

Estimation of the phytoremediation potential of *Alisma plantago-aquatica* L. taken from different stations during water contamination by Cu and Pb (Russia, Vologda region)

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Abstract. Accumulation of Cu and Pb by *Alisma plantago-aquatica* L. plants under laboratory conditions on separate solutions (3 MPC) and changes in the content of heavy metals (HM) when placed on distilled water (control) were studied. The phytoremediation potential of *Alisma plantago-aquatica*, taken from different stations, is assessed: from conditionally clean habitat located in the middle course of Yagorba river (Cherepovets region) and from conditionally polluted habitat located on the bank of Serovka river within Cherepovets city. It is established that irrespective of what stations are taken plants of *Alisma plantago-aquatica*, they actively accumulate heavy metals, but plants from conditionally clean habitat accumulate more. More effective in the purification of water from heavy metals is *Alisma plantago-aquatica* from conditionally clean habitat. *Alisma plantago-aquatica* specimens from different areas are capable of excretion of Cu and Pb ions, the leaves being the most important in this. *Alisma* has a good phytoremediation potential and is suitable for inclusion in the composition of bioplato to clean the water of small rivers from Cu and Pb.

One of the main problems in the implementation of phytotechnologies in the processes of water purification from pollution is the problem of selecting plants to create a biocenosis. It is indicated that candidates for phytoremediation should be indigenous to the region and have certain survival mechanisms that correspond to pollutants, soil and climate conditions and other stresses [1, 2]. In addition, the use of local plants avoids the introduction of potentially invasive species that can pose problems for regional biodiversity [1]. From the analysis of the list of plants hyperaccumulators presented on the basis of generalization of a significant number of works [3], it follows that most species cannot survive in Northern climates, for example, is widely used for phytoremediation water *Eichhornia sp.*; other types are not suitable for dynamic river conditions, such as *Lemna sp.* and submerged plants, which possess higher absorption capacity according to the available experimental

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data [4, 5], characterized by the desorption of heavy metals in 10-12 days. We believe that the ability to desorption, which generally reduces the efficiency of water purification, is less pronounced in semi-submerged macrophytes, since the process of phytovolatilization is possible for them. Therefore, we started work on the assessment of the phytoremediation potential of wild semi-submerged macrophytes of the Vologda region with their further use on polluted small rivers of the region.

It is known that the absorption of mineral substances by plants is due to species specificity [6], and the difference can be very significant. And will there be a significant difference in the absorption of the same substances in plants of the same species, but grown in different stations: under conditions of anthropogenic stress (industrial node) and in natural habitats (in the absence of industrial pollution)? Should this factor be taken into account when selecting macrophytes for phytoremediation? The object of the study was a semi-submerged macrophyte-*psyllium* plantain, which is found in the reservoirs of the Vologda region everywhere, including in conditions of anthropogenic pressure (pollution, urban environment). For the experiment 08/04/2019 were selected (dug) not blooming plants of the water plantain roughly the same size of 15 individuals from relatively clean habitats located in the area of the middle reaches of the river Yagorba (near the village Solmanskoe), and 15 of plants from dirty shareware habitat, located on the banks of the river Serovka within the city of Cherepovets. All plants were placed in a moist environment and on the same day were delivered to the Laboratory of Plant Physiology and Microbiology of the Department of Biology of Cherepovets State University, where the experiment was conducted. The selected plants were mechanically cleaned with tap water as recommended [7] and aged in distilled water in fifty-liter barrels for three days. In the future, some plants from 08/07/2019 to 08/15/2019 were placed in separate containers with 3 liters of distilled water (control), and experimental plants-in the same containers with solutions of copper acetates (option 1) and lead (option 2). The experiment was carried out in three-fold repetition for each habitat. To model the contamination conditions, solutions of metal acetates were prepared based on the MPC of metals in water [8], taking into account regional pollution data: 3 MPC for Cu, respectively, 3 mg/l; 3 MPC for Pb – 0.03 mg/l. A day after the start of the experiment (August 8), the HM solutions were replaced with new ones of the same concentration (dynamic system simulation).

