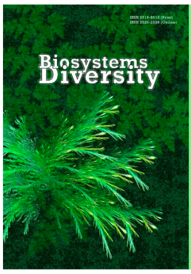

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Resistance of seedlings of native and alien species of the genus Bidens (Asteraceae) from different geographic populations to the action of heavy metals

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One approach to assessing the competitiveness of invasive species is a comparative analysis of the morphological, physiological, and reproductive traits of this species with native species of the same genus. The invasive species *Bidens frondosa* L. from the Asteraceae family, included in the list of the 50 most common and most aggressive invasive species in Europe, occupies the same ecological niche as the native species *B. tripartita* L., and displaces it from natural habitats. There is an obvious and growing interest in the sustainability of *B. tripartita* and *B. frondosa* in extreme conditions of existence, one of which is the action of heavy metals. Our research was performed in laboratory conditions with seedlings that developed from seeds collected from populations of the Upper and Middle Volga region. The seeds were germinated in Ni^{2+} and Cu^{2+} solutions at various concentrations. At the end of the experiment, morphometric parameters were measured and the index of tolerance was determined. Seedlings from different populations under the influence of nickel ions developed at concentrations of 1–50 mg/l, under the influence of copper ions – at 1–100 mg/l. The nickel and copper ions had the greatest toxic effect on the growth and development of the root system – at 25 mg/l and above, the main root was completely necrotic, while the action of copper ions simultaneously increased the number of adventitious roots. The tolerance index ("root test") under the action of nickel ions was higher among the seedlings from the population of the Middle Volga region, while under the action of copper ions there were no significant differences among the seedlings from different populations. However, it decreased with the action of both heavy metals at a concentration of 10 mg/l. High concentrations of both metals significantly reduced the length of the hypocotyl, cotyledon, and the true leaf. It was also found that copper ions are more toxic for the root system (main root and adventitious roots), nickel ions – for above-ground organs (hypocotyl, cotyledons, and true leaves). We noted differences between the populations to the action of nickel and copper. From the population of the Upper Volga region, the seedlings of *B. frondosa* were more stable. For seedlings from the population of the Middle Volga, a smaller toxic effect was confirmed for *B. tripartita*. It can be assumed that the resistance of *B. frondosa* to the action of heavy metals as a stress factor in the Upper Volga region is one of the reasons for the suppression of *B. tripartita* by the invasive species.

Keywords: heavy metals; *Bidens frondosa*; *Bidens tripartita*; morphometric indicators; tolerance index

Introduction

Nowadays, the foreign species are actively introduced into natural ecosystems, occupying stable positions in the composition of the flora, driving local species from the plant communities due to their high competitive ability (Vinogradova et al., 2014; Galkina et al., 2015). An example of such species is *Bidens frondosa* L. (Devil's beggarticks) – a North American invasive species of the Asteraceae family (Abramova & Nurmieva, 2013). *B. frondosa* has had a negative effect on a native species *B. tripartita* L. (three-lobe beggarticks), occupying the same ecological niche, leading to disappearance of *B. tripartita* in the places of occurrence of *B. frondosa* (Vinogradova et al., 2010; Vasilyeva & Papchenkov, 2011; Kovalchuk & Tokhtar, 2013).

The *Bidens* genus is one the most common alien taxa. According to the data of the DAISIE programme, *B. frondosa* is listed as one of 50 commonest and most aggressive invasive species in Europe (Chytry et al., 2008; Vinogradova et al., 2010; Van Kleunen et al., 2015). Its biology and ecology in the conditions of its secondary range have been studied in a number of works (Brandel, 2004; Pyšek & Richardson, 2008; Borisova, 2010; Hovick et al., 2012; Kostrakiewicz-Gierałt & Zając, 2014; Vinogradova et al., 2014; Galkina et al., 2015; Khapugin et al., 2016; Yan, 2016). One of the approaches to the assessment of competitive ability of invasive species is a comparative analysis of morphological, physiological and reproductive features of these species with native species of the same genus (Pyšek & Richardson, 2012). Often studies focus on the reaction of these species to the impact of unfavourable factors (consumption by pests, drought, deficiency of mineral substances, etc.) (Pyšek & Richardson, 2008; Hovick et al., 2012).

