Localization of Lead in Root tip
of *Dianthus carthusianorum*

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The distribution of lead in root tips of *Dianthus carthusianorum* was compared in populations from a zinc-lead waste heap in Bolesław near Olkusz and from a natural stand in the Botanical Garden in Lublin. The analyses were made at two developmental stages: seedlings (after 8 days of incubation in 5 mg/dm^3 Pb^{+2} from PbCl$_2$ in 1/8 Knop medium) and mature plants (after 23 days of incubation in 10 mg/dm^3 Pb^{+2} from PbCl$_2$ in 1/2 Knop medium). Histochemical methods (rhodizonate and dithizonate) of lead detection revealed significant accumulation of this metal on the root surface of the examined plants. The site of next-strongest lead accumulation in root tips of plants from both populations was in cell walls of the pericycle. The layer of meristematic pericycle cells seemed to be a strong barrier against penetration of lead to the deepest cells of the procambium. Histochemical methods and tissue sections revealed no differences in lead distribution between root tips from the waste heap and from the natural population, but differences were detected on the ultrastructural level. There were numerous lead deposits in the cytoplasm of cells from ground meristem in the natural population, and none in specimens from the waste heap, indicating that lead had a higher toxic effect on the natural population of *D. carthusianorum*.

Key words: *Dianthus carthusianorum*, lead, pericycle, roots, ultrastructural localization.

INTRODUCTION

Dianthus carthusianorum is a common plant throughout Poland and is also the dominant species on lead-zinc (calamine) waste heaps in Bolesław near Olkusz. These waste heaps are characterized by a water deficit, intensive insolation, and elevated levels of heavy metals in the soil (lead: 1650 mg/kg) (Dobrzańska, 1955; Godzik, 1991).

In previous studies on *D. carthusianorum*, significant morphological differences were shown between the population of plants growing on the waste heap and plants from areas not contaminated with heavy metals. The described differences point to the adaptation of waste-heap plants to xerothermic conditions. Moreover, plants from the calamine waste heap were characterized by higher tolerance to zinc and lead than plants from unpolluted stands (Wierz-bicka and Rostański, 2002; Załęcka and Wierzbicka, 2002).

Immobilization of metal in the cell wall and detoxification of its cytoplasmic pool by sequestration in vacuoles are important processes influencing plants' tolerance to lead; both processes protect the cell metabolism from its toxic effects (Siedlecka et al., 2001; Seregin and Ivanov, 2001). Up to 96% of the metal taken up by plants can be accumulated in these cellular compartments (Antosiewicz and Wierzbicka, 1999; Seregin and Ivanov, 2001). Lead ions enter plants mainly through roots, and most of them are retained in the tissues of this organ. The presence of lead may stimulate the synthesis of cell-wall polysaccharides in root tip meristems, which thicken, providing space for nontoxic accumulation of lead (Wierzbicka 1998). To a certain extent, the processes occurring in the roots protect the

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aerial parts of plants from the toxic effects of lead. Accumulation of lead in the cell wall of the endodermis having Casparian strips plays a major role in reducing transport of lead to the stele and vascular bundles (Wierzbicka, 1987a; Heumann, 2001).

This study compares the distribution of lead in the apical root meristem of *Dianthus carthusianorum* from natural and waste-heap populations. The results should provide information on the features of *D. carthusianorum* that allow this species to colonize the zinc-lead waste heap and to tolerate such high lead pollution.

MATERIALS AND METHODS

The experiment was conducted on *Dianthus carthusianorum* seedlings and mature plants with well-developed leaf rosettes and root systems. The plants were from two populations. The one termed the waste-heap population was cultivated from seeds collected from the waste heap in Bolesław near Olkusz. The one termed the natural population was grown from seeds obtained from the Botanical Garden in Lublin.

EXPERIMENTS ON SEEDLINGS

After germination in Petri dishes containing 1/8 Knop medium, 5-day-old seedlings of both populations (30 plants per treatment) were transferred to 25-liter plastic containers and preincubated in 1/8 Knop medium with trace elements (Michalak and Wierzbicka, 1998), for adaptation to hydroponic culture. After 24 h preincubation, control plants were transferred to 1/8 Knop medium without PO_4^{-3} ions, and the experimental plants to 1/8 Knop medium without PO_4^{-3}, to which 5 mg/dm^3 Pb^{+2} from $PbCl_2$ had been added. The incubation media were aerated constantly during the experiment. The plants were incubated in the presence of lead for 12 days in a growth chamber at 25°C under a 16 h photoperiod. During incubation, root length was measured to assess the level of tolerance of the plants to lead (Wilkins, 1957).

EXPERIMENTS ON MATURE PLANTS

Plants were cultivated to maturity as follows: after the seeds germinated in Petri dishes, seedlings of both populations (waste-heap and natural) were grown for three months in a greenhouse at 25°C in garden soil (Universal Kronenerde finished, fer-

tilized and moistened potting soil, pH 5-6), and then transferred to hydroponic culture in 61 containers, 5 plants per container, in 1/2 Knop medium for 2 weeks. The lead-treated plants were incubated in 1/2 Knop medium without PO_4^{-3} ions, to which lead in the form of Pb^{+2} from $PbCl_2$ had been added at a concentration of 10 mg/dm^3. Control plants were cultivated at the same time. The incubation media were continuously aerated and replaced every 3 days. Five plants per treatment were cultivated. These plants were weighed before and after incubation.

DETERMINATION OF LEAD

After the end of treatment with lead, the plant material was separated into roots and aerial parts, dried, and mineralized for 20 min at maximum 180°C in 69% HNO_3. After mineralization, 35% H_2O_2 was added to each sample in a 1:9 ratio. Ten seedling roots from each treatment (or the entire root system of the mature plant) were used, in 2 or 3 replicates. The lead content in samples was determined by atomic absorption spectrometry (AAS) using an M600303 v1,13 spectrometer at a detection limit of 0.1 mg/l lead.

