EFFECTS OF NICKEL AND ITS COMBINATION WITH OTHER HEAVY METALS (Cd, Pb, Zn) ON COMMON CARP (*Cyprinus carpio* Linnaeus, 1785)

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ABSTRACT

We aimed to study ex-situ the effects of acute toxicity test with Ni²⁺, Ni+Cd, Ni+Pb and Ni+Zn on behavior, survival and oxygen consumption of common carp, *Cyprinus carpio* L. Behavioral effects were more pronounced in fish exposed to 0.2 mg/l Ni²⁺, 0.3 mg/l Ni²⁺ and 0.45 mg/l Ni²⁺. Common stress reactions, such as anxiety, jumps and quick movements, and also accelerated movements of the fish gill covers were observed. Fish behavior from the lower Ni²⁺ concentrations (0.05 and 0.1 mg/l Ni²⁺) and from the test combinations of Ni+Cd, Ni+Pb and Ni+Zn was quite different. In general, during the first hours of the experiment the fish were anxious, but after 48 hours they started to be lethargic, which was expressed in their slow movements. Fish survival was 90% under intoxication with 0.2 mg/l Ni²⁺ and combination of Ni+Pb. In the rest of the tested heavy metal concentrations it was 100%. Data on the respiration intensity rate and oxygen consumption of fish exposed to 0.2 mg/l Ni²⁺, 0.3 mg/l Ni²⁺, Ni+Pb and Ni+Cd were lower in comparison with the control group. This result indicated that the Ni²⁺ ions impacted the fish respiratory system. Depending on their toxic effect the descending row of the studied heavy metals could be presented as follows: Ni²⁺ > Ni+Pb > Ni+Cd > Ni+Zn. Overall, such experiments could be successfully applied in environmental monitoring and risk assessment programs for metal-contaminated aquatic ecosystems and toxic effects on fish.

Key words: Heavy metals, Acute test, Survival, Behavior, Oxygen consumption, Fish, *Cyprinus carpi*

INTRODUCTION

Heavy metal pollution has begun to grow at an alarming rate [1] because heavy metals cannot degrade, and they are continuously deposited and incorporated into water, sediments, and aquatic organisms. Pollution of natural waters by metals released by different anthropogenic activities such as increased population, urbanisation, domestic, industrial, and agricultural processes is an environmental problem of global significance which have been further aggravated [2, 3, 4].

In the Annual Report of the Basin Directorate – Plovdiv, 2009, Section 2 from the management plan for the river basin in the East Region 2010-2015, Volume 2 – River Arda, heavy metals are pointed as the most significant contributors to water pollution. Common contaminants are copper (Cu), manganese (Mn), zinc (Zn), and other

priority substances according to Directive 2013/39/EU [5] of the European Parliament and of the Council regarding priority substances in the field of water policy – cadmium (Cd), lead (Pb) and nickel (Ni). Contamination with these toxic elements has been found throughout the whole catchment area of River Arda until the borderline with Greece. Their distribution also suggests accumulation in the food chain and possible environmental consequences that this freshwater ecosystem could face are still unknown.

Among aquatic organisms, fish are generally considered to be the most relevant organism for pollution monitoring in aquatic ecosystems [6]. They are also relatively situated at the top of the aquatic food chain; therefore, fish normally can accumulate heavy metals from food, water and sediments [7, 8]. Additionally, they are low in cholesterol and contain beneficial poly-unsaturated fatty acids [9] thus the contaminant levels in fish present a considerable concern for human health [10-13].

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Elevated concentrations of metals can be toxic to aquatic life because they are able to induce oxidative stress by accelerating the generation of highly reactive oxygen species (ROS), including superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen species (1O_2). If not detoxified, these ROS can oxidize proteins, lipids and nucleic acids, often leading to enzymatic and morphological changes, altered physiological processes and even cell death [14].

There is a large amount of literature regarding heavy metals such as Cd, Cu, Hg, Pb and Zn, and their toxic effects on biota [15-18]. However, there is insufficient data on Ni^{2+} toxicity and in particular, on its combination with other heavy metals on the degree of expression of oxidative stress. Hence, the aim of the present study is to examine ex situ the impact of: (i) increasing Ni^{2+} concentrations; (ii) and combined effect of Ni+Cd, Ni+Pb and Ni+Zn, on the survival, behavior, respiratory processes and oxygen consumption in common carp (*Cyprinus carpio* L.) under acute metal exposure.

MATERIAL AND METHODS

Experimental set up

The experiment was performed at the laboratory of Lyuben Karavelov Branch, University of Plovdiv. Duration of the toxicity

tests was 96 h. Nine aquaria of 100 l volume were used for each Ni^{2+} concentration and its combination with other heavy metals – 5 with Ni^{2+} in different concentrations (shown below), 3 for Ni+Cd, Ni+Pb and Ni+Zn and 1 for control with no heavy metals added in the water. Water temperature was maintained constant by a heater monitor (Sharc-H-229). Water was continuously aerated by pumps (Atec-AR-850 and Hailea-HT-BT 2000). Water properties such as: temperature, pH, oxygen saturation, conductivity and water hardness was measured with a combined field-meter (WTW) (Table 1).

