

Full Length Research Paper

Lead-induced changes in plant morphology, cell ultrastructure, growth and yields of tomato

Shouping Zhao, Xuzhu Ye and Jici Zheng*

Institute of Quality Standards for Agro-products, Zhejiang Academy of Agricultural Sciences, Hangzhou Center for Inspection and Testing for Quality and Safety of Agricultural and Genetically Modified Products, Ministry of Agriculture, Hangzhou 310021, China.

Accepted 19 May, 2011

A pot experiment in plastic greenhouse was conducted to investigate the effect of Pb toxicity on development and ultrastructure of tomato (*Lycopersicon esculentum* Mill. cv. ZZ203) by four levels of Pb addition [0, 150, 450, and 900 mg/kg Pb in soil as Pb(NO₃)]. The results showed that excess lead resulted in dwarf plants, wilting and misshapened leaves accompanied by less number, less numbers of the calyx, and longer-smaller fruits. At the same time, the condensed cytoplasm accompanied by the department of plasma from cell wall, swollen mitochondrial and chloroplast including thylakoid, fewer cristae in mitochondrial, loosely combined thylakoid to form askew grana, disruption plasma membrane and also followed with dilation organelles were all the typical toxic symptoms of Pb stress. Otherwise, plant biomass and fruit yields decreased as Pb increased: treatment or addition of Pb (150 mg/kg) decreased the aforesaid parameters, but these differences did not approach significance at 5% level of probability; however, Pb (450 or 900 mg/kg) treatment, significantly decreased the aforementioned parameters. Excess lead (900 mg/kg) inhibited biomass and fruit yields by 71 and 51% as compared to the zero Pb control treatment respectively. Concentration of Pb in plant tissues decreased in the following order: fruits, leaves, stems and roots.

Key words: Lead, tomato, plant morphology, ultrastructure, growth, yield.

INTRODUCTION

Lead (Pb) as an environmental pollutant is particularly important (Salt et al., 1998) because it is toxic to many plants and organisms, and causes harmful effects on the health of children and adults (Lanphear, 1998). Lead is not an essential element for the growth of plants, nor does it participate in the process of cell metabolism. Lead pollutions in the environments are mainly brought about by industrial activities (mining and smelting activities and so on), motor vehicles and also by the use of chemical fertilizers and municipal sewage sludge (Jackson and Watson, 1977; Levine et al., 1989). Many fertilizers contain Pb and therefore, it is found that vast areas of agricultural land contain high concentrations of Pb (Shull, 1998).

Lead contamination may bring about a serious problem

for agriculture, for example, the primary effect of Pb toxicity on plants is a rapid inhibition of root growth due to the inhibition of cell division in the root tip (Eun et al., 2000). Plants are poisoned by Pb, and Pb-contaminated soil may result in a great decrease in crop productivity (Buchauer, 1973; Johnson and Eaton, 1980). Previous studies revealed that soybean has the highest sensitivity to excessive lead among vegetables such as horse bean, white mustard and cucumber (Chwill, 2000). Furthermore, Pb is also accumulated in the human body through our foodstuff and damages the brain and the nervous system (Body et al., 1991).

Although, we all recognized that lead is an environmental pollutant that interferes with plant growth; unfortunately, the mechanisms of lead toxicity on plants are still poorly understood. Nowadays, most studies are still focused on the physiological process and seldom report about the growth characteristics and the ultrastructure of vegetable leaves with excess Pb. The purposes of this study were to investigate the

*Corresponding author. E-mail: zjpaper@yahoo.cn. Tel: +86 0571 86415206. Fax: +86 0571 86419052.

physiological damage and distorted ultrastructure in the leaves of tomato plants caused by Pb toxicity and to determine the effects of different Pb dose on growth and fruit yields of tomato.

MATERIALS AND METHODS

Plant material and growth medium

A greenhouse experiment was conducted at Hangzhou city (31° 37' N and 120° 30' E), Zhejiang province, China. The growth medium was a mixture of 1/2 soil and 1/2 sawdust. The soil was collected in Haiyan county, Zhejiang province which belonged to typical paddy soil derived from river and sea sediments with physico-chemical properties as follows: pH 6.71, organic matter 22.7 mg/kg, alkali-hydrolyzed N 93.4 mg/kg, available P 76.0 mg/kg, available K 112.3 mg/kg, Pb 19.6 mg/kg. Watertight-pot with 20 cm height × 30 cm diameter and installed with 2.3 kg of the mixture medium was the growing medium in our experiment. The variety of tomato (*Lycopersicon esculentum* Mill.) used in this study was ZZ203. Seeds of tomato were germinated in the salvers filled with the mixture medium. 25-day-old tomato seedling was transplanted to the watertight-pot with different amounts of Pb, and cultivated up to 100 days. The experiment was carried out in a plastic greenhouse.

Experimental design and treatments

Lead was added as Pb(NO₃)₂ at four levels: 0, 150, 450, and 900 mg/kg soil. Treatments were arranged in a randomized block design with one tomato plant per pot and three replications. 100 ml of solution with different concentrations of Pb was added into the growing medium in watertight-pots and then incubated for one month at the room temperature and 70% humidity. The compound fertilizer (15-15-15) was applied at 4 g/plot, of which 75% was added as pre-transplanting and 25% as top fertilization in early spring at 55-day-old plant. The humidity of the growth medium was maintained at field capacity during the whole growth period.