Therefore, the stability of *B. tripartita* and *B. frondosa* in extreme conditions of existence is a matter of obvious interest, in particular under the impact of heavy metals. Pollution with heavy metals is one of the consequences of anthropogenic impact on ecosystems of water bodies. The response reactions of plants to the impact of heavy metals depend on the species, ecotype, stage of development, concentration of metals, and manifest at different levels of organisation (Yruela, 2005; Chukina & Borisova, 2010; Yusuf et al., 2011). The physiological role and toxic effect of ions of nickel and copper have mainly been studied for terrestrial plants (Yruela, 2005; Seregin & Kozhevnikova, 2006; Chen et al., 2009; Yusuf et al., 2011). The question of the impact of heavy metals on the development of seedlings of shoreline-aquatic plants, which we have studied, has been insufficiently described in the literature (Krylova, 2011; Timofeeva et al., 2016; Lapirov et al., 2017). Earlier, we analyzed the impact of heavy metals on the initial stages of ontogenesis of the representatives of *Bidens*, which grew in the same geographic conditions (Yaroslavl Oblast) (Krylova & Vasilyeva, 2011a, b). The objective of this study was to determine the response reaction to the impact of nickel and copper ions of seedlings of *B. tripartita* and *B. frondosa* developing from seeds collected in different geographic populations.

Materials and methods

Seeds of *B. tripartita* and *B. frondosa* were collected from the outskirts of Kostroma (Upper Povolzhye – first population) (57º46' N, 40º54' E) and Mariinsky Posad (Middle Povolzhye – second population) (56°07′ N, 47°43′ E). In the places where the seeds were collected, we observed the differences in the duration of vegetative period, annual amplitude of temperatures and mean annual amount of precipitations.

After cold humid stratification in distilled water at a temperature of 4–8 °C, the seeds were cultivated in Petri dishes in a luminostat at 50 seeds per dish at the temperature of 20–25 °C on filter paper moistened with solutions of Ni^{2+} and Cu^{2+} in the volume of 15 ml per dish in different concentrations (1, 10, 25, 50 and 100 ml/g). The control was distilled water. The replication of the experiments was three times, the illuminance – 3200 λк, photoperiod 9/15 (light:dark), the duration of the experiment – 15 days. In the experiment, 900 seeds of each species were used. At the end of the experiment, 20 seedlings of each variant, where the seeds sprouted, were examined: we measured the length of the main root, the hypocotyl and the true leaf, and calculated the number of root initials. The tolerance index (TI) was determined according to the formula: $TI = L_{experiment}/L_{control} \times 100\%$, where TI – tolerance index (%), $L_{experiment}$ – length of sprouted roots grown under the influence of heavy metals (mm), $L_{control}$ – length of roots of the control seedlings (mm) (Willkins, 1978; Samantarey et al., 1997).

The data were presented as mean values and their standard deviations (x ± SD). The statistical reliability of the values was determined according to ANOVA at the significance level of $P < 0.05$.

Results

The influence of nickel on the development of the seedlings of B. tripartita and B. frondosa from different populations. The seedlings of both species of the population from the Upper Povolzhie did not develop at 100 mg/l. Nickel showed the highest toxic effect on the root system (Fig. 1, 2). At 10 mg/l, the seedlings of *B. tripartita* were observed to have a statistically reliable decrease in the length of the main root and the number of root initials. Root initials at 10 mg/l were found only among 1/5 of the seedlings. For the seedlings of *B. frondosa*, the length of the main root was reliably different from the control at 10 and 25 mg/l, root initials were recorded only at 1 mg/l. According to the length of the main root, the index of tolerance to the influence of nickel was determined (Table 1).

