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Histological Aspects of Mercury Contamination in Muscular and Hepatic Tissues of *Hoplias malabaricus* (Pisces, Erythrinidae) from Lakes in the North of Rio de Janeiro State, Brazil

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Abstract

Ultrathin sections of muscular and hepatic tissues of *Hoplias malabaricus* from contaminated and non-contaminated areas by mercury were compared in order to observe histological and ultrastrutural perturbations, under transmission electron microscopy (Zeiss 900 Electron Microscope). The hepatocytes of control site (Cima Lake) showed endoplasmic reticulum, mitochondria and nucleus with classical morphological aspects and cytoplasm distribution. However, the hepatocytes of contaminated area (Campelo Lake) showed altered mitochondria distribution with morphological change in the mitochondrial cristae. Therefore the endoplasmic reticulum elements were dilated. The muscular tissue of fish from Cima Lake showed a symmetric and parallel miofibrils distribution with defined sarcomeres. The contaminated samples of Campelo Lake developed large lysed areas, myofibrils disruption, discontinued I band and undefined sarcoplasm. Our results strongly suggest that mercury contamination observed in *Hoplias malabaricus* caused hepatic and muscular alterations.

Keywords: fish, mercury, ultrastructure, hepatocytes, skeletal muscle.

Introduction

The concern about mercury (Hg) pollution in aquatic systems has increased in the last decade due to high levels of mercury observed in fish and the associated risks to human health. This element is one of the few metal pollutants that had already caused human deaths due to ingestion of contaminated food. The aquatic biota is the main mercury pathway from a contaminated environment to humans, since this metal suffers biomagnification through food chains, presenting its highest concentration in higher trophic level organisms like fish (8). Although potential human health effects from mercury exposure have received considerable attention, as well as studies involving fish exposition to high levels of mercury in laboratory conditions (7, 13), relatively few studies have dealt with the effects of this contaminant on fish tissues in field conditions.

The North of the Rio de Janeiro State (Figure 1) presented two main sources of anthropogenic mercury contamination: the use of mercurial fungicides in sugar cane plantations and the gold mining activities. Although both stopped in the beginning of the eighties, they caused the contamination of soils, sediments and the biota of this region (3, 16, 12).

The aim of this study is to establish the total mercury concentrations in muscular and hepatic tissue of *Hoplias malabaricus* (BLOCH 1794) from four lakes (Cima Lake, Campelo Lake, Feia Lake, Taquaruçu Lake) and from the Campos Municipal Market (CMM) observing differences in histological aspects in both tissues.

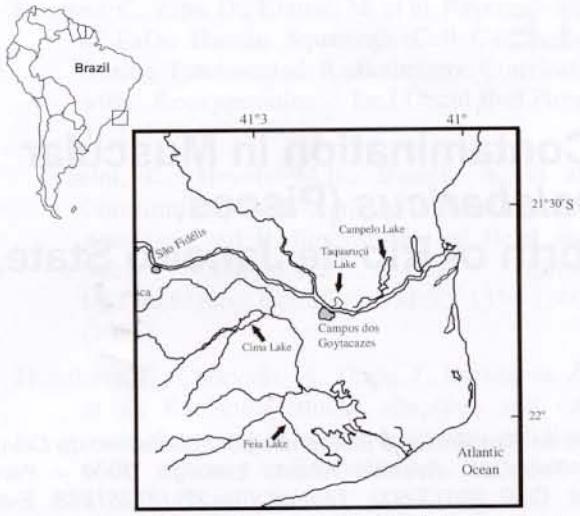

Figure 1. Map of the study area on North of Rio de Janeiro State.

Materials and Methods

Fish tissues samples were collected during the dry season (July-September/2000). Muscular and hepatic tissues aliquots were dissected for analyses with the help of a stainless steal stiletto. The acid extraction methodology for Hg determination in 1.00 g of biological samples (1) followed an addition of 1 ml H_2O_2; 3 ml $1H_2SO_4$: $1HNO_3$; hot plate (60 °C/4 h); 5 ml $KmnO_4$ 5%; hot plate (60 °C/30 min); NH_2OHHCl 12 % and filtration (Wattmann 41).

