table 1). This suggests that the formation of lateral roots is more sensitive to Pb than the elongation of already formed roots. Malone et al.(1978) showed that the growth of lateral roots of *Zea mays* and *Glycine max* was more sensitive to Cd and Pb than primary root growth. Pb caused disturbed mitosis in onion root tip cells with a series of abnormalities (Mukherji and Maitra, 1976). It can be assumed that Pb may inhibit root growth by inhibiting cell division and/or cell elongation (Godbold and Kettner, 1991b).

In the present study, root growth inhibition was stronger than shoot growth inhibition (Figs. 2 and 4). The effect of Pb treatment was more on the roots of broad beans and the least affected part by Pb was the stem of plant (Salim, 1992). It has been reported that Pb accumulation in roots is significantly higher than in shoots, possibly because of the low Pb translocation from roots to shoots (Huang and Cunningham, 1996). In general, root accumulate more Pb than shoot of *Picea abies* (Godbold and Kettner, 1991b) and a differential accumulation of Pb was found in *Sesamum indicum* (Bharti and Singh, 1993). Heavy metal toxicity is avoided by higher plants through a general principle of forming complexes between phytochelatins and the metals.

With the histochemical method Pb was detected along the entire root during the incubation time (Table 2). As expected, the rate of uptake is dependent on the Pb concentration and exposure time of the roots to lead salts. Rapid and almost complete lead uptake from lead nitrate by *Allium cepa* roots was also reported by Wierzbicka (1987a). He confirmed the findings that Pb can be taken up from the surrounding solution against concentration gradients and deposited in large amounts in the roots. The deposits are not easily washed out even from fixed material, suggesting that most of the Pb present in roots is bound. Lead was first deposited on the root surface, and then was translocated radially through the middle lamella of root cap cells. Based on autoradiographic and ultrastructural studies, it was determined that Pb is taken up from solution with the same intensity along the length of *Allium cepa* roots (Wierzbicka, 1987b). The lead reaches protodermal cells and meristematic cells of the hypodermis, where it penetrates into the symplast. Longer Pb administration resulted in some differences in Pb distribution within ground meristem zones, showing accumulation within cortex or endodermis (Fig. 5). Lead uptake by onion root was most intense during the first 4 h of incubation, with accumulation mainly within the cortex. Several authors have suggested that the endodermis functions as a barrier to the radial transport of Pb in the root (Jentschke, 1991). The chemical composition of both the root tissues and cell compartments considerably affects Pb localization. This fact can be concluded from the results of other authors. During a longer administration nonuniform distribution of Pb may result from differences in Pb transport within the consecutive layers of the tissue (Wierzbicka, 1987b). Significant nonuniformity of Pb distribution within the particular root tip cell layers suggests that Pb pathways in the particular root tip tissues are different. Huang and Cunningham (1996) demonstrated that plant species differ significantly in Pb uptake and translocation. In coleoptile segments of *Triticum vulgare*, Pb reduced plastic and elastic extensibility of cell walls (Burzynski and Jacob, 1983). In roots of *Anthoxanthum oderatum* (Quereshi, 1986) and *Picea abies*(Jentchke, 1991) exposed to Pb, the highest levels of Pb were detected in cell walls of the cortex or endodermis, respectively. This suggests that in ecosystems with high Pb concentrations negative effects of Pb on root

growth and nutrient uptake may be expected.

Pb supply not only influenced root growth patterns but also affected leaf function. A progressive reduction in chlorophyll content with increasing Pb concentrations was observed (Table 3). The results show that Pb is a potent inhibitor in the chlorophyll biosynthesis during seedling development and in the growth of new leaves where active chlorophyll production occurs. Inhibition of chlorophyll content by Pb has been reported in maize (Sinha, 1988) and oats (Moustakas, 1994). Chlorophyll biosynthesis may be inhibited due to inhibition of δ-amino-levulinic acid dehydratase, an important enzyme in the biosynthesis of heme compounds including chlorophyll (Shibata and Ochiai, 1976). A drastic decrease in ALAD activity with increasing Pb supply was observed at all levels tested (Table 3). The results suggest that Pb is a powerful inhibitor of photosynthetic system under these conditions and support the hypothesis of a coordinated reduction of the photosynthetic machinary (Schafer, 1992). Pb induced structural changes in the photosynthetic apparatus of *Elodea canadensis* (Stoyanova and Tchakalova, 1993). There was more pronounced reduction of ALAD activity than that of chlorophyll content in Pb-stressed leaves. These observations support that there is a regulatory system for ALAD activity which may participate in regulating chlorophyll synthesis. In oat plants grown in Pb contaminated site Pb induced a pronounced reduction in chlorophyll content, accompanied by proportional changes in RuBPCO activity, an important factor in regulating the rate of leaf photosynthesis (Moustakas, 1994). It has been reported that protease and α-amylase were much affected by Pb, whereas RNase and DNase exhibited little inhibition from control in rice seedlings (Mukherji and Maitra, 1976).

