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Original scientific paper

DETERMINATION OF SPECIATION AND BIOAVAILABILITY OF COPPER IN THE LAKE MODRAC WITH GEOCHEMICAL BIOTIC LIGAND MODEL (BLM)

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Abstract: The results of investigation of inorganic and organic speciation of copper compounds in the lake Modrac, using the geochemical equilibrium model BLM (Biotic Ligand Model; USEPA 2007) and other geochemical models are presented, as well as toxicity and bioavailability of copper compounds in the lake Modrac for chosen biotic ligand of fish fathead minnow (*Pimephales promelas*). Speciation and bioavailability of copper complexes was investigated and toxicity levels for chosen biotic ligand determined. The influence of water chemistry on copper toxicity is predominant, and application of sophisticated BLM model enables the prediction of toxicity and bioavailability of copper.

Key Words: copper speciation, geochemical models, bioavailability, toxicity.

Introduction

The artificial lake Modrac was constructed in 1964 and it extends about 17 km^2 and presents great potential not only for the supply of industrial plants with water, but for the factory for drinking water. Low depth and slow flow are the basic features of the accumulation of Modrac, which mostly affects the ecological balance.

The content of heavy metals, particularly copper (Cu) is of great importance for biological and aquatic animal systems, as well as for evaluation of Cu entering into the food chain, since copper has the highest binding potential for biotic ligands.

Total content of heavy metals is usually regularly measured, and it is not an adequate indicator of bioaccumulation of heavy metals in aquatic organisms.

To evaluate the toxicity of copper, it is necessary to know the information about the speciation - coexisting physical and chemical forms of ionic Cu species in the tested aquatic systems.

Speciation of copper ionic species in natural aquatic systems depends on the distribution of ionic Cu species and complex compounds and interactions present in the aquatic system (soil-colloids, suspended solids-aqueous phase).

In natural waters only a small percentage of trace metals exist as free metal ions, while the majority is adsorbed on colloidal particles or forms complex compounds (inorganic complexes, organic complexes).

When determining the bioavailability and toxicity of Cu, it is of particular importance to determine the dissolved fraction of metal ions (toxic fraction), which can be transferred to the biological membrane - such as gill cells of fish, which are used as biological indicators, i.e. the biotic ligands. (Batley, 2004).

Special risk to aquatic organisms present copper complexes which are soluble in lipids and can quickly diffuse through bio-membrane and enter the metal and ligand into the cells, resulting in increased bioavailability and intoxication (Blanchard, 2009). In small quantities copper presents the essential element for most organisms, however, with the increase of concentration it can become toxic.

Geochemical cycle of metals includes transport, adsorption and precipitation, while the biological cycle implies bioaccumulation, bioavailability and toxicity. The toxicity of copper depends of temperature, presence of natural ligands- inorganic and organic, cation content, pH, dissolved organic carbon (DOC).

Most metal speciation studies are based on the application of geochemical equilibrium models that include software thermodynamic databases. Bioavailability depends on the presence of free copper ions and labile copper complexes, namely the content of toxic forms of copper (Cu^{2+}; $CuOH^+$, $[Cu_2(OH)_2]^{2+}$). (Kramer, 2004).

The toxicity of copper is influenced by dissolved organic carbon (DOC), which often competes with inorganic ligands for free copper by building strong copper complexes. Concentration and strength of binding organic ligands are critical factors in determining the toxicity of copper.

The gills of fish regulate the balance of major ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, NH_3, H^+) through water intake and are exposed to the potential source of copper toxicity. The adsorption of Cu^{2+} ions on fish gills disturbs the balance of Na ions, with the result of toxic effect of copper.

In aquatic systems copper can exist in three different forms: particulate, colloidal and dissolved. Total concentration of dissolved copper in the natural environment can be described by the following equation: (Schamphelaere, 2003).

$$[Cu]_{total} = [Cu^{2+}] + \sum_i \left(\beta'_{CuL}[L'_i][Cu^{2+}]\right) + \sum_i \left(\beta'_{CuX_n}[X'_i]^n[Cu^{2+}]\right) \quad + \sum \left(\beta'_{CuOH_n}[Cu^{2+}] / [H^+]^n\right) \tag{1}$$

$[Cu]_{total}$ represents the total concentration of total organic copper including free ionic copper and copper associated with organic, $[L_i']$ and inorganic substances, [Xi']. β'_{CuL} and β'_{CuX} represent conditional stability constants for all organic and inorganic copper-ligand complexes. β'_{CuOH} is the acidity constant in natural waters, and *n* represents the stoichiometric coefficient for a given ion.