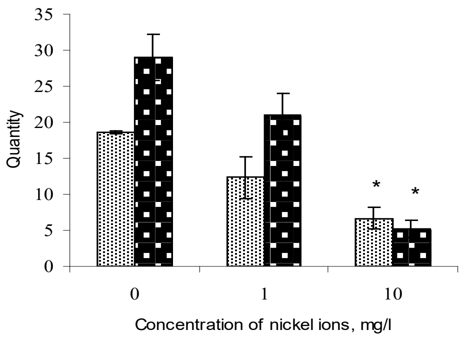

Fig. 1. The influence of nickel ions on the length of the main root of the seedlings from the first population (x ± SD, n = 20): light columns – *B. tripartita*, dark columns – *B. frondosa*; * – reliable differences compared to the control

Table 1
Change in the tolerance index of the seedlings of *B. tripartita* and *B. frondosa* from the first population under the influence of nickel ions

Concentration of nickel ions, mg/l	Tolerance index, %	
	B. tripartita	*B. frondosa*
1	66.68	72.41
10	36.03	18.32

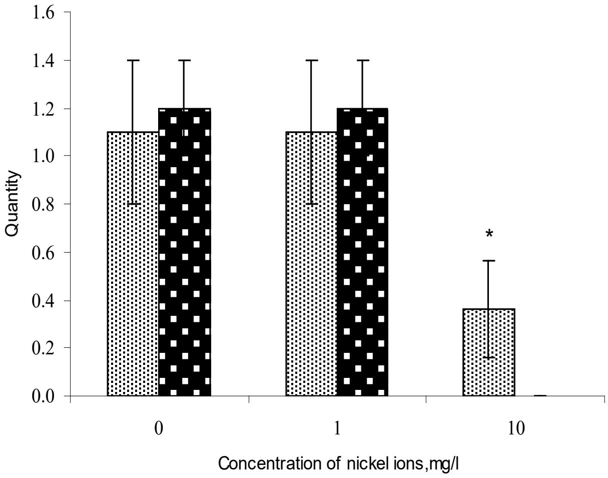

Fig. 2. The influence of nickel ions on the number of root initials of the seedlings from the first population (x ± SD, n = 20): light columns – *B. tripartita*, dark columns – *B. frondosa*: * – reliable differences compared to the control

The length of the hypocotyl of both species decreased significantly compared to the control at 50 mg/l, partial necrosis was observed at 10 mg/l (Table 2). Length of the true leaf decreased significantly at 25 mg/l, at 50 mg/l, it did not form. Length of the cotyledon differed from the values of control at 25 and 50 mg/l, partial necrosis was observed at 25 mg/l.

Table 2
Change in morphometric parameters of the above-ground parts of the seedlings of *B. tripartita* and *B. frondosa* from the first population under the influence of nickel ions at the 15th day (x ± SD, n = 20)

Concentration of nickel ions, mg/l	*Bidens tripartita*			*Bidens frondosa*		
	length of hypocotyl, mm	length of cotyledon, mm	length of true leaf, mm	length of hypocotyl, mm	length of cotyledon, mm	length of true leaf, mm
control	27.51 ± 2.92	8.70 ± 0.21	1.70 ± 0.36	32.52 ± 1.91	9.29 ± 0.28	1.66 ± 0.31
1	21.94 ± 4.11	8.20 ± 0.29	1.26 ± 0.42	30.20 ± 1.12	9.52 ± 0.21	1.87 ± 0.18
10	26.20 ± 4.83	8.32 ± 0.32	1.31 ± 0.41	29.71 ± 2.28	9.20 ± 0.73	1.24 ± 0.43
25	22.52 ± 4.42	7.51 ± 0.27*	0.50 ± 0.23***	27.10 ± 2.77	7.20 ± 0.41*	0.20 ± 0.11***
50	7.30 ± 3.41***	6.80 ± 0.41**	–	5.83 ± 3.01***	2.72 ± 0.49***	–

Note: * – significant differences compared to the control at P < 0.05, ** – significant differences at P < 0.01, *** – at P < 0.001; dash indicates absence of the organ.

The seedlings of both species of the second population also did not develop at 100 mg/l. In the seedlings of *B. tripartita* the length of the main root was significantly higher than *B. frondosa*, the main root of which underwent complete necrosis at 25 mg/l (Fig. 3, 4).

On the whole, the seedlings of both species were observed to develop equally at high concentrations, while nickel was toxic already at 1 mg/l. Using the parameters of the main root length, we determined the tolerance index to the influence of nickel (Table 3).

Table 3
Change in tolerance index among the seedlings of *B. tripartita* and *B. frondosa* from the second population under the influence of nickel ions

Concentration of nickel ions, mg/l	Tolerance index, %	
	B. tripartita	*B. frondosa*
1	92.5	72.1
10	53.1	28.9

In the remaining variants, no differences from the development of the seedlings of the first population were observed (Table 4).

