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Biochemical and cellularchanges in *Oreochromis niloticus* related to the water pollution of a degraded river

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ABSTRACT. The effects of polluted water at three sites in the Marinho River, Brazil, on *Oreochromis niloticus* (Nile tilápia) were investigated using histological, hematological and biochemical approaches. Fish exposed to the impacted water demonstrated that histological changes in gills were accompanied by nuclear and micronuclei abnormalities in cells. The activity of liver and plasma biomarkers (alkaline phosphatase (ALP), acid phosphatase (ACP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and liver glutathione S-transferase (GST)) showed an expressive change due to the. The results were also correlated with the highest levels of Cu^{+2}, Zn^{+2} and Mn^{+2} in the water. The data of this study evidenced the importance of using a set of biomarkers to quantify pollution in lentic ecosystems. Additionally, histological analyses of gills and erythrocytes have proven to be an important instrument for signaling the impact of pollutants in rivers.

Keywords: aminotransferases, phosphatases, glutathione-*S*-transferase, histology, cell abnormality, Tilapia.

Alterações bioquímicas e celulares em *Oreochromis niloticus* relacionadas com a poluição da água de um rio degradado

RESUMO. Os efeitos da poluição da água de três locais do Rio Marinho, Brasil, *em Oreochromis niloticus* foram investigados usando técnicas histológicas, hematológicas e bioquímicas. Peixes expostos à água impactada demonstraram que alterações histológicas nas brânquias foram acompanhadas de anomalias nucleares e micronúcleo nas células. A determinação da atividade de biomarcadores em fígado e plasma de tilápia (fosfatase alcalina (ALP), fosfatase ácida (ACP), alanina aminotransferase (ALT), aspartato aminotransferase (AST) e glutationa S-transferase (GST)) mostrou uma substancial alteração em função da poluição. Os resultados são correlacionados com os níveis mais elevados de Cu^{+2}, Zn^{+2} e Mn^{+2} na água. Os dados deste estudo demonstram a importância de utilizar um conjunto de biomarcadores para quantificar a poluição em ecossistemas lênticos. Adicionalmente, as análises histológicas das brânquias e de eritrocitos têm provado ser importante instrumento para sinalizar a impactação de poluentes ao longo de rios.

Palavras-chave: aminotransferases, fosfatases, glutationa-*S*-transferase, histologia, anormalidades celulares, Tilápia.

Introduction

Water pollution resulting from industrial, agricultural and anthropogenic activities has become a major problem affecting ecosystems. The pollutants include organic and inorganic xenobiotics that can accumulate in animal tissues (MOREIRA et al., 2004; CHANDON et al., 2005) and affect several physiological functions (BORKOVIC et al., 2008; KROUPOVA et al., 2008; FRIAS-ESPERICUETA et al., 2008).

The biological responses of aquatic organisms to pollutants frequently involve metabolic, hematological and histological alterations (MOREIRA et al., 2006). Fish have been used as bioindicators in toxicological studies and may act as 'sentinel' organisms for detecting genotoxic chemicals in the environment (STEGEMAN; LECH, 1991; AL-SABTI; METCALFE, 1995). In this context, *Oreochromis niloticus* (tilápia) is a species commonly used as a fish model to estimate the toxicological level in a given polluted environment by analyzing its enzymatic activities (COSTA-PIERCE; RAKOCY, 1997). Viarengo et al. (2007) suggested the integrated use of different biomarkers to facilitate the detection of alterations at molecular, cellular, tissue and organism levels. Using a variety of markers, one can correlate the amount of pollution and the effects on micronucleus and gills. In addition, the micronucleus analysis is an alternative to detect chromosomal damages and may be applied in different cell types

exposed to clastogenic substances (HOOFTMAN; RAAT, 1982; AL-SABTI; METCALFE, 1995).

Other disturbances induced by pollutants include an imbalance between the oxidant defense capacity and the production of reactive oxygen species (ROS), resulting in membrane lipid peroxidation, DNA oxidative damage and subsequent changes in the activity of enzymatic complexes (GERET et al., 2003; ORBEA; CAJARAVILLE, 2006). In this way, the Glutathione *S*-Transferase (GST) is an enzyme playing an important role in antioxidant defense, thereby supporting the detoxification and prevention of lipid peroxidation (HERMES-LIMA, 2004). GST also catalyzes the conjugation of glutathione with a variety of endogenous and xenobiotic substances (SUREDA et al., 2006). However, its activity varies according to different organism species and environmental contaminants (MOREIRA; GUILHERMINO, 2005).