Determination of growth parameters and the concentration of Pb in plant tissues

The plant height and leaf number were recorded at 43-, 48-, 53-, 58-, and 64-day-old. The biomass of roots, stems, and leaves were recorded at 100-day-old. Fruits were harvested in the red stage at morning every 3 days and the fresh weight (FW) of each fruit was determined directly after harvest, then the sum of fresh weight was calculated as fruit yield.

The samples of roots, stems, leaves and fruits at 100-day-old were taken, to measure the concentrations of Pb in plant tissues. The Pb concentrations in plant digests were determined by microwave digestion and graphite furnace-atomic absorption spectrometer Thermo MKII-M6 (Soil Science Society of China, 1999).

Preparations of sample for transmission electron microscope (TEM)

The fragments of the 100-day-old leaves and calyx with Pb, 0 and 900 mg/kg soil treatments were treated as follows:

Fixation

The fragments were fixed in a fixative containing 2%

paraformaldehyde and 2.5% glutaraldehyde, buffered at PH 7.0 in phosphate buffer. Fixation was performed at room temperature for 4 h, followed by 12 h at 4°C. After being fixed, the samples were rinsed three times with a phosphate buffer and then osmium-treated with 1% OsO₄ for 1 h and washed three times in the phosphate buffer.

Dehydration

The specimen was first dehydrated by a graded series of ethanol (50, 70, 80, 90, 95, and 100%) for 15~20 min at each step, transferred to absolute acetone for 20 min.

Infiltration

The samples were placed in a 1:1 mixture of absolute acetone and the final Spurr resin for 1 h at room temperature and then transferred into a 1:3 mixture of absolute acetone and the final resin mixture for 3 h and finally into the final Spurr resin mixture for overnight.

Embedding and ultrathin slice

The samples were placed in capsules which contained embedding medium and heated for 9 h at 70°C. The samples were stained by uranyl acetate and alkaline lead citrate for 15 min, respectively. The samples were observed in transmission electron microscope of Model JEM-1230.

Statistical analysis

Statistical analysis of experimental data was performed with Microsoft[®] Excel 2000 and SPSS10.0 software. The significant difference analysis was carried by one way anova and the Duncan system.

RESULTS

Changes in morphological characteristics

The typical changes in morphological characteristics of tomato plants induced by lead toxicity included dwarf plants, wilting and misshapened leaves accompanied by less number, less numbers of the calyx, and longer-smaller fruits as shown Figure 1. The 100-day-old tomato plants grown in Pb-containing growing medium were characterized by a retarded development in leaf blades especially with higher Pb addition treatment. With Pb addition amount at 450 or 900 mg/kg, the leaf blades became shrunken and smaller, while Pb (150 mg/kg) treatment made no significant effect on the shape and size of leaf blades as compared with the control (addition of Pb, 0 mg/kg) (Figure 1A).

Pb toxicity at 900 mg/kg level made a significant damage in leaf-shape, especially on top height of the plants where leaves crinkle up or down and then became acicular leaves (Figures 1A, B, C and D). Otherwise, earlier death of adult leaves was also obvious under

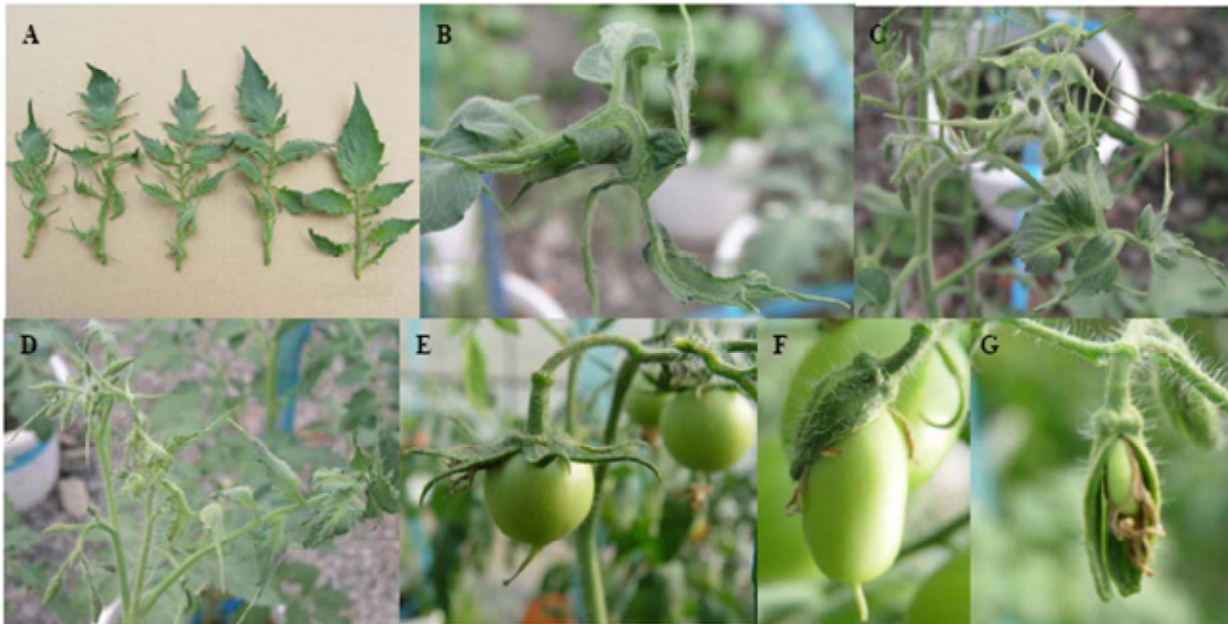


Figure 1. The morphologic characteristics of leaves, fruits, and calyx of 100-day-old plants with different Pb levels. (A): The leaves from right to left are the ones of the plants treated with Pb (0, 150, 150, 450, and 900 mg/kg soil, respectively); (B): The leaves on the middle part of the plant treated with Pb (900 mg/kg); (C and D): the leaves on the top of plants treated with Pb (900 mg/kg); (E): the fruits of the plants treated with Pb (0 mg/kg); (F and G): the fruits of the plants treated with Pb (900 mg/kg soil).

higher Pb addition (450 and 900 mg/kg) treatment.