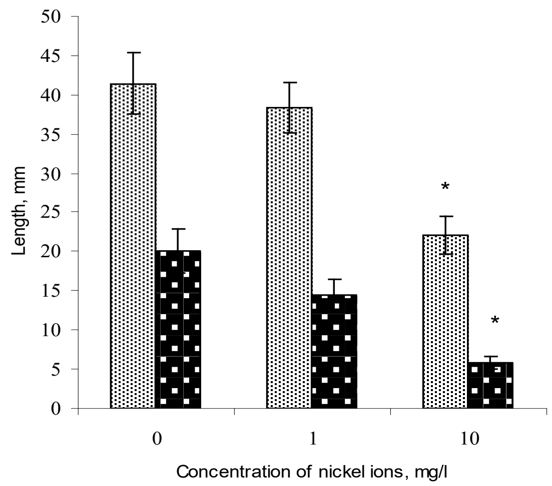

Fig. 3. Influence of nickel ions on the length of the main root of the seedlings from the second population (x ± SD, n = 20): light columns – *B. tripartita*, dark columns – *B. frondosa*; * – significant differences compared to the control

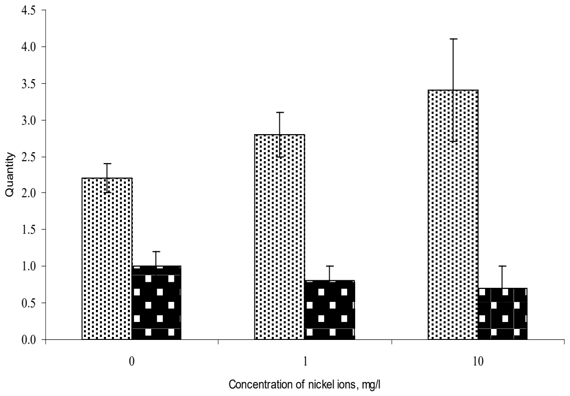

Fig. 4. Influence of nickel ions on the number of root initials from the second population (x ± SD, n = 20): light columns – *B. tripartita*, dark columns – *B. frondosa*

Table 4
Change in morphometric parameters of the above-ground part of the seedlings of the second population under the influence of nickel ions at the 15th day (x ± SD, n = 20)

Concentration of nickel ions, mg/l	*Bidens tripartita* length of the hypocotyl, mm	length of the cotyledon, mm	length of the true leaf, mm	*Bidens frondosa* length of the hypocotyl, mm	length of the cotyledon, mm	length of the true leaf, mm
control	36.18 ± 2.02	9.29 ± 0.18	2.04 ± 0.31	30.37 ± 1.67	8.96 ± 0.34	1.42 ± 0.41
1	29.91 ± 2.08*	9.33 ± 0.42	2.01 ± 0.28	34.64 ± 1.22	9.10 ± 0.18	1.65 ± 0.63
10	35.86 ± 1.54	8.96 ± 0.26	1.40 ± 0.23	32.92 ± 1.07	9.34 ± 0.59	0.70 ± 0.22
25	18.71 ± 1.88**	9.10 ± 0.69	0.22 ± 0.09***	24.80 ± 3.78	7.82 ± 0.72	0.40 ± 0.21*
50	6.50 ± 2.42***	7.71 ± 0.61*	0.30 ± 0.32***	3.10 ± 0.91***	1.90 ± 0.48*	–

Note: * – significant differences compared to the control at P < 0.05, ** – significant differences at P < 0.01, *** – at P < 0.001; dash indicates absence of the organ.

Influence of copper on the development of B. tripartita and B. frondosa from different populations. The seedlings of both species from the first population developed at all concentrations. At 100 mg/l *B. tripartita* and *B. frondosa* at 25–50 mg/l were observed to have root initials performing the basic function, for the main root had undergone complete necrosis (Fig. 5, 6).

