

Ecological Consequences of Pollution of Freshwater Reservoirs with Metals: Role of Cu(II) Binding with Low-Molecular Organic Substances

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Abstract

Mesosimulation of the chemical forms of Cu(II) with variation of their distribution in solution was used to study the ecological consequences of the transformations of these forms caused by pollution of a natural freshwater reservoir. Copper (II) and substances that serve as models of natural low-molecular organic compounds with different structures (benzyl- and *n*-hexadecyl malonic acids, BMAH₂, HDMAH₂, and phthalocyanine CuPhC) were introduced into mesocosms arranged directly in a water reservoir (Novosibirsk water reservoir). The distribution of Cu(II) over chemical forms in solution was specified based on the results of the preliminary computer simulation and realized by varying pH of water. The residual concentration of Cu in solution and on suspended particles, as well as the response of phyto- and zooplanktons, were monitored for 8–15 days in the mesocosms. It was discovered that in the presence of other forms of copper in solution, binding of Cu(II) into CuBMA complexes had no effect on metal removal to bottom sediments and on the productive capacity of phytoplankton, but stimulated growth of the zooplankton mass. The rate of copper removal to bottom sediments increased when the polar fragment of CuBMA was replaced by a hydrophobic unit (CuH-DMA); however, zooplankton was suppressed. The introduction of the CuPhC complex into water has stimulated the development of both phyto- and zooplanktons. The presence of low-molecular organic compounds able to bind Cu(II) in solution generally weakens the adverse ecological consequences of water pollution with copper salts.

INTRODUCTION

The metals (Me) dissolved in natural waters exist in various chemical forms: Me^{z+} aqua ions, complexes with inorganic ligands, and MeL forms bound with various organic solute (OS) substances. They differ in their ability to be sorbed on suspended particles, bioavailability, and toxicity for organisms living in water [1–5]. The aqua ions are the most toxic species, while the MeL forms bound with high-molecular OS are less toxic. As to other Me forms (in particular, MeL forms bound with low-molecular OS), the literature data are contradictory.

The same is also true of the ability of different metal forms to be sorbed on suspended particles, whose sedimentation is the major mechanism of Me removal from water to bottom sediments. Investigations were mostly carried out with laboratory model systems, substantially simplified relative to real water reservoirs, which is one of the reasons of ambiguity of the results.

This circumstance hinders the prediction of the ecological consequences of pollution of real freshwater reservoirs with Me compounds. Evidently, the behaviour of pollutant metals entering water reservoirs, namely, the dyna-

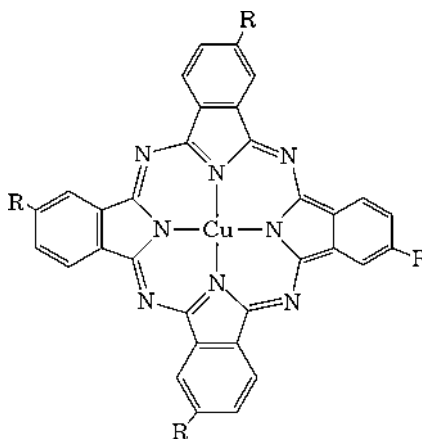
mics of Me removal into bottom sediments and effects on biota, depend on the forms into which Me compounds are transformed under specific conditions. The parameters of chemical composition, as well as those of biohydrocenes, are individual and variable for real hydroecosystems. To take into account the entire set of these parameters it was appropriate to arrange special on-site experiments in the water reservoir using mesocosms – small sections isolated in the water reservoir and conserving the natural composition of water, bottom sediments, biota, and other parameters (temperature, illumination), including their dynamics and gradients. The necessary portions of Me were introduced into the mesocosms; the ratio of the chemical forms of Me in a solution of specific composition (including the inorganic and organic subsystems) was selectively varied by adjusting pH of water to a value chosen on the basis of preliminary computer simulation [6–8]. Since CO_3^{2-} and OH^- are the major inorganic ligands forming Me complexes in freshwater, variation of pH leads to substantial changes in the concentrations of these species and to a redistribution of the metal forms inherent in these water reservoirs. Field experiments with mesocosm water acidified to pH 6–6.5 were carried out to investigate the ecological consequences of an increase in the fraction of aqua ions due to a decrease in the fraction of the inorganic complexes of Cu(II), Pb(II), Cd(II), and Zn(II) in the water of a real water body [6–8]. This methodology was used to study the role of Cu(II) binding with low-molecular organic compounds modeling the natural OS substances.

Two classes of compounds were chosen as model OS substances: carboxylic acids (benzyl- and *n*-hexadecylmalonic acids, BMAH_2 and HDMAH_2 , respectively) and phthalocyanines (PhC). Since the protonation constants of malonic acid derivatives are close in magnitude to the stability constants of their Cu(II) complexes, these derivatives were chosen in [9] as ideal models to study the toxicity effects of the metal-binding polar (BMAH_2) and hydrophobic (HDMAH_2) fragments of OS on the organisms living in water. Laboratory experiments established that the presence of CuBMA and CuHDMA com-

plexes (in addition to the inorganic forms of copper) in water had no toxic effects on mature fish, while CuHDMA suppressed embryonal development. Preliminary investigation of the behaviour of the same substances under conditions of an entire water ecosystem showed that both complexes had no appreciable effects compared to the suppressive action of the copper aqua ions on phytoplankton [10]. At the same time, the presence of a hydrophobic fragment in CuHDMA led to more efficient removal of copper from water to bottom sediments due to better sorption on suspended particles.

Compounds with a tetrapyrrole structure are another class of OS substances able to bind metal ions in natural water. Porphyrins, chlorophylls, cobalamins, and other biological molecules in which a macrocycle of four pyrrole molecules acts as a ligand for Me (Mg, Fe, Co, Cu) play a vital role in the life of animals, plants, and bacteria. Due to their structural similarity, these compounds have common metabolites and similar routes of transformation [11]. As a model OS of this class we chose copper phthalocyanine (tetrabenzoporphyrine) (CuPhC), which was sulphonated ($\text{R} = \text{SO}_3\text{H}$) to increase the solubility of the complex in water (Scheme 1).

The goal of the present work was to study the role of Me binding with organic compounds serving as models of low-molecular natural OS substances of varying structure and composition under conditions of a real natural freshwater reservoir polluted with Cu(II) salts.



Scheme 1.

