MERCURY BIOACCUMULATION IN TISSUES OF FRESH WATER FISH *CARASSIUS AURATUS GIBELIO* (SILVER CRUCIAN CARP) AFTER CHRONIC MERCURY INTOXICATION

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Abstract

The objective of the present study was to determine the mercury bioaccumulation level in various organs of a cyprinid fish, namely the silver crucian carp (Carassius auratus gibelio), after a 21 days chronic mercuric chloride exposure. We used a mercury sublethal dose of 0.25 ppm from a $ClHg_2$ stock solution. Mercury concentration in the tissue samples (gill, skin, intestine, muscle, cord, brain, liver, kidney) was assayed with the aid of a Spectr AA atomic absorbtion spectrophotometer, using the cool steam technique, and the results were given as mg/kg wet weight. In order, mercury bioaccumulation was: gill>liver> intestine and kidney>muscle>skin>brain>cord. High levels of mercury bioaccumulation in any tissues of the intoxicated animals definitely relieve that, mercury contamination of the aquatic ecosystems affect the life of the fish species, altering their metabolic functions. Hence, a scientific detoxification method is essential to improve the health of economic species of fish in any stressed environmental conditions (accidental or induced discharges of heavy metal.

Key words: chronic mercury intoxication, bioaccumulation, fresh water fish

INTRODUCTION

Chemical pollutants presence in water may induce severe ecological consequences, generating reorganizations of the biocenosis, changing it and consequently affecting aquatic ecosystems integrity ([3], [8], [21]).

Heavy metals are dangerous pollutants for aquatic inhabitants by themselves or by their toxic salts that have a great stability. Heavy metals contamination (pollution) of the surface waters happens widely by discharge of mill effluents.

Mercury is one of the most hazardous environmental pollutants. Mercury tends to concentrate in various organisms including fish due to reduced biodegradation of its derivatives. Consequently, fish are widely used as biomarkers for assessing heavy metal contamination level of aquatic environment and the health state of aquatic ecosystems.

MATERIAL AND METHOD

The experiment was performed on two groups of silver crucian carps (first group serving as control and second group as test group), with a body weight of 36.40 g ±1,2 g, that were collected from Cornesti' Fishfarm (Timiş county) and acclimated 2 weeks in laboratory conditions. Each group was housed in a 120 l capacity glass aquarium containing at a rate of 20 fishes/aquarium. Siphoning three quarters aquariums was done twice a week for replacing water by an equal volume of containing stored dechlorinated water with an adequate dose of contaminant respectively. Fishes were feed twice a day with commercial dry pellets and were starved for 24 h prior to the experimentation. Test specimens were selected to be ecologically representative.

The physico-chemical parameters of the laboratory water (during the experimental period) were as follows: dissolved oxygen 8,28±0,10 mg/l, water temperature

19.7±0.3°C, NO^-_2 0.2± mg/l, NO^-_3 3.5±0.5 mg/l, pH 8±0.5, hardness of water 6 dH° (soft water) and a 12 h illumination was maintained throughout the experiment. All these parameters were measured daily: (water temperature and dissolved oxygen - Hanna Hi 9145 oxygen-meter with water resisting microprocessor; pH, NO^-_2, NO^-_3, hardness of water – Germany TERMATEST kits).

The tested group (20 fishes) was exposed for 21 days to a chronic mercuy intoxication in concentration of 0.25 ppm from a $HgCl_2$ stock solution. Mercury concentration was obtained by following formula: heavy metal salt/heavy metal = molecular weigth/atomic weight = 1 g metal + 1 l distilled water = 1000 ppm stok solution.

The sublethal treatment dose (25% of LC_{50}) was calculated from percentage mortalities of fish as described by Veena et al. [19]. Mercury circulation in the aquarium was ensured by two AC 9904 air pumps.

Gill, kidney, intestine, skin, liver, brain, muscle and cord specimens (from control and tested group) were collected after stunning the subjects by a blow to the head. Bratu`s procedure [5] was used for tissue samples digestion.

A SpectrAA atomic absorbtion spectrometer was used to determine Hg concentration in tissue samples of the fish and the results were given as mg/kg wet weight.

Data analysis implied evaluation of mercury bioacumulation magnitudine between the experimental and control group tissues.

RESULTS AND DISCUSSION

Mercury concentration in control group tissues had less values (<0.5 mg/kg)than those allowed in the announcement of the Romanian Ministry of Health No. 1.145/2005 for fish meat and fish products.

