The effect of manganese ions (II) on representatives of aquatic biota

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Abstract. The work is devoted to the consideration of the peculiarities of the impact of manganese (II) on representatives of freshwater biota. Manganese plays an important role in the aquatic environment, since it determines the course of the most important metabolic processes in hydrobiont organisms of varying degrees of organization. The analysis of processes involving manganese has shown that the main chemical factors affecting the accumulation of manganese in the aquatic environment are anaerobic biochemical processes caused by active silting of the riverbed due to the washout of organic substances from agricultural fields. The use of various physiological endpoints in daphnids is considered as an inexpensive and simple alternative, the criterion of which corresponds to the rule "replacement, reduction, refinement". It has been shown that manganese (II) ions in the concentration range of 0.1-1000 LOCvr do not have an acute toxic effect on D. magna crustaceans, chronic toxicity is manifested in samples with concentrations of more than 10 LOCvr. The acute toxicity of solutions of manganese (II) salts in relation to S. Quadricauda was revealed by the change in the fluorescence level of microalgae at a concentration of Mn²⁺ from 0.05 mg/l.

1. Introduction
The purpose of the work is to provide a systematic analysis of Mn²⁺ conversion processes in the aquatic environment, its influence on the formation of toxicity effects in relation to different types of aquatic organisms and the possibilities of using the latter in biotesting of open water sources. To do this, we have analyzed data on the forms of the existence of manganese in the aquatic environment, as well as their toxic effect on groups of freshwater and other organisms, and also revealed patterns of changes in the toxic effect in relation to Daphnia magna and S. Quadricauda from Mn²⁺ concentration in model media.

In solutions Mn can exist in several valence forms: Mn²⁺, Mn³⁺ and Mn⁴⁺. At the same time, Mn²⁺ exists mainly in a soluble form, Mn³⁺ – both in soluble and suspended form, Mn⁴⁺ – only in the form of a precipitate. In the absence of complexation processes, the Mn³⁺ ion undergoes reduction to Mn²⁺...
[1,2]. The equilibrium between the most stable forms of Mn$^{2+}$ and Mn$^{4+}$ is realized in abiotic or microbiologically mediated redox reactions. The ecological chemistry of manganese is largely determined by the pH of the medium and redox conditions. The content of manganese in water depends on the contributions of surface and underground effluents to the migration of ions in water, the effectiveness of its participation in photosynthesis, destruction of phytoplankton and zooplankton, as well as the processes of its complex formation in silt and soil [3,4]. The solubility of soil manganese is also determined by the pH of the medium and the redox potential [3,4]. Manganese is absorbed by aquatic plants, undergoing a heterogeneous metabolic distribution through their tissues. For algae, the average concentrations of manganese in biomass are 130-735 mg/kg, for shellfish – 3-660 mg/kg, freshwater fish - 0.2-19 mg/kg. Manganese in a living organism has mutagenic and cumulative properties [5].

Test objects used in assessing the toxicity of environmental objects due to the presence of manganese. Most tests for the toxicity of manganese are carried out using its ionic form: little is known about the toxicity of waters contaminated with manganese in colloidal or solid forms, as well as in the form of complexes. It is assumed that the toxicity of such forms of manganese is lower than that of the ionic form in an aqueous solution.

A specific effect on the action of manganese is inherent in echinoderms Paracentrotus lividus. It was shown [6] that, unlike other heavy metals, a moderate increase in the concentration of manganese is not perceived by the cells of embryos of this species as a stressful situation, but on the contrary, an increased tolerance to this element is detected: low concentrations of manganese stimulate the growth of embryos, probably as a result of their participation in metabolic-enzymatic reactions. Bacteria and some fungi can oxidize Mn (II) under aerobic conditions. Mn$^{2+}$ ions in the presence of microbial oxidases of fungi and bacteria are oxidized under aerobic conditions to Mn$^{3,4+}$ [7, 8]. In addition to atomic oxygen, hydrogen peroxide functions as an oxidizer in the Mn$^{2+}$ to Mn$^{3+}$ manganese conversion reaction in water. The resulting Mn$^{3+}$ is reduced by lignin to Mn$^{2+}$ [9].