Nickel (Ni^{2+}) was used as $Ni(NO_3)_2 \cdot 6H_2O$; cadmium (Cd^{2+}) as $Cd(NO_3)_2 \cdot 4H_2O$; lead (Pb^{2+}) as $Pb(NO_3)_2$ and zinc (Zn^{2+}) as $Zn(NO_3)_2 \cdot 6H_2O$, respectively (Merck). The increasing concentrations of Ni^{2+} were 0.05 mg/l, 0.1 mg/l, 0.2 mg/l, 0.3 mg/l and 0.45 mg/l.

Test concentrations of the combined heavy metals were determined after preliminary testing and single exposure to different concentrations of Cd, Pb and Zn. In the present experiment the lowest observed effect concentration (LOEC) for each metal: Cd – 0.01 mg/l, Pb – 0.05 mg/l and Zn – 3.0 mg/l, was chosen. Endpoint was the death of fish.

Table 1. Physico-chemical properties of the water in every tank used in the experiment

Metal concentration (mg/l)	0.05 Ni^{2+}	0.1 Ni^{2+}	0.2 Ni^{2+}	0.3 Ni^{2+}	0.45 Ni^{2+}	Ni+Cd	Ni+Pb	Ni+Zn	control
pH	7.5±0.22	7.2±0.07	7.2±0.08	7.1±0.06	7.2±0.06	7.4±0.07	7.6±0.17	7.3±0.09	7.5±0.21
t°C	22.4±0.4	23.0±0.1	21.0±0.9	22.2±0.7	22.9±0.3	21.9±0.1	22.1±0.1	21.9±0.1	22.2±0.8
O ₂ , mg/l	7.2±0.15	7.5±0.11	7.3±0.18	7.3±0.08	7.5±0.14	7.3±0.03	7.3±0.05	7.2±0.2	8.2±0.05
Conductivity $\mu S/cm$	119.1±11	129.6±16	133.2±23	127.1±17	154.7±25	101.1±6	142.3±11	108.4±12	112.6±11

Experimental animals

Common carp (*Cyprinus carpio* L.) is a cyprinid species used in aquaculture and recreational fishing. It is also one of the most preferred protein source through Europe and particularly, in Bulgaria. Furthermore, common carp is a very resilient to water contamination and that is why, it is very often recommended for the purposes of ecotoxicological research [19-21]. Common carps of equal size and age (12±2 cm length and 9.5±25 g weight) were used. They were purchased from the certified fish farm 'Venikol' Ltd. (Kardzhali, Bulgaria). Fish had no external lesions and injuries. After acclimatisation in de-chlorinated (by

evaporation) water for 7 days, 10 fish were placed in each experimental aquarium. We monitored the fish behavior – mobility, location in the tank and opening and closing of their gill covers twice daily. Every 24 hours during the experiment the number of survived fish in each aquarium was recorded and the dead ones (if any) were removed.

Respiration intensity was examined after the 96-hour exposure to the test concentrations ended. Two marked fish of each experimental tanks were chosen, measured, weighed and placed in a new aquarium, filled with dechlorinated water. The aquarium was isolated from the air through stretch film and the fish were left for 1 hour [22]. Oxygen

content in the water was measured at the beginning (Q) and at the end (Q_1) of the experiment with an oximeter (pHenomenal-OX-4000H-VWR). Respiration intensity was determined by the following formula:

$$I = Q_2/G,$$

where I is the intensity of respiration (oxygen quantity in mg O_2 on 1 g fish weight for 1 hour); G – fish weight in g; Q_2 – oxygen consumption for 1 h (oxygen quantity in the begging minus oxygen quantity at the end, as follows $Q_2 = Q - Q_1$).

Fish resistance to oxygen deficiency was examined directly following our previous experiment [23] according to the method by Tsekov [22]. We also monitored the fish behaviour.

The present study was performed in accordance with national and international guidelines of the European Parliament and the Council on the protection of animals used for scientific purposes (Directive 2010/63/EU) [24].

Statistical analysis

All data were statistically processed by calculating the Pearson correlation coefficient ($P < 0.05$) with software packet Statistika 7.0 StatSoft Inc. [25].

RESULTS AND DISCUSSION

Behaviour

Immediately after the Ni^{2+} was added in the first five aquaria, we observed sharp spasmodic contractions of the muscle of the fish mouth and frequent gill cover movements. Some fish were anxious, jumping quickly towards the water surface. These effects were most severe in the fish exposed to higher Ni^{2+} concentrations. After the first 24 hours, the fish from the lower Ni^{2+} concentrations (0.2, 0.3 and 0.45 mg/l) were apatic, they formed groups close to the bottom of the aquaria with slow and lethargic movements, excreting mucus. During the first hours of the experiment the fish were anxious, but after 48 hours they started to be lethargic, which was expressed in slow movements close to the bottom of the aquaria. On the bodies of some of the fish from the tested Ni^{2+} concentrations we found a thick layer of mucus, most likely secreted because of the metal-contaminated water. Menne *et al.* [26] explain this fact with the strong irritant capacity that soluble nickel salts have towards the fish skin. Similar results were also reported by El-Saieed and Mekawy [27].

