Distinctive features of changes in biological parameters of rainbow trout exposed to heavy metal model mixture

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The effect of long-term (28 days) exposure of rainbow trout (*Oncorhynchus mykiss*) to heavy metal (Cu, Zn, Ni, Cr, Pb, Cd, Mn) model mixture was studied evaluating fish survival, morphological (length, weight, specific growth rate, tissue weight and tissue-somatic indices), physiological parameters (gill ventilation frequency, "coughing" rate), haematological parameters (erythrocyte, haemoglobin, white blood cell concentrations, haematocrit level). It was found that most sensitive to this toxicant were tissue-somatic indices from the morphological, gill ventilation frequency from the physiological, and leucocyte concentration from the haematological parameters studied. Significant changes in these parameters were found at low concentrations (1.25–0.6%) of the HMMM studied.

Key words: fish, heavy metal model mixture, morphological, physiological, haematological parameters

INTRODUCTION

Heavy metals are commonly distributed in industrial and municipal discharges, and numerous studies are performed to evaluate their toxic effects to aquatic organisms. However, the majority of these works have focused on the acute toxicity of a single metal to aquatic animals [1, 6, 10, 11]. Meanwhile heavy metals in an aquatic environment are mostly distributed in mixtures, where different interactions among metals are possible; besides, metal mixtures could affect aquatic animals through long periods of their life, causing different sublethal effects.

Studies evaluating the responses of different functional systems or changes in physiological parameters of fish caused by the impact of a mixture of heavy metals are scanty [2–5, 8, 9]. However, the data obtained demonstrated a different response of the physiological parameters studied, confirming the necessity to evaluate a set of biological parameters for a better understanding of the toxic effects of heavy metals on fish.

The aim of the study was to determine lethal and sublethal effects of a heavy metal model mixture (HMMM) on rainbow trout, evaluating the fish survival as well as alterations in the morphological, physiological and haematological parameters.

MATERIALS AND METHODS

Tests were carried out at the Institute of Ecology. Test fish were yearling rainbow trout (*Oncorhynchus mykiss*) obtained from the Žeimena hatchery. They were kept in holding tanks of about 2000 l capacity, supplied with aerated flow-through artesian water. Randomly sorted fish were transferred into 7 aquaria 100 l each for the experiments. Fish were kept until they got acclimated to the new environment and started swimming freely and feeding well. Water in the aquaria was changed three times a week (on Monday, Wednesday, and Friday). Fish were weighed and measured before experiments. The physical and chemical characteristics of water ranged: water temperature from 6 to 10 °C, oxygen concentration from 9 to 10 mg/l, pH 6.8–7.5, average total hardness was 250 mg/l $CaCO_3$. Fish were fed on standard fish meal.

The formation of the heavy metal model mixture (HMMM) was carried out based on the available analytical data on the content of seven representative heavy metals in the annual discharges of wastewater from the Ignalina Nuclear Power Plant to Lake Drūkšiai. Chemically pure metal salts $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $NiSO_4 \cdot 7H_2O$, $K_2Cr_2O_7$, $Pb(NO_3)_2$, $Cd(CH_3COO)_2 \cdot 2H_2O$, $MnSO_4 \cdot 5H_2O$, were used in making the model mixture solution, the final con-

centration being recalculated according to the amount of heavy metal ions. The concentration of HMMM solution considered to be equal to 1% was: Cu 0.008, Zn 0.06, Ni 0.002, Cr 0.003, Pb 0.014, Cd 0.0002, Mn 0.01 mg/l. Six HMMM concentrations (10, 5, 2.5, 1.25, 0.6, 0.3%) were tested. During 28 days of the test period the morphological and respiratory indices were registered, and at the end of exposure haematological and morpho-physiological parameters were measured.

Survival of fish. Dead individuals were removed from aquariums, dried with paper, weighed and measured.

Morphological indices. Body length and weight of the fish were measured three times during the experiments (at the beginning, after two weeks and at the end of exposure). Mean specific growth rate was calculated using the formula:

$r = \ln m_{t_2} - \ln m_{t_1}/t_2 - t_1 \times 100$, where r is the mean specific growth rate, m_{t_2} is the weight (g) of fish at t_2. m_{t_1} is the weight (g) of fish at t_1. The r was calculated for a period of 28 days.

