Letter to Editor

Characteristic Features of the Effect of Cu and Zn Mixtures on Rainbow Trout *Oncorhynchusmykiss* in Ontogenesis

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Abstract

The effects of 96-hour exposure to 0.25, 0.125 and 0.06 portions of the 96-hour LC_{50} of Cu, Zn singly and Cu + Zn mixture on biological (survival, gill ventilation frequency, gill-somatic index) parameters of rainbow trout were studied depending on the stage of development of the fish. Hatching embryos had the greatest sensitivity to the affect of metals singly and in admixture (the highest percentage of mortality). Marked changes were recorded in gill ventilation frequency during exposure to these metals singly and in admixture, which corresponded to changes in their gill-somatic indices. Changes to certain parameters studied (mortality of embryos, especially hatching embryos, gill-somatic index of juveniles) indicated enhanced toxicity of the metal mixture to rainbow trout in ontogenesis.

Keywords: copper, zinc, binary mixture, fish, ontogenesis, toxicity

Introduction

Copper and zinc are essential constituents of many enzymes. However, copper is toxic to aquatic animals at levels marginally in excess of those found in many unpolluted aquatic environments [1]. The background concentrations of Cu and Zn in Lithuanian water bodies are comparatively low (Cu concentrations ranged from 0.38 to 3.85µg/l; Zn from 6.1 to 19.6µg/l). Levels of these metals, especially Cu, can frequently increase from industrial pollution or from anthropogenic sources (e.g. up to 13.6µg/l) [2]. The toxic effects of Cu and Zn singly have been widely studied [3, 4]. Nevertheless, environmental contaminants are frequently encountered as mixtures, and the behavior of chemicals may not correspond to data predicted for pure compounds [5]. Data for Cu and Zn mixture toxicity depend largely on different organisms/methods applied, sensitivity of parameters studied etc. [6, 7]. The aim of this study was to undertake investigations into the effects of Cu, Zn singly and to ascertain characteristic features of the effect of Cu + Zn mixtures depending on development stage and parameters studied in exposed fish.

Experimental Procedures

Toxicity tests were conducted at the Laboratory of Ecology and Physiology of Hydrobionts (Institute of Ecology of Vilnius University). Rainbow trout embryos and larvae were obtained from the Žeimena hatchery. Dilution water was 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 12° ± 0.5°C and the oxygen concentration was between 8 to 10 mg/l. The effects of the 0.25, 0.125 and 0.06 portion

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of the LC_{50} of Cu, Zn singly (96 h LC_{50} of Cu for embryos – 0.86, for larvae – 0.36 and for adult fish 0.65 mg/l; 96 h LC_{50} for Zn – for embryos – 1.2, for larvae – 0.48, for adult – 3.8 mg/l [8, 9] and their mixture (Cu + Zn) were analyzed (Table 1). Toxicity tests on fish at early stages of their development (continuously from "eye-egg" embryos to 1-day-old larvae) and on juveniles were conducted under semi-static conditions and two replications were performed. The samples at the relative concentrations were collected at

1) "eye-egg" stage embryos (beginning 8 days before hatching),

2) "eye-egg" stage embryos (from the beginning of hatching),

3) 1-day old larvae (newly hatched),

4) juvenile fish after 96 h of exposure.

Biological [survival, morphological and physiological (gill ventilation frequency – GVF)] parameters of the embryos, larvae and juvenile fish in addition to Gill somatic index (GSI) of juvenile fish were evaluated using routine methods [10]. The significance of all results was verified by Student's t-test at $P \leq 0.05$.

Table 1. Concentrations of Heavy Metals Studied

Concentration, Portion of LC_{50}	Embryos		Larvae		Adult	
	Heavy metal concentration (mg/l)					
	Cu	Zn	Cu	Zn	Cu	Zn
1	0.86	1.2	0.36	0.48	0.65	3.8
0.25	0.22	0.3	0.09	0.24	0.16	0.95
0.125	0.11	0.15	0.045	0.12	0.08	0.48
0.06	0.06	0.075	0.02	0.06	0.04	0.24

Results

The mixture of Cu + Zn at concentration 0.25 portion of the LC_{50} increased significantly the mortality of "eye-egg" stage embryos up to 41.3% as compared to the effect of Cu and Zn singly. The mortality of embryos exposed to the same concentration of mixture at the commencement of hatching was also elevated (58%), whereas the metals, singly induced effects were less (Table 2). The mortality of newly hatched larvae was 16.0% and was similar to larvae exposed to Cu and Zn separately. The mortality of controls ranged from 8.2 to 10.2% (Table 2). No significant increase in the mortality of juvenile fish exposed to this concentration of Cu + Zn mixture was recorded (30%) as compared to the effect of single metals (20.0%, 30.0%, respectively). Cu + Zn mixture and single metals at concentration 0.125 portion of the LC_{50} did not affect significantly the mortality of "eye-egg" stage embryos as compared to controls (Table 2). Hatching embryos were more sensitive to this concentration of Cu + Zn mixture and single metals. Their mortality exceeded that of controls. No significant alterations in the mortality of newly hatched larvae and juvenile fish exposed to this and lower (0.06 portion of the LC_{50}) concentrations of metal mixture and single metals were recorded.

