SUBLETHAL EFFECTS OF NICKEL TO RAINBOW TROUT *ONCORHYNCHUS MYKISS* BIOLOGICAL PARAMETERS

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Abstract. *The aim of this study was to assess sublethal effects of nickel on rainbow trout juveniles evaluating alterations in fish biological parameters - behavioral responses, hematological, morphological parameters and physiological indices. The study of the responses of rainbow trout to different concentrations of nickel demonstrated their different effect on fish organism. Feeding behavior of test-fish showed their adaptation to lower nickel concentrations (0.1 mg/L, 0.2 mg/L). Higher concentration of nickel (0.4 mg/L) had affected fish feeding irreversibly. There was no significant effect on fish ventilation frequency during exposure to 0.1 mg Ni/L concentration. Ventilation frequency decreased during exposure to 0.2 mg Ni/L, and 0.4 mg Ni/L, which leaded to increase in coughing rate. Hematological indices of fish were affected during exposure to 0.2 mg Ni/L, and 0.4 mg Ni/L concentrations. Erythrocyte, leucocyte count, haematocrit level and haemoglobin concentration had decreased significantly at 0.2 mg Ni/L, and 0.4 mg Ni/L concentrations. No significant changes in glucose concentration were found during the exposure to all nickel concentrations tested. Present investigation confirmed different sensitivity of the morphophysiological parameters studied, the most sensitive ones were: gill somatic > liver somatic = spleen somatic indices. This study showed that nickel even at low concentrations can affect biological parameters of fish. This phenomenon can lead to revision of Maximum-Permissible-Concentration of nickel in ambient water.*

Keywords: *fish, nickel, sublethal effects, biological parameters*

Introduction

Environmental pollutants such as heavy metals, pesticides, and organic compounds pose serious risks to many aquatic organisms. 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 [1, 2, 3]. The data on Nickel toxicity to fish is very scarce, however impact of this metal was partially investigated for other freshwater organisms [4]. The metabolic role of nickel in aquatic organisms is poorly known, however, recent studies of Chowndhury [5] had shown that nickel maintained homeostasis of rainbow trout (*Oncorhynchus mykiss*), while high concentrations of Ni^{2+} are toxic and disturbed fish homeostasis [6]. The ionic form a bivalent nickel (Ni^{2+}) was found to disturb the gills ventilation of fish [7]. The majority of studies on nickel toxicity had been carried out only with high concentrations of this metal in order to determine the LC50 of this pollutant, while in unpolluted waters Ni^{2+} concentrations rarely exceed 0.001 - 0.2 mg/L ranges [4, 8]. In acute exposures nickel caused decreasing locomotors activity [9]. Remarkable changes in the behavior of fish exposed to lethal and sublethal concentrations of nickel were observed (10, 11]. Many scientists consider that the many toxicants disrupt complex fish behaviors, such as predator avoidance, reproductive, and social behaviors [12]. Many behavioral test involving predator avoidance, feeding behavior, learning, social interactions, and a variety of locomotors behaviors show promise but have been insufficiently studied to judge their sensitivity or utility [13]. Changes in certain fish behaviors, especially cough rate and avoidance reactions, are very sensitive indicators of sublethal exposure to metals [13]. According to Little [10] behavioral measurements may be useful indicators of sublehal contamination due to concentrations even being lower than those that affect growth. Behavioral changes usually occur much earlier than mortality. After exposure to sublethal concentration of metals (nickel, copper and zinc) some behavioral changes in fish were observed due to stress, such as mucus like secretion over gills, excessive excretion, and increased distance between gills and operculum [14].

Stress response of the fish organism is an integrated reaction with behavioral, neural, hormonal and physiological elements all combined together to provide fish with the best possible chance of survival [15]. Physiological effects of toxicants in the literature include disruption of sensory, hormonal, neurological, and metabolic systems, which are likely to have profound implications for many fish behaviors [12]. However, little toxicological research has sought to integrate the behavioral effects of toxicants with physiological processes. Those studies that take this multidisciplinary approach add important insight into possible mechanisms of behavioral alteration [12].

The aim of this study was to assess nickel (Ni^{2+}) sublethal effects on rainbow trout juveniles evaluating alterations in fish biological parameters - behavioral responses, haematological, morphological parameters and physiological indices.