Before exposure in water/solutions and at the end of the experiment, samples of leaves and roots were taken from the plants. After sample preparation in the MINOTAUR-2 mineralizer, the HM content in the samples (2-5 measurements) was determined by atomic absorption spectrometry using the MGA-915MD spectrometer (with an autosampler).

To calculate the efficiency of water purification from ions of HM used the formula [9]:

$$E = [(c_0 - c_t) / c_0] * 100, \quad (1)$$

where E is the efficiency of water purification from ions, HM (%); c_0 is the initial concentration of HM ions (mg/l); c_t is the concentration of HM ions in the solution at the end of the exposure (mg/l). For statistical processing of the obtained data, Microsoft Excel and Statistica programs were used.

In the course of the work, the water content in the plant organs was determined, it averaged 84 ± 4 % for the leaves (coefficient of variation 4.6 %) and 91 ± 4 % for the roots (coefficient of variation 4.2%). Neither in natural habitats, nor during the experiment, the plants did not experience a lack of water, so the concentrations of elements in the work are given without translation to absolutely dry weight.

At the beginning of the experiment, it can be seen (Table 1), that regardless of the habitats in which *Alisma plantago-aquatica* plants grow, most of the Cu can be found both in the leaves and in the roots. The average copper content is slightly higher in plants from a polluted habitat (PH) compared to plants from a conditionally clean habitat (CCH) (Mann-

Whitney test, $p=0.19$). The variance analysis also shows that the variance within groups is greater than between plants from different habitats.

Table 1. Copper content in leaves and roots of *Alisma plantago-aquatica* L. (mg / kg, option 1)

Habitat (station)	Organs	Start of experiment (08/07/2019)				End of experiment (08/15/2019)			
		repetitions			average	repetitions			average
		1	2	3		1	2	3	
conditionally clean habitat (CCH)	leaf	3.91	7.50	3.21	4.87	191.34	229.30	150.34	190.33
	roots	4.88	6.58	4.37	5.28	10.49	54.13	84.05	49.56
	plants *	4.395	7.04	3.79	5.07	100.915	141.715	117.195	119.94
poluted habitat (PH)	leaf	3.75	8.91	16.49	9.72	44.30	46.13	67.22	52.55
	roots	8.68	8.94	5.12	7.58	205.39	64.37	60.33	110.03
	plants *	6.215	8.92	10.81	8.65	124.845	55.25	63.775	81.29

Note: leaves - leaf blades without signs of aging, taken from the middle part of the rosette; plants * - the average result for a plant, obtained from adding the content in leaves and roots and dividing by 2. The stem was not taken into account, because it is greatly shortened in the chastuha.

All plants of *Alisma plantago-aquatica* actively extract metal ions from the solution, and individuals from CCH accumulate more (Fig. 1) and mainly in the leaves (Fig. 2). Plants from PH are inferior in copper content, localizing it mainly in the roots. The content of copper in specimens of *Alisma plantago-aquatica* from CCH increased 20 - 31 times compared to the beginning of the experiment, and in plants from PH - 6 - 20 times. According to the Mann-Whitney test, the samples of plants from different stations did not differ significantly in the Cu content at the time of the end of the experiment ($p = 0.38$). However, there is a significant difference in the content of copper in leaves: individuals from CCH accumulate more than leaves from plants from PH (Mann-Whitney test, $p = 0.049$).

Table 2. Lead content in leaves and roots of *Alisma plantago-aquatica* L (mg / kg, option 2)

Habitat (station)	Organs	Start of experiment (08/07/2019)				End of experiment (08/15/2019)			
		repetitions			average	repetitions			average
		1	2	3		1	2	3	
conditionally clean habitat (CCH)	leaf	2.89	1.44	3.46	2.60	45.06	33.88	27.91	35.62
	roots	1.85	0.60	0.78	1.08	87.05	152.08	124.82	121.32
	plants *	2.37	1.02	2.12	1.84	66.055	92.98	76.365	78.47
poluted habitat (PH)	Leaf	1.31	3.02	2.00	2.11	29.57	45.12	20.96	31.88
	Roots	2.56	2.74	0.93	2.08	136.34	33.5	16.47	62.10
	plants*	1.935	2.88	1.465	2.09	82.955	39.31	18.715	46.99

Note: see table 1.