A signaling cascade mediated by enzymatic activities can be developed in freshwater fish exposed to environmental pollutants, e.g. significant changes on phosphatases (ACP and ALP) and aminotransferases (ALP and AST) activities can be observed (ATLI; CANLI, 2007; BORKOVIC et al., 2008). The phosphatases hydrolyze a large set of phosphomonoester substrates at alkaline pH (ALP) and acidic pH (ACP) and also act as transphosphorylase (BLASCO et al., 1993). Previous studies have described an important role of ALP in the mineralization of the skeleton of aquatic animals (OLSEN et al., 1991; BLASCO et al., 1993). The aminotransferases ALT and AST are used as specific indicators of hepatotoxicity and histopathological changes. It was reported that tissue damages of liver, kidney and gills of fish were correlated with high levels of AST and ALT activities (OLUAH, 1999).

In the present work, the interaction of the histology of gills, enzymes analysis and abnormality in erythrocyte micronucleus combined were used as biomarkers to assess the impact of pollution by industrial and domestic discharges along the Marinho river (Espírito Santo State, Brazil). Nile tilápia (*Oreochromis niloticus*) was used as biological indicator of environmental contamination. The activity of phosphatases (ALP and ACP), aminotransferases (AST and ALT) from plasma and liver and Glutathione *S*-transferase from liver of *Oreochromis niloticus* was analyzed, in order to characterize the enzymatic response to different levels of pollution.

Material and methods

Study area

The hydrographic basin of the Marinho river is located at an altitude of 750 m and the rivers flows 119 km until the estuarine area in Vitória Bay in the State of Espírito Santo, Brazil. The water source region is characterized by low urban occupation, but along its course, the Marinho river banks is exposed to industrial pollution in addition to high levels of domestic drainage. A reduced aquatic life can be observed along the river mainly close to industrial discharges. Our risk classifications were based on the impact generated by industrial and human activities along the river. Subsequently, we collected samples at three exposed sites and one reference was the dechlorinated water. The site S1 was characterized by being a low risk area showing only vegetation on the banks and free of industrial pollution. It was similar to the reference (20º20'15,9"S 40º21'28,7"W); site S2, classified as a high risk area due to industrial and human impact (20º21'0,2"S 40º21' 26,4"W); and site S3 (20º23'58,9"S 40º21'53,1"W), classified as having intermediate risk.

Experimental protocol

Oreochromis niloticus Linnaeus, 1758 (Osteichthyes: Cichlidae) were obtained from commercial aquaculture, selected by weight and length (mean body weight 24.8 ± 8 g; length 11.6 ± 4 cm) and then immediately taken to the laboratory. They were acclimated in a 250 L tank of sterile water for 5 days. During the acclimation period, the ambient temperature was kept at 20 ± 1ºC with a 12:12 light/dark cycle. Fish were fed daily *ad libitum* with commercial dry pellets (30% crude protein). The dissolved oxygen levels in the water were kept at 5 mg L^{-1} by continuous aeration, and the pH was monitored to be at 7.2 ± 0.2. The physical-chemical characteristics (temperature, electrical conductivity, dissolved oxygen, salinity) of the water were analyzed *in situ* using an YSI 85 multiparameter probe. pH measurements were performed using a Li-300 pH meter (Table 1). The water of each site was collected in sterile glass vials and transported at -4ºC, and taken to CenterLab Ambiental (http://www.centerlabambiental.com.br) for analysis of metal concentrations. In the laboratory, the collected water samples were filtered through a 55 μm nylon mesh and transferred to glass aquariums (30 L) for experimental analysis. During the three independent experiments, fish were randomly transferred to four aquariums with 12 repetitions (n = 12), kept for 96h at 20 ± 1°C without feeding, and under gentle aeration. At the end of the experiment, fish were transferred to a solution of 3% menthol, and after 1 minute, body length and weight were measured, and blood and tissues were collected for hematological, histological and biochemical studies.