OBJECTS AND METHODS OF INVESTIGATION

Field experiments with model OS introduced into mesocosms were carried out in summer 2002 at the site of the Novosibirsk water reservoir according to the procedure described in [7]. In each experiment, one of the mesocosms (volume 2 m³, height 3 m) served as a base, while the others were charged with the necessary portions of Cu(II) and model OS. Water was either kept at the natural level of pH or was acidified by bubbling CO₂ until the chosen pH value within the range 6.5–7.2 has been achieved. The scheme of experiments is shown in Table 1. The parameters monitored during experiment included the residual concentration [Cu]_w in solution, [Cu]_s on the suspended particles, and the dynamics of the functional and structural parameters of plankton; after the experiment had been completed (in 8–15 days) we measured the concentration [Cu]_{res} of the metal in the precipitate that settled in the sediment scavengers. The [Cu]_w concentration in the filtrates of averaged water samples from three depth points (50 ml aliquots from the surface and from depths of 1.5 and 2.5 m) was determined by inversion voltammetry (IVA-3 AK analyzer, Yekaterinburg) immediately after sampling and filtering the samples through MFA-MA filter No. 5 (pore diameter 0.5 mm). The filters were dried for determining

[Cu]_s. Determination was carried out by atomic absorption spectroscopy in a stationary laboratory (Perkin-Elmer 3030 spectrometer with an HGA-600 graphite atomizer) after the precipitate had been dissolved in HNO₃. The same procedure was used to analyze the sediments from the sediment scavengers. The concentrations [Cu]_w and [Cu]_s were correlated with the copper content in 1 l of water, and [Cu]_{res} was correlated with the copper content in 1 g of the air-dry sediment.

An integrated functional characteristic of plankton, namely, an overall daily primary product *P* in the mesocosms, was estimated every day. This parameter was determined from the diurnal dynamics of oxygen dissolved in water using the procedure described in [12]; for this, [O₂] was measured on the surface and at depths of 1.5 and 2.5 m of the mesocosm during daylight hours. In addition, the dynamics of the population and specific composition of the zooplankton organisms was monitored by catching over a water column in the mesocosm with a Jady net (inlet diameter 18 cm). The sample concentrated in a volume of 50 ml was fixed with formalin; the population of the main groups was visually counted using the Bogorov chamber. These data were used to estimate the biomass *B*, which was correlated with the mass of zooplankton in 1 m³ of water.

TABLE 1

Scheme of field experiments at the site of the Novosibirsk water reservoir (summer 2002)

Date	Mesocosm charge			
	M1	M2	M3	M4
23.06–08.07	Background, pH 8.5 ± 0.6	BMAH ₂ 10 ⁻⁵ M, pH 7.2 ± 0.3	CuSO ₄ (250), BMAH ₂ 10 ⁻⁵ M, pH 7.2 ± 0.3	CuSO ₄ (250), pH 7.2 ± 0.3
10.07–23.07	Background, pH 8.7 ± 0.7	CuSO ₄ (310), HDMAH ₂ 5 · 10 ⁻⁶ M, pH 6.5 ± 0.5	CuSO ₄ (310), HDMAH ₂ 5 · 10 ⁻⁶ M, pH 7.3 ± 0.5	CuSO ₄ (310), pH 6.5 ± 0.5
25.07–06.08	Background, pH 8.7 ± 0.7	CuPhC (50), pH 6.5 ± 0.5	CuSO ₄ (200), CuPhC (50), pH 6.5 ± 0.5	CuSO ₄ (250), pH 6.5 ± 0.5
26.08–02.09	Background, pH 8.7 ± 0.7	CuPhC (5), pH 8.1 ± 0.4	CuPhC (20), pH 8.1 ± 0.4	CuPhC (32.5), pH 8.1 ± 0.4

Note. The content (in µg/l) of Cu introduced with the given reagent is given in parentheses.

The initial concentrations of Cu(II), BMAH₂, HDMAH₂, and CuPhC in the mesocosms (see Table 1) were created by introducing solutions of known concentrations. A solution of Cu(II) was prepared from a CuSO₄ sample of analytical grade ("ch. d. a"); BMAH₂ was prepared from a reagent of ultrapure grade ("os. ch."). Hexadecylmalonic acid was synthesized by alkylation of diethylmalonic ester with 1-hexadecyl bromide. The product was isolated and purified by recrystallization from benzene; the purity of the product was confirmed by elemental analysis data and by the melting point (122.4 °C) which coincided with the literature data (122–123 °C) [13]. In view of the low solubility of HDMAH₂ in water, the latter was alkalized to pH 12 to prepare an initial solution of this acid [9]. Sulphonated copper phthalocyanine was obtained by treating the initial copper phthalocyanine with oleum with heating [14]. The solution was neutralized with an alkali and then evaporated several times to remove excess Na₂SO₄ by filtering. Finally, we obtained an aqueous solution of sulphonated copper phthalocyanine with a Cu concentration of 0.52 mg in 1 ml; this solution was used to introduce CuPhC into mesocosms.

The equilibrium distribution of Cu(II) over chemical forms in solution for experiments I and II (with BMAH₂ and HDMAH₂ introduced into the mesocosms) was calculated using WATEQ4f software [15]. Our analytical data on the ion composition of water and the inte-

grated parameters of copper binding with natural OS (conventional constant K_{CuL} and concentration [L] of binding centres) for the water of the Novosibirsk reservoir were published previously [7]. The protonation and Cu(II) binding (with BMAH₂ and HDMAH₂) constants are taken from [9]. The results of calculations are shown in Table 2. The distribution of Cu(II) over aqua ions and hydroxo and carbonate complexes depends on pH of water. The fraction of the former species increased substantially as pH decreased from 7.2 to 6.5 due to a decrease in the fraction of the latter species. The fraction of the copper forms bound with natural OS changed only slightly in all mesocosms in both experiments. The introduction of a model OS (BMAH₂) into mesocosm M3 in experiment I slightly decreased the content of other copper forms due to the formation of the CuBMA complex; its fraction was somewhat larger than the fraction of aqua ions but remained substantially smaller than the fraction of CuL forms bound with natural OS. In the second experiment, the fraction of CuHDMA was noticeably larger than the fraction of Cu²⁺, but comparable to the fraction of CuL.

In experiments III and IV, the equilibrium distribution of Cu(II) over forms in solution was not calculated because of the absence of the stability constants for the copper complex with phthalocyanine. In experiment III, decomposition of this complex in natural water was clearly evidenced by the gradual (within 2 or 3

TABLE 2

Fractional distribution of Cu(II) over its main forms in the water of mesocosms in experiments I and II (mean pH value of water given in parentheses), %

Copper form	Mesocosms				
	Experiment I		Experiment II		
	M3 (pH 7.2)	M4 (pH 7.2)	M2 (pH 6.5)	M3 (pH 7.3)	M4 (pH 6.5)
Cu ²⁺	5.0	5.3	17.5	4.6	27.8
CuOH ⁺	0.9	0.9	0.5	0.8	0.8
Cu(OH) ₂	33.3	36.0	3.0	31.4	4.7
CuHCO ₃ ⁺	2.5	2.8	9.3	2.4	14.7
CuCO ₃	24.6	26.6	14.0	23.2	22.2
CuL	27.4	28.3	27.1	25.8	29.8
CuBMA	6.3	0	0	0	0
CuHDMA	0	0	28.5	11.7	0

days) disappearance of the blue colouring of water in mesocosms M2 and M3 (M4 with the same total concentration of copper was not coloured blue) (see Table 1). For the initial solution of CuPhC, a few months had passed before this effect showed itself; it may be assumed, therefore, that decomposition of this compound in a natural water reservoir occurs with participation of living organisms. To verify this, we performed an additional experiment (IV) by varying the concentration of CuPhC (no other changes were induced in the hydroecosystem, *i. e.*, there were no copper additions and no changes in pH). It was confirmed that the CuPhC complex decomposed; as will be shown below, its individual addition to water stimulated the vital activity of plankton organisms, which was suppressed in other experiments.

