In: Pešić, V. & Hadžiablahović, S. (Eds.) Proceedings of the Symposium,
II International Symposium of Ecologists of Montenegro. Kotor, 20-25.09.2006

A STUDY ON SOME PHYSIOLOGICAL PARAMETERS OF THREE HYDROBIOTIC SPECIES UNDER THE INFLUENCE OF COPPER

ILIANA VELCHEVA[1], ATANAS ARNAUDOV[2], GANA GECHEVA[1], IVELIN MOLLOV[1]

[1]University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Ecology and Environmental Conservation, 24 Tsar Assen Str., 4000 Plovdiv, BULGARIA, e-mail: anivel@pu.acad.bg; [2]University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Human Anatomy and Physiology, 24 Tsar Assen Str., 4000 Plovdiv, BULGARIA, e-mail: arny87@yahoo.co.uk

Abstract

A study on the effects of five increasing copper concentrations on three hydrobiotic species *ex situ* was carried out. The individuals of this research are gathered from various habitats of the Maritsa River, from unpolluted ponds. The researched indices are as follow: chlorophyll content of the aquatic bryophyte species *Amblystegium riparium*; hematological parameters in the blood samples of *Carassius auratus gibelio* and *Rana ridibunda*, particularly erythrocite alterations. The current paper commented alterations of the studied parameters depending on the different copper concentrations in the solutions. The results could be used and applied in future biomonitoring surveys of copper contaminated aquatic ecosystems.

Key words: ecological physiology, copper, hydrobionts, *Amblystegium riparium, Carassius auratus gibelio, Rana ridibunda*

Introduction

The assessment of sub-lethal heavy metals concentrations includes both studies on the elements accumulation in hydrobionts and occurring alterations in the organism structure and processes (Radwan *et al.* 1990; Marek, 1990; Annabelle & Baylosis, 1990; Gorokin, 1991; Wong & Wong, 1999). For the purpose of the ecological monitoring there have been applied laboratory screening tests which give opportunity to study the heavy metals effects on hydrobionts and their adaptable reactions.

Aquatic bryophytes play an important role in the uptake, storage, and recycling of metals. The freshwater habitats pollution negatively affects aquatic plants in diverse directions. Whitton *et al*. (1981) suggested the use of ten macrophyte species including the moss *Amblystegium riparium* (syn. *Leptodictyum riparium*) to monitor the levels of metals in European river and stream waters. Bryophytes pigment ratios have also been used to monitor pollution (Peñuelas, 1984; López & Carballeira, 1989). The chlorophyll content in the aquatic species *Fontinalis antipyretica* is used as a part of the biomonitoring in Maritsa River, Bulgaria and two species (*Fontinalis antipyretica* and *Amblystegium riparium*) were recommended as suitable biomonitors in different parts of the river (Yurukova & Gecheva, 2003, 2004).

The morphological blood cells characteristics of fish and amphibians enable the assessment of the compensatory adaptable processes in condition of pollution.

According to different investigations, the blood picture of fish and amphibians changes in cases of illness, intoxications, diverse stress (Chubinishvili, 1998; Peskova, 2004).

Studies on copper in the fresh water ecosystems are less intensive comparing to other heavy metals as cadmium, lead, mercury or zinc. There are insufficient researches following copper distribution in the trophic chain and its influence over the organisms (Moore & Ramamoorthy, 1984).

Due to the fact that until now a complex study on the copper effects simultaneously on species from different hydrobionts groups hasn't been conducted, the purpose of this paper is to survey the influence of the increasing copper concentrations on the chlorophyll *a* and *b* content in the aquatic moss *Amblystegium riparium* and erythrocite morphology in *Carassius auratus gibelio* and *Rana ridibunda*.

Material and Methods

The experiment was conducted in the Department of Ecology and Environmental Conservation's scientific laboratory at the Faculty of Biology, University of Plovdiv. We used copper solutions with five increasing concentrations: 0.1; 0.5; 1.0, 1.5 and 2.0 mg/l Cu and dechlorinated tap water for the control exposition respectively. The test duration was 96 hours.

For the present study the species *Amblystegium riparium* was collected from the middle stream of the Maritsa River, before the town of Plovdiv (South Bulgaria). Tufts of *Amblystegium riparium* were washed in stream to remove sediments and attached invertebrates. The plant was placed into containers with capacity of 1 liter and exposed to copper solutions. Temperature and light conditions were moderate. After 96 hours the samples consisted upper fragments 3 cm long, covered to protect from the light (Peñuelas, 1984b) were collected for analysis. Determination of the chlorophyll content followed the method of Shlyk (1968). The calculated pigment ratios are presented in mg/g wet weight.

