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INFLUENCE OF COPPER AND ZINC ON THE ERYTHROCYTE-METRIC PARAMETERS OF *CARASSIUS GIBELIO* (PISCES, CYPRINIDAE)I. INFLUENCE OF COPPER ON THE ERYTHROCYTE-METRIC PARAMETERS OF *CARASSIUS GIBELIO* (PISCES, CYPRINIDAE)

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Abstract

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Some metric parameters of the erythrocytes (big cell diameter *Dc*, small cell diameter *dc*, big nuclear diameter *Dn*, small nuclear diameter *dn*) in the blood of *Carassius gibelio* after influence of different copper concentrations were established. The beginning of processes of atrophy in the low copper concentrations and necrosis in the higher copper concentrations were found out. There was an advent of the cell hypertrophy in the higher copper concentrations. The higher copper concentrations also caused anisocytosis.

Key words: Copper, fish, erythrocytes, metric parameters, Prise –Jones curves

Introduction

Environmental pollution with heavy metals is recognized as a worldwide problem. While following up the sub-lethal chronic concentrations of heavy metals, observations are being done over the heaping of this group of chemical elements in the hydrobionts, as well as over the current changes in some structures and processes in them. Laboratory screening tests are also applied for the purposes of ecological monitoring. These tests give the opportunity for following up the influence and the adaptive reactions which occur in hydrobionts organisms in cases of different heavy metal concentrations. This relates to the early diagnostics of the pathological changes that allows using some groups of hydrobionts like test-objects for finding the level of heavy metal pollution in water eco-systems.

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Copper is one of the most important heavy metal pollutants of the reservoirs. At present, copper content in freshwater increase because of many industrial, as well as agricultural pollutants. Researching the toxic effect of copper on fish is mainly related to determining the processes of cumulating and lodgments in the organism (Carpene at al., 1989; Papagiannis et al., 2004; Cogun and Kargin , 2004; Bielmyer et al., 2005) and to following up the changes in some biochemical features (Tishinova–Nanova, 1980; Cyriak et al., 1989; Arellanî et al., 1999; Chavez-Crooker et al., 2003; Monteiro et al., 2005; Carvalho and Fernandes, 2006; Hoyle et al., 2007).

In our previous study (Velcheva et al., 2006) various pathological changes in the morphology of carps' erythrocytes under the influence of increasing copper concentrations were found out.

In order to give a quantitative expression of these changes, it was set the task with this study to follow up the influence of increasing copper concentrations on the erythrocyte-metric parameters of *Carassius gibelio.*

Material and Methods

The stagnant tap water was put in aquariums with capacity of 25 liters. For the aim of the experiment series of increasing concentrations of copper sulphate ($CuSO_4.5H_2O$) were used. Test concentrations were respectively 0.1, 0.5 and 1.0 mg/l copper. The initial concentration (0.1 mg/l) is under the Limited Permitted level of Concentration by Bulgarian Standards for copper in waters. Stagnant tap water was used as control sample. Water for all experimental and for the control samples were with possessed the following parameters: temperature – 20°C, ðÍ – 7.0-7.5 and water hardness – 9.5 dH (German degrees).

As experimental fish were used 10 specimens from the species *Carassius gibelio*. The fish were taken from a clean water pond in fish breeding farm. The specimens had no external pathological changes and they were of the same size and age group (length 10-12 cm). The fish were not being fed during the experiment. The duration of the test for each concentration was 96 hours. For each specimen the following erythrocyte indices were determined:

- big diameter of the cell (Dc), small diameter of the cell (dc), big diameter of the nucleus (Dn) and small diameter of the nucleus (dn);
- ratio big cell diameter/small cell diameter (Dc/dc)
- ratio big nuclei diameter/small nuclei diameter (Dn/dn)
- type of *Prise-Jones* curves of big cell diameter, small cell diameter, big nuclei diameter and small nuclei diameter respectively.
- a width on the bases of the curves.

The blood samples were taken through heart punctures. The samples were collected in monovets with anticoagulant (EDTA). The blood smears were colored for the morphology investigation using a set for instant coloring of blood smears DKK COLOR – *200 (VIVA MT*).

An eyepiece- and object-micrometer were used for defining the metric indices (Dc, dc, Dn and dn) (Ibrishimov and Lalov, 1984).

For determination of the anisocytosis and the type of the anemic damages the percentage proportion of the various types of *Prise-Jones* curves (preliminary constructed) was calculated (Ibrishimov and Lalov, 1984).