Materials and methods

Exposure of fish for the toxicity studies was performed at the Laboratory of Ecology and Physiology of Hydrobionts (Nature research center Institute of Ecology). Rainbow trout (*Oncorhynchus mykiss*) juveniles were obtained from the Žeimena hatchery (Lithuania). The toxicity tests were undertaken under semi-static conditions. Deep well water of high quality was used for storing control fish. Control water and Ni^{2+} in aquaria were renewed on alternant days. The temperature of water in the aquaria was maintained at 10 ± 0.5°C. Average hardness of water was approximately 250 mg/L as $CaCO_3$, dissolved oxygen concentration and pH were not less than 7 mg/L and 7.9–8.1, respectively. During these studies, juvenile fish were fed with commercial DANA FEED fish food *ad lib*. Studies with juvenile were performed in two replications (N = 7 in a group). The concentrations of Ni introduced into the aquarium water were 0.1; 0.2; 0.4 mg/l. Ni^{2+} concentration 0.2 mg/L is accepted as the Maximum-Permissible-Concentration in water bodies according to the European Parliament and Council Directive 2000/60/ec. Long-term (10 days) toxicity tests of Ni^{2+} were conducted using juvenile rainbow trout (*Oncorhynchus mykiss)* according to ISO 10229:1994 [16]. The average total length of test fish was 150 ± 10 mm and the total weight was 40 ± 2 g (mean ± SEM). For rainbow trout juvenile nutritional and behavioral responses were recorded. Nutrition responses were measured every day for each test fish individually and were estimated in %: 0% - not ate; 50% - ate a half food; 100% - ate the all food. The following behavioral responses of juvenile rainbow trout were investigated: two types of fish behavioral-respiratory responses – gill ventilation frequency (GVF) and coughing rate (CoR) in counts per minute [17]. Gill ventilation frequency (counts/min) and coughing rate (counts/min) of juvenile fish were measured during 3-min periods for each test fish individually and the mean value was calculated. Morphological parameters studied were: mean body weight (Q, g; q, g), mean body length (L, cm; l, cm), weight of spleen, gills, liver (g). Morphophysiological parameters evaluated: spleen-somatic index (SSI), gill-somatic index (GSI), liver-somatic index (LSI). Tissue-somatic indices were calculated using the formula [18].

For each fish, the erythrocyte count (RBC, 10^6 mm^{-3}), the haemoglobin concentration (Hb, g l^{-1}), the haematocrit level (Hct, l l^{-1}) the leucocyte counts (WBC, 10^3 mm^{-3}) were determined using routine methods [19]. Glucose concentration (Mmol/l) was determined with „EKSAN-G“ [20].

Differences between the measured characteristics were tested by Student's t-test ($p < 0.05$) using the programme GraphPAD InStat (USA).

Results and Discussion

Feeding behavior. Feeding behavior of test-fish showed their adaptation to lower nickel concentrations (0.1 mg/L, 0.2 mg/L). Sublethal concentrations of Ni^{2+} after the first days of exposure caused a stressful state - fish feeding behavior reduced in all tested Ni^{2+} concentrations. Mid-term examination at 0.1 and 0.2 mg/L Ni^{2+} concentrations demonstrated that fish feeding behavior returned at the control level. Feeding behavioral assessment showed the fish organism's adaptability to permissible pollutant concentration in water bodies. Higher concentration of nickel (0.4 mg/L) had affected fish feeding irreversibly.

Comparison our data obtained with those of other authors revealed not only similarities but also differences. Atchison and co-authors [13] investigated the feeding behavior of various fish, including rainbow trout, exposed to different toxicants (Cu, Zn, Ni, Pb) and found that during the first days of exposure to sublethal concentrations the feeding behavior has been disturbed. Meanwhile Vosylienė and Kazlauskienė [21] study with rainbow trout juveniles, 10 days exposed to mixture of five heavy metals (Cu, Zn, Ni, Cr, Fe) showed that at the first days the feeding behavior of the fish did not changed. Midway through the study 50-60% of the fish stopped eating. Meanwhile at the end of the study feeding behavior of test-fish gradually restored.

Respiratory parameters. The gill ventilation frequency (GVF) of juvenile exposed to 0.2; 0.4 mg/L concentrations of Ni^{2+} was significantly ($P \leq 0.01$) lowered (59.6 ± 4.3; 61.5 ± 2.8 counts/min) as compared to the controls (72.0 ± 1.0 counts/min) from the initiation of exposure till the conclusion of the tests (Table 1).

The significantly ($P < 0.0001$) decreased coughing rate was found in the groups of juveniles exposed to 0.2; 0.4 mg/L concentrations of Ni^{2+} for 10 days (Table 1).

