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Hematological Parameters in Nile Tilápia, *Oreochromis niloticus* Exposed to Sub-letal Concentrations of Mercury

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ABSTRACT

Mercury toxicity in tilapia, Oreochromis niloticus, (Linnaeus, 1758) was investigated by the hematological parameters after long-term (14 days) exposure to various Hg concentrations (0.02, 0.002, 0.0002mg/L Hg). Test groups were set up with three replicates for each concentration, plus the control group. Blood samples were collected from six individuals for each concentration at 0, 3, 7, 10 and 14 days of exposure. The hematological parameters analyzed were: total red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cell count (WBC) and differential leukocyte counts and total thrombocyte count (Tr). There were no significant differences among the mean hematological values at the different Hg concentrations indicating that Hg at the concentrations studied was not toxic to tilapia.

Key words: Mercury, heavy metal pollution, fish, *Oreochromis niloticus*, hematology

INTRODUCTION

The discharge of effluents containing mercury (Hg) into the environment has caused grave contamination problems in communities of various ecosystems and in human populations. Cases such as Minamata and Niigata in Japan, during the 1950s and 1960s, and in Iraq in the 1970s, reflect a high risk from this type of pollution. This stirred the attention of health authorities in relation to the contamination of rivers by industrial waste containing mercury, because besides being the most toxic among heavy metals, it is also accumulated in animals for long periods of time.

Aquatic organisms produced in breeding systems are constantly exposed to pollutants, since the water utilized in the production process originates from rivers, lakes or sources that could be contaminated with Hg. Tavares (1995) showed that for aquatic organisms to develop and survive well, the conditions of the environment in which they live is of fundamental importance. The introduction of any substance in the water produces changes in its quality, which are not always favorable to the development and survival of aquatic organisms. Aquacultural organisms may bioaccumulate trace metals in their tissues and consequently threaten themselves directly. Standard toxicity tests laboratory performed are used extensively to predict the effects of chemicals in aquatic ecosystems (Tsai et al., 2005). According to Oliveira-Ribeiro et al. (1996) and Machado and Fanta

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(2003), toxicity studies utilizing fish under controlled conditions provide, through the evaluation of mortality, behavioral, reproductive success, hematological changes and damage to tissues, important information corresponding to the effects of pollutants on the biota of a natural aquatic ecosystem.

The study of different biochemical and cellular constituents in blood is of fundamental importance in the physiological and physiopathological evaluation of animals, because morphological and quantitative variations in blood parameters can be induced by pollutants and other environmental factors (Juneja and Mahajan, 1983; Ranzani-Paiva et al., 1997). According to Nussey et al. (1995), the study of the hematological picture is frequently utilized for the detection of physiopathological changes in different stress conditions such as exposure to heavy metals. Changes in the hematological profile of the fish exposed to mercury have been observed in *Hoplias malabaricus* (Oliveira-Ribeiro et al., 2006), *Tilapia mossambica* (Menezes and Quasim, 1984), *Oreochromis aureus* (Allen, 1994), *Acipenser baeri* (Mikryakov and Lapirova, 1996), *Ctenopharyngodon idella* (Shakoori et al., 1994), *Pleuronectes platessa* (Fletcher and White, 1986), *Channa punctatus* (Juneja & Mahajan, 1983; Sastry and Sharma, 1980), *Aphanius dispar* (Hilmy et al., 1980).

Tilapia (*Oreochromis* sp.) is one of the most important freshwater finfish in aquaculture world. Among the numerous regions now inhabited by tilapia, many are under threat from metal pollutants including mercury. There are many works using this species in bioassay tests in order to learn the effects of chemicals in aquatic ecosystems. Effects of some heavy metals in tilapia have been reported (Shaw and Handy, 2006; Tsai et al., 1995; Nussey et al., 1995; Allen, 1994; Cuvin-Aralar, 1994; Dangé, 1986; Menezes and Quasim, 1984). This fish species is commonly used in experimental work for its rusticity and good adaptation to the captivity conditions (Ranzani-Paiva et al., 2004, Baccarin and Camargo, 2005, Gordon et al., 2005; Molina et al., 2005).