At the same time, some of the plants of *Alisma plantago-aquatica* were kept in solutions with lead ions (Table 2). It was found that plants from CCH are characterized by general regularities in the localization of Pb and changes during the experiment: if before the experiment there is a little more lead in the leaves, then after the end of the experiment a significant part of the ions is confined to the roots (Fig. 3). And although, on average, for plants from PH, most of the lead at the time of the end of the experiment is also confined to the roots, the replicates reflect the variety of responses of individuals. In general, plants from CCH accumulate Pb more than plants from PH (Fig. 1).

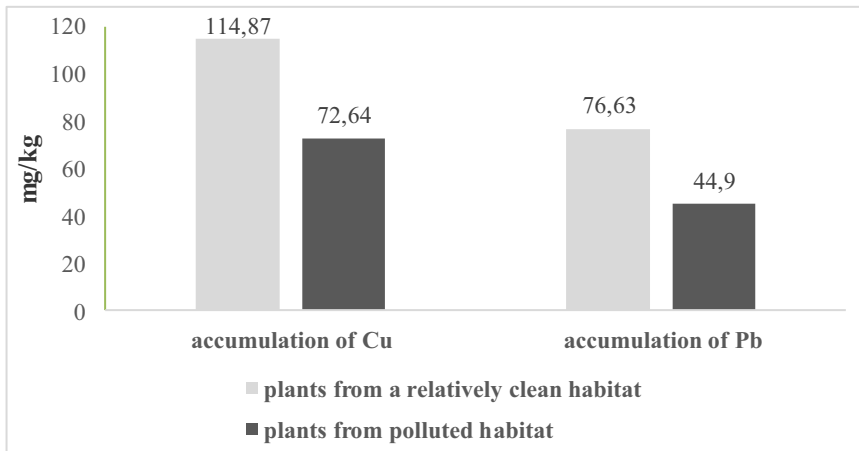


Fig. 1. Accumulation of Heavy Metals (mg / kg) by *Alisma plantago-aquatica* L. plants from different stations

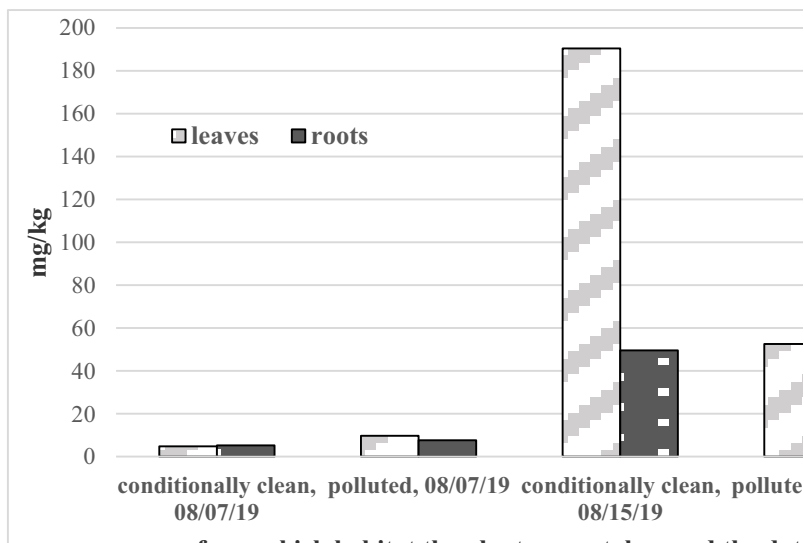


Fig. 2. Cu content in leaves and roots of *Alisma plantago-aquatica* L. plants from different stations before (08/07/2019) and after (08/15/2019) experiment

