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The influence of lead on structure of *Cucumis sativus* L. leaves

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ABSTRACT

The cucumber seedlings were cultivated in the aquaculture at the Pb presence. Lead was applied as $PbCl_2$ at four following rates: 0, 10, 20 and 40 mg dm^{-3}. At every Pb level the comparison of the leaf blade morphology as well as density of stoma and trichomes distribution was made. The structure of the epidermis surface covering leaves was analysed in the scanning electron microscope. The plants examined at 40 mg $PbCl_2$ dm^{-3} presence developed thinner leaf blades of considerably limited area as against the control group. Lead induced the changes in stoma distribution and trichomes. Area of epidermis cells covering the cucumber leaves showed less abundant cutin in relation to the control.

INTRODUCTION

Transportation of lead taken by the plants from the substrate up the roots to the leaf blades proceeds by the conducting bundles. Then the metal penetrates from the tracheary elements through pits to the pith and further, through the cell walls to the other leaf tissues (Książek et al. 1984). A lead content uptaken from the soil solution diminishes in the root system and hypocotyls while approaching the cotyledons (Gzyl et al. 1997) that in case of *Cucumis sativus*, accumulated about 1% of this element (Burzyński 1988). The *Allium cepa* plants developing from the reserve organ cumulated 25% of the taken lead in the leaves. The highest Pb content was recorded in their apical part and the lowest in the basal (Michalak and Wierzbicka 1997). In the conditions of the environment polluted with heavy metals, some disturbances in leaf development were observed. Some parts of *Pinus silvestris* L. needles displayed an accelerated process of the surface cell decay (Kurczyńska et al. 1994). The lettuce leaf blades formed a loose head or remained at the leaf rosette phase (Baluk et al. 1988). Among the studied various plant taxons, cucumber was classified into the third group in respect of lead-sensitivity (Wierzbicka 1999). The objective of the present studies was to determine the impact of the lead uptaken by the cucumber root system on the leaf epidermis structure, stoma distribution density and trichomes as well as leaf blade morphology.

MATERIAL AND METHODS

The three-year investigations were conducted on the lead influence on *Cucumis sativus* L. 'Cezar F_1' leaf structure. The plants were grown in the water culture in the modified Knop medium of pH 5.5 with KH_2PO_4 exclusion to prevent lead phosphate precipitation. Owing to a short experimental period (16 days) potassium phosphate was not substituted with another compound. Lead was introduced as $PbCl_2$ at four concentrations: 0, 10, 20 and 40 mg dm^{-3} and 35 plants were analyzed in each object. The measurements of cotyledons (length, width and area) were performed after 7 days of seedling growth and of young leaves on the day of experiment closing. A number of trichomes and stomata was stated while stoma cell size determined in the abaxial leaf epidermis (n = 30). To measure the thickness of leaf blade, and the main nerve as well as to observe the epidermis surface (abaxial, adaxial) in the scanning electron microscope the sections from the central part of leaf blade were fixed. The samples were fixed in 4% glutaraldehyde and 0.1 M cacodylate buffer of pH 7.4 at 4˚C temperature for 12 hours. Afterwards they were dehydrated, placed in acetone and dried at the critical point in liquid CO_2 to be finally dusted with gold by CS 100 Sputter Coater. The observations were run in the scanning electron microscope (BS-340 Tesla). The variance analysis and Tukey confidence intervals were computed for the results of the morphometrical measurements.

RESULTS

The morphological symptoms of Pb^{+2} ion activity were manifested by the reduction of leaf blade size of cucumber. Its intensity depended on a leaf position on a plant and a toxicant concentration. Due to the Pb operation the cotyledon length got reduced to a higher degree than its width, whereas the area diminished significantly and made up 70-60% of this value at the control (Fig. 1). The cotyledons of seedlings grown at the Pb presence exhibited slightly downward curved edges particularly noticeable at the highest Pb rate (40 mg $PbCl_2$ dm^{-3}). These organs were characterized with dark green colour whose intensity grew with the succeeding toxicant levels as opposed to the leaf colour (Figs 2 and 3). The cotyledons dried off at the control the quickest, while the increasing Pb doses kept back their ageing process.

