ECOLOGIA BALKANICA

2010, Vol. 2 December 2010 pp. 19-23

Influence of Zinc on Gill Morphology of Gibelio Carp (Carassius gibelio)

Iliana G. Velcheva[1], Atanas D. Arnaudov[2], Elenka S. Georgieva[3]

University of Plovdiv "Paisii Hilendarski", Faculty of Biology,
1 - Department of Ecology and Environmental Conservation,
2 - Department of Human Anatomy and Physiology,
3 - Department of Developmental Biology,
24 Tsar Assen Str., BG-4000 Plovdiv, BULGARIA
E-mail: *anivel@uni-plovdiv.bg*

Abstract. The influence of increasing concentrations of Zinc sulfate (Zn SO_4x7H_20) on the hystostructure of Gibelio carp gills was investigated. Changes were observed even in the lowest concentration (0.1 $mg.l^{-1}$) – degenerating, cirulation and hyperplastic processes. With the increasing of the Zinc concentration, the hyperplasic processes were predominant over the degenerating and cirulation ones.

Key words: histopathology, zinc, gills, fish, Gibelio carp

Introduction

By the sub-lethal chronic heavy metal concentrations observations were made on the changes appearing in various cells, tissues, organs and processes in the hydrobionts.

This is connected to the early diagnosis of the pathologic changes which allows the use of certain groups of hydrobionts as test-objects to determine the level of heavy metal pollution of the water ecosystems.

As to its toxicity to fish, Zinc is of middle strength – less toxic than Mercury, Copper and Cadmium and more toxic than the Nickel and Lead. Its toxicity is expressed mainly in morphofunctional damage of respiratory organs (MOORE & RAMAMAURTI, 1987).

Although Zinc is a necessary microelement, the sub-lethal concentrations of this metal change the biochemical parameters in fish organism which can lead to a change of the normal cell function GIODA *et al*. (2007), TYAGI & SRIVASTAVA (2005).

Bioaccumulation of Zinc in the fish's gills is proven as well (SERRA *et al*., 1996; VELCHEVA, 2006; MURUGAN *et al*., 2008; KORI-SIAKPERE & UBOGU, 2008; ARNAUDOVA *et al*., 2008).

According to SAPPAL *et al*. (2009) the gills namely play an important role for the absorption of Zinc from the water and hence why physiological and morphological changes often appear in them (CAVAS *et al*., 2005; SAPPAL *et al*., 2009; DOBREVA *et al*., 2008).

The aim of this investigation is to trace *ex-situ* the histological changes in the gills of *Carassius gibelio* in water with increasing Zinc concentration.

Material and methods

Experimental set-up. In aquariums of 25 l filled with dechlorated tap water were set the

following increasing Zinc sulfate ($ZnSO_4.7H_2O$) (produced by MERC) concentrations - 0.1, 0.5 ,1.0, 1.5, 2 mg.l^{-1}. The chosen concentrations are below LC50 for this species and are in consideration with the LAC by the Bulgarian legislation.

As a control environment dechlorated tap water was used.

In each aquarium 10 specimens of the experiment fish from the species *Carassius gibelio* was used. The experiment species were with no exterior pathological changes and from the same size (10 – 12 cm) and age group (1 year old). The fish was acclimatized before the test for 1 week in clean dechlorated tap water. During the experiment the fish were not fed. The duration of the experiment for each concentration was 96 hours. In the process of the experiment the following parameters of the water were maintained – oxygen content – 8.3 mg.l^{-1}, pH – 7.0-7.5, temperature - 17.5-19.0°C and hardness 9dH for each of the experiment concentrations (BATHE & FREI, 1985).

Histological study. The biopsied material from the gills was fixed in a 10% neutral formalin solution for 12 hours. The samples were treated with increasing concentrations of ethyl alcohol (70%, 80%, 86%, 96%, 100%) and they were put in paraffin with a melting point of 54-56°C by the method described by ROMEIS (1989), 0.6 μm wide paraffin cuts were made and put for colouring in hematoxyline and eosin (H&E). The morphologic variations were examined through a light microscope (Olympus CX21), using as reference TAKASHIMA & HIBIYA (1995), and photographed using a digital camera.

Results

Compared to the control group, the changes (Fig.1a) in the gills were observed even under the influence of the lowest concentration (0.1 mg.l^{-1}). The following major changes were found out.

1. Degeneration of the secondary lamella.

The degenerative changes observed were caused only by the influence of lower concentrations (0.1 and 0.5 mg.l^{-1}). Such changes were not present by the higher concentrations.

A thinning of the secondary lamella walls, shortening in length and increasing of the distance between two contiguous lamellas was observed (Fig. 1 a, b).

2. Circulation changes

Changes of a hyperaemic type were found. In the capillary of some secondary lamellas tens of erythrocytes were observed (norm is 1-2) and the lacunas had disappeared. (Fig. 1c).

