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LOCALIZATION OF LEAD ACCUMULATED BY BARLEY (Hordeum distichon L.) ROOT TIPS AND ITS EFFECTS

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ABSTRACT

Barley roots were traeted for 1-24 hours with 0.1-10 mMol/1 lead nitrate sclutions and examined by transmission electron microscope. Lead deposits were observed in the middle lamella, cell walls and in the pinocytotically formed vesicle at the first and third hours, at low concentrations. The plasmalemma acts as a barrier to the influx of lead intc the protoplasm. But this was not considered as a complete protection mechanism. With increasing concentrations and time, lead has migrated to the cytoplasm, and even to the nucleus and the nucleolus. The epidermal and the cortical cells were highly susceptible to the lead. They were seriously injured exhibiting plasmolysis and disorganized cell structure. The endodermis has protected the central cylinder for a short time. The electron micropgraphs made after 6 and 24 hours of lead treatments in the all experimental combinations have shown dense lead deposits in all tissues. Lead has been taken up and has accumulated by barley roots and caused structural damages in root cells.

INTRODUCTION

Hundreds of thousands of tons of lead are discharged annually into the earth's soil, atmosphere and water by various sources (NRIAGU, 1979). From the atmosphere, this metal, being largely oxides and salts, is washed down by rain to the surface of the earth (SCHULZ-BALDES and LEWIN, 1976). The increasing problem of environmental pollution by heavy metals necessitates the study of toxicity of heavy metals in plants as well as in animals. The toxicity of heavy metals in crop plants varies from metal to metal and from crop to crop (HARA and SONODA, 1979). Lead and cadmium are not so much interesting because of the phytotoxicity, but rather because of its uptake and transportation into the food chain (FOY et al., 1978).

The analysis of pollutants in organisms has been shown to be a suitable method for the pollution monitoring. Plants, mosses and

lichens clearly reflect deposition levels owing to their high accumulating rates of a number of pollutants (LAGERWERF, 1971; VAN STEVENICK et al., 1976; THOMAS et al., 1984; RODERER, 1984; VASQUEZ et al., 1987). The intake of lead is accumulated chiefly in the roots, occuring to a lesser degree in the upper parts of the plants (WONG et al., 1984). Plants absorb considerable quantity of lead through the roots, from where it is being transported to the parts above the ground (stem and leaves) with limited quantities (KLOKE and RIEBARTSCH, 1964; MARTEN and HAMMOND, 1966).

The researchs on lead uptake by plants done so far have generally been carried out on a practical agronomic basis and have focused on the physiological character of this uptake (BAUMHARDT and WELCH, 1972; PETTERSSON, 1976; WONG et al., 1986; RAPPAPORT et al., 1987). But any fraction or extraction procedure used will introduce the possibility of measuring only the existance of lead. Lead is an electron dense metal and it can be easily detected in the cells with electron microscope (MALONE et al., 1974; SHARPE and DENNY, 1976; WIERZBICKA, 1987; STOCKING and ONGUN, 1962).

The aim of present study is to determine the uptake and the physical localization of lead in barley root tip sections.

MATERIAL and METHODS

Barley *(Hordeum distichon* L. *var cultuvar union*) has been grown by hydroponics procedure in a clima chamber (TOKER, 1988). One-week-old seedlings have been used for the experiments and the electron microscobic studies have been carried out in the root tip tissues.

Lead ions have been supplied with $Pb(NO_3)_2$ which is the highly water soluble compound of lead and the pH has been adjusted to 6.6 with 0.01 N tris buffer. The lead concentration have ranged from 0.1, 0.2, 1, 5 to 10 mMol / 1. The seedlings have been immersed in one liter of the lead solutions for each concentration for the periods of 1–3–6 and 24 hours. The roots for the control have been cut out from the seedlings just before the lead treatment and have been fixed.

After the periods mentioned above, the roots have been rinsed in distilled water for 1 minute, then have been cut 2–3 mm in length from the root tips. They have been fixed in 2.5 % glutaraldehyde in 0.01 N NaOH buffer at pH 6.6 for 24 hours. The root tips have been

rinsed with bidistilled water 2–3 times for one minute, then post-fixed with 1 % OsO_4 (1 % OsO_4– 1 % $KMnO_4$– m / 200 $CaCl_2$) for 24 hours. After the post-fixation, the root tips have been rinsed with bidistilled water 2–3 times for one minute and were stained with 2 % uranylacetate for 24 hours. After dehydratation in a graded series of ethylalcohol and propylene oxide, the root tips have been embedded in a 1 / 1 mixture of epon–spurr.

The blocks have been trimmed with the Reichert TM 60 specimen trimmer and the ultrathin cross sections of the root tips have been prepared with the Reichert OMU 3 ultramicrotome. The sections which were not stained with lead have been examined in a Philips EM 301 electron microscope.

RESULTS

After 1 hour of incubation in 0.1 mMol Pb / 1 solution (low concentration) lead was present in all the root tip tissues examined. Numerous lead crystals were visible in the pinocytotic vacoules of the outher side of the epidermal cell (Fig. 1). There were many crystals seen in the inner vacuoles. The granular lead deposits were observed in the middle lamella of the cell walls. Large deposits were also occured on the plasmodesmatas. Though the sections were not stained with lead to obtain contrast, as it is done for regular preparations for electron microscopy, the epidermal cells showed strong contrast and the nucleolus and the chromatin structure in particular were very darkly stained. These results showed that lead was accumulated in huge amounts by epidermal cells. The cortical cells showed the same appearance (Fig. 2). There were profuse small granular deposits in the cell wall. The same structures were observed in the protoplasm but they were denser than that of the plasmalemma. The mitochondria also exhibited the same deposits.