The aim of this study was to evaluate the hematological effects of mercury in tilapia, *Oreochromis niloticus*, exposed to different sublethal concentrations.

MATERIALS AND METHODS

The experiments were performed in the laboratory of Aquatic Toxicology of the Fisheries Institute – SP, in an acclimatized environment (10L:14D photoperiod and water temperature of 25.3 ± 1.06 C). The fish utilized were tilapia, *Oreochromis niloticus* (with mean weight of 27.13 ± 4.67 g and mean length of 12.44 ± 0.84 cm), acquired from a commercial fish farm. The acclimation period was 96 h in a tank of 2,500 L of capacity and during this period the fish were fed with commercial ration and observed to examine for possible variations that could interfere with the experiment. After this period, the fish were transferred to 250 L tanks and acclimated again for 48 h, maintaining the same conditions of bioassay (location x density). At the end of this second acclimation, the fish were transferred to aquaria capable of holding 40 L of water, equipped with an artificial aeration system and coated internally with transparent plastic bags.

The chemical product employed was mercuric chloride (99%, $HgCl_2$) in the salt form, obtained from Synth Laboratory ^{TM}. A stock solution of 10 mg/L mercury was made by dissolving the salt in double-distilled water. This solution was diluted directly into aquaria in sufficient amounts to provide the following mercury concentrations in water: 0.02, 0.002 and 0.0002mg/L, which were estimated from the $LC_{50\text{-}96h}/10$, $LC_{50\text{-}96h}/100$ and $LC_{50\text{-}96h}/1000$ (Ishikawa, 2003). The lowest concentration is the maximum concentration recommended by Conama (2005) for water to be used in the cultivation of aquatic organisms. An aquarium was maintained without the addition of Hg for the control group. This bioassay with the three different Hg concentrations and the control group was performed in triplicate. The exposure time to mercury was 14 days at a density of 16 fish per aquarium.

The fish were fed with extruded ration once each three days corresponding to approximately 1.5% of the bodyweight, followed by siphoning of the excrement. Subsequently, 1/3 of the water was replaced by a solution previously prepared of the same Hg concentration. Every 24 h, the temperature (^oC) of the water was recorded using a mercury thermometer; pH values were obtained with a pH meter and the electrical conductivity was measured (μS/cm). At the end of the experiment, the water was

tested for total hardness ($mgCaCO_3/L$), total alkalinity ($mgCaCO_3/L$) and total ammonia (mg/L).

For hematological analyses, at the beginning of the experiment, blood was collected from six individuals before the distribution of the fish into the aquaria for time zero samples. Blood was collected from six individuals per concentration test at intervals of 3, 7, 10 and 14 days of exposure. Fish were anesthetized with benzocaine and the blood samples were obtained with a heparinized syringe from the caudal vein. The blood samples were used for determinations of the red blood cell (RBC) after dilution with Natt and Herrick (1952) solution and counted in a Neubauer chamber; hematocrit (Ht) by the microhematocrit technique; hemoglobin level (Hb) by the cyanomethemoglobin method; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC); total thrombocyte and leukocytes counts (Hrubec and Smith, 2000), and differential leukocyte count in blood smears stained with May Grünwald-Giemsa, according to Rosenfeld (1947).

The water utilized in these experiments, with the exception of controls, was stored and treated before being discharged into the urban sewage system. This was done by adding sodium hydroxide until the solution reached a pH between 9 and 10. Subsequently, a solution of sodium sulphate was added, which resulted in the crystallization of Hg and the consequent sedimentation of the chemical product. The water was discharged and the sediment was recovered and sent to the Center for Nuclear Energy in Agriculture – CENA, in Piracicaba, SP – Brazil, for final recycling. The data obtained from the hematological tests were submitted to analysis of variance to determine significant differences between the treatments and the control group for all of the exposure times.