Addition of Pb, 900 mg/kg also induced the long-smaller fruits and a lesser number of calyx compared to control treatment (Figures 1E, F and G). Except for fruits, the calyx were also misshaped to incassated and connected in the presence of Pb at 900 mg/kg level (Figures 1F and G). In a word, the contractive, curly and spiculate leaves, elongated fruits, and macrocephalic calyx occurred on the plants treated with Pb 900 mg/kg.

Change in ultra-structure of the leaves and calyx

Result of transmission electron microscope (TEM) on leaves and calyx treated with Pb 900 mg/kg, showed that the ultrastructure of chloroplast, mitochondria, and cell membrane in the leaves were all disturbed (Figure 2). The cells of leaves under 0 mg/kg of Pb addition showed well-developed ultrastructure (Figure 2A), these cells contained plenty of cytoplasm, the plasma membrane was tightly clung to the cell wall, the chloroplast membrane was integral, the mitochondria kept normal configuration and contained abundant cristae, and the cell wall showed a normal uniform color. While in the malformation, leaves were treated with Pb 900 mg/kg (Figures 2B, C and D), the concentrated cytoplasm, enlarged chloroplast and thylakoid, and swollen mitochondria were all investigated. The disorder phenomena also include the loose structure of grana

lamellae in the chloroplast, the declined or disappeared cristae in mitochondria, and the ruptured or disappeared plasma membrane, and markedly dark stains occurred on the cell wall.

The cells of calyxes showed normal ultrastructure with 0 mg/kg of Pb treatment as leaves (Figures 2E and F), such as plasma membrane was almost clung to cell wall, the chloroplast membrane was integral, arrangement of grana lamella was tight and parallel, mitochondria contained abundant cristae, and the cell wall was thick (Figures 2G, H and I) and so on. The ultrastructure of calyx cells was also damaged by treating with Pb 900 mg/kg (Figures 2E and F). In the cell of the malformed calyx, intensely condensed cytoplasm induced the department of cytoplasm completely from the cell wall. Chloroplasts and plasma were near the cell wall in most cases. The swollen chloroplast was covered by fragmentized membrane and the thylakoid overlap each other. The chloroplast membrane was not integral and swollen. In the chloroplast, the thylakoids integrated closely to form grana lamellae. The inner membrane of the mitochondria was strewn with plentiful cristae. The cell wall became thin and stained. The mitochondria were swollen (Figures 2G, H and I).

