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Reaction of Aquatic Plants of Small Rivers of the Turkestan Region of Kazakhstan to Heavy Metal Ions

Akmaral Issayeva[1], Zhanar Myrzabayeva[2*], K. Kidirbayeva[3], Talgat Ibragimov[3], G. Baitasheva[2], Assel Tleukeyeva[3]

[1] Shymkent University, Zhybek Zholy St 131, Shymkent 160031, Kazakhstan
[2] Kazakh State Woman Pedagogical University, Almaty 050000, Kazachstan
[3] M. Auezov South Kazakhstan University, Tauke Khan Avenue 5, Shymkent, Kazakhstan
* Corresponding author's e-mail: myrzabayeva1976@mail.ru

ABSTRACT

Heavy metals are some of the environmental pollutants that have a serious impact on the environment. The analysis of hydromacrophytes growing in small rivers of the south of Kazakhstan with different contents of heavy metal salts revealed that the morphometric indicators of the same plant species differ significantly, depending on the level of total mineralization of the aquatic environment in different rivers of the Turkestan region. It has been established that two plant species can be used to bioindicate the content of lead ions in the aquatic environment: *Azolla caroliniana* Willd. and *Veronica beccabunga* L., which must be introduced into the tested aqueous solutions in the amount of 1.0 kg/m^3 and 1.5–2.0 kg/m^3, respectively, to fully cover the water column at different depths. The first morphological changes in plants, in the form of destruction of the structure of chloroplasts along the edges of unfolded leaves in *A. caroliniana* Willd. and slight withering of the lower underwater leaves in *V. beccabunga* L., occur already at a 1.5 mg/l Pb^{2+} content in water, and a further increase in the content of lead ions in water to 600.0–800.0 mg/l leads to the death of plants.

Keywords: hydromacrophytes, heavy metals, bioindication, morphological changes, accumulation, lead content.

INTRODUCTION

The problem of rational use of water resources is relevant for all territories of our planet, especially for countries with arid climate, including Kazakhstan. Therefore, the issues of water pollution by various pollutants, including heavy metal ions, remain open and sometimes require an individual solution for each object. On the other hand, due to various circumstances, it is not always possible to record the facts of contamination of water bodies with heavy metal ions, which requires the use of alternative methods of ascertaining or indicating the level of water pollution. Biological objects, such as microorganisms or higher vascular plants, are most often used for such dual purposes [Eid, 2020a].

Studies [Glibovytska, 2019] show the possibility of using *Salix viminalis* L., *Helianthus tuberosus* L. and *Medicago sativa* L. for the reclamation of oil-contaminated areas. The authors have identified the factors limiting the growth of *Salix viminalis* L. on recultivated soils, both dry air and lack of moisture in the soil, and *Medicago sativa* turned out to be very sensitive to the content of petroleum products in the soil and can serve as a bioindicator of the level of soil pollution. According to the results of studies [Samecka-Cymerman, 2011], it was revealed that the needles and bark of *Taxus baccata* are capable of accumulating Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb from polluted atmospheric air.

A high purifying effect of higher aquatic plants or hydromacrophytes is achieved where water flows through a community of semi-submerged, floating and submerged plants. The mucus (periphyton) present on the surface of plants, as well as a decrease in the velocity of fluid flow in the overgrowth zones, contribute to the deposition of suspended substances of organic and mineral origin of water. For water purification and disposal of waste sludge, lake reeds as well as narrow- and broad-leaved cattails are used [Obarska-Pempkowiak, 2003]. Lake reed intensively cleanses water from phenols. In addition to phenol, its derivatives (pyrocatechin, resorcinol, xylene, etc.) are also absorbed. Macrophytes play no less a role in photosynthetic aeration processes than phytoplankton [Osei, 2019]. They are able to accumulate various elements in their body [Eid, 2020b]. In the process of mineral nutrition, higher aquatic plants absorb and utilize a significant amount of substances in their organs. The ability of reed tissues to accumulate various heavy metals, such as nickel and lead, is very valuable. The coefficient of movement of Ni and Pb from the lower to the aboveground organs was usually > 1. Thus, this type of reed is a potential candidate for phytoextraction of Ni and Pb [Klink, 2017].