Bivalves are capable of bioaccumulating trace elements to concentrations 10 times higher than their content in the habitat [10]. Of the two daphnia species common in the northern hemisphere, D. magna and D. galeata, the first one is more sensitive to Mn$^{2+}$ and Hg$^{2+}$, the second - to Ag$^+$, As$^{3+}$, Cr$^{6+}$, Fe$^{3+}$, Ni$^{2+}$ and Pb$^{2+}$, and in relation to ions Cu$^{2+}$, Cd$^{2+}$ and Zn$^{2+}$, both species showed the same toxic sensitivity [11, 12]. The use of D. magna is very informative in the toxicological assessment of bottom sediments of Lake Ladoga, localized in the iron-manganese province on the Karelian Isthmus. Iron-manganese inclusions are formed on the surface of the sediment, in which oxidized forms of manganese predominate [13, 14]. The microalga Scenedesmus quadricauda is exposed to acute toxic effects of solutions of manganese (II) salts at their content in concentrations from 5 LOCvr. The presence of manganese compounds in river water may cause an increase in the number of larvae of some insects. Thus, an increase in the occurrence of larvae of T. Nebusola, S. Pallipes is caused by an increase in the manganese content in the waters of small rivers of the Bashkir Cis-Urals [15]. Representatives of the biota of the Majdanska River (Poland) have a good ability to bioaccumulate Mn, Fe, and Ti: algae Audouinella sp. and Spirogyra sp., with freshwater snails Radix labiata, diatoms and bacteria co-existing with them. The mechanism of bioconcentration in this case assumes biomineralization of these metals as products of extracellular deposition on cell walls [16]. The use of Dafnia Magna to assess the toxicity of water due to the presence of manganese and other heavy metals.

The theory of combined risk underlies the ecotoxicological approach to a comprehensive assessment of the toxicity of open reservoirs contaminated with metals, based on data on their maximum permissible content in fishery reservoirs and the lethal effect of these metals on daphnia. Using this approach, it is possible to assess the effects of metal pollution in reservoirs of different climatic zones, as well as to consider the factor of seasonal fluctuations in the content of metals in reservoirs [17,18]. The toxicity of water by biotesting using daphnia can be based on a change in their
trophic activity against Chlorella, recorded by a change in the fluorescence signal of the latter [19]. Daphnia magna and Lemna minor refer to the difference in sensitivity of individual aquatic species, the methods of toxicity assessment based on them can accurately determine the ecotoxicological effects of emerging pollutants that are not removed by conventional water purification procedures [20].

Thus, using the example of the Bitha River, a comprehensive approach to the organization of system monitoring of the impact of landfill runoff on natural ecosystems is proposed, including an assessment of water toxicity by biotesting using Dafnia Magna, due to the content of manganese [21, 22]. Biotesting using Dafnia Magna is informative when studying the influence of sediments containing heavy metals on the hydrochemical composition of cooling ponds of nuclear power plants [23]. The effect of low concentrations of copper and cadmium on Dafnia Magna in model solutions is considered in [24]. Biotesting with the use of daphnia is advisable to use in conditions of medium-long-term forecasts of changes in water toxicity [25]. To obtain urgent, current information about the quality of drinking water in the sources, it is advisable to use test objects that give faster responses (for example, infusoria, ceriodaphnia dubia). The culture Dafnia Magna can be successfully used to test the toxicity of water contaminated with peretroids and other insecticides [26, 27]. The cumulative ability of Daphnia magna to copper, zinc, selenium and arsenic is affected by age sensitivity: mortality decreases, and cumulative reproduction increases with age. At the same time, the peak of sensitivity from exposure to copper and zinc corresponds to the age of four days for 4-day-old organisms exposed to Cu and Zn, and organisms aged 2-3 days show increased sensitivity to As and Se [28,29]. The use of various physiological endpoints in daphnids is considered as an inexpensive and simple alternative, the criterion of which corresponds to rule 3R (replacement, reduction, refinement) [30]. Toxictants, including heavy metals, can lead to inhibition of chlorella uptake, and this effect depends on pollutant concentration [31].

2. Materials and methods

Objects of research: protococcal green alga Scenedesmus quadricauda (Turp.) Breb.; cladocerans Daphnia magna Straus.

Reagents: MnSO4·H2O (“p”, GOST 435-77, LLC "LABTECH", Russia); MnCl2·4H2O (“p” according to GOST 612-75, CJSC "VECTON", Russia), distilled water.