Effects of lead on plant height and leaf numbers

The decreases in plant height and leaf numbers were not

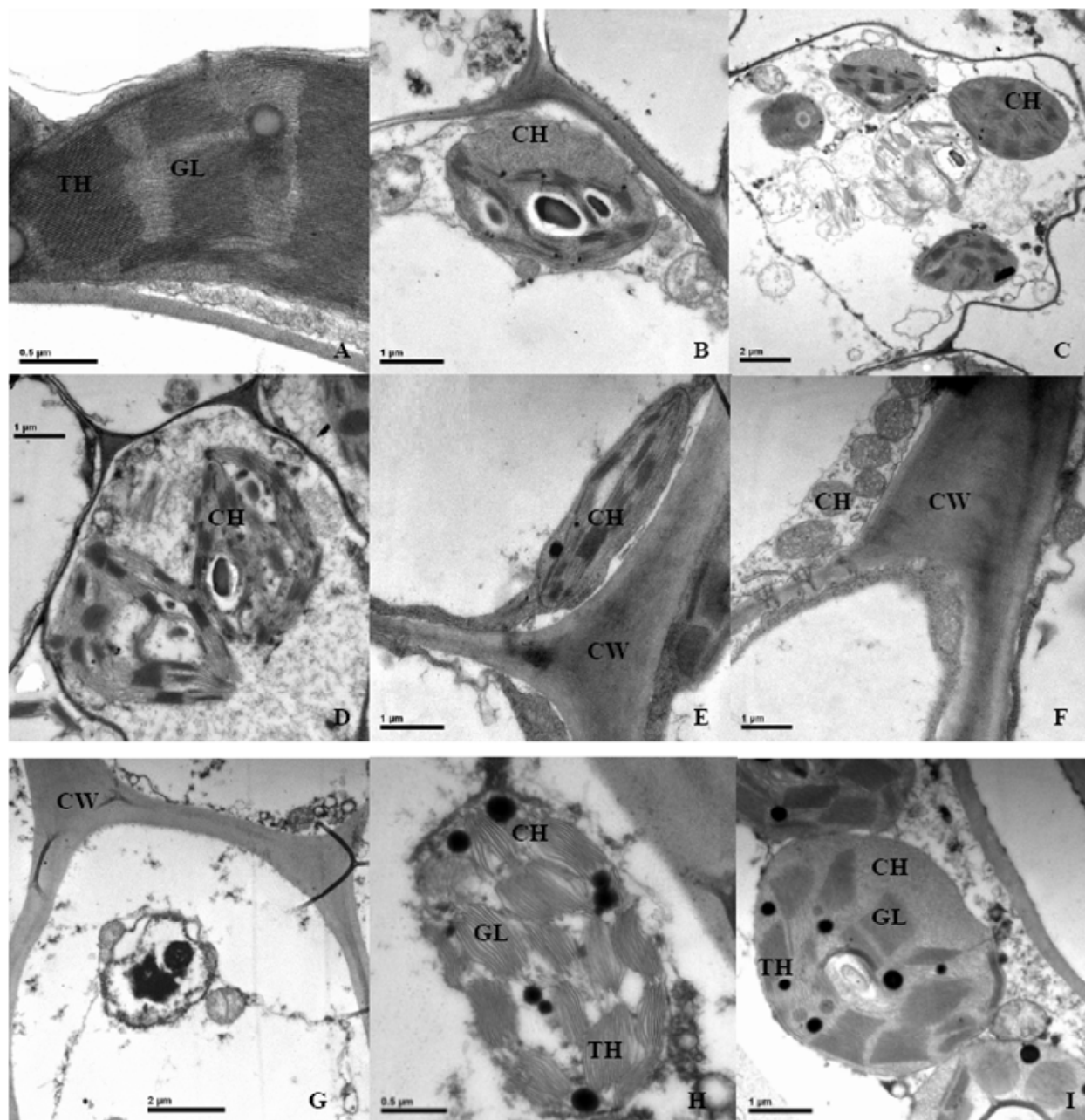


Figure 2. Transmission electron microscope (TEM) of leaves and calyx sampled from 100-day-old plants. The tomato was planted in pot culture experiment in plastic greenhouse treated with Pb (0 and 900 mg/kg soil); sample the leaves and calyx of the two treatments at 100 days old for transmission electron microscope analysis. (A): leaves treated with Pb (0 mg/kg); (B, C and D): leaves treated with Pb (900 mg/kg); (E and F): calyx treated with Pb (0 mg/kg); (G, H, and I): calyx treated with Pb (900 mg/kg). CW: Cell wall, CH: chloroplast, GL: grana lamella, TH: thylakoid.

only related to the Pb concentrations, but also to the growth duration under Pb stress. The height was reduced proportionally to the increase of Pb levels and the plant age (Figure 3). Height of 64-day-old plant decreased by 10, 26, and 29% in treatment containing Pb (150, 450 and 900 mg/kg) compared to control treatment respectively, while leaf numbers decreased by 9, 36, and 49% under the same condition respectively. Height and

leaf numbers were decreased by addition of Pb (150 mg/kg) at 43- and 48-day-old plants compared to control, but the difference did not approach significance at 5% level of probability. Addition of Pb, 450 or 900 mg/kg dose, significantly reduced the plant height and leaf numbers as compared with (0 mg/kg) Pb treatment, especially for leaf numbers at 64-day-old (Figure 3).