While investigating the ability to accumulate trace elements in two chelophytes *Typha latifolia* and *Phragmites australis,* it was found that Mn, Fe and Cd accumulated in large amounts in the leaves of P. australis, while Pb, Zn and Cu accumulated in the leaves of *T. latifolia*. It was found that both plants accumulated the Fe, Cu, Zn, Pb and Ni metals along the following gradient: roots > rhizomes > leaves > stems. The author found that high bioaccumulation coefficients and low translocation coefficients for zinc, manganese, lead and copper show the prospects of using *T. latifolia* and *P. australis* in phytostabilization of aquatic ecosystems.

When studying the possibility of copper, nickel and cadmium accumulation by hydromacrophytes, it was found that cadmium accumulates the least in plants [Senze, 2017]. The accumulation of nickel and copper depended on the content of these metals in polluted waters, so the content of the three metals in plants varied between Cd<Ni<Cu (average 0.46 < 1.91 < 5.56) in one year and Cd<Cu<Ni (0.0036 < 0.0107 < 0.0115) the following year. Statistically significant differences were revealed between the pH of water and the content of all metals in ode and the content of cadmium in plants.

In studies [Eid, 2020b] plants: *Eichhornia crassipes* (Mart.) Solms, *Ludwigias tolonifera* (Guill. &Perr.) P.H. Raven, *Echinochloa stagnina* (Retz.) P. Beauv. and *Phragmites australis* (Cav.) Trin. exSteud.) were considered as promising bioindicators of the state of water pollution with cadmium, nickel and lead ions of wetlands [Abdelaal, 2021]. The possibility of cleaning the polluted waters of three rivers in the Nile Delta from a number of heavy metals using seven types of hydromacrophytes was studied: *Cyperus alopecuroides, Echinochloas tagnina, Eichhornia crassipes, Ludwigia stolonifera, Phragmites australis, Ranunculus sceleratus* and *Typha domingensis*. It was found that the heavy metal contents in the sediments of the three drains were ordered as follows: Fe>Mn>Zn>Cu>Pb>Cd>Ni>Co, depending on the metal content in the sediments. At the same time, the sediment exceeded the world limit concentrations for Cu, Zn, Pb, but was within safe limits for manganese, cadmium, nickel and cobalt. It was found that *P. australis* accumulated the highest concentrations of iron, cobalt, cadmium and nickel from the studied composition of hydromacrophytes, while *E. crassipes* contained the highest concentrations of copper, zinc, manganese and lead.

It should be noted that the behavior of heavy metals in the environment and their potential threat are influenced by both geographical landscape and weather and climatic conditions. Thus, the analysis of chemical speciation of a number of metals in the surface sediments of the Wujiang River (China) [Cai, 2021] showed that the average total concentrations of all heavy metals, except arsenic, were higher than their background values. At the same time, Cu, Zn, Pb, As and Hg were present in the residual fraction. According to the riskassessmentcode (RAC) classification, copper, zinc, lead, arsenic and mercury presented relatively low risk in this research area; in contrast, manganese and cadmium presented high risk in most sampling sites. The levels of heavy metal contamination in surface sediments are in the following sequence: Cd>Zn>Cu>Pb>Hg>Mn>As.

Thus, it can be summarized that the role of hydromacrophytes in the accumulation of heavy metals from the aquatic environment is undeniable, but the reaction of plants themselves to the influence of individual heavy metal ions depends on many factors and this can serve as one of the criteria for bioindication of the level of water pollution.

OBJECTS AND METHODS OF RESEARCH

The object of the study involved the small rivers Badam (42001`24` s.w., 70003`26` v.d.) and Koshkar-Ata (42052`27"s.w., 70025`01`v.d.), the location of which relative to the territory of the city of Shymkent, a megalopolis located in the Turkestan region, are shown in Figure 1. The Badam River is a left tributary of the Arys River. Water consumption: 4.51 m^3/s, length – 141 km. The river basin is 4239 km^2. The Koshkar-Ata River originates in the center of Shymkent, flowing through different districts of the city after 12 km it flows into the Badam River.