Table 1. Physical-chemical parameters (temperature, conductivity, dissolved O_2, salinity and pH) and levels of metals measured in the water from three impacted sites (S1 to S3) of the Marinho River. Dechlorinated water was used as reference. Mean values followed by the same capital letter, in the same column, are not significantly different by Tukey's test at $p < 0.05$.

Sites	Temperature (°C)	Conductivity (μS cm^{-1})	O_2 dissolved (mg L^{-1})	Salinity (g L^{-1})	pH	Metals (mg L^{-1})							
						Cd	Co	Cr	Cu	Fe	Mn	Pb	Zn
Reference	24.90 A	111.70 D	6.42 A	-	7.25 D	-	-	-	-	-	-	-	-
S1	25.30 A	203.30 C	0.55 B	0.20 B	8.35 C	0.0010	0.101	0.251	0.112	0.220	0.100	0.0004	0.00033
S2	28.30 A	1267.0 A	0.13D	0.90 A	8.80 A	0.0012	0.125	0.278	0.112	0.450	0.279	0.001	0.00637
S3	25.40 A	739.00 B	0.28 C	0.40 B	8.55 B	0.0006	0.073	0.126	0.112	0.560	0.186	0.0002	0.0025

Site 1: low risk, site 2: high risk, site 3: intermediate risk.

Biochemical parameters

Liver and plasma collected after exposure were used to quantify the levels of: GST- protection against xenobiotic–induced lipid peroxidation; ACP- immune defense; ALP- for metal sensitivity; ALT and AST- detection of tissue damage in the liver. Each fish liver was cut, processed, and separated for enzyme determination. GST activity was determined according to the method described by Habig et al. (1974) and adapted by Moreira et al. (2006).

ACP and ALP activity were determined according to Barred (1972), but using pNPP (Sigma-Aldrich, Gillingham, UK) as substrate, ALT and ASP were determined according to Yatzidis (1960). The protein content of the samples was determined according to Lowry et al. (1951) using γ-bovine globulin as standard.

Histological, cell abnormality, micronucleus presence and morphometric procedures

For histological preparation, the second right gill arches were excised and washed in cold saline solution (0.9% NaCl). After fixation in Bouin's solution for 24 h, the gills were decalcified, dehydrated in alcohols, cleared in xylol and embedded in paraffin. Histological sections (5 μm thick) were cut on an American optical rotative microtome and mounted on optical microscope slides. Samples were dried at 37°C for 24h and stained with haematoxylin and eosin modified by Putt (1948). In the erythrocyte analysis, a volume (0.5 mL) of whole blood was collected from the caudal vein with a syringe containing heparin. A sample drop was, immediately, used in the preparation of thin plate using the smear technique. After 24h, samples were fixed in absolute methanol, for 10 minutes, and stained with 5% Giemsa for 20 min. Morphometric analysis was applied to plan metric integration of interlamellar tissue in gills, were counted the superposes camps using a reticle composed of hexagonal areas (S = 3L.r) with 1.82 mm^2 each (1820 μm^2) calculated to optical complex of 400 x. In this method, each integration of area of interlamellar tissue were individualized the chlorite, the mucocyte and superficial planar cells (CASTRO et al., 1994).

Statistical analysis

All data were analyzed by one-way ANOVA to compare the mean values (considering "reference water", site S1, S2 and S3 as main factors), which was validated by residual analysis, and whenever necessary combined with Tukeys' test for multiple comparison. The differences plan metric of areas between reference and site S2 were done and values expressed in % were analyzed by two-way ANOVA (considering chloride, mucocytes, flat and other cells as factors). The confidence intervals were calculated for the mean difference, in order to ensure an overall 95% confidence level. The results were expressed as mean values and the numbers of repetitions were given in each figure legend. All statistical analyses were conducted in R program and the level of significance was set at $p < 0.05$ (SNEDECOR, 1956; IHAKA; GENTLEMAN, 1996).

