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RESEARCH PAPERS

Maize Plant Growth and Accumulation of Photosynthetic Pigments at Short- and Long-Term Exposure to Cadmium

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Abstract—A wide range of cadmium concentrations (from 4 to 200 μM for seedlings and up to 2 mM for germinating kernels) was used to assess Cd toxic effects on maize (*Zea mays* L.) plants at the different developmental stages: germinating kernels, seedlings (4–9 days), and juvenile plants (34 days). Cd accumulation in plant organs was followed, and its lethal concentration was elucidated. In maize, cadmium was accumulated predominantly in roots; in shoots it was mainly accumulated in the lower leaves, and the higher was leaf position the lower was Cd content in it. At high concentrations (80 and 200 μM), kernels became the substantial cadmium depot. Germinating kernels manifested the lowest sensitivity to cadmium; seedlings were more sensitive; the inhibition of juvenile plant growth attained 90% and more. In the tested range of concentrations, cadmium suppressed shoot mass accumulation harder than that of roots. In 34-day-old plants, water content in shoots was stronger reduced than in roots. Plant death was also manifested earlier in shoots. It was concluded that maize plant sensitivity to cadmium increases with plant growing and that, under conditions of normal mineral nutrition, cadmium inhibits shoot growth more severe than root growth.

Keywords: Zea mays, cadmium, accumulation, distribution between organs, stress

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INTRODUCTION

Cadmium is one of the most toxic heavy metals (HM). As a result of human industrial activity, rather great areas were contaminated with cadmium [1]. Cadmium inhibits activities of some enzymes, disturbs processes of respiration and photosynthesis, cell division; it suppresses plant growth and development [2–4]. The main strategy of plant adaptation to HM, cadmium in particular, is the restriction of their translocation to metabolically active cell compartments. Plants bind cadmium ions by the cell wall polysaccharides, whereas cadmium ions transported across the plasmalemma are directed to the vacuole [2, 4]. Phytochelatins, metallothioneins, amino acids, and organic acids participate in detoxification of intracellular cadmium [2, 4]; some plants excrete cadmium into trichomes [5, 6].

Plants are subdivided into three groups in accordance with their relation to HM: excluders, accumulators, and indicators [7, 8]. Excluders retard HM translocation to the shoot and accumulate them predominantly in the roots. Accumulators, in contrast, accumulate HM mainly in the shoots. In indicators, the content of HM in the roots and shoots is similar and reflects metal content in soil. In addition, plants demonstrate a variety of other defense mechanisms and the ways of their application. Therefore, they differ markedly in HM accumulation (cadmium in particular), tolerance to them, and manifestation of their toxic effects; considerable differences may be found even between cultivars of a single plant species [9, 10].

Maize is an important agricultural crop and a model for laboratory investigations. Maize is established to be excluder [11–14]. Maize and other cereals are more tolerant to cadmium action than many dicotyledonous plants [15] but less tolerant than some dicotyledonous hyperaccumulators [16–18].

We decided to study (under unified conditions of the experiment) cadmium accumulation and its toxic effect in the process of young maize plant growth. For this purpose, a wide range of Cd concentrations was applied and its physiological effects on germinating kernels, seedlings, and juvenile plants were assessed; cadmium accumulation in various maize organs was also followed.

MATERIALS AND METHODS

Experiments were performed with maize (*Zea mays* L.) plants, cv. Luchistaya. Plants were grown at 25°C, an illumination of 100–120 μmol photon/(m^2 s) and photoperiod of 12 h in the vessels 1.2 L in volume under continues aeration on modified Hoagland medium containing 3 mM KNO_3, 2 mM $Ca(NO_3)_2$, 1 mM $NH_4H_2PO_4$, 1 mM $FeSO_4$, 0.5 mM $MgSO_4$,

Abbreviations: Car—carotenoids; Chl—chlorophyll; DW—dry weight; FW—fresh weight; HM—heavy metals.

25 μM H_3BO_3, 2 μM $ZnSO_4$, 2 μM $MnSO_4$, 1 μM KCl, 0.1 μM $CuSO_4$, 0.1 μM $(NH_4)_6Mo_7O_{24}$, 2 mM MES, pH 6.5. Cadmium was added as $CdSO_4$. It was not added to the nutrient medium for control plants. For one week experiments, 13–15 seedlings (sometimes from 6 to 21) were cultivated in a vessel; for five week experiments – 4–5 plants. Media were replaced once a week. At the end of an experiment, vessels were washed 3–5 min with detergent and running water; however, such procedure did not completely remove cadmium contamination, and control plants contained substantial amount of Cd (see Table 7). Complete vessel cleaning was attained using weak solutions of EDTA or HCl and long (8–16 h) Cd desorption; in this case, Cd content in control plant became the lowest.

Plants from each vessel were analyzed individually or an united sample was made from corresponding organs of all plants.

All experiments were repeated at least three times; when the parameter was very variable, the number of independent experiments attained 10–12. One long-term experiment was performed in May and two others in July–August.

Various schemes of experiment were used; however, in all cases, plant age was determined from the start of germination at 25°C.

Scheme 1. For studying cadmium impact on maize kernel germination, they were first immersed in the Hoagland solution for 30 min, then kept for 2 days at 4°C in Petri dishes with Hoagland solution containing corresponding cadmium concentration, placing 15 kernels in 15 mL of the solution per dish. Imbibed kernels were transferred to the chamber for plant growing in the same solutions for 3 days at 25°C and illumination.

