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The assessment of cadmium nitrate effect on morphological and cytogenetic indices of spring barley (*Hordeum vulgare*) seedlings

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Abstract

The influence of different concentrations of the heavy metal (Cd) – $Cd(NO_3)_2$ – on seedlings of four cultivars of the spring barley was studied (the length of shoots and roots, the percentage of germinated seeds and strong seedlings were estimated). The topic of this work is investigation of effects of cadmium nitrate doses range on seedlings of several cultivars of spring barley of different geographical origin and searching of the Cd critical dose. For realization of these goals, an analysis of visible changes of the plants morphology and cytogenetical analysis (for searching of the discovered morphological changes causes) are suitable. Cadmium enters plants organism by the root path, and with this, morphological and cytogenetic effects in the seedlings roots have the highest value. Suppression of growth processes was described with an increase in the dose of Cd, and the appearance of morphological anomalies of seedlings was noted. Thus, average shoots length was decreased twice on the Cd^{2+} concentrations range 0.15–0.4 mg mL^{-1}; at the range from 0.4 to 0.7 mg mL^{-1}, this index was maintained on the plateau, and at doses higher than 1 mg mL^{-1}, a total suppression of shoots development was noted. Cytogenetic analysis was performed to clarify the reasons for the formation of these reactions. The increase in the yield of cytogenetic disorders was revealed with increasing cadmium concentration, and the blockage of proliferation in the late stages of mitosis was marked. High frequencies of multipolar mitoses, anomalous dividing of chromosomes, chromosome agglutinations and pathological anaphases have been indicated. At maximum dose, the frequency of cells with cytogenetic anomalies was 19 times higher than at control variant. Doses of Cd, which can be considered as not so harmful for plants, were estimated as 0.1–0.2 mg mL^{-1}. 0.3–0.7 mg mL^{-1} caused significant inhibition of growth processes, while doses higher than 1 mg mL^{-1} can be considered as conditionally lethal ones. It is shown that the barley cultivars have a significant differentiation in their resistance to cadmium, and the probable causes of the formation of observed effects were considered on the basis of literature data. With this, the Cd dose of 0.35 mg mL^{-1} can be considered as critical and can be used for searching of barley cultivars, which have the high tolerance to Cd influence. In perspective, such cultivars can be used for agricultural production in the territories with the high rates of Cd soil pollution.

Keywords Barley cultivars · Cytogenetic anomalies · Heavy metals · Resistance to cadmium · Roots and shoots length

1 Introduction

In conditions of constant growth of the Earth population, the food issue became especially acute. However, at the same time, the intensive development of industrial production leads to progressive contamination of the environment. Contamination of agrosphere is one of the most dangerous for human health, because chemical wastes can easily enter the food chain of humans and domestic animals. Moreover, many harmful substances suppress agricultural plants vital processes, which lead not only to decreasing of productivity of the agriculture, but also to a lack of quality of its production. Currently, more than 1 million hectares of agricultural land of Russia are contaminated with highly toxic (hazard class I—according to the Russian standard classification) and 2.3 million hectares—toxic elements (class II danger) (Aleksakhin 2004).

Heavy metals (HM) are important group of pollutants, being chemical elements with a metal crystal lattice with a density over 5 g cm^{-3}. Some of HM are essential plant micronutrients, which are needed for the maintenance of

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plant homoeostasis, while an excess of these elements leads to serious disturbances of plant functions. Such HM include, for example, copper and cobalt (Clemens 2006; Mishra et al. 2019). Biological role of some other HM is not completely clear, but they also can be accumulated in soil and water of natural and agricultural ecosystems in significant amounts. Some of these elements, such as lead, cadmium and mercury, are highly toxic for plants and animals.

One of the most dangerous heavy metals is cadmium. It is a widespread element, which probably has no essential functions for plant and animal metabolic processes. Its accumulation in the environment is associated with industrial production. Its molecular form or compounds are widely used in electroplating, as a mineral pigment in paint and varnish materials, in power cells. It is also a byproduct of mining and smelting of lead and zinc. Soluble cadmium compounds such as nitrate or chloride can easily enter into the plant organism from the soil solution, causing a disruption of the morphology and various physiological processes. The mechanisms of toxic influence of this HM are not completely clear (Murzaeva 2004; Wang et al. 2010; Dandan et al. 2011; Wang and Bjorn 2014; Derakhshani et al. 2020); it is suggested that Cd accumulation does not lead to a substantial increase in free radical production (Garg and Manchanda 2009; Çatav eat al. 2020), but processes of lipid peroxidation in cell membranes are induced soon after appearance of Cd ions in the environment (Verma and Dubey 2003; Zhang et al. 2009). At the same time, it is reported (Liu et al. 2009a, b) that injection of free radical scavengers and antioxidants in harmed organism weakens toxical stress. With this, more advances at the field of HM influence mechanisms on the plant organisms are required.

Thus, modern agriculture is faced the significant problem. For aforementioned reasons, it has a challenge to identify donors of HM resistance genes, which could be useful for breeding of new cultivars of major crops. These cultivars must have a high tolerance to the pollutants and as lowest as possible HM accumulation rate in edible parts. The agricultural production from the polluted sites/areas must be safe for humans and animals. The phenomenon of HM hyper accumulation in plants tissue is also of interest for cleaning contaminated soils. The studies of natural plant populations (Baker 1987; Barnatt 2004; Demkova et al. 2017; Khan et al. 2017), grown in contaminated areas for decades (e.g. areas of mining of polymetallic ores), indicated the possibility of using plants for phytoremediation in Cd-polluted areas. Spring two-row barley (*Hordeum vulgare* L.) is well suited as a model object for such studies. Such choice is determined by the fact that barley is one of the main crops, known since ancient times and cultivated worldwide in different climatic and soil conditions. This culture is well studied at different levels of plants organization—genetic, cellular, biochemical (Pan 2001; Wu 2003; Geras'kin 2005; Chen et al. 2007; Lentini et al. 2018).

With this, the hypothesis can be proposed. Some of the barley cultivars have a high resistance to the cadmium harmful influence, and it is bound with the specific mechanisms of tolerance and low rates of this pollutant accumulation in plant tissues. For discovering of these cultivars, the critical dose of Cd must be found and with using of this dose, a screening of wide range of barley cultivars must be carried out with using of morphological and cytogenetic indices.

Therefore, the topic of this article is investigation of effects of cadmium nitrate doses range on seedlings (changing of roots and shoots length, number of strong seedlings and germinative seeds) of several cultivars of spring barley of different geographical origin. In order to estimate sensitivity of different barley cultivars to Cd stress, critical dose of cadmium provoking a significant damage to barley seedlings must have been found. Such dose was verified by using of morphological and cytological methods and can be used at future works for screening a wide range of barley cultivars for those having a high tolerance to cadmium stress. The ultimate aim of this work is discovering a bunch of barley cultivars, which have a high Cd tolerance and low rates of Cd accumulation in the edible parts.