Results

Histological, cell abnormality and micronucleus presence analysis

The histological alterations in the gills subjected to bioassays with Marinho river water showed substantial differences between different regions. The most severe changes were found when exposed to water of S2, followed by S3 and S1 (Figure 1). In the reference assay, the gill tissue of *O. niloticus* followed the standard described for teleost fish with equally spaced secondary lamellae, with little or no distortion/swelling observed on the filamental epithelium of primary gill lamellae (Figure 1). The histological changes were observed in gills of all individuals subjected to the water of the three sites and to reference water during 96 h. The common morphological changes were hypertrophy of epithelial cells and degeneration of respiratory secondary lamellae with loss of support and reduction of interlamellar space (Figure 1). The morphometric analysis of interlamellar area in gills tissue presented numeric alterations in chloride, mucocytes, flat and other cellular components when fish were exposed to water of the site S2 (Figure 2). Fish subjected to sites S1 and S3 revealed only mild injuries in the secondary lamellae with loss of support and reduction of the interlamellar space (data not shown).

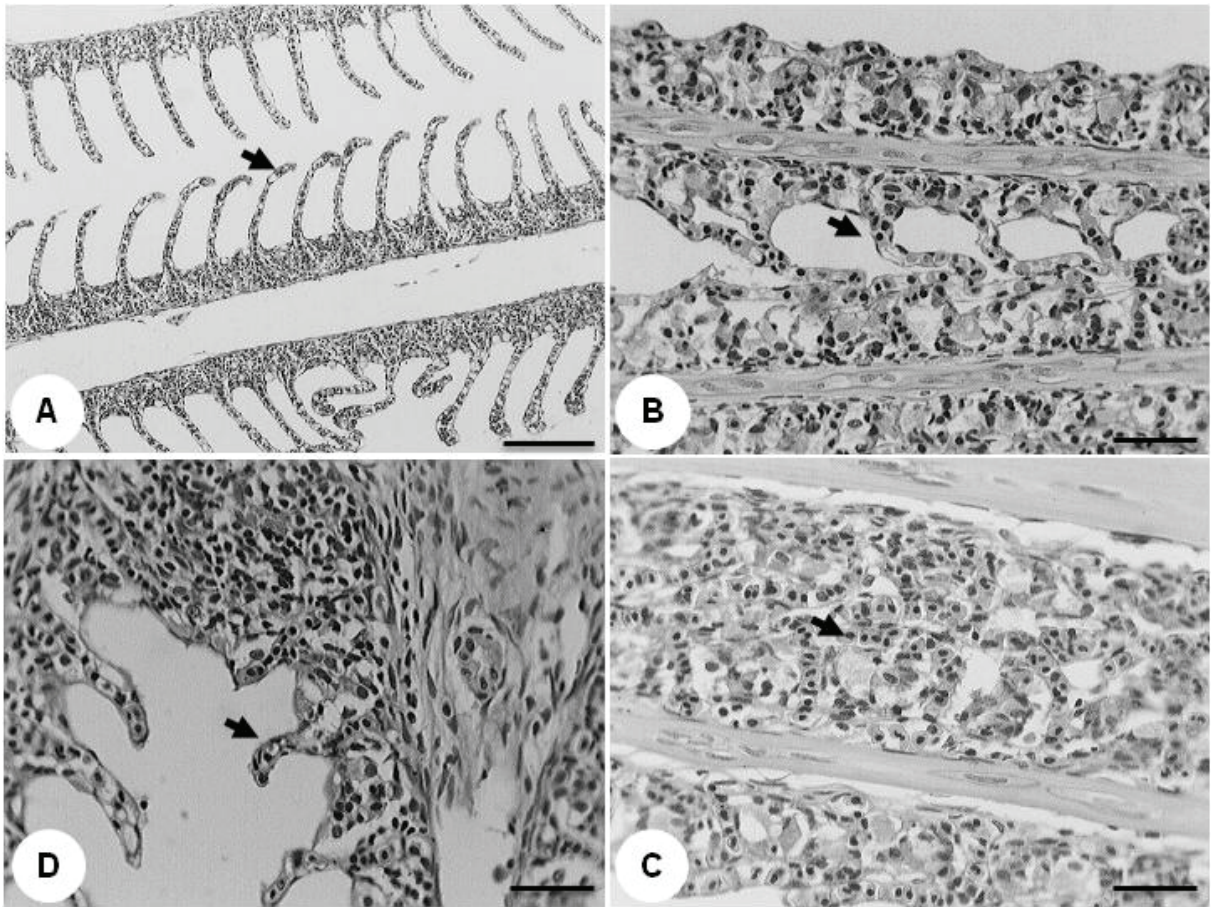

Figure 1. Photomicrographs illustrating four stages of the continued apoptosis in gill filaments (arrows). A, histological section of a gill from a fish in normal conditions (dechlorinated water – reference). Scale bar = 50 μm. B, C and D represent the gill of fish exposed to the water of the sites S1, S2 and S3 at the Marinho River, respectively. In these cases the experimental process of apoptosis is carried to term with juxtaposition of the filament, preventing thus the water flow inside the inner filamentous zone. Scale bar = 50 μm.

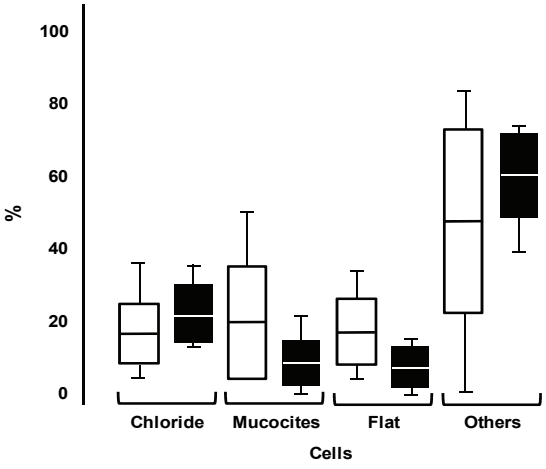

Figure 2. Morphometric analysis of the interlamellar area in gill tissue presenting numerical changes in chloride, mucocytes, flat and other cellular components when exposed to the water of the site S2.