After 6 hours of incubation in 0.1 mMol Pb / 1 solution, the amount of lead in the root cells was high. A huge amount of lead crystals were observed in the cell wall and the intercellular spaces of the root's cortical cells (Fig. 3). The accumulation of lead crystals was so abundant at the cell wall that the width of wall was enlarged.

After 24 hours incubation in 0.1 mMol Pb / 1 solution, there was a massive lead precipitation in the central cylinder cells (Fig. 4). The organelles, nucleolus and protoplasm of these cells were stained dark

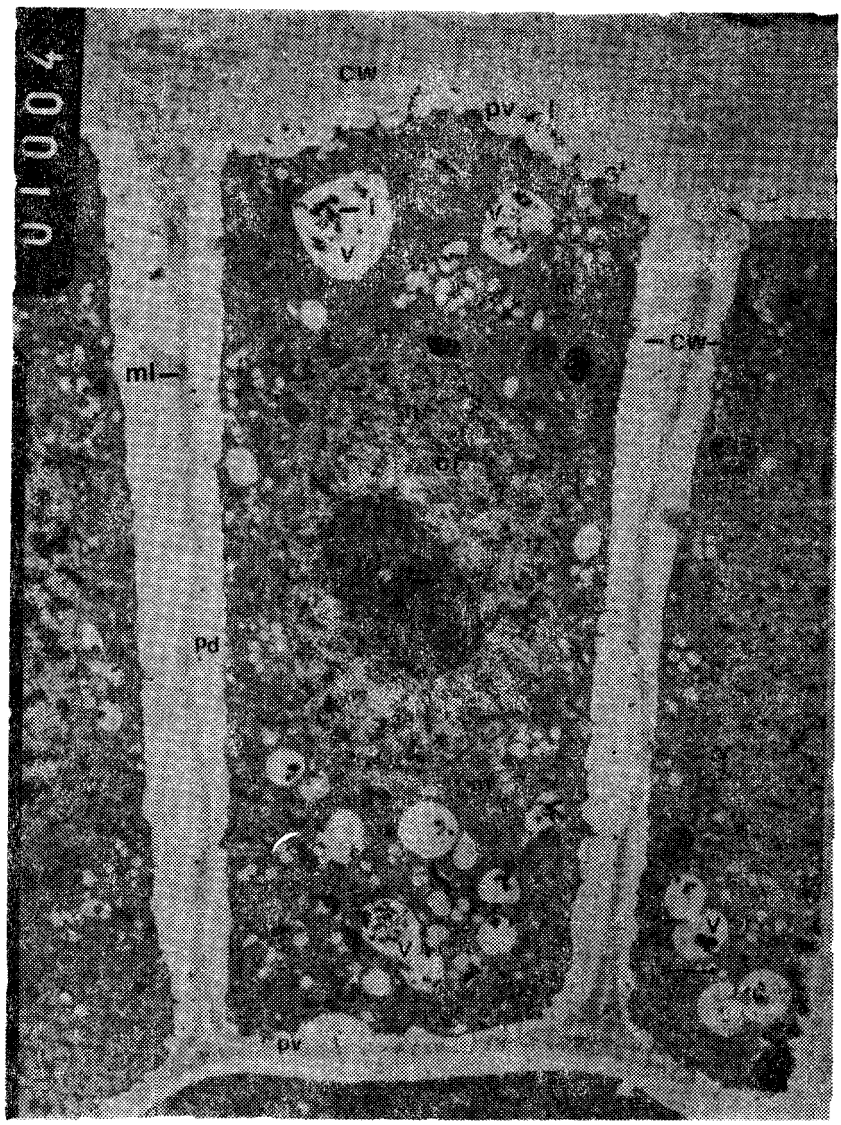


Figure 1. Lead deposits in the epidermal cells of the root tip. 1 hour incubation in 0.1 mMol/1 $Pb(NO_3)_2$. Bar = 1 μm.

and the protoplasm in particular appeared sandy in aspect. The black accumulations accured between the cell walls and the protoplasm were heavy lead deposits. The cells developed pinocytotic vacuoles for the protection, but it was not successful.

