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# Bioaccumulation and oxidative stress parameters in silver catfish (*Rhamdia quelen*) exposed to different thorium concentrations

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ABSTRACT

The objective of this study was to evaluate the effect of chronic thorium (Th) exposure on bioaccumulation, metabolism (through biochemical parameters of the muscle) and oxidative parameters (lipidic peroxidation levels and antioxidant enzymes in the gills and in the hepatic and muscular tissues) of silver catfish (*Rhamdia quelen*). Silver catfish juveniles were exposed to different waterborne Th levels (in $\mu g\ L^{-1}$): 0 (control), 25.3 ± 3.2, 80.6 ± 12.0, 242.4 ± 35.6, and 747.2 ± 59.1 for 30 d. The gills and skin were the organs that accumulated the highest Th levels. The increase in the waterborne Th concentration corresponded to a progressive increase in the Th levels in the gills and kidney. Chronic Th exposure causes alterations in the oxidative parameters of silver catfish gills, which are correlated with the Th accumulation in this organ. The levels of GST decreased in the gills of fish exposed to 747.2 $\mu g\ L^{-1}$ Th and SOD activity decreased in silver catfish exposed to 242.4 and 747.2 $\mu g\ L^{-1}$ Th. In addition, the increase in the LPO in the gills exposed to 242.4 and 747.2 $\mu g\ L^{-1}$ Th suggests that higher oxidative damage occurred in the gills. However, in the liver and muscle, these alterations occurred mainly in the lowest waterborne Th level. Metabolic intermediates in the muscle were altered by Th exposure, but no clear relationship was found.

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## 1. Introduction

The trace elements are an important group of environmental toxic compounds capable of causing physiological damage to organisms (Flowler, 1975). Contamination by trace elements has been studied in different organisms, and several investigations have been performed with fish (Poston, 1982; Paine et al., 2000; Lemos et al., 2001; Barillet et al., 2005; Buet et al., 2005; Ahmad et al., 2006). Thorium (Th) has potential use as a nuclear fuel and is a natural trace element present in the aquatic ecosystem. Brazilian surface and groundwater present very low Th levels (0.009–14 $\mu g\ L^{-1}$) (Lauria and Godoy, 2002; Casartelli and Miekeley, 2003; Godoy and Godoy, 2006), but water contaminated by uranium and iron ore mines drainage in Southeast Brazil may contain Th levels up to 800–1400 $\mu g\ L^{-1}$ (Veado et al., 2006; Ladeira and Goncalves, 2007). Thorium can occurs predominantly as a tetravalent cation, being a trace constituent in phosphates (simple and multiple oxides) and silicates (Gascoyne, 1982). For example, phosphogypsum, a waste by-product derived from the wet process production of phosphoric acid by Brazilian producers, contains high levels of Th, but is bound to insoluble compounds such as sulphates, phosphates and silicates and therefore do not represent a threat to the surrounding aquatic environment (Santos et al., 2006). Since Th is present in minerals of low solubility, Th generally has been considered insoluble in the water. In freshwater environments, Th isotopes are relatively unavailable for biological uptake, because they adsorb strongly to inorganic sediment in lakes (Cowart and Burnett, 1994; Veado et al., 2006). Most environmental transport of Th is through physical processes where Th adheres to particulate material (inorganic and organic) suspended in water (Langmuir and Herman, 1980).

Cellular biomarkers are important tools for evaluating an organism's exposure to toxic agents that may cause death or alterations in the structure and function of vital organs (Au et al., 1999). Studies showed that fish exposed to xenobiotics experience changes in the oxidative balance of different tissues (Radi and Matkovics, 1988; Di Giulio et al., 1989; Mather-Mihaich and Di Giulio, 1991; Ahmad et al., 2000). Exposure to elevated metal concentrations (higher than the maximum levels allowed by governmental agencies) may stimulate the development of reactive oxygen species (ROS), like superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$), and

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hydroxyl radical (HO·), which may damage DNA, lipids and proteins (Halliwell and Gutteridge, 1999). Oxidative stress is defined as a disruption of the prooxidant–antioxidant balance in favor of the prooxidants, leading to potential damage (Sies, 1991). The antioxidant defense system is formed by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), and by the non-oxidant defense system, like glutathione (GSH), E vitamin, and uric acid (Storey, 1996; Trenzado et al., 2006).