Exposure to more than one metal may have antagonistic or synergistic effect on plant growth. In seedlings grown at same levels of Pb and Al in combination, Al reversed the inhibitory effect of Pb on root and shoot growth, but the growth in Al-treated plants never reached the level of control ones (Figs. 2 and 4). The combination of Al and Pb also caused an increase in the chlorophyll content and ALAD activity, although not up to the levels of the control plants (Table 3). The data presented in this paper are consistent with the suggestion that in nature Al could have a protective effect against the generally inhibiting action of Pb on the root growth and chlorophyll biosynthesis. Unfortunately this action of Al may be limited because of Al toxicity for plants at higher concentrations. The data suggest that Al tolerance mechanism must first be induced by exposure to Al. This may be compared with metal tolerant grasses in which tolerance to metals has been shown to be constitutive (Godbold, 1984). Not only did the induction of the Al tolerance of the *Vigna angularis* seedling protect against Al, but also tolerance to Pb was induced. Co-tolerance has been demonstrated in a number of plant species (Baker and Walker, 1989). The copper tolerance mechanism in *Silene cucubalus* provided a degree of tolerance against Zn (Verkleji and Bast-Cramer, 1985). The protection afforded by the Al tolerance mechanism against Pb suggests that Pb and Al may in part inhibit plant growth and photosynthetic system by a similar mechanism. It is clear that the role of Pb and Al in plants is still uncertain and that additional research needs to be done with concern for interactions which may arise when many such elements are available to the plant.

References

Arnon, D. I., 1949, Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*, Plant Physiol., 24, 1-15.

Baker, A. J. M. and P. L. Walker, 1989, Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity, Chem. Spec. Bioavail., 1, 7-17.

Bharti, N. and R. P. Singh, 1993, Growth and nitrate reduction by *Sesamum indicum* cv PB-1 respond differentially to lead, Phytochem., 33, 531-534.

Body, P. E., P. R. Dolan and D. E. Mulcahy, 1991, Environmental lead: a review, Critical Reviews in Environmental Control., 20, 299-310.

Burzynski, M. and M. Jacob, 1983, Influence of lead on auxin-induced cell elongation, Acta Soc. Bot. Pol., 52, 231-239.

Carlson, R. W. and F. A. Bazzaz, 1997, Growth reduction in American sycamore (*Platanus occidentalis* L.) caused by Pb-Cd interaction. Environ. Pollut., 12, 243-253.

Garland, C. J. and D. A. Wilkins, 1981, Effect of calcium on the uptake and toxicity of lead in *Hordeum vulgare* L. and *Festuca ovina* L., New Phytol., 87, 581-593.

Glateer, F. A. B. and L. Hernandez, 1972, Lead detection in living plant tissue using a new histochemical method, J. Air Pollut. Control Assoc., 22, 463-467.

Godbold, D. L., W. J. Horst, J. C. Collins, D. A. Thurman and H. Marschner, 1984, Accumulation of zinc and organic acids in roots of zinc tolerant and non-tolerant ecotypes of *Deschampsia caespitosa*, J. Plant Physiol., 116, 59-69.

Godbold, D. L. and C. Kettner, 1991a, Use of root elongation studies to determine aluminium and lead toxicity in *Picea abies* seedlings, J. Plant Physiol., 138, 231-235.

Godbold, D. L. and C. Kettner, 1991b, Lead influences root growth and mineral nutrition of *Picea abies* seedlings, J. Plant Physiol., 139, 95-99.

Huang, J. W. and S. D. Cunningham, 1996, Lead phytoextraction: species variation in lead uptake and translocation, New Phytol., 154, 75-84.

Jentschke, G., E. Fritz and D. L. Godbold, 1991, Distribution of lead in mycorrhizal and non-mycorrhizal Norway spruce seedlings, Physiol. Plantarum, 81, 417-422.

Koo, S. Y. and J. H. Hong, 1996, Effects of aluminium on growth, chlorophyll content, ALAD activity and anatomy of root and shoot in azuki bean(*Vigna angularis*) seedlings, J. Kor. Environ. Sci. Soc., 5(6), 813-826.