Fish tissue weights were measured and tissue-somatic indices were calculated at the end of exposure as described by Vosylienė and Svecevičius [9].

Respiratory parameters. Gill ventilation frequency (GVF, count/min) and "coughing" rate (back-flushing of gills, count/min) were measured at the beginning of experiments, after two weeks and at the end of experiments. They were measured during 3-minute periods for each fish individually, from which the mean value for 15 fish was calculated [9].

Haematological parameters. Erythrocyte (RBC, T/l) and haemoglobin concentrations (Hb, g/l), haematocrit level (Hct, l/l), white blood cell concentration (WBC, G/l) and percentage of different leucocytes were determined using methods and techniques described by Svobodova and Vykusova [7].

The significance of all the data obtained was determined by Students' test.

RESULTS AND DISCUSSION

The mortality of fish was recorded at 10% and 5% concentrations of HMMM; it occurred within the first week after initiation of exposure: two fish at 10% and five fish at 5% a concentration of HMMM had died within 72 h. The mortality of fish exposed to these two concentrations of HMMM was registered till the end experiments (Table 1). There was no mortality in control fish and in fish exposed to 2.5–0.3% concentrations of HMMM.

Absolute length of fish (L) at the initiation of exposure ranged from 15.0 ± 0.3 to 16.4 ± 0.1 cm and total weight (Q) from 680.0 to 835.0 g. No significant differences of these parameters in fish exposed to 2.5–0.3% concentrations of HMMM and control individuals were found at the end of exposure: mean length of control fish was 17.8 ± 0.3 cm, that of exposed fish ranged from 17.0 ± 0.2 to 18.4 ± 0.2 cm. The weight of fish exposed to highest concentrations (10–5%) of HMMM at the end of exposure was lower as compared to controls, however, the differences were marginally significant ($t = 1.560$) ($P > 0.05$). Mean specific growth rate

Table 1. **Mortality of fish exposed to different concentrations (%) of heavy metal model mixture (HMMM)**

HMMM concentration, %	Number of dead fish				Survival %
	Days of exposure				
	7	14	21	28	
10	8	1	0	0	40
5	9	0	0	1	33
2.5	0	0	0	0	100
1.25	0	0	0	0	100
0.6	0	0	0	0	100
0.3	0	0	0	0	100
Control	0	0	0	0	100

Table 2. **Effect of exposure to heavy metal model mixture (HMMM) on tissue weight (g) and tissue-somatic indices (SI %) of rainbow trout**

HMMM concentration, %	Number of fish	Gills		Liver	
		Weight, g	SI, %	Weight, g	SI, %
10	6	1.51 ± 0.2*	2.87 ± 0.2*	0.70 ± 0.1	1.41 ± 0.9
5	5	1.66 ± 0.1*	2.91 ± 0.1*	0.68 ± 0.1	1.18 ± 0.1
2.5	15	1.89 ± 0.1	2.49 ± 0.4*	0.77 ± 0.1	1.16 ± 0.04*
1.25	15	1.83 ± 0.2	2.93 ± 0.2*	0.80 ± 0.1	1.27 ± 0.1
0.6	15	2.02 ± 0.2	3.00 ± 0.1	1.03 ± 0.1	1.41 ± 0.1
0.3	15	1.99 ± 0.1	2.92 ± 0.1	0.78 ± 0.04	1.40 ± 0.1
Control	15	2.08 ± 0.2	3.52 ± 0.2	0.81 ± 0.1	1.35 ± 0.1

* Significantly different from control ($P \leq 0.05$).

(*r*) of individuals differed: *r* in control fish was 1.39, while in fish exposed to 10 and 5% concentrations of HMMM was 0.78.