Scheme 2. Another scheme was used for the assessment of cadmium impact on plant growth. Maize kernels were immersed in 0.25 M $CaCl_2$ for 30 min and then kept for 2 days at 4°C in darkness on filter paper moistened with 0.25 M $CaCl_2$. Imbibed kernels were germinated for 2 days under the same conditions but at 25°C. Germinated kernels were transferred to vessels for plant growing containing Hoagland medium; cadmium was added next day. The analyses were performed on 4–9-day-old seedlings (1–6 days of cadmium exposure) and 34-day-old plants (31 days in the presence of cadmium). The death of seedlings exposed to 250 μM Cd was recorded until the age of 34 days (31 days in the presence of cadmium). In this case, two different cadmium salts were used, sulfate and chloride. Plants were considered dead when they were completely dry and had no spots of green tissue.

Plant height was determined as the distance between kernel and the highest shoot point. The area of the leaf blade was calculated according to the equation: $S = 2/3\ Ld$, where S is the leaf area, L is the length of the leaf blade, and d is the leaf width in the middle of the blade [19].

To determine the content of photosynthetic pigments, 1-cm leaf segment was cut. In 9-day-old plantlets, the terminal 1-cm segment was removed and the following 1-cm segment was analyzed. In 34-day-old plants, the segments from the leaf middle were analyzed. Pigments were extracted with 80% acetone, and concentrations of chlorophylls (Chl *a* and Chl *b*) and carotenoids (Car) were estimated according to equations presented in [20].

For determination of water content and cadmium content, tissues were dried at 60°C overnight. Roots were preliminary washed in distilled water for 10 min, as described in [5, 9, 15]. Dried samples (50–100 μg) were incubated overnight in the solution containing 1.5 mL of 64% nitric acid and 0.6 mL of 65% perchloric acid. On the following day, samples were heated for 1.5 h at 150°C, then for 2 h at 180°C; thereafter, 50 μL of concentrated hydrogen peroxide was added and samples were left for a night. Afterward, samples were adjusted to the final volume (10 mL) with distilled water. Cadmium concentration was determined with a Formula FM400 atomic-absorption spectrophotometer (Labist, Russia)

The data were processed using Excel (Microsoft) software. Significance of differences between mean values was verified with the Student's *t*-criterion.

RESULTS

First experiments were performed to study the effect of cadmium on kernel germination (Table 1). Inhibition of germination was observed only at the highest concentration used – 2 mM Cd. Inhibition of a radicle elongation became significant, starting from the concentration of cadmium of 500 μM and rose along with further increase in the concentration.

Then, the earliest stage of shoot development was studied (the leaves were analyzed from the time of their appearance).

Cadmium concentration of 80 μM significantly inhibited the growth of the shoot since the age of 5–6 days (Fig. 1a). A significant decrease in the content of Chl *a* under the influence of cadmium was found in 8-day-old seedlings, and the content of Chl *b* and Car – in 9-day-old seedlings only (Fig. 1b). The ratio of Chl *a* to Chl *b* changed slightly with seedling growth, but cadmium did not affect this process (data not shown).

The elevated cadmium content was observed in the shoots already in a day after its addition to growth medium, and than its content increased (Fig. 2). At this stage, a linear dependence between shoot growth and cadmium concentration was observed. The relation between shoot height and cadmium accumulation may be approximated by the equation $y = 0.0148x + 1.8229$ ($R^2 = 0.96$)), the relation between length and area of the second leaf and the content of cadmium may be approximated by the equations: $y = 0.0108x + 1.8582$ ($R^2 = 0.99$) and $y = 0.0093x + 0.6894$ ($R^2 = 0.99$), respectively.

Table 1. Effect of Cd^{2+} on germination of maize kernels

Material and parameter	Control	Cd concentration, μM						
		4	20	80	200	500	1000	2000
Kernels at radicle protrusion, %	94.4	94.4	90.0	86.7	93.3	93.3	93.3	93.3
Germinated kernels, %	77.8	76.7	72.2	71.1	75.3	76.7	78.9	65.2
Radicle length of germinated kernels, cm	12.5 ± 0.8^a	12.1 ± 0.8^a	11.3 ± 0.8^a	11.6 ± 0.8^a	10.9 ± 0.7^a	9.2 ± 0.5^b	7.9 ± 0.4^c	5.7 ± 0.4^d

Notes: The medium composition (here and in other tables) is presented in the Materials and Methods section. Kernels were germinated for three days. Different letters at the figures designate significant differences ($\alpha < 0.05$). Means and their standard errors are presented. Kernels were considered at radicle protrusion when the length of emerged radicle was no less than 1 mm; kernels were considered as germinated when the root length was no less than 5 mm.

Table 2. Cadmium toxicity and its accumulation in 9-day-old maize seedlings (6 days of cadmium exposure)