LOCATION OF LEAD

Histochemical detection of lead in plant roots was performed using rhodizonic acid disodium salt (Glater and Hernandez, 1972) and dithizone (diphenylthiocarbazone) (Seregin and Ivanov, 1997). Both of these reagents form products with lead that range in color from red to blue-black. The seedling roots for these procedures were collected on the 8th day of incubation with lead at a concentration of 5 mg/dm^3 Pb^{+2} from $PbCl_2$, and mature plants roots after 23 days of incubation with lead at a concentration of 10 mg/dm^3 Pb^{+2} from $PbCl_2$.

Lead was located using rhodizonic acid disodium salt as follows: after rinsing in deionized water, the roots were transferred to a 0.2% aqueous solution of the reagent for 24 h in the dark (Wierzbicka, 1987a,b). Specimens were observed in a drop of the stain. To locate lead using dithizone, roots rinsed in deionized water were placed in a solution of the reagent (30 mg dithizone dissolved in 80 ml 80% acetone containing a few drops of acetic acid) for 1.5 h. The roots were viewed after being rinsed several times with deionized water. The slides were viewed with a Nikon light microscope in transmitted light and in a dark field.

Ultrastructural analysis used root tips from seedlings treated for 8 days with 5 mg/dm^3 Pb^{+2} from PbCl$_2$, and from mature plants treated for 23 days with 10 mg/dm^3 Pb^{+2} from PbCl$_2$. Ten root apical meristems 1.5 mm long were fixed per treatment. The samples were prepared by rinsing control and lead-treated root tips in deionized water, followed by fixation in 2% glutaraldehyde (pH 7.3) in 0.1 M cacodylate buffer for 2 h at room temperature. After four rinses in cacodylate buffer (4×10 min) the root tips were postfixed in 1% OsO$_4$ at 4˚C for 2 h (seedlings) or 15 h (mature plants). The material was again rinsed in cacodylate buffer (4×10 min) followed by deionized water (2×2 min), and dehydrated in a series of aqueous solutions of acetone (25%, 50%, 70%, 96%) for 15 min each, followed by three 15 min rinses in 100% acetone. After dehydration, the root tips were transferred to mixtures of propylene oxide and Epon-Spurr resins (3:1, 1:1, 1:3) for 2 h in each, then embedded in pure Epon-Spurr resins (Antosiewicz and Wierzbicka, 1999). Semithin central and longitudinal sections of the roots were made and stained with toluidine blue. The sections were viewed with a light microscope. Ultrathin sections contrasted with uranyl acetate and lead citrate (Reynolds, 1963) and uncontrasted sections were viewed with a BS-500 Tesla Brno (60 kV) transmission electron microscope.

RESULTS

HISTOCHEMICAL DETECTION OF LEAD IN PLANT TISSUES

The presence of lead in the tissues of the analyzed plants was confirmed by the appearance of a strong stain ranging in color from red-brown through brown to black. The intensity of staining pointed to significant accumulation of this metal in the roots of the studied plants, but no differences in accumulation between plants from the waste-heap and natural populations (data not shown). In both cases, lead was detected mainly on the root surface (data not shown). The second site of intense staining, indicating intense accumulation of lead, was in the cell walls of a cell layer in the central part of the root. A string of cells containing lead deposits commenced in the root meristematic zone and continued along its entire length (Fig. 1).

The cells containing the lead deposits previously detected by histochemical methods were located precisely in longitudinal semithin sections showing apical root meristem structure. The main site of lead accumulation in root tips was the walls of meristematic pericycle cells (Figs. 2–4).

Three tiers of initials are visible: (I) the lowest tier containing the root cap and protoderm initials; (II) the middle tier with the ground meristem initials; and (III) the highest tier generating the procambium (Fig. 4). The borders of the procambium on both sides are clearly distinguished by a pillar of cells undergoing anticlinal division (8th layer of cells counting from the root tip surface); other visible adjoining pillars (7th layer of cells) originate from tier II of initials, and are undergoing both anti- and periclinal divisions (Fig. 4). The mutual orientation of the cell pillars shows that the first of them, originating from tier III of initials, is formed by meristematic pericycle cells, whereas the neighboring pillar is formed by meristematic endodermis cells (Fig. 4) (Kuraś, 1978; Kuraś, 1980; Hejnowicz, 2002).

Lead deposits in meristematic pericycle cells were clearly visualized in the form of spherical opalescent structures (Figs. 3, 4) in longitudinal sections of apical meristems from both the natural and waste-heap populations. Such structures were not found in control roots. Lead was located in the anti- and periclinal walls of meristematic pericycle cells (Fig. 3). In this layer, lead deposits can be seen starting from the 9th cell counting from the root initials. The number of deposits increases toward the basal area of the meristem (Fig. 2). In that part of the meristem, lead deposits can be found not only in the meristematic pericycle cells but also in the adjoining meristematic endodermis (Fig. 2).

Lead deposits were found in meristematic pericycle cells in the roots of both seedling and mature plants subjected to lead treatment. This layer retained the largest amounts of lead in the root tips. Lead accumulation of this magnitude was not seen in the cell walls of any of the remaining root cell layers.

ULTRASTRUCTURAL DETECTION OF LEAD IN CELLS

Analysis of the meristematic cells of the root tips of mature *D. carthusianorum* plants treated with lead revealed the presence of electron-dense deposits. This type of deposit usually was not visible in control roots (Fig. 12), indicating that these were lead deposits. The lead deposits were found to be located mainly in vacuoles, the endoplasmic reticulum (ER) and cell walls (Tab. 1; Figs. 5, 9, 14–19, 21).