Exposure of rainbow trout to HMMM induced changes in fish gill and liver weight and tissue-somatic indices (Table 2). Most pronounced changes in gill weight were determined in fish exposed to 10% and 5% of HMMM, meanwhile changes were not significant in liver weight in fish exposed even to the highest concentrations of mixture. Exposure of fish to 10–1.25% concentrations of HMMM caused a significant decrease ($P \leq 0.05$) in gill-somatic indices. Decreased liver-somatic indices were determined in fish exposed to 5–2.5% of HMMM (Table 2).

The mean values of respiratory parameters of control fish did not fluctuate widely during the period of exposure (Table 3). Gill ventilation frequency of fish exposed to 10–1.25% concentrations of HMMM significantly decreased after 2 weeks of exposure ($P \leq 0.05$); similar changes in this parameter were obtained after 3 weeks of exposure, as well as a significantly decreased gill ventilation frequency ($P \leq 0.05$) was found in fish exposed to 5–1.25% of HMMM at the end of experiments. Increase in ventilation frequency at the end of experiments was observed only in fish exposed to a 10% concentration of HMMM. This increase was probably induced by social interaction among the individuals, as the number of fish in this aquarium was lower as compared to other aquaria. The "coughing rate" in fish slightly, however, significantly increased after 3 weeks of exposure to highest concentrations of HMMM (10–5%) ($P \leq 0.05$), mostly at the end of exposure (Table 3).

A decrease in RBC, Hb concentrations and Hct level were measured in rainbow trout exposed to 10 and 5% of HMMM (Table 4). Changes in lymphocyte counts were most pronounced in rainbow trout exposed to 10%, 5% and 2.5% concentrations of HMMM. A study of the effect of HMMM on the fish organism demonstrated the importance of assessment of a set of biological parameters. The highest concentrations of HMMM tested (10% and 5%) which were 0.33 and 0.17 of 96 h LC_{50} of the mixture caused the death of more sensitive fish. However, only these concentrations induced alterations in some parameters studied: changes in gill weight, "coughing" rate, parameters of RBC (erythrocyte count, haemoglobin concentration, haematocrit level). No changes were observed in liver weight of fish exposed to these concentrations. The negative effect of various toxicants on the growth and development of fish could be explained by a lower food assimilation or an increased demand of food resources for detoxication and maintenance of the normal physiological status of the organism. Our data did not demonstrate any impact of exposure to HMMM on the total weight of fish meanwhile a slightly lower-

Table 3. **Effect of exposure to heavy metal model mixture (HMMM) on gill ventilation frequency and "coughing rate" (count/min) of rainbow trout**

Days of exposure	HMMM concentration, %						
	Control	10	5	2.5	1.25	0.6	0.3
	Gill ventilation frequency						
14	91.8 ± 1.2	83.0 ± 0.9*	82.4 ± 2.5*	83.4 ± 1.9*	79.4 ± 1.3*	93.8 ± 1.3	102.8 ± 3.7
21	85.7 ± 1.7	97.8 ± 1.1*	82.1 ± 1.2	79.9 ± 1.9*	81.8 ± 1.8	89.2 ± 2.2	88.8 ± 0.6
28	88.8 ± 1.3	93.6 ± 1.8*	84.3 ± 1.2*	81.6 ± 2.3*	81.9 ± 1.7*	85.3 ± 1.9	88.6 ± 0.6
	"Coughing" rate						
14	1.5 ± 0.2	2.2 ± 0.4	1.8 ± 0.6	1.6 ± 0.5	1.8 ± 0.2	1.2 ± 0.6	1.2 ± 0.2
21	0.8 ± 0.1	1.4 ± 0.3*	1.4 ± 0.3*	0.6 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	0.8 ± 0.1
28	0.6 ± 0.1	1.7 ± 0.3*	2.1 ± 0.4*	0.4 ± 0.2	0.8 ± 0.2	0.6 ± 0.1	0.6 ± 0.2

* Significantly different from control ($P \leq 0.05$)

Table 4. **Effect of exposure to heavy metal model mixture (HMMM) on haematological parameters of rainbow trout**