RESULTS AND DISCUSSION

Experiment I

Figure 1, *a-d* demonstrates the influence of additional binding of some Cu(II) with BMAH₂ in solution on the dynamics of the residual copper concentration in water and on the overall primary product of plankton. Initially, the copper concentration in solution decreased rapidly, primarily due to sorption of copper on the suspended particles; sedimentation of the latter led to gradual removal of copper into bottom sediments. Toward the end of the experiment, both [Cu]_w and [Cu]_s decreased more smoothly; there were no noticeable differences between mesocosms without BMAH₂ (see Fig. 1, *a*) and with it (see Fig. 1, *b*). In 340 h after the mesocosms had been charged

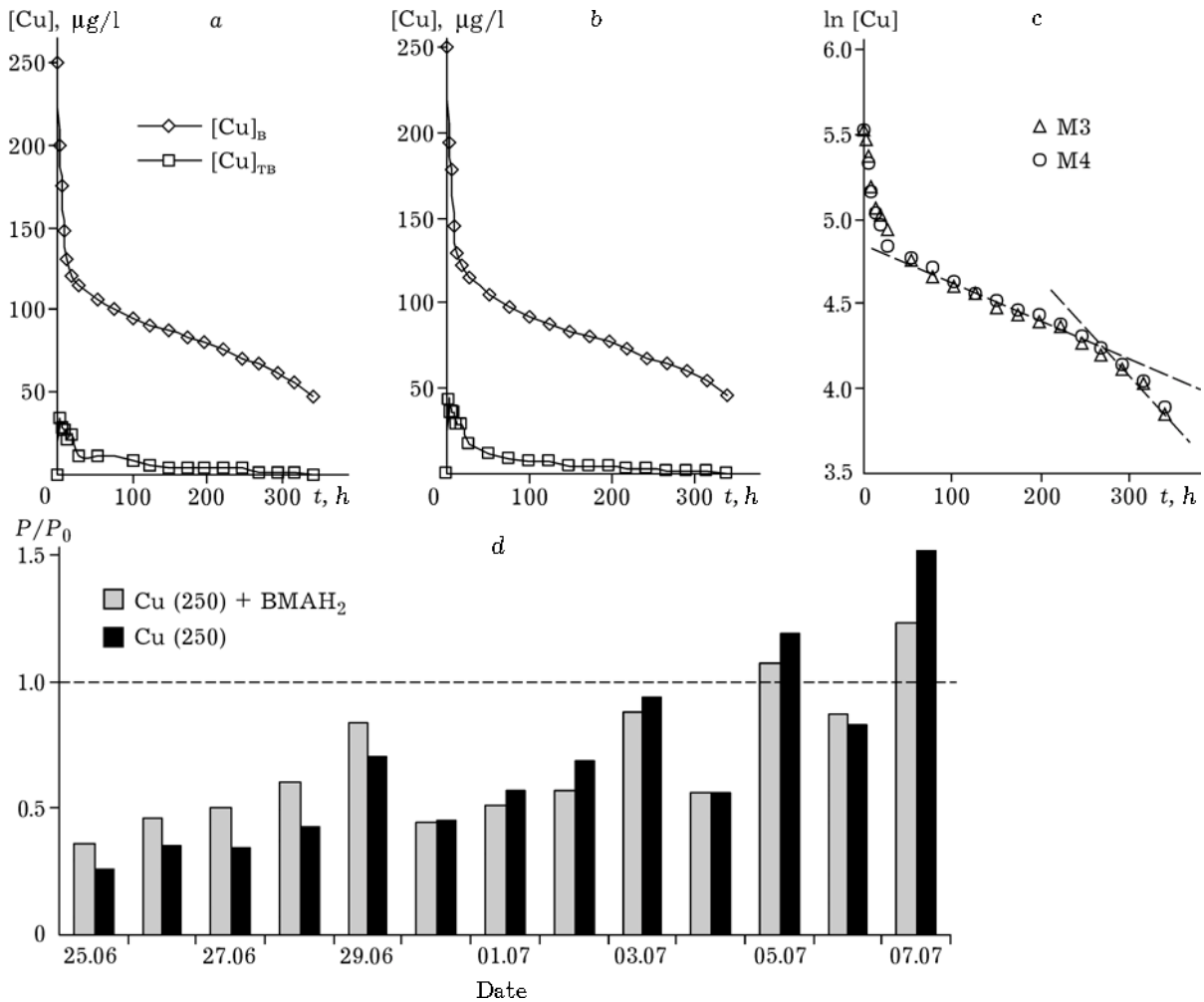


Fig. 1. Dynamics of copper concentration in the primary product in mesocosms M3 and M4 in experiment I: *a* - [Cu]_w and [Cu]_s; *b* - the same in M3; *c* - ln ([Cu]_w + [Cu]_s) in M3 and M4; *d* - the ratio of P in M3 and M4 to P₀ in M2.

with copper, the residual concentration of copper in water was ~20 % of the initial level, and its major part was in solution. Two linear segments can be distinguished on the line of the kinetic dependence of the logarithm of the total concentration $[Cu] = [Cu]_w + [Cu]_s$ (see Fig. 1, c): one extending from 50 to 270 h; and the other, from 270 to 340 h. The dynamics of copper removal from the water volume coincides for the two mesocosms and is identically accelerated at the final stage of the experiment, which can be concluded from the calculated rate constants k_1 and k_2 of this process in its formal description in terms of a first order equation: $k_1 = (0.0024 \pm 0.0001) \text{ h}^{-1}$ and $k_2 = (0.0049 \pm 0.0001) \text{ h}^{-1}$ for the first and second regions, respectively. Therefore, binding of some Cu(II) with BMAH₂ in the presence of other forms of Cu(II) in solution did not have any effect on the sorption of the pollutant on suspension particles and on the dynamics of its removal into bottom sediments. This was reported in our previous experiments with a different distribution of copper forms in solution [10].

The character of the kinetic dependence of copper removal from water (see Fig. 1, c) is determined by the presence of two different kinds of suspended particle: mineral particles and dead plankton organisms. Under the conditions of a mesocosm where water is not stirred up, the mineral particles settle to the bottom within a few hours and do not reappear in water. Thus this mechanism manifests itself only during the first day of the experiment. The plankton organisms that died directly after copper introduction into the mesocosm need longer periods of time to settle; this mechanism of Cu removal is concurrent with sedimentation of the mineral particles. Unlike the latter, the dead organisms can be replaced by newly born ones; their number reaches 10^6 and more individuals in 1 litre of water, while their life cycle lasts for 2 or 3 days. The plankton mechanism of Me removal from water into bottom sediments explains why it takes long for the Me concentration to decrease in the mesocosms [6–8]. The linear regions of the $\ln [Cu] - t$ dependence (see Fig. 1, c) correspond only to the plankton channel, while the non-linear region observed during the first

hours of the experiment corresponds to co-sedimentation of the mineral particles and dead plankton. This process may be decelerated or accelerated due to the irregular reproduction of phytoplankton. Indeed, the period of decreased primary product P in M3 and M4 mesocosms with respect to P_0 in M₂ without copper additions but with the same pH of water gives way to a period of recovery of the plankton vital activities (see Fig. 1, d). The increased rate of copper removal from water correlates with this period (see Fig. 1, c). In this process, the binding of some Cu(II) with BMAH₂ does not appreciably affect the kinetics of $[Cu]$ and the dynamics of P/P_0 .