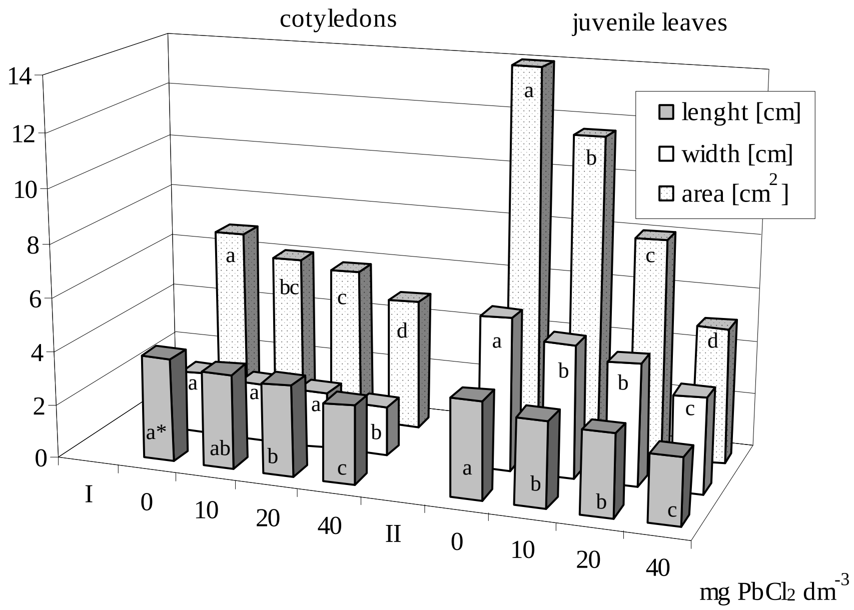
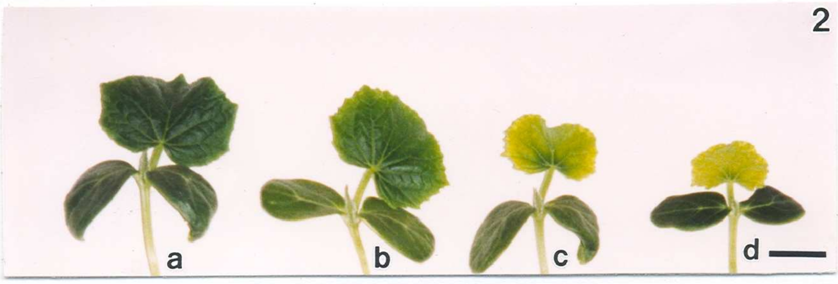
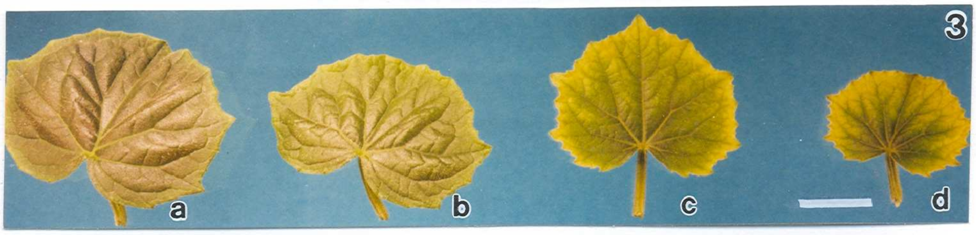


Figure 1. Cotyledon size in first (I) and juvenile leaves of cucumber in second (II) measurement date

* Means marked with the same letters do not differ significantly for p = 0.05





Figures 2, 3. Shoots of cucumber seedlings after 16-day incubation in different rates lead: a - 0; b – 10; c - 20; d - 40 mg PbCl2 dm-3; Bar = 2 mm (Fig. 2); Bar = 5 mm (Fig. 3).

The effect of lead on the leaf blade size depended on a leaf position on a plant. Cotyledons, the primordial leaves collecting nutrients for a developing seedling functioned for a short time, withered early and fell off, thus shortened the time of toxicant penetration into their tissues. Therefore, the effect of lead on morphometric parameters studied was less pronounced for cotyledons in comparison to juvenile leaves, which intensive development proceeded throughout the experimental period (sixteen days).