A characteristic trait of the root apical meristems of both populations was that the cell walls, in
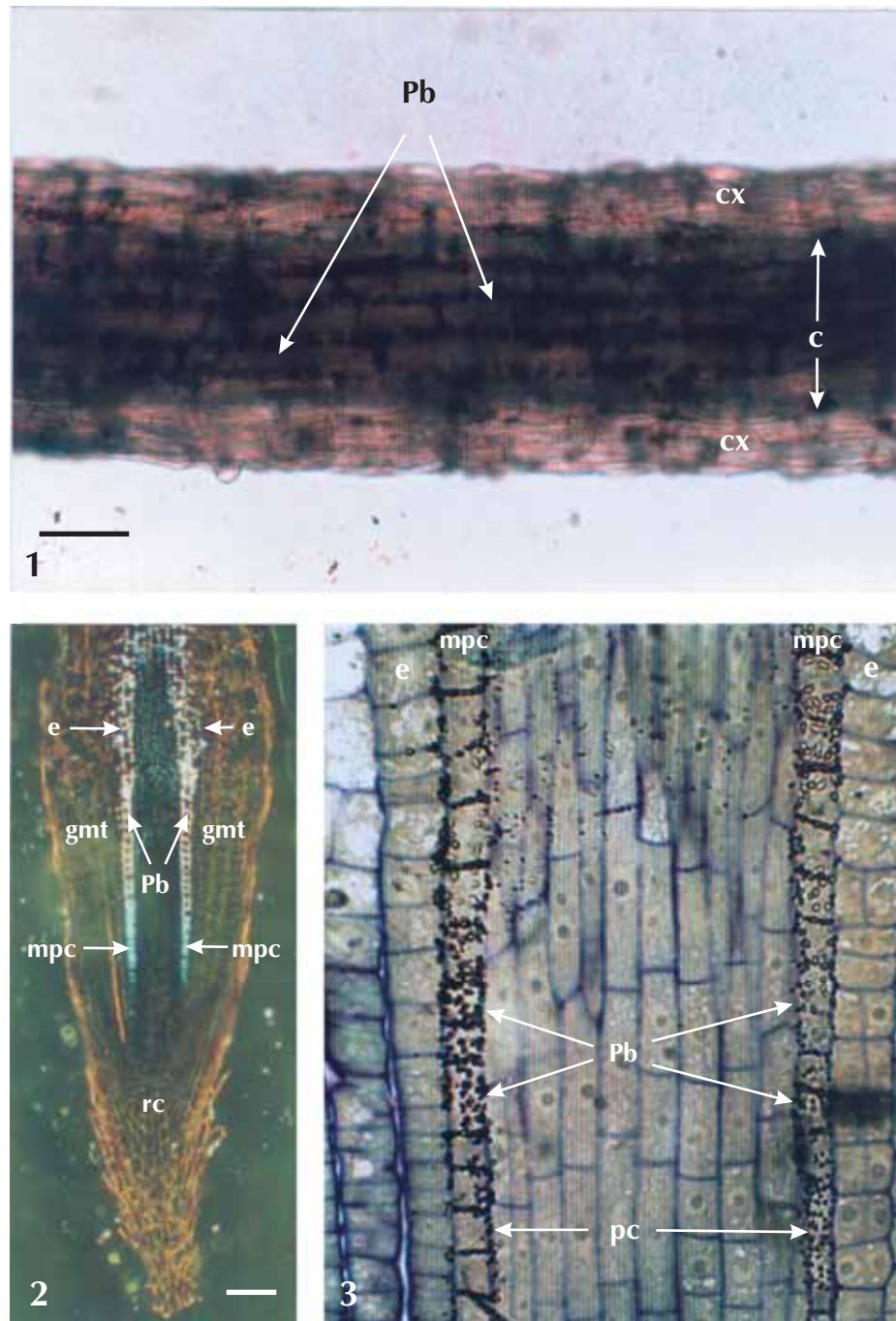
Figs. 1–3. Lead localization in root tissues of seedlings and mature plants of *Dianthus carthusianorum*. **Fig. 1**. Lead deposits (Pb, arrows) visible in cell walls in central cylinder (c) of elongation zone of root. *D. carthusianorum* seedling after 8 days of treatment with 5 mg/dm^3 Pb^{+2}. Waste-heap population. Lead detected with dithizone method. Bar = 0.1 mm. **Fig. 2.** Central longitudinal semithin section through *D. carthusianorum* mature plant root tip after 23 days of treatment with 10 mg/dm^3 Pb^{+2}. Lead accumulation (Pb) visible as bright points in cell walls of meristematic pericycle cells (pmc) and in meristematic endodermis cells (e). Natural population. Section stained with toluidine blue. Dark field. Bar = 0.1 mm. **Fig. 3.** Large lead deposits (Pb) in cell walls of meristematic pericycle cells (mpc) in meristematic zone of *D. carthusianorum* mature plant root after 23 days of treatment with 10 mg/dm3 Pb^{+2}. Natural population. Central longitudinal semithin section stained with toluidine blue. Bar = 0.01 mm. gmt – ground meristem tissue; cx – cortex, c – central cylinder; rc – root cap; e – cells of meristematic endodermis; mpc – meristematic pericycle cells; pc – procambium.
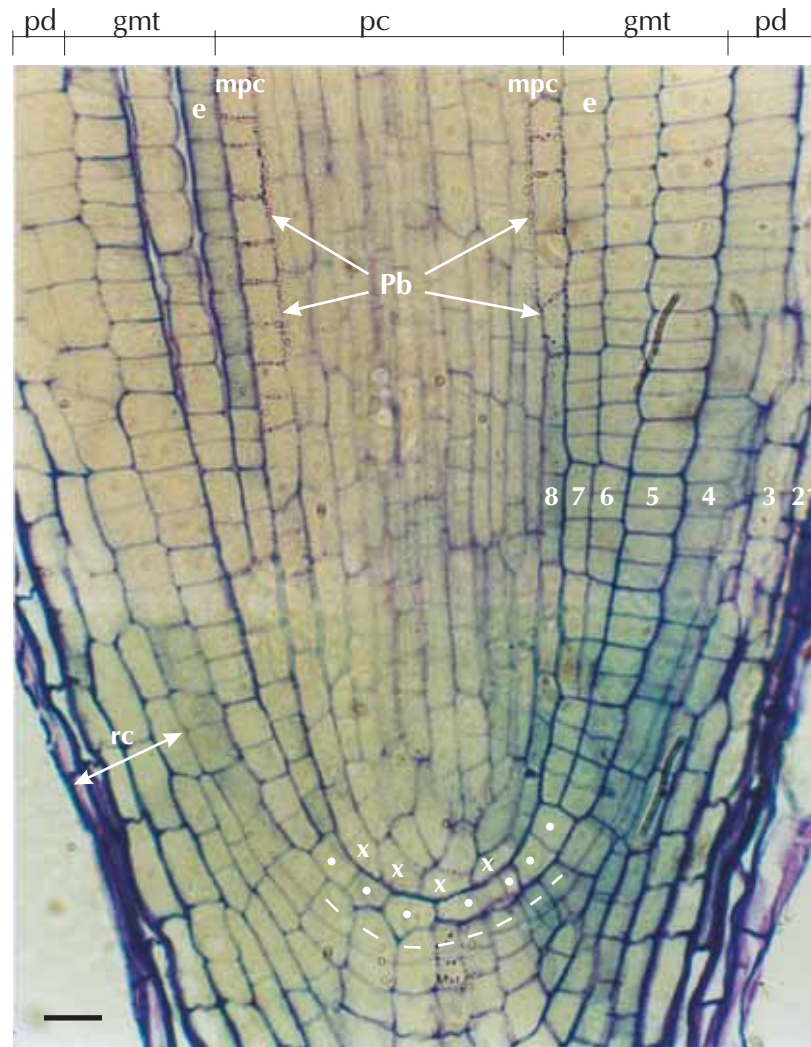
Fig. 4. Structure of meristem of *D. carthusianorum* root tip. Central longitudinal semithin section through *D. carthusianorum* mature plant root tip stained with toluidune blue. Bar = 0.02 mm. (⎯) – tier I containing initials of root cap and initials of protodermis; (•••) – tier II containing ground meristematic tissue initials; (xxx) – tier III of procambium initials; pd – protodermis; gmt – ground meristem tissue; e – cells of meristematic endodermis; mpc – cells of meristematic pericycle; pc – procambium; rc – root cap; Pb – lead deposits.