Statistical analysis evidenced a significant stimulation in the frequency of nuclear abnormality in cells and cell micronucleus at the site S2 compared with the reference (Table 2, $p < 0.001$).

Table 2. Nuclear and micronuclei abnormality observed in erythrocytes of *Oreochromis niloticus* exposed to the water from three different sites of Marinho river. Mean values followed by the same capital letter, in the same column, are not significantly different by Tukey's test at $p < 0.05$.

Treatment	Samples	Normal Cells	Nuclear abnormality	Micronucleus abnormality
Control	Frequency (%) (n=1974)	99.70 B	0.26 C	0.03 C
Site 1	Frequency (%) (n=1985)	99.26 B	0.68 B	0.06 C
Site 2	Frequency (%) (n=1976)	98.85 A	0.96 A	0.19 A
Site 3	Frequency (%) (n=1986)	99.30 B	0.58 B	0.12 B

Site 1: low risk, site 2: high risk, site 3: intermediate risk.

In terms of nuclear abnormality frequencies the sites S1 and S3 have also induced significant changes (Table 2, $p < 0.045$), but less than observed at the site S2. However, the frequency of micronuclei alterations were higher in fish exposed to water from S3 compared to those exposed to S1 (Table 2, $p < 0.01$). These results have also supported our experimental approach

and the exposure time to the polluted water was enough to observe hematological and histological changes in the bioindicator organism.

Liver Glutathione-S-transferase activity

The GST enzymatic activities were significantly superior in fish exposed to S2 and S3. No differences were found between reference water and S1 (Figure 3; $p < 0.076$). The GST activity was increased in S1 (17.83%), S2 (93.19%) and S3 (33.30%). However, the highest stimulation on GST activity was detected at the site S2 ($p < 0.015$), the most impacted site. These data clearly showed that the GST activity underwent a differential regulation when incubated with polluted water from the Marinho river. In our experimental conditions, a possible relationship between the GST activity and the pollution level can be speculated.