Aquariums with capacity 25 l were used for the experiments with the species *Carassius auratus gibelio* and *Rana ridibunda*. During the exposition the water temperature was kept at 25°C, pH from 7 to 7.5 and the water hardness 9.5 dH. Ten individuals from each species (*Carassius auratus gibelio* and *Rana ridibunda*) collected from unpolluted habitats of the Maritsa River were used as test animals for each concentration. They had no external pathological alterations and were from equally size-age group. During the test they were not fed. The blood samples received by the means of heart puncture were collected in monovets with an anticoagulant (EDTA). The blood smears for the morphological studies were colored by composition for express coloring DKK Color – 200 (VIVA-MT, Bulgaria) by the method of Ibrishimov & Lalov (1984). Under the microscope were determined: the erythrocite shape; presence of young cells from the erythrocite row; pathological changes in the core, protoplasm and membranes.

Results and Discussion

Chlorophyll content of the aquatic bryophyte species *Amblystegium riparium*

In spite of long residence time in the solutions, the moss specimens were considerably not affected, showing a former green color and without dead strands. The concentration values of the pigments in the moss are presented at Fig. 1. Primary effect of the initial four copper concentrations is an increase in the chlorophyll concentration. The pigments, especially chlorophyll *a* show highest levels at the medium Cu concentration probably due to the fact that the copper is an essential trace element for all higher plants and has several roles in metabolic processes (Pätsikkä *et al.,* 2002). After 96 hour treatment with 2 mg/l (Cu^{2+}) the pigment ratios slightly decreased compared to the control sample. Contrary to expectation, the aquatic moss *Amblystegium riparium* treated with Cu did not show significant decreasing in the above parameters suggesting that the chosen range of copper concentrations did not affect the physiology of the plant. The chlorophyll *a* and *b* values measured in the control specimens (0.6 and 0.4 mg/g w.w. respectively) are similar to the pigment content in the aquatic moss *Fontinalis antipyretica* from Maritsa River habitats (Yurukova & Gecheva, 2003).

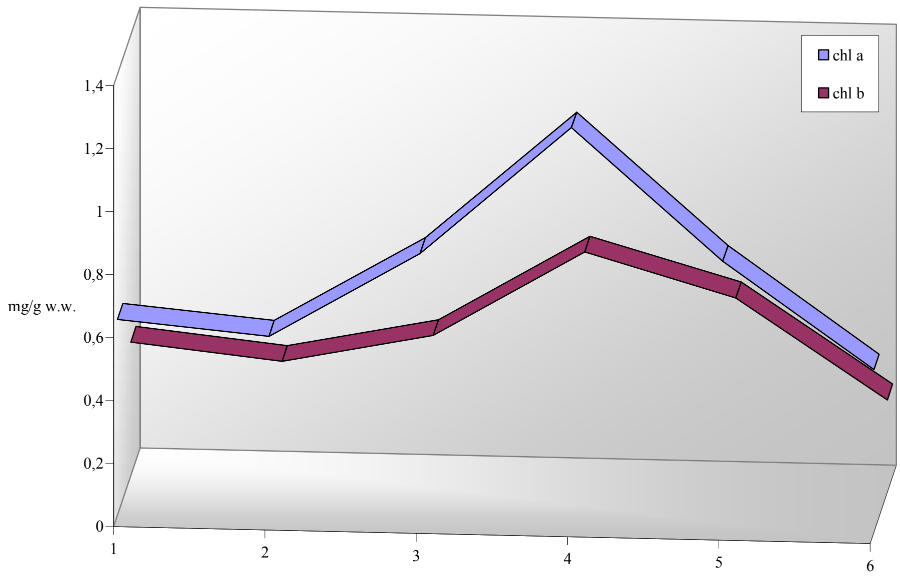

Fig. 1. Changes in chlorophyll *a* and *b* content, mg/g w.w. in *Amblystegium riparium* after 96-h treatment with different copper concentrations: point 1 – control; point 2 – 0.1 mg/l; point 3 - 0.5 mg/l; point 4 – 1 mg/l; point 5 – 1.5 mg/l and point 6 – 2 mg/l (Cu^{2+}).

Erythrocites changes in *Carassius auratus gibelio*

The prevailing part of the cells was with normal morphology at the copper solution with concentration of 0.1 mg/l. There were established changes in the shape – oval or with mucronate edge; destroyed cell membrane; bifurcated core at single cells.