But spectrometer analysis revealed increases of the mercury concentrations in every tissue sampled from the tested group at the end of the experimental period (table 1). Thus, in gill tissue its concentration increased 36 fold towards control group (from 0.0006 mg/kg ww to 0.026 mg/kg ww). Shah and Altindag, 2005 [18] and Houserova et al. [12] instead, found that the lowest concentration of total mercury accumulated in the gills of chub. Nor Wen-Bin Huang et al. [22] mention gills as predilect tissue for mercury bioaccumulation. However gill represent not only an important input site for heavy metals access into fish organism but also, the site where mercury induces structural lesions, affecting their function; this fact was signalized by Bols et al. [4] and Nicula et al. [17].

Table 1
Hg concentration in tissues of tested and experimental group and its magnitude of bioaccumulation

Tissue	C group (mg/kg ww)	E group (mg/kg ww)	Magnitude of bioaccumulation
Gill	0.0006	0.022	x 36.66
Liver	0.0005	0.013	x 26
Intestine	0.0007	0.005	x 7.14
Kidney	0.0022	0.014	x 6.36
Muscle	0.0005	0.003	x 6
Skin	0.0002	0.001	x 5
Brain	0.9949	0.013	x 2.65
Cord	0.0012	0.002	x 1.66

Hg concentration raised from 0.0005 mg/kg ww to 0.013 mg/kg ww in the hepatic tissue, which means a 26 fold increase, liver beeing a storage and detoxification organ for metals (Avenant-Oldewage and Marx cited by Wen-Bin Huang et al. [22]) and also acts as an active site of pathological effects induced by contaminants. Actually, chronic mercury intoxication leads to severe hepatic damages ([10], [15], [17]) in fish. This is the reason why liver is more often recommended

as an indicator of water pollution than any other organs in fish ([1]).

Hg concentration in intestinal tissue of experimental group exceeded 7 fold aproximately that found out in the same tissue of the unintoxicated group (from 0.0007 mg/kg ww to 0.005 mg/kg ww). Relative high mercury concentration in fish intestine were reported by Chen and Chen [6] and Lundebye et al. [14]. According to Farrar et.al. [9], it seems that intestinal mucosa often contains high concentration of inorganic mercury because excretion is initially through the gastrointestinal tract, howewer most mercury is eventually excreted by the kidney.

The kidney and muscle accumulated 6 fold more Hg in the experimental group versus its extremely low level in control animals (from 0.0022 mg/kg ww to 0.014 mg/kg ww and from 0.0002 mg/kg ww to 0.003 mg/kg ww respectively). If gills has a role in heavy metal acces in the fish body, kidney apparently is at the bottom of their „exit" control. Kidney being the main excretory organ, is affected in all species by all mercurials and generally contains high concentration after exposure. But even though approximately 50% of mercury in the body is absorbed by the kidney, only 10% is readily excreted (Markel et al., cited by Farrar et al. [9]). This is due to mtallothionein of renal tubular epithelium that binds mercury causing it to accumulate [9], with subsequent tubular damage ([13], [16], [17]).

However muscle is the tissue that usually has the lowest essential and non-essential metal concentrations in fish [23], a 6 fold increase of Hg accumulation in the muscular tissue of tested fish, advertises about the danger of its biomagnification into human beings (the last level of the trophic chain) consuming contaminated fish. But there are opposite cases as well; thus, Voigt [20] found higher mercury concentrations in muscle than in liver of smelt *Osmerus eperlanus* and perch *Perca fluviatilis*, due to the charactersitics of the fish habitat. Also, the other authors ([2], [7], [12]) observed high concentrations of total mercury in muscle tissues of various fish species, where mercury is bound to cysteine rich proteins.

Metal concentration in skin increased 5 fold after chronic exposure (from 0.0002 mg/kg ww to 0.001 mg/kg ww). Generally, this tissue accumulates less metals than others – gill, liver, intestine or gonads ([23], [12]).

Brain and cord have the least storage level of Hg (x 2.65 and x 1.66 respectively); but the function of these tissues is not less disturbed. After absorbtion, mercury is distributed throughout the body and tends to concentrate in kidney and brain as well [11]. The brain retains mercury longer than other organs, except kidney (David et al., 1976, cited by Farrar et al. [9].

CONCLUSIONS

1. In ours experiment, mercury was readily accumulated in every tissues sampled from chronic intoxicated fish.
2. Magnitude of mercury bioaccumulation was in following decreased order:
gill>liver>intestine and
kidney>muscle>skin>brain>cord

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