Total Hg concentrations in all the studied samples were measured with the help of an inductively coupled plasma atomic emission spectrophotometer (ICP-AES, Varian model Liberty II) with vapor generating accessory (VGA 77).

For tissue ultrastructural analysis, the cells were fixed in routine solution for transmission electron microscopy that included 1% glutaraldeyde; 4% paraformaldeyde; 5mM $CaCl_2$ in 0.1M cacodylate buffer containing 5% sucrose, pH 7.2 (17). The tissues were postfixed for 1h in OsO_4 at room temperature. The cells were rinsed with cacodylate buffer, dehydrated in acetone and embedded in Epon.

Ultrathin sections were examined using Zeiss 900 Electron Microscope.

Results and Discusion

The average Hg concentrations in all the sample areas are summarized in the Figure 2. The highest Hg concentration in muscle and liver were respectively 1366 and 831 $\mu g.Kg^{-1}$ in samples from Campelo Lake. The lowest concentrations were observed in specimens collected in Cima Lake, 63.6 and 57.2 $\mu g.Kg^{-1}$ in muscular and hepatic tissue respectively. The samples that presented the highest Hg concentrations were used to histological analyses for electron microscopy and the lowest were used as a control.

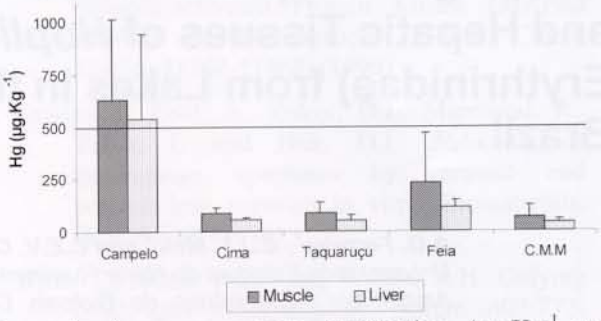

Figure 2. Average mercury distribution ($\mu g.Kg^{-1}$ wet weight) in all the studied lakes and the Campos Municipal Market (CMM). 500 $\mu g.Kg^{-1}$ = Brazilian Consumption Advisories for Hg concentration in fish.

Ultrathin sections from the contaminated site (Campelo Lake) and the uncontaminated sites (Cima Lake) were compared in order to observe histological and ultrastructural perturbations in muscle and liver.

Our results showed ultrastructural modifications in livers and muscle *of H. malabaricus* between both sites. When compared with Campelo Lake, the fish hepatocytes of Cima Lake (control site) showed some organelles with classical morphological aspects: disperse endoplasmic reticulum with short and small cistern, a small number of mitochondria and weakly contrasted nucleus (Figure 3f). However, the hepatocytes of contaminated fish (Campelo Lake) showed altered mitochondria distribution, always grouped near the nuclei. These organelle presented diverse forms with prominent number. Morphological change in the mitochondrial cristae it was observed with internal membrane apparently more evident. Large and elongated endoplasmic reticulum may be seen frequently close to mitochondrial organelles (Figure 3a, 3b, 3c, 3d and 3e).

Similar results were observed in liver of *Brachydanio rerio* (11) with ultrastructural perturbations when exposed to sublethal concentrations of copper sulfate in laboratory conditions. The same endoplasmic reticulum proliferation and modified mitochondria in hepatocytes were observed in *Trichomycterus brasiliensis* under acute exposure of inorganic mercury (14). *In vitro* results (4) suggest that the formation of superoxide anions in the mitochondria during mercury contamination might be involved in the mercury cytotoxicity mechanism and the formation of antioxidant defenses, a typical response against the oxidative stress.