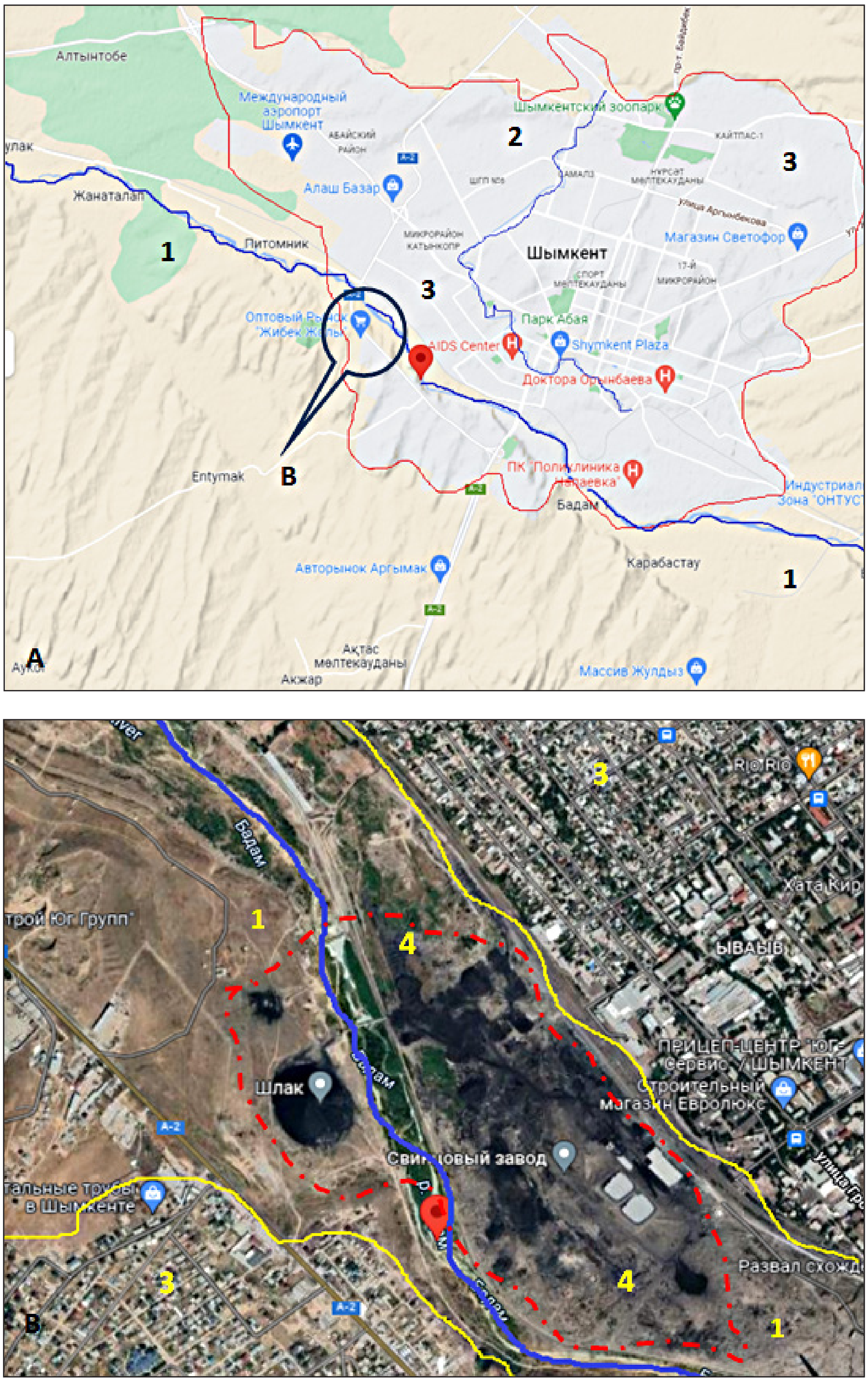

Figure 1. Location of the Badam and Koshkar-Ata rivers relative to the territory of the city of Shymkent. A. General view of the territory of the city of Shymkent and the location of the Badam and Koshkar-Ata rivers; B. View of the Badam River flowing through the territory of the Yuzhpolymetal Joint Stock Company (JSC); 1. Badam River, 2. Koshkar-Ata River, 3. Shymkent city territory, 4. Yuzhpolymetal JSC territory (former lead plant)

Sampling and hydrochemical analyses of waters were carried out according to the conditions of GOST-ov18826-73, 4388-72, 18293-72, 18309-72, 4245-72, 3351-74, 4979-49, 4151-72 and 18293-73, as well as according to the generally accepted method [Reznikova, 1979].