Behavior of the fish from the combined metal exposures was less pronounced compared to

the high concentrations of single Ni^{2+} exposures. The most anxious were the carps of the combination $Ni+Pb$. This behavior lasted up to 24 hours after the experiment started. After 48 hours until 96-th hour, it was replaced by lethargy, expressed in slow movements, mainly oriented to the bottom of the tank. Fish of the last two combinations – $Ni+Cd$ and $Ni+Zn$ made slight movements towards the bottom of the aquarium in the last 72 hours of exposure. We consider that such behavior is a possible way to reduce or delay the flow of metal-contaminated water over the gills.

Survival

Fish survival was 90% under intoxication with 0.2 mg/l Ni^{2+} and its combination with Pb . In the rest of the tested heavy metals concentrations it was 100%.

Respiration Intensity Rate

In our research the data on the respiration intensity rate of the fish exposed to single Ni^{2+} were higher in comparison with the control group (Table 2). In addition, the data showed lower absorbed oxygen results compared to the control group. This result indicated that the Ni^{2+} ions impacted the fish respiratory system. We consider that such increase in the respiration intensity rate could be a compensatory mechanism, most likely connected with altered respiratory functions of the gills and oxygen transport. On the other hand, the results Javid *et al.* [28] found a positive correlation between the Ni^{2+} concentrations and quantities absorbed oxygen. On the opposite, our results showed no such trend. In this sense, Abou- Hadeed *et al.* [29] provided data on morphological and functional changes in the gills after Ni^{2+} exposure.

Data for absorbed oxygen and respiration intensity of the fish exposed to Ni^{2+} and its combination with Cd , Pb and Zn for the fish exposed to $Ni+Cd$ and $Ni+Pb$ showed a lower respiration intensity rate and absorbed oxygen levels compared to the control. In addition, those for fish exposed to $Ni+Zn$ showed similar results to the control, showing that this metal combination is the least stressful.

Resistance to oxygen deficiency

The exposed fish dropped in the following order: $Ni+Pb$ at 0.07 mg/l O_2 ; $Ni+Zn$ at 0.05 mg/l O_2 ; $Ni+Pb$ at 0.04 mg/l O_2 ; 0.45 Ni^{2+} at 0.03 mg/l O_2 ; 0.2 Ni^{2+} at 0.02 and 0.00 mg/l O_2 . The individuals from the other Ni^{2+} concentrations and its combinations with Cd , Pb and Zn dropped over the next 40 minutes at concentrations of 0.00 mg/l O_2 . After aeration for 15 minutes 100% from the control fish

recovered and 50% from the fish exposed to 0.05 Ni²⁺, 0.1 Ni²⁺ and Ni+Cd, as well as 50% from the fish exposed to 0.3 Ni²⁺ and Ni+Zn.

Table 2. Data on absorbed oxygen and respiration intensity levels of common carp under metal exposure

Metal concentration (mg/l)	0.05 Ni ²⁺	0.1 Ni ²⁺	0.2 Ni ²⁺	0.3 Ni ²⁺	0.45 Ni ²⁺	Ni+Cd	Ni+Pb	Ni+Zn	control
Absorbed oxygen (mg O ₂)	1.55	2.3	1.55	1.6	2.2	1.81	1.84	2.02	2.2
Respiration intensity (mg/g)	0.01	0.0099	0.01	0.012	0.009	0.007	0.008	0.009	0.009

The order of lethal outcome for fish in this study showed that despite of the high survival rate for 96-hour exposure of all tested concentrations, the Ni²⁺ caused damage to the respiratory system. This damage was more serious in one of the combinations and higher Ni²⁺ concentrations. In general, the results for resistance to oxygen deficiency confirmed those for respiration intensity. According to Ololade and Oginni [30], the lethal outcome after Ni²⁺ exposure is due to stress, which weakens the fish body's resistance or due to the cumulative impact of Ni²⁺ toxicity.

CONCLUSIONS

Depending on their toxic effects on the fish the descending row of the studied heavy metals could be presented as follows: Ni > Ni+Pb > Ni+Cd > Ni+Zn. In general, we can conclude that Ni²⁺ toxicity is moderate to fish (and other aquatic organisms) in comparison with other heavy metals. This opinion is in agreement with Nebeker *et al.* [31] and Khangarot and Ray [32]. Phenology and abnormal behavior of carp such as constant umping and gulping of air, increased opercular activities, sharp mouth opening, sudden surface to bottom movements, restlessness and lethargic movements are sensitive indicators of sub-lethal toxic effects. They can be used for early diagnosis of lesions. Overall, such fish experiments are relatively fast, inexpensive and reliable, thus they could be successfully included in environmental monitoring and risk assessment programs for metal-contaminated aquatic ecosystems.

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