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The effect of heavy metal salt anions on their toxicity to higher aquatic plants

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The toxic effects of nickel and copper salts with different anions were compared at the initial stages of the ontogenesis of *Bidens cernua*. No toxicity limits of the studied salts for the seed germination have been found, which suggested a high resistance of this species to the effect of HM in comparison with other representatives of aquatic plants. It has been shown that acetates of both metals are more toxic than chlorides for seed germination at high concentrations (500–1000 mg·L^{-1}), as evidenced by a significant decrease in laboratory germination. For the seedling growth, nickel acetate was more toxic than its chloride, as evidenced by a significantly shorter main root and hypocotyl comparing to control. Meantime, copper chloride turned out to be more toxic than copper acetate, which was observed for the development of seedlings. The study indicated the need to take into account anions when comparing the effects of HM salts on plants during the experiments.

Keywords: nickel, copper, chloride, acetate, seed germination, seedling development, *Bidens cernua.*

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Introduction

The issue of controlling the content of heavy metals (HM) in various parts of aquatic ecosystems is becoming of great importance in regard to the increase of anthropogenic environmental impact. Heavy metals enter water bodies with hydrochemical runoff, they are distributed rather quickly over their water masses, accumulating both in water and bottom sediments in various concentrations, sometimes hundreds of times higher than the MPC, and have a toxic effect. Plant organisms as the primary link of the trophic chain are the first to experience the HM effects. The resistance of plants to their effects depends on the species, ecotype, growth stage, metal concentration, exposure period, and it is manifested at various levels of organization of plant organisms (Chukina and Borisova, 2010; Kositsyna et al., 2010; Maleva et al., 2012; Yruela, 2009; Yusuf et al., 2011).

A wide range of concentrations of various HM is studied under experimental and natural conditions in order to determine the adaptive capabilities of aquatic plants. Essential HMs (e.g., copper and nickel) are of particular interest; in high concentrations, they are toxic to the physiological processes of plants (Seregin and Kozhevnikova, 2006; Yruela, 2013). Earlier, the effect of nickel and copper has been considered for several species of aquatic plants (Krylova, 2012, 2013).

Large doses of nickel inhibit growth, productivity, and photosynthesis; promote chlorosis and necrosis of leaves, lead to the browning and inhibition of growth of the root system (Seregin et al., 2003; Titov et al., 2011). Excess copper alters photosynthesis and respiration processes; the changes are especially pronounced in young tissues and organs (Kositsyna et al., 2010).

Some authors believe that the cation plays the main role in the toxic effect of HM salts. An acid radical influences on this effect slightly due to the changes in the solubility or degree of dissociation of particular salt (Dmitrieva et al., 2002). However, there is an evidence indicating that the degree of oxidation of the main element of the anion may affect the toxicity of salts. For example, the toxicity of anions containing chlorine and bromine increases with an increase in their oxidation state, and the anions containing nitrogen and sulfur decrease with an increase in valency (Levina, 1972). Therefore, the study aims to determine the effect of anions of different salts of nickel and copper on the seed germination and morphophysiological indicators of seedlings of nodding beggarticks (*Bidens cernua* Linnaeus, 1753), native hygrophyte, common in wet meadows, in swamp-meadow and coastal-water communities (Brändel, 2004; Lisitsyna and et al., 2009).