2 Materials and methods

Evaluation of the morphological indices – In a preliminary experiment to determine the range of effective doses, the solutions with the following concentrations of $Cd(NO_3)_2$ were taken: 0; 0.005; 0.01; 0.02; 0.04; 0.08; 0.16; 0.20; 0.32; 0.40; 0.50; 0.60; 0.70; 1.0; 1.50 mg mL^{-1}. Barley seeds (Zazerskii 85 cultivar) (100 seeds per each variant) were germinated in these solutions (200 mL) for 5 days in filter paper rolls under temperature 20 °C (The National Standard of Russia 2010). At the end of this period, the rolls were unfolded and each of the following indices was evaluated: average length of roots and shoots (mm), the percent of germinated seeds and the percent of strong seedlings. As a «strong seedling», we consider such seedling that had at least 3 normal roots (length not less than 5 mm) and which shoots were occupied not less than a half of the coleoptile. This was followed by an additional experiment using 4 cultivars: Zazerskii 85 (Belarus), Jelen (Serbia), Chelyabinskii 1 (the Chelyabinsk region of Russia) and Zarya (the Kirov region of Russia). Seeds of used cultivars were received from the N.I. Vavilov All-Russian Institute of Plant Genetic Resources, aka VIR, St. Petersburg, Russia (Zazerskii 85 catalogue number К-26,965; Jelen—К-30,955; Chelyabinskii 1—К-30,819; Zarya—К-4731). In this case, we used the following concentrations: 0; 0.1; 0.3; 0.5; 0.7; 1.0; 1.3 mg mL^{-1} of $Cd(NO_3)_2$. The large and small concentrations were excluded as uninformative. At this experiment, the same methodic and indices were used, as at the previous.

Cytogenetic analysis – Cytogenetic analysis of the roots apical meristem cells was performed using anaphase method on the seedlings of the cultivar Zazerskii 85 (Atabekova 1971; Pausheva 1974). Seeds were germinated by the roll method on solutions of $Cd(NO_3)_2$ with the following concentrations: 0; 0.25; 0.5; 0.7; 1; 1.5 mg mL^{-1}. Roots (10 mm of length) at the stage of the first mitosis were fixed in the Clark solution (ethanol and glacial acetic acid in the ratio 3:1). Temporary squashed preparations of the barley seedlings root apical meristem were stained with the acetic orcein and analyzed at the Nikon Eclipse E200 microscope (Japan). In each variant, 6–7 thousand of anaphases were analyzed. The frequencies of the following cytogenetic disorders were scored: bridges, fragments, lagging of chromosomes and multipolar mitoses. To evaluate the proliferative activity of cells of the apical meristem, a mitotic index was calculated. For this task, 1–1.5 thousand cells per each variant were analyzed. At the same time, the visually observed disruptions of the cell and root ultrastructure were noted.

The validity of the results was ensured by calculating of the optimal sample size, variance, standard error and deviation, the confidence interval by using of MS Excel 2003 and STATISTICA 6.0 software. The significance of differences between control and Cd treatments was evaluated using Student t test.

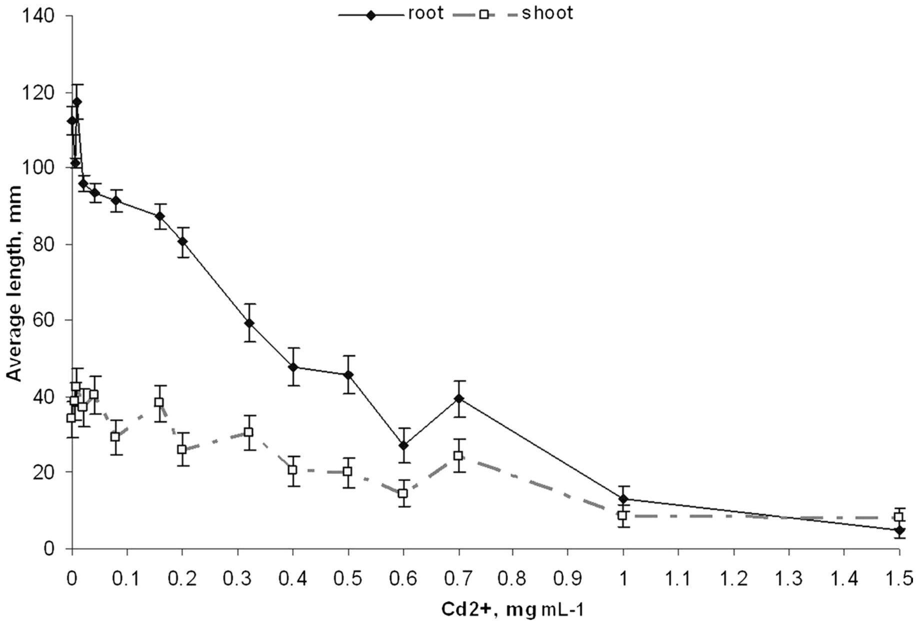
3 Results

The preliminary evaluation of barley seedlings reactions to cadmium stress – No significant influence on the length of the roots and shoots of Zazerskii 85 seedlings under low (from 0.005 to 0.080 mg mL^{-1}) Cd^{2+} concentrations was found (Fig. 1). However, the shoot length decreased with the increase in Cd concentrations. The four well-defined segments with the different slope angle were evident on the concentration curve (Fig. 1). The shoots length decreasing was developing slowly to 0.2 mg mL^{-1}, but in the range of 0.2–0.4 mg mL^{-1} average roots length was reduced more than half. Mean values of this index did not change significantly up to 0.7 mg mL^{-1}, forming a plateau. The final decrease in the shoot length took place at the range of 0.7–1.0 mg mL^{-1} of Cd, and further increase in HM dose practically had no effect.

Shoots were less sensitive to cadmium. Up to 0.32 mg mL^{-1}, their length decreased insignificantly, and only after this dose, more pronounced tendency to decrease was noted. At the dose 0.7 mg mL^{-1}, shoot length was reduced 1.3 times in comparison with the control variant. The subsequent increase in the Cd concentration did not change root length. Two others indices used (the number of strong seedlings and percent of germinated seeds) did not show reliable dependence on the dose of HM, meaning that they will not be considered in the future research.

These data confirm that the main barrier to HM ions entry into the plant organism is the root. While its barrier function handles HM stress, preventing the uptake HM from the

Fig. 1 The effect of the different Cd $(NO_3)_2$ concentrations on 5 day seedlings of Zazerskii 85 cultivar



soil, the plant continues to grow and develop. However, it is possible that productivity of plants may be reduced due to the costs of energy to protect from intoxication. When the excess of pollutant (Cd^{2+} 0.6–0.8 mg mL^{-1}) penetrates the protective barriers of the root, the oppression of all vitality functions occurs.