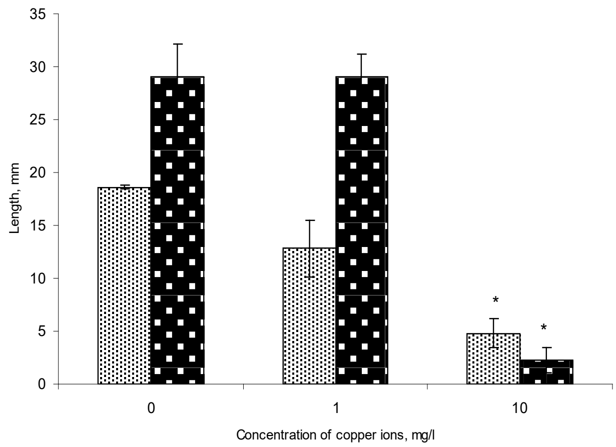

Fig. 5. Influence of copper ions on length of the main root of the seedlings of first population (x ± SD, n = 20): light columns – *B. tripartita*, dark columns – *B. frondosa*; * – significant differences compared to the control

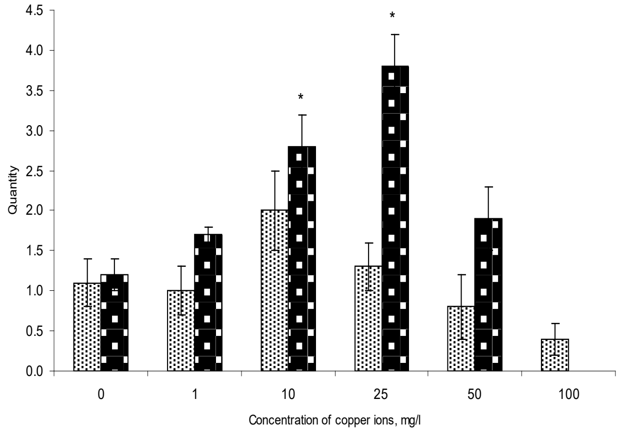

Fig. 6. Influence of copper ions on the number of root initials of the seedlings of the first population (x ± SD, n = 20): light columns – *B. tripartita*, dark columns – *B. frondosa*; * – significant differences compared to the control

On the basis of the parameters of the main root length, the index of tolerance to the influence of copper was determined (Table 5).

Table 5
Change in tolerance index among the seedlings of *B. tripartita* and *B. frondosa* of the first population under the influence of copper sulphate solutions

Concentration of copper ions, mg/l	Tolerance index, % *B. tripartita*	*B. frondosa*
1	68.8	100.3
10	25.8	7.9

Statistically reliable differences in the main root length and the cotyledon were observed at lower concentrations (Table 6). At 100 mg/l, the cotyledons and the true leaves were absent. The seedlings of both species of the second population also developed under all concentrations. Starting from 25 mg/l, the development of root initials was observed at complete necrosis of the main root (Fig. 7, 8). By the parameters of the main root length, the index of tolerance to the influence of copper was determined (Table 7). The hypocotyl significantly differed from the control at the same concentrations, the cotyledon – at higher concentrations, and leaves – lower compared to the first population (Table 8).

Discussion

In previous studies, we demonstrated that the toxic effect of ions of nickel and copper was at its greatest on the growth and development of the root system of the seedlings of the studied species (Krylova & Vasilyeva, 2011a, b). In the seedlings of the first population, nickel was more toxic for *B. tripartita* – the main root underwent necrosis at 25 and 50 mg/l. However, at 10 mg/l, the root initials this species remained, while among *B. frondosa*, they were observed only at 1 mg/l. This indicates stimulation of the protective reaction of the seedlings by maintaining the total area of roots, and therefore decrease in the influence of heavy metals. Such effect of nickel on the root initials was also observed in other species (Samantarey at al., 1997). The tolerance index allows one to assess the resistance of species to heavy metals. The tolerance index decreased by 1.9 and 3.9 times respectively. Resistance to nickel at the level higher than 50% was maintained by both species only at 1 mg/l.

