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ASSESSMENT OF HEAVY METAL (Ni, Cu) INTERACTIONS IN BINARY MIXTURE TO RAINBOW TROUT (*Oncorhynchus mykiss*) AT EARLY LIFE STAGES

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Abstract. *Lethal toxicity test and sublethal short-term and long-term toxicity tests on embryos and larvae of rainbow trout Oncorhynchus mykiss (beginning from "eye-egg" embryos and from 1-day old larvae) were performed. Lethal and sublethal toxic effects of the 1.0, 0.5, 0.25, 0.125 and 0.06 portion of the LC50 of Cu, Ni singly and their binary mixtures (Cu+Ni) were analyzed. The mixtures according to 96-hour LC50 for embryos and 96-hour LC50 for larvae were prepared. During exposure such biological parameters of the embryos and larvae as: mortality; gill ventilation frequency, behavioral responses of larvae were evaluated. Lethal toxicity test showed that the 96-hour LC50 values for embryos and larvae were different, and depended on the mixture preparation mode, heavy metal concentration in the mixture, and development stage of organism. Sublethal exposure of mixture disordered gill ventilation function in larvae, affected behavioral reactions, and depended on the mixture of preparation mode, heavy metal concentration in the mixture, duration of exposure and affected development stage of organism. Obtained data showed that toxicity assessment in fish at early stages of their development is important for evaluation of water pollution with heavy metals and prediction of their risk to aquatic animal populations.*

Keywords *toxicity, nickel, copper, binary mixture, fish*

1. Introduction

Copper is toxic to aquatic animals at levels marginally in excess of those found in many unpolluted aquatic environments [1]. Nickel as a water-soluble metal is easily absorbed and accumulated by the aquatic organisms resulting in metal-induced disturbances in the structure and function of various tissues and organs [2, 3]. The background concentrations of copper and nickel in Lithuanian water bodies are comparatively low. Levels of these metals, especially copper, can frequently increase from industrial pollution or from anthropogenic sources [4]. The toxic effects of copper alone have been widely studied [5, 6]. The data on nickel toxicity in fish is very scarce, however impact of this metal was partially investigated for other freshwater organisms [7]. Nevertheless, environmental contaminants are frequently encountered as mixtures, and the behavior of chemicals may not correspond to data predicted for pure compounds [8]. Data for heavy metal binary mixture toxicity depend largely on different organisms/methods applied, sensitivity of parameters studied etc. For the majority of the binary combinations, the interactions were of synergistic nature [9, 10, 11, 12 and 13]. The aim of this study was to undertake investigations into the lethal and sublethal effects of copper, nickel singly and to ascertain the toxic effect of Cu+Ni mixtures depending on the mixture preparation mode, on the heavy metal concentration in the mixture, on the duration of exposure and on affected development stage of organism; to assessment of copper and nickel interactions in their binary mixture to rainbow trout (*Oncorhynchus mykiss*) at early life stages of development.

1. Materials and methods

Toxicity tests were conducted at the Laboratory of Ecology and Physiology of Hydrobionts Institute of Ecology of Nature Research Centre. Rainbow trout embryos and larvae were obtained from the Žeimena hatchery. Dilution water was from high quality artesian water. The average hardness of dilution water was approximately 284 mg/L ($CaCO_3$), alkalinity 244 mg/L (HCO_3^-), the mean pH was 8.0, temperature was 10° ± 0.5°C and the oxygen concentration was between 8 to 10 mg/L. The lethal and sublethal toxic effects of the 1.0, 0.5, 0.25, 0.125 and 0.06 portion of the LC50 of Cu, Ni singly and their binary mixtures (Cu+Ni) were analyzed (Table 1). The mixtures according 96-hour LC50 for embryos and 96-hour LC50 for larvae were prepared (Table 2) [14, 15]. The mixtures were equal to 1%. Solutions were made from chemically pure copper and nickel salts ($CuSO_4 \cdot 5H_2O$; $NiSO_4$ $7H_2O$). Concentrations of Ni and Cu 0.2; 0.5 mg/L, respectively are accepted as the Maximum-Permissible-Discharge into inland water bodies according to the European Parliament and Council Directive 2000/60/EC [16]. Control water and solutions were renewed on alternate days.

Table 1
Concentrations of heavy metals studied

Concentration, portion of LC50	Heavy metal concentration (mg/L)			
	Embryos		Larvae	
	Cu	Ni	Cu	Ni
1	1.1	3.4	0.4	1.2
0.5	0.5	1.7	0.2	0.6
0.25	0.25	0.8	0.1	0.3
0.125	0.125	0.4	0.05	0.15
0.06	0.06	0.2	0.025	0.08

Lethal toxicity test (96-hour) and sublethal short-term (96 hour) and long-term (38, 24 days) toxicity tests on embryos and larvae (beginning from “eye-egg” embryos – for the first test and beginning from 1-day old larvae – for the second test) were performed. All tests were conducted under semi-static conditions. One hundred embryos were exposed to each concentration of HM, and their mixtures. Studies with embryos and larvae were performed in three replications. Biological [mortality; gill ventilation frequency (GVF, counts/min); behavioral responses of larvae (the number of individuals making nests in % and the number of individuals responding to external stimuli in %); parameters of the larvae were evaluated using routine methods [14].

