

## Decreasing the Measurable Concentrations of Cu, Zn, Cd, and Pb in the Water of the Experimental Systems Containing *Ceratophyllum demersum*: The Phytoremediation Potential

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Development of V.I. Vernadsky's theory of the biosphere has revealed new examples of how organisms affect the physical and chemical parameters of the environment [1, 2], including the characteristics of the aquatic environment [3, 4]. Natural aquatic ecosystems have complex self-purification mechanisms, in which the biota, including macrophytes, plays an important role [5–11]. Macrophytes are being studied in order to develop ecological technologies for purifying environmental components [12, 14]. Our previous studies dealt with the role of macrophytes as potential components of systems for purification of the aquatic environment polluted with perchlorate [14] and the synthetic surfactant sodium dodecyl sulfate [12, 13]. It was interesting to study systems containing other pollutants, heavy metals ranking high among them. Many heavy metals have various deleterious effects on organisms, including membranotropic effects.

The purpose of this study was to collect data on the changes in the concentrations of metals (Cu, Zn, Cd, and Pb) in the aquatic medium of microcosms containing the macrophyte *Ceratophyllum demersum*.

Tap water settled for a week was used to prepare aquatic medium containing metals. State reference standards (SRSs) with a mass concentration of  $1 \text{ mg/cm}^3$  at a temperature of  $20^\circ\text{C}$  were used as the original aqueous solutions of metal ions. We used the following SRSs: Zn SRS 7770–2000 in 1 M hydrochloric acid, Pb SRS 7778–2000 in 1 M nitric acid, Cd SRS 7773–2000 in 1 M nitric acid, and Cu SRS 7764–2000 in 0.5 M sulfuric acid. By using successive dilutions and definite aliquots, we obtained a solution 1 l of which contained 2 mg of each Zn and Cu, 0.1 mg of Pb, and 0.02 mg of Cd. The concentrations of all these elements exceeded the maximum allowable concentrations (MACs) for domestic/potable and public water consumption. Fe ( $0.1 \text{ mg/l}$ ) was added as a com-

ponent of mineral nutrition of plants. For this purpose, we used the SRS of  $1 \text{ mg/cm}^3$  Fe in 1 M HCl.

The macrocomponent (anion) content was the following: 73 mg/l chloride ion, 96 mg/l sulfate ion, and 12 mg/l nitrate ion; this corresponded to the allowable levels. The pH of the resultant solution of salts of the metals studied was 6.0. The pH increased in the course of incubation of macrophytes in the solution.

The macrophytes *C. demersum* were collected in a fresh water body (pond) in the Moskva River floodplain (Odintsovo raion, Moscow oblast). This area is characterized by fairly good environmental conditions, the water being free of industrial sewage. The collected macrophytes were placed into laboratory microcosms and washed with settled tap water several times to remove contaminating suspensions and decrease the possible surface pollution.

Shoots of *C. demersum* were put into microcosms containing salts of the metals (Cu, Zn, Cd, and Pb). Table 1 shows the initial biomass of the macrophytes. Each microcosm contained 500 ml of the solution.

Microcosms containing the same solution but no macrophytes and microcosms containing macrophytes in water without additional metals served as control groups.

Incubation was carried out under the conditions of the natural photoperiod and temperature. After three

**Table 1.** Biomass of the macrophytes *C. demersum* in experimental microcosms

Variant no.	Numerical mark of the microcosm in a specific experiment	Presence of macrophytes	Wet biomass of macrophytes, g
1	15	+	12.7
2	16	+	17.8
3	17	–	–
4	18	–	–

**Table 2.** The state of the macrophytes *C. demersum* in microcosms containing metals (Cu, Zn, Cd, and Pb)

Incubation time, days	State (brief characteristic)	Comment
0	Plants are alive	Start of incubation
7	Signs of worsening	Some leaves have detached from the stems; a mucous film is observed on some plants
10	Distinct signs of worsening; the shoots are dead or dying	The leaves have detached from the stems. There is a mucous film on the water surface. The phytomass has sunk to the bottom part of the water column; the upper part of the water column, free of plants, is 1–2 cm in depth
19	The plants have died	The same; the upper part of the water column, free of plants, is 1–2 cm in depth
28	The same	The same; since the phytomass has sunk, the upper part of the water column, free of plants, is 3–4 cm in depth; in microcosm 15, the shoots of dead macrophytes are greenish; the water in microcosm 16 is turbid, with a musty odor