which the largest deposits of lead are usually found in other species (Woźny et al., 1995a), were relatively free of deposits of this metal, except for the cell walls of the root cap (Fig. 18).

The largest and most numerous lead deposits, however, were found in the walls of meristematic pericycle cells (Fig. 5). They can be seen in characteristic swellings of the cell wall (Figs. 8, 9). Large amounts of lead were also visible in the vacuoles of meristematic pericycle cells. (Fig. 9). Lead deposits were also detected in the vacuoles and cell walls of the procambium (Tab. 1), though less numerous

than in the meristematic region of the cortex. In seedlings, as in mature roots, very large deposits of lead were found in swellings of the walls of meristematic pericycle cells. The deposits seen here were larger than the corresponding structures in mature roots (Figs. 10, 11).

Aside from the walls of meristematic pericycle cells, large lead deposits were seen in vacuoles in both studied populations, especially in the ground meristem (Figs. 13–15). Increased vacuolization of cells in the cortex meristem was found in the roots of plants from the natural population. It was more

TABLE 1. Presence of electron-dense deposits in meristematic cells of mature *D. carthusianorum* plants treated for 23 days with 10 mg/ dm^{+3} Pb^{+2} from PbCl$_2$. Data based on analysis of 20 micrographs per experiment

Root region	Cell walls		Vacuoles		Cytoplasm		ER		Nucleus		Golgi apparatus	
	N	W	N	W	N	W	N	W	N	W	N	W
Root cap	+	+	+	+	+	+	+	+			+	
Ground meristem tissue	+	+	+	+	+		+	+			+	
Procambium: cells of meristematic pericycle	+	+	+	+	+				+		+	+
Procambium: other cells	+	+	+	+								

N – natural population; W – waste-heap population; + – presence of lead deposits; ++ – largest lead deposits in a given cell layer

TABLE 2. Lead content (mg kg^{-1} dw average ± SD) in roots of *D. carthusianorum* plants from waste-heap and natural populations after treatment with lead. Seedlings – 8 days of incubation in 5 mg/dm^3 Pb^{+2} from PbCl$_2$ in 1/8 Knop's medium; mature plants – 23 days of incubation in 10 mg/dm^3 Pb^{+2} from PbCl$_2$ in 1/2 Knop's medium

Stage of development	Population	Lead content in root tissues mg kg^{-1} dw
Seedlings	Waste-heap population	8481 ± 1691
	Natural population	11285 ± 635
Mature plants	Waste-heap population	51693 ± 13011
	Natural population	55963 ± 2904

pronounced than in the control and waste-heap populations. Some of the observed vacuoles in the root cells of the natural population were autophagic (Fig. 16).

There were also differences between populations in the localization of lead in the protoplast. In both populations, lead was found in the cytoplasm of the root cap (Tab. 1; Figs. 19, 20). Lead deposits in the cytoplasm were visible in the ground meristem cells of the natural population, and there the deposits were most numerous (Fig. 13). The meristematic pericycle cells also contained lead deposits (Fig. 7). In these cells, lead was also found in the nuclear envelope (Fig. 7). In the root cells of the waste-heap population, no lead deposits were found in the cytoplasm of the ground meristem cells or meristematic pericycle cells (Tab. 1).