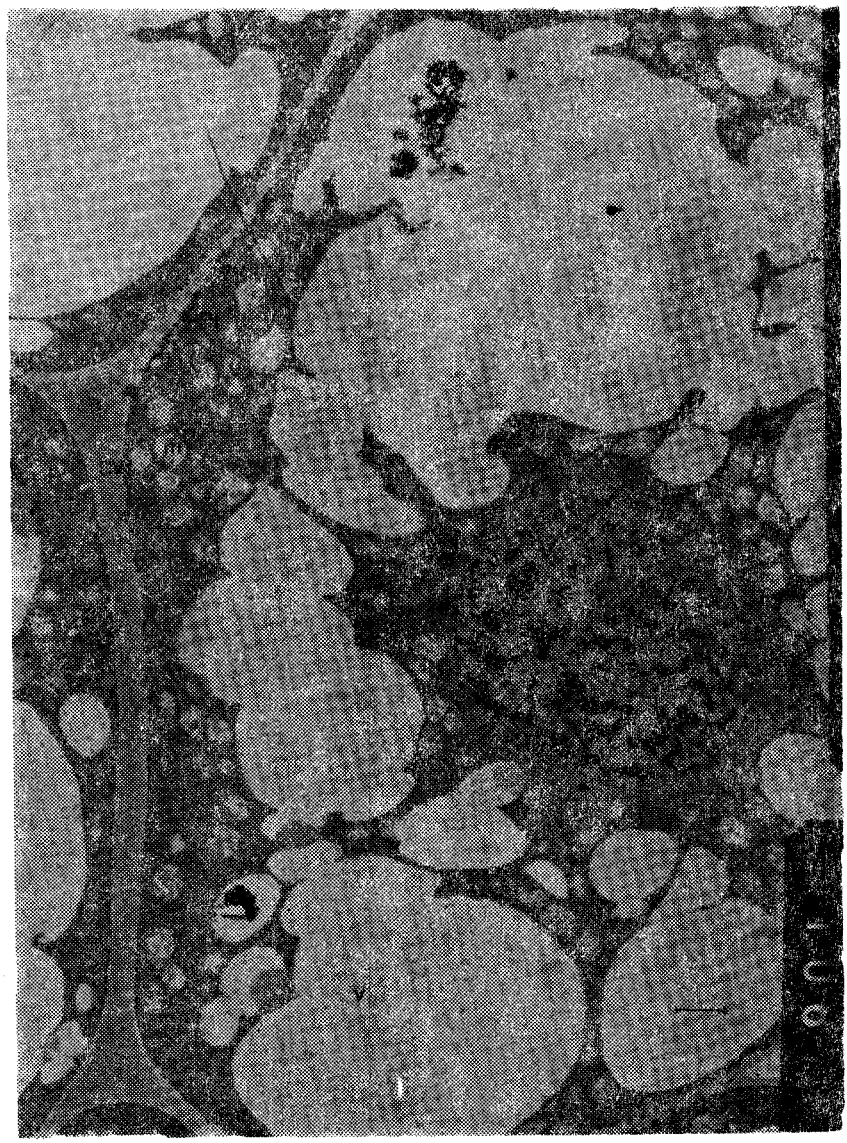


Figure 2. Lead deposits in the cortical cell of the root tip 1 hour incubation in 0.1 mMol/l $Pb(NO_3)_2$. Bar = 1 μm.

When the concentration was increased (1 mMol Pb/l), a great amount of deposition was observed at the sections prepared after one hour (Fig. 5). Lead deposits were seen in intercellular spaces. The middle

Figure 3. Dense lead deposits in the cortical cell walls and in the intercelluler spaces. 6 hours incubation in 0.1 mMol/1 $Pb(NO_3)_2$. Bar = 1 μm.

lamella, the cell organelles, the nucleus, the nucleolus and the protoplasm showed dense granular structure. The results of this concentration after 24 hours (1 mMol Pb/1) showed that the epidermal cells and all

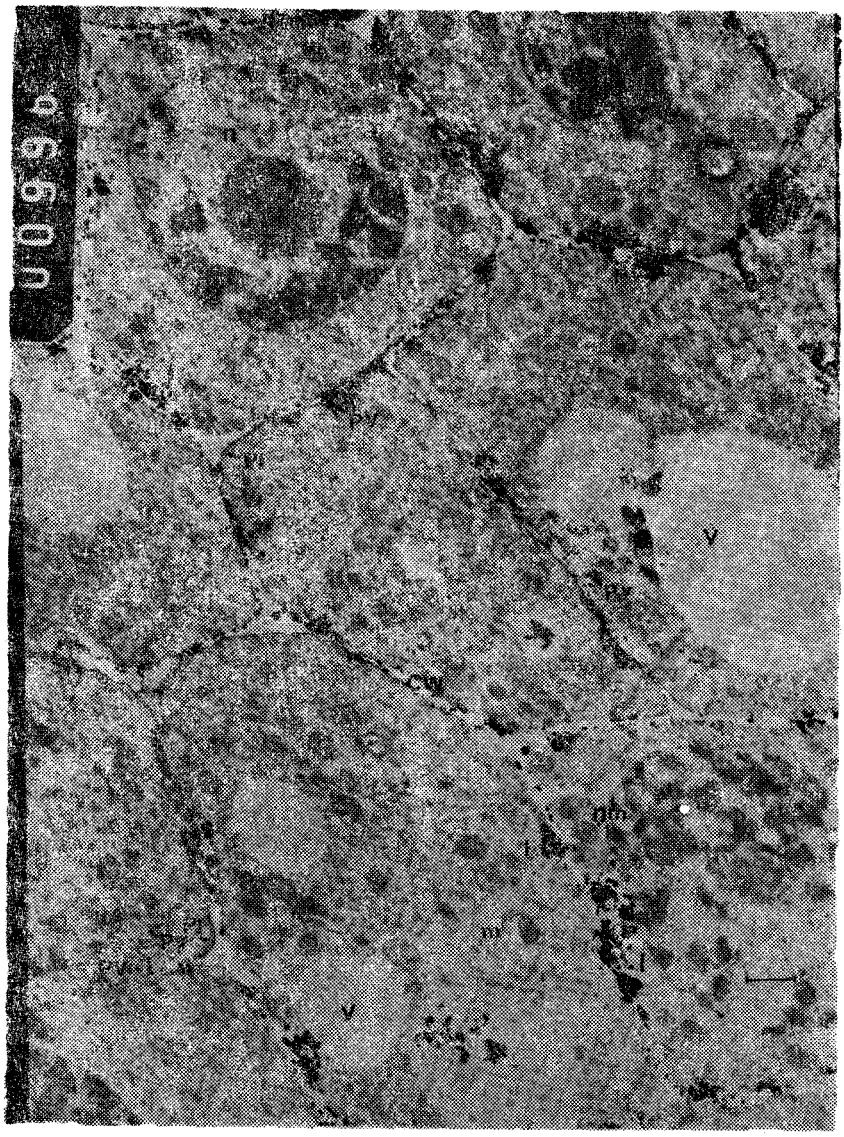


Figure 4. Lead deposits in the cell walls of the central cylinder cells. 24 hours incubation in 0.1 mMol/1 Pb $(NO_3)_2$. Bar = 1 μm.

the cortical cells were completely damaged (Fig. 6–7). Behind the endodermis, the central cylinder cells by comparison with cortical cells look rather healhty. But their dark coloring proves that lead has