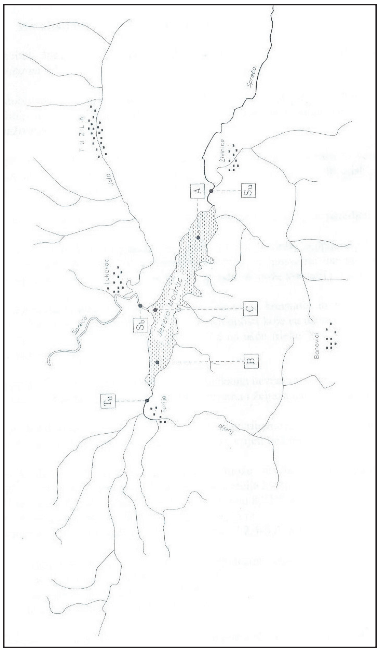
Organic matter in natural water (NOM-Natural organic matter) is present as dissolved organic matter (DOM- Dissolved Organic Matter) and particulate organic matter (POM).

Most abundant natural organic compounds are humic and fulvic acids, which are strong complexing agents for copper ions, and affect the concentration of bio-available copper. Studies have shown that most of the dissolved and suspended organic matter is derived from humic substances and their organic carbon represents 40-70% of total organic carbon.

Copper complexes with organic matter are different in structure, depending on the binding sites of compounds with different molecular weights and stability constants, so that the application of geochemical models enables the study of complex interactions of metal with dissolved organic matter, DOM. (Bryan, 2002).

Materials and Methods

Sampling and investigations of physical and chemical characteristics of the lake Modrac water on six sampling localities for two seasons (spring, summer, 2011), have been performed using certified methods in accordance with the applicable legislation and AOAC methods in the accredited laboratory of Institute for chemical engineering SODA SO Tuzla, during regular monitoring of the lake Modrac. Analyses of concentrations of heavy metals, including copper, were performed with spectroscopic methods: atomic absorption spectroscopy (AAS) and plasma emission spectroscopy (ICP-OES). Figure 1 presents the schematic presentation of the lake Modrac and six testing Localities:



- Locality A - area of the river Spreča (Locality 1)
- Locality B - area of the river Turija (Locality 2)
- Locality C - dam area-entrance (Locality 3)
- Locality Su - Spreca River (Locality 4)
- Locality Tu - Turija-river (Locality 5)
- Locality Sb - Spreca River downstream from the dam reservoir (Locality 6)

Figure 1: Lake Modrac with six testing Localities

The obtained data of water quality parameters of the lake Modrac on six testing localities are used as input data for software of BLM geochemical model (BLM-Biotic Ligand Model). The basis of geochemical equilibrium models lies in the application of ion-association approach, known as the Debye-Huckel's model. Sorption of ions in the aqueous phase of the specific surface areas can be described using electrostatic or non-electrostatic model.

Non-electrostatic sorption is described using a simple distribution coefficient, (K_D) of a given component between the aqueous phase and solid phase or by Langmuir's and Freundlich's model.

The application of geochemical software models is based on the balance of chemical components expressed by a set of equations for all chemical species in the equilibrium system (Paquin, 2002).

$$T_j = \Sigma_i S_i a_{i,j} \qquad (2)$$

Concentrations of individual chemical species are presented with S_i while the stoichiometric coefficient marked with $a_{i,j}$.

Inorganic speciation involves aquatic hydroxyl and carbonate complexes. The values of thermodynamic stability constants used are from the NIST (National Institute of Standards and Technology, 1995) database. Competitive reactions are simulated as balance of simultaneous reactions and equilibrium speciation of Cu, which involves the creation of organic and inorganic complexes.

It is believed that the dissolved organic molecules exist as negatively charged groups with a continuous distribution of different binding affinity (NICA-Donnan's model) or as a mixture of ligands that can be described as two discrete groups with a wide range of affinities characterized by proton binding intrinsic constants (pK_{HA}; pK_{HB}) as it is proposed in the models WHAM V and Win Humic V. (Ying, 2005).