The response of the zooplankton community, whose biomass increased in the base mesocosm during the experiment, to copper additions in the presence of BMAH₂ was similar to the response in the absence of the latter (Fig. 2). The initial total concentration of Cu in the mesocosms is shown in parentheses in Figs. 3–8. Acidification of water and addition of BMAH₂ had almost no effects on the biomass dynamics in the main groups of zooplankton B in which the Copepoda group of crustacean predators were predominant, while the introduction of copper suppressed these organisms. The presence of the CuBMA complex in water has slightly weakened the negative action of copper; at the final stage of the experiment, the biomass of Copepoda in this mesocosm turned out to be even larger than that in the base mesocosm.

After the experiment, the mass of the sediment collected in the sediment scavengers in the M2–M4 mesocosms was 2.2–2.5 times smaller than that in the base mesocosm, which reflects the general suppression effect of the plankton organisms. The amount of copper present in 1 g of the sediment was larger in M4 without BMAH₂ additions (836 $\mu\text{g/g}$) than in M4 with this addition (556 $\mu\text{g/g}$), but the total amount of the metal removed from water into bottom sediments was approximately the same in both mesocosms due to the difference in the sediment mass.

Thus Cu(II) binding with the carboxyl groups of the model OS with a polar fragment does not markedly affect the efficiency of the plankton mechanism of Cu removal from water into

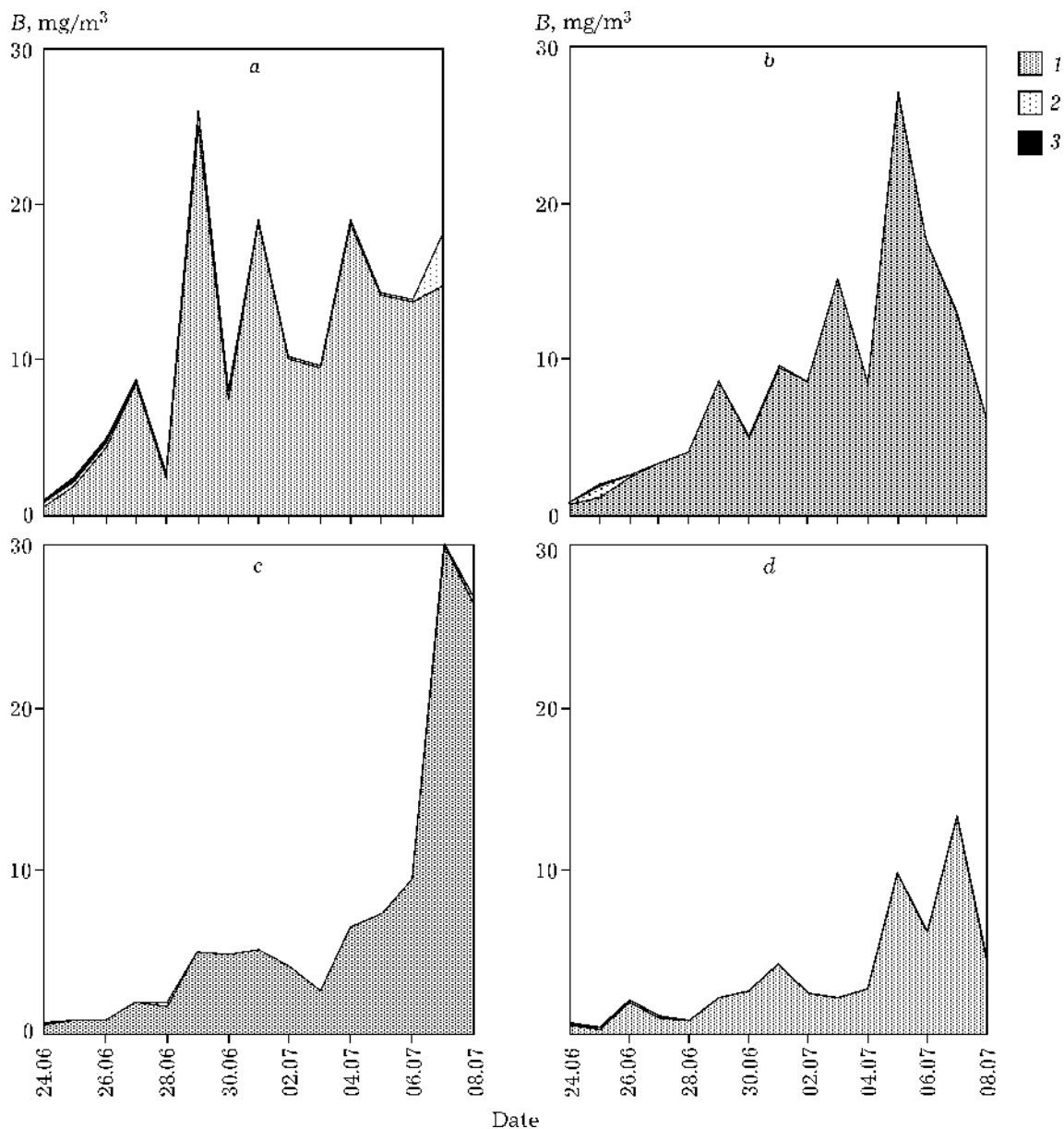


Fig. 2. Effect of binding of some copper with BMAH₂ in solution on changes in the biomass of the main groups of zooplankton (pH 7.3): a - background, b - BMAH₂, c - Cu (250) + BMAH₂, d - Cu (250); 1 - Copepoda, 2 - Cladocera, 3 - Rotatoria.

bottom sediments. Compared with other copper forms in solution, CuBMA is no more toxic for phytoplankton and exhibits lower toxicity for zooplankton.