After the 16-day incubation in Pb the cucumber seedlings formed the leaves of far more limited area making up 16-83% of the studied feature in the control (Fig. 1). At the highest Pb rate (40 mg $PbCl_2$ dm^{-3}) the plants developed a leaf blade thinner by 14% as compared to the control plant leaves (Table 1).

Table 1. The thickness of leaf blade and main nerve tissue on transverse section of leaves of cucumber seedlings cultivated at various Pb rates

Investigated traits			$PbCl_2$ rates (mg dm^{-3})			
			0	10	20	40
Thickness of	leaf blade	(μm)	190.25 a*	192.20 a	182.39 a	162.93 b
		(%)	100	101	96	86
	main nerve	(μm)	593.18 a	578.35 a	572.89 a	588.89 a
		(%)	100	98	97	99

* Means in lines marked with the same letters do not differ significantly for p=0.05

In the peripheral parts of leaves, particularly at 20 and 40 mg $PbCl_2$ dm^{-3}, chloroses appeared. In numerous plants the leaf blade turned yellow at the edge, while green colour maintained exclusively at this organ base (Figs 2 and 3). Some seedlings showed necroses at the apical parts of their leaves.

In the leaf epidermis of the cucumber plants cultivated at the increased Pb there was noted a marked density of mechanical and glandular hairs per area unit. At the highest toxicant rate their number was four times larger than the control. The above mentioned relation was also recorded while recounting the number of trichomes into the whole leaf area. The frequency of these outgrowths occurrence following the lead treatment proved to be higher by 50-80% as against the control plant leaves (Table 2, Figs 4 and 5). At the Pb presence the trichome basal cells developed more clear cuticular lines on the wall surface as well as less abundant cutin in a form of rare granular structures (Figs 8 and 9).

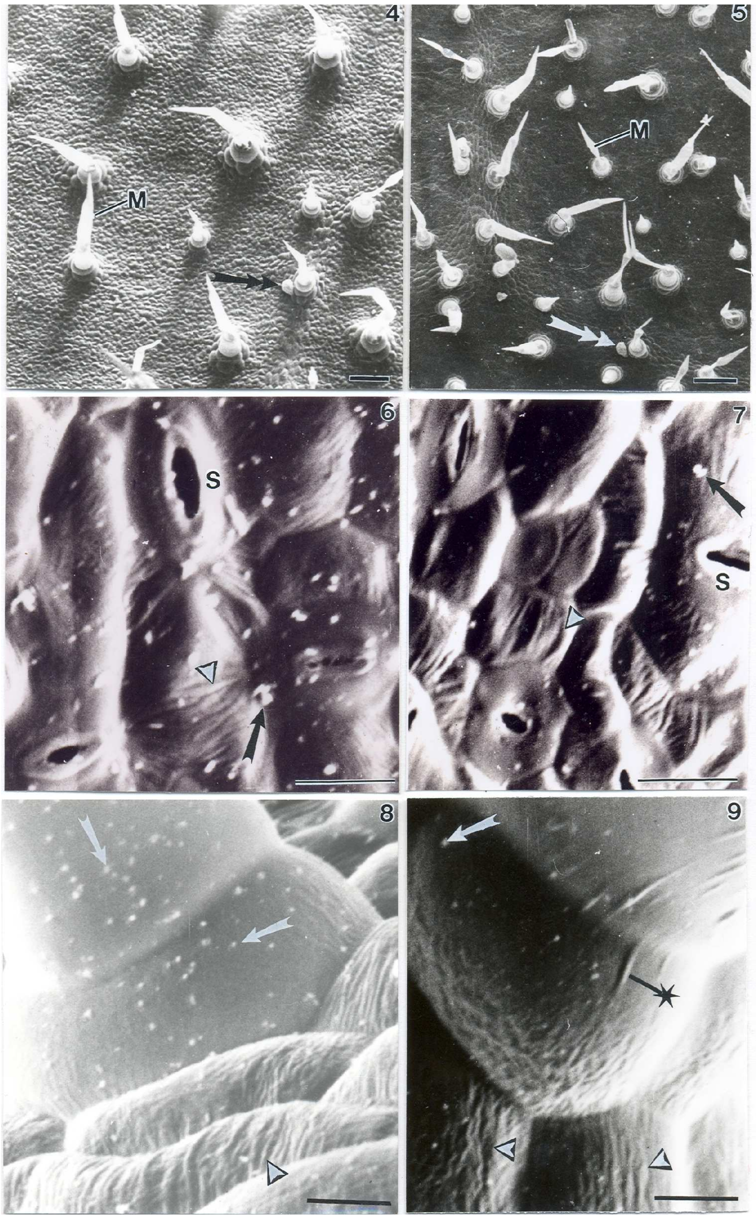
Table. 2. Number of trichomes and stomata and stoma cell size in abaxial epidermis of leaves of cucumber seedlings cultivated at various Pb rates