These models demonstrate multiligand character of humic substances, taking into account the competitive binding of functional groups and non-electrostatic interactions in the diffuse layer, and they are included in BLM model. On the basis of experimental data on the content of dissolved organic carbon (DOC), modeling and estimation of organic speciation is performed. Geochemical software Visual Minteq was applied for the determination of inorganic and organic speciation. This model is combines the following models: (Di, 2001).

1. Gaussian model for the multiligand distribution of humic acids
2. NICA-Donnan model simulating electrostatic interactions
3. SHM model (Stockholm Humic Model) for metal-proton interactions

Inorganic and organic speciation and toxicity of copper was determined with BLM model, which includes several of the above mentioned models with associated thermodynamic databases for the stability constants of complexes of copper with inorganic and organic species, and with selected biotic ligand of fish fathead minnow (*Pimephales promelas*).

Water quality parameters: pH, Ca, Mg and Na influence the toxicity of Cu in natural waters. Taking into account the effect of these factors on the interactions of metals with biological receptor, called biotic ligand (BL), Biotic Ligand Model (BLM) provides the predictions of copper toxicity for selected biotic ligands. This model includes thermodynamic data for the simulation of competitive interactions of copper with hydrogen ions, alkalinity ions, and natural organic matter in the aqueous phase and calculates copper inorganic and organic speciation and toxicity (Hydroqual, 2007). Figure 2 presents a schematic representation of the BLM model for copper.

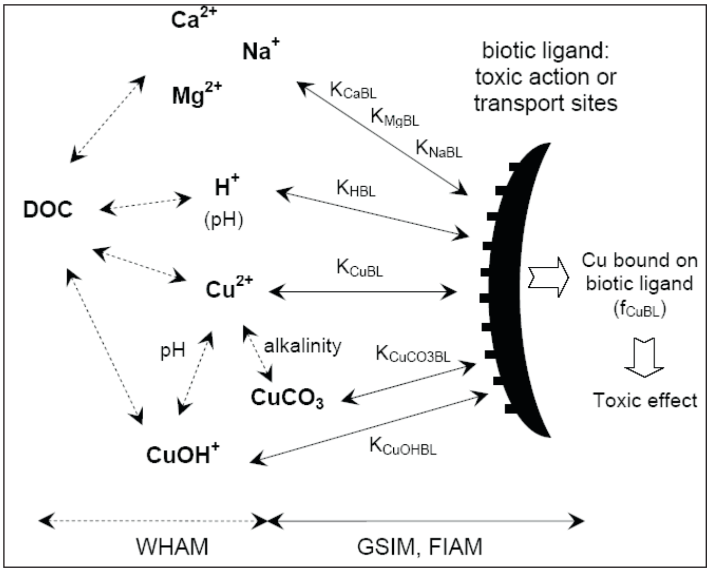


Figure 2: Schematic representation of BLM for copper(9)

Dashed lines present speciation reactions (WHAM, Tipping, 1994), while the solid lines present the binding of the biotic ligand. Free copper ions, Cu^{+2}, build complexes with inorganic ligands such as OH^- and CO_3^{2-}. Ions Cu^{2+} and $CuOH^+$ build complexes with dissolved organic carbon (DOC). Ca^{2+}, Mg^{2+} and H^+ compete with copper for binding sites on DOC. Ions Cu^{+2}, as well as $CuOH^+$ and $CuCO_3$ bind to biotic ligand, where the concentration of Cu^{+2} on the bound ligand determines the toxic effects on biotic ligand.

Ions Ca^{2+}, Mg^{2+}, Na^+ and H^+ can compete with Cu^{2+} ions for places on biotic ligand, thereby reducing toxicity. This concept was first formulated in the models FIAM (Morel, 1983) and GSIM (Pagenkopf, 1983). Based on the results of extensive studies of Cu accumulation at the site of toxic action (biotic ligand-fish gills) and Cu speciation tests, toxic effects can be predicted (Meador, 1991).