LEAD CONCENTRATION IN ROOTS

Lead accumulation in the apical meristem of plants treated with this metal was confirmed by AAS. Exceptionally large amounts of lead were accumulated in the root tissues of both seedlings and mature plants, about five times more in the tissues of mature plants than in seedlings (Tab. 2). This result reflected the higher dose and longer treatment of mature plants (seedlings were incubated with 5 mg/dm^3 Pb^{+2} for 12 days, whereas mature plants were incubated with 10 mg/dm^3 Pb^{+2} for 23 days).

The root tolerance index of the plants treated with lead declined the most between days 2 and 5 of the experiment. Later the root tolerance index of *D. carthusianorum* seedlings from both the waste-heap and natural populations ceased to decline and remained at 30%.

The biomass of mature plants treated with lead reached 39% of the control in plants from the waste-

Figs. 5–11. Lead distribution in meristematic pericycle cells of *D. carthusianorum* mature plants and seedling root tips. Mature plants were treated for 23 days with 10 mg/dm^3 Pb^{+2}; seedlings were treated for 8 days with 5 mg/dm3 Pb^{+2} from PbCl$_2$. **Fig. 5.** Layer of meristematic pericycle cells (mpc) with large deposits of lead (Pb) in cell walls. Natural population. Section not stained. Bar = 1 μm. **Fig. 6.** Deposits of lead (Pb) in Golgi apparatus. Natural population. Section stained with uranyl acetate and lead citrate according to Reynolds (1963). Bar = 0.5 μm. **Fig. 7**. Deposits of lead (Pb) in cytoplasm, Golgi apparatus and nucleus membrane. Natural population. Section stained with uranyl acetate and lead citrate according to Reynolds (1963). Bar = 0.5 μm. **Fig. 8.