The assessment of cadmium influence on the 4 barley cultivars – The next step was to compare two considered indices in the selected range of doses for 4 spring barley cultivars of different geographical origin. The assessment of the shoot length of 4 barley cultivars (Fig. 2a) showed that at 0.1 mg mL^{-1}, Cd^{2+} values of this index were reduced comparing with the control. The only exception was the cultivar Zazerskii 85, which at this dose showed a stimulation of shoot growth (t test, $P \leq 0.05$). At dose 1 mg mL^{-1}, the value of the index (shoot length for Zazerskii 85) was significantly higher than the control level (t test, $P \leq 0.05$). It is known (Soudek 2010) that such effects can be observed at low HM doses. With the further increase in HM dose, a gradual decrease in shoots length for all 4 cultivars was shown. This process developed rather slowly, and only above 1 mg mL^{-1} this index became significantly lower than the control level for all 4 cultivars. At the maximal dose, growth process of

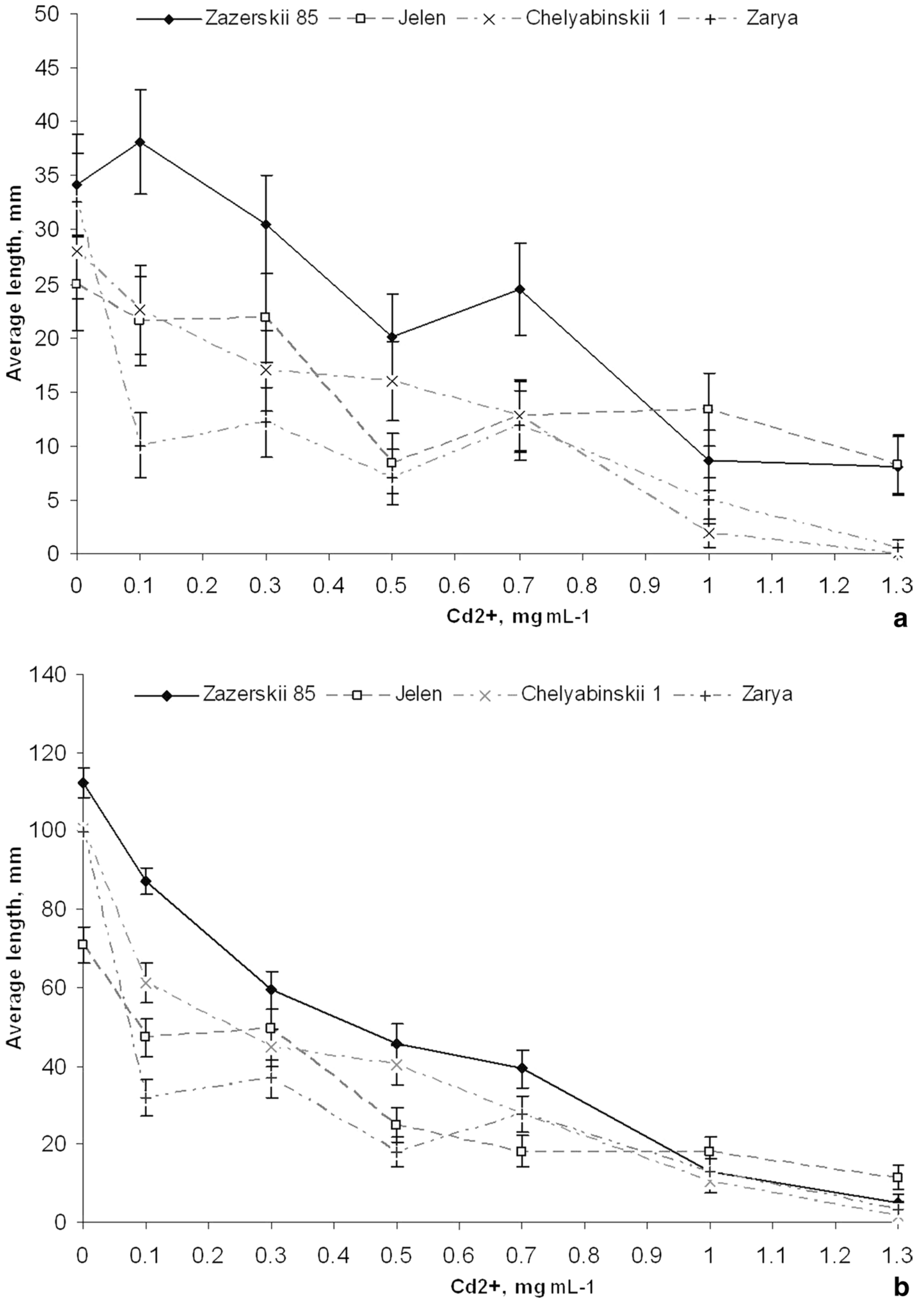


Fig. 2 The effect of cadmium concentrations on the length of shoots **a** and roots **b** of cultivars of spring barley

seedling was almost completely stopped at two cultivars—Zarya and Chelyabinskii 1. Based on the analysis of average length of shoots, we can say that the most resistant to cadmium was Zazerskii 85 cultivar, and the least—Zarya. The other two cultivars slightly differed from each other.

The length of roots (Fig. 2b) proved more reliable indicator to study the effect of cadmium on the barley seedlings. Its value gradually decreased with increase in HM concentration. From 1 mg mL^{-1}, it was changed slightly and at the maximal dose root length was close to zero. At the same time, the average length of shoots was characterized by sharp fluctuations of values, although in general there was a clear tendency to decrease. Based on the average length of the roots, Zazerskii 85 cultivar was considered as the most resistant to cadmium. The other 3 cultivars showed similar values of the index for all concentrations.

Besides the root length decrease, pathologic changes of root's morphology were also observed (Fig. 3), such as tissue hardening, coloration changes and geotropism disruption (which was especially obvious in Jelen cultivar at doses higher than 0.7 mg mL^{-1}). Similar effects were reported in (Kopittke 2007). All these data suggest that the basic mechanisms of plant organism protection from the harmful ions are primary associated with the root.

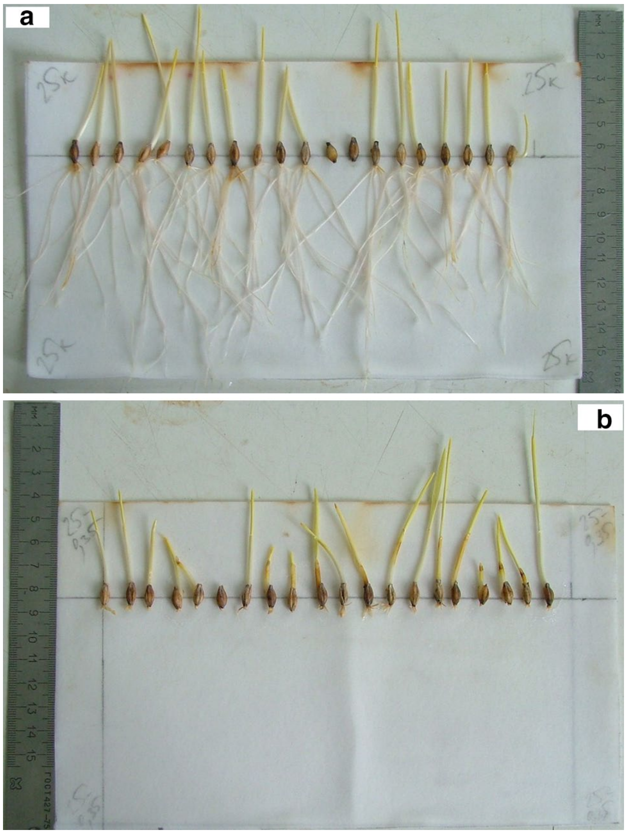


Fig. 3 The outer view of 5th day old seedlings of the Zazerskii 85 cultivar **a**–control, **b**–Cd^{2+} 0.3 mg mL^{-1})

Cytogenetic effects in cells of the root apical meristem under the cadmium stress.

To better understand the processes occurring in the plant organism under cadmium exposure and the possible ways of formation of tolerance to this HM, it is not enough to use only the biometric parameters. For identification of adverse effects that occur in the tissues of the plants before they affect the whole organism, more delicate methods must be used. One of such methods is cytogenetic analysis.