Di Toro et al have developed the concept of BLM predictions of acute toxicity of copper to fish (Di, 2001). According to this model, it is assumed that the toxicity of copper to aquatic organisms occurs as a result of reaction of metal with physiologically active binding sites, resulting in the formation of complex copper-biotic ligand. BLM concept is based on the hypothesis that the interaction of the copper-biotic ligand can be displayed in the same manner as any kind of metal reactions with organic or inorganic ligands. The concentration of a metal- biotic ligand is determined based on the equation of mass balance:

$$[M_iL_b^+] = K_{MiLb}[M_i^{2+}][L_b^-] \qquad (3)$$

K_{MiLb} represents the stability constant for the metal-ligand complex. It is also assumed that the protonation can occur with the formation of the complex proton-biotic-ligand, HL_b with concentration $[HL_b]$ and the stability constant K_{HLb}.

$$[HL_b] = K_{HLb}[H^+][L_b^-] \qquad (4)$$

Mass balance equation associated with biotic ligand is L_b^- :

$$[L_b^-]_T = [L_b^-] + [HL_b] + \Sigma_i{=}_1^{NMi}[M_iL_b^+] \qquad (5)$$

$[L_b^-]_T$ represents the total density of the binding sites on the biotic ligand (eg vacancies nmol / g tissue), $[HL_b]$ is the concentration of protonated sites while N_{Mi} is the number of metal complexes, $M_iL_b^+$, eg CuL_b^+ with biotic ligand. Analogous equations for metal cations and other aquatic ligands that form metal-ligand complexes are characterized by their stability constants. K_{MiLj} and K_{HLj} are the stability constants for these ligands. Mass balance equations for metal cations in solution are:

$$[M_i^{2+}]_T = [M_i^{2+}] + [M_iL_b^+] + \Sigma_j{=}_1^{NLj}[M_iL_j^+] \qquad (6)$$

N_{Lj} is the number of metal-ligand complexes, including hydroxyl complexes with form M_i^{2+}. According to BLM model, it is assumed that the amount of metal bound to the biotic ligand is negligible in relation to aquatic species. Therefore the previous equation takes the form:

$$[M_i^{2+}]_T = [M_i^{2+}] + \Sigma_j{=}_1^{NLj}[M_iL_j^+] \qquad (7)$$

The required, specific for BLM model, are conditional stability constants, K_{HLb} and K_{MiLb} , i=1 ..... N_{Mi} for complexes of biotic ligand with proton and metal, with total density of sites, $[L_j^-]_T$. The model is based on the idea that the mortality rate (or other toxic effects) occur if the concentration of metals on biotic ligand reaches a critical concentration C_{Mi}^*.

$$C_{Mi}^* = [M_iL_b^+] \qquad (8)$$

This critical concentration for mortality can only be determined from the toxicity experiments in which LC $_{50}$ concentration is determined for different concentrations of toxic metals and competing cations. When the density of binding sites and stability constants are known, critical concentration can be determined by calculating the concentration of the biotic ligand corresponding to aquatic LC_{50} concentration. This critical concentration is called LA_{50} or lethal accumulation of metals on biotic ligand associated with 50% of mortality (Welsh, 1996).

LA_{50} is expressed in the units of nmol / g wet weight of bioticligand. As BLM includes inorganic and organic speciation of metals, as well as competitive complexation with biotic ligand, amounts of dissolved metals necessary to achieve this threshold value will vary, depending on the water chemistry. Therefore, in addition to calculating chemical speciation, BLM can be used to predict the concentrations of metals that would cause acute toxicity in a given aqueous system. It was found that variations in water quality affect the speciation, which affects the composition and type of metal ion species that determine the bioavailability and toxicity of the aquatic world. Differences in the activity of free copper ions (Cu^{2+}) can explain the influence on the toxicity. Mass balance for the total concentration of ionic forms of copper at the biotic ligand is given by the equation:

$$[BL\text{-}TOT] = [CuBL^+] + [CaBL^+] + [MgBL^+] + [NaBL^0] + [HBL^0] + [Bl^-] \qquad (9)$$

Concentrations of the complex cation-biotic ligand are expressed in mol / l. Equilibrium equation for cation binding can be expressed as the conditional stability constant (l / mol) in the form:

$$K_{CuBL} = \frac{[CuBL+] \quad [CuBL+]}{\{Cu+2\}[BL-]\{Cu+2\}[BL-]} \qquad (10)$$

Activity of copper ions, the concentration of the complex copper-biotic ligand and unoccupied places on biotic ligand are expressed in mol / l., and by replacing in the upper equation, total concentration of binding sites is expressed by:

$$[BL\text{-}TOT]=[BL^-](1+K_{CuBL}\ \{Cu^{+2}\}+K_{CaBL}\ [Ca^{+2}]+K_{MgBL}[Mg^{+2}]\ +K_{NaBL}\ [Na^+]\ +K_{HBL}\ [H^+]) \qquad (11)$$

Results and Discussion

Test results of investigations of water quality of the lake Modrac on six localities in two seasons, spring and summer 2011 are given in Table 1.

