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Cadmium (Cd) affects the gill structure and respiration rate of Common Carp (*Cyprinus carpio* L.)

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Abstract. The main purpose of the present study was to provide some preliminary data on the effects of Cd, which is considered as priority toxic substance in surface waters according to Directive 2008/105/EO on the gill structure and respiration rate of common carp (*Cyprinus carpio* L.) under ex situ conditions. We observed significant histological changes, which were grouped as proliferative and degenerative ones, as well as increase in the respiration rate index in the treated with Cd fish, compared with the control. In general, the tested fish species proved to be sensitive to Cd exposure in terms of the studied parameters.

Key words: cadmium, fish gills, respiration rate.

Introduction

Cadmium (Cd) is a major metal present in the earth's crust and is usually associated with other metals such as zinc, copper and lead. It is a non-essential trace metal, which has no known essential role in living organisms; exhibit extreme toxicity even at very low (trace) exposure levels and have been regarded as the main threats to all forms of life (Järup 2003).

Biota such as fish are used as indicators of trace metal contamination of aquatic environments because they are large and easily identified; have longer life-span and high position in the aquatic food chains; and have been recommended as valuable biological indicators in aquatic environmental pollution assessment (Kumolu-Johnson *et al.* 2010; Naigaga *et al.* 2011).

The aim of the present study was to give some preliminary data on the effects of Cd, which is considered as priority toxic substance in surface waters according to Directive 2008/105/EO on the histology of the gill structure and respiration rate of common carp (*Cyprinus carpio* L.) under ex situ laboratory conditions.

Materials and Methods

Ten Common Carps of the same size-group (10.5 cm ± 1.5; 20 gr ± 0.5) were used in the experiment. All animals were collected in the spring of 2015 from one of the basins at the Institute of Fisheries and Aquaculture in Plovdiv, Bulgaria where fish are usually reared under strict toxicant-free conditions. After transportation the fish were acclimatized for a week. The water was kept oxygen saturated, the animals were maintained under a natural light/dark cycle (12:12 hours) and they were not fed prior or during the experiment.

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After acclimatization the fish were divided into two groups in 50 l tanks – control, untreated and test variants, which were treated with soluble Cd ($Cd(NO_3)_2.xH_2O$) for 72 hours (acute exposure). The metal concentration represented the maximum permissible levels set by the national and EU law. According to the Bulgarian legislation based on the EU Directive, the maximum permissible concentration of Cd in inland surface waters is 0.45 µg/l. This means that for 50 L tanks in our experiment 22.5 µg were applied. No fish mortality was recorded during the exposure period.

The physico-chemical characteristics of the aquarium water (pH, temperature, conductivity and oxygen level), were measured once at the start of the experiment (0 h), as well as on the 72nd h according to a standard procedure (APHA 2005).

To study the gill histological structure a standard histological technique was performed (Suvarna *et al.* 2012). The histological alterations were studied using a light microscope and photographed.

To measure the respiration rate the method of Stroganov (1962), whith modification by Tsekov (1989) was applied. The respiration rate was calculated according the formula: I = Q2/G, where I – respiration rate index; G – weight of the animals, in grams, Q2 – oxygen consumed by the animals between the two measurements (the difference between the oxygen levels before and after the 1-hour Q2 = Q-Q1 hour). Q was calculated by the following formula: Q = V x q, where: Q – total oxygen level in the tank; V – volume of the water in the tank, l; q – level of dissolved oxygen in 1 liter of water (mg/l). The oxygen levels were measured, using oximeter "Oxi 315i/SET". The fish were weighted, using digital scale "WTW".

Results

The physico-chemical properties of the water showed relatively constant values in the control and experimental tanks. These for the control groups were as follows: pH – 8.1 ± 0.5; conductivity – 435 µS/cm ± 1.5, temperature – 20.5 ˚C ± 1.5 and oxygen level – 6.8 mg/l ± 0.5, and these for the experimental tank - pH – 7.9 ± 0.3; conductivity – 461 µS/cm ± 3.5, temperature – 20.5 ˚C ± 1.5 and oxygen level – 6.5 mg/l ± 1.5, respectively. Therefore, we think that the changes, which we observed in the fish were not due to physico-chemical properties, but due to the Cd exposure.

We found histological alterations associated with proliferative changes in the gill epithelium, resulting in epithelial lifting, edema, hypertrophy and hyperplasia of squamous epithelium and glandular cells, proliferation of epithelial covering the secondary lamellae, as well as degenerative changes in the gill epithelium and changes affecting the circulatory system (Fig. 1). The proliferative changes prevailed over the degenerative and those in the circulatory system. The most common of proliferative changes were lamellar lifting, proliferation of squamous epithelium, and this covering the secondary lamellae. Similarly to Fernandes & Mazon (2003), we consider that the presence of lamellar lifting increases the distance between the gill surface water and violates the absorption of oxygen. Therefore, the frequency of respiration increases as well. On one hand, high presence of proliferative changes is an indicator of enhanced compensatory-adaptive mechanisms that represent the body's response due to the negative effects of the toxicant in order to increase the distance between the circulatory system of the body and the effects of Cd in our case. This in turn is an indicator of activation process of mitotic division concerning the epithelial cells of the body. On the other hand, degenerative changes indicate the process of cell death, in parallel with those of cell division. The changes in the circulatory system were expressed in vasodilation, which was localized mainly in the venous sinus and placed in the basal part of the secondary lamellae.