Table 6
Change in morphometric parameters of the above-ground part of the *B. tripartita* and *B. frondosa* seedlings of the first population under the influence of copper on the 15th day (x ± SD, n = 20)

Concentration of copper ions, mg/l	*Bidens tripartita*			*Bidens frondosa*		
	length of the hypocotyl, mm	length of the cotyledon, mm	length of the true leaf, mm	length of the hypocotyl, mm	length of the cotyledon, mm	length of the true leaf, mm
control	27.51 ± 2.92	8.70 ± 0.19	1.70 ± 0.37	32.52 ± 1.91	9.29 ± 0.33	1.66 ± 0.32
1	20.93 ± 4.08	8.91 ± 0.32	1.42 ± 0.41	32.21 ± 2.18	10.28 ± 0.19*	2.43 ± 0.21
10	18.29 ± 2.83**	8.24 ± 0.28	1.34 ± 0.39	28.34 ± 2.13	9.90 ± 0.72	2.50 ± 0.53
25	19.68 ± 1.12*	6.70 ± 0.83*	0.70 ± 0.22**	20.79 ± 1.29**	7.61 ± 0.28*	1.46 ± 0.29
50	4.93 ± 2.86***	6.64 ± 0.92*	0.20 ± 0.08***	13.02 ± 1.37***	6.92 ± 0.51*	0.30 ± 0.08***
100	5.41 ± 1.62***	–	–	5.55 ± 1.89***	–	–

Note: * – significant differences compared to the control at P < 0.05, ** – significant differences at P < 0.01, *** – at P < 0.001; dash indicates absence of the organ.

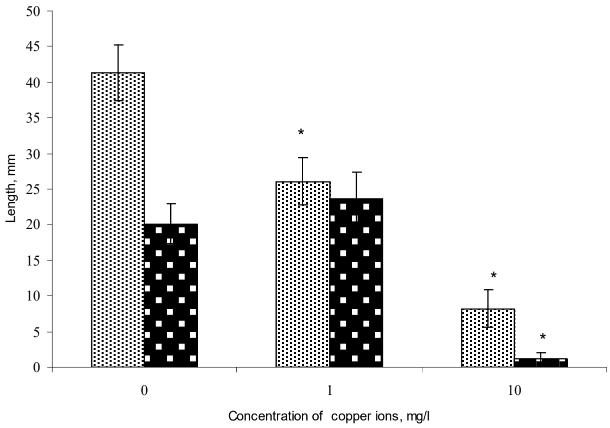

Fig. 7. Influence of copper ions on length of the main root of the seedlings of the first population (x ± SD, n = 20): light columns – *B. tripartita*, dark columns – *B. frondosa*; * – significant differences compared to the control

The above-ground part reacts to the heightened concentrations of heavy metals in the environment to a lesser extent. At 50 mg/l, the seedlings of both species were observed to show development of both the hypocotyl and cotyledon, and only the true leaves failed to form. That is, the above-ground parts of both species developed in the same way.

The *B. tripartita* seedlings of the second population were observed to be more resistant than *B. frondosa* to the impact of nickel – they had a longer main root and more root initials, which maintained at 10 mg/l for both species. The tolerance index decreased by 1.7 and 2.5 times respectively, and remained higher than 50% for the first species at 1 and 10 mg/l, whereas for the second species – only at 1 mg/l. However, the concentration of 25 mg/l proved toxic for both species. With the above-ground part, the higher toxic effect was observed for *B. tripartita* – 1 mg/l concentration was toxic for the hypocotyl. However, the cotyledon and the leaves were more affected by nickel in the case of *B. frondosa*.

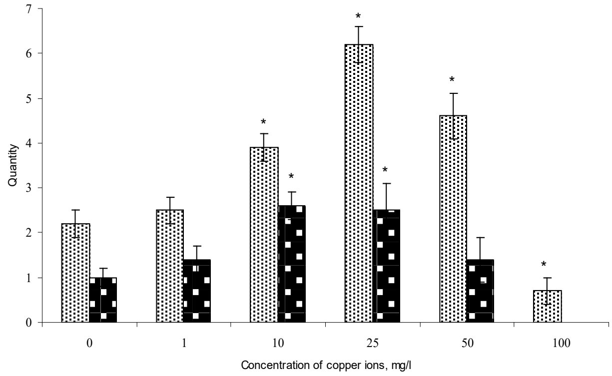

Fig. 8. Influence of copper ions on the number of root initials of the seedlings of the second population (x ± SD, n = 20): light columns – *B. tripartita*, dark columns – *B. frondosa*; * – significant differences compared to the control