Mitochondria increase numbers were described as a common reaction against pollutants (15). Low concentrations of mercury may lead to depletion of mitochondrial glutathione and enhances hydrogen peroxide formation under conditions of impaired respiratory chain electron transport (18). The increase hydrogen peroxide may

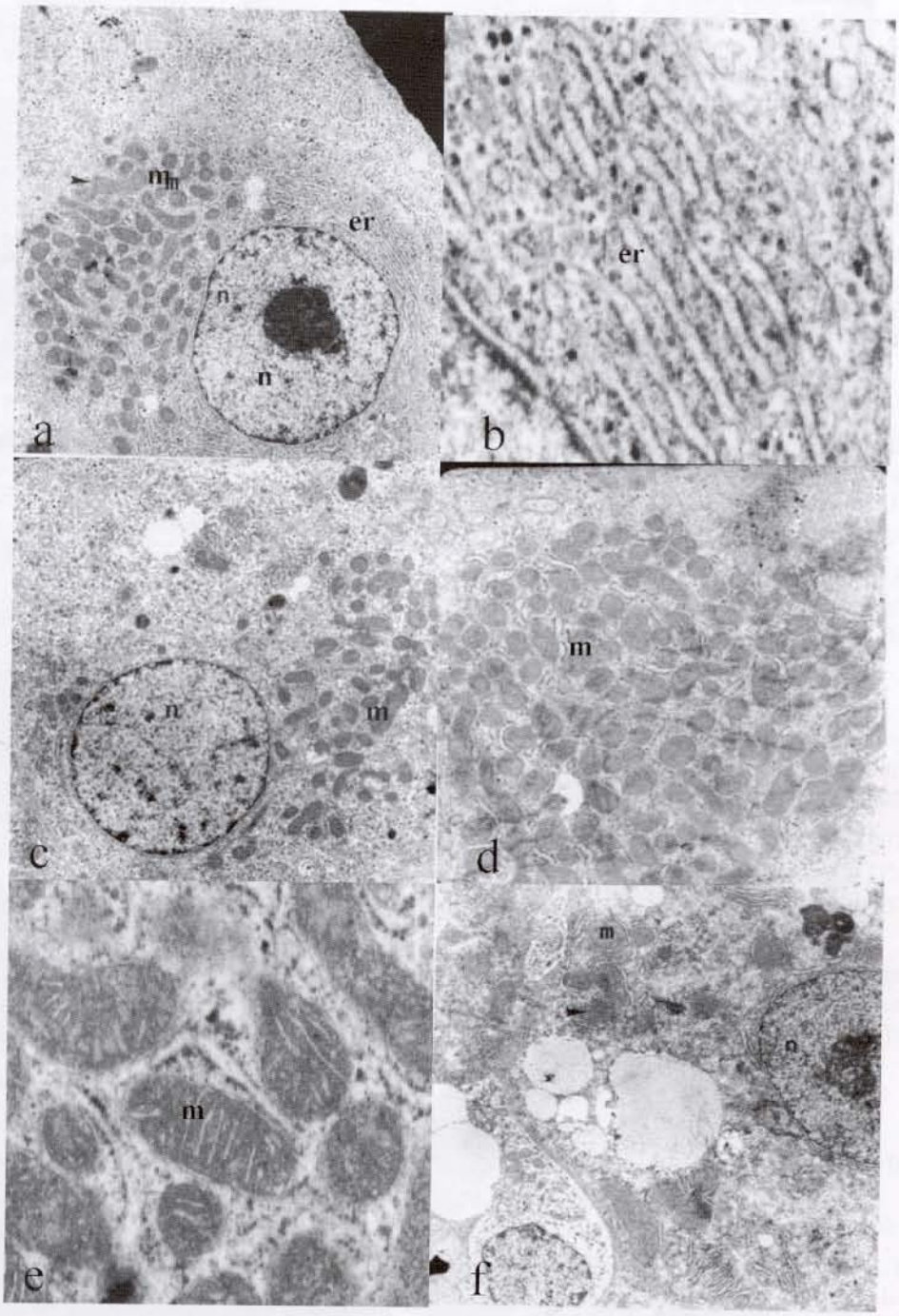

Figure 3 a) .x 7000. Hg contaminated hepatic cell with development endoplasmic reticulum; b) .x 20000. Endoplasmic reticulum detail;.c) x 7000. Hg contaminated hepatic cell with mitochondria aggregate; d) .x 12000. Mitochondria aggregate detail; e) .x 20000 Mitochondria detail; f) .x 7000 Hepatic control cell. **n** = nucleus; **m** = mitochondria; **er** = endoplasmic reticulum.

lead to oxidative tissue damage, including lipid peroxidation, resulting in mercury-induced hepatotoxicity.

Our results, in accordance with these authors suggest that the morphological perturbations on mitochondria and rough reticulum, are results of adaptive changes to heavy metal contamination in fish hepatocites.

The muscular tissue in fish of Cima Lake showed a symmetric and parallel miofibrils distribution with defined sarcomeres limited by the sarcoplasm (Figure 4e and 4f).

Figure 4a) .x 4400. General aspect of muscular contaminated tissue; 4b) x 12000. Lised area (black head arrow) and sarcoplasmic reticulum grouping (black arrow) in Hg contaminated tissue. Note the discontinue H and I band.; 4c) .x 20000 Sarcoplasmic reticulum inclusion; 4d) .x 24000. Sarcoplasmic reticulum; 4e) .x 12000. General aspect of muscular control tissue; 4f) . x 32000 Sarcoplasmic reticulum control. **I** = I bamd; **H** = H band; **sr** = sarcoplasmic reticulum.

The contaminated samples of Campelo Lake developed lysed areas with myofibrils disruption, discontinued H and I band, large endoplasmic reticulum and undefined sarcoplasm (Figure 4a, 4b, 4c and 4d). Similar aspects

were described in muscular tissue of human under occupacional chronic exposure (9). The authors related loss of myofibrils or complete disappearance in some fibres, in accordance with our findings.