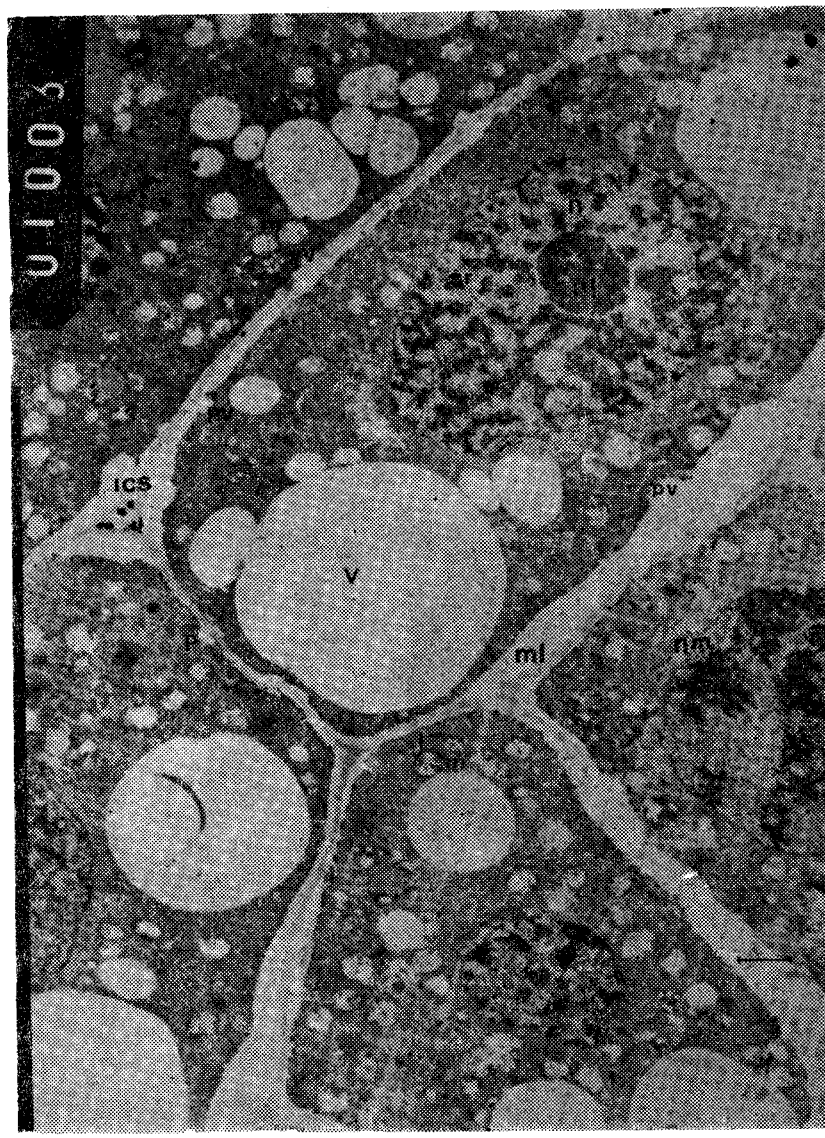


Figure 5. Lead deposits in the middle lamella and nucleus of the cortical cells. 1 hour incubation in 1 mMol/1 Pb $(NO_3)_2$. Bar = 1 µm.

already reached these cells as well. The nucleus and the organelles of these cells were darkly stained and the whole protoplasm of the cells have been in granular nature.

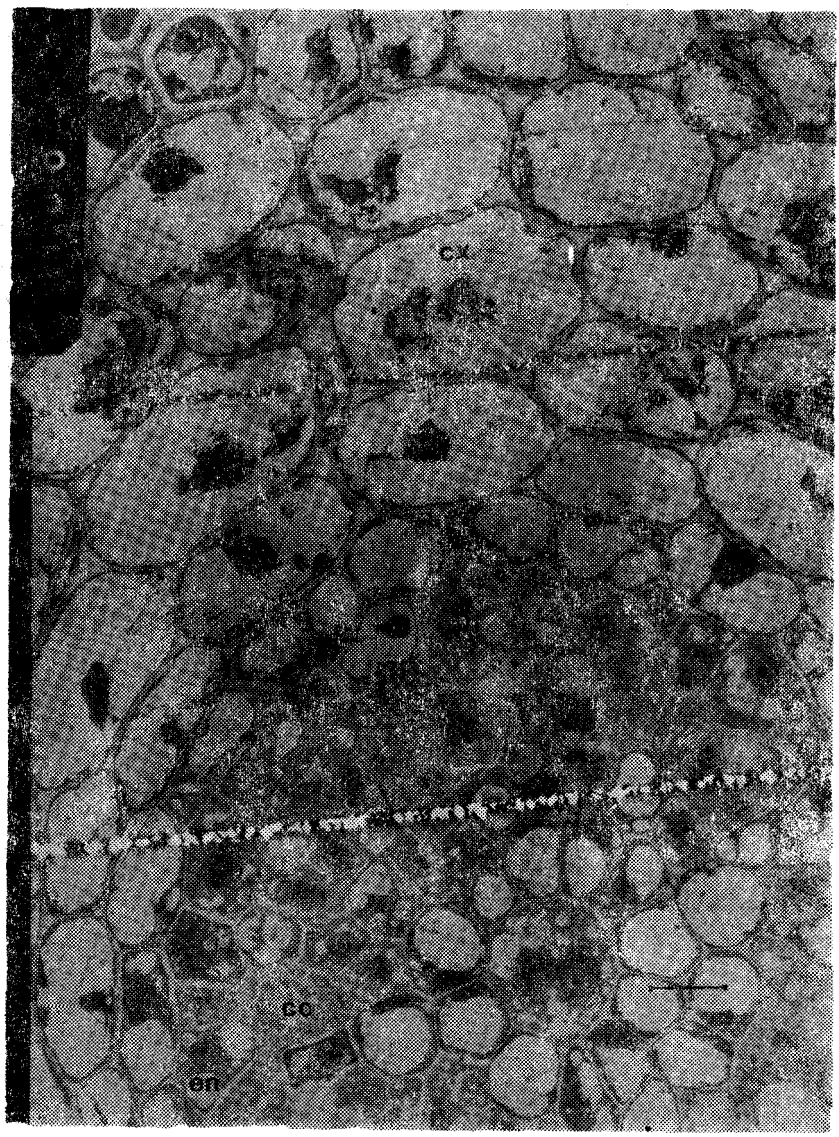


Figure 6. The effects of lead on the cross section of the root tip. Deformed cortical cells and dark stained central cylinder cells are seen. 24 hours incubation in 1 mMol / 1 Pb $(NO_3)_2$. Bar = 10 μm.