By comparing the examined parameters on five

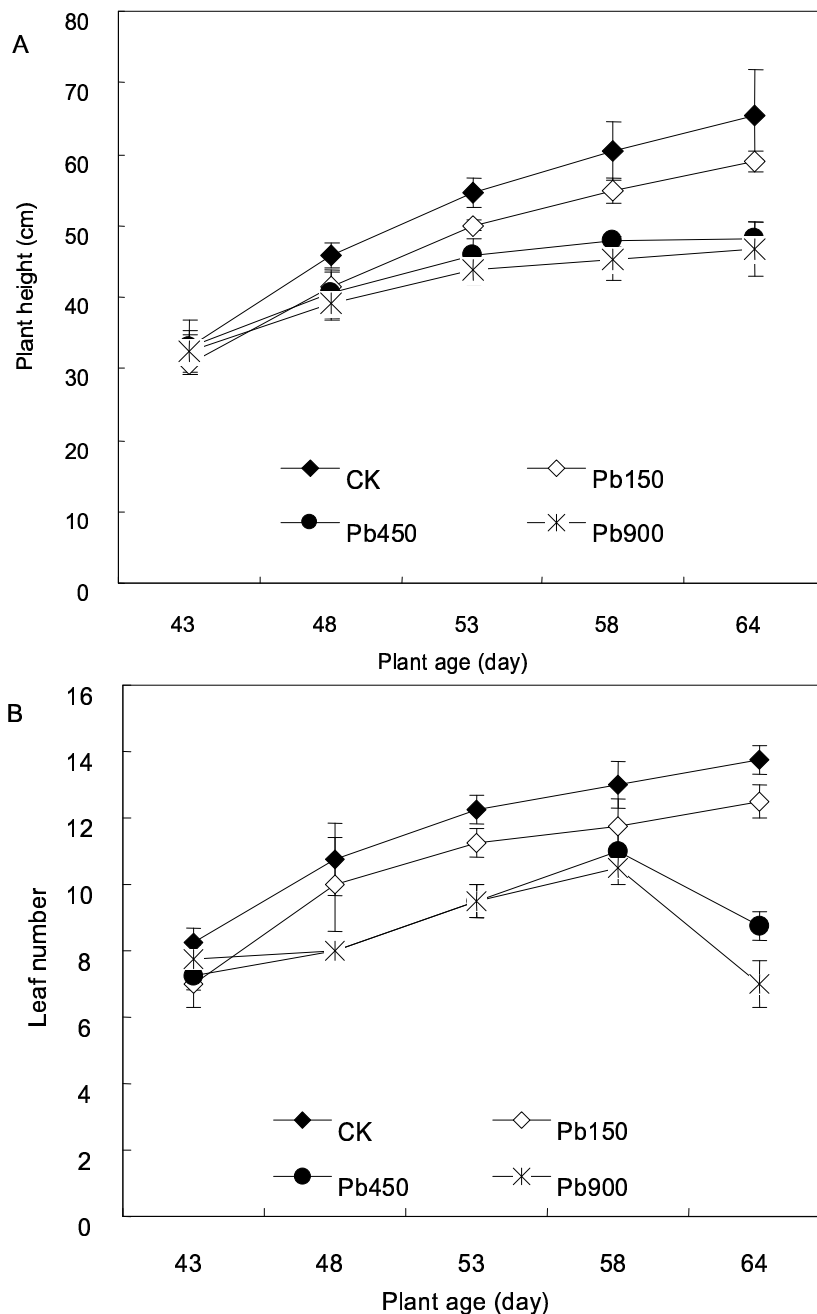


Figure 3. Effects of different Pb treatments on height (A) and leaf numbers (B) of tomato plants. The bars represent SE based on three replicates. Pb₀, Pb₁₅₀, Pb₄₅₀, and Pb₉₀₀ indicate the treatments of Pb addition as 0, 150, 450 and 900 mg/kg soil, respectively.

sampling points, it was found that the negative effect of Pb on plant development was more pronounced in adult plants than in young plants; and in another word, the influence of Pb toxicity mainly exhibited on adult growing period (Figures 1A and 3). Maybe these should be due to the accumulative effect. The plant height treated with Pb (900 mg/kg) were 90, 85, 80, 74, and 71% of those under control treatment at 43-, 48-, 52-, 58-, and 64-day-old

respectively, whereas the leaf numbers were 95, 74, 77, 81, and 51% of those with (0 mg/kg) Pb treatment, respectively.

Fruit yields and biomass of tomato

Treatment of Pb (150 mg/kg) reduced the biomass of

Table 1. The effects of addition of Pb on Pb concentrations in the different organs of 100 day-old tomato plants.

Addition Pb (mg/kg)	Root		Leave		Stem		Fruit	
	(mg/kg)	(%)	(mg/kg)	(%)	(mg/kg)	(%)	(mg/kg)	(%)
0	5.8c	100	3.69c	100	0.52d	100	0.023b	100
150	44.2b	762	4.420b	120	1.59c	306	0.028ab	122
450	115.4a	1990	5.12ab	139	2.32b	446	0.045ab	196
900	98.6a	1700	5.55a	150	5.35a	1028	0.056a	244

Each value is the average of three replicates. The different letters in the same column indicate a significant difference at the 0.05 level using Duncan's Multiple Range Test.

roots, leaves, stems and fruit yields of tomato compared with the control (0 mg/kg Pb) treatment, but all these decreases did not approach significance at 5% level of probability except for biomass of stems. Addition of Pb (450 or 900 mg/kg) significantly decreased biomass of roots, leaves, and stems as well as total fruit yields of tomato as compared with the control treatment except for biomass of stems treated with Pb (450 mg/kg soil) (Figure 3). Biomass of roots, leaves, and stems as well as total fruit yields of tomato in the presence of Pb (150 mg/kg) were 90, 85, 80, and 71% of those under control treatment, respectively, whereas these parameters in the presence of Pb (900 mg/kg) were 95, 74, 77, and 51% of those with the control treatment, respectively.

Concentrations and accumulation of Pb in the different organs of tomato

According to different organs of plant, the Pb concentrations decreased followed by roots, leaves, stems and fruits (Table 1). The Pb concentrations in roots were 250~2564 times greater than that in fruits. Among different treatments, the Pb concentrations increased with Pb rate increased in root, leaves, stem and fruit (Table 1). By comparing the increase step of Pb concentrations among different organs, it was found that the roots and stems seem easy to accumulate Pb than leaves or fruits. The Pb concentrations increased by 16.0 and 1.44 times in root and fruit respectively, because the Pb addition amount increased from 0 to 900 mg/kg. Addition of Pb significantly increased the Pb concentration in the root, leaves and stem as compared with the control, while only 900 mg/kg Pb treatment, induced the significant increment in Pb concentration in fruit. These suggested again that the fruit is insensitive to Pb addition (Table 1).

The study of correlation showed that there was a significant correlation between Pb concentrations in plant and Pb dose added into the soil. Correlation coefficients between Pb concentrations and Pb addition dose in roots, leaves, stems and fruits were 0.6492*, 0.8959**, 0.9594**, and 0.9661**, respectively. Concerning the total Pb accumulation amount, dwindle order change to fruits,

leaves, stems and roots (Table 2). Although, Pb concentration in the fruits was much lower than that in the roots, the biomass of the fruits was much greater than that of the roots. So, the accumulation amounts of Pb in the fruits were much greater than that in the roots. In the presence of Pb (0, 150, 450, and 900 mg/kg soil), accumulation amounts of Pb in fruits were 17.7, 27.4, 9.4, and 19.3 times greater than that in roots.