The results were variably-statistically processed by methods described by Sepetliev (1986). Test with 95% confidence limited was applied to compared the means whenever the date were significant ($p<0.05$).

Results

Table 1 shows the results about the dimensions of the diameters of the erythrocytes of *Carassius gibelio* under the influence of various copper concentrations.

The big diameter of the fish cells from both experimental groups was significantly smaller than the cells of fish from control group ($p<0.001$). It has the lowest values by erythrocytes of fish under the influence of low copper concentrations 0.1 mg/l and 0.5 mg/l. By the highest copper concentration value an increase of the size was found out, but it did not reach this of

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Table 1
Metric erythrocytes parameters of *Carassius gibelio* after influence on different copper concentrations

Metric parameters	Control	0.1 mgl^{-1} Cu	0.5 mgl^{-1}Cu	1.0 mgl^{-1}Cu
Dc	13.3±0.3	11.1±1.1***	11.2±0.5 ***	12.4±0.6 ***
dc	7.8±0.5	6.2±0.7 ***	6.7±0.4 ***	7.4±0.4 *
Dn	7.8±0.5	5.3±0.6 ***	5.2±0.3 ***	5.0±0.4 ***
dn	3.7±0.6	2.5±0.4 ***	2.8±0.3 **	2.5±0.2 ***

Dc-big cell diameter * (p<0.1), **(p<0.02), ***(p<0.001).
dc-small cell diameter
Dn-big nuclei diameter
dn-small nuclei diameter

Table 2
Ratio: big cell diameter/small cell diameter" (Dc/dc)

Control	0.1 mgl^{-1}Cu	0.5 mgl^{-1}Cu	1.0 mgl^{-1}Cu
1.7 (1.60-1.80)	1.77 (1.60-2.00)	1.65 (1.60-1.70)	1.68 (1.50-1.80)

Table 3
Ratio: big nuclei diameter/small nuclei diameter (Dn/dn)

Control	0.1 mgl^{-1} Cu	0.5 mgl^{-1}Cu	1.0 mgl^{-1}Cu
1.94 (1.60-2.10)	2.1 (2.00-2.20)	1.84 (1.70-2.00)	2 (1.70-3.30)

Table 4
Prise-Jones* curves of the big and small erythrocyte diameter of *Carassius gibelio

Type of the curves, %	Control		0.1 mgl^{-1} Cu		0.5 mgl^{-1} Cu		1.0 mgl^{-1} Cu	
	big diameter	small diameter	big diameter	small diameter	big diameter	small diameter	big diameter	small diameter
One-peak	60	80	60	80	80	60	60	80
Two - peak	0	0	30	0	20	20	0	20
Three-peak	0	20	10	0	0	0	0	0
Parabolic	40	0	0	0	0	20	40	0
Ascending	0	0	0	0	0	0	0	0
Descending	0	0	0	20	0	0	0	0

Table 5
Prise-Jones* curves of the big and small nuclear diameter of *Carassius gibelio

Type of the curves, %	Control		0.1 mgl^{-1} Cu		0.5 mgl^{-1} Cu		1.0 mgl^{-1}Cu	
	big diameter	small diameter	big diameter	small diameter	big diameter	small diameter	big diameter	small diameter
One-peak	40	80	50	100	100	90	60	100
Two - peak	40	0	0	0	0	0	0	0
Three-peak	20	0	0	0	0	10	0	0
Parabolic	0	0	50	0	0	0	30	0
Ascending	0	20	0	0	0	0	0	0
Descending	0	0	0	0	0	0	0	0

Table 6
***Prise-Jones* curves of the erythrocytes of *Carassius gibelio*. A width on the bases- µm**

Cu concentrations	Dc	dc	Dn	dn
Control	4.8	4.5	5.3	3.8
0.1 mgl^{-1}	5.7	4.9	4.5	3.6
0.5 mgl^{-1}	4.9	4.6	4.3	3.8
1.0 mgl^{-1}	5.5	6.3	5.1	3.5

Dc-big cell diameter
dc-small cell diameter
Dn-big nuclei diameter
Dn-small nuclei diameter

the control group. The same tendency for the small diameter of the cell was found.

The big and small diameters of the nucleus decrease reliably in the experimental groups in comparison with the control samples.