Investigated traits	$PbCl_2$ rates (mg dm^{-3})			
	0	10	20	40
Number of trichomes per (mm^2) of leaf area	15.88 a*	34.17 b	42.40b c	66.80 d
Number of stomata per (mm^2) of leaf area	1243.40 a	1297.60 a	1714.80 b	1979.56 c
Length of stomatal cells (μm)	19.41 a	17.56 b	16.19 c	13.81 d

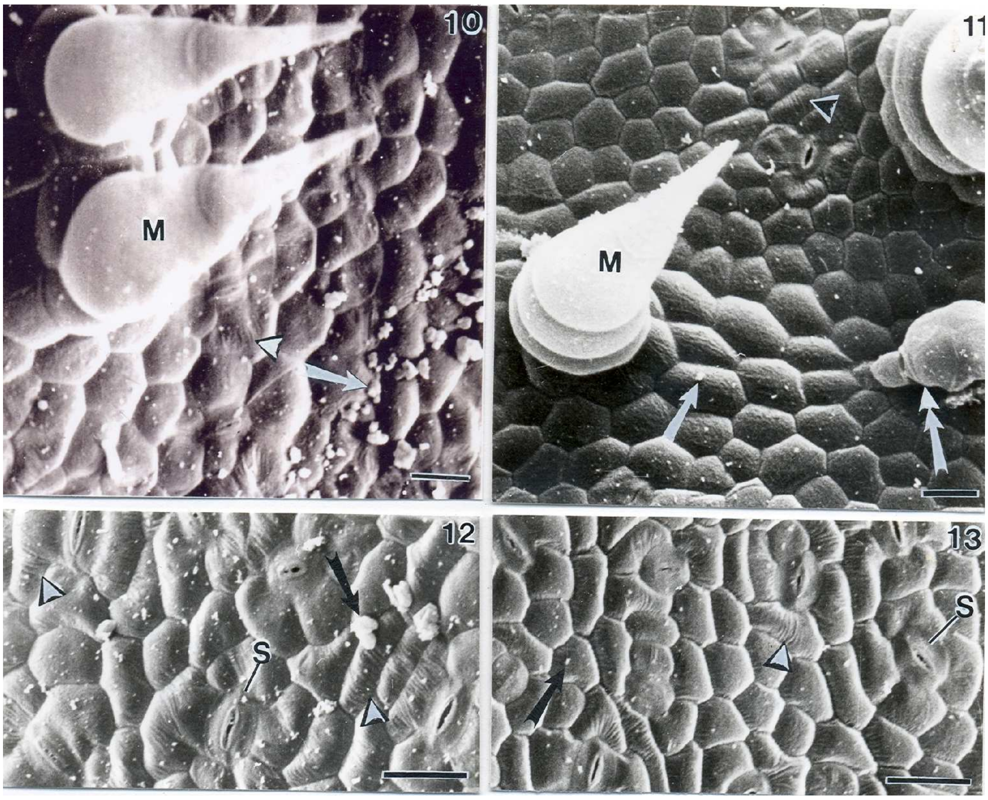
* Note – see Table 1

Alike, in the toxicant presence the surface of adaxial and abaxial epidermis cells exhibited less profuse cutin compared to the control level, where in some regions the wax formed agglomerations as granule complexes. The epidermis cells of cucumber leaves in both, control plants and treated with lead displayed the cuticular ornament taking shape of the lines arranged perpendicularly with their long axis (Figs 6, 7, 10-13).