Materials and methods

Seed germination was carried out according to the generally accepted methodology (Mezhdunarodnye pravila..., 1969). After 4–5 months of cold wet stratification, 30 seeds were germinated in a luminostat in Petri dishes (90-mm diameter) on filter paper moistened with 15 mL of $NiCl_2$, $Ni(CH_3COO)_2$, $Cu(CH_3COO)_2$, $CuCl_2$ aqua solutions in concentrations of 1, 10, 25, 50, 100, 250, 500, 750, and 1000 $mg{\cdot}L^{-1}$ at a temperature of 20–25 °C. The salt concentrations were recommended by the colleagues from V.L. Komarov Botanical Institute of Russian Academy of Sciences and calculated for each salt per metal ion. High concentrations were used to identify the toxicity limit of nickel and copper for seed germination (i.e., the concentration above which seeds did not germinate). The experiments with acetates and chlorides were carried out at different periods, hence the differences in control parameters, but not in the nature of the manifestation of the effect of different HMs. The experiment lasted for 15 days in triplicates. Distilled water served as control. At the end of the experiment, the germination percentage was calculated as a laboratory germination (Shipley and Parent, 1991). After 15 days, 10 seedlings were randomly selected from each experimental dish to assess the main morphological parameters: the length of the roots (main and secondary), of hypocotyl, of cotyledons, and that of leaves. Microsoft Office Excel 2003 was used for statistical data processing and plotting. The data were presented as means and their standard deviations ($x \pm SD$). Normality of distribution was assessed using Statistica 8.0 software. The significance of differences was evaluated by Student's *t*-test at a significance level of $p \le 0.05$.

Results and discussion

The effect of nickel and copper chlorides and acetates on the seed germination

The seeds in the experiment sprouted together, and their germination in the control and at low concentrations of HM was high, which indicates their normal ripening and ability to germinate (Krylova et al., 2014, 2015).

No significant differences in laboratory germination have been found under the effect of nickel chloride and in control (Fig. 1). No significant stimulation or inhibition of the germination process was observed regardless of the HM concentration. The fluctuant pattern (sequential increase and decrease in the indicator) of changes in laboratory germination should be noted with increasing concentration. This pattern reflected the internal developmental rhythm of this plant species and indicated that the germination process was governed by the interaction of the mechanisms of seed dormancy termination with potential environmental capabilities that met the conditions for germination (Ivanova, 2006). Under the influence of nickel acetate at 25 $mg{\cdot}L^{-1}$ and higher concentrations, a significant decrease by 1.1–3.7 times in laboratory germination was noted comparing to control. This pattern evidenced on a gradual inhibition of the germination process with increasing concentration of the applied salt. Despite the changes observed in laboratory germination, no toxicity limit for nickel for germination of *B. cernua* seeds has been found. The acceleration of germination at 50 $mg{\cdot}L^{-1}$ (chloride) and at 25 and 50 $mg{\cdot}L^{-1}$ (acetate) was probably associated with activation of the mechanisms of protection and detoxification of HM (Rozentsvet et al., 2003). In general, nickel acetate is significantly more toxic than nickel chloride at high concentrations of HM (250–1000 $mg{\cdot}L^{-1}$).

Copper chloride and copper acetate were more toxic for the seed germination than the nickel salts (Fig. 2).

Similar (fluctuant) pattern of laboratory germination has been observed for copper chloride. Its threshold concentration for seed germination was 25 $mg{\cdot}L^{-1}$. At higher concentrations, a significant decrease in percentage germination was noted. Significant differences were noted at 50 $mg{\cdot}L^{-1}$ and higher (1.2–1.7 times) comparing to control. Under the influence of copper acetate, a significant decrease in laboratory germination by 1.1–6.1 times was observed at higher concentrations (100 $mg{\cdot}L^{-1}$). At 1–10 $mg{\cdot}L^{-1}$, the number of germinated seeds exceeded that in control.