Table 2. The effects of Cu, Zn and their mixture on mortality and gill ventilation frequency of rainbow trout.

Chemicals	Mortality,% (N = 200)			Gill ventilation frequency, count/min			
	Embryos		Larvae	1-day old larvae (N = 10)		Juvenile fish (N = 10)	
	1	2	3	Before exposure	After 4 days	Before exposure	After 4 days
0.25 portion of the 96-hour LC_{50}							
Cu	24.7	40.7	11.3	98.8 ± 0.4	88.0 ± 0.4 [a]	70.0 ± 2.6	83.0 ± 3.4 [a]
Zn	21.3	32.7	9.3	100.4 ± 0.4	98.8 ± 0.4	70.2 ± 3.3	84.5 ± 4.2 [a]
Cu + Zn	41.3	58.0	16.0	100.2 ± 0.6	82.2 ± 0.4 [a]	78.8 ± 1.9	82.5 ± 2.0 [a]
Control	8.2	8.4	10.2	100.2 ± 0.8	102.2 ± 0.6	73.5 ± 1.7	77.5 ± 1.2
0.125 portion of the 96-hour LC_{50}							
Cu	12.3	24.0	10.4	102.0 ± 0.8	104.4 ± 0.4	73.3 ± 2.8	82.7 ± 4.4 [a]
Zn	9.3	20.7	8.6	100.4 ± 0.6	100.4 ± 0.4	76.4 ± 2.6	84.3 ± 1.4 [a]
Cu + Zn	16.0	29.3	11.4	100.2 ± 0.6	98.6 ± 0.4	73.8 ± 3.3	83.9 ± 3.3 [a]
Control	8.6	8.6	8.8	100.2 ± 0.8	102.2 ± 0.6	76.0 ± 1.3	76.8 ± 2.8

[a] values significantly different from controls ($P<0.05$), [b] 1 – "eye-egg" stage embryos (beginning 8 days before hatching), 2 – "eye-egg" stage embryos (from the beginning of hatching), 3 – 1-day-old larvae (newly hatched)

A significant decrease in the GVF for one-day old larvae affected by 0.25 portion of the LC_{50} of a Cu + Zn mixture and Cu singly was observed after four days of exposure. On the contrary, this parameter for juvenile fish was significantly increased (Table 2). The differences in the character of the changes in GVF of larvae and juveniles, probably, were caused by metal-induced lesions of gills in newly hatched larvae. No significant changes were determined in GVF of 1-day-old larvae exposed to 0.125 portion of the LC_{50} of Cu + Zn mixture and Cu. Exposing juvenile fish to this concentration of metal mixture and metals singly induced the significant elevations in GVF (Table 2). Only the concentration 0.06 portion of LC_{50} of Cu + Zn mixture and metals singly induced transient increases in the GVF of juvenile fish. This stress-induced response is explained by the action of toxicants during short-term exposure. Fish embryos were affected from the "eye-egg" stage to their hatching, and then larvae were exposed to the yolk-sac resorption. Moreover, the duration of study with juveniles was four days. According to previous data [8] the GVF of fish can increase after short-term exposure to Cu, whilst long-term exposure usually induced a decrease in this parameter.

Significant alterations were observed in GVF of exposed juvenile fish, corresponding with changes in their GSI. GSI of fish exposed to 0.125 portion of the 96-hour LC_{50} of Cu + Zn mixture consisted of 3.58 ± 0.11 and was significantly reduced when compared to control (4.21 ± 0.13) and to fish exposed to Cu and Zn single (3.82 ± 0.19, 3.88 ± 0.09, respectively).

Discussion of Results

Our previous investigations of the impact of heavy metals (Cu, Zn, Ni, Cr, and Fe) on test-organisms of different phylogenetic and ontogenetic level showed that in most cases Cu is the more toxic metal for rainbow trout (*Onchorchyncus mykiss*) at all stages of development [8, 9]. The sensitivity of different life stages of rainbow trout (based on 96-h LC_{50}) might be indicated in the following sequences: larvae>adult fish>eggs [9]. Copper influenced survival during hatching and hatched larvae were the most sensitive as compared with embryos [10]. The difference in sensitivities is most likely due to the chorion protecting the embryonic fish [11]. Our previous data demonstrated that there were greater-than-additive toxic effects of metal mixtures on embryos and larvae of rainbow trout and the partial additive toxic effect on adult fish [12]. However, the present study indicated that the enhanced toxicity of metal binary mixtures was only related to changes in some of the parameters studied (mortality of embryos, especially hatching embryos, gill-somatic index of juvenile fish). One of the more probable explanations for these data could be that at high concentrations of dietary Zn it can interact antagonistically with Cu uptake, internal organ metal concentrations and other physiological parameters [4]. The results obtained demonstrated that toxicity studies performed using only one level of parameters and stage of development of an organism are insufficient and produce controversy in scientific data relating to the toxicity of binary mixtures of metals.

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