It was shown that mitotic index (MI), characterizing the activity of cell division in meristematic zone of the root, gradually, although not significantly, increased up to a dose of 0.5 mg mL^{-1} along with the increase in cadmium concentrations (Fig. 4). After this dose, MI curve reached a plateau and then reduced at range 0.7–1 mg mL^{-1}. However, at the maximal dose of cadmium MI increased again, becoming higher than the control level. Noteworthy, the shape of MI curve was almost identical to the curve of the prophase's frequency (Fig. 4). From this observation, we can make the conclusion that majority of dividing cells was in the prophase stage. Because both indices reached maximum at the highest HM's dose, it can be assumed that frequencies of cells at later mitosis stages became equal zero. It is well known that metaphase chromosomes are distributed within equatorial plate by microtubules, which further form a division spindle.

Frequencies curves of later mitosis phases are shown on separated figure (Fig. 5), because their values were significantly lower than for prophase. The frequency of metaphases gradually decreased compared to the control level, becoming almost 2 times lower at 1 mg mL^{-1}. At the maximal dose, frequency of metaphases changed insignificantly. Frequencies of ana-telophases were insignificantly increased at 0.25 mg mL^{-1} in comparison with the control variant, but slowly decreased further. On the maximal HM concentration, this index was strongly decreased. The obtained results probably indicate that there is a secondary blockage of the process of proliferation on the late phases of mitosis. Therefore, cells cannot complete the division and proceed to the interphase, which results in the accumulation of prophase cells. It can be supposed that these effects are also connected with microfiber breach of microtubules. Thus, mitotic spindle cannot properly perform its function of moving the chromosomes to the poles of the cell. At the same time at maximal concentrations of cadmium, the process of dividing practically suppressed, which leads to the almost complete absence of late phases.

Multiple structural chromatin breaches were found in the cells of root apical meristem (Table 1). At the cytogenetic practice (for example, under the influence of ionizing radiation) are commonly observed such aberrations, as the

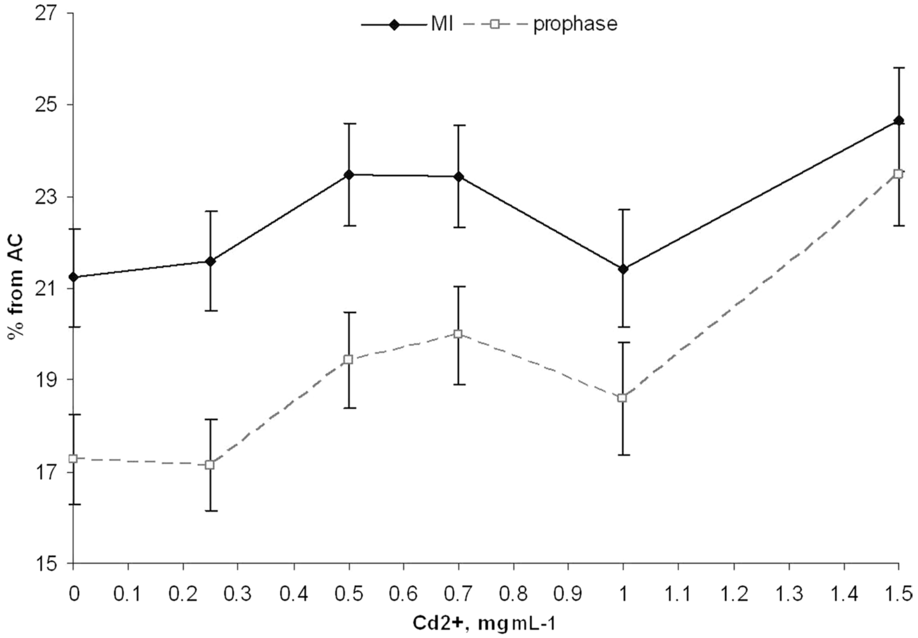


Fig. 4 Mitotic index and prophase frequencies in root apical meristem cells of Zazerskii 85 cultivar seedlings under rising cadmium doses (AC–all cells)

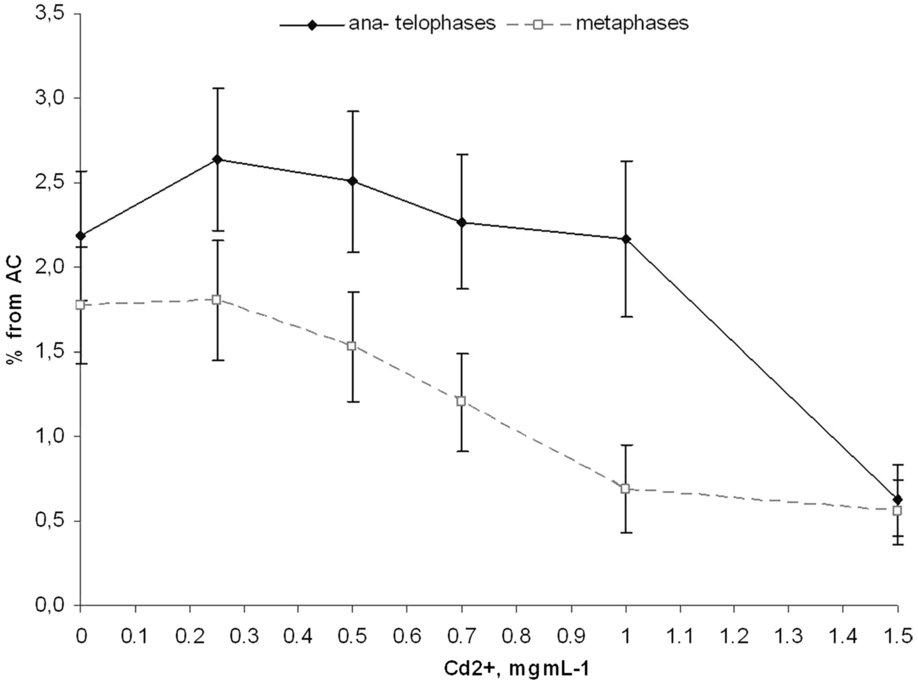


Fig. 5 Frequencies of meta- and ana-telophases in root apical meristem of Zazerskii 85 seedlings

bridges and fragments (Geras'kin et al. 2005). But in our study were not too many of these aberrations found. Frequencies of these anomalies at all studied doses were not higher than in control variant. Moreover, these anomalies were discovered in the control variant, but disappeared after exposure to HM. The most common types of cytogenetic aberrations were lagging of chromosomes and multipolar mitoses, agglutinations of chromosomes, pathologic anaphases, the anomalous dividing of chromosomes. Thus, frequency of chromosome lagging at maximal Cd^{2+} dose was increased at 5.6 times as compared with the control variant, and for multipolar mitosis at 51.7 times. Other types of aberrations were not found in control, but were appeared at Cd^{2+} 0.25–0.7 mg mL^{-1} and disappeared at maximal doses (1–1.5 mg mL^{-1}). However, frequencies of these anomalies at doses 0.25–0.7 mg mL^{-1} did not show any tendencies.