The circulation changes usually accompanied the degenerative but were seen by higher concentrations as well, but only in areas with no hyperplastic changes. Hyperaemic changes were found in the primary lamellas blood vessels, too.

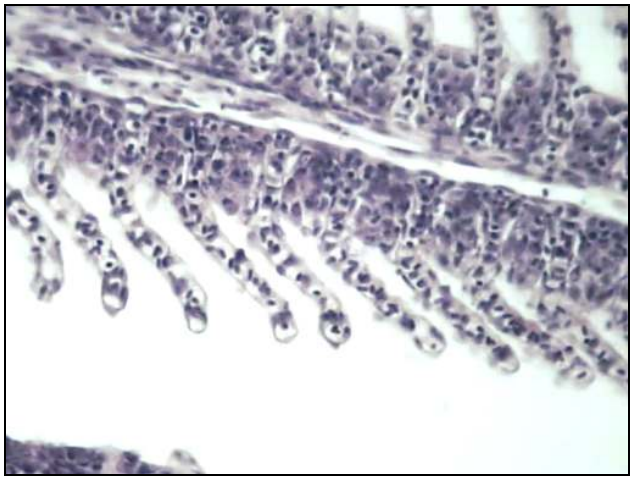
3. Hyperplasic processes

They were observed even under the influence of the lowest concentration (0.1 mg.l^{-1}) and were found in all other ones in a progressive manner. By the lower concentrations investigated (by 0.1, 0.5 and partly by 1.0 mg.l^{-1}), the hyperplasia revealed itself as proliferation of the intra-lamellas epithelial cells (Fig. 1d). Epithelia tissue growth was partial and it did not fill the intra-lamella space. By the 1.5 mg.l^{-1} concentration, the hypertrophy processes occurred in the distal part of the primary lamella (the so-called Club-shaped) which denotes the progressing nature of the damages (TAKASHIMA & HIBIYA, 1995) (Fig. 1e).

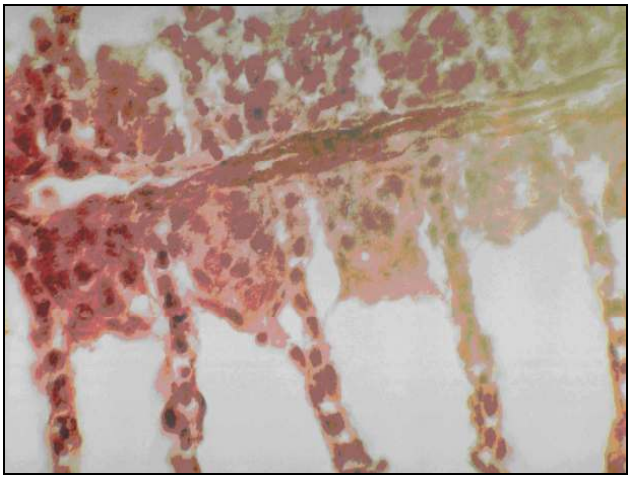
By the specimens treated with the highest concentration (2.0 mg.l-1) and partly with the lower ones (1.0 and 1.5 mg.l^{-1}) the proliferation of the intra-lamella epithelia cells causes full filling of the space between contiguous (fusion). Secondary lamellas had merged and the distance between them had disappeared (Fig. 1f).

Discussion

Our results show that probably the zinc influence is connected to an initial oxidative stress in fish.

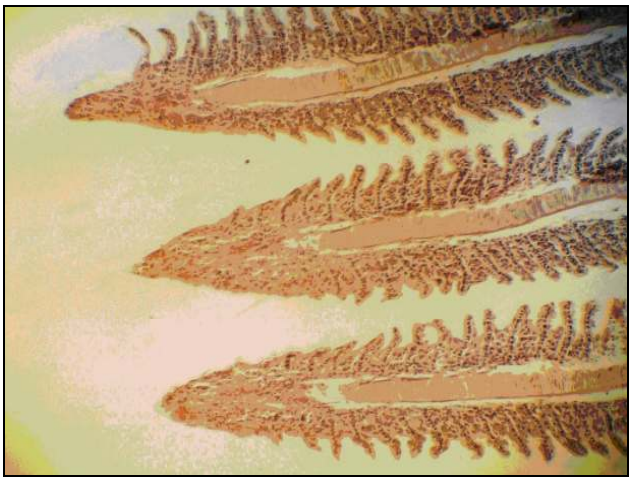


a. Normal structure. Control group. H&E x 400.