RESULTS AND DISCUSSION

The physical and chemical parameters of the water utilized in the chronic toxicity test in tilapia (Table 1) did not show any changes that would interfere with the results among the treatments. Except for the high level of total ammonia, as determined according to Noga (1995), the quantity of unionized ammonia (UIA) were calculated as a function of pH, temperature and total ammonia concentration. The UIA showed a mean of 0.08mg/L. Hargreaves and Kucuk (2001) noted that concentrations of UIA below 0.28mg/L had no significant effects on the growth rate, feed conversion and survival on the tilapia *O. aureus*. Xu et al. (2005) reported no behavior alteration in *O. niloticus* exposed to 0.13 mg/L of unionized ammonia. However, decreases in circulating erythrocytes in *O. niloticus* exposed to ammonia were reported by Ahamed et al. (1992). Hence, the observed decrease of RBC and Ht in the present study could be affected by the high level of ammonia.

In the present study the fish were deprived of food for two days before the blood collection and after feeding a commercial diet at 1.5% bodyweight.

The results obtained for the erythrocyte parameters of *O. niloticus* exposed to different concentrations of Hg did not differ significantly in relation to the concentrations tested over 14 days (Figure 1). However, when comparing separate exposure times, some treatments showed significant differences in relation to control. The reduction in mean of Ht values is due to a decrease in circulating RBC number, as reflected in the MCV values, occurring primarily at the highest concentration examined (0.02 mg/L). The mean values for total Hb varied between 4.0 and 6.2 g/dL, with a greater difference at a concentration of 0.0002 mg/L Hg, compared to control. In determining Hb content (MHC) and concentration (MCHC) in erythrocytes, a slight rise in mean values was observed during the course of the experiment, suggesting a small increase in erythrocytes volume and of the hemoglobin content inside.

The variations of the mean values for the red blood cell parameters are shown in Fig. 1, which demonstrated a decrease in RBC count and Ht for all groups exposed to different concentrations, including the control group. Shakoori et al. (1994), in studying grass carp, *Ctenopharyngodon idella* exposed to $HgCl_2$ (0.005mg/L), observed a decrease in RBC count, Ht and Hb level during the first two weeks of the experiment and a later increase in MCV after four weeks of exposure. Similar results were described for carp, *Cyprinus carpio* exposed to $HgCl_2$ (0.30 mg/L) for 90 h (Beena and Viswaranjan, 1987) and *Aphanius díspar* exposed to the same product (1.0 mg/L) for 96 h and 30 days (Hilmy et al., 1980). *Pleuronectes platessa* exposed to toxicity tests with $HgCl_2$ (0.3 mg/L) showed same hematological changes, following splenomegaly.

This organ is important to the phagocytic organ in the plaice, with avidity for erythrocytes (Fletcher and White, 1986). O'Connor and Fromm (1975) reported a decrease in RBCs number and Ht, due to hemolysis as a consequence of Hg toxicity. On the other hand, some authors (Benfey and Biron, 2000; Affonso et al., 2002) suggested that inexperiment of toxicity a lowered Ht level could be related to the conditions of confinement or stress induced by the lack of food.

Different and divergent results could occur due to various factors, such as differences in body size, species, concentration of the test substance and the time of exposure (Fletcher and White, 1986). In *Channa punctatus* (Juneja and Mahajan, 1983) and *O. aureus* (Allen, 1994) exposed to sub-lethal concentration (0.034 – 0.136 and 0.5 mg/L, respectively) of $HgCl_2$ in water, was reported increase of RBC count and Ht. O'Connor and Fromm (1975) observed at the beginning of their experiments a fall in Ht and at the end of 12 weeks the opposite. There was mention of the possibility that erythropoietic tissues could have been stimulated in response to the decline in RBCs caused by hemolysis as a consequence of exposure to Hg. In addition, Oliveira-Ribeiro et al. (2000) described that fish exposed to inorganic Hg dissolved in the water causes hypoxia, as a result of cellular hyperplasia in the secondary lamellae of the gills, diminishing the surface area for gas exchange. In response to respiratory difficulty, the organism stimulates an increase in RBCs, Hb and MCHC as mechanism to enhance oxygen transfer (Affonso et al., 2002).