The culture of daphnia crustaceans, in accordance with the method of PND F T 14.1:2.4.12-06; FR.1.39.2007.03222, was grown in a climatostat R-2 at a temperature of 20±1°C, with constant artificial lighting (1000-1500 lux for 12 hours during the day). A suspension of culture S. Quadricauda was used as daphnia feed daily [32].

Microalgae S. Quadricauda were cultured, in accordance with the method of FR.1.39.2007.03223 on a Prat medium in a climatostat under artificial lighting conditions (3000-10000 lux for 24 hours) and shaking at intervals 1-2 times during the day.

Acute toxicity of the studied water samples was determined by the formula:

$$\Lambda = \frac{X_k-X_0}{X_k} \times 100\%,$$

where \(X_k\) is the number of surviving organisms in the control, \(X_0\) is the number of surviving organisms in the sample under study [32].

3. Results and discussion

Acute toxic effects of Mn2+ ions in the water on D. magna was absent in the range of 0.1-1000 LOCvr. Chronic toxic effects were manifested at concentrations of more than 10 LOCvr. The relative mortality in this range of Mn2+ ions was 40-60% for MnSO4 and 30-60% for MnCl2. A statistically significant decrease in the fertility of daphnia compared with the control was observed at concentrations of Mn2+ in 1, 3 and 10 mg/l (22.2-82% of the control for MnSO4 and 25.4-74.6% for MnCl2). The above is also
typical for MnCl₂ solution with a concentration of Mn²⁺ equal to 0.1 mg/l (a decrease in the number by 20.6%) [60].

The survival rate of *D. magna* offspring decreased at Mn²⁺ ion content of 0.01 mg/l, this process acquired a statistically significant character at Mn²⁺ ion content of 0.05 mg/l and higher. The decrease in the number of offspring compared to the control is 9.5-82.5% for MnSO₄ and 12.7-90.5% with MnCl₂. Statistically valid (significant) decrease in the filtration activity of crustaceans compared to the control is, respectively, 28-61% and 28-62% for MnSO₄ and MnCl₂.

**Figure 1.** Dependence of relative mortality D (%) of *D. magna* from the content of Mn²⁺ ions in water (1- MnSO₄, 2- MnCl₂).

By changing the fluorescence level of microalgae *S. quadricauda* the effect of acute toxic action of Mn²⁺ ions at their content of 0.01 mg/l was detected, but statistically significant differences were obtained only at concentrations of Mn²⁺ from 0.05 mg/l and higher.

**Figure 2.** Dependence of relative mortality D (%) of *S. quadricauda* from the content of Mn²⁺ ions in water (1- MnCl₂, 2- MnSO₄).
Figure 3. Dependence of relative mortality D (%) of *D. magna* from the duration of exposure (1- MnSO₄, 2- MnCl₂).

Figure 4. Dependence of relative mortality D (%) of *S. quadricauda* of the exposure duration (1- MnCl₂, 2- MnSO₄). The decrease in the fluorescence level compared to the control for these concentrations was, respectively, 22-66% with MnSO₄ and 15-69% for MnCl₂.

4. Conclusion
The analysis of processes involving manganese has shown that the main chemical factors affecting the accumulation of manganese in the aquatic environment are anaerobic biochemical processes caused by active silting of the riverbed due to the washout of organic substances from agricultural fields. The marine diatomaceous algae *Ditylum brightwelli* and freshwater algae *Scenedesmus quadricauda* are highly sensitive to manganese toxicity in water. *Daphnia Magna* and *Lemna minor* are highly sensitive to the presence of manganese in water. Increasing the concentration negatively affects the natural process of bioaccumulation of metal ions in the cells of hydrobionts. Soy is promising in biotesting water contaminated with manganese: excess Mn hinders plant growth and increases the accumulation of Mn in the form of brown spots on soybean leaves, and this effect increases with the age of the plant. The use of various physiological endpoints in daphnids is considered as an inexpensive and simple alternative, the criterion of which corresponds to the rule "replacement, reduction, refinement". It has been shown that Mn²⁺ ions in the range of their content in water, which is 0.1-1000 LOCvr, do not
show acute toxic effects on *D. magna*, the effect of chronic toxic action is manifested in samples with Mn$^{2+}$ ion content of more than 10 LOCvr. The acute toxicity of solutions of manganese (II) salts in relation to *S. quadricauda* was revealed by the change in the fluorescence level of microalgae at a concentration of Mn$^{2+}$ from 0.05 mg/l.

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