**Table 3.** Concentrations of metals (Cu, Zn, Cd, and Pb) in the aquatic medium used in microcosms (mg/l)

Aquatic medium	Elements	Microcosm 15 (containing macrophytes)	Microcosm 16 (containing macrophytes)	Microcosm 17 (control)	Microcosm 18 (control)
Aquatic medium for plant incubation containing salts (nominal concentrations)	Zn	2	2	2	2
	Cu	2	2	2	2
	Cd	0.02	0.02	0.02	0.02
	Pb	0.1	0.1	0.1	0.1
The same aquatic medium; measurements made a day before the experiment, before macrophytes were introduced	Zn	1.8	1.8	1.8	1.8
	Cu	2.0	2.0	2.0	2.0
	Cd	0.04	0.04	0.04	0.04
	Pb	0.09	0.09	0.09	0.09
After 3 days of incubation	Zn	1.5	1.4	1.8	1.9
	Cu	1.3	0.3	1.3	1.6
	Cd	0.01	0.01	0.03	0.02
	Pb	0.001	0.003	0.03	0.02
After 6 days of incubation	Zn	0.43	0.49	1.8	1.8
	Cu	0.3	0.3	1.3	1.5
	Cd	0.005	0.005	0.03	0.03
	Pb	0.003	0.003	0.02	0.02
After 10 days of incubation	Zn	0.2	0.2	0.9	1.1
	Cu	0.14	0.12	1.00	0.95
	Cd	0.002	0.003	0.010	0.009
	Pb	0.002	0.002	0.018	0.016
After 19 days of incubation	Zn	0.2	0.2	0.9	1.0
	Cu	0.066	0.053	1.20	1.12
	Cd	0.003	0.002	0.008	0.011
	Pb	<0.001	<0.001	0.016	0.012

days of incubation, the pH was 7.3 in all experimental variants and in control microcosms (salt solution without macrophytes).

Samples of water for chemical analysis were taken from the incubation microcosms. The mass concen-

trations of metal ions in the solution were measured by inversion voltamperometry. We used an AKV-07MK voltamperometric analyzer (Aquilon, Russia) with a three-electrode system consisting of (1) a rotating carbon–glass-ceramic measuring electrode; (2) a silver

**Table 4.** Comparison of the initial (nominal) metal concentrations and their MACs

Element	Amount added, mg/l	MAC (drinking water), mg/l	MAC for domestic/potable and public water consumption, mg/l
Zn	2	0.01	1
Cu	2	0.01	1
Cd	0.02	–	0.001
Pb	0.1	–	0.03

chloride reference electrode filled with saturated KCl solution; and (3) a glass-carbon melting pot in which electrolysis took place (it served as the third electrode).

The Polar-3 software was used for data management and processing. The method is based on electrochemical concentration of copper, lead, cadmium, and zinc on the surface of the measuring carbon-glass-ceramic electrode in the form of Hg amalgam followed by electrochemical dissolving at a set potential, with a voltamperogram being recorded. The mass concentrations of the metals were determined by the method of additives. The zinc, cadmium, and lead concentrations were measured in a medium buffered with ammonium acetate (pH 4.8) by adding triethanolamine. The copper concentration was measured against the background of 1 M hydrochloric acid.

The main advantages of metal assay using inversion voltamperometry are a high sensitivity (from  $10^{-10}$  to  $10^{-8}$  M), satisfactory selectivity and resolving power, a high reproducibility, quickness, and simple preparation of specimens (in most cases, it is not necessary to concentrate samples or remove contaminating substances that could affect the results) [15].

Table 2 shows the changes in the state of the macrophytes; Table 3, changes in the metal concentrations in the aqueous solution.

The metal concentrations used in the experiment were selected so that they exceeded the MACs for both drinking water and for sources of domestic and public water consumption (Table 4). Thus, these concentrations definitely went beyond those allowable in terms of sanitary and hygienic safety. If metals at these concentrations are found in water supply sources, it is certainly necessary to find a practicable way of decreasing them.

The results of the experiment (Table 3) showed the concentrations of all metals assayed by the given method decreased with time. The concentrations of all metals became several times lower after incubation. The zinc, copper, and lead concentrations decreased below the respective MACs for domestic/potable and public water consumption. The cadmium concentration was substantially decreased: the initial cadmium concentration was 20 times higher than its MAC,

whereas that at the end of the experiment was, on average, only 2.5 times higher than it.

Note that aquatic plants died after less than 18 days of incubation. Distinct signs of worsening of the general state of the plants were observed after seven days of incubation (Table 2).

It is interesting to compare the time courses of the decrease in metal concentrations in water and the state of the plants. The metal concentrations began to decrease within the period of viability of the plants. However, when the state of the plants had already become considerably worse (with the leaves detached from the stems), the metal concentrations continued to decrease. Macrophytes remained a potent factor in decreasing the metal concentrations even after they died.

Note that metals are present in water in various forms [15]. The method used in this study (anodic inversion voltamperometry, AIV) detects the so-called free metal ions and complexes, which can dissociate in the diffusion layer near the surface of the electrode [15]. To detect Zn and Pb complexes more completely, we performed the measurements in a medium buffered with ammonium acetate, with triethanolamine added to neutralize the interfering effect of iron. The decrease in the metal concentrations in the control microcosms was probably related to their sorption on the walls at pH 7. Comparison of the patterns of changes showed a significant effect of macrophytes on the decrease in metal concentrations in the solution measured by the AIV method. To analyze the details of the macrophyte effect, it would be reasonable to perform analysis using other methods for detecting metals.

Thus, (1) the results of this study agree with the assumption that the presence of the macrophyte *C. demersum* may accelerate the decrease in the water content of pollutants (the metals Cu, Zn, Cd, and Pb), which has implications for the development of phytotechnologies of water purification (phytoremediation); (2) the subject requires further analysis using a wider range of methods for measuring metal concentrations and testing various possibilities of metal redistribution in components of microcosms.

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