The total number of thrombocytes showed a tendency towards an increase at the end of 14 days of exposure to the metal. However, there were no significant differences between mercury-exposed animals and control that would demonstrate an effect by the concentrations tested (Fig. 2).

In the present study, no statiscal significance was found in leukocyte counts for all treatments. However, in all groups, the decrease observed in the total leukocytes number including the control group, was result of lymphopenia and neutropenia occurring in the first three days of exposure. Subsequently, stabilization was observed up to the end of the experiment at 14 days (Fig. 3). Similar finding was reported for *Channa punctatus* exposed to 0.25 mg/L of $HgCl_2$ (Misra and Behera, 1992). Gill and Pant (1985) attributed the leucopenia to the stress caused by the bioassay type, which had also been demonstrated in several other vertebrates. *Oncorhynchus mykis*, and *Salvelinus fontinalis*, submitted to the stress of confinement and manipulation showed a reduction in number of WBC and a slight increase in thrombocytes total number (Benfey and Biron, 2000). Then, the stress leads to a redistribution of lymphocytes, mainly to lymphoid organs (thymus and anterior kidney), diminishing in the blood circulation, or to the destruction of lymphocytes in response to high levels of cortisol.

The monocytes number showed a variation at the beginning and to increase at the end of the experiment. No significant difference was noted between the treatments and the control group. Basophils and eosinophils were found in only a few fish, disable any interpretation of the effects of Hg in these cells.

Based on the hemogram results obtained in the present study, it was seen that mercury had no significant hematological effects.

Dawson (1990), testing $HgCl_2$ on *Scophthalmus aquousus* at concentrations (0.005 and 0.01mg/L) similar to those utilized in the present study, obtained the same results. It was, therefore, possible that these two species were resistant to the toxic effects of Hg at these concentrations and more vulnerable to experimental stress.

Table 1 - Physical and chemical parameters of water in the bioassay with *O.niloticus* exposed to $HgCl_2$

Trial (mg/L)	pH	Conductivity (µS/cm)	Water Temperature (^oC)	Hardness ($mgCaCO_3/L$)	Alkalinity (mg $CaCO_3$/ L)	Total Ammonia (mg/L)
Control	6.77 ± 0.25 a	126.73 ± 39.19 b	25.43 ± 1.10 a	29.96 ± 2.92 a	62.35 ± 7.44 a	14.90 ± 0.71 a
0.02	7.03 ± 1.02 a	112.88 ± 31.29 c	25.28 ± 1.12 a	28.70 ± 0.00 a	50.16 ± 3.28 a	11.30 ± 1.09 b
0.002	7.03 ± 0.72 a	133.28 ± 40.38 ab	25.34 ± 1.01a	26.79 ± 1.91 a	63.06 ± 4.96 a	15.21 ± 1.82 a
0.0002	7.22 ± 0.39 a	140.56 ± 47.73 a	25.48 ± 1.02a	28.07 ± 1.10 a	56.61 ± 6.91 a	15.81 ± 0.55 a
CV (%)	3.97	3.91	0.43	6.43	10.14	8.08

CV= Coefficient of variation

Means, in same column, followed by the same letter are not significantly different ($p<0.05$).