Table 1

The effect of Ni^{2+} for gill ventilation frequency and coughing rate of the juvenile rainbow trout (*Oncorhynchus mykiss*) (X ± SE).

Concentration, mg/L	GVF, counts/min	CoR, counts/min
Control	72.0 ± 1.0	0.2 ± 0.7
0.1	70.2 ± 2.6	0.6 ± 0.3
0.2	59.6 ± 4.3*	3.3 ± 0.9*
0.4	61.5 ± 2.8*	6.3 ± 0.6*

* different from the control (P ≤ 0.01).

In summary, we may suggest that respiratory parameters are among the most sensitive biological indicators of heavy metals effects. The respiratory-frequency fluctuations we consider to result from the difference in toxicant concentrations. As well as, the data obtained coincide with Vosylienė and Kazlauskienė [21] conducted studies on the toxicity of model mixture of seven heavy metals (Cu, Zn, Cd, Ni, Cr, Cd, Fe) to fish. Sublethal concentrations of the mixture induced the ranges in fish respiratory frequency, which increased or decreased, while the "coughing" rate increased. Fish "coughing" rate is also considered as a one of the most sensitive indicators, which alterations are among the first signs of intoxication and usually are observed at very low concentrations of toxicants when other indicators are not registered [17]. The data suggest that 0.2 mg/L and a 0.4 mg/L Ni^{2+} concentration adversely affect fish respiratory system, causing a significant decrease in respiratory frequency and the increase in "coughing" rate.

Haematological parameters.

Changes in haematological parameters of rainbow trout blood are presented in the Table 2. Statistically significant decrease in erythrocyte count was observed at 0.2 mg/L and 0.4 mg/L Ni^{2+} concentrations.

Table 2

The effect of Ni^{2+} for haematological parameters of the juvenile rainbow trout (*Oncorhynchus mykiss*) (X ± SE).

Haematological parameters				
Eritr., $1x10^{-6}$ mm^{-3}	**Hb, g/l**	**Hct, l/l**	**Leucoc., $1x10^{-3}$ mm^{-3}**	**Glucose Mmol/l**
Control				
1.06 ± 0.04	73.6 ± 1.5	0.31 ± 0.01	18.4 ± 1.2	2.27 ± 0.15
0.1 mg/L				
1.08 ± 0.03	68.5 ± 3.5	0.33 ± 0.02	17.6± 1.4	1.93 ± 0.13
0.2 mg/L				
0.92 ± 0.06*	64.5 ± 3.1*	0.18 ± 0.01*	16.1 ± 1.6*	2.04 ± 0.13
0.4 mg/L				
0.83 ± 0.06*	62.9 ± 2.8*	0.21 ± 0.02*	14.6 ± 1.1*	2.09 ± 0.11

* different from the control (P ≤ 0.01).

Changes in erythrocyte count in blood of fish showed a sensitivity of this parameter to Ni^{2+} exposure. Haemoglobin concentration in metal-affected fish decreased as compared with controls, and significant changes were observed at the 0.2 mg/L and 0.4 mg/L concentrations of Ni^{2+}. Haemoglobin concentration decrease can be attributed to the drop in erythrocyte count in blood. Studies on the effect of heavy metals (Cu, Zn, Cd) on red blood cell count changes demonstrated direct proportional alterations in haemoglobin concentration [22, 23, 24]. Significant changes in haematocrit also were determined in the blood of fish at 0.2 mg/L and 0.4 mg/L concentrations of Ni^{2+}. The decrease in haematocrit level is associated with reducing of red blood cells counts and haemoglobin concentrations. PANE and co-authors [25] studies on effects of high Ni^{2+} concentrations (11-16 mg/L) on rainbow trout showed the significant increase in haematocrit level accompanied by an elevation in hemoglobin concentration. At 0.2 and 0.4 mg/L Ni^{2+} concentrations significant decrease in leucocytes count in blood of rainbow trout was recorded. Such reducing in leucocytes count at higher metal concentrations indicated disturbance in homeostasis and immune functions in fish. Similar data were obtained affecting rainbow trout with sublethal concentrations of Cu and Zn [26, 27, 28, and 29]. No significant changes were observed in glucose concentration in blood of fish after exposure to 0.1 mg/L; 0.2 mg/L and 0.4 mg/L concentrations of Ni^{2+} (Table 2).

Morphophysiological parameters.