Parameter			Control	Cd concentration, μM			
				4	20	80	200
Plant height, cm			16.1 ± 0.27^a	15.1 ± 0.26^b	12.0 ± 0.25^c	10.7 ± 0.16^d	7.5 ± 0.16^e
L2 length, cm			10.4 ± 0.23^a	9.5 ± 0.24^b	7.3 ± 0.20^c	6.9 ± 0.15^c	4.8 ± 0.18^d
L2 area, cm^2			8.8 ± 0.27^a	7.7 ± 0.25^b	5.6 ± 0.22^c	5.0 ± 0.15^d	3.0 ± 0.16^e
L2 DW, mg			9.7 ± 0.44^a	9.8 ± 0.47^a	6.7 ± 0.31^b	6.1 ± 0.30^b	3.4 ± 0.48^c
Shoot DW, mg			36.7 ± 1.90^a	35.4 ± 1.10^a	–	28.4 ± 0.76^b	–
Root DW, mg			12.2 ± 0.32^a	12.5 ± 0.48^a	12.1 ± 0.56^a	12.0 ± 0.22^a	8.1 ± 0.48^b
Water content, %		whole shoot	93.3 ± 0.07^a	93.0 ± 0.07^b	–	91.4 ± 0.16^c	–
		L2	92.3 ± 0.13^a	92.3 ± 0.21^a	91.5 ± 0.19^b	90.7 ± 0.29^c	89.0 ± 0.50^d
		roots	95.0 ± 0.10^a	95.0 ± 0.13^a	94.4 ± 0.18^b	93.5 ± 0.17^c	91.0 ± 0.37^d
L2 pigments (μg/g FW) and their ratios		Chl *a*	1609 ± 25^a	1510 ± 29^b	1446 ± 25^b	1356 ± 26^c	1292 ± 22^c
		Chl *b*	412 ± 9^a	408 ± 10^a	380 ± 7^b	372 ± 8^b	368 ± 8^b
		Car	247 ± 5.0^a	240 ± 4.6^a	234 ± 3.7^{ab}	226 ± 4.3^b	228 ± 4.4^b
		Chl *a*/*b*	3.9 ± 0.03^a	3.8 ± 0.05^{ab}	3.9 ± 0.03^a	3.8 ± 0.02^b	3.5 ± 0.05^c
		Chl/Car	8.22 ± 0.07^a	8.14 ± 0.05^a	7.89 ± 0.07^b	7.82 ± 0.04^b	7.31 ± 0.06^c
Cd accumulation	μg/g DW	L2	10.6 ± 4.6^a	87 ± 9^b	117 ± 16^b	337 ± 29^c	430 ± 37^c
		roots	52 ± 16^a	1240 ± 80^b	2320 ± 32^c	4496 ± 233^d	9306 ± 519^e
		roots/L2	4.9	14.2	19.9	13.3	21.6
	by a single plant, μg	whole shoot	2.4 ± 1.1^a	41.2 ± 1.6^b	–	60.7 ± 7.1^c	–
		roots	7.3 ± 4.1^a	80 ± 8^b	–	283 ± 19^c	–
		roots/shoot	3.1 ± 3.0^a	2.0 ± 0.4^a	–	4.7 ± 1.4^a	–

Notes: L2—the second leaf. Means and their standard errors are presented. Different letters at the figures designate significant differences ($\alpha < 0.05$). A dash designates the absence of measurement.

Because of all parameters studied were inhibited significantly by the 9th day of seedling development, just this age was chosen for the analysis of the concentration dependence of its action.

Linear parameters of growth and the area of the second leaf of 9-day-old seedlings were reduced significantly already at the lowest of used concentrations – 4 μM $CdSO_4$ (Table 2). For most growth characteristics, the degree of inhibition increased along with the increase in the cadmium concentration in medium. The only exclusion was root dry weight (DW), which was not affected significantly up to the 80 μM Cd. Cadmium inhibited mass accumulation of the aboveground seedling parts more severe than that of roots (Fig. 3a). Water content in the second leaf and roots

Table 3. Cadmium effect on growth and the content of photosynthetic pigments in 34-day-old maize plants (31 days of cadmium exposure)

Parameter			Control	Cd concentration, μM		
				20	80	200
Plant height, cm			82.0 ± 1.00[a]	36.3 ± 1.96[b]	28.5 ± 1.47[c]	17.0 ± 1.83[d]
Average leaf number			7.7 ± 0.36[a]	6.4 ± 0.19[b]	5.4 ± 0.31[c]	4.6 ± 0.33[c]
Shoot DW, mg			1685 ± 15[a]	273 ± 42[b]	169 ± 37[bc]	85 ± 14[c]
Root DW, mg			226 ± 86.1[a]	62.3 ± 13.9[a]	40.1 ± 12.2[ab]	18.7 ± 7.9[b]
Kernel DW, mg			206 ± 5.5[ab]	186 ± 13.9[a]	207 ± 5.3[ab]	239 ± 13.1[b]
Pigments (μg/g FW) and their ratios	Chl *a*	L4	1344 ± 67[a]	519 ± 65[b]	617 ± 35[b]	1086 ± 40[c]
		L5	1364 ± 51[a]	489 ± 18[b]	709 ± 37[c]	854 ± 41[d]
	Chl *b*	L4	343 ± 14[a]	180 ± 24[b]	194 ± 12[b]	367 ± 25[a]
		L5	341 ± 11[a]	152 ± 6[b]	228 ± 18[c]	355 ± 17[a]
	Car	L4	174 ± 12[a]	96 ± 8[b]	117 ± 6[b]	213 ± 12[a]
		L5	179 ± 11[a]	91 ± 5[b]	124 ± 8[c]	186 ± 14[a]
	Chl *a*/*b*	L4	3.9 ± 0.1[a]	2.9 ± 0.1[b]	3.2 ± 0.1[c]	3.0 ± 0.2[bc]
		L5	4.0 ± 0[a]	3.2 ± 0.1[b]	3.2 ± 0.2[b]	2.4 ± 0.1[c]
	Chl/Car	L4	9.8 ± 0.3[a]	7.2 ± 0.4[b]	7.0 ± 0.2[b]	6.8 ± 0.1[b]
		L5	9.6 ± 0.3[a]	7.1 ± 0.3[bc]	7.6 ± 0.2[b]	6.6 ± 0.3[c]

Notes: L4—the fourth leaf; L5—the fifth leaf. Means and their standard errors are presented. Different letters at the figures designate significant differences ($\alpha < 0.05$).