The taxonomic analysis of plant species was carried out according to the determinant of V.M.Katanskaya.

The content of Pb^{2+}, Cd^{2+}, Cu^{2+}, and Zn^{2+} ions in the aqueous medium was determined on the STA – 1 complex, using the method of inversion voltammetry and atomic adsorption method on the AAS 1 spectrophotometer. Ions of chlorides, sulfates, nitrates and nitrites were determined by using the photocolorimetric method on the KFC photometer -3-01- ZOMZ and the ionometric method on the I-500 ionomer.

Statistical processing of the obtained results was carried out by calculating the arithmetic mean and the value of the standard deviation at $0.95 > P > 0.80$. All determinations were carried out in 3- and 5-fold repetition. The data was processed using an IBM Pentium personal computer based on Excel application software packages.

RESULTS AND DISCUSSION

The natural factors determining the species composition of the hydrophytocenoses of small rivers of the Turkestan region are the hydrological, hydrothermal and hydrochemical parameters of the watercourse, which change significantly as the geographical level decreases, in the direction of which all the rivers of the region flow. In addition, in the same direction, the level of technogenic load on the water sources associated with the receipt of surface wastewater from settlements, agricultural landscapes and industrial territories of enterprises is growing. The combination of these factors significantly affects the formation of aquatic vegetation communities.

The results of the studies conducted over a number of years have established that the main sources of pollution of water sources in the south of Kazakhstan are unorganized dumping of garbage and wastewater from settlements, surface runoff from agricultural facilities and industrial wastewater, and pollutants are nitrates, nitrites, petroleum products, sulfates, chlorides, magnesium ions, copper and a number of heavy metals, the average annual concentrations of which exceed the norm of 1.4–5.8 maximum permissible concentrations (MPC). At the same time, the nature of pollutants and the degree of contamination of the aquatic environment of the studied reservoirs are strictly dependent on the influence of the above-mentioned sources of pollution. For example, the main source of pollution of the waters of the Badam River with heavy metal ions is wastewater and groundwater of the production area of Yuzhpolymetal JSC, a former lead plant. Up to the city limits, the main length of the river falls on the flat territories of the region, where the water temperature in the winter and summer months of the year in the river ranges from 3–4 °C to 16–18 °C, respectively. A sharp increase in the level of water mineralization occurs in a section of about 20 km long, which falls on the territory of the industrial zone of Shymkent. In addition to magnesium, calcium and iron ions characteristic of all rivers of the region, ions of heavy metals such as lead and cadmium also enter the river water, the concentrations of which, depending on the time of year, exceed the MPC by 2.1–3.5 times. The results of chemical analyses of the waters showed that the content of heavy metal ions in the river water up to the city limits does not exceed the MPC value. At this site, the main pollutants of the river waters are nitrates and nitrites (11.5 ± 1.1 and 0.034 ± 0.001 mg/l by N), which come from the waters of the Togus and Lengersai rivers polluted from agro-industrial areas of the Turkestan region. After passing through the territory of Yuzhpolymetal JSC, the river waters are polluted with lead, cadmium and zinc ions, while the concentrations of pollutants begin to exceed the MPC – for sulfates by 3.5 times; for nitrites – 2.2; for magnesium – 2.1; for lead – 3.5; for cadmium – 2.8 and for zinc – 3.4. In general, the aquatic environment of the greater extent of the Badam River is characterized by moderate water temperature and high contamination with minerals, which is a specific habitat for most species of aquatic plants.

By its geographical location, by the species composition of hydrophytocenosis and the level of technogenic load, the Koshkar-Ata River is a unique model watercourse, since it originates from large underground sources in the center of Shymkent. The hydrological characteristics of the watercourse are uniform throughout the river. The channel is 5–12 m wide with a sandy-muddy bottom, the banks are flat, the current is moderate. The water temperature at the sources during the year ranges from 8–14 ± 1.3 °C. The source and initial section of the river, 1.5–2.5 km long, characterized by low water temperature and moderate pollution of the aquatic environment. At this site, the indicators of ISV are 3.89 ± 0.2, BPK5 – 2.55 ± 0.2 mg O_2/dm^3. The content of nitrites is 0.02 ± 0.0015 mg/l, nitrates – 9.5 ± 0.11 mg/l, copper ions – 0.9 ± 0.05 mg/l, zinc – 0.4 ± 0.04 mg/l, lead – 0.001 ± 0.00 mg/l, cadmium – 0.001 ± 0.00 mg/l.