The frequency curve of cells with cytogenetic aberrations (FCA) for the considered range of doses of cadmium is shown on Fig. 6. There was a gradual increase in the frequency of aberrations. To 0.5 mg mL^{-1} development of this

Table 1 Spectrum of cytogenetic anomalies in cells of root apical meristem of Zazerskii 85 seedlings ($P \leq 0.05$)

Cd^{2+}, mg mL^{-1}	Frequencies of different anomalies, %									All
	f′	f″	m′	m″	g	mp	agg	pata	and	
0	0.041 ± 0024	0.151 ± 0045	0.260 ± 0060	0.055 ± 0027	0.383 ± 0072	0.014 ± 0014	0	0	0	0.904 ± 0111
0.25	0.076 ± 0.054	0.038 ± 0.038	0.306 ± 0.108	0.038 ± 0.038	0.650 ± 0.157	0.038 ± 0.038	0.497 ± 0.137	0.612 ± 0.152	0.268 ± 0.101	2523 ± 0.307
0.50	0	0.055 ± 0.055	0.109 ± 0.077	0	0.712 ± 0.197	0.219 ± 0.109	0.985 ± 0.231	0.712 ± 0.197	0.602 ± 0.181	3394 ± 0.424
0.70	0	0	0.168 ± 0.119	0.168 ± 0.119	0.672 ± 0.237	0.252 ± 0.145	0.336 ± 0.168	0.840 ± 0.264	0.084 ± 0.084	2519 ± 0.454
1.00	0	0	0	0.281 ± 0.198	1264 ± 0.419	0.281 ± 0.198	0	0	0	1826 ± 0.502
1.50	0	0	0.483 ± 0.341	0	2174 ± 0.717	0.725 ± 0.417	0	0	0	3382 ± 0.889

f′—single fragment, f″—double fragment, m′—single bridge, m″—double bridge, g—chromosome laggings, mp—multipolar mitosis, agg—chromosome agglutinations, pata—pathologic anaphases and—anomalous dividing of chromosomes

tendency was slow (about 4 times in this period as compared with control variant), in interval 0.5–1 mg mL^{-1} rising of FCA value was accelerated (a value almost 3 times higher than at first interval) and rose even more at the maximal dose (about 2 times). In general, the observed pattern coincides with that noted in the analysis of roots lengths changes. Morphology breaches that were observed on organism level can be provoked by the increase in cytogenetic anomalies frequency. If cytogenetic anomalies frequency is relatively low, plant organism can cope with toxic stress and yield is not significantly decreased. However, discussed above indices of plants and their productivity can be reduced. With the further HM dose increase, efficiency of protective mechanisms gradually drops, and signs of life processes deep oppression can be discovered. HM ions in dose more than 1 mg mL^{-1} can penetrate all protective barriers and negatively affect all organism functions. Thus, the Cd^{2+} dose of 1 mg mL^{-1} can be considered lethal.

At the high cadmium doses (0.7–1.5 mg mL^{-1}), the crystals of some insoluble Cd compounds were noted (Fig. 7), which were situated on the cell walls and into the intercellular space. At the control variant and at the low doses (Cd^{2+} 0.25–0.5 mg mL^{-1}), such crystals were not observed.

4 Discussion

With the progressing development of the industry, heavy metals content in water and soil has significantly increased and, at the present time, it often exceeds permitted limits for the wide range of chemical elements. Accordingly, income of HM ions in biological cycles and living organisms is also significantly increased. (Clemens 2006; Huybrechts et al. 2019). Cadmium is a trace element, which is not so common in the lithosphere and normally can be found in the same minerals as zinc. An average content of Cd in soil is around 0.1–2 ppm (usually below 1 ppm) (Kabata-Pendias and Pendias 2001). Sewage sludges, smelting, phosphate fertilizers, mining and fuel combustion are the major sources of Cd depositions, from which this HM enters to agricultural ecosystems and includes to food chain (Lugon-Moulin et al. 2004; Seifikalhor et al. 2020). However, not all of Cd content is available for plants, but only a part of it. Normally, available for uptake Cd exists at the form of inorganic and organic complexes, or soluble salts (Clemens 2006). Amount of Cd^{2+}, which is available for plants, is depended from many characteristics of soil, such as pH and the organic matter content (Sauve et al. 2000).

Uptake and distribution of Cd^{2+} into roots and its entry into cells —An availability of different metals for plants uptake is not strictly correlated with their content in soil. Thus, metals such as Cr, Ag or Sn have few soluble salts, and because

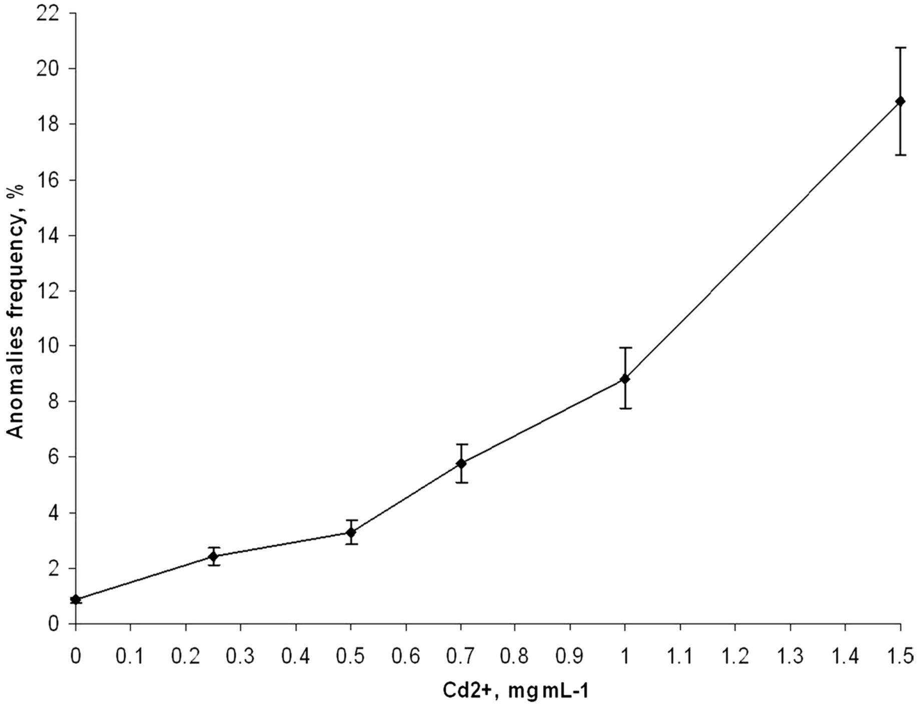


Fig. 6 Frequency of cells with cytogenetic anomalies in Zazerskii 85 cultivar seedlings root apical meristem