The microscope analysis of the samples at the second concentration (0.5 mg/l) showed membrane damages – cell puncture and pouring the core content out; presence of single symplasts (Fig. 2a). We found also changes in the shape similar to the described from the first concentration. The represented alterations were with different intensity among the individuals. At the concentration 1 mg/l most of the cells had oval form or mucronate edge (preparation for cell division) and destroyed membrane (Fig. 2b).

Pear-shaped erythrocites with destroyed cell membrane were established as well as cells with two cores at the 1.5 mg/l Cu solution (Fig. 2c).

At the highest concentration (2 mg/l) the blood smears consisted erythrocites with membrane in pieces and poured protoplasm out (Fig. 2d). Both shape changes (pear-shaped or rarely with mucronate edge) and single cells with two and more cores were more peculiar.

Erythrocites changes in *Rana ridibunda*

The amphibians tend to form constantly core, oval, plane, double protuberanced erythrocytes. The cells cytoskeleton is responsible for their morphogenetic transformation from sphere to ellipse and affects the cell mass alteration under stress (Glomski *et al*., 1997).

According to Peskova (2004) the increased relative number of the smaller erythrocytes with oval shape should be considered as a compensatory reaction in *Rana ridibunda* under the influence of heavy metals salts (copper, cadmium, zinc) with high concentration (3 mg/l for seven days experiment). These changes in the erythrocytes morphology are due to short-term adaptation.

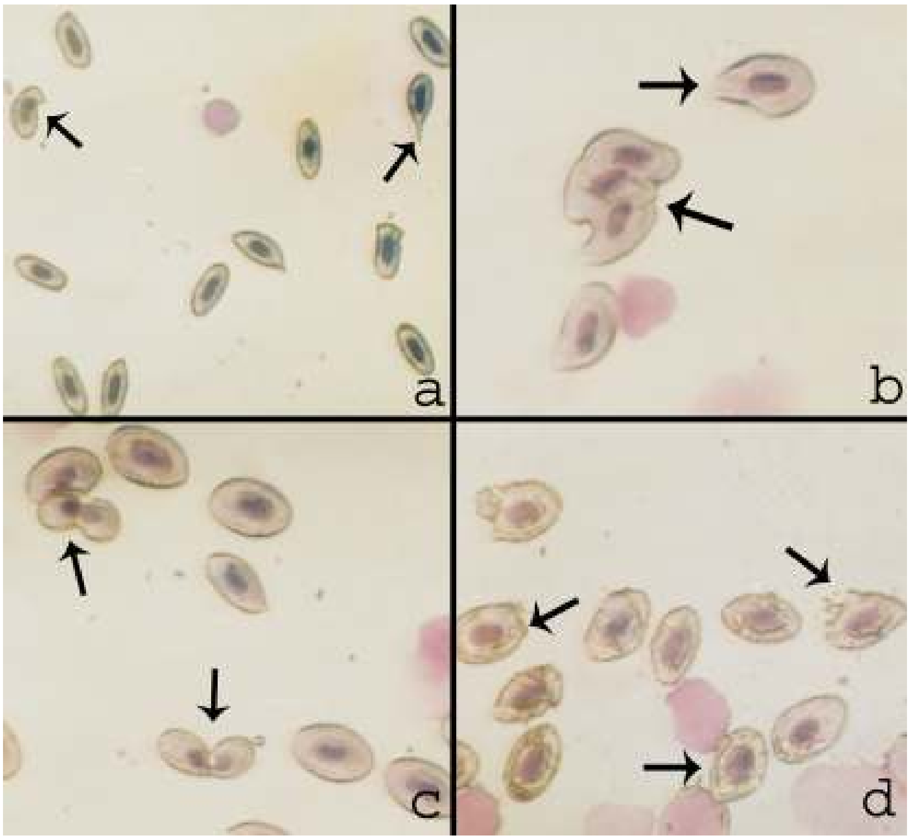

Fig. 2. Changes in the erythrocytes of *Carssius auratus gibelio* at different copper concentrations: a – 0.5 mg/l (x400); b – 1 mg/l (x600); c – 1.5 mg/l (x600); d – 2 mg/l (x600).