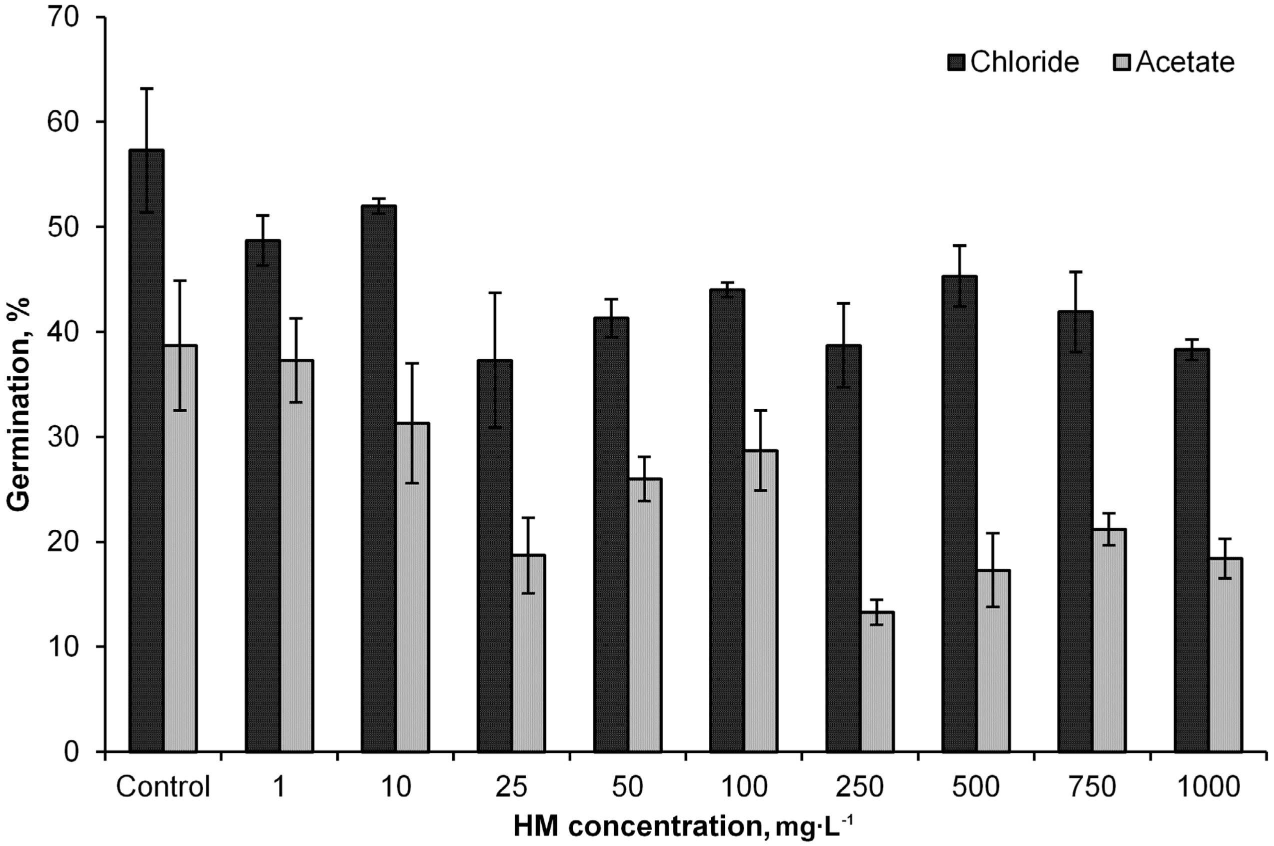

Fig. 1. The effect of nickel salts on the seed germination of *Bidens cernua* under the laboratory conditions.