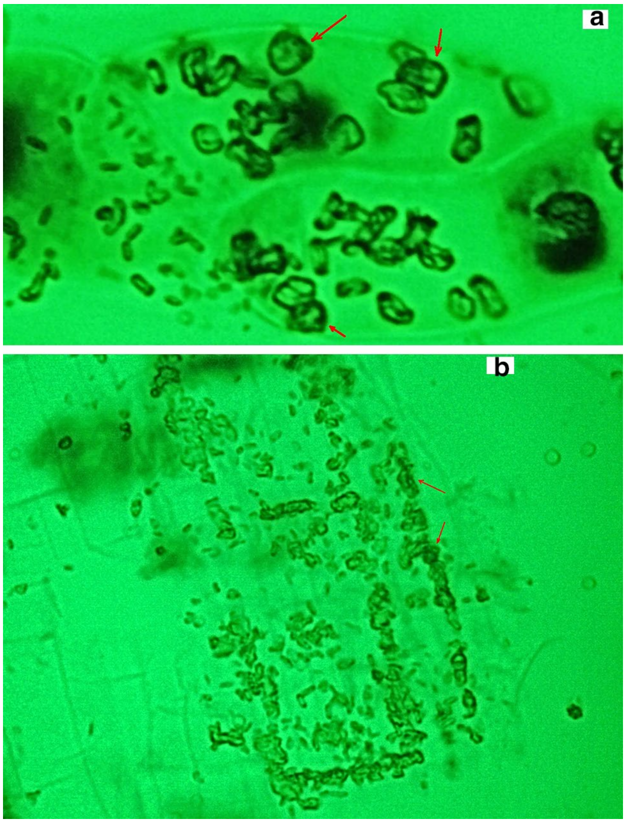


Fig. 7 The insoluble depositions in the barley (Zazerskii 85) seedlings roots apical meristem (Cd^{2+} 1.5 mg mL^{-1}), magnification × 400–600: **a**–in the cytoplasm and vacuoles, **b**–into the intercellular space

of this even a high concentration of these HM in soil does not provoke a significant harm to plants. Pb and Cd also can be widely spread in the ecosystems, but low solubility of their compounds and bounding with the soil particles make them less available for plants.

It is well known that major source of HM entry to the human organism is the accumulation of harmful ions in edible parts of agricultural plants (Kramer and Clemens 2005). In particular, cadmium can relatively easy migrate from the roots to the overground parts of plant (e.g. leaves or fruits, seeds) and from there enters food-chain (Sauve et al. 2000). Thus, considering the bioavailability of metal ions, two processes have a significant meaning: uptake velocity and translocation activity. The toxicity of HM to roots is determined by the number of ions, which is accumulated inside the root cells, and toxicity to shoots is determined by the number of ions, which is translocated from roots to the shoots and after that, is entered shoot cells.

Plant species have differences at the Cd distribution among the tissues (Clemens 2006; Huybrechts et al. 2019). Normally, cadmium can be found at the maximal concentrations in the roots, at lesser in the overground tissues, and at the minimal—in the fruits and seeds (Clemens 2006). The Cd distribution rates are depended of the metal amount, which is being uptaken by the roots and can reach the xylem. Activity of the protection systems, which defend the plant organisms from harmful ions translocation, determines the rate of Cd^{2+} transfer from roots to the overground tissues. The degree of accessible Cd^{2+} into the roots also plays a significant role at this process. The third factor is radial symplastic passage efficiency, and the last is the activity of ion efflux into the xylem vessels from parenchyma cells (Clemens et al. 2002).

From the 1990 s, a significant amount of data has been collected at area of the molecular mechanisms of Cd^{2+} accumulation into plant cells. It is well known that calcium and zinc ions can restrict cadmium from entering to the plant cells. (Clemens 2006; Huybrechts et al. 2019). The cause of this phenomenon is the competition at the ion uptake process. With this, a question can be asked: are the molecular transport systems for the essential cations (Fe^{2+}, Zn^{2+}) uptake also have an ability to transfer Cd^{2+}? Because it is obvious that Cd^{2+} as nonessential ion has no specific transporters, cadmium and some other nonessential ions transport must be processed by the same systems, as essential ones (Maser et al. 2001; Dho et al. 2010; Wang et al. 2014; Demkova et al. 2017).

Some plant species have the ability to bind and fix the HM ions in cell walls and because of that inactivate these toxic agents (Clemens 2006). That ability is determined by chemical and physical properties of some substances, which can be found into the cell walls. The major site for the HM ions binding is the matrix near the cell wall fibrils. Such binding leads to a change in properties of cell walls: they get thickened and mineralized, and their permeability is diminished (Liu et al. 2009a, b).

For example, the cell walls of *Allium cepa* are enriched by pectins (42.4%) and have a significant lesser amount of hemicelluloses (36.6%) in comparison with other monocotyledons (Vanstraelen et al. 2009). It is known (Saxena et al. 2009) that cadmium has an ability to bind with pectins and organic acids, but such affinity to hemicelluloses is lesser and other substances of cell walls practically have no that feature.

Considering this, changing of roots coloration and thickening of their tissues observed at the present study can be determined by the high rates of polysaccharides synthesis, and this process must restrict an uptake of the harmful ions. The binding of Cd^{2+} by polysaccharides of cell walls plays a significant role at regulation of polysaccharide synthesis. The more HM ions are bind at cell walls by polysaccharides, the more such substances are synthesized and excreted by cells. (Wierzbicka 1998).

Changes of roots cell structure – In accordance with Wierzbicka (1998) and Dandan et al. (2011), an uneven thickening of cell walls occurred in response to the HM influence at high doses, while rates of pectines synthesis were increased at any doses. Observed at our research, changes of roots morphology and insoluble deposits in cell walls formation testify that above-mentioned processes are occurred under Cd^{2+} influence. The Cd compounds deposition process has following phases. At first, intensification of the polysaccharide synthesis in the cell wall matrix occurs, and then, deposition of Cd–pectines complexes at proper places of the cell walls began. However, at high HM doses second process can be interrupted. The uneven thickening of the cell wall, which is occurred at the high cadmium doses, can be explained by the last phenomenon. Probably, by reducing the number of root hairs and saturating the cell walls with such substances as callose and suberin (Clemens 2006), the organism tends to eliminate the contaminant from the nutritious solution, which is being uptaken through the root.

Some other works describing cytogenetic and ultrastructural effects of HM on root meristem cells of *A. sativum* L. (Liu et al. 2009a, b) justify our conclusions. The new material, which is needed for new cell wall construction, under HM stress conditions is deposited between sister nuclei, while normally such compounds can be found in the plane of the cell plate (Wade 2009). The accumulation of the cell wall material is processed normally, while its translocation into the cell wall is disturbed.

When the plant organism has the first contact with the pollutant, HM ions can be uptaken by two ways. First of these is the penetration of root tips with the soil (or cultural) solution through apoplast. Second one is the migration through membranes of root tip protoderm and prohypoderm cells by symplast (Antosiewicz and Wierzbicka 1999). HM ions, which enter roots by second mechanism, are provoked manifestation of the harmful effects very early (at few hours), because symplast transport process works much faster than apoplast. (Sougur et al. 2008). However, after some time, that toxic effect diminishes, suggesting that the detoxification is took place (Sougur et al. 2008).

For the first time, process of the cell membrane surface increase and connection of this process with the symplast and apoplast transport was studied at work (Harris et al. 1982) on the model of the germinating barley grains scutellum. Authors found tubular evaginations of the cell membrane that were named ″plasmatubules″, having a 16–23 nm diameter. Later works proved that these plasmatubules are not artifacts (Chaffey and Harris 1985; Soltys et al. 2011), but are the counterparts of cell wall transfer evaginations. The only exception is the case, when the plasmatubules are created for fastening a translocation process between apoplast and symplast (Soltys et al. 2011).

The increase in cell membrane surface is directly connected with the increase in transport rate through it. Herewith, this transport is mainly processed by symplast mechanism (Wade 2009). However, under condition of the HM stress the direction of that transport can be changed. The formation of much evaginations onto the rhizodermis cell membranes signalizes about strong efflux of protons from roots that promotes Fe^{3+} uptake (Clemens 2001). These structures can be found in the roots of different plants, for example, *A. cepa* (Eleftherios et al. 2011).