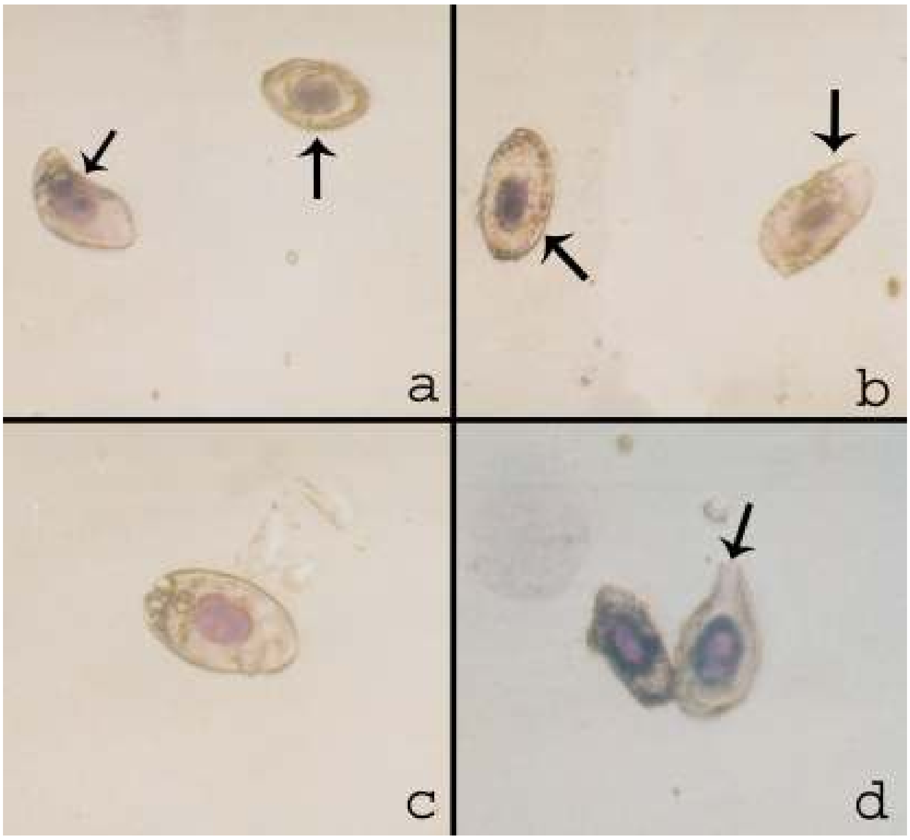

Fig. 3. Changes in the erythrocytes of *Rana ridibunda* at different copper concentrations: a – 0.1 mg/l (x600); b – 0.1 mg/l (x600); c – 0.5 mg/l (x600); d – 0.5 mg/l (x600).

At the initial concentration (0.1 mg/l) most of the erythrocytes had normal shape although there were cells with angular or mucronate form (Fig. 3a). The cytoplasm changes consisted in the presence of granules in the part of the cells (Fig. 3b). Thin fractional membrane breaks were found only in single erythrocytes (Fig. 3d).

The established changes in the erythrocytes morphology at the copper concentration 0.5 mg/l were more indicative. The cytoplasm granulation and appearance of vacuolization were much more common (Fig. 3c). Single cells were with increased amount of protoplasm causing anomalous cell shape. The erythrocytes number was decreased strongly (anemia). At concentrations of 1.0; 1.5 and 2.0 mg/l the established changes in the erythrocytes morphology were similar consisted in presence of granulation in the cytoplasm, vacuolization, breaking of the membrane and various changes in the cell shape, including more oval (young) cells (Fig. 3). The observed changes were established in the majority of the cells.

The observed irregular changes in the erythrocyte morphology in the blood samples of *Carassius auratus gibelio* and *Rana ridibunda* are a result of the destroyable heavy metal effect and are connected with processes not typical of that type cells. We consider that the assessed attempts for cell division, increasing of the protoplasm and the presence of young cells (in *Rana ridibunda*) and symplasts (at *Carassius autratus gibelio*) is a compensatory reaction for both species with the purpose of cell mass growth for transporting the higher amount of oxygen. Inspite of the different classes to which belong the hydrobionts the studied morphological alterations are similar. It is our opinion that the influence of the copper on the vertebrate hydrobionts is similar regardless of their taxonomy affiliation. In contrast with the terrestrial mammals the main physiological reaction to copper influence is so called „hemolytic crisis".

Conclusion

Until now a parallel screening-tests for copper contamination with specimens from three different taxonomic groups is not mentioned in the accessible literature. The conducted experiment shows that there are differences in the stability of the hydrobionts' physiological state after treatment with identical copper concentrations.