Biomass was reduced by Pb toxicity so dramatically that the accumulation amounts of Pb decreased with the increasing dose of Pb addition (Figure 4, Table 2). The decrease in Pb accumulation in root, leaves and fruit did not approach significance at 5% level of probability, as compared between Pb, 0 and 150 mg/kg treatment, while significant reduction occurred when Pb dose was increased from 0 to 450 or 900 mg/kg. Total accumulation amounts of Pb of the whole plant treated with Pb (150, 450, and 900 mg/kg) were 85, 33, and 32% of that in the plants treatment with Pb as 0 mg/kg respectively with only 19.6 mg/kg Pb as the background value.

DISCUSSION

Our result showed that the changes in morphological characteristics of plants induced by excess lead included the reduction in size and thickness of leaf blade, shrink and distortion of the leaves, less calyx, and the longer and smaller fruits. Study on soybean have indicated that the lead toxicity induced a histological change in leaves, and made a thin leaf blade, minified the xylem and phloem in the vascular bundles, and also reduced the diameter of the xylem vessels (Elzbieta and Miroslawa, 2005). The results of our study also showed that excess lead decreased leaf area and plant height as well as fruit size dramatically.

Although, the heavy metals used in previous studies were different, the common poison symptoms were, department of the plasma from the cell wall, swollen chloroplast and mitochondrial, disruption of the plasma membrane, and dilation of organelles. It was previously

Table 2. Accumulation of Pb in the different organs of 64 day-old tomato plants treated with different rates of Pb addition.

Addition of Pb (mg/kg)	Root		Leave		Stem		Fruit		Total	
	(g/plant)	(%)	(g/plant)	(%)	(g/plant)	(%)	(g/plant)	(%)	(g/plant)	(%)
0	35.2a	100	298.3a	100	149.1b	100	658.4a	100	1141.0a	100
150	34.2a	69	249.5a	84	117.6c	79	569.3a	84	970.7a	85
450	9.0b	26	111.8b	38	163.5a	110	94.0b	14	378.3b	33
900	9.1b	26	125.5b	42	122.1c	82	111.3b	17	368.0b	32

Each value is the average of three replicates. The different letters in the same column indicate a significant difference at the 0.05 level using Duncan's Multiple Range Test.

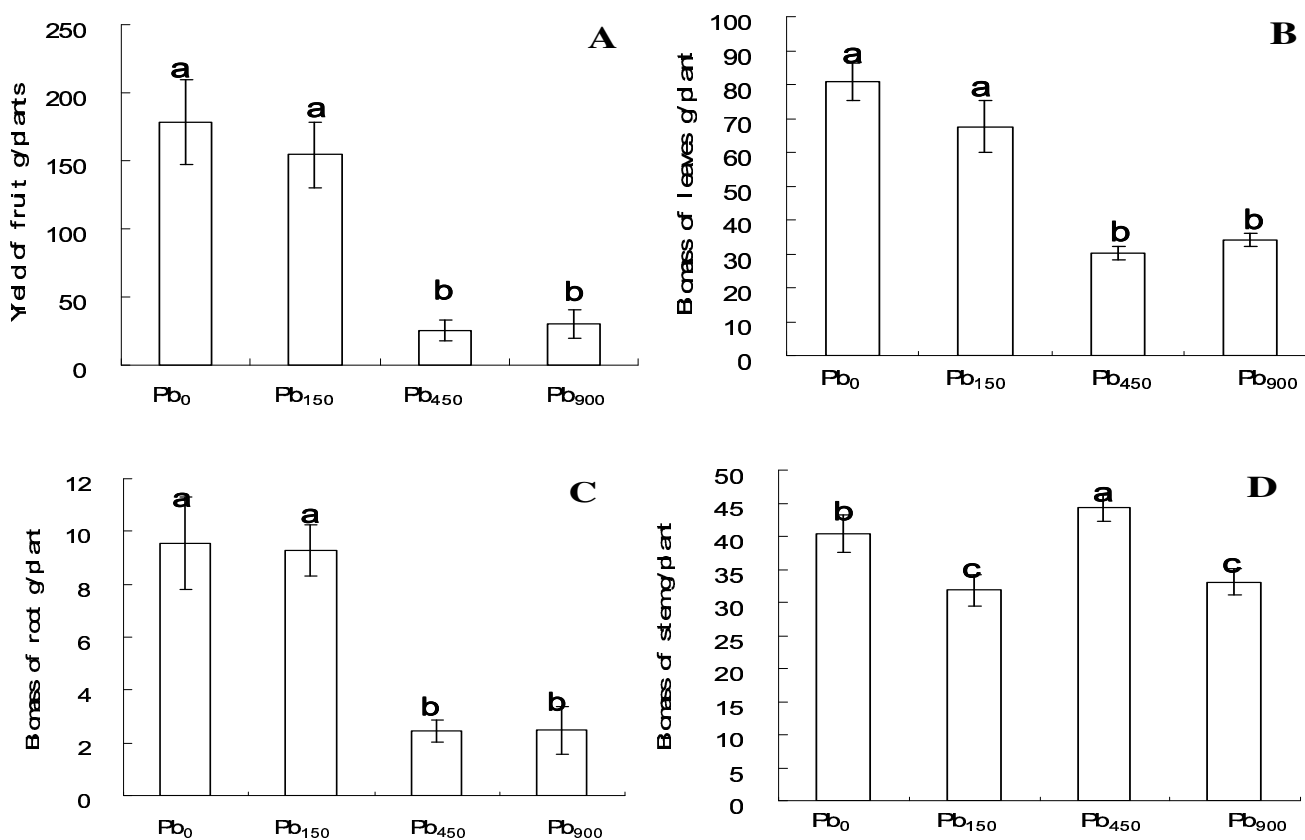


Figure 4. The effects of different amount of Pb treatments on fruit yields (A) and plant biomass (B, C and D) of tomato. The different letters on the bar charts indicate significant difference at 5% level of probability using Duncan's Multiple Range Test, the bars represent SE based on three replicates, Pb₀, Pb₁₅₀, Pb₄₅₀, and Pb₉₀₀ indicate the treatments of Pb addition as 0, 150, 450 and 900 mg/kg soil, respectively.

