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Metabolic Glycogen Activity in Hepatocytes of Fish from a Water Source Containing Heavy Metals

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By a histochemical research it was investigated what the glycogen concentration in the hepatocytes of three species freshwater fish (bleak, perch and rudd) is. By this it is proven that there is bioaccumulation of lead, zinc and cadmium in their livers as a result of the fish's inhabiting water basins containing the same metals.

It was established that heavy metal bioaccumulation in the fish liver causes decrease of glycogen and its uneven distribution in the organ compared to fish in clean water basins. Resulting from the presence of heavy metals, species peculiarities in the disorders of glycogen metabolism were found.

Key words: glycogen, freshwater fish, heavy metals, histochemistry.

Introduction

In the hydrobiomes there is a bioconcentration of persistent pesticides and other xenobiotics including of heavy metals. Metals are not biodegradable and are referred to as mail environment polluters that have a mutagenic and carcinogenic effect on living organisms (More et al., 2003). Dural et al. (2007) and Ploetz et al. (2007) report that the highest levels of cadmium, lead, copper and zinc accumulation can be found in livers and gills of the fish investigated.

Down the Arda river valley there are the reservoirs 'Studen Kladenets' and 'Kardzhali' that are located near sources of strong anthropogenic pollution with heavy metals. Velcheva (1998) indicates morphological changes in liver cells of bleak, carp and perch fish from 'Studen Kladenets' reservoir by increased cadmium, lead, copper and zinc content in this organ. Concerning the physiological role of liver in carbohydrates metabolism, as well as the property of hepatycytes to accumulate polysaccharates in their cytoplasms it is of interest to comparatively study the morphofunctional characteristics of glycogen activity in cells containing accumulated heavy metals.

The aim of the research was to determine the quantitative parameters of the glycogen synthesized in the liver cells of fish inhabiting the basin of 'Studen Kladenets' reservoir down the Arda river containing heavy metals and to compare the results with those of fish of the same species but from unpolluted basins.

Materials and Methods

The study is conducted in the 'Studen Kladenets' reservoir region(Southern Bulgaria). In the investigations are included 30 freshwater fish of each of the 3 species: bleak (Alburnus alburnus L., Cyprinidae), rudd (Scardinius erythrophtalmus L., Cyprinidae) and perch (Perca fluviatilis L., Cyprinidae). As control specimens were used 3 to 5 fish of the same species from the 'Varbitsa' river in a region with no anthropogenic pollution.

The chemical analysis of heavy metals content in the soil and sediment was conducted using the method flame atomic absorbance spectrophotometry air–acetylene (2100-2300°C) with an apparatus "Perkin Elmer 3030 B".

The content of zinc, lead and cadmium in organs samples of the freshwater fish investigated was determined by the atomic absorbance spectrophotometry (AAS „PERKIN-ELMER 3030 B"). The results are calculated in $mg.kg^{-1}$ air-dry sample and are in conformity with the Threshold Limit Value (TLV) regulation for the concentration of toxic stuff in foodstuffs (Regulation No 5 of Health Department 1984).

Treating the histological samples for proving the presence of glycogen in the hepatocytes is conducted using the PAS histochemical method.

Results and Discussion

The permissible lead, zinc and cadmium level in soil depends on the active reaction of sail to water suspension. In the soil around 'Studen Kladanec' reservoir by a pH=8.0 the norm of the concentration Pb is <80 $mg.kg^{-1}$, of Zn - <370 $mg.kg^{-1}$ and Cd - 3 $mg.kg^{-1}$. According to our data the quantity of Pb and Cd is more than the norm. The measured Pb concentration is higher than TLV by 60.845 $mg.kg^{-1}$ and the quantity of Cd is a little more than it by 0.13 $mg.kg^{-1}$. Unlike these, the content of Zn is under the TLV by 31.189 $mg.kg^{-1}$.

The data about the heavy metal concentration in the sediment show higher values of those in the soil but our country does not have regalement norms for TLV in it. This is of big methodological importance as water organisms are capable of accumulating heavy metals from different sources including sediment (Labonne et al., 2001).