Experiment II

In this experiment, HDMAH₂ was used as the model OS; this compound contains a hydrophobic fragment instead of the polar fragment of BMAH₂. The dynamics of [Cu]_w and

[Cu]_s concentrations in mesocosms with different distributions of Cu(II) over its forms in solution is shown in Fig. 3, a; the kinetics of the total concentration of copper in water is given in Fig. 3, b. The binding of some Cu(II) into the CuHDMA complex resulted in [Cu]_w decreasing more effectively by the end of the experiment and led to more complete removal of copper from water into bottom sediments, especially in the M3 mesocosm in which pH of water was higher than that in M2 (see Tab-

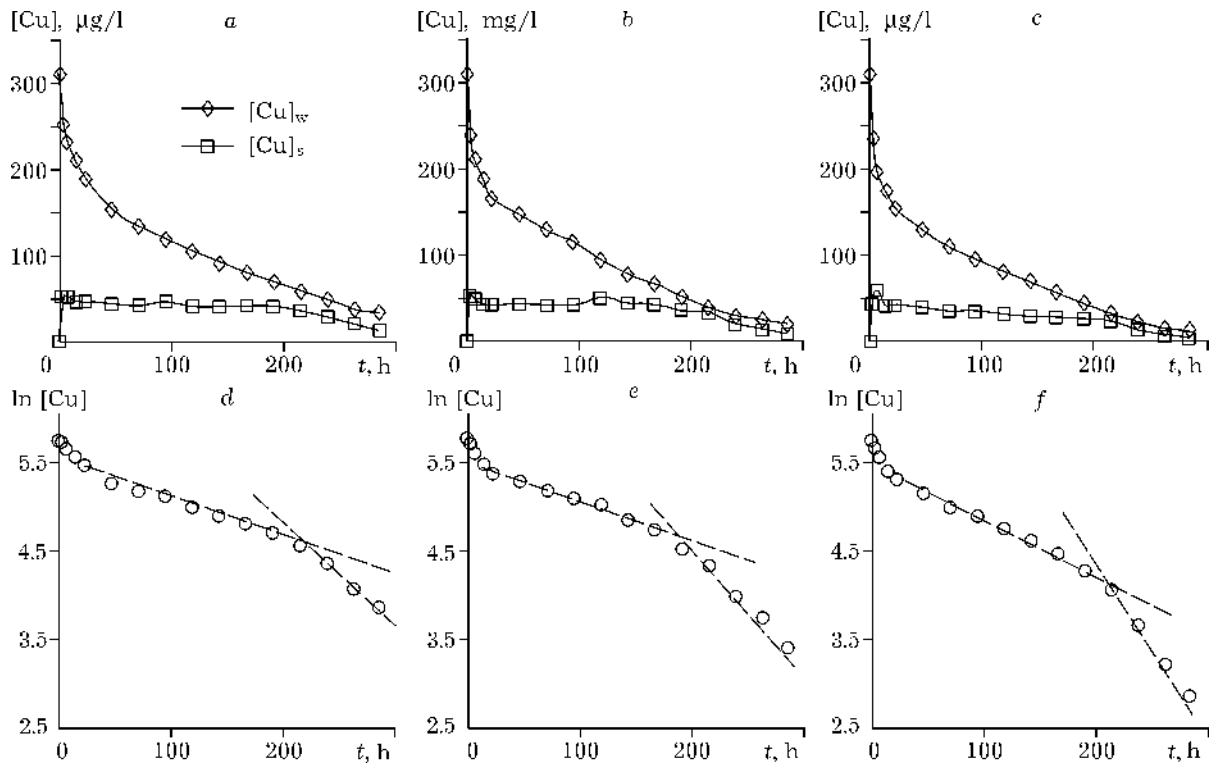


Fig. 3. Dynamics of copper concentration in mesocosms M2-M4 in experiment II: *a-c* - $[Cu]_w$ and $[Cu]_s$; *d-f* - $\ln([Cu]_w + [Cu]_s)$; *a, d* - Cu (310), pH 6.5; *b, e* - Cu(310) + HDMAH₂, pH 6.5; *c, f* - the same, pH 7.2.

le 1). This seems strange because the fraction of this complex in M2 is more than doubled relative to its fraction in M3 (see Table 2). However, the fractions of other copper forms also differ between the two mesocosms. The higher efficiency of copper removal from water in M3 correlates with the decreased fraction of Cu^{2+} and $CuHCO_3^+$ and with the increased fraction of the neutral $Cu(OH)_2$ and $CuCO_3$ complexes. Evidently, redistribution between these inorganic forms of copper in water produced

a stronger effect on the dynamics of copper removal into bottom sediments than did changes in the concentration of the model CuHDMA complex.

If we compare the dynamics of $[Cu]_s$ in the first and second experiments (see Figs. 1 and 3), we can see that the fraction of copper sorbed on the suspension particles is higher in experiment II. The rate of Cu removal from water into bottom sediments was also higher. The values of k_1 were equal for the M2 and

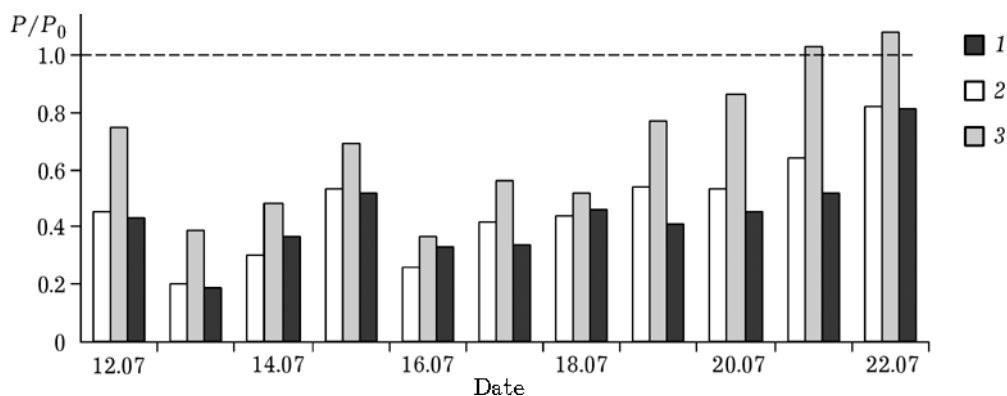


Fig. 4. Effect of binding of some copper with HDMAH₂ on changes in the primary product of plankton: 1 - Cu (310), pH 6.5; 2 - Cu (310) + HDMAH₂, pH 6.5; 3 - the same, pH 7.2.