Table 2
Lethal toxicity of heavy metals to rainbow trout embryos and larvae [14]

Heavy metal	96-hour LC50 (mg/L)	95% confidence interval (mg/L)
Embryos		
Cu	1.13	1.0 ÷ 1.27
Ni	3.39	2.98 ÷ 3.85
Larvae		
Cu	0.44	0.39 ÷ 0.49
Ni	1.25	1.07 ÷ 1.47

Median acutely lethal concentration (LC50) values and their 95% confidence intervals were estimated by use Spearman-Karber methods [17]. The toxic effect of the mixtures was calculated according to the mixture toxicity index (MTI), and it was estimated according to the presented classification [18]. The significance of all the data obtained was determined by use of Student‘s t-test with P ≤ 0.05.

Table 3
Lethal toxicity of Cu+Ni mixtures, mixtures toxicity indexes (MTI) and effects of the mixtures to rainbow trout embryos and larvae

HM concentration in mixture (portion of LC50)	96-hour LC50 (%/mg/L)	95% confidence interval (%)	MTI	Effect of mixture
Embryos (mixture prepared according to 96-hour LC50 for embryos, the acute test beginning from "eye-egg" embryos till hatching period)				
1:1	0.32/0.36+1.08	0.29 ÷ 0.44	1.65	more-than additive
0.5:0.1	0.43/0.48+1.46	0.48 ÷ 1.46	1.23	more-than additive
Larvae (mixture prepared according to 96-hour LC50 for larvae, the acute test beginning from "eye-egg" embryos including hatching period)				
1:1	0.28/0.11+0.35	0.22 ÷ 0.36	1.92	more-than additive
1:0.1	0.42/0.18+0.5	0.36 ÷ 0.58	1.27	more-than additive
Larvae (mixture prepared according 96-hour LC50 for larvae, the acute test beginning from 1-day larvae)				
1:1	0.38/0.17+0.45	0.30 ÷ 0.42	1.42	more-than additive
1:0.1	0.48/0.21+0.60	0.39 ÷ 0.50	1.06	more-than additive

3. Results and Discussion

2.1. Lethal toxicity

The data obtained showed that the 96-hour LC50 values for embryos and larvae were different, and depended on the mixture preparation mode, on the heavy metal concentration in the mixture, and on affected development stage of the organism (Table 3). Hatching larvae were more sensitive to Cu+Ni mixture as compared to embryos and 1-day old larvae.

2.2. Sublethal toxicity

Short-term effect of 0.125 portion of the 96-hour LC50 for larvae concentration of Cu and Ni did not induce alterations in the GVF of the larvae. Meanwhile, the same concentration of Cu+Ni mixture induced significantly increase in the GVF of the larvae at the end of the first test (Table 4). Short-term effect of 0.125 portion of the 96-hour LC50 for larvae concentration of.

Table 4
Sublethal toxic effects of Cu, Ni and their binary mixture on GVF and behavioral responses of rainbow trout larvae (mean±SEM)

Chemicals	GVF (counts/min)		The number of individuals making nests (%)		The number of individuals responding to external stimuli (%)	
	After 4 days	After 38 days	After 4 days	After 38 days	After 4 days	After 38 days
0.125 portion of the 96-hour LC50 for larvae						
Beginning from “eye-egg” stage embryos						
Cu	90.0 ± 4.1	112.8 ± 3.2	86 ± 4.6	86 ± 6.2	88 ± 3.8	88 ± 3.8
Ni	92.6 ± 5.7	104.2 ± 2.6*	80 ± 4.4	56 ± 4.8*	86 ± 4.2	48 ± 4.2*
Cu+Ni	98.4 ± 4.8*	106.0 ± 2.2*	72 ± 4.0*	42 ± 4.4*	78 ± 4.4*	46 ± 4.2*
Control	**87.4 ± 4.4**	**115.0 ± 3.4**	**91.6 ± 8.4**	**90.4 ± 9.4**	**92.4 ± 7.6**	**94.4 ± 5.6**
	After 4 days	**After 20 days**	**After 4 days**	**After 20 days**	**After 4 days**	**After 20 days**
0.125 portion of the 96-hour LC50 for larvae						
Beginning from 1 day old larvae						
Cu	90.2 ± 2.8	112.4 ± 2.4	86 ± 4.6	86 ± 6.2	88 ± 3.8	88 ± 3.8
Ni	90.4 ± 2.6	114.8 ± 3.0	80 ± 4.4	88 ± 4.8	86 ± 4.2	84 ± 4.2
Cu+Ni	92.4 ± 2.4	127.6 ± 2.2*	82 ± 4.0	72 ± 4.4*	90 ± 4.4	66 ± 4.2*
Control	**87.4 ± 4.4**	**115.0 ± 3.4**	**91.6 ± 8.4**	**90.4 ± 9.4**	**92.4 ± 7.6**	**94.4 ± 5.6**