In the course of floristic studies of the plant community of water sources in the Turkestan region in the south of Kazakhstan, it was found that morphometric indicators of the same plant species differ significantly depending on the level of total mineralization of the aquatic environment [Issayeva, 2021]. Thus, a comparative analysis conducted between five plants of the same species growing in the area contaminated with heavy metals in the aquatic environment of the Badam River (cadmium ion content – 0.005 ± 0.00, lead – 0.07 ± 0.00, copper – 3.6 ± 0.02 and zinc – 3.8 ± 0.13 mg/l) and the initial section of the Koshkar-Ata River showed that morphometric indicators of all vegetative parts of plants are significantly lower. The plants were characterized by a decrease in overall habit, a decrease in biomass, seed productivity, stem length, and a change in the typical colors of vegetative parts for this species (Table 1).

As shown by the results of screening studies under model conditions conducted to study the reaction of aquatic plants to the action of ions of a number of heavy metals, according to the degree of increase in toxic effects, the studied types of heavy metals are arranged in the following order: cadmium → zinc → lead → copper. At the same time, if the acute toxic effect of copper and lead manifests itself, respectively, on the 2–3 and 5–6 days of the experiment, in the form of a sharp withering of the leaves, followed by their drying, then this period of time takes 8–9 days for zinc and cadmium. According to the degree of additivity of test signs for the toxic effect of heavy metal ions, two plant species are distinguished: *Azolla caroliniana* Willd. and *Veronica beccabunga* L. At concentrations of copper – 3.50 ± 0.03 mg / l, lead – 1.51 ± 0.02 mg / l, cadmium – 1.01 ± 0.02 mg / l and zinc – 3.12 ± 0.32 mg / l, there is a violation of the integrity of the body. On the fourth day of exposure to ion-heavy metals, the blackening of the edges of the leaves begins, followed by their separation from the stems, and on the eighth day, the plants of Azolla Karolinska completely disintegrate into separate components, which soon undergo damage. For a similar period of action of heavy metal ions, Veronika porucheyna has a process of withering of plants, which begins

Table 1. Reactions of dominant plant species to the increase in the level of total mineralization of the aquatic environment

№	Plant names	Indicators of morphological parameters of plants								Description
		Biomass, kg/m²		Transverse dimensions of mixed plant formations, m		Length of the stem of an individual plant, m		Seed productivity, pcs		Special signs of deviation from control (indicators of plants growing in an average polluted environment)
		Control	Experience	Control	Experience	Control	Experience	Control	Experience	
1	*Veronica beccabunga* L.	2.71± 0.13	1.32± 0.06	2.16± 0.15	0.85± 0.09	0.55± 0.00	0.22± 0.00	125.11± 8.91	56.02±5.23	The color of the leaves is light green and reddish
2	*Chara vulgaris* L.	1.80± 0.06	0.99± 0.07	1.51± 0.05	0.42± 0.01	0.26± 0.02	0.12± 0.01	-	-	Plants are very small.
3	*Pjlygonumamphibium* L.	0.71± 0.02	0.38± 0.02	1.52± 0.04	0.32± 0.03	0.56± 0.01	0.28± 0.02	34± 5.63	18±2.35	The plants are completely reddish in color
4	*Siumsizaroideum* DC.	3.51± 0.21	2.62± 0.15	1.78± 0.03	0.78± 0.07	0.86± 0.01	0.38± 0.01	36± 6.23	8±0.23	The plants have a compact habit and are distinguished by dense and durable stems
5	*Cardamine densiflora* N. Gontsch.	3.72± 0.18	2.23± 0.13	2.51± 0.12	0.59± 0.04	0.67± 0.02	0.43± 0.01	85.01± 7.45	23.20±2.12	The plants are densely green in color, the leaves have a reddish border around the edges

Note: The control is the initial section of the Koshkar-Ata River with moderately clean water, the experiment is the waters of the Badam River contaminated with heavy metals.

with the lower leaves and ends with the death of the entire organism. Analysis of the experiment results showed that when growing *A. caroliniana* Willd. and *V. beccabunga* L. in an aqueous medium contaminated with heavy metal ions, the following concentrations can be indicated: copper – 3.51 ± 0.12 mg/l, lead – 1.50 ± 0.11 mg/l, cadmium – 1.02± 0.01 mg/l.