All tested concentrations of Ni^{2+} caused significant decrease in fish liver weight and at 0.4 mg/L Ni^{2+} significant reducing in the liver somatic index (LSI) was calculated (Table 3, 4). These results are confirmed by our previous data [21]. The alterations in liver weight could be explained by detoxifying function of the liver, which, furthermore, regulates excretion of heavy metals from the organism [30]. According to Lowe-Jindu and Niimi [31] the liver weight changes may also be associated with liver enzyme synthesis and carbohydrate metabolism changes. Changes in liver size and changes in the LSI are sometimes associated with liver glycogen level changes [32]. Liver weight and LSI decrease show that nickel caused adverse changes in the liver structure.

Table 3

The effect of Ni^{2+} for morphological parameters of the juvenile rainbow trout (*Oncorhynchus mykiss*) (X ± SE).

Concentration, mg/L	Mass, g		
	Liver	Spleen	Gills
Control	0.57 ± 0.05	0.07 ± 0.007	1.22 ± 0.10
0.1	0.47 ± 0.04*	0.06 ± 0.006	1.41 ± 0.10
0.2	0.46 ± 0.05*	0.08 ± 0.015	1.48 ± 0.07*
0.4	0.45 ± 0.05*	0.09 ±0.007*	1.58 ± 0.09*

* different from the control ($P \leq 0.01$).

Spleen weight and SSI significantly increased only at 0.4 mg/L Ni^{2+}concentration (Table 3, 4). Spleen enlargement and SSI increase respectively could be induced by the stress state of the fish after exposure to toxicant.

Table 4

The effect of Ni^{2+} for morphophysiological parameters of the juvenile rainbow trout (*Oncorhynchus mykiss*) (X ± SE).

Concentration, mg/L	Somatic indices		
	LSI	SSI	GSI
Control	1.13 ± 0.09	0.18 ± 0.01	3.00 ± 0.14
0.1	1.00 ± 0.01	0.17 ± 0.03	2.67 ± 0.21
0.2	1.15 ± 0.16	0.19 ± 0.01	3.86 ± 0.29*
0.4	1.05 ± 0.20*	0.23 ± 0.02*	3.89 ± 0.34*

* different from the control ($P \leq 0.01$).

Gill weight was mostly influenced by 0.4 mg/L, while GSI was significantly elevated by 0.2 and 0.4 mg/L Ni^{2+} concentrations (Table 3, 4). According to Pane and co-author [33], high (16 mg/L) nickel concentration caused the gill epithelial cell necrosis, secondary lamella increase, and disorders in gas and ion metabolism. According to the sensitivity to nickel the morphophysiological parameters may be arranged in the following sequence: gill somatic > liver somatic = spleen somatic indices. Results obtained by Pane [25, 33], and Leonard [34] showed that gills were mostly affected by nickel as compared to other organs and tissues.

In this study the toxicity of Maximum-Permissible-Concentration of nickel (0.2 mg/L) in water bodies [35] was investigated. Under this Directive, this concentration should not cause any noticeable changes in fish organism. However, our study demonstrated that this concentration can exert toxic effects upon all studied biological (feeding behavior, respiratory, morphophysiological, haematological) parameters of fish. We consider, after more detailed studies, the revision of the nickel "safe" standards for aquatic life will be proposed.

Conclusions

- Feeding behavior of test-fish showed adaptation of organisms to lower nickel concentrations (0.1 mg/L, 0.2 mg/L). Higher concentration of nickel (0.4 mg/L) had affected fish feeding behavior irreversibly.
- No significant effect on fish gill ventilation frequency during exposure to 0.1 mg Ni/L concentration was observed. Gill ventilation frequency decreased during fish exposure to 0.2 mg Ni/L, and 0.4 mg Ni/L, in contrast, the increase in "coughing" rate was registered.
- Haematological indices of fish were affected of 0.2 mg Ni/L, and 0.4 mg Ni/L concentrations. Erythrocyte, leucocyte count, haematocrit level and haemoglobin concentration had decreased significantly at 0.2 mg Ni/L, and 0.4 mg Ni/L concentrations. No significant effect on glucose concentration during the exposure to all test concentrations of nickel was determined.
- Investigation confirmed different sensitivity of the morphophysiological parameters studied to nickel. Gill somatic index was the most sensitive parameter to nickel toxic effect.
- This study showed that nickel even at low concentrations can affect biological parameters of fish. This phenomenon can lead to revision of Maximum-Permissible-Concentration of nickel in ambient water.

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