reported that when lead-organic - compounds were combined with *Chara fragillis*, one of the structural elements of the cells, the chloroplasts would sustain the most severe damage (Heumann, 1987). Studies about the effects of heavy metal copper (Cu^{2+}) and nickel (Ni^{2+}) on the ultrastructure of lichen (*Bryoria fuscescens*) found that thylakoid and mitochondrial cristae were swollen, then chloroplasts and mitochondria were greatly damaged (Tarhanen, 1998). When Scots pine seedlings were treated with Ni 25 mg/kg and Cu 50 mg/kg,

plasmolysis, sparse cytoplasm, dark staining of the central vacuole, markedly the light-colored, and swollen thylakoids were observed (Kukkola et al., 2000). The ultrastructure of root cortical cells of pigeonpea (*Cajanus cajan*) exposed to zinc and nickel showed alterations both at the cellular and the organelle level (Sresty et al., 1999).

In the study of Elzbieta and Mirosława (2005), remark-

able anomalies in chloroplasts of soybean mesophyll cell were observed under Pb stress. Similar pathological changes in ultrastructure level were reported on other plant species by Patel and Devi (1986) and Wozny et al. (1991). In present results, the similar distorted cellular ultrastructure in leaves or calyx treated with Pb (900 mg/kg) was observed. Excess lead in the soils and environment can cause a sharp decrease in crop productivity (Buchauer, 1973; Johnson and Eaton, 1980; Wallace et al., 1977). Application of municipal solid waste materials containing heavy metal resulted in decreased yield and increased Zn and Pb concentration in calcareous soils in tomato (Hampton et al., 1994). The study of Lagerwerff et al. (1998) showed that the yields of rye decreased, accompanied by the increasing rates of sludge containing Cd, Cu, Pb, and Zn inputment.

The results of the present study also confirmed that the yield and biomass of tomato decreased by increasing Pb dose, and the negative effect also behaved as the disruption of the growth or development of the roots and the aboveground parts of plants. At the same time, all these damage could disturb many plant activities including antioxidative system, photosynthesis, respiration, mineral nutrition, membrane structure and properties and gene expression (Bittell et al., 1974; Bazzaz et al., 1975; Burzynski, 1985; Jones and Harwood, 1993; Kastori et al., 1992; Jones et al., 1987; Maksymiec, 1997; Rama and Prasad, 1999; Smith et al., 1985; Tyler, 1990). Previous studies also confirmed that the damage to plant root system and the decrease in transpiration intensity caused by excess lead (Kastori et al., 1992) brought about a reduction in water uptake, and then insufficient supply of water to the above-ground plant parts. Lead causes disorder in the composition of both the lipid membrane and the protein fraction, facilitating its permeation into cells (Kastori et al., 1992).

The results of this study showed that the Pb concentration in the roots was the highest among all the organs of tomato, but accumulation amounts of Pb in the fruits was the greatest because the biomass of the fruits was the greatest, which suggested that accumulation amounts of Pb in the organs of tomato plants were highly dependent on the biomass.

CONCLUSION

Lead toxicity modified morphologic characteristics, growth, biomass, and yield of tomato. The typical symptoms included the curly acicular leaves that are narrow in areas and less in number, fewer conjoint incrassated calyx, smaller elongated fruits and dwarf plants. The excess lead (Pb 900 mg/kg) resulted in the abnormal ultrastructure in cells of leaves and calyx, which is represented by condensed cytoplasm, department of plasma from cell wall, swollen mitochondrial and chloroplast, loosely combined thylakoid to form askew

grana, disruption of plasma membrane, and dilation of organelles.

Under Pb stress, the roots and stems seem easier to absorb Pb than leaves or fruits, especially in root. Biomass take an important part in Pb accumulation, so application of Pb (450 and 900 mg/kg) significantly decreased the amounts of Pb accumulation in the plant because of the dramatically reduced biomass. Also, the accumulation amounts of Pb in plants decreased by fruits, leaves, stems and roots.

ACKNOWLEDGEMENT

The present investigation was financially supported by a grant from forward-looking project of Key Laboratory and Innovation, to improve the project of Zhejiang Academy of Agricultural Science.