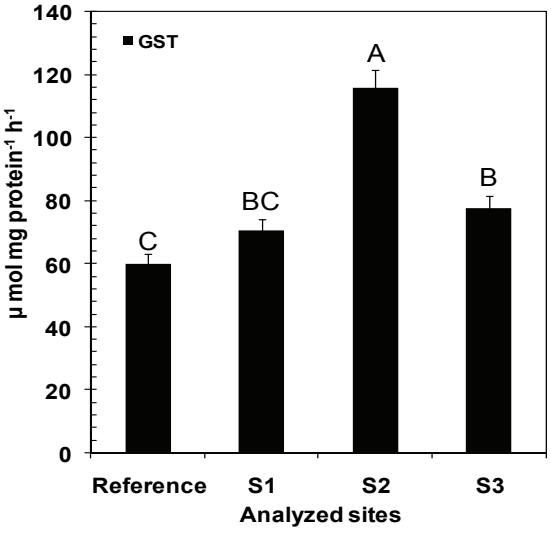

Figure 3. Glutathione S-transferase (GST) activity in the liver of *Oreochromis nilotticus* analyzed in the sites S1, S2, S3. The reference was dechlorinated water. Bars followed by the same letter are not significantly different by Tukey's test at $p < 0.05$. Values represent the mean value of 12 individuals and corresponding standard deviation bars (n = 12).

Liver transaminase and phosphatase activities

Results obtained with liver transaminase activities showed that fish exposed by 96h to the S2 water had a significant reduction in the AST (26.9%) and ALT (43.8%) activities (Figure 4A and B). For both, AST and ALT activities, no significant differences were observed between the site S1 and S3 ($p < 0.062$), although a significant reduction in these two sites (S1 and S3) was observed in comparison with the reference water (Figure 4A and B).

Substantial inhibition was detected in the activity of ACP (68.4 ($p < 0.023$)) and ALP (44.4% ($p < 0.036$)) in fish exposed to water from the site S2 (Figure 4C, D). For ACP results, no difference was verified when comparing the sites S1 and S3 (Figure 3C), AST and ALT data also had no differences (Figure 4C; $p < 0.068$). Nevertheless, ALP activity showed a behavior similar to ACP, except for site S1, which had no significant difference compared with the reference (Figure 4D, $p < 0.053$).

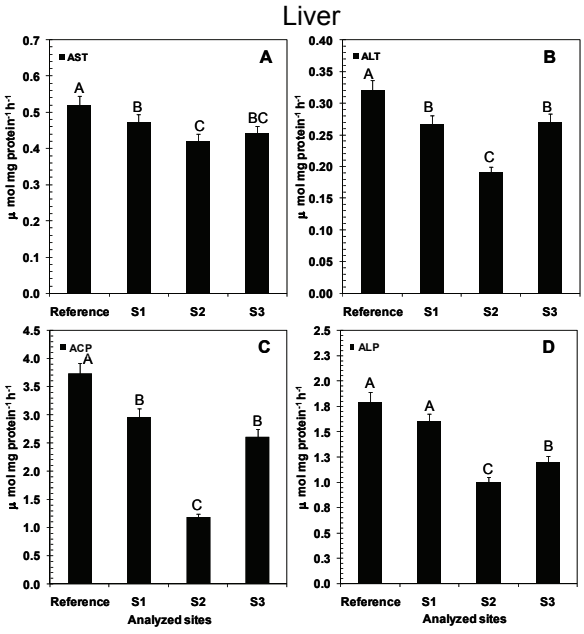

Figure 4. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and acid phosphatase (AcP) in the liver of *Oreochromis niloticus* analyzed in the sites S1, S2, S3. The reference was dechlorinated water. Bars followed by the same capital letter are not significantly different by Tukey's test at $p < 0.05$ (n = 12).

Plasma transaminase and phosphatase activities

Plasma transaminases (AST and ALT) and phosphatases (ACP and ALP) showed an opposite behavior in fish exposed to the S2 water. In plasma, the activities of these enzymes were extremely high in fish exposed to water from S2 (Figure 5). Plasma ALT activity of S2 fish were increased by 125% ($p < 0.001$), while no difference was detected between site S1 and reference (Figure 5B; $p < 0.057$). For the activity of plasma AST, the results indicated a 60% increase in the site S2 ($p < 0.01$) and the other sites had behavior similar to described to ALT activity, where no statistical difference was found between site S1 and reference (Figure 5A and B; $p < 0.051$).

The activities of plasma ACP and ALP were significantly stimulated exclusively after the exposure to the water from the site 2 ($p < 0.01$) and no statistical difference was detected between the sites S1, S3 and reference (Figure 5C and D; $p < 0.069$).