BLM (Biotic Ligand Model) was applied for determination of inorganic and organic speciation of copper and toxicity of copper, for biotic ligand of fathead minnow (*Pimephales promelas*). This biotic ligand is chosen because it is 30% more sensitive to copper toxicity than other biotic ligands of fish present in the lake water.

Water quality parameters, which are input data in BLM model, are: temperature, pH, dissolved organic carbon, major cations (Ca, Mg, Na and K), major anions (SO_4 and Cl^-), alkalinity and sulfides. The content of organic matter is expressed as the concentration of dissolved organic carbon (DOC), it is calculated from the equation:

$$DOC = \frac{DOM\,DOM}{1,7 \quad 1,7} \qquad (12)$$

Table 1: Limiting values of indicators of water quality of the lake Modrac on six testing Localities

Indicator	Spring 2011.	Summer 2011.
pH	8,21-8,42	7,7-9,07
Dissolved O_2	10,8-11,5mg/l	2,2-12,3mg/l
Consumption of $KMnO_4$	17,31-20,10mg/l	5,4-16,0mg/l
NH_4	0,1000-0,4000mg/l	0,0020.0,5200mg/l
NO_2	0,0139-0,0195mg/l	0,0028-0,1393mg/l
NO_3	0,5481-0,7065 mg/l	0,0571-0,1170mg/l
Fe	0,1350-0,3500mg/l	0,1400-0,9120mg/l
Mn	0,0106-0,0226mg/l	0,0124-0,3230mg/l
Cu	0,0070-0,0090mg/l	0,0060-0,0150mg/l
Zn	0,0138-0,0350mg/l	0,0123-0,0341mg/l
Pb	0,0170-0,0368mg/l	0,0051.0,0370mg/l
Ni	0,0270-0,0310mg/l	0,0055-0,0210mg/l
Cr	0,0270-0,0310mg/l	0,0090-0,0210mg/l

The approximation is used that dissolved organic matter (DOM) is approximately equal to the chemical oxygen demand (COD), namely DOM ≈ COD.

Table 2: Values of DOC (Dissolved Organic Carbon) for spring and summer period 2011

DOC (mg/l)	Locality 1	Locality 2	Locality 3	Locality 4	Locality 5	Locality 6
Spring	39,41	10,76	5,64	61,94	5,64	11,23
Summer	25,58	10,76	153,47	51,17	46,05	20,47

According to BLM model, inorganic carbonate species include carbonate (CO_3), bicarbonate (HCO_3) and carbonic acid (H_2CO_3). The sum of these species is called dissolved inorganic carbon (DIC). Bicarbonates are usually the most important DIC species in natural waters, since they are dominant fraction in lake water with pH from 6.35 to 10.33. In BLM model the critical parameter is inorganic carbon, since many metals, including copper, form carbonate complexes. Values for DIC are estimated in accordance with the empirical expression related to the alkalinity.

By entering the water quality parameters given in Table 1 and values of DOC and DIC into BLM software, inorganic and organic speciation can be determined, as well as acute toxicity criteria (Criterion Maximum Concentration, CMC) and chronic toxicity (Criterion Continuous Concentration, CCC). With entering the input data of the content of dissolved copper, BLM also calculates the acute toxic units (TU), as the ratio of dissolved copper (Cu) and CMC, i.e. (TU = Cu / CMC). If the values of TU are greater than 1, it indicates that values of copper bioavailability are in the range of chronic toxicity for the selected biotic ligand.

BLM predicts the final value of acute toxicity, FAV (Final Acute Value), wherein the CMC = FAV / 2 .The following toxicity criteria: CCC; CMC, FAV, TU and ACR (ACR-ratio of acute to chronic toxicity) are introduced into the Water Quality Criteria of USEPA (US Environmental Protection Agency, 1985).

Results of BLM simulation and speciation of copper for six sampling sites of the lake Modrac are presented in Table 3.