The plasmatubules can be considered as a marker of active transfer through the root tip cell membrane. It can be assumed that observed in our work HM deposits are situated above plasmatubules, through which Cd^{2+} is being transported. The presumed direction of this transfer may be considered as from the symplast into apoplast of the root tips. This hypothesis can be confirmed by the following arguments.

1. As mentioned in Wierzbicka (1999), plasmatubules, which are the marker of active cross-membrane transportation, can be found after not less than 24 h treatment of roots in HM-enriched solution. It may be supposed that when the cadmium enters into root tip cells, its toxic influence is manifested. Transport at the opposite direction can lead to decreasing of toxic effects, and that phenomenon can be indeed found at some cases (Wierzbicka 1998).
2. At first, the HM insoluble compounds can be found above cell membrane, and after that, in the cell wall. Same structures, which were found at this work (Fig. 7), were described at the literature (Wierzbicka 1998; Antosiewitz and Wierzbicka 1999) into the tissues of *Allium cepa* L., treated by Pb^{2+} and at our previous work, in which response of the barley seedlings to the lead stress was investigated (Dikarev et al. 2014, 2018). The second case is well suited for our study, because such deposits were observed after more than 24 h treatment, and this time is enough for transfer of Cd^{2+} from the protoplast to the cell wall.
3. 3. If the HM treatment continues, the concentration of pollutant in the periplasmatic space constantly rises. In some cases, this process did not end even if the plant was removed from HM-enriched solution (Wierzbicka 1999). However, cadmium disappeared from the primary cell walls, middle lamellas and intercellular spaces. In this case, plasmatubules with the cadmium deposits in their tips can be found. Their formation is marked the process of HM ions transfer into the periplasmatic space through the membranes of cells by symplast mechanism. Some researchers considered this process as the exocytosis by dictyosome vesicles, in which cell wall materials are involved, but others assumed that this is connected with endocytosis (Maluszynska and Juchimiuk 2005; Soltys 2011).

Cytogenetic effects at the root apical meristem – The cytogenetic analysis was well proven as objective, reliable and operative method for bioindication of different xenobiotic effects in living organisms (Qin et al. 2015). Zazerskii 85 cultivar was selected for investigation of barley plants reactions to different cadmium doses on cytogenetic level, because it was found as the most tolerant of Cd^{2+} on the base of previous biometric tests. The choice of more sensitive cultivars could be risky, since the analysis of maximal HM concentrations could be difficult because of the possible suppression of the proliferation process.

The cell mitosis defines root growth. It is reported (Souguir et al. 2008) that at many sensitive to HM influence plant species the disruption of mitosis process occurred. At our study, we collected enough evidence that under Cd^{2+} stress the frequencies of cytogenetic anomalies are raised and mitotic index depression is observed. Herewith, mainly these breaches were connected with the disturbance of the mitotic spindle apparatus (chromosome laggings and agglutinations, pathologic anaphases, etc.), but frequencies of the anomalies, which are normally can be found at the cytogenetic practice (fragments, bridges) did not shown a noticeable increase. Such results can be explained by anomalous condensation of chromosomes and pathologic changes of their conformation. Some authors (Liu et al., 2003; Souguir et al. 2008; Saxena et al. 2010) attribute that phenomenon to the damage to proteins (for example, histones), which control the normal chromosomal arrangement. Considered processes can provoke the appearing of severe cytogenetic anomalies (chromosome laggings and agglutinations, pathologic anaphases, etc.), which in comparison with C-mitosis and chromosome bridges, mark a heavy damage to cells and usually become a cause of cell death (Liu et al. 2004). As concerns the blocking of mitosis process, such effects can provoke the root growth suppression, which was observed in our study.

All eukaryotic cells have some microscopic structures—the microtubules, which are formed by α-tubulins and β-tubulins (Eleftherios and Ioannis-Dimosthenis 2012). They are constructed the cytoskeleton—the micromachinery of cell that controls many processes in living cells (construction of the mitotic spindle and the phragmoplast, cytokinesis, etc.). The microtubules play a significant role in dividing cells, which are changed in dependence of the cell cycle stage. Such functions are: chromosome translocation, expansion and differentiation of cells and motility (Wade 2009; Soltys et al. 2011). Different works (Eleftherios and Ioannis-Dimosthenis 2012; Shimizu et al. 2013) confirmed that microtubules (their synthesis and arrangement) have a high sensitivity to many harmful agents, such as metal ions, temperature and pesticides. Consequently, the cadmium causes destruction of microtubules or prevents their synthesis (Their et al. 2003; Liu et al. 2004; Hattab et al. 2009). Perhaps this is due to the fact that cadmium poisons the active centers of enzymes responsible for microtubules synthesis, or violates their native conformation. Similar results were obtained in our previous study, which was devoted to lead influence on barley plants (Dikarev et al. 2014, 2018), and supported the other researchers works (Wierzbicka 1998; Antosiewitz and Wierzbicka 1999).

In the present study, we observed a high rate of mitotic anomalies, which are connected with the damage to the mitotic spindle (chromosome laggings, multipolar mitosis and anomalous dividing of chromosomes). However, at the highest doses of Cd^{2+} (1–1.5 mg mL^{-1}) proliferation process is practically stopped at the prophase stage, and therefore, such anomalies disappeared. Obviously, it is connected with the ceasing of work of the mitotic spindle apparatus. Notably, frequency of cells with the cytogenetic anomalies is constantly raising to the last dose (1.5 mg mL^{-1}).

The occurrence of multipolar mitoses and pathological anaphases confirms the earlier suggestion (Qin et al. 2015) that cadmium ions cause a violation of the mitosis spindle and the process of cytokinesis. Similar effects were observed under influence of colchicine and other toxins on cells (Pausheva 1974). On the other hand, the appearance of chromosomes agglutinations can be explained by the fact that cadmium causes denaturation of chromatin, and as a result, a large part of the chromosomes turns into an indefinite mass. Such effects were described for other heavy metals (Sharma and Dubey, 2005). The appearing of severe anomalies (chromosome agglutinations, pathologic anaphases and anomalous dividing of chromosomes) are connected with violations of cytokines process. Chromosome agglutinations indicated a high toxic potential of cadmium, because this is irreversible aberration (Qin et al. 2015), which leads to cell death. At maximal doses (1–1.5 mg mL^{-1}), the proliferation process is practically stopped and with this above-mentioned anomalies disappeared. Thus, such Cd^{2+} concentrations can be considered as lethal.