Table 4. Water content (%) in various organs of 34-day-old maize plants (31 days of cadmium exposition)

Organ		Control	Cd concentration, μM		
			20	80	200
Leaf	8	88.0 ± 1.02			
	7	84.3 ± 3.34	88.5 ± 0.33		
	6	88.3 ± 0.59	89.6 ± 0.48	87.8 ± 0.23	87.9**
	5	88.8 ± 0.81	90.8 ± 0.51	88.4 ± 0.56	84.7 ± 1.82
	4	88.9 ± 0.65	90.4 ± 0.32	87.3 ± 1.06	82.9 ± 3.18
	3	55.7 ± 10.53	87.4 ± 2.26***	70.2 ± 6.06	80.7 ± 0.80
	1 + 2	24.8 ± 15.18	81.1 ± 0.88***	33.2 ± 16.02	31.5 ± 18.24
Stem*		91.1 ± 1.53	90.2 ± 1.27	86.3 ± 2.99	83.5 ± 3.33
Whole shoot		89.3 ± 0.36	89.6 ± 0.78	85.2 ± 1.26***	81.5 ± 2.46***
Kernel		18.0 ± 6.62	15.7 ± 1.56	14.9 ± 9.89	16.2 ± 2.64
Root		93.7 ± 1.50	93.6 ± 0.37	92.6 ± 0.49	90.5 ± 1.15

Notes: Mean values and their standard errors are presented. Leaves are numerated in the order of their emergence, correspondingly, from lower to upper leaves. Empty cells designate that the leaf of a given storey was absent from all or most plants.

* Stem with leaf sheaths.

** Leaves of this storey were present in one experiment only.

*** Difference from control is significant ($\alpha < 0.05$).

reduced significantly starting from 20 μM Cd (Table 2). Among photosynthetic pigments, the content of Chl *a* was most inhibited and the content of Car – the least (Table 2, Fig. 3b). The Chl *a*/*b* ratio changed faintly; the Chl/Car ratio reduced along with the increase in cadmium concentration in nutrient solution (Table 2).

Cadmium was accumulated predominantly in roots (Table 2). Cadmium accumulation in roots

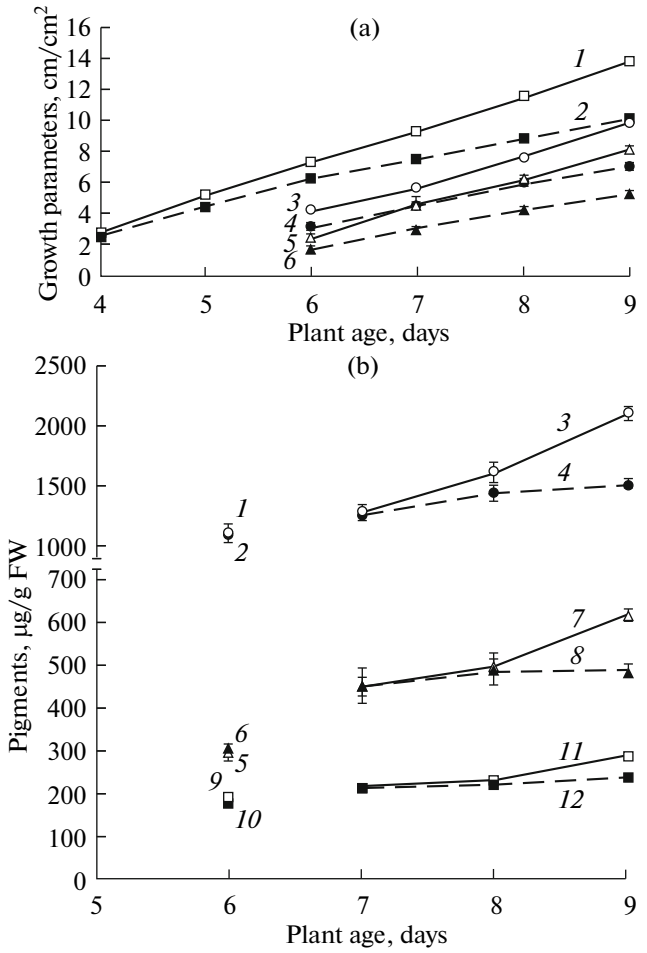

Fig. 1. Dynamics of the $CdSO_4$ (80 μM) effect on growth (a) and the content of photosynthetic pigments (b) in maize seedlings (4–9 days after the start of germination).

Cadmium was added to medium in three days after the start of kernel germination. During the fourth day, the first leaf broke through coleoptile. (a) Shoot height: (*1*) control; (*2*) + cadmium; the length of the second leaf: (*3*) control; (*4*) + cadmium; area of the second leaf: (*5*) control; (*6*) + cadmium. (b) Chlorophyll *a* content: in the first leaf (*1*) control; (*2*) + cadmium; in the second leaf (*3*) control; (*4*) + cadmium; chlorophyll *b* content: in the first leaf (*5*) control; (*6*) + cadmium; in the second leaf (*7*) control; (*8*) + cadmium; carotenoid content: in the first leaf (*9*) control; (*10*) + cadmium; in the second leaf (*11*) control; (*12*) + cadmium.

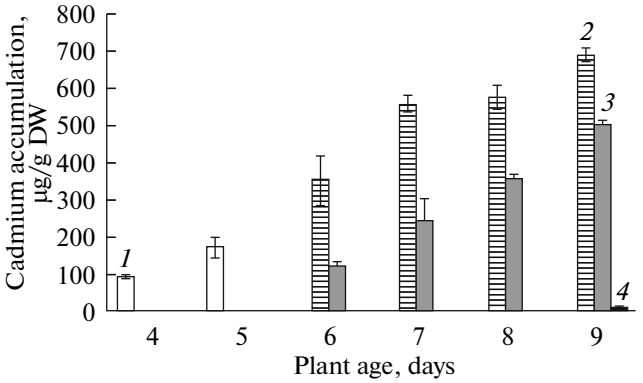

Fig. 2. Dynamics of cadmium accumulation in the shoots of maize seedlings exposed to 80 μM $CdSO_4$.