Researches on heavy metal accumulation in organs of fish from 'Studen Kladenets' reservoir show species peculiarities. The Lead content in the three fish species' organs show that the metal accumulates in the highest concentration in the hepatopancreas of rudd and less in the same organ in the bleak followed by perch but by all three fish the content is above the Threshold Limit Value. Bioaccumulation of zinc is also changed to more than the Threshold Limit Value in all three fish species. Among them, the zinc concentration is highest by perch followed by rudd and bleak. The cadmium content in liver of all three species shows that the metal accumulates in a considerably high level in perch liver. There is an increase over the Threshold Limit Value indicated as well, although in lower grades, in the hepatopancreases of the other two species. The found quantity accumulation and the regularities coincide with some previous researches of ours. Similar results have been reported by other authors when examining heavy metal bioaccumulation in different organs of freshwater fish (Dural et al., 2007; Ploetz et al.,

2007). It is considered that lead and chromium bioaccumulation in liver is due to protein affinity of the protein metallothionein towards these elements (Ikem et al., 2003).

The found accumulation of heavy metals and its species specificity proves right the statements of Tawari-Fufeyin and Ekaye (2007) and Karadede-Akin and Unlu (2007) that fish can serve as a bioindicator for pollution of the water they inhabit.

Normal metabolic activity of liver cells of bleak, rudd and perch

The parenchyma of the hepatopancreas is built by prismatic cells with a light-coloured cytoplasm, distinctive borders between them and centrally located nucleuses. The density of the parenchyma cells cytoplasm is due to the glycogen in them which is the form of granules. By the histochemical investigation on availability of glycogen in the hepatocytes of the control specimens there can be observed that the glycogen granules are present on the whole surface of the histological sections. In the organ are found vacuolised hepatocytes and a great number of lipocytes around them which gives the parenchyma a netlike character (fig. 1a, b).

In the perch liver the hepatocytes are with a more homogeneous cytoplasm and the lipocytes between them are less in quantity which provides the organ with a relative density (fig. 1c).

Metabolic activity of liver cells of bleak, rudd and perch from the 'Studen Kladenets' reservoir

By acquiring the glycogen concentration in the hepatocytes of bleak, rudd and perch from the "Studen Kladenets' reservoir a degranulation in the cell cytoplasm can be observed. (fig. 2). In the bleak samples the positively displayed areas with glycogen are lighter coloured. The vacuolization of hepatocytes is in higher rate while the quantity of lipocytes between them is reduced (fig. 2a).

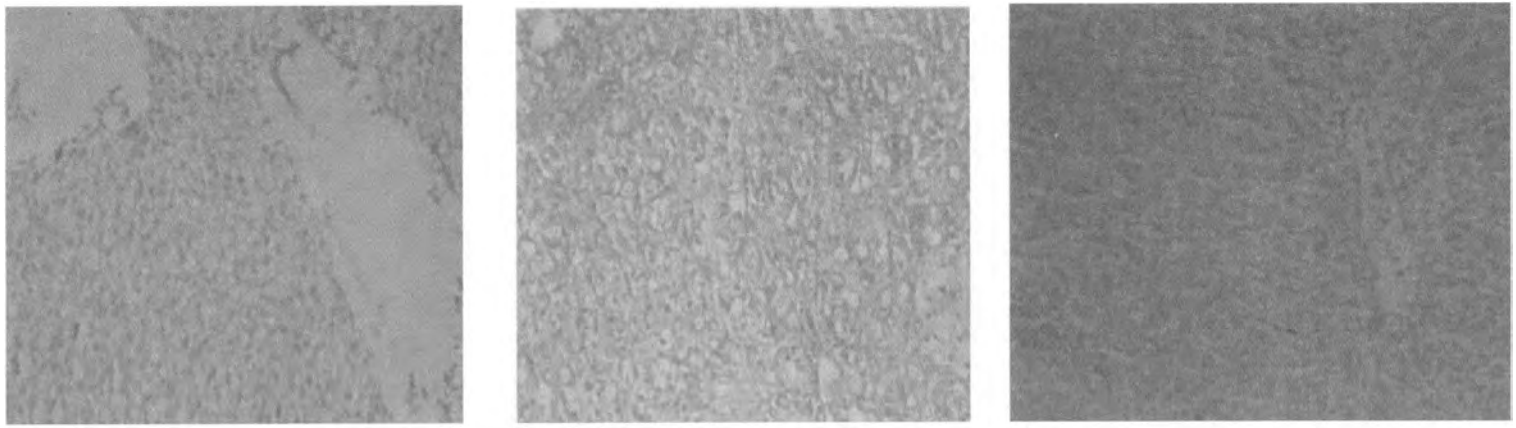
Glycogen by rudd fish is evenly distributed but has a weaked intensity and the parenchyma acquires a looser appearance(fig. 2b). In the perch samples the degranulation of glycogen is increased and the parenchyma density is decreased (fig. 2c).