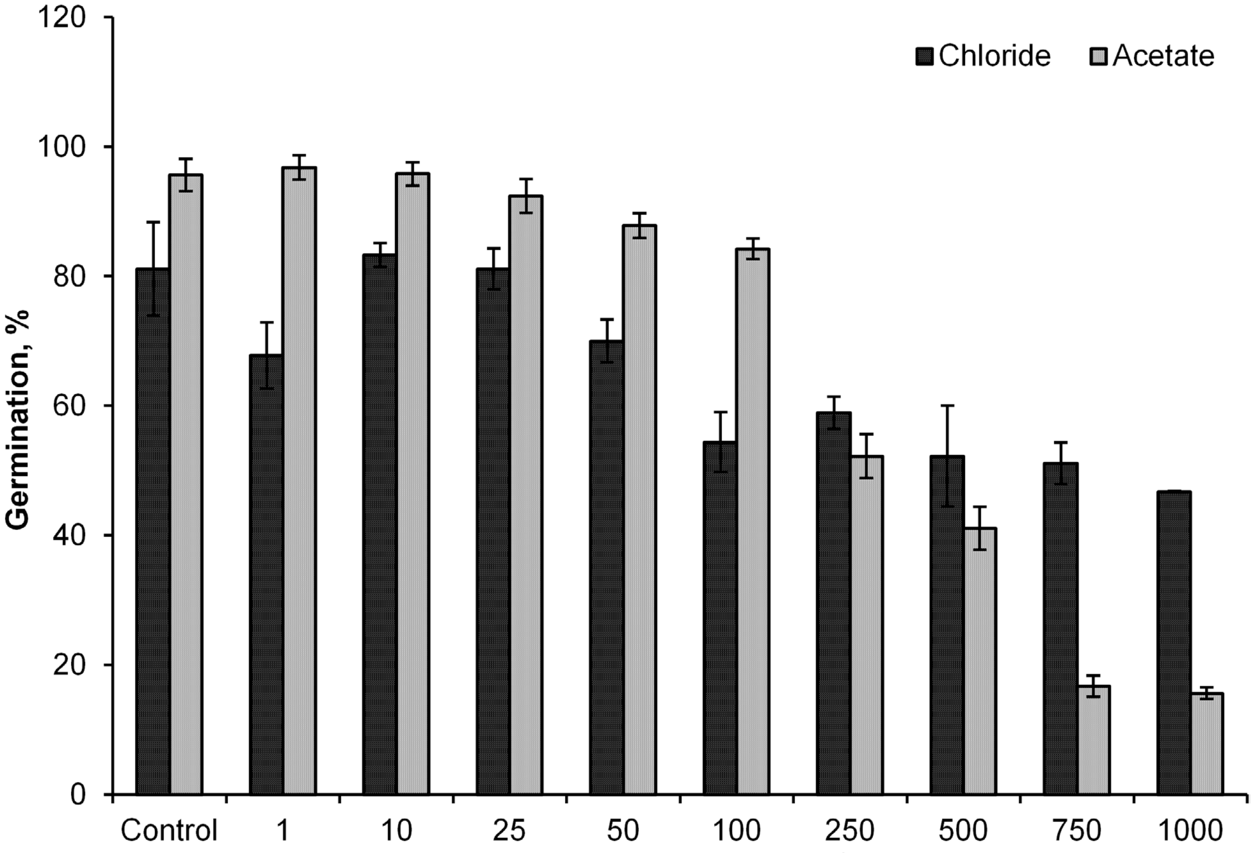

Fig. 2. The effect of copper salts on the seed germination of *Bidens cernua* under the laboratory conditions.