REFERENCES

- Bazzaz FA, Carlson RW, Rolfe GL (1975). Inhibition of corn and sunflower photosynthesis to lead concentration. *J. Environ. Qual.*, 34:156-1588.
- Bittell JE, Koeppel DE, Miller RJ (1974). Sorption of heavy metal cations by corn mitochondria and electron and energy reactions. *Physiol. Plant.* 30:226-230.
- Body PE, Dolan PR, Mulcahy DE (1991). Environmental lead: a review. *Crit Rev Environ. Control.*, 20:299-310.
- Buchauer MJ (1973). Contamination of soil and vegetation near a zinc smelter by zinc, cadmium, and lead. *Environ. Sci., Technol.*, 17:121-123
- Burzynski M (1985). Influence of lead on the chlorophyll content and on initial steps on its synthesis in greening cucumber seedlings. *Acta Soc. Bio. Pol.*, 54:95-105.
- Chwill M (2000). Sensibility of three plant species on increasing lead chloride rates in medium. *Biu. Magnezol.*, 5:135-142.
- Elzbieta WC, Chwil M (2005). Lead-induced histological and ultrastructural changes in the leaves of soyben (*Glycine max* (L) Mee.) *Soil Sci. Plant Nutr.*, 51:203-212.
- Eun SO, Youn HS, Lee Y (2000). Lead disturbs microtubule organization in the root meristem of *Zea mays*. *Physiol. Plant.* 98: 611-620.
- Hampton MO, Schaffer B, Hanlon EA (1994). Nutrient concentrations, growth, and yield of tomato and squash in municipal solid-waste-amended soil. *HortScience.* 29: 726-835.
- Heumann HG (1987). Effects of heavy metals on growth and ultrastructure of *Chara fragillilis*. *Protoplasma.* 5: 187-191.
- Jackson DR, Watson AP (1977). Disruption of nutrient pools and transport of heavy metals in a forested watershed near a lead smelter. *J. Environ. Qual.*, 6:331-338.
- Johnson MS, Eaton J (1980). Environmental contamination through residual trace metal dispersal from a derelict lead-zinc mine. *J. Environ. Qual.*, 9:175-179.
- Jones AL, Harwood JL (1993). Lipid metabolism in the brown marine alage *Fucus vesiculosus* and *Ascophyllum nodosum*. *J. Exp. Bot.*, 44: 1203-1210.
- Jones CJ, Nichols PD, Johns RB, Smith JD (1987). The effect of mercury and cadmium on the fatty acid and sterol composition and cadmium on the fatty acid and sterol composition of the marine diatom *Asterionella glacialis*. *Phytochem.*, 26:1343-1348.
- Kastori R, Petrovic M, Petrovic N (1992). Effect of excess lead, cadmium, copper, and zinc on water relations in sunflower. *J. Plant Nutr.*, 15: 2427-2439.
- Kukkolaa E, Rautiob P, Huttunen S (2000). Stress indications in copper- and nickel-exposed Scots pine seedlings. *Environ. Exp. Bot.*, 43: 10124 Afr. J. Biotechnol.

197-210.

- Lagerwerff JV, Biersdorf GT, Milberg RP, Brower DL (1998). Effects of Incubation and Liming on Yield and Heavy Metal Uptake by Rye from Sewage-Sludged Soil. *J. Environ. Qual.*, 6: 427-431.
- Lanphear BP (1998). The paradox of lead poisoning prevention. *Sci.*, 281: 1617-1618.
- Levine MB, Stall AT, Barrett GW, Taylor DH (1989). Heavy metal concentration during ten years of sludge treatment to an old-field community. *J. Environ. Qual.*, 18: 411-418.
- Maksymiec W (1997). Effect of copper on celler processes in higher plants. *Photosynthetica*. 34: 321-342.
- Patel JD, Devi GS (1986). Variations in chloroplasts of lead mesophyll of *syzygium cumini* L. and *Tamarrindus indica* L. Growing under airpolution stress of a fertilizer complex. *Indian J. Ecol.*, 13:1-4.
- Rama DS, Prasad MNV (1999). Membrane lipid alterations in heavy metal exposed plants. Springer. Berlin
- Salt DE, Smith RD, Raskin I (1998). Phytoremediation. *Annu Rev Plant Physiol Plant Mol Biol.*, 49: 643-668.
- Shull LR (1998). Non-Technical Summary: Development of risk-based concentration for arsenic, cadmium, and lead in inorganic commercial fertilizers. In: The California Department of Food and Agriculture and the Heavy Metal Task Force, Newfields Inc., West acramento, CA
- Smith KL, Bryan GW, Harwood JL (1985). Changes in endogenous fatty acids and lipid synthesis associated with copper pollution in *Fucus* spp. *Environ. Exp. Bot.*, 63: 663-669.
- Soil Science Society of China (1999). Soil Science Society of China, Soil Physical and Chemical Analysis. Agricultural Science and Technology Press (in Chinese), Beijing, pp. 146-226.
- Sresty T, Rao VS, Madhava KV (1999). Ultrastructural alterations in response to zinc and nickel stress in the root cells of pigeonpea. *Environ. Exp. Bot.*, 41:3-13.
- Tarhanen (1998). Ultrastructural responses of the lichen bryoria fuscescens to simulated acid rain and heavy metal deposition. *Ann. Bot.*, 82:735-746.
- Tyler G (1990). Bryophytes and heavy metals: a literature review. *Botanical. J. Linn. Soc.*, 104: 231-253.
- Wallace EM, Romney GV, Alexander SM, Patel P, Soufi M (1977). Some Interactions in plants among cadmium, other heavy metals, and chelating agents. *Agron. J.*, 69:18-20.
- Wozny A, Jerczynska E, Liberska B, Schneider J (1991). Influence of lead and kinetin on greening and cut leaves of "Polluted environment and plant's pysiology." *Warsaw* (in polish), 10-11:May:109-116.