(*1*) In the whole shoot; (*2*) in the first leaf; (*3*) in the second leaf; (*4*) in the second leaf of control plants (without $CdSO_4$ addition to medium).

depended linearly on its concentration in the medium ($y = 42.828x + 878.98$; $R^2 = 0.98$)). In the second leaf, the dependence was more complex. An increase in Cd concentration in the solution from 4 to 80 μM resulted in proportional increase of its accumulation in the leaf. Further increase up to 200 μM Cd gave proportionally smaller increment in cadmium accumulation in leaf tissues. In 9-day-old seedlings grown in the presence of 200 μM Cd, very great variability in the accumulation of this HM in the second leaf was observed; therefore substantially more replications of the experiment was aplied to measure cadmium content in the leaf at this concentration.

Long-term (31 days) cadmium exposure revealed plant development retardation. Most control plants were in the phase of the 8th leaf emergence; at 20 μM Cd – in the phase of the 7th leaf emergence; at 80 μM Cd – in the phase of the 6th leaf emergence; at 200 μM Cd – in the phase of the 5th leaf emergence (Table 3). As well a tendency to delay leaf dying in treated plants was noted. In control plants, the second and third leaves were died and dried, whereas in experimental plants these processes occurred slower and corresponding leaves contained more water (Table 4). Significant difference from control was observed in the presence of 20 μM Cd only; however, this tendency was observed also at higher cadmium concentrations.

In long-term experiments, cadmium toxicity was manifested very hard (Table 3, Fig. 4a). Thus, fresh weight (FW) of 34-day-old plants grown in the presence of 200 μM Cd comprised only 3.3% of the weight of control plants (data not shown). It should be noted that shoots lost much water as well (Table 4).

The data concerning the cadmium impact on the content of photosynthetic pigments in the leaves at long-term exposure of maize plants turned out to be unexpected (Table 3, Fig. 4b). In plants grown in the presence of 20 μM Cd, the content of pigments per gram of fresh weight was twice lower than in control. At 80 μM Cd, the degree of inhibition was less, whereas in plants grown in the presence of 200 μM Cd, the content of pigments was approximately similar to that in control plants. Such a pattern was observed for all pigments (Chl *a*, Chl *b*, and Car).

When cadmium action lasted for 31 days, the relationship between cadmium accumulation and its concentration in nutrient medium was well approximated by the linear equation for both roots ($y = 101.87x + 1892.7$; $R^2 = 0.97$) and for the entire shoot ($y =$

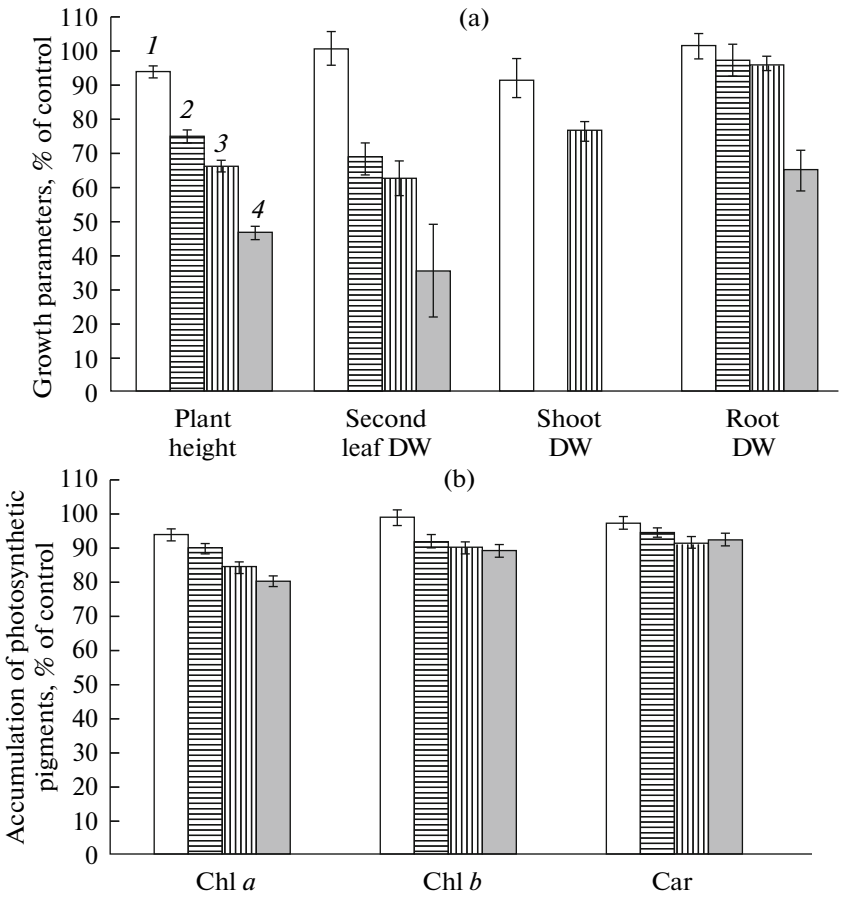

Fig.3. Effect of cadmium on growth (a) and the content of photosynthetic pigments in the second leaf (b) of maize 9-day-old seedlings (6 days of cadmium exposure).
(*1*) 4 μM; (*2*) 20 μM; (*3*) 80 μM; (*4*) 200 μM $CdSO_4$.

