

## **LDH ACTIVITY IN COPPER INTOXICATION OF CARASSIUS AURATUS GIBELIO GILLS AND INTESTINE**

### **ACTIVITATEA LDH IN BRANHII SI INTESTIN LA CARASSIUS AURATUS GIBELIO IN INTOXICATIA CU CUPRU**

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*The pathological effects of two sublethal concentrations (100 µg/l and 250 µg/l) of copper (CuSO<sub>4</sub>·5H<sub>2</sub>O) on goldfish Carassius auratus gibelio were studied for 7, 14 and 21 days. The specific activity of LDH in gills and intestine, two target organs that uptake the metal from the water were assayed. In gills at 100 µg Cu<sup>2+</sup>/l the specific activity of LDH was gradually decreasing, while in the intestine, after 7 days of exposure, the enzymatic activity was distinct significantly increased. LDH activity demonstrated a hypoxic condition and a stimulation of glycolysis. In the both organs, the 250 µg Cu<sup>2+</sup>/l concentration generated a decrease of LDH specific activity after 7 days followed by an increase of this after 14 and 21 days of exposure. Histologically, the modifications are, generally, directly correlated with the toxicant dose and exposure time.*

**Key words:** *gold fish, copper toxicity, LDH, histopathology.*

#### **Introduction**

Copper (Cu) is an essential element found in all living organisms in the oxidised Cu (II) and reduced Cu (I) states. Cu is specifically required as a catalytic cofactor in redox chemistry for proteins that carry out fundamental biological processes such as respiration, normal cell growth and development. However Cu also participates in the redox reactions that generate hydroxyl radical which causes considerable damage to lipids, proteins and DNA (Puig and Thiele, 2002).

Aquatic organisms can take up Cu directly from water and elevated ambient copper concentrations can lead to excess Cu accumulation in several tissue (Kamunde et al., 2002). Oxidative stress is generally accepted as one of the major effects of excessive cellular copper concentrations. Fish are relatively sensitive to changes in their surrounding environment including increased pollution. Fish

health may thus reflect and give a good indication of the health status of a specific aquatic ecosystem.

Biochemical indicators, such as enzymes, could be used (as biomarkers) in order to identify possible environmental contamination before the health of aquatic organisms is seriously affected (Barnhorn and van Vuren, 2004). In toxicology and clinical chemistry, the cytoplasmic enzyme LDH is widely used as marker of organ or tissue lesions. It has been used also to demonstrate the tissue damage in fish. Therefore, LDH has also been used as an indicator of hypoxic conditions in organisms and plays an important role in glycolysis (Das et al., 2004).

The aim of this study was to evaluate some biochemical and histological effects of the copper intoxication in the gills and intestine of *Carassius auratus gibelio*.

### **Materials and Methods**

Freshwater goldfish *Carassius auratus gibelio* of length 13.5-16.5 cm and weight 20.0-30.0 g were obtained from Fishery Research Station Nucet and kept in three glass tanks (each tank of 60 l) at 20-21<sup>0</sup>C. Prior to exposure, fish were held for 15 days for acclimatization and evaluation of overall health under laboratory conditions. The water from the control and experimental tanks was changed every 3 days. For the intoxication experiments the fish were divided in three groups: one as control and the others two as experimental groups. In both experimental tanks, concentrations of 100 µg Cu<sup>2+</sup>/l or 250 µg Cu<sup>2+</sup>/l were used. During the experiment, fish were exposed for 7, 14 and 21 days. Ten individual lots were used for every period of exposure. During the experiment the fish were not fed. After the selected periods, the gills and intestine were excised and prepared for biochemical and histological analysis.

Enzymatic assay. LDH activity was determined by the rate of oxidation of NADH measured by the change in extinction at 340 nm (Bergmeyer and Bernet, 1974).