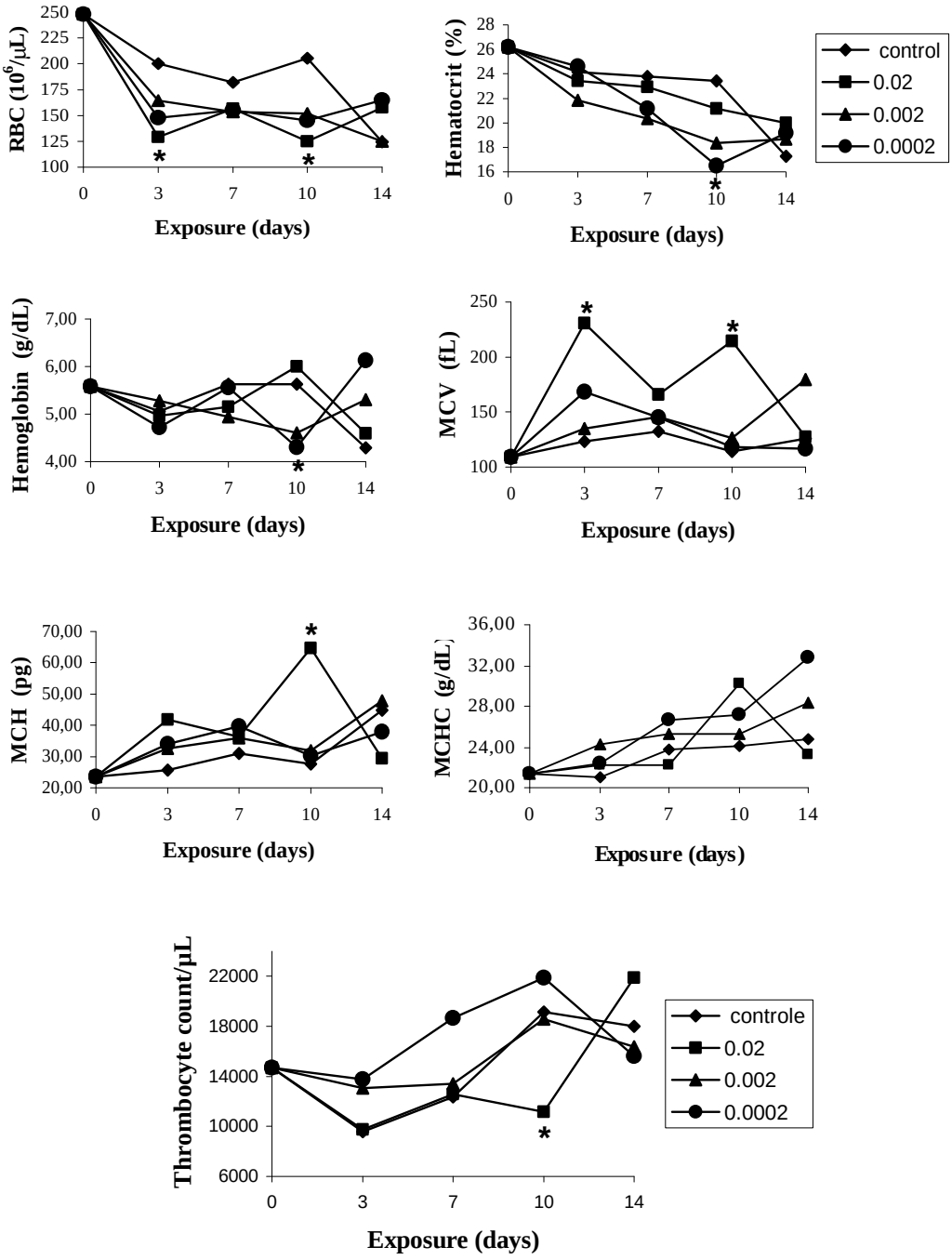

Figure 1 – Variation of mean values for erythrocyte parameters in *O. niloticus,* exposed to different concentrations of $HgCl_2$, at different sampling times
(*) Significant different in relation to control group ($p < 0.05$)

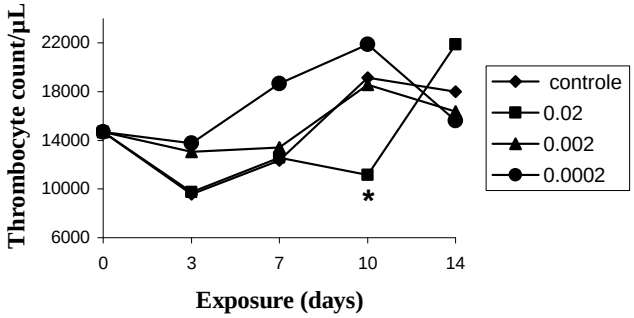

Figure 2 - Mean values of the total number of thrombocytes of *O. niloticus*, exposed to differents concentrations of $HgCl_2$, at different sampling times.
(*) Significant different in relation to control group ($p < 0.05$)