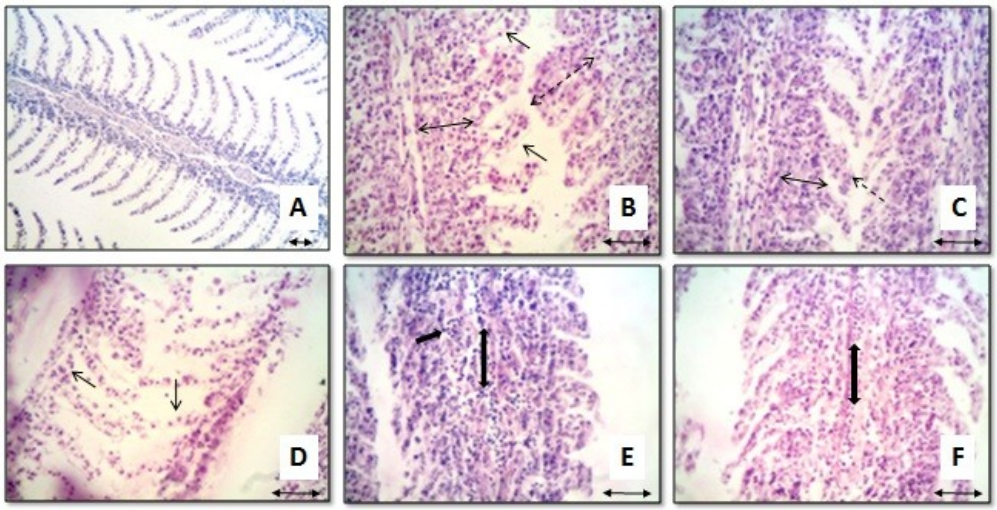

Fig. 1. Histological alteration in common carp gills (H&E) after Cd exposure. A - normal gill histological structure, x200; B - lamellar lifting (→), proliferation of filamentous epithelium (↔), fusion of secondary lamellae (↔), x400; C - proliferation of filamentous (↔) and secondary lamellae epithelium (↔), x400; D - degenerative changes, x400; E - vasodilatation in blood vessels of secondary lamellae (➡) and gill filament (↔), x400; F - vasodilatation in gill filament blood vessels, x400.

The results from the respiration rate measurements are presented in Table 1. At the beginning of the experiment (0 hour) we found that the respiration rate index in fish from the test tank was higher than the control, which remained unchanged until the end of the experiment (after 72 h of exposure).

Table. 1. Index of respiration rate of common carp after Cd exposure at the beginning (0 hour) and the end of the experiment (72nd hour).

Test variant	Water volume, l	Weight, g (G)	Total oxygen level (mg/l)				Total (Q_2)	Index of respiration rate (I)
			Beginning		End			
			q	Q	q_{1h}	Q_{1h}		
Beginning (0 hour)								
Control	30	102.95	7.9	237.00	7.7	231.00	6.00	0.058
Test	30	152.43	7.8	234.00	7.4	222.00	12.00	0.079
72nd hour								
Control	30	128.78	6.0	180.00	5.5	165.00	15.00	0.116
Test	30	139.74	8.0	240.00	7.4	222.00	18.00	0.129

Similarly to us Todorova *et al.* (2015) studied the respiration rate of Common Carp under Ni exposure, as well as its combination with Cd, Pb and Zn, and found that the results for the fish exposed to single Ni2+ were higher in comparison with the control group. This result indicated that the Ni2+ ions impacted the fish respiratory system. Data for the respiration rate of the fish exposed to Ni2+ and its combination with Cd, Pb and Zn for the fish exposed to Ni+Cd and Ni+Pb showed a lower rate compared to the control. Furthermore, those for the fish exposed to Ni+Zn showed similar results to the control, showing that this metal combination is the least stressful. Dobreva *et al.* (2008) also studied the respiration rate of *Carassius gibelio* after 96-hour Cu exposure, but found that the respiration rate was weaker compared to the control (lower values of oxygen uptake). Both observed reactions could be related to interference with the respiratory processes caused by disruption of oxygen access in the fish body.

Overall, in our experiment the fish reacted immediately to the Cd presence with changes of the respiration rate, which probably led the observed gill alterations. One explanation for such changes is most likely that the organism tries to deliver more oxygen to all tissues and organs triggered by the stress caused by the metal. Thus, we suggest that further research in this field should be carried out, including concentrations less than the maximum permissible ones set by law.

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