Owing to the stoma occurrence in the abaxial and adaxial epidermis the cucumber leaves can be classified as an amphistomatic type. As the Pb concentration increases in the substrate the progressing growth of anomocytic stoma number per abaxial epidermic area unit is observed, its value went up by around 63% at the 40 mg $PbCl_2$ dm^{-3} rate compared to the control. Having recounted a number of stomata per leaf blade area of seedling grown at $PbCl_2$ dm^{-3} presence, it was established that their amount was eight times larger than in the control. However, at two higher doses 20 and 40 mg $PbCl_2$ dm^{-3} a number of stomata decreased and constituted 80 and 57% respectively, of the control plants number.



Figures 4-9. Sections of abaxial area of cucumber leaf epidermis; 4, 5, 8, 9-glandular (double arrow) and mechanical (M) hairs; 6, 7-stomata (S), visible cells with cuticular ornament shaped as lines (arrow-head), wax granules (arrow). 4, 6, 8 - control; 5, 7, 9 – after 16-day incubation in 40 mg $PbCl_2$ dm^{-3}, observeable smaller cells of epidermis with greater number of stomata as against the control Oblong reliefs of a cell wall trichome (star). Bar = 100 µm (Figs. 4, 5); Bar = 10 µm (Figs. 6, 7); Bar = 10 µm (Figs. 8, 9)



Figures 10-13. Sections of adaxial area of cucumber leaf epidermis; 10, 12 - control; visible wax as granules (arrow) and cuticular ornament shaped as lines (arrow-head) and mechanical (M) hairs. 11, 13 - after 16-day incubation in 40 mg $PbCl_2$ dm^{-3}; observeable smaller cells of epidermis with a greater number of stomata (S) with slightly flattened or concave area. Glandular hair (double arrow). Bar = 50 µm (Figs. 10, 11); Bar = 20 µm (Figs. 12, 13).

This fact was associated with distinct reduction of leaf blade area. The stomata in the abaxial epidermis of cucumber plants cultivated at the highest lead concentration were built of shorter by 29% stoma cells as against the control plants (Table 2). These cells were also of a changed shape and the surface often flattened or slightly concave (Figs 6 and 7). Having been treated with lead most leaves exhibited their stomata with a smaller aperture or closed, contrary to the control (Figs 6, 7, 12, 13).

DISCUSSION

The abnormalities in the leaves of the studied seedlings *Cucumis sativus* and other plant taxons emerging from lead treatment (Weryszko-Chmielewska and Chwil 2005, Kosobrukhov et al. 2004) are likely to be related with many metabolic and structural changes in various plant organs. The previous investigations showed that strongly reduced root system of *Cucumis sativus* of a tolerance index 24% in the Pb presence (Chwil 2000) had hindered the appropriate uptake of water and nutrients as well as their transportation to the upper tissues. In the deformed roots of broadbean lead induced some disorders in the cell divisions (Weryszko--Chmielewska 2001). Wierzbicka (1998) found that the enhanced polysaccharide synthesis in the cell walls of *Allium cepa* affected by Pb ions facilitate more intensive fixation of the toxicant. However, the Pb deposit in a wall brings about its thickening and flexibility diminishment. These changes may induce inhibition of leaf blade growth, as observed in the case of cucumber studied in this work.

It may be stated that the chlorotic leaf discoloring as observed by the author, were connected with a decreased photosynthesizing pigment content and low iron contents as found by Fodor et al. (1996) in the case of cucumber. The earlier investigations revealed that iron deficiency causes the damage of tylacoid membranes (Baszyński 1996). The disorganization and the varied directional tylacoid system as well as the plastid capsule cracking and poorly compacted acini were recorded in the *Glycine max* leaves at the Pb presence (Weryszko--Chmielewska and Chwil 2005).

A number of changes noted in the plastids of various plant leaves may account for the physiological ageing of the cells treated with lead. The occurrence of numerous plastoglobules in the chloroplasts of *Elodea canadensis* (Stoyanova and Tchakalova 1993), *Hordeum vulgare* (Woźny 1995) and *Glycine max* (Weryszko-Chmielewska and Chwil 2005) in the Pb presence may be considered a symptom of activated cell ageing. The plastoglobule presence proves the lipid release from tylacoid membranes and electron transportation disturbances (Baszyński 1996).