The high affinity of methyl-mercury to skeletal muscle of fish (responding for approximately 75-95% of the total mercury concentration in this tissue) was described (2). These findings suggest that methyl-mercury, which has high affinity for thiol groups, turn proteins and peptides susceptible to structural modifications in subcellular compartments and tissues as in skeletal muscle.

Some authors have already observed that mercury alters calcium homeostasis (18, 19). Inhibitory effects of mercury on Ca^{+2}-ATPase activity from sarcoplasmic reticulum in rabbits (6) and frogs muscle tissues (10) were described. Elemental and morphological alterations in cultured myoblasts as reflection of mercury contamination altering the membrane permeability were also described (20). The anionic phospholipids as the major targets for methyl-mercury binding in biological membranes were cited (5), resulting in large structural effects, as fluidity changes and integrity loss that could induce several functional perturbations at the biomembrane level and finally at the tissue level. Our findings leave us to suppose that the structural modifications in the muscular tissue of *Hoplias malabaricus* at the contaminated site might be associated to change at the membrane level that implied in tissue perturbations.

Conclusion

Our results showed the singular aspect of *in vivo* sublethal mercury contamination in muscular and hepatic tissues of *Hoplias malabaricus*, a carnivorous fish with large distribution in tropical rivers and lakes and very consumption by human populations. Despite *in vivo* responses often consist of multiple steps, including several environmental variables, the histological aspects observed among the contaminated and the non-contaminated fish samples of Campelo Lake, point to adaptative mechanisms against the cytotoxic effects of mercury contamination in both, muscle and liver.

Furthermore, other studies are necessary to understand how these histological changes can influence and modify the cellular metabolism and the fish health. Tests that are capable to measure and find specific intracellular targets of mercury will allow to better predict the toxicological potencial of xenobiotics as mercury and other heavy metals to tropical fishes.

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