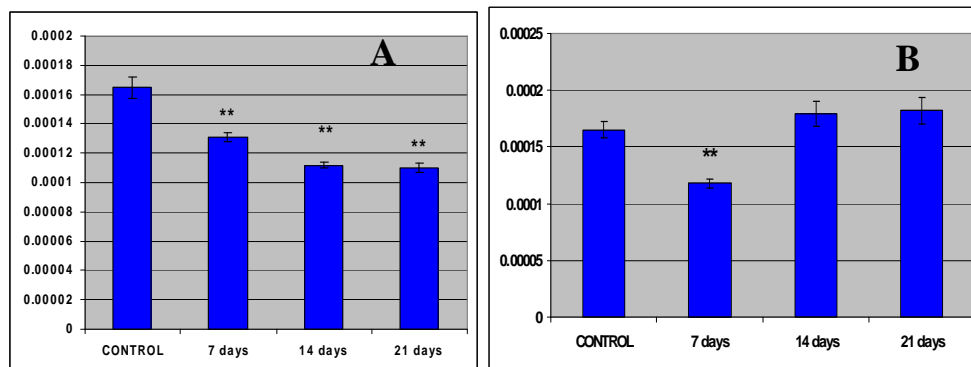
Protein concentration was determined according the method of Lowry (1951) with bovine serum albumin (BSA) as standard.

Histological procedures. Small pieces of gills and intestine were immersed in Bouin's solution (overnight), dehydrated in ethanol, cleared in xylene and embedded in paraffin. The sections were stained with hematoxylin-eosin. The photos were made with Zeiss Axyostar Plus Microscope.

### **Results and Discussions**

Fish are unique among the vertebrates in having two routes of metal uptake, the gills and the intestine (Nadella et al., 2006). LDH is generally associated with cellular metabolic activity, which is inhibited or elevated under oxidative stress, especially after exposure to heavy metals (Das et al., 2004).

The gill is a target organ, contributing significantly to osmoregulation in fish. In our experiment it can be seen that in the case of the first concentration (100  $\mu\text{g Cu}^{2+}/\text{l}$ ) the LDH specific activity decreased. Thus, after 7 days of treatment, the LDH activity decreased by 20.6%, after 14 days by 32.1%, while after 21 days by 33.3%, compared to the control (fig.1A). This fact determined a decrease of glycolysis rate.



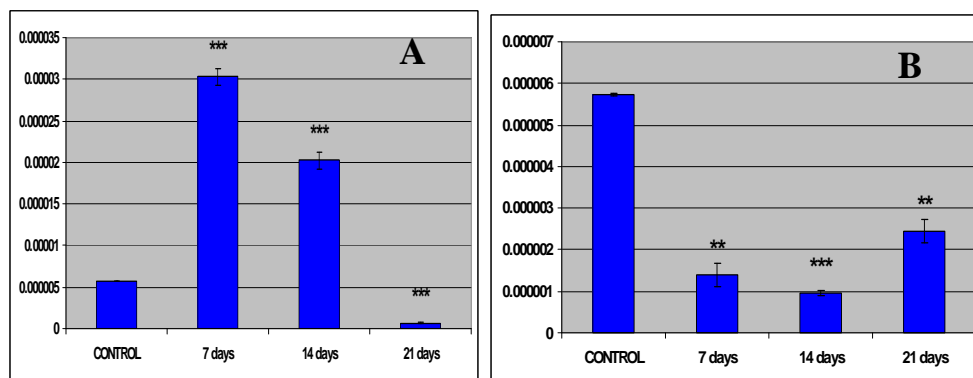
**Figure 1.** The variation of gill LDH specific activity in gills of *Carassius auratus gibelio* exposed to 100  $\mu\text{g Cu}^{2+}/\text{l}$  (A) and 250  $\mu\text{g Cu}^{2+}/\text{l}$  (B).

According to Almeida et al (2001), the inhibition of the activity of LDH may be due to changes in the activity of mitochondrial membrane function. They reported that mercuric chloride inhibited the activity of LDH in the gills of the fish *Channa punctatus*. At the second concentration (250  $\mu\text{g Cu}^{2+}/\text{l}$ ) the enzymatic activity decreased after 7 days by 28.5 %, compared to control, but increased after 14 and 21 days by 51.7 % respectively 54.2 % compared to the first period of exposure (fig. 1B). After 7 days of copper intoxication the  $\text{Na}^+$ ,  $\text{K}^+$  pump is inhibited and as a consequence, the glucose entrance in the chloride cells decreases and glycolysis rate is diminished. So the inhibition of Na transport across the gills co-incident with inhibition of the Na/K-ATPase enzyme (Lauren and McDonald, 1987). Probably, after 14 and 21 days, the copper accumulation is significant enough to stimulate glycolysis and to increase LDH activity.