Table 3: BLM model- Speciation of Cu for spring and summer period in 2011

Indicators	Locality 1	Locality 2	Locality 3	Locality 4	Locality 5	Locality 6
pH	8,320	8,390	8,280	7,840	8,020	8,000
Cu,diss;mol/l	1,264E-05	3,604E-06	1,694E-06	1,208E-05	1,292E-06	2,530E-06
Cu,free,mol/l	2,890E-10	2,728E-10	3,042E-10	3,485E-10	3,264E-10	3,505E-10
Cu,org,mol/l	1,261E-05	3,566E-06	1,659E-06	1,206E-05	1,265E-06	2,502E-06
Bl-u,nmol/gw	2,313E-02	2,237E-02	2,433E-02	2,927E-02	2,787E-02	2,803E-02
Bl-CuOH	1,080E-02	1,157E-02	9,611E-03	4,665E-03	6,059E-03	5,912E-03
DOC,mg/l	3,941E+01	1,076E+01	5,640E+00	6,194E+01	5,640E+00	1,123E+01
HA%	1,000E+01	1,000E+01	1,000E+01	1,000+01	1,000+01	1,000+01
DIC	7,882E-02	2,152E-02	1,128E-02	1,239E-01	1,128E-02	2,246E-02
BL-Ca	2,486E-10	2,637E-10	2,744E-10	2,615E-10	2,124E-10	2,705E-10
BL-Mg	1,280E-10	1,408E-10	1,410E-10	9,862E-11	1,909E-10	1,364E-10
Bl-H	1,338E-13	9,785E-14	1,172E-13	4,619E-13	2,263E-13	2,361E-13
Bl-Na	3,206E-11	2,783E-11	2,556E-11	2,591E-11	3,172E-11	2,694E-11
CuOH	7,082E-10	7,539E-10	5,772E-10	2,220E-10	2,671E-10	2,951E-10
$Cu(OH)_2$	1,325E-10	1,591E-10	8,366E-11	1,078E-11	1,663E-11	1,900E-11
$CuSO_4$	1,506E-11	1,304E-11	1,132E-11	1,022E-08	1,008E-11	1,652E-11
$CuCO_3$	2,562E-08	2,728E-08	2,339E-08	1,022E-08	1,008E-11	1,652E-11
$Cu(CO_3)_2$	1,114E-09	1,340E-09	8,831E-10	1,455E-10	3,134-10	2,997E-10
CuCl	6,885E-14	5,503E-14	8,055E-14	1,047E-13	4,239E-14	7,939E-14
$CuHCO_3$	9,881E-09	8,959E-09	9,892E-09	1,188E-08	1,118E-08	1,175E-08
Ionic strength	4,865E-02	2,005E-02	1,497E-02	7,099E-02	1,464E-02	2,078E-02

Organic and inorganic speciation of Cu was investigated with application of other geochemical models as well, Visual Minteq-model and CHEAQS-model. The results showed satisfactory correlation with the results of BLM model, and are presented on Figures 3 and 4.

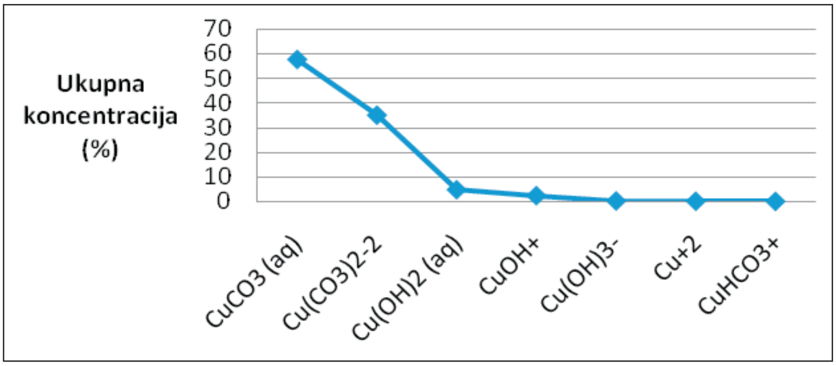


Figure 3: Inorganic speciation on the Locality 1 (summer season 2011) with Visual MINTEQ model

Carbonate fraction prevails in inorganic speciation on all localities in spring and summer period, but due to increased alkalinity in summer period, concentration of carbonate species is much higher.