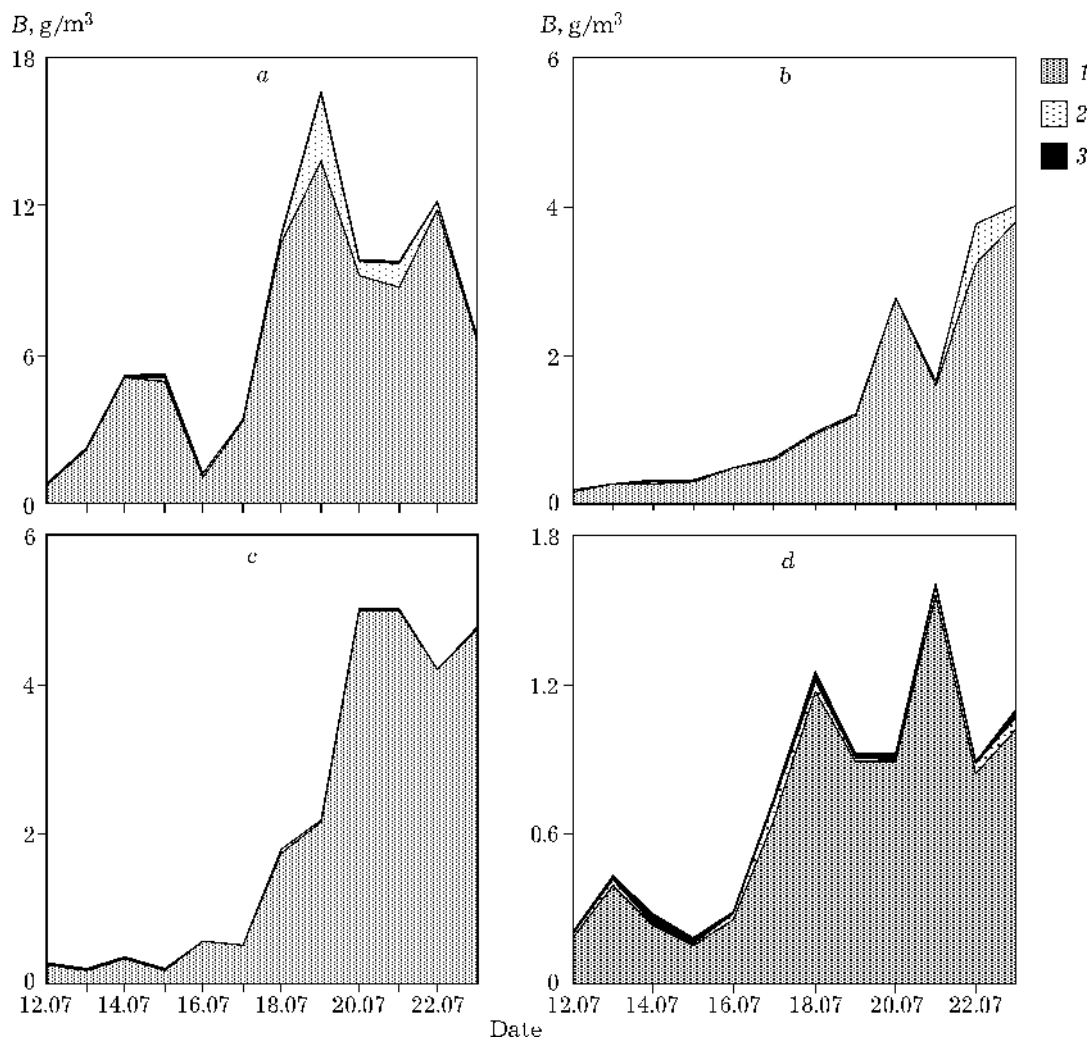


Fig. 5. Effect of binding of some copper with HDMAH₂ on changes in the biomass of zooplankton: *a* - background; *b* - Cu (310), pH 6.5; *c* - Cu (310) + HDMAH₂, pH 6.5; *d* - the same, pH 7.2; 1 - Copepoda, 2 - Cladocera, 3 - Rotatoria.

M4 mesocosms with identical pH values of water ($0.0043 \pm 0.0003 \text{ h}^{-1}$), but slightly higher ($0.0057 \pm 0.0001 \text{ h}^{-1}$) for M3 with higher pH. At the final stage of the second experiment we also observed accelerated copper removal from water; the values of k_2 increased to (0.0102 ± 0.0006), (0.0127 ± 0.0007), and (0.0171 ± 0.0003) h^{-1} for M4, M2, and M3, respectively. The increased fraction of $[\text{Cu}]_s$ in the water volume and the nearly doubled rate of copper removal into bottom sediments are associated with the higher efficiency of the plankton mechanism due to the larger number of phytoplankton organisms in the water of the reservoir. The average value of P in the base mesocosm (2.26 mg of Cu in 1 l of water) in

this experiment was two times higher than the corresponding value in experiment I.

At the second stage of experiment II, the higher rate of copper removal into bottom sediments correlated with the dynamics of production processes in the mesocosms, which is shown in Fig. 4 as the ratio of P to P_0 in the base mesocosm. These processes proved to be less suppressed, while the intensity of the recovery was higher in the M3 mesocosm (pH 7.3), where some Cu(II) was bound into the CuHDMA complex, the fraction of Cu^{2+} and CuHCO_3^+ was the lowest, and that of $\text{Cu}(\text{OH})_2$ and CuCO_3 was the highest.

In contrast, in the zooplankton community, the distribution of copper over its forms in

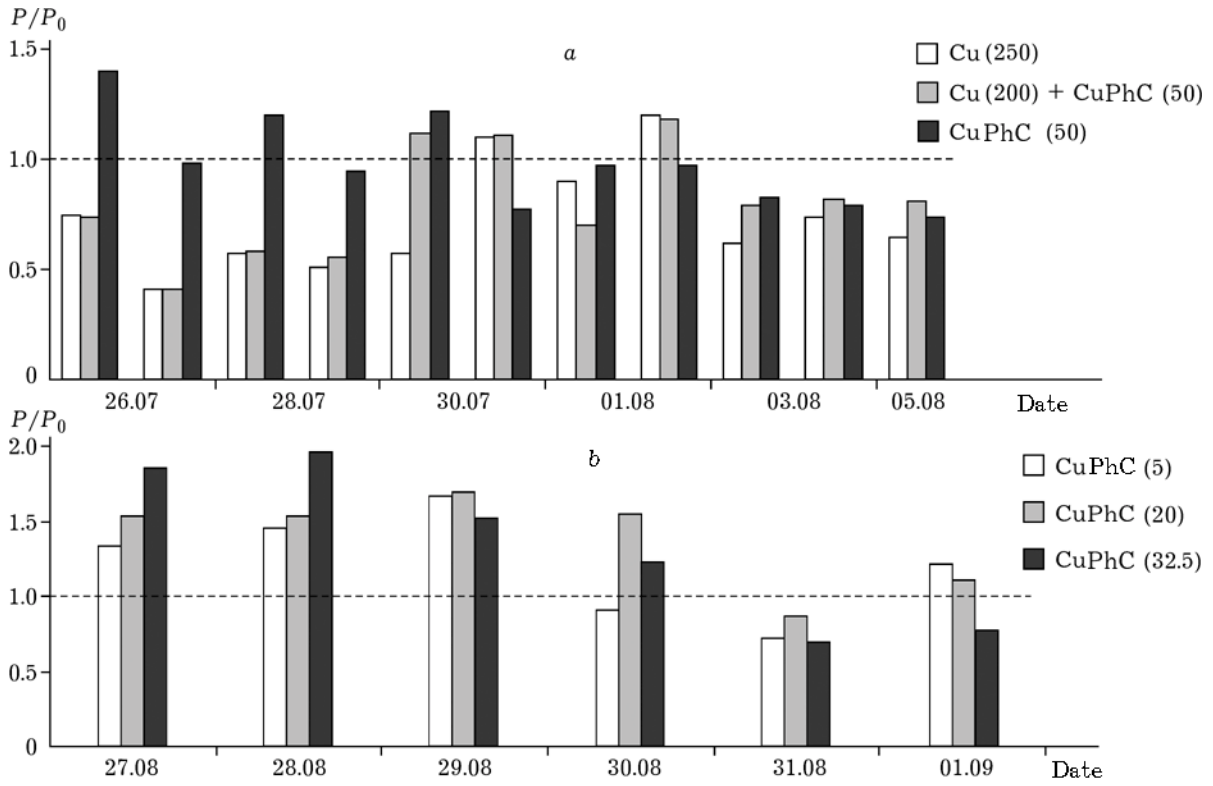


Fig. 6. Dynamics of the primary product of plankton in mesocosms M2-M4 in experiments III (a) and IV (b) with CuPhC additions.