In the epidermis of the cucumber leaves and soybean as mentioned before (Weryszko-Chmielewska and Chwil 2005) more trichomes developed. These formations occurring in various plant species grown in the polluted environment cumulated the heavy metals (Salt et al. 1995, Tung and Temple 1996). In the *Nympaea* leaves the toxic elements were detected round the degenerated trichome head and within the glandular cells of a hair. At the heavy metal presence in this taxone trichomes there were also established polyphenoles (Lavid et al. 2001 a, b), whereas in the *Phaseolus vulgaris* trichomes-metallothioneins (Foley and Singh 1994). This fact implies that the accumulation of heavy metals and other compounds in the epidermis hairs may be related with the detoxification processes

(Foley and Singh 1994, Salt et al. 1995, Lavid et al. 2001b). Development of larger amount of trichome amount in the epidermis of plants in the contaminated environment, as observed in cucumber plants in the present work, may result from the plant defensive mechanisms towards the toxic agents.

Increased number of stomata with shorter cells formed in the leaf epidermis of cucumber cultivated at the Pb presence corresponds to the results of the investigations on *Helianthus annuus* (Kastori et al. 1992), *Plantago major* (Kosobrukhov et al. 2004) and *Glycine max* (Weryszko-Chmielewska and Chwil 2005). Some similar experiments showed that in spite of higher frequency of stomata occurrence, a level of *Plantago major* leaf transpiration was lower by 40% (Kosobrukhov et al. 2004). It was probably connected with the hindered water transportation at the toxicant presence due to xylem vessel number reduction and lowered degree of stomata opening (Chwil 2001, Kosobrukhov et al. 2004). This statement agrees with numerous observations on the epidermis surface of cucumber plant realized in the scanning electron microscope that confirmed a tendency of stoma closing at the Pb presence. According to Woźny (1991) after lead treatment the closed stomata in the *Hordeum vulgare* epidermis made 87%. A factor regulating their opening turned out to be the increased ABA supply in the heavy metal environment (Woźny 1995, Talanova 2000). Another reason for the stoma closing could be constituted by decreased turgor of stoma cells due to the disturbed transportation of potassium ions from the adjacent cells and the growing permeability of plasmalemma (Woźny 1995, Raduła et al. 2001).

As indicated in the present work, the diminishment of wax secretion on the leaf epidermis surface of cucumber plant induced by lead treatment deprives it of the biological protective barrier. The other studies indicate that wax on the leaf epidermis surface in the polluted environment fixes the heavy metals coming down with the dusts in the atmosphere and limits their foliar penetration into further tissues (Woźny 1995).

CONCLUSION

1. The leaves of cucumber seedlings cultivated at 40 mg $PbCl_2$ dm^{-3} showed thinner leaf blades.
2. In the lead presence at the surface of the leaf epidermis cells less abundant cutin was detected.
3. Having been treated with lead the epidermis covering cucumber leaves exhibited a greater number of trichomes and stomata per area unit.
4. The stoma cells in the Pb environment were shorter and flatter. There were observed stomata of limited aperture or completely closed.

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WPŁYW OŁOWIU NA STRUKTURĘ LIŚCI *CUCUMIS SATIVUS* L.

Streszczenie: Siewki ogórka uprawiano w kulturach wodnych w obecności Pb. Ołów zastosowano w postaci $PbCl_2$ w czterech dawkach: 0, 10, 20 i 40 mg dm^{-3}. W poszczególnych poziomach ołowiu porównano morfologię blaszek liściowych oraz gęstość rozmieszczenia aparatów szparkowych i włosków. W skaningowym mikroskopie elektronowym analizowano strukturę powierzchni epidermy okrywającej liście. Badane rośliny w obecności 40 mg $PbCl_2$ dm^{-3} wykształciły cieńsze blaszki liściowe o znacznie ograniczonej powierzchni w porównaniu z grupą kontrolną. Pod wpływem ołowiu stwierdzono zmiany w rozmieszczeniu aparatów szparkowych oraz włosków. Powierzchnia komórek skórki okrywającej liście ogórka charakteryzowała się występowaniem mniej obfitego nalotu woskowego w stosunku do obiektu kontrolnego.

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