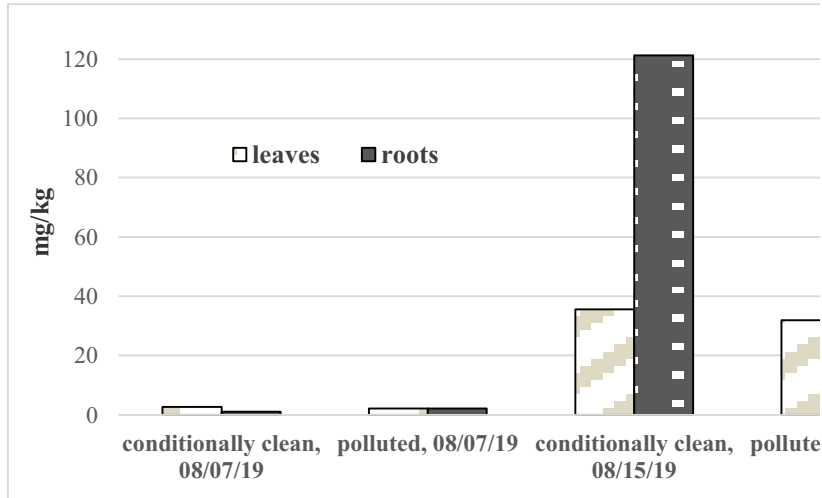


Fig. 3. Pb content in leaves and roots of *Alisma plantago-aquatica* L. plants from different stations before (08/07/2019) and after (08/15/2019) experiment

Table 3. Change in the content of heavy metals in solutions before and after exposure of *Alisma plantago-aquatica* L. and the efficiency of water purification

Habitat (station)	repetitions	Cu			Pb		
		c_0 , mg/kg	c_t , mg/kg	E, %	c_0 , mg/kg	c_t , mg/kg	E, %
CCH	1	6.000	0.0037	99.94	0.060	0.0045	92.50
	2	6.000	0.0041	99.93	0.060	0.0063	89.50
	3	6.000	0.0064	99.89	0.060	0.0064	89.33
	Average	6.000	0.0047	99.92	0.060	0.0057	90.50
PH	1	6.000	0.0026	99.96	0.060	0.0092	84.67
	2	6.000	0.0074	99.88	0.06	0.0145	75.83
	3	6.000	0.0032	99.95	0.06	0.0103	82.83
	average	6.000	0.0044	99.93	0.06	0.0113	81.17

Note: for designations, see formula 1, by which the efficiency of water purification is determined; c_0 , the initial solution is the total concentration of two successively replaced solutions.

The efficiency of water purification from the ions of heavy metals (Table 3) is quite high, for copper it is close to 100%, for lead about 90% when using plants from CCH and significantly lower (about 81%) when using chastoids from PH, according to the Mann-Whitney criterion differences in the efficiency of water purification from lead between plants from different stations are significant at $p = 0.08$.

Control plants of *Alisma plantago-aquatica*, kept from 08/07/2019 to 08/15/2019 in distilled water (Tables 4, 5) allow us to evaluate the peculiarities of heavy metal ion release. At the beginning of exposure, plants from different stations do not show significant

differences in the content of heavy metals (Mann-Whitney test, $p = 0.66$ (Cu), $p = 1.00$ (Pb)), possibly because all individuals were previously kept in distilled water for 3 days. At the time of the end of the experiment, the copper content in plants taken from CCH decreases by 26 - 75% from the initial level, and in plants from PH - by 54 - 72%. The lead content decreases significantly in plants taken from CCH, by 58-61% of the initial level. In plants from HP, the content of lead decreases weakly, by 39%, or it is redistributed in the plant, which is reflected in an increase in the average index for replication. This assumption requires additional verification. It was recorded that a part of heavy metals passes into distilled water (Table 6). The Cu content in water after exposure is from 0.02 to 0.07% of the initial average content in the plant, and Pb is from 0.11 to 2.20%.