Table 7
Change in tolerance index among the *B. tripartita* and *B. frondosa* seedlings of the second population under the influence of copper

Concentration of copper ions, mg/l	Tolerance index, %	
	B. tripartita	*B. frondosa*
1	63.1	117.4
10	19.8	6.0

Table 8
Change in morphometric parameters of the above-ground parts of the *B. tripartita* and *B. frondosa* seedlings of the second population under exposure to copper ions on the 15th day (x ± SD, n = 20)

Concentration of copper ions, mg/l	*Bidens tripartita*			*Bidens frondosa*		
	length of the hypocotyl, mm	length of the cotyledon, mm	length of the true leaf, mm	length of the hypocotyl, mm	length of the cotyledon, mm	length of the true leaf, mm
control	36.18 ± 2.01	9.29 ± 0.23	2.04 ± 0.33	30.37 ± 1.73	8.96 ± 0.32	1.42 ± 0.38
1	31.82 ± 1.13	9.63 ± 0.29	1.12 ± 0.19**	32.83 ± 2.02	9.43 ± 0.31	2.41 ± 0.62
10	28.98 ± 2.09	8.80 ± 0.41	1.10 ± 0.31**	28.34 ± 1.14	9.60 ± 0.19	1.34 ± 0.21
25	20.60 ± 1.78*	8.15 ± 0.38	1.03 ± 0.38**	17.46 ± 2.01**	8.74 ± 0.28	1.59 ± 0.44
50	17.14 ± 1.47**	6.92 ± 0.39*	0.82 ± 0.29***	17.38 ± 1.98**	4.72 ± 0.31**	0.63 ± 0.32
100	4.90 ± 1.11***	–	0.24 ± 0.09***	4.22 ± 1.49***	–	0.34 ± 0.18***

Note: * – significant differences compared to the control at P < 0.05, ** – significant differences at P < 0.01, *** – at P < 0.001; dash indicates absence of the organ.

On the whole, for the seedlings of both populations, we observed that they developed at metal concentrations of 1–50 mg/l. At the same time, the root system was formed at 1 and 10 mg/l, the tolerance index was higher among the seedlings from the second population.

Increasing doses of heavy metals in plants cause first of all inhibition of the growth of the roots (Titov et al., 2014). This occurs because the roots are the first barrier in the path of the metals and perform the basic function in their accumulation and detoxication. A number of researchers have proven that apart from general metabolic disorder, nickel causes decrease in flexibility of the cellular walls (Seregin et al., 2006). It was also mentioned that the toxicity of nickel manifests in chlorosis and necrosis, and also inhibition of growth of the root and the shoot due to the cessation of cell division in the meristem zone (Moya et al., 1993; Molas, 1997; Ivanov et al., 2003). Inhibition of the formation of side roots distinguishes the toxic effect of nickel from the impact of other heavy metals (Ivanov et al., 2003; Seregin et al., 2006). Negative impact of heavy metals manifests also in inhibition of growth of the above-ground part of the plants, though to a lesser extent compared to the roots. Similar processes were observed in our experiments, the growth was inhibited both in the underground and above-ground organs, but to different degrees.

With seedlings of the first population, copper was more toxic for *B. tripartita*. Concentration of 1 mg/l caused stimulation of growth processes in the main root of *B. frondosa*. At 1–25 mg/l, the number of the root initials increased in both species. However, on contrast to nickel, copper turned out to be more toxic at 10 mg/l – the tolerance index fell for both species by 2.7 and 12.7 times respectively. The fact that copper is more toxic is proved also by the development of the root initials at 25–100 mg/l, which were used by the seedlings for reducing the impact of copper ions. As for the above-ground part of the seedlings, we observed an identical impact of copper on both species, at the same time, low concentrations had a stimulating effect on some parameters, and high concentrations significantly inhibited the growth, especially of the hypocotyl.

The *B. tripartita* seedlings of the second population were observed to be more resistant than *B. frondosa*. Despite the stimulation of growth of the main root and increase in the number of the root initials at 1 mg/l, a significant decrease in these parameters was observed at higher concentrations of copper. The tolerance index decreased by 3.2 and 19.6 times respectively. The hypocotyl increased in sizes at 1 and 10 mg/l in the seedlings of *B. frondosa* and decreased in *B. tripartita*. However, significant changes took place mostly at 25–100 mg/l. The cotyledons significantly changed at 50 mg/l and were absent at 100 mg/l. We can state that the reactions of the above-ground parts of the seedlings of both species to the impact of copper was the same.