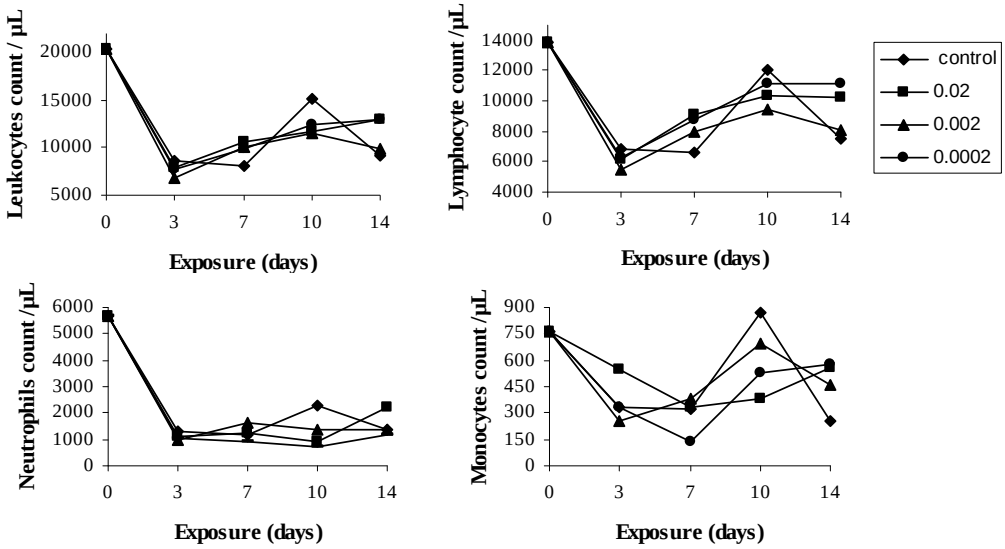

Figure 3 - Variation of mean values for the total number of leukocytes, lymphocytes, neutrophils and monocytes in peripheral blood of *O. niloticus*, exposed to differents concentrations of $HgCl_2$, at different sampling times

CONCLUSIONS

The Hg ($HgCl_2$) concentrations tested were insufficient to cause hematological changes in *O. niloticus*. Therefore, further studies of Hg toxicity would be need to be carried out at higher concentrations or using long exposure times.

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RESUMO

A toxicidade do mercúrio foi avaliada em tilápia, *Oreochromis niloticus* (Linnaues, 1758) através da

análise dos parâmetros hematológicos após exposição a diferentes concentrações sub-letais, durante um período de 14 dias. O bioensaio foi conduzido no laboratório de toxicologia do Instituto de Pesca, SP. Foram utilizados alevinos (12.44 ± 0.84 cm, e 27.13 ± 4.67 g) e aquários com capacidade para 50 litros e preenchidos com água declorada e mais a quantidade de solução de mercúrio ($HgCl_2$) correspondendo as seguintes concentrações: 0,02; 0,002; 0.0002 $mg.L^{-1}$ Hg. Foram utilizadas 3 repetições de cada concentração e grupo controle. Amostras de sangue foram coletadas de seis animais de cada concentração nos tempos 0, 3, 7, 10 e 14 dias de exposição. Foram avaliados: a contagem de eritrócitos (RBC), concentração de hemoglobina (Hb), hematócrito (Ht), volume corpuscular médio (VCM), hemoglobina corpuscular média (HCM) e concentração de hemoglobina corpuscular média (CHCM), trombócitos totais (Tr), contagem diferencial e total de leucócitos (Lc). Os resultados demonstram que as concentrações de Hg testadas, não alteraram significativamente os parâmetros hematológicos, permitindo concluir que a quantidade de Hg na água não foram suficientes para afetar o quadro hematológico de *Oreochromis niloticus*.

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