Within one hour period of extreme concentration (10 mMol Pb / 1), lead deposits were seen in the intercellular spaces, the cell walls and especially along with plasmalemma (Fig. 8). After 24 hours of this

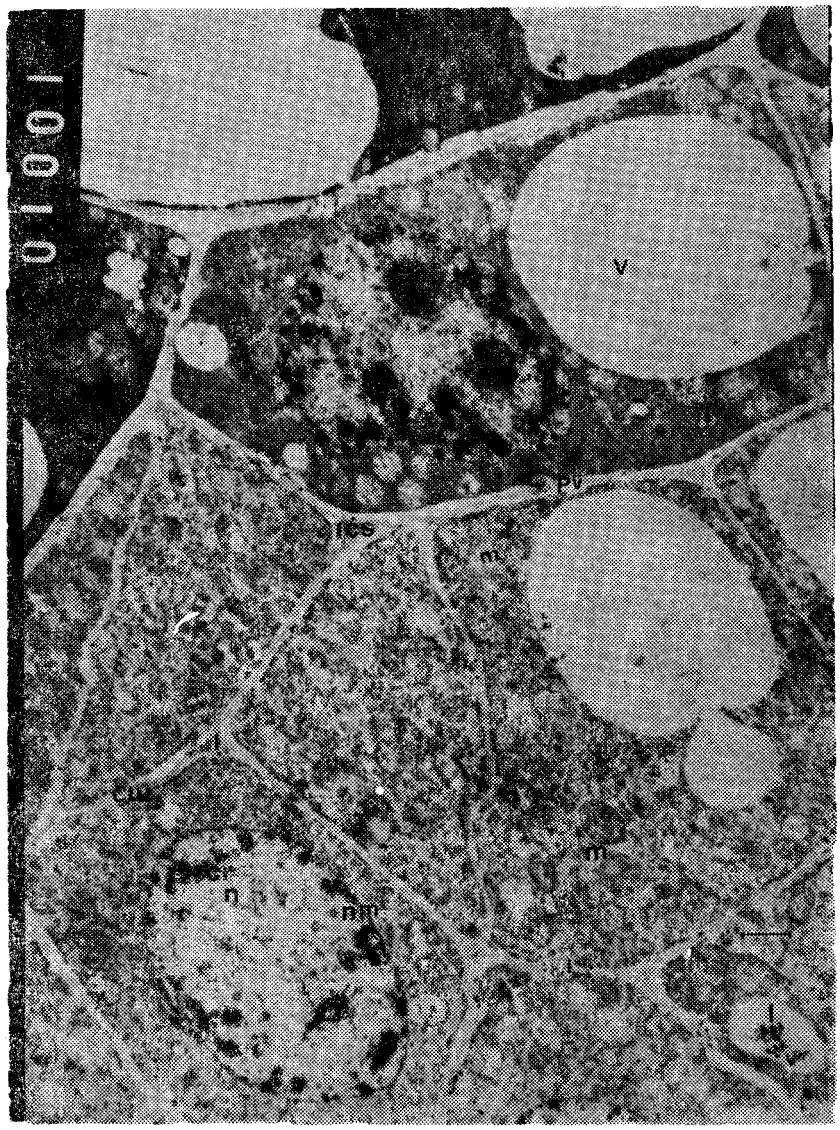


Figure 7. Dense lead deposits in central cylinder cells and nucleus. 24 hours incubation in 1 mMol/l Pb $(NO_3)_2$. Bar = 1 μm.

concentration, lead deposits spoiled all the cell structures and there was no sign of living organelles (Fig. 9).