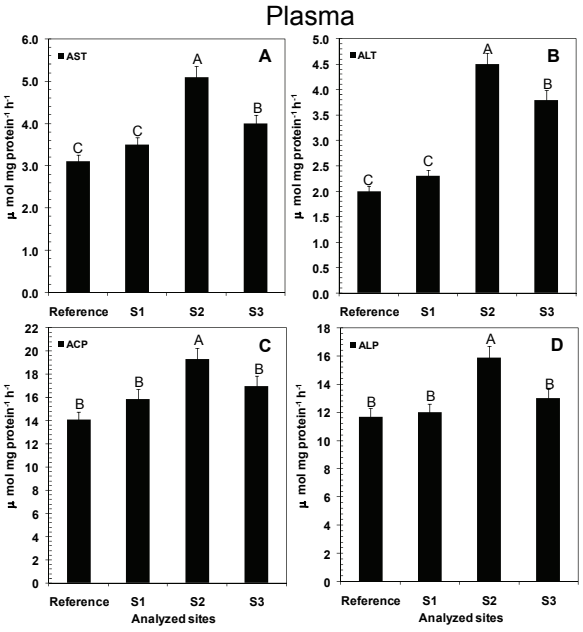

Figure 5. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Acid phosphatase (AcP) in the plasma of *Oreochromis niloticus* analyzed in the sites S1, S2, S3. The reference was dechlorinated water. Bars followed by the same capital letter are not significantly different by Tukey's test at $p < 0.05$ (n = 12).

Discussion

Domestic sewage, metallurgical activities and industrial effluents are the major sources of pollution in brazilian rivers. To the best of our knowledge, this is the first study evaluating physico-chemical parameters, metal pollution and biomarker activity in the Marinho river, Espírito Santo state, Brazil. The present work is the first study focused on water pollution in Marinho river, using a set of biomarkers in Nile tilápia.

Our work showed originally that from ecotoxicological, hematological and histological point-of-view, the polluted areas in the Marinho river have induced significant effects on gills, liver and blood of Nile tilápia. Indeed, at the site S2, considered the most impacted site, higher values of temperature, electrical conductivity, salinity, pH and lower values of O_2 dissolved can be observed (Table 1). In fact, the water quality has proven to be correlated with these parameters (MOREIRA; GUILHERMINO, 2005).

Nile tilápia is one of the aquatic organisms affected by heavy metals, and was used as a metal biological marker in several toxicological studies, because it shows the highest sensitivity to toxic effect (RASHED, 2001). In this study, we found values of the metals Cu^{+2}, Zn^{+2} and Mn^{+2} significantly above the standard set by the Brazilian National Council of the Environment (CONAMA), mainly at the sites S2 and S3 (See Table 1). Probably our results are due to the values of these metals.

The frequencies of histological changes in the gills subjected to bioassays with Marinho river water were also high at the site S2, and lower at the sites S1, S3 and reference water (Figure 1).

In fact, histological changes of gills are well-established as a rapid and valid method for determining damages resulted from the exposure of the test organism to different pollutants (ARELLANO et al., 1999; VAN DEN HEUVEL et al., 2000).

Moreover, the morphometric analysis of interlamellar area in gills tissue has shown numerical changes in chloride, mucocytes, flat and other cellular components at the site S2 (Figure 2). However, fish subjected to the water of sites S1 and S3 revealed only mild injuries in respiratory secondary lamellae with loss of support and reduction of interlamellar space (data not shown).

The abnormalities observed for micronuclei in erythrocytes combined with histological data evidenced a stimulation in the frequency of nuclear and micronucleus abnormality in *Oreochromis niloticus* blood treated with polluted water (S1 to S3) (Table 2). It is in accordance with Tavares-Dias et al. (2002) who demonstrated that variations of erythrocytes of fish are standards to understand the cellular, physiological and ecological responses. On the other hand, the frequency of erythrocytes with nuclear and micronuclei abnormality is an important indicator for genetic alterations in organisms exposed to environmental stressors (GRISOLIA; CORDEIRO, 2003; VIARENGO et al., 2007) in vertebrate and invertebrate animals (AL-SABTI; METCALFE, 1995; BARSIENE et al., 2006). Our data are also supported by Arellano et al. (2000) who reported significant histological changes in gills and liver of *Solea senegalensis* treated with cupper.