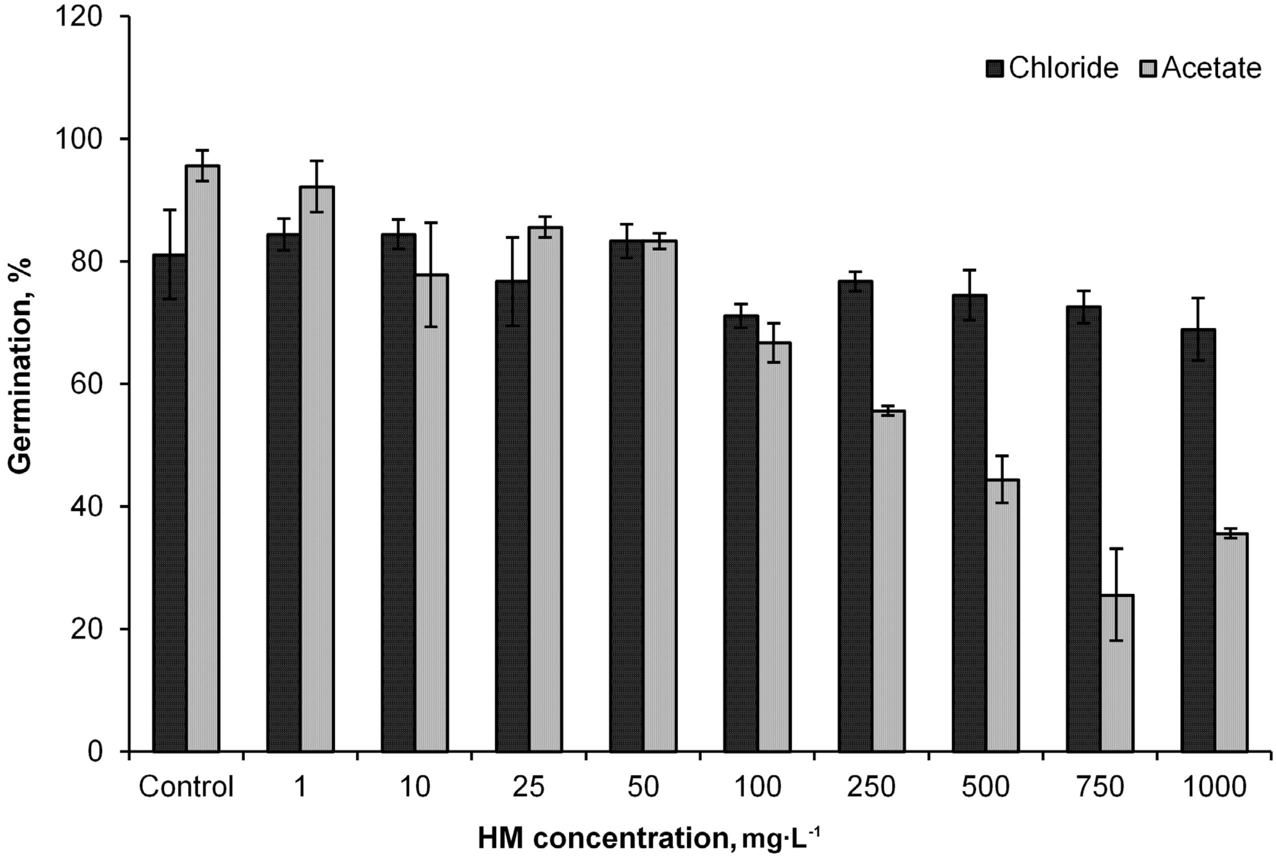

Fig. 3. The effect of nickel salts on the seed germination of *Alisma plantago-aquatica* under the laboratory conditions (Krylova, 2013).

Our data confirmed the literature reports on stimulation of seed germination with low concentrations of HM, which was possibly associated with the activation of cell division and with an increase in cell size (Seregin et al., 2003; Titov et al., 2011). Despite the changes observed in some indicators, no toxicity limit was found for germination of *B. cernua* seeds under the copper effect. Copper acetate was more toxic than copper chloride for seed germination of the studied species at high concentrations (250–1000 mg·L^{-1}), as evidenced by a significant decrease in laboratory germination.

When comparing the effects of nickel and copper acetates with those of chlorides of these HMs, one may easily conclude that acetates are more toxic than chlorides. It should also be noted that in both cases, copper salts are more toxic than nickel salts (at 250–1000 mg·L^{-1}), possibly due to the ability of copper to displace functional metals in enzymes, to interact with biological membranes, and to restore molecular oxygen to its active forms. Copper has an average degree of absorption and may cause a violation of the membrane barriers of the cell, which results in a greater toxic effect compared to nickel (Demidchik et al., 2001). A similar pattern is also characteristic of other species of aquatic plants, for example, *Alisma plantago-aquatica* L., 1753 (Krylova, 2013) (Fig. 3).

In general, seed germination is resistant to the effect of the studied HM. Similar pattern was also noted by other authors and, apparently, was associated with a low permeability for HM of the seed peel of most species; therefore, the period from the beginning of the seed swelling to laceration of the seed peel by the root tip was least susceptible to their toxic effects (Kholodova et al., 2005; Lyanguzova, 1999; Seregin et al., 2006).