A similar response was obtained in the chronic and acute manganese intoxication of *Carassius auratus gibelio* by Munteanu et al.(2002). Sastry et al.(1997) also observed an increase of LDH activity in the gills of the fish *Channa punctatus* exposed to Cd and Cu. The increase may be correlated to Cd and Cu induced damage in gill epithelium, which can reduce oxygen uptake capacity and determine hypoxia in this vital organs.

The intestine represents the second route of metals uptake. In the case of the first concentration (100  $\mu\text{g Cu}^{2+}/\text{l}$ ) the specific activity of LDH increased 4.27 time

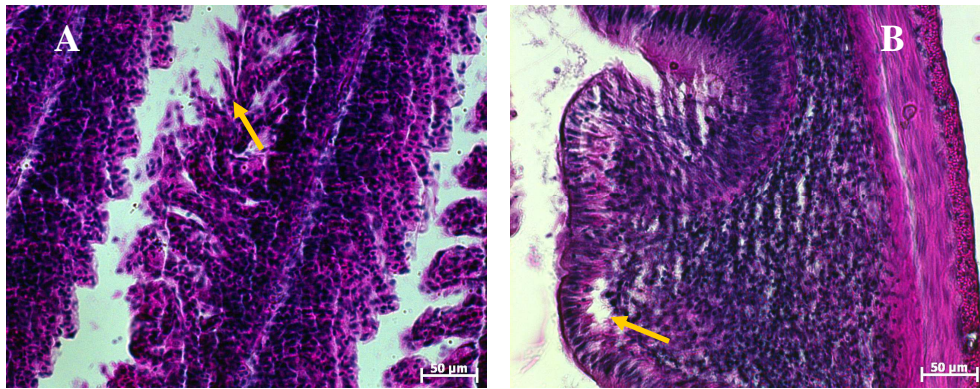
after 7 days, compared to the control, inducing a hypoxic condition. After 14 and especially 21 days the activity decreased by 33.1%, respectively 97.8%, compared to 7 day intoxicated intestine and as a consequence the glycolysis rate could be diminished (fig. 2A). In the case of the second concentration (250  $\mu\text{g Cu}^{2+}/\text{l}$ ) the enzymatic activity of LDH decreased significantly after 7 days (by 75.8%) and 14 day (83.2%) compared to control and increased 2.54 times after 21 days, compared with 14 days intoxicated intestine. Probably, after 21 days, the copper accumulation is significant enough to stimulate glycolysis and to increase LDH activity (fig.2B).



**Figure 2.** The variation of gill LDH specific activity in intestine of *Carassius auratus gibelio* exposed to 100  $\mu\text{g Cu}^{2+}/\text{l}$  (A) and 250  $\mu\text{g Cu}^{2+}/\text{l}$  (B).

These sublethal levels of Cu affect also the morphology and cause pathological changes in the gills and intestine. We have noticed that the toxic induced alterations in the gills and intestine epithelium (fig. 3A, 3B).

The fusion of gills lamellae, aneurysm, mixing of different cell types at lamellae level were noticed. At the level of the intestine the dilatation of villi also occurred.



**Figure 3.** The structure of gills (A) and intestine (B) after 21 days of exposure.

### Conclusions

The goldfish showed various stress responses to 100 µg Cu<sup>2+</sup>/l and 250 µg Cu<sup>2+</sup>/l exposure. Taking in account that the fish have two routes of metal uptake (the gills and the intestine), the biochemical and histological alterations produced by Cu<sup>2+</sup> on these two target organs were studied. Our results provide evidence that LDH can be used as a sensitive indicator of aquatic pollution.

Generally, the histological modifications, were related to the toxicant dose and exposure time. Thus, sublethal levels of Cu cause pathological modifications in the gills and intestine of *Carassius auratus gibelio*.

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