Parameter	HMMM concentration, %						
	Control	10	5	2.5	1.25	0.6	0.3
RBC, T/l	0.91 ± 0.04	0.87 ± 0.03*	0.84 ± 0.1*	0.94 ± 0.1	0.91 ± 0.1	1.02 ± 0.1	1.12 ± 0.06
Hb, g/l	87.2 ± 2.7	80.0 ± 3.6*	77.2 ± 7.0*	89.6 ± 4.2	88.1 ± 3.2	83.1 ± 4.7	91.9 ± 3.0
Hct, l/l	0.43 ± 0.01	0.36 ± 0.02*	0.34 ± 0.02*	0.44 ± 0.0	0.41 ± 0.0	0.42 ± 0.1	0.38 ± 0.01

* Significantly different from control ($P \leq 0.05$).

ed mean specific growth rate of rainbow trout exposed to 10–1.25% of HMMM was calculated. Alterations in tissue-somatic indices revealed the organ – target to heavy metals, which was gills. No changes in fish liver weight were obtained, and changes in the liver-somatic index were found only at 10–2.5% concentrations of HMMM, whereas the both parameters, gill weight and gill-somatic index, changed under the effect of rather low concentrations of HMMM. These data confirm the disturbances occurring in the respiratory system of fish affected by HMMM. During all period of exposure a deceleration in gill ventilation frequency of rainbow trout exposed to 5–1.25% of HMMM was registered ($P \leq \leq 0.01–0.05$).

The high sensitivity of the haematological parameters of fish to intoxication with heavy metals or organic pollutants was described [9]. However, in our study, RBC count as well as haemoglobin concentration changed only in fish exposed to highest concentrations of HMMM, inducing the mortality of fish. However, it should be noted that damaged erythrocytes were found even at low concentrations of the mixture tested. WBC parameters changed in a different way: lymphocyte count decreased at 10–0.6% concentrations of HMMM, meanwhile the neutrophil number was slightly elevated at 10%, 5%, and significantly at 2.5–0.6% concentrations of HMMM ($P \leq 0.05$). Changes in WBC count such as increase in the proportions of monocytes and neutrophils and decrease in relative proportion of lymphocytes was found in fish exposed to oil-shale mine drainage water containing heavy metals [6]. Rainbow trout (*Oncorhynchus mykiss*) exposed to copper exhibited a consistent increase in the percentage of neutrophils and a decrease in the percentage of lymphocytes also at the highest concentration tested [1]. In conclusion, we suggest that a set of various biological indices of fish should be tested for assessment of the effects of exposure to HMMM. Different alterations of indices were obtained, which demonstrated the organs-targets, or the most sensitive parameters to this kind of pollutants.

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YPATINGI VAIVORYKŠTINIO UPĖTAKIO, PAVEIKTO SUNKIŲJŲ METALŲ MODELINIU MIŠINIU, BIOLOGINIŲ RODIKLIŲ POKYČIAI

S a n t r a u k a

Tirtas ilgalaikis (28 parų) modelinio sunkiųjų metalų (Cu, Zn, Ni, Cr, Pb, Cd, Mn) mišinio poveikis vaivorykštiniam upėtakiui (*Oncorhynchus mykiss*), įvertinant žuvų išgyvenimą, morfologinius (žuvų ilgį, masę, specifinį augimo greitį, organų masę ir jų somatinius indeksus), fiziologinius (žiaunų ventiliacijos ir „kosėjimo“ dažnius), hematologinius (eritrocitų, hemoglobino, baltųjų kūnelių koncentracijas, hematokrito lygį) rodiklius. Palyginus tirtų rodiklių jautrumą, nustatyti jautriausi šiam toksikantui – iš morfologinių – žiaunų somatiniai indeksai, fiziologinių – žiaunų ventiliacijos dažnis ir hematologinių – leukocitų koncentracija – rodikliai, kurių statistiškai patikimi pokyčiai buvo užregistruoti esant mažoms (1.25–0.6%) modelinio sunkiųjų metalų mišinio koncentracijoms.

Raktažodžiai: žuvys, sunkiųjų metalų modelinis mišinys, morfologiniai, fiziologiniai, hematologiniai rodikliai