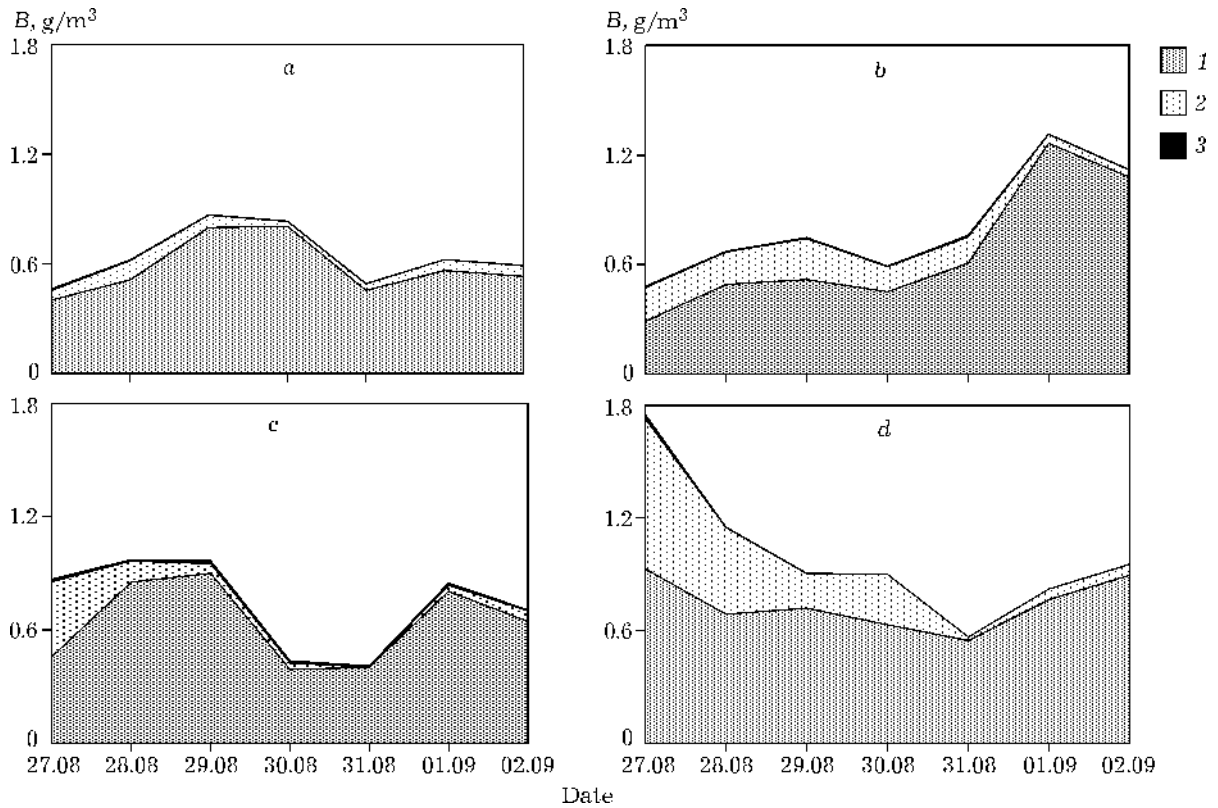


Fig. 7. Effect of CuPhC concentration on the dynamics of the biomass of zooplankton: a - background, b - CuPhC (5), c - CuPhC (20); 1 - Copepoda, 2 - Cladocera, 3 - Rotatoria.

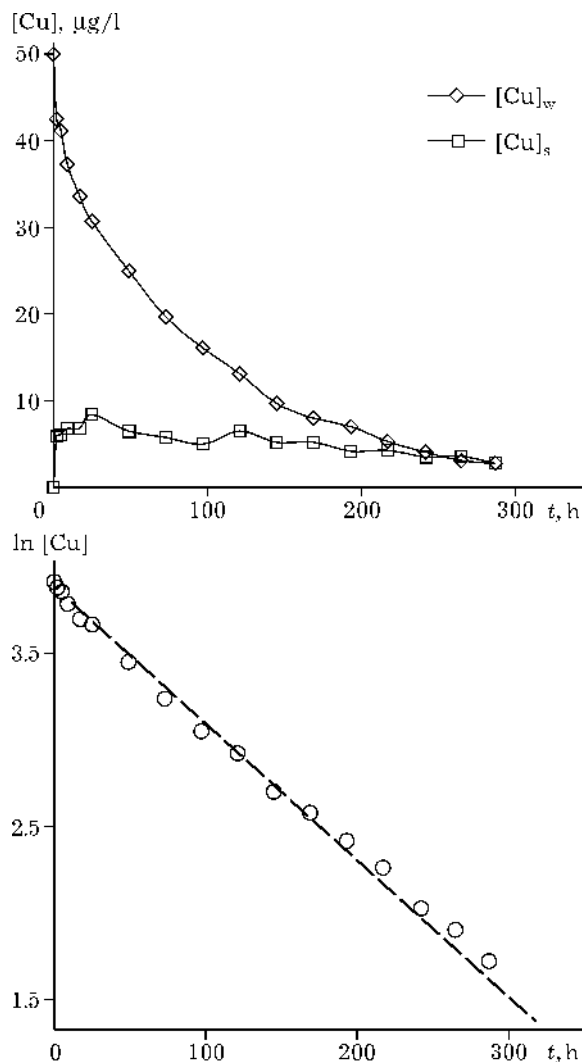


Fig. 8. Dynamics of the residual copper concentration in a water volume in a mesocosm with a CuPhC addition (50 µg/l).

solution was very suppressing because the biomass of organisms decreased tenfold in comparison with that in the base mesocosm (Fig. 5). This was not observed in experiment I for the M3 mesocosm in which the concentrations of the inorganic forms of copper were the same, and the only difference was the presence of the CuBMA complex instead of CuHDMA (see Table 2). Suppressed life activity of zooplankton under the action of Cu(II) additions was also observed in the absence of CuHDMA in M4. However, suppression was less pronounced. The response of zooplankton in experiment I to Cu(II) additions in the absence of CuBMA (see Fig. 2, mesocosm M4) was even less noticeable. Comparing these data with the distribu-

tion of copper over its forms in solution (see Table 2) we can notice that the negative effect of copper weakened when the fraction of the Cu^{2+} and CuHCO_3^+ cations decreased and that of the neutral $\text{Cu}(\text{OH})_2$ and CuCO_3 complexes increased. This leveled the suppressing action of CuHDMA in M2, where its concentration was the highest and the total decrease in the zooplankton biomass was smaller than that in M3 (see Fig. 5).