Such effects can be explained by the depletion of microtubules pool and damage to the mitotic spindle, which occur after Cd^{2+} treatment. Such hypothesis can be evidenced by previous studies (Buljan et al. 2001; Dho et al. 2010; Soltys et al. 2011). In addition, the work (Qin et al. 2015) showed that under HM stress an expression of one of the microtubules components—α-tubulin—is decreased. Such effects have a negative influence to the microtubules structure and tend to disrupt their functions. HM influence can provoke an oxidative stress, and damaging of microtubules proteins by the reactive oxygen species can also be the cause of cytogenetic anomalies appearing (Rudrappa et al. 2007). Progressing damage of the microtubules is connected to the diminishing of mitotic activity (Eleftherios and Ioannis-Dimosthenis, 2012). Soltys (2011) observed the damage to the mitotic spindle, which was provoked by depolymerization and disarrangement of microtubules. Such disturbances in the mitotic spindle can explain high frequencies of the mitotic anomalies and proliferation blocking, and eventually, the suppression of root growth. Similar effects were discovered in our work. Contrariwise, such anomalies as fragments are provoked by breaches of the DNA chain, which can be made, for example, by the high-energy particles. HM do not have such ability; therefore, frequencies of these anomalies were not changed with the Cd dose increasing. Moreover, at the high doses (0.7–1.5 mg mL^{-1}) fragments disappeared.

Mechanisms of Cd tolerance – There were many studies of the HM toxicity, which were carried on different plant species and under various conditions. Many of these researches used high Cd^{2+} doses and described effects of the severe Cd stress (Sanita di Toppi and Gabbrielli 1999; Clemens 2006). Visually detectable non-specific symptoms of HM intoxication can manifest as the rapid inhibition of root growth, stunting and chlorosis. Cd influence induces such effects as suppression of enzyme activity, disruptions of mineral nutrition and water balance, changes of hormonal status and membranes permeability (Sanita di Toppi and Gabbrielli 1999). High HM concentrations in the medium lead to death of cells and even the whole plant (Clemens 2006). On the cell level, Cd also suppresses activity of enzymes having–SH functional group (Clemens 2001). HM also inhibit seeds germination and suppress growth process of seedlings. HM ions decrease number of germinating seeds and index of germination, diminish roots and shoots length, tolerance index and dry mass of roots and shoots. In our study, a suppression of roots growth was also observed, which was progressing with the Cd^{2+} concentration rising, and negative changes in root morphology occurred (coloration changes, thickening of roots tissues and roots fibrils disappearing). At high Cd doses, development of the shoots was also suppressed. Apart from these, the plant water balance was disturbed; stomatal opening was inhibited (Sanita di Toppi and Gabbrielli 1999). Cd has no redox activity (it has one oxidize degree 2+) and cannot be drawn in Fenton and Haber–Weiss reactions, but it can provoke the oxidative stress.

In our study, we discovered that certain cadmium dose (0.3–0.4 mg mL^{-1}) allowed separating the different barley cultivars according to their tolerance to this HM. Such dose is high enough for provoking the significant suppression of the barley organism living processes, but it does not cause the severe damage. All these observations are quite consistent with those described in the literature (Murzaeva 2004; Wang et al. 2010; Dandan et al. 2011; Wang and Bjorn 2014). Consequently, different lines or cultivars of the same plants at the equal conditions can have a various potential of tolerance to the harmful agents. In the present study, the most tolerant cultivar was the Zazerskii 85, and the most sensitive was the Zarya. With that, a question may be asked—what factors identify the tolerance of the plant organisms to Cd stress?

It seems that all plant species have some level of the basal Cd tolerance and it can present the significant threat to human health. This threat occurs due to the ability of Cd to be accumulated in the edible parts of some crops without causing visible symptoms of phytotoxicity with the subsequent transfer into human organism (Clemens 2006). Moreover, different lines or cultivars of the same plant have different levels of the HM tolerance. The mechanisms of plants tolerance have a strong connection with the pathways for uptake, accumulation and partitioning of HM. One of these mechanisms is the chelation and sequestration of the harmful ions, during which the HM are removed from the tissues that have tendency to its accumulation. Different activities of the ion efflux also can tend to accumulation in specific sites. Thus, the essence of the basal tolerance decreases the availability of uptaken HM. Apart of that, not so much critical systems of the plant cell can enter the chemical reactions with toxic ions. Generally, it is reached by the two ways sequestration and efflux (Clemens 2001). Both these ways are connected with proteinaceous or low molecular weight compounds synthesis, which play a role of metal chelators. Such substances mediate sequestration and efflux of the HM ions as the intracellular metal buffers and/or interactors of transport proteins. However, tolerance to the specific HM usually has some nuances due to specific chemical properties of specific metal.

In plants, the synthesis and processing of phytochelatins (PC) is the major pathway of Cd toxic influence deceasing. It was evidenced by the inactivation of the phytochelatins synthesis genes on the model of three organisms (*Arabidopsis thaliana* L., *Caenorhabditis elegans* L., *Schizosaccharomyces pombe* L.). At all these cases, the knock-out of the proper gene provoked Cd^{2+} hypersensitivity due to the lack of phytochelatins (Ha et al. 1999). The Cd detoxification pathway by PC includes two stages. At first, the PC–metal complex is synthesized from peptides and metal ions (in this case–Cd^{2+}), and after that, developed complex is transferred into the vacuole. Thus, the combining chelation and transport have equally high meaning for cadmium detoxification. In article (Cobett and Goldsbrough 2002), it is reported that other chelators (such as metallothioneins) in plants do not play a significant role at the Cd detoxification. In plants, the majority of toxicants are finally deposited into the vacuoles. This circumstance stimulates the search of other pathways of the HM sequestration in the vacuoles. Thus, the involvement of divalent cation/H^+ transporters (CAX) in heavy metal/H^+ antiport was evidenced (Hirschi et al. 1996). There is another protein family the cation diffusion facilitator (CDF), which also plays significant role in transfer of Cd^{2+} into the vacuoles (Maser et al. 2001).

From these data, it may be supposed that discovered in our study cadmium tolerant barley cultivars have a higher rates of the chelating compounds synthesis and higher rates of specific gene expression than at sensitive ones. The assessment of the PC (and other metal-binding substances) activity and searching of the genes, which are controlled PC synthesis, can be the topic of future works at area of the barley cadmium tolerance investigation.

A complex of biometric and cytogenetic indices was studied at current work. These indices characterize plant responses to heavy metal stress using cadmium as an example. The reaction of the seedlings of 4 barley cultivars of different geographical origin to a range of increasing concentrations of Cd was studied. It was defined that cadmium provoked suppression of roots growth and the disruption of their morphology. All the HM doses were classified as (1) easily endured by the plants, (2) provoked a significant suppression and (3) presumably lethal. New experimental data about the various aspects of the cadmium influence on processes of the seed germination and seedling development on morphologic and cytogenetic levels were collected. It was revealed that the increase in HM dose led to an increase in cytogenetic anomalies, which were mainly represented by the anomalies associated with the formation of spindle and karyokinesis. Cadmium suppressed the proliferation process and stopped it on the early mitosis phases. With this, the Cd dose of 0.35 mg mL^{-1} can be considered as critical.

It was shown that the reaction of different barley cultivars on the cadmium stress was in general similar. However, it is possible to identify such cultivars (not only barley), which are characterized by high tolerance or sensitivity to the HM influence. Described methods are well proven by our research as suitable for this task. Tolerant ones, in perspective, can be used for agricultural production in the territories with the high rates of Cd (or some other HM) soil pollution. It can help to increase in the main crops productivity and make it yield safe for human health. Sensitive to the HM influence cultivars are interesting object for future works in the field of the plants tolerance mechanisms investigation.

Using literature data, feasible causes of effects discovered in our study were analyzed. Such effects are likely not random and tend to be reproduced on the various test-objects. In general, described effects are common not only for the cadmium influence, but for the large bunch of heavy metals.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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