$6.0012x + 45.584$; $R^2 = 0.97$). Most cadmium was accumulated in the roots (Table 5). In the shoot, cadmium accumulated predominantly in the oldest leaves. An enlargement of cadmium concentration in nutrient solution resulted in the increase in its content per FW in the leaves of first three storeys and in stem (with leaf sheaths), whereas, in the leaves of the 4th–6th storeys, such tendency was not observed (Table 5). Cadmium distribution between the shoot and root did not depend on concentration of this HM in nutrient medium. However, at high cadmium concentration, the contribution of kernels in cadmium accumulation increased markedly: from 1.41% at 20 μM Cd to 7.4–10.5% at 80–200 μM $CdSO_4$ (Table 5).

In the presence of 200 μM Cd on the 31st day of its action, all plants were alive. When Cd concentration was increased to 250 μM, some plants perished, starting from the 8th day of cadmium action (Fig. 5). Plants were considered dead when their shoots were completely necrotized; the roots of such plants were not substantially necrotized yet. Mass plant death started since the 18th day of cadmium exposure; by the end of experiment (31st day of exposure), 42% of plants were dead. Similar dynamics of plant death was observed when cadmium was introduced in the form of sulfate or chloride. According to the equation on Fig. 5, the death of 50% of maize plants should be expected as a result of 34-day-long exposure to 250 μM Cd (in 37-day-old plants).

DISCUSSION

We analyzed toxic effect of cadmium and its accumulation in maize plants, cv. Luchistaya, at various developmental stages. The results obtained confirmed that maize accumulated cadmium predominantly in the roots (Tables 2, 5). Our data about cadmium accumulation in the shoots (Table 2) correspond to that of other researchers, who studied cadmium accumulation by maize plants under similar experimental design [12–14], whereas cadmium content in the roots in our experiments was several times higher. It is known that, in the shoots of dicotyledonous plants from the family Cruciferae, cadmium accumulates mainly in young (upper) leaves [5, 17, 21] or distributes between all leaf storeys relatively evenly [17]. In the monocotyledonous plant, maize, cadmium in contrast accumulated mainly in the lower (old) leaves. The higher and thus

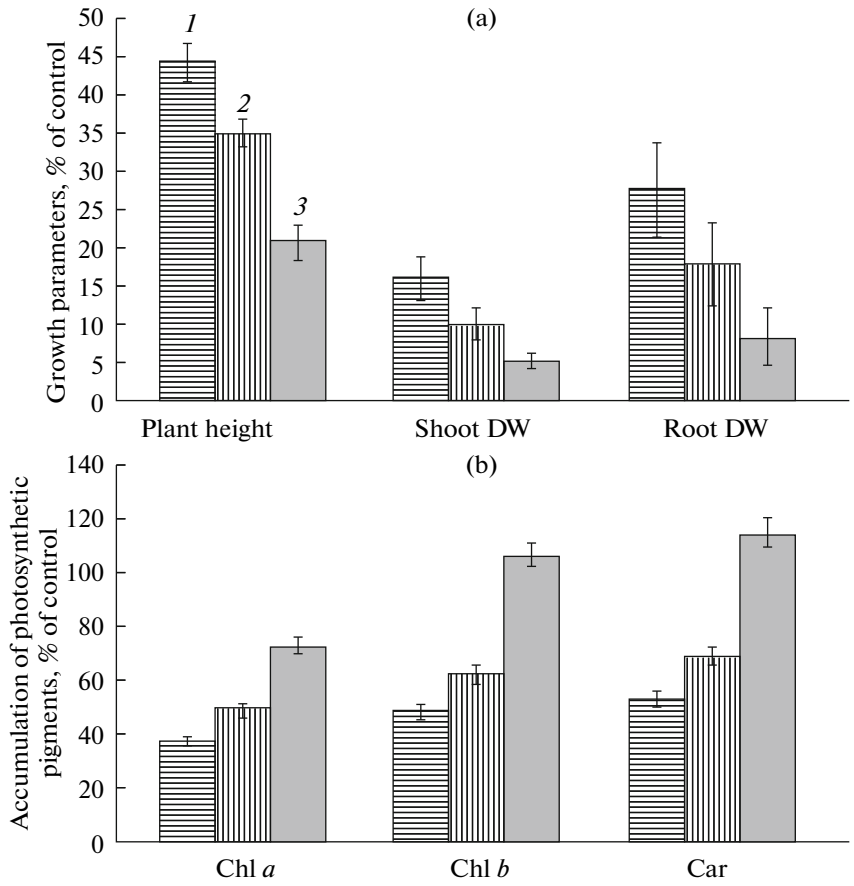

Fig. 4. Effect of cadmium on growth (a) and the content of photosynthetic pigments in the second leaf (b) in 34-day-old maize plants (31 days in the presence of cadmium).

The contents of chlorophylls and carotenoids – average values for 4th and 5th leaves.

(*1*) 20 μM; (*2*) 80 μM; (*3*) 200 μM $CdSO_4$.

younger was the leaf, the less cadmium was accumulated in its blade (Table 5).

It is known that cadmium transported from the root to the shoot can be directed into leaf trichomes [5, 6]; the glands of *Tamarix aphylla* can excrete Cd on the leaf surface [22]. In our experiments, at 80 and 200 μM Cd in medium nearly one-third of Cd overcoming "root barrier" was transported to the kernel (Table 5, kernels did not contact with the nutrient solution). However, in the soil solution the concentration of free cadmium is very low [23]; therefore, it remains unclear how much of cadmium coming from the root may be accumulated in kernels under field conditions.