The effect of nickel and copper chlorides and acetates on the growth and development of seedlings

Under the effect of nickel salts, the growth and development of seedlings were observed only at 1–25 mg·L^{-1} (Table 1). Both metals had the greatest toxic effect on the main root; significant differences from the control values were noted at all concentrations. The root is the first to experience HM, so it is a barrier to their entry into the aerial part of the plant (Nesterova, 1989). The secondary roots were absent at 1 mg·L^{-1} (nickel chloride) and at 25 mg·L^{-1} (nickel acetate). The development of hypocotyl was significantly more inhibited by nickel acetate. The growth of the aerial part of the seedlings was more resistant to nickel than the underground

Table 1. The effect of different nickel salts on the morphophysiological parameters of *Bidens cernua* seedlings. Values that are significantly different from the control are highlighted in bold.

Heavy metal concentration, mg·L^{-1}	NiCl$_2$					Ni(CH$_3$COO)$_2$				
	Plant organ length, mm									
	Main root	Secondary roots	Hypocotyl	Cotyledon	True leaf	Main root	Secondary roots	Hypocotyl	Cotyledon	True leaf
Control	26.6 ± 4.8	2.1 ± 1.0	29.8 ± 3.5	4.2 ± 0.9	0.9 ± 0.5	47.8 ± 13.2	8.0 ± 6.7	20.1 ± 4.2	4.7 ± 0.6	1.2 ± 0.5
1	**2.1 ± 0.6**	0	**17.2 ± 4.4**	4.3 ± 1.0	0	**21.0 ± 7.2**	3.6 ± 2.8	20.2 ± 2.4	4.4 ± 0.5	1.1 ± 0.2
10	**3.6 ± 1.1**	**1.0 ± 0.9**	27.4 ± 4.3	**4.7 ± 1.0**	1.5 ± 0.4	**2.2 ± 0.7**	**0.3 ± 0.1**	15.3 ± 2.4	4.6 ± 0.4	0.9 ± 0.1
25	**3.7 ± 1.1**	**1.0 ± 0.8**	26.0 ± 3.9	**5.0 ± 0.6**	1.6 ± 0.7	**1.0 ± 0.2**	0	**4.2 ± 1.2**	4.3 ± 0.4	0

Table 2. The effect of different copper salts on the morphophysiological parameters of *Bidens cernua* seedlings. Values that are significantly different from the control are highlighted in bold. "–"– no data.

Heavy metal concentration, mg·L^{-1}	CuCl$_2$					Cu(CH$_3$COO)$_2$				
	Plant organ length, mm									
	Main root	Secondary roots	Hypocotyl	Cotyledon	True leaf	Main root	Secondary roots	Hypocotyl	Cotyledon	True leaf
Control	26.6 ± 4.8	2.1 ± 1.0	29.8 ± 3.5	4.2 ± 0.9	0.9 ± 0.5	47.8 ± 13.2	8.0 ± 6.7	20.1 ± 4.2	4.7 ± 0.6	1.2 ± 0.5
1	**8.4 ± 6.6**	2.8 ± 1.6	29.9 ± 4.1	**5.0 ± 0.9**	**1.9 ± 0.7**	40.5 ± 11.8	8.1 ± 5.6	19.7 ± 2,8	4.5 ± 0.4	1.1 ± 0.2
10	**1.2 ± 0.4**	**0.6 ± 0.7**	**16.4 ± 3.4**	4.4 ± 0.6	1.5 ± 0.5	**0.7 ± 0.2**	**1.5 ± 1.2**	**12.9 ± 2.9**	4.3 ± 0.4	1.3 ± 0.5
25	**1.1 ± 0.3**	**0.05 ± 0.4**	**17.2 ± 3.1**	4.3 ± 0.7	1.6 ± 0.6	**0.4 ± 0.1**	**0.7 ± 0.6**	**10.4 ± 1.9**	4.1 ± 0.3	1.0 ± 0.3
50	–	–	–	–	–	**0.4 ± 0.1**	**0.3 ± 0.1**	**3.8 ± 1.4**	**3.5 ± 0.5**	0.8 ± 0.2
100	–	–	–	–	–	**0.3 ± 0.1**	0	**1.7 ± 0.6**	**3.0 ± 0.4**	0

part. No significant decrease in the size of cotyledons was noted; under the effect of nickel chloride, their morphometric indices increased. The length of the first true leaf did not change significantly, but under the effect of nickel chloride (1 $mg \cdot L^{-1}$) and nickel acetate (25 $mg \cdot L^{-1}$), it did not develop. In general, the greater toxic effect of nickel acetate is clearly evident than that of nickel chloride.