** Large deposits of lead (Pb) in cell walls. Waste heap population. Section stained with uranyl acetate and lead citrate according to Reynolds 1963). Bar = 0.5 μm. **Fig. 9.** Large deposits of lead (Pb) in cell walls and smaller deposits in vacuole. Natural population. Section stained with uranyl acetate and lead citrate according to Reynolds (1963). Bar = 1 μm. **Fig. 10.** Order of lead deposition (Pb) in particular parts of meristematic pericycle cell in seedling root tip from waste heap population. Section not stained. Bar = 1 μm. **Fig. 11.** Structure of electron-dense lead deposits in cell wall swellings of meristematic pericycle cell in seedling root tip from waste heap population. Section not stained. Bar = 0.5 μm. mpc – meristematic pericycle cell; CW – cell wall; N – nucleus; Nm – nuclear membrane; ER – endoplasmic reticulum; GA – Golgi apparatus; M – mitochondrion; C – cytoplasm; V – vacuole; Ml – middle lamella; in circles – lead deposits in swellings of cell wall. Figs. 5–9 mature plants, Figs. 10–11 seedlings.
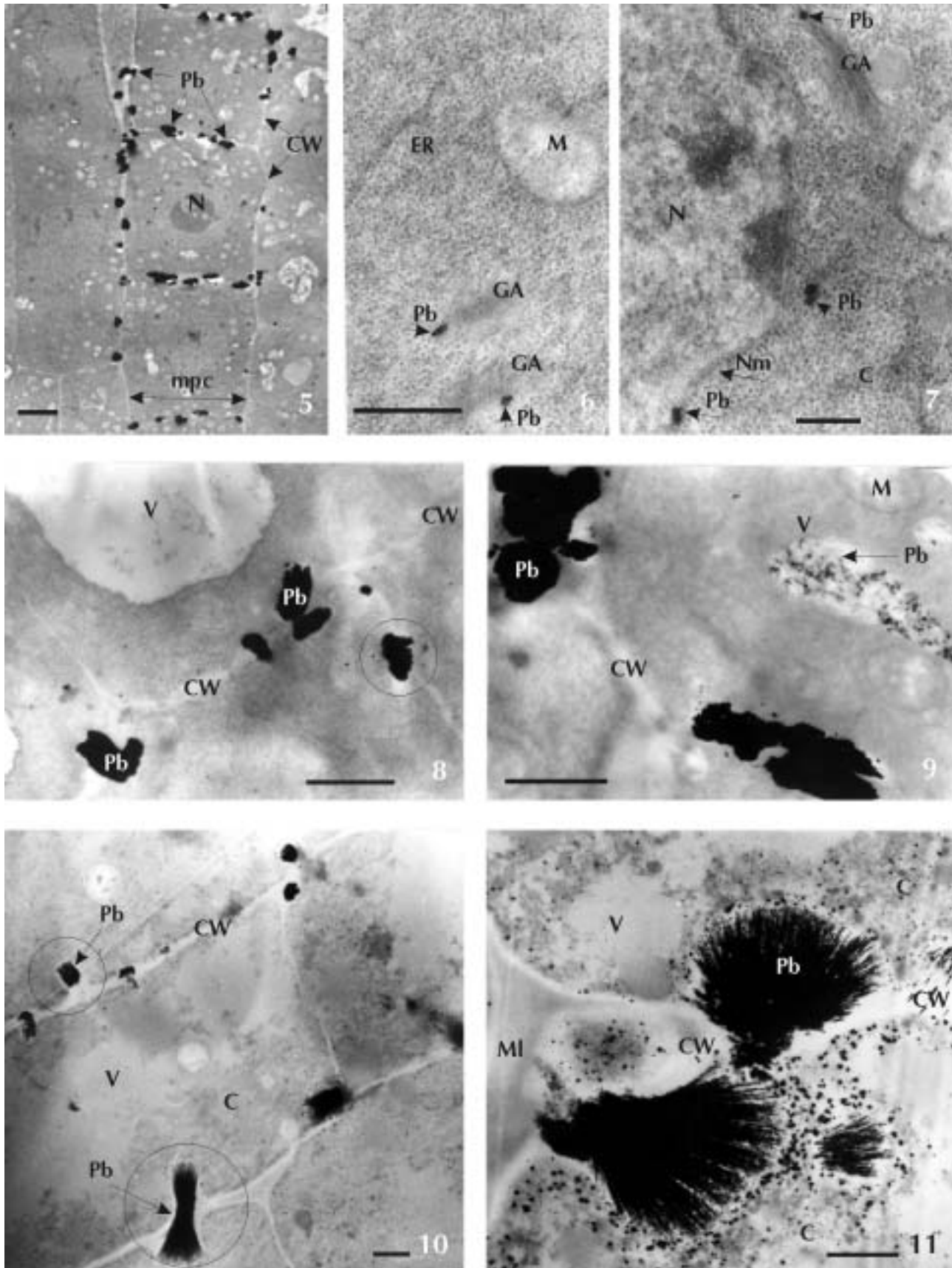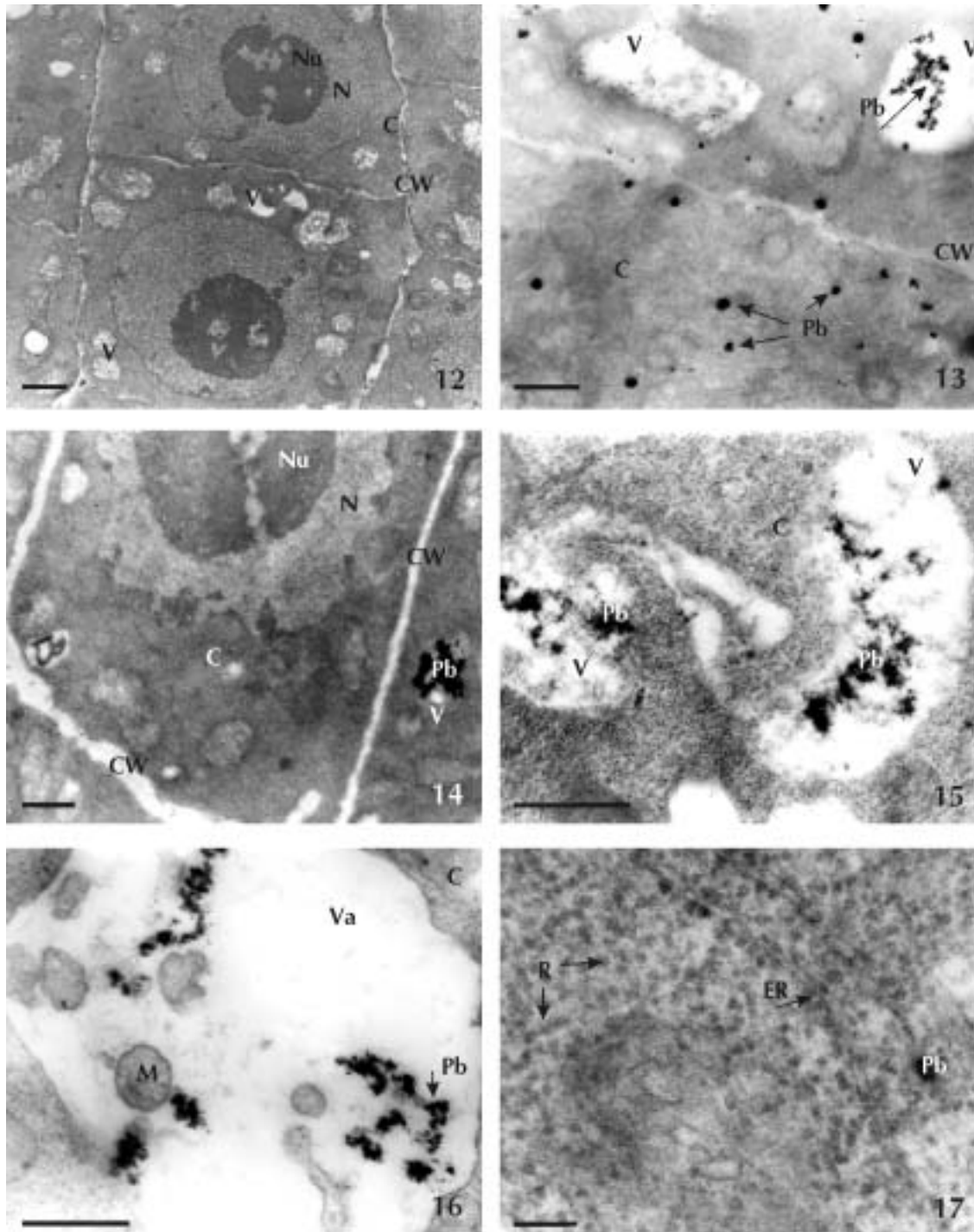
Fig. 12. Ground meristem tissue from control root tip without electron-dense deposits of lead. Natural population. Section not stained. Bar = 2 μm. **Figs. 13–17.** Ground meristem tissue. Lead distribution in cells of *D. carthusianorum* mature plants root tips. Plants were treated for 23 days with 10 mg/dm³ Pb⁺². **Fig. 13.** Numerous small lead deposits (Pb) in cytoplasm of cells. Natural population. Section not stained. Bar = 1 μm. **Fig. 14.** Lead deposits (Pb) in vacuole. Waste heap population. Section not stained. Bar = 1 μm. **Fig. 15.