An enlargement in the cadmium concentration in the nutrient medium induced a proportional increase in its content in the unit of root tissue weight (Tables 2, 5). In the shoot, such correlation was weaker and in the upper leaves of 34-day-old maize plants such concentration dependence was not observed at all (Table 5).

Plant growth and prolongation of treatment time led to the increase in the Cd content per weight (Fig. 2, Tables 2, 5); consequently, total cadmium accumulation by plant organs and the whole plant increased as well. The root to shoot ratio of cadmium content (μg/g DW) decreased with the plant age, whereas the difference in total cadmium accumulation in organs increased (Tables 2, 5). The latter may be because cadmium suppressed increment in the root biomass weaker than shoot biomass (Figs. 3a, 4a).

At 200 μM Cd, 9- and 34-day-old plants demonstrated extremely large variability in cadmium accumulation by the shoot (Tables 2, 5). Perchaps, the regulation of mechanisms of cadmium translocation over the plants at this concentration are disturbed, indicating once more that this concentration is close to lethal one for maize cv. Luchistaya.

Dicotyledonous plants (*Brassica napus, B. juncea, Vigna radiata*) grown under comparable conditions could adapt to the presence of cadmium in medium, and its toxicity decreased with time [23, 24]. In contrast, in maize cadmium toxic effect enhanced with plant age and prolongation of metal action. For example, 200 μM Cd did not affect significantly kernel germination (Table 1), inhibited by 50% growth of 9-day-

old seedlings (Fig. 3a), and inhibited growth of 34-day-old plants by 90% and more (Fig. 4a).

In 34-day-old maize plants, development was retarded. In the presence of cadmium, the emergence of new leaves was delayed (Table 3) and dying the old ones was retarded as well. In plants exposed to cadmium, leaves died slower, saving more water. The analysis of water content made this tendency evident in all leaves; however, significant difference from control was observed in plants exposed to 20 μM Cd only (Table 4). Senescence was not accelerated in either treatment.

Among parameters we tested, cadmium exerted the hardest inhibitory effect on the accumulation of plant biomass, moderate effect – on linear growth, to a lesser extent was affected the content of photosynthetic pigments, and the water content was reduced only by 3–8% (Tables 2–4; Figs. 3, 4).

In the range of concentrations used (4–200 μM), Cd inhibited shoot biomass accumulation more severe than that of roots (Figs. 3a, 4a; Tables 2, 3). In 34-day-old plants, 80 and 200 μM Cd reduced the water content in shoots more than in roots (Table 4). Plant death also began from shoots: at the lethal concentration of 250 μM, shoots were completely necrotized, whereas necroses were not still noted in the roots. Under similar conditions, rye seedlings perished in a similar manner: shoot necrotization started earlier than root necrotization [25]. These data allow a conclusion that cadmium inhibits shoot growth stronger than root growth, at least in maize and related species.

In the review of Seregin and Ivanow [4] devoted to the effects of Cd and Pb on plants, the opposite conclusion was made: "Root growth is more sensitive to heavy metals than shoot growth." The authors relied on experiments, in which plants were grown on distilled or tap water with the introduction of cadmium in the appropriate concentrations (see references in the review [4]). For maize seedlings grown on water at the age of less than a week, the lethal concentration was 10 μM Cd [26], whereas in the present study plants supplied with 200 μM Cd in a nutrient medium did not dye in a month.

Later, the work was carried out on maize plants grown on Hoagland nutrient medium with cadmium. In these studies more severe cadmium suppression of shoot biomass accumulation compared to that of roots was shown [14, 27], although the linear growth of the roots was more inhibited than that of shoots [27]. We should also mention the work [13], which shows the data (Fig. 2b), indicating that 5 or 10 μM Cd inhibited the increment in the fresh weight of shoots without affecting the roots, while at higher concentrations cadmium reduced the biomass of both organs in a similar manner. However, the authors' conclusion contradicts to their findings. We failed to find any data concerning cadmium-induced inhibition of maize root and shoot growth in plants grown in soil; however, such data were obtained in experiments with the member of the same subfamily Panicoideae, *Digitaria sanguinalis*, and related family (*Cyperus difformis*), as well as dicotyledonous plant *Chenopodium ambrosioides*. In these soil-grown plants cadmium suppressed shoot growth (both height and weight) harder than root growth [28]. These works [12–14, 27, 28] showed that cadmium accumulated predominantly in the roots, but its content in the shoots was also rather high (80 μg/g DW and higher). When maize plants were grown on water, only small amounts of cadmium was detected in the shoots (less that 10 μg/g DW) [11]. Therefore, we believe that, under conditions close to natural (illumination, mineral nutrition, and optimal temperature), cadmium suppressed shoot growth more than root growth.

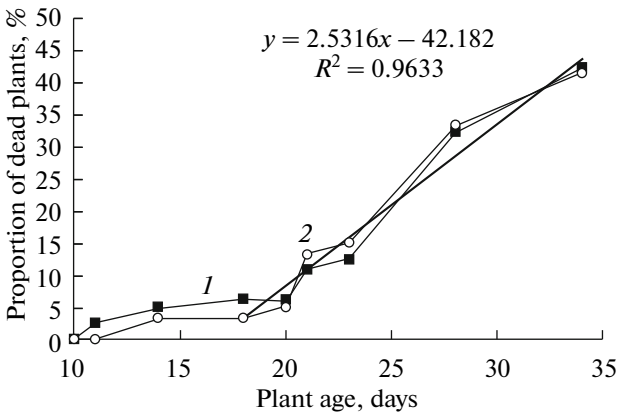

Fig. 5. Dynamics of plant death during their growing on the medium containing 250 μM cadmium salt.
Cadmium was introduced in three days after kernel germination. Diagram presents the proportion of plants perished by a definite day. For the period of intense plant death (from 18 to 34 days) a total approximating dependence (line) is built for both types of anions. (*1*) $CdSO_4$; (*2*) $CdCl_2$.