The aquatic bryophyte *Amblystegium riparium* is a convenient plant for laboratory toxicity bioassays. The results show that changes in the chlorophyll *a* and *b* concentration must always be taken into account when monitoring various stress conditions.

We established a positive correlation between the increasing concentration of copper in the water and the level of the morphological changes in the erythrocytes in *Carssius auratus gibelio* and *Rana ridibunda*. The indicated changes in the erythrocytes morphology of *Carassius auratus gibelio* and *Rana ridibunda* give us the opportunity to recommend them for suitable biomonitors for the purposes of the ecological biomonitoring surveys of copper contaminated aquatic ecosystems.

On the basis of the conducted study, we express the opinion that for fully clarifying the mechanisms of the toxic influence of copper, additional studies are required.

References

Radwan, S., Kowalik, W., Kornijow, R. (1990) Accumulation of heavy metals in a lake ecosystem, *Science of Total Environment*, 96 (1–2): 121 – 129.

Marek, J. (1990) *Metall ciezkie w srodowisku wodnym dolini Baryczu ocean zagrour gospodarki rybackiej,* Lesz. Nauk. AR, Wroclawin. Rozpr. Habie.82, 54 pp.

Annabelle, H., Baylosis M. (1990) Cadmium toxicity on the kidnry proximal tubule of the teleost Tilapia nilotica, *Cell. Biol.: Int. Repts. 3rd Eur. Congr. Cell. Biol., 02 – 07.09.1990.*, Firenze, Italy, London etc., 1990, p. 261.

Gorokin, I. (1991) Ecological-physiological aspects of the bioconcentration of microelements in the hydrobionts in natural environment, *Referativnii Zhurnal* – 07.02.1991, 7:202.

Wong, C., Wong M. (2000) Morphological and biochemical changes in the dills of tilapia (Oreochromis mosambicus) to ambient cadmium exposure, *Aquatic Toxicology*, 48: 517–527.

Chubinishvili, A. (1998) Status of *Rana ridibunda* (Amphibia, Anura) natural populations in the lower Volga River Basin: Morphogenetic and cytogenetic approaches. *Zoologicheskii Zhurnal,* 77(8): 945-946.

Peskova, T. (2004) *Adaptive changeability of the amphibians in anthropogenic polluted environment.* DSc Thesis, Tolyati, 38 pp.

Moore, J., Ramamoorthy S. (1984) *Heavy Metals in Natural Waters,* Springer-Verlag, New York, 286 pp.

Whitton, B., Say, P., Wehr, J. (1981) Use of plants to monitor heavy metals in rivers. *In: Say P. & Whitton B. (Eds.) Heavy Metals in Northern England: Environmental and Biological Aspects.* University of Durham, Dept. of Botany, Durham.

Peñuelas, J. (1984a) Pigments of aquatic mosses of the River Muga, NE Spain, and their response to water pollution. *Lindbergia*, 10: 127-132.

López, J., Carballeira, A. (1989) A comparative study of pigment contents and response to stress in five aquatic bryophytes. *Lindbergia*, 15: 188-194.

Yurukova, L., Gecheva, G. (2003) Active and passive biomonitoring using *Fontinalis antipyretica* in Maritsa River, Bulgaria. *Journal Balkan Ecology*, 6(4): 390-397.

Yurukova, L., Gecheva, G. (2004) Biomonitoring in Maritsa River using aquatic bryophytes. *Journal of Environmental Protection and Ecology*, 5(4): 729-735.

Peñuelas, J. (1984b) Pigment and morphological response to emersion and immersion of some aquatic and terrestrial mosses in N. E. Spain. – *Journal of Bryology*, 13: 115-128.

Shlyk, A. (1968) On spectrophotometric determination of chlorophylls *a* and *b*. *Biochemistry*, 33(2): 275-285.

Ibrishimov N., Lalov, H. (1984), *Clinical-laboratory analysis in veterynary medicine*, Zemizdat, Sofia, 421 pp.

Pätsikkä, E., Kairavuo, M., Sersen, F., Aro, E., Tyystjärvi, E. (2002) Excess Copper Predisposes Photosystem II to Photoinhibition in Vivo by Outcompeting Iron and Causing Decrease in Leaf Chlorophyll. *Plant Physiology*, 129: 1359-1367.

Glomski, C., Tamburlin, J., Hard, R., Chainani, M. (1997) The phylogenetic odyssey of the erythrocyte. IV. The amphibians. *Histology and Histopathology*, 12(1): 147-170.