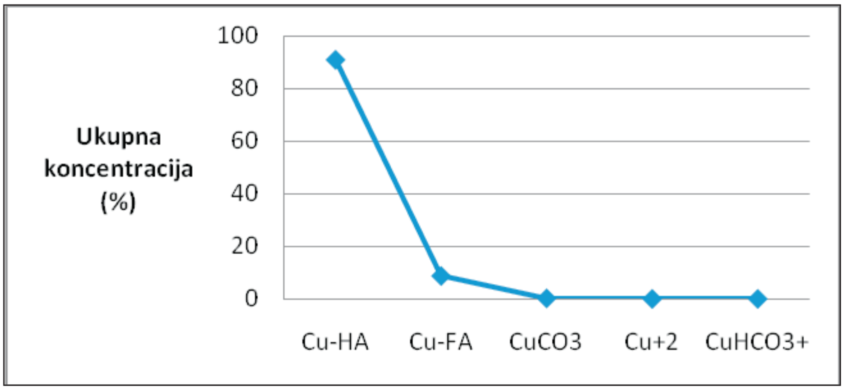


Figure 4: Organic speciation on the Locality 1 (summer season 2011) with CHEAQS model

In organic speciation copper complexes with humic substances dominate in both periods on all testing localities. Toxicity simulations of Cu to biotic ligand (fathead minnow; *Pimephales promelas*) with BLM model for spring period on six testing localities of the lake Modrac are given in Table 4.

Table 4: Results of Cu toxicity investigations using BLM model for biotic ligand of fathead minnow (*Pimephales promelas*) for spring period 2011

Locality	Final Acute Value (FAV) µg/L	CMC (CMC=FAV/2) µg/L	**CCC (CCC=FAV/ACR) µg/L**	**Acute Toxic Units (TU=Cu/CMC)**
Locality 1	803.2214	401.6107	249.4477	0.0174
Locality 2	229.0198	114.5099	71.1242	0.0611
Locality 3	107.6469	53.8235	33.4307	0.1486
Locality 4	767.6357	383.8178	238.3962	0.0287
Locality 5	82.1014	41.0507	25.4973	0.4141
Locality 6	160.7714	80.3857	49.929	0.0995

FAV (Final Acute Value) refers to the lethal concentration of 50%, what means that it is the concentration of Cu ionic species (in water), which has 50% probability to cause death of aquatic organism (fathead minnow). If the value of FAV is lower, it means that the content of copper species is approaching to toxic values. FAV value for copper, for freshwater fish, is generally from 0.1-1.0 mg/l (Welsh, 1996).

Chronic exposure to sublethal levels of copper reduces the survival, growth, and reproduction rate for broad range of aquatic species. According to BLM model, lethal toxicity is defined as the concentration of metal that is essential to result in accumulation at the biotic ligand and that is equal to the value FAV for tested biotic ligand.

These results provide the ability to predict when acute copper toxicity for fathead minnow will occur, at the given physico-chemical parameters of the quality of the natural aquatic system of the lake Modrac, for all tested sites. The obtained FAV (Final Acute Value) values are consistent with values obtained for other tested natural aquatic systems for given biotic ligand, i.e. fathead minnow (*Pimephales promelas*) (Welsh, 1996).

The increase in hardness, pH, alkalinity, and natural organic matter content leads to the decrease in bioavailability of copper, with increasing FAV values for a given biotic ligand, (fathead minnow). (Playle, 1992).

Alkalinity (mg $CaCO_3$ / l) is considered an indicator of copper inorganic complexation and bioavailability. However, investigations of the influence of alkalinity on the biotic ligand of fathead minnow showed no correlation, confirming the complexity of natural aquatic systems and the inability to predict the toxicity of copper on the basis of individual parameter of water quality. It can be concluded that there is a protective effect with synergistic activity of several anions and cations (Ca^{2+} +, Mg^{2+}, Na^+, K^+, CO_3^{2-}, Cl^-, SO_4^{2-}) which form complexes with free copper ions.

BLM model implies the formation of organic complexes with reactive functional groups of organic matter (humic and fulvic acids), which are continuously distributed on the active sites of these acids, and molecular charge is used to determine the electrostatic changes. It was found that for a variety of aquatic organisms there are almost identical models of toxicity, what can be explained by different affinity of metals towards oxygen nitrogen or sulfur. Copper shows affinity towards oxygen and sulfur and is considered to be one of the most toxic metals with high potential of forming strong complexes with biotic cells.