The analysis performed showed that physiological changes were not well correlated with cadmium accumulation in maize organs. Cadmium was accumulated predominantly in the roots (Tables 2, 5) but inhibited shoot growth harder (Figs. 3a, 4a; Tables 2–4). Nine-day-old seedlings grown in the presence of 20 and 80 μM $CdSO_4$ differed markedly in HM accumulation in tissues: almost twice in the root and almost threefold in the second leaf; however, in most parameters tested, differences between these two plant groups were very small (Table 2). We compared the contents of cadmium and photosynthetic pigments in the fourth and fifth leaves of 34-day-old maize plants. The leaves of one and the same storey in plants grown in the presence of 20, 80, and 200 μM Cd did not essentially differ in the cadmium content but differed markedly in the content of photosynthetic pigments. At the same time, leaves of different storeys of these plants, in contrast, differed in the cadmium content but very little

Table 5. Cadmium distribution between organs of 34-day-old maize plants (31 days of cadmium exposure)

Organ		Control		Cd concentration, μM					
				20		80		200	
		μg/g DW	μg/organ	μg/g DW	μg/organ	μg/g DW	μg/organ	μg/g DW	μg/organ
Leaf	8	1.9 ± 0.3^{a1}	0.23 ± 0.13^{a1}						
	7	3.3 ± 1.4^{a1}	0.38 ± 0.13^{a1}	63 ± 33^{a1}	0.47 ± 0.27^{a1}				
	6	6.4 ± 4.3^{a1}	0.70 ± 0.21^{a1}	113 ± 45^{ab1}	3.5 ± 1.2^{a1}	99 ± 18^{b1}	0.92 ± 0.67^{a1}		
	5	7.7 ± 4.1^{a1}	0.65 ± 0.22^{a1}	173 ± 47^{b1}	4.5 ± 1.0^{b1}	187 ± 73^{ab1}	$3.9 \pm 2.5^{ab1,2}$	177 ± 58^{b1}	1.1 ± 0.90^{ab1}
	4	10.9 ± 4.3^{a1}	0.66 ± 0.19^{a1}	246 ± 65^{b1}	7.0 ± 2.0^{b1}	277 ± 45^{b1}	$7.2 \pm 2.0^{ab1,2}$	288 ± 96^{b1}	$3.0 \pm 1.70^{ab1,2}$
	3	23.6 ± 11.8^{a1}	0.79 ± 0.40^{a1}	433 ± 54^{b2}	$9.4 \pm 0.33^{b1,2}$	553 ± 32^{b3}	10.3 ± 2.9^{bc2}	746 ± 49^{c2}	6.8 ± 0.24^{c2}
	1 + 2	110 ± 55^{a1}	2.6 ± 1.10^{a1}	788 ± 48^{b3}	16.9 ± 3.2^{b2}	940 ± 47^{b4}	$22.7 \pm 7.3^{b2,3}$	4861 ± 2947^{ab2}	66.4 ± 41.6^{ab4}
Stem*		5.5 ± 3.2^{a1}	2.2 ± 1.10^{a1}	230 ± 46^{b1}	21.8 ± 0.84^{b2}	$369 \pm 37^{b1,2}$	24.2 ± 2.3^{b3}	656 ± 89^{c2}	22.9 ± 4.7^{ab3}
Whole shoot		10.6 ± 6.8^{a1}	$8.1 \pm 2.5^{a1,2}$	$271 \pm 39^{b1,2}$	63.5 ± 8.1^{b3}	427 ± 31^{c2}	69.3 ± 18.0^{b4}	1275 ± 418^{bc2}	$100 \pm 49^{b4,5}$
Kernel		6.6 ± 2.7^{a1}	1.4 ± 0.57^{a1}	24.4 ± 9.0^{a1}	4.7 ± 2.0^{a1}	200 ± 63^{b1}	$41.1 \pm 12.3^{b3,4}$	288 ± 68^{b1}	58 ± 6.0^{b4}
Root		247 ± 161^{a1}	21.3 ± 7.7^{a2}	4462 ± 641^{b4}	264 ± 20^{b4}	11987 ± 1808^{c5}	445 ± 36.5^{c5}	21435 ± 3181^{d3}	394 ± 125^{bc5}
Root/shoot		21.0 ± 8.4^{a}	2.5 ± 0.4^{a}	16.9 ± 4.9^{a}	4.2 ± 0.6^{a}	28.9 ± 9.9^{a}	7.0 ± 1.9^{a}	22.4 ± 17.5^{a}	4.9 ± 2.9^{a}
Root/leaf (1 + 2)		1.8 ± 0.7^{a}		5.8 ± 2.0^{a}		12.9 ± 3.8^{b}		9.4 ± 9.0^{ab}	

Notes: Notes: Mean values and their standard errors are presented. Groups of values significantly different at $\alpha < 0.05$: (a–d) for a single organ of plants exposed to different cadmium concentrations; (1–5) for different organs of plants grown at one and the same Cd concentration. Leaves are numerated in the order of their emergence, correspondingly, from lower to upper leaves. Empty cells designate that the leaf of a given storey was absent from all or most plants.

* Stem with leaf sheaths.

differed in the content of photosynthetic pigments (Tables 3, 5).

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