The lead, cadmium and zinc found in hepatocytes of the fish investigated play a deactivating role on the glycogen synthesized in them. These data are in a correlation with the observed by More et al. (2003) metabolic deficit of DNA and hypofunction of the organism as a result of accumulation of heavy metals in it. Hayat et al. (2007) prove it that these metals have a growth hold-back influence of carp fish. They probably are the cause and consequence of the found morphological changes in fish hepatocytes (Velcheva, 1998). Depending on their aptitude towards bioconcentration, heavy metals take part in the metabolic chains on the highest levels of ecological pyramids where they create critical concentrations. This accumulation can lead to a long-lasting effect and changes in the biological lifecycle of fish.

Conclusion

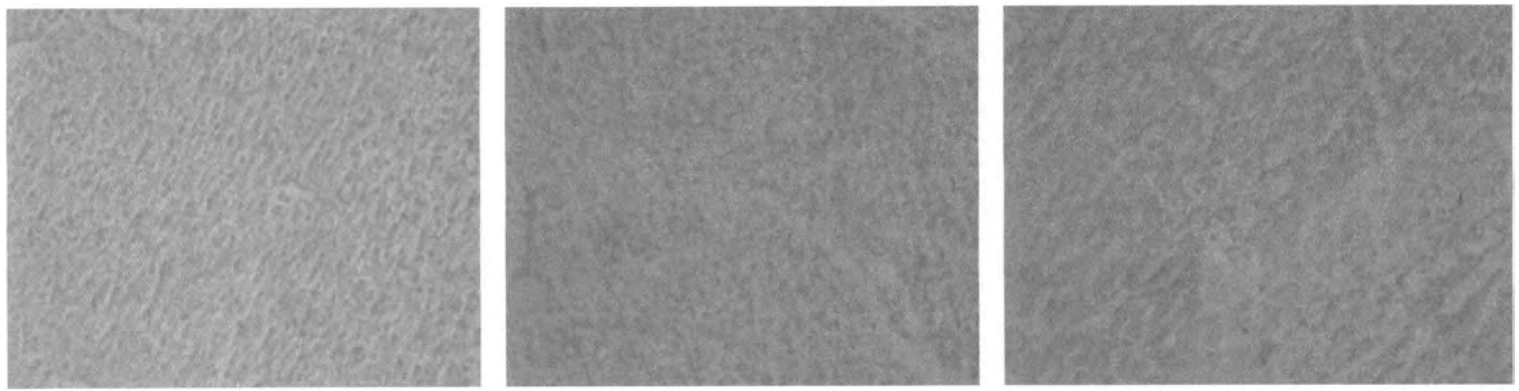
Bioaccumulation of lead, zinc and cadmium in the hepatocytes of bleak, perch and rudd causes the decrease of the glycogen content in them.

Species peculiarities exist in the glycogen metabolism disorder in the hepatocytes of fresh water fish caused by the presence and action of heavy metals in their organisms.



(a) (b) (c)

Figure 1. Expresson of glycogen by a PAS reaction in a hepatopancreas of bleak (a), rudd (b) and perch (c) from a clean water basin. × 40.



(a) (b) (c)

Figure 2. Expresson of glycogen by a PAS reaction in a hepatopancreas of bleak (a), rudd (b) and perch (c) from the 'Studen Kladenets' reservoir. × 40.

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