Under the effect of copper salts, there was a difference in the toxic effect between chlorides and acetates of this metal, expressed more than that of nickel (Table 2). Under the effect of copper chloride, seedlings also developed at 1–25 $mg \cdot L^{-1}$, and under the effect of acetate, at 1–100 $mg \cdot L^{-1}$. Both salts had the same effect on the length of the main root; the length of the secondary roots significantly decreased at high concentrations of both salts. At the same time, the number of secondary roots increased, which indicates the stimulation of the protective reaction of plants by increasing the total area of the secondary roots and, therefore, reducing the influence of HM. Copper caused a significant decrease in the length of the hypocotyl, which, together with its noticeable curvature, led to a change in orientation in the space of the main root of the seedling. The linear sizes of cotyledons significantly decreased at 50 and 100 $mg \cdot L^{-1}$ under the effect of copper acetate. However, in general, copper chloride was more toxic than acetate. By the end of the experiment, under the effect of copper, in contrast to nickel, leaf death was noted. Due to the lower mobility of copper, some authors noted its retention in the lower leaves, which later led to their death (Ivanova et al., 2010).

Despite the fact that the growth of the aerial part of the plant turned out to be more resistant to the effect of HM in comparison with the growth of the main root, the HM content in the aerial part noticeably increases with an increase in their concentrations, since root protective barriers cannot cope with a large flow of toxic ions (Titov et al., 2003). This may lead to the loss of seedlings.

At the moment, it is nearly impossible to say which salt, acetate or chloride, of which heavy metal, nickel or copper, is the most toxic for the growth and development of seedlings. Nickel acetate is more toxic than its chloride, but the toxic effect manifested itself in different ways. Nickel chloride significantly inhibited the growth and development of seedlings at a concentration of 1 $mg \cdot L^{-1}$, but an increase in the concentration up to 25 $mg \cdot L^{-1}$ caused an increase in the studied parameters. When studying nickel acetate, inhibition progressed gradually and manifested itself to a greater extent by the end of the experiment. Copper chloride is more toxic than copper acetate; under its effect, the development of seedlings was observed only at concentrations of 1–25 $mg \cdot L^{-1}$. The effect of both salts occurred gradually in regard to their concentrations.

Conclusions

A study of the influence of different salts of nickel and copper (1–1000 $mg \cdot L^{-1}$) on the seed germination and the initial stages of development of *B. cernua* seedlings made it possible to draw the following conclusions:

1. No toxicity limits of the studied HM salts for seed germination have been found, which suggests a high stability of this species compared to other representatives of aquatic plants.
2. Acetates of both metals are more toxic than chlorides for seed germination at high concentrations (500–1000 $mg \cdot L^{-1}$), as evidenced by a significant decrease in the seed germination under laboratory conditions.
3. Nickel acetate is more toxic than nickel chloride for the growth of seedlings (at 10–25 $mg \cdot L^{-1}$), as evidenced by a significant change in the length of the main root and hypocotyl.
4. Copper chloride is more toxic than copper acetate for the growth and development of seedlings (at 10–100 $mg \cdot L^{-1}$).

It is believed that the studied salt group is a derivative of biologically active cations and relatively inactive acid residues (Cl^-, NO_3^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-}, etc.), and the intrinsic toxicity of anions may be neglected (Korshun and Krasnokutskaya, 2011). However, our experiments confirm the conclusion made by other authors that both ions are important for the toxic effect of salts (Levina, 1972). This indicates the need to consider anions when comparing the effects of HM salts on plants.

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