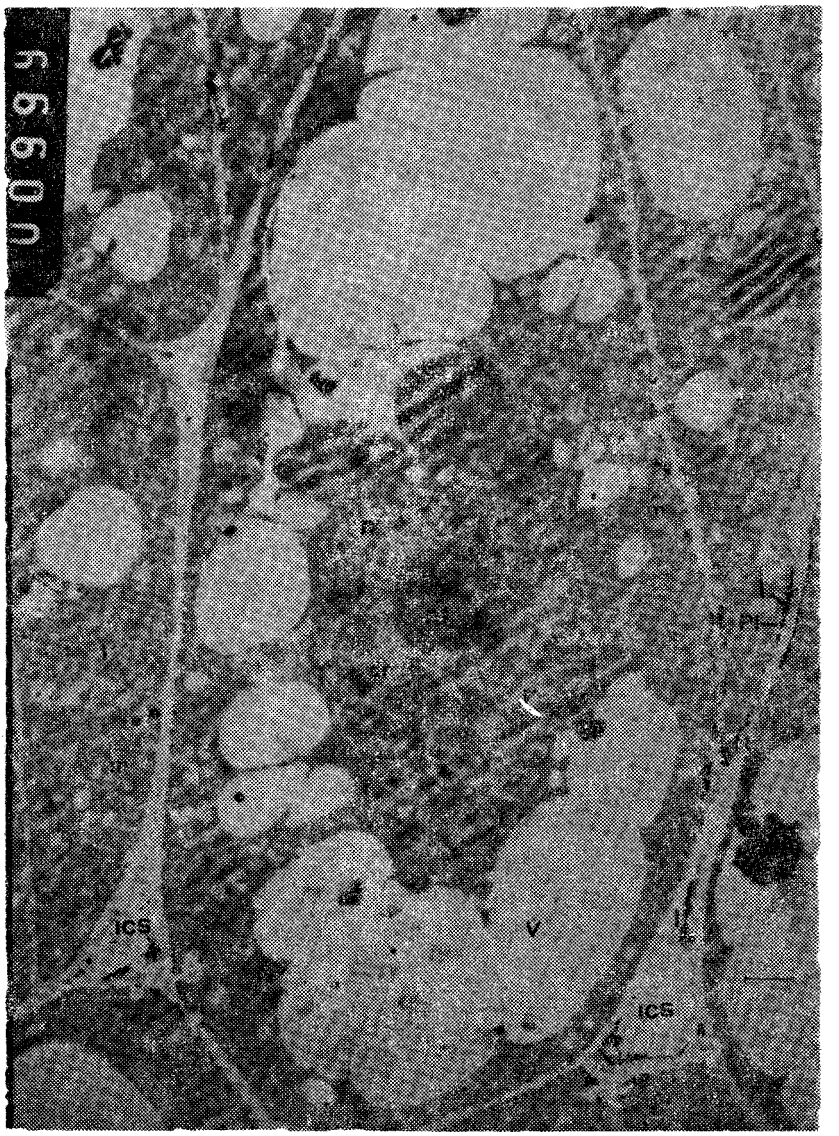
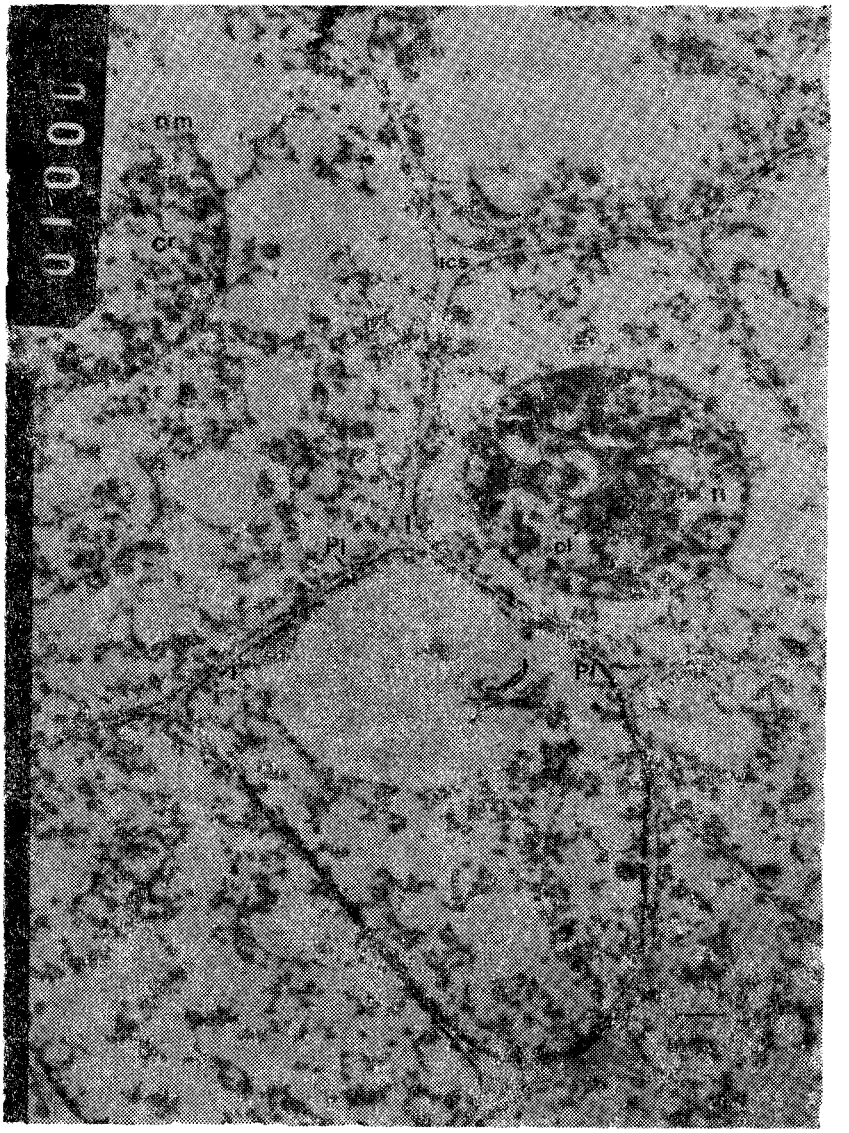


Figure 8. Lead deposits in cortical cells. 1 hour incubation in 10 mMol/1 Pb $(NO_3)_2$. Bar = 1 μm.

All the roots grown in lead nitrate solutions showed that lead was taken up and then deposited at every kind of root cells, causing the cell deformation.

The control cells contained no electron–dense deposits (Fig. 10).



Fgure 9. Effects of lead in central cylinder cells. 24 hrs. incubation in 10 mMol / l $Pb(NO_3)_2$. Bar = 1 μm.