It was found that the accumulation of copper in the biotic ligand (BL) is directly dependent on the activity of Cu^{2+}. FAV values and acute toxicity of copper increases with increasing the pH value of the lake water due to intense formation of copper complexes, especially Cu-carbonate and Cu- hydroxide, and with stronger adsorption of copper to DOC (Dissolved Organic Carbon).

With the increase of alkalinity, and at elevated concentrations of Ca^{2+} and Mg^{2+} ions, there is an intensive competition in the process of adsorption of ions on the biotic ligand (gills of fathead minnow), resulting in higher values of FAV, so that additional amounts of Cu^{2+} ions are necessary to reach the level of acute toxicity, and that population of fathead minnow is not exposed to toxic levels of copper ionic species.

Binding of Mg^{2+} ions to biotic ligand is significantly weaker than binding of Cu^{2+}, so that Mg^{2+} affects the toxicity less than Ca^{2+} ions. Similarly, Na^+ ions compete for the active sites on the biotic ligand, so that the increased concentration of Na^+ results in higher values of acute toxicity, FAV. This is especially true with biotic ligand of fathead minnow (*Pimephales promelas*). Impact of copper on the acute toxicity can be explained by the difficulties in ion-regulation of copper on gill epithelium, i.e. reduction of the Na^+ / K^+-ATP activity. (Playle, 1992).

With the application of BLM model for fathead minnow, FAV values are obtained in broad range, in spring period from 0.0821 to 0,803 mg / l, and in summer period from 0,436-1,117 mg/l, what can be explained by the difference in chemical composition of water on six different localities. Also, the values of toxic units, TU are less than 1 in all localities, meaning that it is sublethal exposure.

It can be concluded that the values of acute toxicity of copper, i.e. the final acute values FAV, which could cause death of half the population of fathead minnow in all localities, are in expected range for tested biotic ligand, with exception of some localities in summer period, where FAV values are over 1.0 mg/l, but the value for toxic units, TU are less than 1. Higher values of FAV in summer can be explained due to the higher pH values, and increased content of organic matter, and because of the increased alkalinity, as well as because of the specific ratio of concentrations of the competing ions (Ca, Mg, Na and K) for complexation on the biotic ligand. FAV values indicate the predicted limiting values of copper content which can be tolerated on specific site until the value of toxicity (LA_{50}) is reached.

Final toxicity of copper is lower in spring period, what means that the possibility for toxic activity of copper toward fathead minnow is higher than in case of summer period when higher concentrations of organic copper complexes and carbonate fraction in inorganic speciation prevail.

Conclusions

1. In natural aquatic systems, bioavailability is a complex function of many factors, including the total concentration and speciation of metals, mineralogy, pH, redox potential, temperature, total content of organic matter, content of suspended matter, water volume, time availability.
2. Key water parameters, such as, alkalinity, cation content, ionic strength, redox potential, dissolved organic matter and pH, affect the speciation of copper in natural waters, and consequently the bioavailability and adsorption on the gills of fish.
3. Ionic forms of Cu^{2+} and $CuOH^+$ are considered the most toxic ionic species in inorganic speciation, while Cu-carbonates are considered to be less toxic complexes, and they prevail in inorganic speciation. Of particular importance is the content of DOC (dissolved organic carbon) matter, which competes with inorganic ligands for free copper ions in aquatic systems.
4. Ions that contribute to the increase in hardness reduce acute toxicity of copper to aquatic organisms (fathead minnow) through a complex mechanism of action. Hardness ions of Ca and / or Mg and protons inhibit copper binding on the surface of the biotic ligand, what results in formation of carbonates.
5. With applying of BLM model, the values of acute and chronic toxicity are determined for biotic ligand of fathead minnow (*Pimephales promelas*) which vary for spring and summer periods in the range from 0.08 to 1.12 mg / l, depending on the physico-chemical parameters of water, reaching the upper limit for values of final toxicity (FAV), but with toxic units (TU) less than one, indicating that state of chronic toxicity is not reached.
6. Regular monitoring on the basis of simple physico-chemical characterization of water criteria as input parameters of BLM model can give credible predictions of the bioavailability and toxicity of copper for specific biotic ligand on the basis of simulation of inorganic and organic speciation. This approach is introduced in USEPA legislation and is in process of adoption into the EU water quality standards.

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