The ratio: big diameter/the small diameter (Table 2) changes in the experimental groups in comparison with the control samples. In the lowest concentration it is higher than this of the control samples, but it decreases in the other two concentrations. This shows that the erythrocytes get slightly sharper in the lowest concentration, and they become rounder in higher concentrations.

A tendency in the changes of the ratio big diameter/small diameter of the nucleus in the experimental copper concentrations was not determined (Table 3).

At the big diameter of erythrocytes, the percentage of one-peak *Prise-Jones* curves increases at the expense of two- and three-peak curves, and there are no rising and falling curves (Table 4).

At the small diameter of erythrocytes, the percentage of one-peak *Prise-Jones* curves increases and the percentage of two-peak and parabolic curves decreases (Table 4).

At the curves of the big diameter of the nuclei, an increasing percentage of one-peak curves at the expense of the rest (except the parabolic ones) when the experimental concentrations are higher was found. At the curves of the small diameter of the nucleus, a high percentage (80%) of one-peak curves was found, and at the control samples, their percentage is 100 (Table 5).

The bases of *Prise-Jones* curves (Table 6) gave the following results:

- At the big diameter of erythrocytes, anisocytosis in concentrations of 0.1 and 1.0 mg/l. were found
- At the small diameter of erythrocytes, there is a widening of the curves bases, especially well-expressed in 1.0 mg/l concentration.
- At the big diameter of the nuclei, there was a tendency of decreasing anisocytosis.
- At the small diameter of the nuclei, the fluctuations are near to the values of the controls.

Discussion

Our results allow us to point out some peculiarities, related to some changes in the metric features of erythrocytes of *Carassius gibelio* under the influence of different copper concentrations.

It can be accepted that decreasing of erythrocytes sizes under the effects of the examined copper concentrations is a result of atrophic changes in them. Probably this is an early reaction to the toxic copper effect, since it reacts even in 0.1 mg/l concentration. Increasing the erythrocytes sizes with increasing copper concentration is a fact that we can explain with the appearance of hypertrophied cells, most probably as a compensatory reaction. It correlates to the bigger anisocytosis found in 1.0 mg/l concentration (the presence of small size cells, as well as big size cells).

Decreasing the sizes of erythrocyte nuclei together with the low level of anisocytosis shows that some necrotic changes also occur in erythrocytes.

In conclusion, it could be pointed out that the toxic effect of copper in low concentration causes atrophy in erythrocytes and in higher concentrations – necrobiotic changes as well.

Retrospectively, only a research that concerns typical changes in hematology features of fish under the influence of copper can be found. (Lotti et al., 1988; Gill et al., 1991; Tejendra et al., 1991; Cavas, 2005).

There are few studies similar to ours that concern the metric values of fish erythrocytes under the effects of various toxicants and they refer either to other species, or to other toxicants (Vosyliente and Jankaite, 2006). Witeska and Kosciuk (2003) says that if carps are put for 96 hours in water with zinc sulphate, a stress-reaction can be seen which is expressed by reducing the frequency of abnormal erythrocytes and compensatory releasing of immature erythrocytes as well. Zhelev et al. (2006) specify metric changes in erythrocytes in *Rana ridibunda* from an industrial region in Bulgaria. As the investigation has been done with species from another systematic class, in natural conditions and it is related to pollution with nitric oxides, it cannot be juxtaposed to ours. The present study is part of a number of similar studies of the team (Velcheva et al., 2006; Tomova et al., 2008). The studies examine the toxic effects of zinc and copper on hematological features of *Carassius gibelio* As a consequence, a difference in pathogenesis of the toxic effect of both metals has been proved.

The research done by the authors about the influence of increasing copper concentrations on the metric parameters of *Carassius gibelio* gave the opportunity, the results to be used for the purposes of biomonitoring. Storelli and Marcotrigiano (2001) give a similar opinion, as well as Brumbaugh åt al. (2005), who consider that blood samples of fish may also be used for that purpose.

Conclusion

The copper concentrations which we used lead to the following changes in erythrocytes of *Carassius gibelio*:

- Decreasing sizes of erythrocytes and their nuclei as a result of the atrophic-necrotic changes that occurred.
- Compensatory reaction in hemopoietic organs of the examined species causing progressive hypertrophic processes in erythrocytes.
- By 0.1 mg/l concentration, erythrocytes and their nuclei become slightly sharpened in low doses of copper. The changes in erythrocytes and their nuclei are not accompanied by developing anisocytosis, whereas the applied highest concentration causes anisocytosis.

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