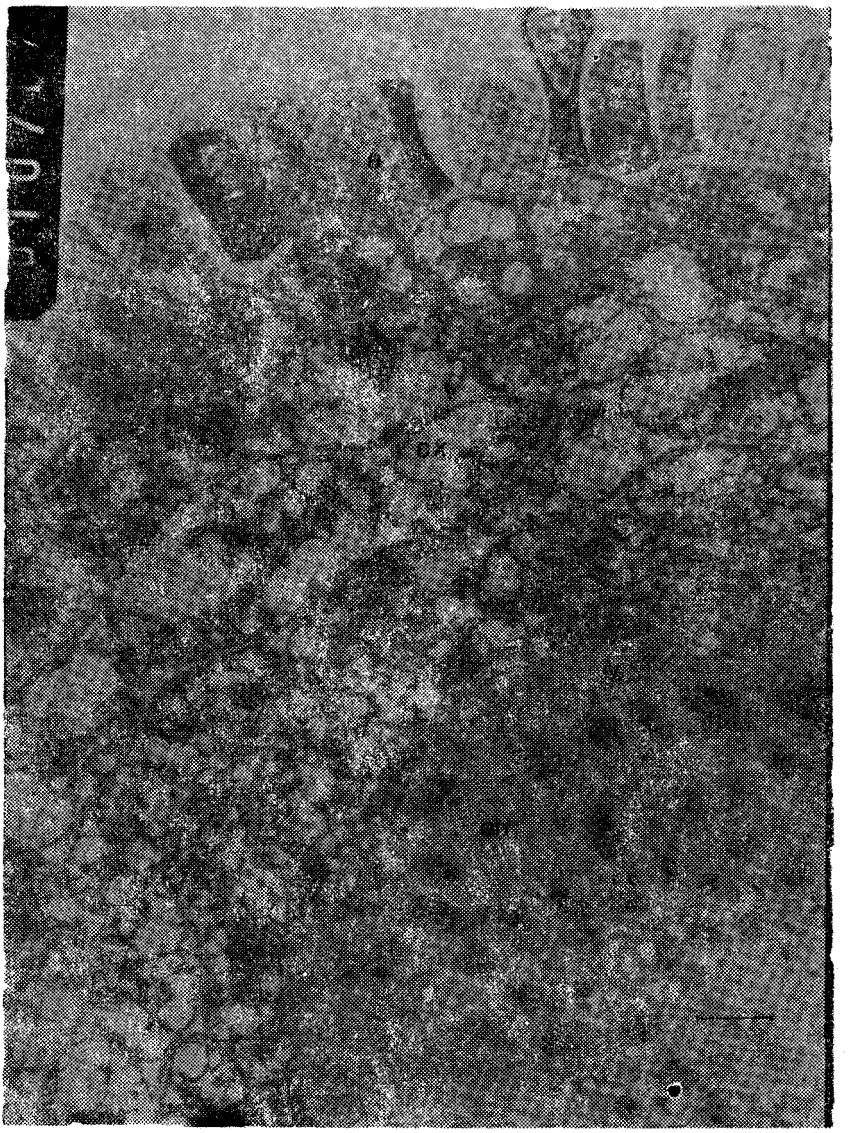


Figure 10. Cross section of the control root. Bar = 10 μm.

DISCUSSION

Although there have been many studies on the physiological pathway of heavy metal and lead uptake by plants and animals (CANNON and BOWLES, 1962; BAUMHARDT and WELCH, 1972; JONES et al., 1973a; JONES et al., 1973b; SCHULZE and BRAND, 1978; CHAHAL et al., 1979; GODBOLD and HUTTERMANN, 1986) the cytological and the histological researches are insufficient. The localization of heavy metals in tissues and cells is easily made by using electron microscobic and autoradiographic techniques (WAISEL et al., 1970; MALONE et al., 1974; SHARPE and DENNY, 1976; VAN STEVENINCK et al., 1976; WIERZBICKA, 1987).

There are a few early communications done on the subject. HAVESY (1923) has suggested that laed was not translocated appreciably from the roots to the shoots even it was used as toxic quantities. HAMMETT (1928a) has reported that lead was absorbed from the solution of Pb $(NO_3)_2$ $PbCl_2$, and Pb $(C_2H_3O_2)_2$ through the roots. Lead was accumulated largely at the areas showing maximum mitotic activity in roots, just behind the root cap of onions, beans and corns. He has also noted that the areas of the largest lead accumulation were blackened, but the black deposits were soluble in very dilute nitric acid. In addition to these results the root elongation was inhibited when the concentration had been increased (HAMMETT, 1928b). MILES and PARKER (1979a, b) have worked on the uptake and the effects of lead solutions on plants. These researchers have reported that heavy metals are largely accumulated in the roots, while in the upper parts of the plants the concentration of the heavy metals was much lower (MARTEN and HAMMOND, 1966; JONES et al., 1973a). This shows that the transport of heavy metals from the roots to the shoots is limited.

Cadmium, a well-known environmental pollutant, is bound to the plant cell wall with a nonmetabolitic bond at first and then is transported to the protoplasm through the diffusion (CUTLER and RAINS, 1974). Lead is bound to the cell wall, especially to the middle lamella at the beginning of the treatment (MALONE et al., 1974; SHARPE and DENNY, 1976; FOY et al., 1978; WIERZBICKA, 1987). In the cell wall there are many sites for lead binding. The middle lamella incorporates many chemical substances (i.e. pectates) for lead binding, and lead is readily bonded to the anionic parts of polyuronic acid in the

cell wall (SHARPE and DENNY, 1976). In the present study, the lead treatment made at even lower concentrations and for a short periods of time has showed lead deposits in the cell wall in all electron micrographs (Fig. 1–9). Lead was accumulated in the middle lamella and it was easily visible there due to its dark color. Also, small granular lead deposits were observed in the cell wall of the epidermal cells (Fig. 1).