Thus replacement of a polar fragment by a hydrophobic one in the CuBMA and CuHDMA complexes resulted in changes in the toxic properties of these complexes with respect to zooplankton. As mentioned above, this difference in the effects of the two copper complexes was discovered in experiments with test fish and embryos [9]. At the same time, binding of some Cu(II) into CuHDMA weakened the suppressive action of Cu(II) additions on the productivity of phytoplankton and led to more efficient copper removal into bottom sediments. The amount of Cu detected in 1 g of the sediment collected in sediment scavengers in M2 and M3 was 1016 and 975 mg, which is much more than that discovered in experiment I with benzylmalonic acid additions.

Experiments III and IV

In these experiments, the model OS introduced into the mesocosms was a Cu(II) complex with sulphonated phthalocyanine and copper additions in the form of CuSO_4 (see Table 1). The introduction of this complex led to blue colouring of water, and the gradual disappearance of the colour indicated that the complex decomposed. Unfortunately, the mechanisms and kinetics of this process remain unclear. Nevertheless, the presence of CuPhC at the start of the experiment had a pronounced effect on the plankton organisms.

The dynamics of P/P_0 in the M2–M4 mesocosms with water acidified to pH 6.5 in experiment III is shown in Fig. 6, a (with respect to the base mesocosm). Initially, production processes in mesocosms with the total contents of copper additions of 250 µg/l were suppressed, but then they were stimulated by additions of 50 µg/l of Cu in the form of CuPhC. This

difference vanished in the second half of experiment III, when P started to recover to the level of the base mesocosm. Even stronger initial stimulating action on primary production was produced by CuPhC additions in experiment IV, when no more CuSO_4 was introduced and water was not acidified (see Fig. 6, b). This effect was stronger for higher concentrations of CuPhC (from 5 to 32.5 $\mu\text{g}/\text{l}$ based on Cu).

The effect of CuPhC on zooplankton is shown in Fig. 7 for experiment IV. During this experiment (August 26 – September 2) the zooplankton biomass in the water reservoir was noticeably smaller than in the previous experiments. The fraction of detritus-eating Rotatoria remained insignificant, but the fraction of phytoplankton-eating Cladocera increased. Introduction of copper only in the form of CuPhC into the mesocosms immediately initiated growth of biomass B with respect to the base mesocosm, which is especially evident for the Cladocera group. The Copepoda crustaceans, feeding on Cladocera, seem to be responsible for the subsequent reduction in the fraction of the latter. The stimulating action of CuPhC additions on zooplankton was also evident from the presence of the additional amount of copper (200 $\mu\text{g}/\text{l}$) in water in experiment III. This response of zooplankton to CuPhC additions differs from that observed in experiments I and II with other model OS (see Figs. 2 and 5). On the one hand, this points to the absence of a direct suppressing action of CuPhC on different groups of zooplankton. On the other, the increased population of phytoplankton creates favourable conditions for the development of Cladocera and thus for Copepoda; as a result, CuPhC additions stimulated the life activity of the whole zooplankton.

The high intensity of production processes in mesocosms with CuPhC additions determined the efficiency of the plankton channel of copper removal from water to bottom sediments. Figure 8 shows changes in the concentration of copper in water for the mesocosm with an initial addition of copper (CuPhC) of 50 $\mu\text{g}/\text{l}$ (pH of water 6.5, experiment III). At the end of the experiment, the residual concentration of copper in water was 5.6 $\mu\text{g}/\text{l}$ with an equal proportion between $[\text{Cu}]_w$ and $[\text{Cu}]_s$. This concentration is close to the copper concentration

in the water of the Novosibirsk water reservoir; consequently, almost all copper introduced in the form of CuPhC was removed from water to bottom sediments. The plot of $\ln [\text{Cu}]$ versus t in 20 h after the mesocosm had been charged is described by one linear segment, and it was calculated that $k = (0.0072 \pm 0.0001) \text{ h}^{-1}$.

Thus the introduction of CuPhC into the mesocosms has stimulated the development of both phyto- and zooplanktons. This gives us grounds to believe that gradual decomposition of copper phthalocyanine in natural water is associated with biotic processes. Taking into account that the metal is rather efficiently removed from water to bottom sediments, it may be concluded that Cu(II) binding with low-molecular OS having a tetrapyrrole structure alleviates the hazard of water pollution with copper salts.

Similar effects of Cu(II) binding with carboxyl groups of low-molecular OS are less pronounced and are structure-dependent. The formation (in water) of a CuBMA complex with a polar fragment did not change the rate of copper removal into bottom sediments, but stimulated an increase in the biomass of zooplankton. The increased activity of these organisms probably leveled the similar action of CuBMA on phytoplankton because the latter reacted to copper additions in the same way whether the complex was present or not. Copper binding (in water) into CuHDMA with a hydrophobic fragment accelerated copper removal into bottom sediments and reduced suppression of phytoplankton, but had a negative effect on zooplankton.

CONCLUSIONS

The ecological consequences of pollution of natural freshwater reservoirs with metal compounds depend on many interrelated factors. One of them is variability of the chemical composition of water, which determines the distribution of Me over chemical forms. Another factor is quantitative and qualitative variability of the community of aquatic organisms. To investigate changes in the pollutant and the response of biota to its action, experiments should be conducted under conditions

maximally approximating those of a natural hydroecosystem. The methodology of these experiments allows one to study the effects of definite substances present in water and affecting the distribution of Me over chemical forms. Among these substances may be organic compounds able to bind copper. In freshwater reservoirs, OS are represented by various compounds, either entering from outside or formed in the reservoirs. These compounds are prone to transformations in various processes. These circumstances, as well as the different abilities of OS to bind Me, create certain difficulties for mesomodeling used to investigate the role of the organic subsystem of natural waters in their pollution with metals.

Two directions of investigation are possible in this situation. The first involves on-site experiments under the conditions of variability of natural OS with identification of individual organic compounds. The second involves the use of model compounds of definite composition whose properties are known or may be examined. This approach allows one to reveal the role of the specific features of OS (for example, molecular mass, functional groups, hydrophobicity) that may be essential to the behaviour of Me bound with these compounds in a hydroecosystem. In the present study we have investigated the role of metal (copper) binding with low-molecular organic compounds from two classes: carboxylic acids and phthalocyanines. Both classes are typical natural OS. The results of this study prove that the presence of these substances in water diminishes the adverse ecological consequences of water pollution with metals. They accelerate pollutant removal from water to bottom sediments and lead to weaker suppression of phyto- and zooplanktons. These effects, however, depend on the nature of the functional groups and on the hydrophobicity of OS. We suppose that the methodology of active chemical and eco-

logical experiments at the site of a natural water reservoir may also be used to study the effects of other features of OS on the behaviour of metals bound with these substances in natural water reservoirs.

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