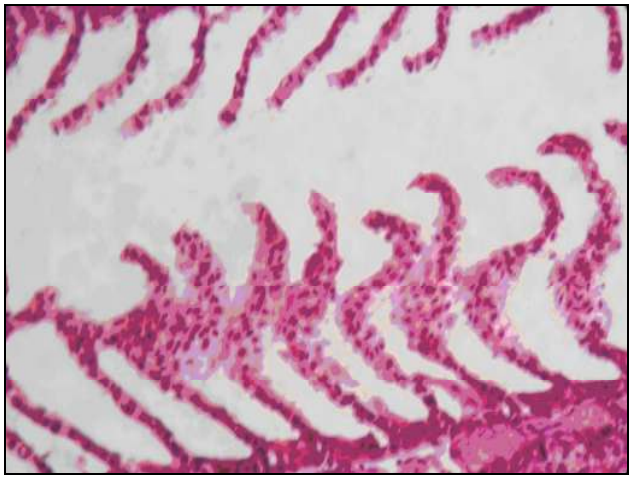


d. Proliferation of the intralamellar epithelial cells. 0.5 mg.l^{-1} Zn SO_4x7H_2O. H&E x 400.

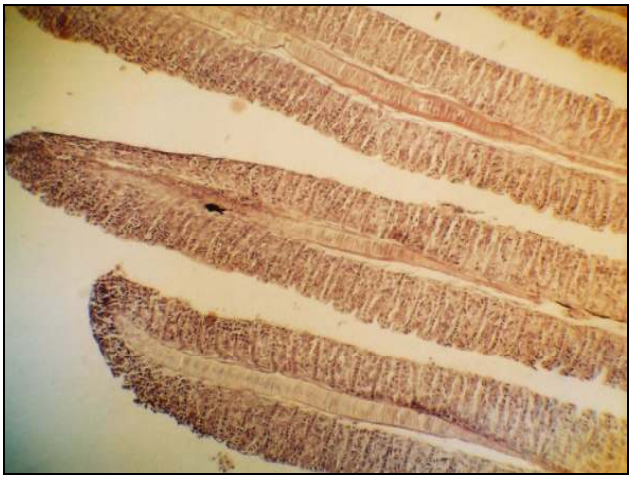
b. Degeneration of the secondary lamellae. 0.1 mg.l^{-1} Zn SO_4x7H_2O. H&E x 400.



e. Club-shaped primary lamellae. 1.5 mg.l^{-1} Zn SO_4x7H_2O. H&E x 200.



c. Hyperemia of the secondary lamellae. 0.1 mg.l^{-1} Zn SO_4x7H_2O. H&E x 400.



f. Fusion of the secondary lamellae.2.0mg.l^{-1} Zn SO_4x7H_2O. H&E x 200.

Fig. 1. Morphological alteration in gills of Gibelio carp under influence of zinc

Verification for this are the results from some past investigations of ours (DOBREVA *et al*., 2008) where we found a decrease of breathing intensity of *Carassius gibelio*, decrease of its sustainability towards Oxygen deficit, as well as changes in the hematological indicators (ARNAUDOV *et al*., 2009). These processes lead to disorders of blood circulation and a full or big foreclosure of the gas diffusion between gills and water. Similar to our opinion, SKIDMORE & TOVELL (1972) show that the initial changes in the gill tissue under the influence of Zinc are typical for an acute inflammatory infection accompanied by blood circulation disorder and a death possibility at a longer exploitation.

The gills epithelia damage leads to impeding of other vital processes – the maintenance of the alkaline-acid balance, ion regulation and the excretion Nitrogen metabolites. This, combined with the caused hypoxia, is to us the probable reason for the high mortality which the Zinc ions cause, which has been found by us in previous investigations. (DOBREVA *et al*., 2008).

Unlike our results, the investigations of FERNANDES *et al*., (2007) show changes in the gills of the leaping grey mullet (*Liza saliens*) that are mostly of the circulation – aneurysms, hyperplasia, lifting and dilatation of the vessels but degenerative and hyperplastic changes are missing in the secondary lamellas.

Changes of gills histology of different fish species under the influence of Zinc are reported in the works of CERQUEIRA & FERNANDES (2002), TKATCHEVA *et al*., (2004), FERNANDES & PERNA–MARTINS (2001) as well but they do not track the relation between the metal content and the degree of the changes found.

However, according to our results, such a relation is present.

By the lower concentrations (0.1 and 0.5 mg.l-1) were detected mainly destructive changes (Fig. 1b).

By increasing the Zinc concentration (1.0, 1.5 $mg.l^{-1}$) were observed mainly hyperplastic changes that were reaching the final phase of adhesion of the gill plates (2.0 $mg.l^{-1}$). We consider that this is due to a compensatory reaction towards the Oxygen deficit related to formation of new epithelia cells in the gills aiming to improve the gas exchange.

We can come to the conclusion that the Zinc influence on the tissue structure of Gibelio carp gills is expressed by the causing of degenerative, circulation and hyperplastic changes. By increasing the Zinc concentration the hyperplastic processes predominate over the degenerative and circulation ones. The probable reason for this is the capillaries compensation of the enlarged epithelia tissue by the higher Zinc concentrations.

Acknowledgements

The present investigation is supported by the "Scientific Research" fund of the Applied Science Activities at the University of Plovdiv "Paisii Hilendarski".

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Received: 17.07.2010
Accepted: 18.08.2010

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