Various researchers have found that enzymes in gills, liver and plasma undergo a significant regulation in the presence of organic or inorganic pollutants (ATLI; CANLI, 2007; JING et al., 2006). Among these enzymes, GST has been studied in animals and used as biomarker under laboratory and field conditions (Moreira et al., 2004, 2006). The GST is an important enzymatic family of the phase II of the detoxification process and plays a key role in the biotransformation and excretion of a variety alkylating agents, which are detoxified via GSH, a reduced form (CLARK, 1989; SUREDA et al., 2006). In this study, fish exposed to water from Marinho river, showed a GST activation. This result strongly indicates a response to the contamination of river water. Considering the three sites, fish exposed to water from the site S2 exhibited a higher activity of the liver GST compared to reference fish (Figure 3), pointing an enhancement of the detoxification process in fish cells. These results are in agreement with Rao (2006), who exposed *O. mossambicus* to

organophosphorus insecticide and found GST stimulations about 79%, much lower than found in this study (93%; Figure 3). The lower effects observed in Nile tilápia exposed to water from site S1 reflected the reduced urbanization near this site, while at the site S3, probably, this effect was a result of water dilution by seawater in the high tide (Figure 3). Canesi et al. (1999) also showed a regulation of glutathione metabolism in mussels exposed to heavy metals.

Indeed, aminotransferases, namely ALT and AST, are specifically used as indicators of hepatotoxicity and histopathological changes. Also, previous studies reported that tissue damages in liver, kidney and gills of fish were correlated with high levels of AST and ALT activities (OLUAH, 1999). The stimulation on ALT and AST activities was registered only at the sites S2 and S3, similarly to Oluah (1999), when *Clarias albopunctatus* was exposed to sublethal doses of zinc.

In the liver, we also analyzed the alkaline phosphatase (ALP) and acid phosphatase (ACP). ALP is a non-specific enzyme in the liver that plays an important role in the dephosphorylation of organic compounds. In higher animals, this enzyme is involved in bone formation and in membrane transport (MOLINA et al., 2005). Furthermore, when Nile tilápia was exposed to heavy metals the liver ALP was modulated by a donation of 5 μM of Cu and Zn (ATLI; CANLI 2007). The high concentrations of Cu^{+2}, Zn^{+2} and Mn^{+2} in Marinho river water may have caused these differential regulation in biomarkers. As shown in this study, the different levels of pollution can be sensitively predicted by quantifying enzymatic activities in plasma and liver of exposed fish. Our results are consistent with this affirmative since plasma ALP activity, after exposure to water from site S2 underwent a significant stimulation, compared with the reference water, sites S1 and S3 (Figure 4 and 5). However, it is important to observe that the differences in plasma ALP in response to metals or an organic xenobiotics are indicative of the possible use of ALP as biomarkers for river ecotoxicology as already established in the literature (JIRAUNGKOORSKUL et al. 2003; LI et al., 2004). These enzymatic changes are in agreement with the results of Atli and Canli (2007) and Canesi et al. (1999).

Another phosphatase enzyme regulated by environmental pollution is the acid phosphatase (RAJALAKSHMI; MOHANDAS, 2005; JING et al., 2006). Nevertheless, the comparison of ALP, ACP, ALT and AST in plasma had similar responses, where the activities had strong stimulations mainly at the site S2 and S3 (Figure 5).

The present study pointed out that enzymatic responses in fish exposed to environmental contamination can be used as both indicators of tissue damage and biomarkers of pollution. Further studies are necessary in order to provide new information about the level of pollution of Marinho river and for the brazilian environmental policies for river protection.

Conclusion

The enzymatic changes observed in O. *niloticus* exposed to water of different regions of the Marinho river suggested the presence of different contaminants from human activities. These alterations were related to the physiochemical characteristics of the water. Significant differences in enzymatic activities after exposure to water from the site S2 have revealed the effect of pollutants in the intermediary metabolism of tilápia. The use of enzymes as biomarkers in ecotoxicology with tilápia combined with histological analysis including the abnormality of erythrocytes data was important to determine the commitment level as for the pollution of Marinho river that induces physiological changes in fish organisms analyzed.

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