In general, for the seedlings from both populations, we determined that under the impact of copper, they developed at concentrations of 1–100 mg/l. The main root formed at 1 and 10 mg/l, the root initials – at 1–100 mg/l. At the same time, there were no significant differences in tolerance index between the seedlings from different populations.

The literature mentions that copper is less mobile compared to nickel, therefore it is stored in the roots and slowly transfers to the above-ground part of plants (Ivanova et al., 2010). In the presence of copper, growth of the root becomes inhibited due to its impact on the growth of cells manifested in stretching and due to shortening of the cells which stop growing (Obrucheva et al., 1997). This may also be related to damage to the penetrability of the membrane due to increase in the amount of active forms of oxygen and increase in lipid peroxidation (Maksymiec, 2007; Sharma et al., 2009; Yruela, 2009; Rozentsvet et al., 2011). Stimulation of growth processes in the root system at low concentrations of copper can be caused by increase in mitotic activity of meristematic cells. One of the reasons for growth inhibition at high concentrations is disorder in donor-acceptor relations between the organs of a plant. Being a donor of the most important substances for the functioning of the shoot (nucleic acids, amino acids, cytokinin and abscisic acids), the root system can affect the course of its growth processes through changes in qualitative and quantitative composition of xylary exudate.

The abovementioned reasons of both inhibition and stimulation of growth processes of the root system apply substantially to the shoot as well. However, the shoot is less sensitive to the impact of metal than the root system, which is seen in its lower degree of inhibition. The symptoms of negative impact of copper on the shoots are chlorosis of leaves and the maroon colour of the basal part of the stem (Makarova, 2009).

Conclusion

The seedlings of both populations developed under the impact of nickel in concentrations of 1–50 mg/l. For the *B. tripartita* seedlings from the first population, nickel was more toxic than for *B. frondosa* – the main root of the first species necrosed at 25 and 50 mg/l. The tolerance index decreased by 1.9 and 3.9 times respectively, and remained at the level higher than 50% in both species at 1 mg/l. The seedlings of *B. tripartita* from the second population were more resistant than the *B. frondosa* seedlings – they had a longer main root and a higher number of the root initials. The tolerance index was over 50% in the first species at 1 and 10 mg/l, in the second – at 1 mg/l. At 50 mg/l, in the seedlings of both species from the first population, the hypocotyl and cotyledon developed, and the true leaves did not form. The tolerance index decreased by 1.7 and 2.5 times respectively and remained over 50% for the first species at 1 and 10 mg/l, for the second – only at 1 mg/l. For the above-ground part of the seedlings from the second population, a significant toxic effect was observed for *B. tripartita* – the hypocotyl significantly decreased at 1 mg/l. However, the cotyledon and the leaves of *B. frondosa* were also affected by nickel.

Under the impact of copper, the seedlings of both populations developed at concentrations of 1–100 mg/l. Copper affecting the seedlings from the first population was more toxic for *B. tripartita*. At 1 mg/l, *B. frondosa* was observed to show stimulation of growth processes in the main root. However, copper was more toxic at 10 mg/l – the tolerance index fell for both species by 2.7 and 12.7 times respectively. The *B. tripartita* seedlings from the second population were more resistant compared to the *B. frondosa* seedlings. The tolerance index decreased by 3.2 and 19.6 times respectively. For the above-ground part, a similar effect of copper on both species was observed, low concentrations stimulated some parameters, and high concentrations significantly inhibitted the development of the seedlings, especially the hypocotyl.

In general, it was observed that copper is more toxic for the root system and nickel – for the above-ground organs. There were also found populational differences for two species: the *B. frondosa* seedlings were more resistant to the impacts of nickel and copper. As for the second population, the *B. tripartita* seedlings were more resistant. We can assume that tolerance of *B. frondosa* to the impact of heavy metals in the Upper Volga region is one of the reasons for suppression of *B. tripartita* by the invasive species. Continued research is necessary in order to confirm the data of the results presented in this article.

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