Le Pabic, C., M. Hoffelt and J. Roussaux, 1987, Effect of 6-benzylaminopurine and potassium on δ-aminolevulinate dehydratase activity of excised cucumber cotyledons, Plant Cell Physiol., 28, 431-437.

Lidon, F. C. and F. S. Henriques, 1993, Changes in the contents of the photosynthetic electron carriers, RNAse activity and membrane permeability, triggered by excess copper in rice, Photosynthetica, 28, 99-118.

Malone, C. P., D. E. Koeppe and R. J. Miller, 1978, Root growth in corn and soybeans: effects of cadmium and lead on lateral root initiation, Can. J. Bot., 56, 277-281.

Mauzerall, D. and S. Granick, 1956, The occurrence and determination of aminolevulinic acid and porphobilinogen in urine, J. Biol. Chem., 219, 435-446.

Moustakas, M, T. Lanaras, L. Symeonidis and S. Karataglis, 1994, Growth and some photo-

synthetic characteristics of field grown *Avena sativa* under copper and lead stress, Photosynthetica, 30, 389-396.

Mukherji, S. and P. Maitra, 1976, Toxic effects of lead on growth and metabolism of germimating rice (*Oryza sativa* L.) seeds and on mitosis in onion (*Allium cepa* L.) root tip cells, Indian J. Exp. Biol., 14, 519-521.

Naito, K., T. Ebato, Y. Endo and S. Shimizu, 1980, Effect of benzyladenine on δ -aminolevulinic acid synthetic ability and δ -aminolevulinic acid dehydratase: differential responses to benzyladenine according to leaf age, Z. Pflanzenphysiol., 96-102.

Ouzounidou, G., 1993, Changes in variable chlorophyll fluorescence as a result of Cu-treatment: dose-response relations in *Silene* and *Thlaspi*, Photosynthetica, 29, 445-462.

Pahlsson, A. M. B., 1989, Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants, Water, Air, Soil Pollut., 47, 287-319.

Quereshi, J. A., K. Hardwick and H. A. Collin, 1986, Intracellular localization of lead in a lead tolerant and sensitive clone of *Anthoxanthum odoratum*, J. Plant Physiol., 122, 357-364.

Rolfe, G. L. and F. A. Bazzaz, 1975, Effect of lead contamination on transpiration and photosynthesis of loblolly pine and autumn olive, For. Sci., 21, 33-35.

Salim, R., M. M. Al-Subu, A. Douleh and S. Khalaf, 1992, Effects on growth and uptake of bread beans (*Vicia fabae* L.) by root and foliar treatments of plant with lead and cadmium, J. Environ. Sci. Health, A27, 1619-1642.

Schafer, C., H. Simper and B. Hofmann, 1992, Glucose feeding results in coordinated changes of chlorophyll content, ribulose-1,5-biphosphate carboxylase-oxygenase activity and photosynthetic potential in photoautrophic suspension cultured cells of *Chenopodium rubrum*, Plant Cell Environ., 15, 343-350.

Shibata, H. and H. Ochiai, 1976, Studies on δ -aminolevulinic acid hydratase in radish cotyledons during chloroplast development, Plant Cell Physiol., 17, 281-288.

Sinha, S. K., H. S. Srivastava and S. N. Mishra, 1988, Nitrate assimilation in intact and excised maize leaves in the presence of lead, Bull. Environ. Contam. Toxicol., 41, 419-426.

Stoyanova, D. P. and E. S. Tchakalova, 1993, The effect of lead and copper on the photosynthetic apparatus in *Elodea canadensis* Rich., Photosynthetica, 28, 63-74.

Van Assche, F. and H. Clijsters, 1990, Effects of metals on enzyme activity in plants, Plant Cell Environ., 13, 195-206.

Verkleji, J. A. C. and W. B. Bast-Cramer, 1985, Cotolerance and multiple heavy metal tolerance in *Silene cucubalus* from different heavy metal sites, *In* Int. Conf. Heavy Metals Environ., Vol. 2, CEP Consultants, Edinburgh, pp. 27-29.

Wierzbicka, M., 1987a, Lead accumulation and its translocation barrier in roots of *Allium cepa* L.: autoradiographic and ultrastructural studies, Plant Cell Environ., 10, 17-26.

Wierzbicka, M., 1987b, Lead translocation and localization in *Allium cepa* roots, Can. J. Bot., 65, 1851-1860.