An experiment conducted in glass aquariums showed that *A. caroliniana* Willd. and *V. beccabunga* L. react with various morphological deviations to the changes in the content of lead ions in water. At the same time, even with a concentration of lead ions above 1.00 ± 0.00 mg/l, there is a violation of the structure of chloroplasts along the edges of the unfolded leaf, as in A. caroliniana Willd. or slight wilting of the lower surface leaves as in *V. beccabunga* L. (Table 2). A further increase in the concentration of lead ions to 0.60 ± 0.00 g/l leads to the death of plants on the eighth day, an increase in the content of heavy metal in the aquatic environment to 0.80 ± 0.05 g/l reduces the time of plant death to four days.

The effect of lead ions in water on the studied plants is due to their toxic effect on plants, which disrupts the normal course of metabolic processes. As a result, the structural integrity of the plant organism is destroyed.

The testing properties of plants established in model experiments were confirmed in the studies conducted under extreme weather and climatic conditions, conducted in a storage pond of Yuzhpolymetal JSC, where test plants were grown for 10 days in sections of a 1.0 m^3 pond fenced off with plastic blocks, in fourfold repetition. The content of copper, lead, zinc and cadmium ions in water ranged from 11.80 ± 1.20 – 16.42 ± 1.40 MPC. At the same time, *A. caroliniana* Willd. on the 8–10 day of the experiment, was completely dismembered into small vegetative parts, which subsequently rotted, and *Veronica beccabunga* L. by the end of the experiment, it completely wilted.

It was found that the introduction of *A. caroliniana* Willd. is necessary for biotesting the lead concentrations above MPC in the aquatic environment in an amount of 0.5–1.0 kg/m^3 and *V. beccabunga* L. in an amount of 1.5–2.0 kg/m^3, enabling to cover different depths of the water column: 3–5 cm and 68–82 cm, respectively. The use of such bioindicators is possible to monitor the level of pollution of natural waters with lead ions.

CONCLUSIONS

It was established that morphometric indicators of the same plant species differ significantly depending on the level of total mineralization of the aquatic environment. As a result of the conducted studies on the degree of additivity of test signs for the toxic effect of lead ions, two plant species were identified: *Azolla caroliniana* Willd. and *Veronica beccabunga* L., which must be introduced into the tested aqueous solutions in the amount of 1.0 kg/m^3 and 1.5–2.0 kg/m^3, respectively, contributing to the coverage of the water column up to 75.5 ± 7.2 cm.

Table 2. Biotesting of various concentrations of lead by *Azolla caroliniana* Willd plants and *Veronica beccabunga* L. (after 8 days of experiment)

Content Pb^{2+}, mg/l	Morphological description	
	Azolla caroliniana Willd.	*Veronica beccabunga* L.
Control 0	There are no visible morphological changes	There are no visible morphological changes
1.0	There are no visible morphological changes	There are no visible morphological changes
1.5	The destruction of the chloroplast structure was noted along the edges of the leaves	Slight wilting of the lower surface leaves
10.0	Obvious signs of chloroplast destruction on half of the leaf blade	Complete withering of the lower surface leaves
30.0	Complete destruction of chloroplasts in unfolded terminal leaves	Dying and drying of individual lower leaves
100.0	Destruction of chloroplasts and darkening of all unfolded leaves	Withering of all unfolded leaves
300.0	Signs of the death of the whole plant, the death of more than 50% of plants	Withering of all vegetative organs of plants
600.0	Complete death of plants, signs of tissue decomposition	The death of all surface leaves, signs of softening of the tissues of underwater parts of plants, the death of 30% of plants
800.0	The death of all plants on the fourth day after planting	The death of all plants on the fourth day after planting

It was found that at a water content of 1.5 mg/l Pb^{2+}, the first morphological changes in test plants are noted as the destruction of the structure of chloroplasts along the edges of the unfolded leaves in *A. caroliniana* Willd. a slight wilting of the lower underwater leaves in *V. beccabunga* L., an increase in the content of lead ions in water leads to an increase in the scale of plant damage up to their death at 600.0–800.0 mg/l Pb^{2+} content.

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