Lead precipitation in protoplasm first appeared between the plasmalemma and the cell wall since plasmalemma acts as a selective organelle in the cell (NISHIZAWA and MORI, 1977; WIERZBICKA, 1987). In the leaf cells of *Potamegoton* ssp., the electrochemical potential gradients between cell vacuoles and the bathing solution range from –150 to –240 mV (SHARPE and DENNY, 1976) and this would favor a passive influx of lead into vacuoles in course of treatment. In the electron micrographs made by SHARPE and DENNY (1976) this influx was not observed. The reason for this was that the plasmalemma acted as a barrier to simple passive diffusion. This was said to be clearly the exclusion of lead. HIATT and LEGGET (1974) have argued that plasmalemma is a membrane which restricts the ion influx to the protoplasm. This effect has resulted from the cell wall precursors and PO_4 anions. The dictyosomes closer to the cell wall, would have high phosphate content since they contain acidphosphatase. PO_4, that forms insoluble precipitates with lead, could act as a sink for lead. At the outset of the treatment in the present study, the presence of lead deposits was shown in the cell wall and plasmalemma (Fig. 2). It was finely grained in the cell walls but the deposition was more more dense near the plasmalemma. After high concentrations and long periods of time much denser accumulation of lead were observed in the plasmalemma and between the plasmalemma and cell wall (Fig. 3).

Univalent and bivalent cations have induced pinocytosis (WHEELER and HANCKEY, 1971). SUTCLIFE (1962) has suggested that the pinocytosis was a mechanism of ion absorption by plant cells. But it has been shown that pinocytotic vacuoles inhibited the metal ions and saved the cells against their toxic effects (WHEELER and HANCKEY, 1971; SHARPE and DENNY, 1976; NASSERY and JONES, 1976; NISHIZAWA and MORI, 1977). These researchers have said that this protection by inhibition was limited, since the increase at concentration and time could have caused deposition of lead in the protoplasm, even in the vacuoles. Furthermore, lead had penetrated into the chloroplast membrane and had been deposited

along its grana (SHARPE and DENNY, 1976). Lead, like other toxic metals, caused membrane system deformation in the cell (VAZQUEZ, 1987). The ion fluxes through the plasmalemma in root cells must have been studied in short term (max. 3–4 hrs.) experiments (PATTERSON, 1976). Since pinocytosis was in action only for a short period and at low concentration and it was not regarded as a protection mechanism. The data obtained in the present study showed that lead accumulation has occured at first in the cell wall and then caused pinocytotic vacuoles in the plasmalemma in the periods of 1–3 hours (Fig. 3–4). After high doses of lead for long durations was applied the large and the uniform lead accumulation were observed in the inner structure of the cells. Toxic uranyl salts (0.1–1 mMol / 1) were absorbed by oat roots rapidly and were crystalized in the cell walls and in the intercellular spaces. That, after 4–6 hours uranyl crystals had migrated pinocytotically into protoplast had been stated by WHEELER and HANCKEY (1971). Metal ions penetrating the protoplast have been concentrated inside it, due to the fact that they had found many binding sites there (cysteinyl, hystidyl side chain of proteins, purines, pteridines and porphyrins) (MATHYS, 1975). Our electron micrographs (Fig. 1–9) had a deep contrast that has come from lead which was absorbed by the protoplasm.

Protoplasm exhibits a granular structure because of lead. Dense lead accumulation has occured in the nuclear membrane and even in nucleolus (Fig. 9). OPHUS and GULVAG (1974), in their studies on lead uptake by moss Rhytidiadelphus squarrosus using electron microscopy and X-ray microanalysis, have demostrated the presence of lead within the nuclear and chloroplast inclusions. According to SHARPE and DENNY (1976), after the long term treatment of Potamegoton pectinatus with lead, electron micrographs clearly have shown the accumulation of lead, mainly in the cell walls and also symptoms of senecens, autolysis and plasmolysis have been observed. Biochemical studies on the similarly treated tissue have showed increases in nucleic acid breakdown products and the free amino acids indicating that the nuclei had been effected by lead. Both the nucleus and the nucleolus have had a strong affinity for heavy metals and inos (VAN STEVENINCK, 1976).

MALONE et al. (1974), who have conducted a similar research on roots of *Zea mays*, have reported that lead accumulation occur first at the cell wall and then at the plasmalemma. These deposits were

granular at the outset but later turned to large crystals. They had observed the same pinocytotic vacuoles as those seen in the barley roots in the present study. They had indicated that lead was taken up by the root system of corn, transported and precipitated throughout the plant. WIERZBICKA (1987) have investigated lead accumulation and translocation in *Allium cepa* roots using electron microscope and autoradiographic methods. Lead transport in the roots was possible, either through the apoplast or symplast. He has suggested by cytochemical estimations that lead probably migrates through the plasmalemma of the protoderm cells and was then symplastically transported by the endoplasmic reticulum. After one hour of incubation lead had appeared in all root tissues except for the central cylinder. He has pointed out that lead was deposited in the cell wall, especially in the middle lamella, but endodermis cells had acted as a barrier against the lead. In the present study, after 24 hours incubation, the highest concentration of lead has been observed in all root tissues. Also SUCHODOLLER (1976) reported that the cortical cells were more sensitive than the central cylinder. In our experiments of barley root tissues also have the same appearance with respect to the deposition of lead in these cells. The epidermis and the cortical cells of the root were seriously injured exhibiting plasmolysis and disorganized cell structure, with the exception of the cell of central cylinder (Fig. 6). In electron micrographs made after 6 and 24 hours of lead treatment, however, all root cells including the central cylinder ones showed dense lead deposits for all experimental combinations.

This study has shown that lead is taken through the roots of barley and precipitated throughout its cells. The results suggest that lead is available to plants under natural condition and that it would seriously affect organisms further down to the food chain.

Acknowledgment

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ABBREVATIONS:

cc	central cylinder
cl	crystall lead
cr	chromatin
cw	cell wall

cx	cortex
e	epidermis
en	endodermis
ics	intercellular spaces
l	lead
m	mitochondria
ml	middle lamella
n	nucleus
nl	nucleolus
nm	nuclear membran
pd	plasmodesmata
pl	plasmalemma
pv	pinocytotic vacuole
v	vacuole

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