***-** values significantly different from controls (*$P<0.05$; $P<0.001$*)

Cu, Ni and of Cu+Ni mixture did not induce alterations in the GVF of the larvae at the end of the second test

Long-term effect of 0.125 portion of the 96-hour LC50 for larvae concentration of Cu did not induce disorders in the GVF of the larvae. Meanwhile, the same concentration of Ni and mixture induced significantly a decrease in the GVF of the larvae at the end of the first test. Long-term effect of 0.125 portion of the 96-hour LC50 for larvae concentration of Cu and Ni did not induce alterations in the GVF of the larvae. However, the same concentration of Cu+Ni mixture induced significantly increase in the GVF of the larvae at the end of the second test (Table 4).

Other obtained short-term behavioral data (Table 4) could be defined as contradictive. For example, larvae significantly demonstrated only weak response to external stimuli and intensively formed the nests, do not scattering in the test mixtures. Long-term exposures also confirmed vulnerable behavioral responses of larvae. This proves that respiratory responses of larvae, apparently, are much more sensitive indicators than other behaviors of exposure to sublethal or even lethal heavy metal mixtures.

The lethal toxicity test showed that the 96-hour LC50 values for embryos and larvae were different, and depended on the mixture preparation mode, the heavy metal concentration in the mixture, and affected development stage of organism. Our previous results indicated that lethal toxic effects of HM singly and their mixture on fish early life stages of development depended on the type of metal and development stages. The impact of heavy metals (Cu, Zn, Ni, Cr, and Fe) on test-organisms of different phylogenetic and ontogenetic level showed that in the most cases Cu is the more toxic metal to rainbow trout (*Onchorchyncus mykiss*) at all stages of development [14]. The sensitivity of different life stages of rainbow trout (based on 96-h LC50) might be indicated in the following sequences: larvae>adult fish>eggs [15]. Hatching larvae were more sensitive to Cu+Ni mixture as compared to embryos and 1-day old larvae (Table 3). Fish at early stages of development are more sensitive to the impact of HM and their binary and multinomial mixtures than adults [19, 12, 20 and 21]. Mixture toxicity indices (MTI) and effects of Cu+Ni mixtures to rainbow trout embryos and larvae (the lethality tests beginning from "eye-egg" embryos and from 1-day larvae) were similar and did not depend on the mixture preparation mode, on the heavy metal concentration in mixture, and on affected development stage of organism (Table 3). These results are consistent with our previous data. The effects of Cu, Cr, Zn, Ni, and Fe mixtures prepared according to the 96-hour LC50 for embryos and larvae were the same – more than additive and did not depend on the mixture preparation, the ratio of the metals in mixture, heavy metal concentration in the mixture, and affected development stage of organism [22, 19, 12]. According Xua [13] the majority of the binary combinations, the interactions were of synergistic nature, but in ternary or quaternary mixtures, the joint action was mainly concentration additive, while antagonism was only observed for two mixtures (Cu+Pb and Zn+Cd) among all the 11 combinations.

The sublethal short-term and long-term toxicity data showed that HM (Ni, Cu) singly induced effects on larvae gill ventilation frequency and behavior responses (the number of individuals making nests and the number of individuals responding to external stimuli in %) were less as compared to the effect of mixture (Cu+Ni) and depended on the mixture preparation mode, on the heavy metal concentration in the mixture, on the duration of exposure and on affected development stage of organism. Our previous sublethal long-term toxicity test results indicated that the HM (Ni, Cu) singly induced effects on rainbow trout larvae mortality were less as compared to the effect of mixture. Long-term exposure to HM and their mixture not only reduced survival of larvae of rainbow trout, but disordered the function of the most important vital systems (cardio-respiratory), disturbed all early ontogenesis processes (hatching of larvae), their growth, caused various disorders, and affected behavior reactions of larvae. The results indicated that toxic effects of HM (Ni, Cu) singly and their mixture on fish early life stages of development depended on the type of metal, its concentration, and development stages [21].

Obtained data showed that toxicity assessment in fish at early stages of their development is important for evaluation of water pollution with heavy metals and prediction of their risk to aquatic animal populations.

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5. References

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