Table 4. Copper content in leaves and roots of *Alisma plantago-aquatica* L. (mg / kg, control - exposure in distilled water)

Habitat (station)	organs	Start of experiment (08/07/2019)				End of experiment (08/15/2019)			
		repetitions			average	repetitions			average
		1	2	3		1	2	3	
CCH	leaf	3.8946	3.4043	3.8752	3.7247	3.6538	1.1098	1.6635	2.1424
	roots	4.8547	6.5746	4.3692	5.2662	2.7853	2.8473	0.3840	2.0055
	plant*	4.3746	4.9894	4.1222	4.4954	3.2195	1.9785	1.0237	2.0739
PH	leaf	4.5047	6.0124	2.6186	4.3786	2.0101	0.9750	0.3237	1.1029
	roots	8.6787	8.9351	5.1240	7.5793	2.7643	3.2043	3.2533	3.0740
	plants*	6.5917	7.4737	3.8713	5.9789	2.3872	2.0896	1.7885	2.0884

Note: see table 1.

Table 5. Lead content in leaves and roots of *Alisma plantago-aquatica* L. (mg / kg, control - exposure in distilled water)

Habitat (station)	organs	Start of experiment (08/07/2019)				End of experiment (08/15/2019)			
		repetitions			average	repetitions			average
		1	2	3		1	2	3	
CCH	leaf	2.4717	1.0223	1.5586	1.6842	0.8303	0.2127	0.0923	0.3784
	roots	1.8455	0.6041	0.7800	1.0765	0.9718	0.4141	0.3461	0.5773
	plant*	2.1586	0.8132	1.1693	1.3804	0.9011	0.3134	0.2192	0.4779
PH	leaf	0.0533	0.6574	0.8550	0.5219	0.5503	2.6352	0.5282	1.2379
	roots	2.5561	2.7438	0.9256	2.0752	1.0467	1.0536	1.4305	1.1769
	plants*	1.3047	1.7006	0.8903	1.2985	0.7985	1.8444	0.9794	1.2074

Note: see table 1.

Table 6. The content of heavy metals in water after exposure of plants of *Alisma plantago-aquatica* L (control - exposure in distilled water)

Heavy metals	Habitat (station)							
	Conditionally clean (outside the city)				Polluted (in city conditions)			
	repetitions			average	repetitions			average
	1	2	3		1	2	3	
Cu, mg/kg*10 ⁻³	1.56	1.14	2.77	1.82	2.66	1.53	1.68	1.96
Pb, mg/kg*10 ⁻³	2.40	17.86	2.98	7.75	2.78	3.12	2.76	2.89

Thus, excretion of heavy metals by the roots is noted. At the same time, the average values are somewhat higher, but also more variable in plants from CCH. Since a small proportion of heavy metals are released due to excretion by roots, and the content of Cu and Pb in plants decreases significantly, the greatest decrease in heavy metals can be attributed to their excretion with the help of leaves - phytovolatilization. Thus, the use of *Alisma Plantago-aquatica* as part of a bioplato will make it possible to effectively purify water from Cu and Pb in case of exceeding the maximum permissible concentration, and to redistribute the load between different media. This, in turn, will lead to the absence of disturbances in the action of the natural mechanisms of self-healing of aquatic ecosystems and the possibility of preserving bioplato without the subsequent utilization of biomass.

So, regardless of which station the plants of *Alisma plantago-aquatica* are taken from, they actively accumulate heavy metals, but plants from a conventionally clean habitat accumulate more. More effective in water purification from heavy metals is *Alisma Plantago-aquatica* from a conditionally clean habitat. Individuals of *Alisma plantago-aquatica* from different habitats are capable of excreting Cu and Pb ions, the leaves are of the greatest importance in this. *Alisma plantago-aquatica* has a good phytoremediation potential and is suitable for inclusion in bioplato to purify the water of small rivers from Cu and Pb.

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