** Vacuoles with deposits of lead (Pb) in cell. Natural population. Section stained with uranyl acetate and lead citrate according to Reynolds (1963). Bar = 1 μm. **Fig. 16.** Lead deposits in large autophagic vacuole digesting part of cell protoplast with mitochondria. Natural population. Section stained with uranyl acetate and lead citrate according to Reynolds (1963). Bar = 1 μm. **Fig. 17.** Lead deposits in endoplasmic reticulum. Waste heap population. Section stained with uranyl acetate and lead citrate according to Reynolds (1963). Bar = 0.1 μm. N – nucleus; Nu – nucleolus; C – cytoplasm; CW – cell wall; V – vacuole; Va – autophagic vacuole; T – tonoplast; ER – endoplasmic reticulum; Pb – lead deposits.
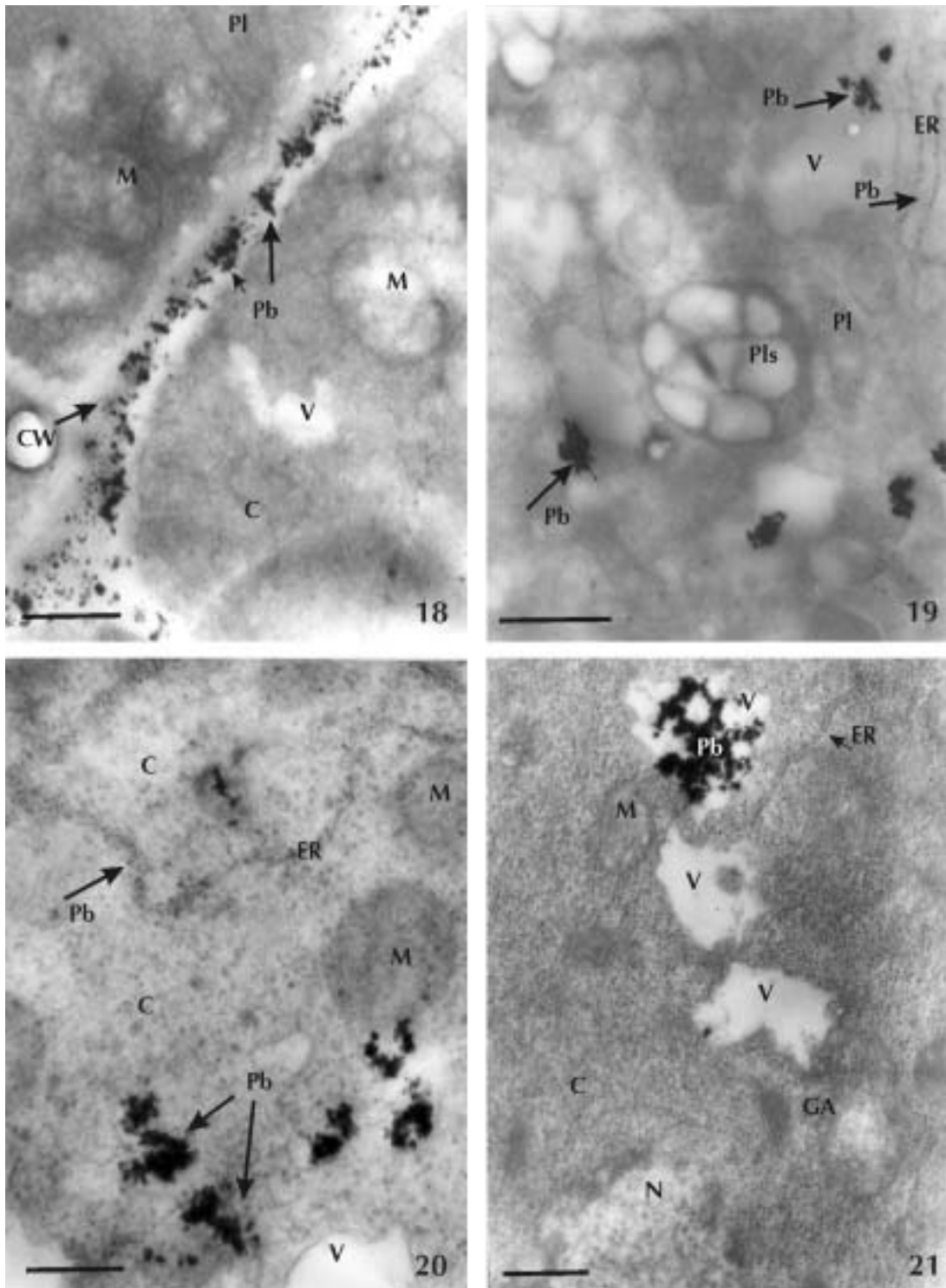
Figs. 18–21. Root cap. Lead distribution in root tip cells of *D. carthusianorum* mature plants. Plants were treated for 23 days with 10 mg/dm³ Pb⁺². **Fig. 18.** Lead accumulation (Pb) in cell wall. Natural population. Section not stained. Bar = 1 μm. **Fig. 19.** Deposits of lead (Pb) in cytoplasm and in endoplasmic reticulum. Waste heap population. Section not stained. Bar = 1 μm. **Fig. 20.** Deposits of lead (Pb) in cytoplasm and in endoplasmic reticulum. Natural population. Section stained with uranyl acetate and lead citrate according to Reynolds (1963). Bar = 0.5 μm. **Fig. 21.** Vacuole with large deposit of lead (Pb). Waste heap population. Section stained with uranyl acetate and lead citrate according to Reynolds (1963). Bar = 0.5 μm. C – cytoplasm; CW – cell wall; M – mitochondrion; Pl – proplastid; Pls – proplastid with statolith starch; V – vacuole; ER – endoplasmic reticulum; GA – Golgi apparatus; N – nucleus.

heap population and 37% of the control in plants from the natural population. These data indicate that the applied lead doses exerted a strong inhibitory effect on the growth of the experimental plants. Despite the large amounts of lead accumulated in root tissues, plants from both populations continued to grow, and the waste-heap plants bloomed continuously during incubation with lead.

DISCUSSION

In the course of evolution, plants have developed a variety of protective mechanisms against heavy metals, allowing them to develop and grow on stands strongly polluted by them (Wenzel and Jockwer, 1999; Clemens, 2001; Siedlecka et al., 2001; Wierzbicka, 2002; Baranowska-Morek, 2003; Szarek-Łukaszewska et al., 2003). Plants differ in their levels of tolerance to metals even within a population belonging to one species. This is true of *Dianthus carthusianorum* (Załęcka and Wierzbicka, 2002), on which this study was conducted, as well as other plants (Wierzbicka, 2002). An important role in tolerance to heavy metals, especially to lead, is played by cellular processes leading to the sequestration of toxic ions and consequently protecting key metabolic sites (Wierzbicka, 1995; Woźny et al., 1995b).

Analysis of the distribution of lead deposits in the meristematic cells of *D. carthusianorum* showed the presence of lead in structures where it has been found in other plants: cell walls, vacuoles, ER and the Golgi apparatus (Woźny et al., 1995a; Samardakiewicz and Woźny, 2000; Glińska and Gabara, 2002). These are cellular compartments in which lead is accumulated in a way that is not toxic to root metabolism. Lead is detected much less frequently in the nucleus and nucleolus (Woźny et al., 1995a). The presence of lead deposits in the protoplast at sites other than those where lead is detoxified causes toxic effects in the root (Wierzbicka, 1995; Glińska and Gabara, 2002). In the analyzed root tips of mature *D. carthusianorum* plants, numerous lead deposits were found in the cytoplasm of ground meristem cells only in plants from the natural population. The ground meristem cells of roots from this population were also characterized by higher vacuolization than in the waste-heap population. Some of the observed vacuoles were autophagic. This type of vacuolization of the protoplast is similar to that described in *Allium cepa* roots as the second degree of cell damage by lead (Wierzbicka 1984). That study

found a distinct correlation between the amount of lead observed in the protoplast and the symptoms of destruction in the ultrastructure of cells and their degree of vacuolization – the more lead in the protoplast, the stronger its toxic effect. In the roots of the natural population of *D. carthusianorum*, lead deposits were present in the protoplast outside of the compartments in which it undergoes nontoxic accumulation. This may indicate that lead exerted a stronger toxic effect on the cells of this population than on those of the waste-heap population. The difference was observed on the ultrastructural level. Inhibition of elongation growth in seedlings and inhibition of biomass increase in mature plants were similar in the two populations at the applied dose of lead. The exceptionally large amount of lead in the roots of plants indicates that the applied dose was probably too high to permit detection of differences in tolerance between the populations at the level of growth measures. The difference was apparent in ultrastructural studies, however, which are much more exact and detailed.

One of the main sites of nontoxic heavy metal sequestration is the cell wall. This applies particularly to lead, since it has the highest affinity for the polygalacturonic acids that are part of the cell wall (Wierzbcka, 1995; Seregin and Ivanov, 2001). Large deposits of lead in this compartment have been described by numerous authors (Wierzbicka, 1987a,b; Samardakiewicz and Woźny, 2000; Gabara and Glińska, 2002; Szarek-Łukaszewska et al., 2004). Accumulation of Pb in cell walls may be a passive process related to apoplastic transport of it, and to its retention by numerous cell wall components. Lead may also be accumulated as the result of being removed from the protoplast through plasmotubules (Wierzbicka, 1998). Transport of lead has also been observed in the dictyosomal vesicle, together with material for the building of cell walls (Malone et al., 1974).

In *Dianthus carthusianorum* a particular role in nontoxic accumulation of lead seems to be played by the walls of meristematic pericycle cells, since the greatest accumulation of lead was found here in the roots of seedlings and mature plants of both investigated populations. The observed pattern of lead distribution in this area indicates that accumulation of lead in this region did not occur as the result of passive apoplastic transport but was related to changes in metabolic processes occurring in the cells. These processes led to changes in the shape of the cell wall and delineation of special areas for lead sequestering. Wierzbicka (1998) observed that the

area of the cell wall in *A. cepa* roots treated with lead increased through stimulation of structural polysaccharide synthesis. The mechanism of formation of lead deposits in the walls of meristematic pericycle cells in *D. carthusianorum* roots requires further study, but this study shows for the first time that meristematic pericycle cells fulfill a protective role in the root tip, as the endodermis does in higher parts.

In angio- and gymnosperms the pericycle always differentiates as the external layer of vascular tissue, whereas the endodermis in all vascular plants has a common origin with the cortex. In seed plants the pericycle remains meristematic for a rather long time and generates lateral roots. In plants with secondary growth it generates part of the vascular cambium and usually phellogen (Hejnowicz, 2002).

The literature data shows that the pericycle cells and the part of the root enveloped by them are protected from lead penetration. In the root meristem of *Allium cepa* L., lead was located mainly in the external parts (protodermis, ground meristem); its central part, the procambium and the quiescent center contained no lead deposits or only small amounts. The cells of the deepest meristematic layers seemed to act as a barrier to lead reaching the procambium (Wierzbicka, 1987a). In maize seedlings the pericycle was shown to be protected against penetration by lead, and consequently the generation of lateral roots was not inhibited in the studied plants (Seregin and Ivanov, 1997b; Obrouchewva et al., 1998)

Many authors have reported that the endodermis with Casparian strips, which block apoplastic transport of water and ions, constitutes a significant barrier to radial transport of lead in the mature part of the root (Wierzbicka, 1987a; Książek and Woźny, 1990; Punz and Sieghardt, 1993; Seregin and Ivanov, 1997b; Wierzbicka and Potocka, 2002). In many plants the hypodermis exhibits properties similar to those of the endodermis (Samardakiewicz and Woźny, 1995). The role of meristematic pericycle cells in blocking transport of lead to the central parts of the root has not been described before. It seems that the walls of meristematic pericycle cells in *D. carthusianorum* have specific properties enabling them to retain the enormous amount of lead taken up by the root, so that it does not penetrate deeper into the procambium. This characteristic of the species and the accumulation of very large amounts of lead on the surface of the roots seem to play a significant role in the tolerance of *Dianthus carthu-*

sianorum to lead. Other specific traits of plant species colonizing a waste heap with heavy metals have been described by Pielichowska and Wierzbicka (2004), Szarek-Łukaszewska et al. (2004), Wierzbicka and Pielichowska (2004) and Wierzbicka et al. (2004).

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