Brazil, Turkey, India, and Egypt have about 70% of the world Th reserves. As studies indicate that Th fuel cycle can be used in most reactor types already operating, Th probably will be a nuclear material much more valuable than uranium in the future (Unak, 2001). Consequently, the waterborne Th contamination on Neotropical aquatic systems from Th mining operations (Ladeira and Goncalves, 2007), as well as during the production of phosphoric acid (Santos et al., 2006) can increase in a near future. The Brazilian legislation does not define any permissive level for Th (CONAMA, 2008).

There are few studies about the effects of waterborne Th in aquatic fauna. Rainbow trout (*Oncorhynchus mykiss*) exposed to Th showed low corporal accumulation (Poston, 1982). We previously found bioaccumulation and biochemical and cytogenetic alterations in the Neotropical silver catfish (*Rhamdia quelen*) exposed to different Th concentrations for 15 d (Correa et al., 2008), but additional studies are needed to determine biomarkers for chronic Th contamination. Therefore, this study aimed to evaluate the effect of longer waterborne Th exposure (30 d) on tissue accumulation and metabolic and oxidative parameters of silver catfish.

## 2. Materials and methods

### 2.1. Experimental protocol

Silver catfish juveniles (6.41 ± 0.17 g and 8.78 ± 0.10 cm) were acquired from the fish culture sector at the Universidade Federal de Santa Maria (southern Brazil). Fish were kept in continuously aerated tanks (250 L) with dechlorinated well water under a natural photoperiod (12 h light and 12 h dark) for at least 2 weeks before experimental use. The temperature and pH of the water were kept at 22 ± 1.0 °C and 7.6 ± 0.2, respectively.

After the acclimation period, fish were transferred into 40 L aquaria (seven fish per tank) and exposed to different waterborne Th concentrations ($\mu g\ L^{-1}$): 0 (below detection limit, 0.05 $\mu g\ L^{-1}$ - control), 25.3 ± 3.2, 80.6 ± 12.0, 242.4 ± 35.6, and 747.2 ± 59.1 (three replicates per treatment) for 30 d. Preliminary experiments revealed that silver catfish exposed up to 2000 $\mu g\ L^{-1}$ Th for 96 h did not provoke mortality. Therefore, the Th levels chosen in the present study were similar to those of a previous study of Correa et al. (2008) that demonstrated some biochemical changes in silver catfish exposed to similar Th levels. The waterborne Th concentrations were adjusted to the appropriate levels using $Th(NO_3)_4 \cdot 5H_2O$ (BDH Chemical Ltda, England Analar®, purity >99%, compound of low radiotoxicity according to the dealer). Water was air-saturated by constant aeration in a semi-static system. Fish were fed to satiety once a day with commercial fish pellets (Supra 42% crude protein, Alisul Alimentos S.A., Carazinho, Brazil). Thorium levels in the food were below detection limits (0.08 $ng\ g^{-1}$). Uneaten food and feces were siphoned daily, and at least 20% of the water in the aquaria was replaced by water with previously adjusted pH and waterborne Th levels.

### 2.2. Tissue collection

After the experimental period, the fish were placed in receptacles with water and ice for 5 min for anesthetizing. Blood was collected from the caudal vein using heparinized syringes. Fish were then killed by spinal section and their tissues and fluids (bile, gills, liver, muscle, brain, skin, and kidney) were removed, weighed separately, and immediately frozen in liquid argon. The tissues were stocked in a −70 °C freezer for subsequent analysis of metabolites and enzymatic activity or in a −20 °C freezer for posterior digestion with concentrated nitric acid ($HNO_3$, 65%, Merck). The Th concentrations in digested samples were determined by inductively coupled plasma mass spectrometry (ICP-MS, Elan DRCII Perkin Elmer SCIEX – Canada) using conditions recommended by the manufacturer. Aqueous calibration standards (0.10, 0.25, 0.50, 0.75, 1.00, 1.50, and 2.00 $\mu g\ L^{-1}$ Th) were prepared by sequential dilution of a stock solution of Th (10 $\mu g\ L^{-1}$, Spex CertiPrep, Metuchen, USA). Sample digests were diluted when necessary. Accuracy was evaluated by the analysis of two biological certified reference materials: IRMM BCR 668 (mussel tissue, 10.7 ± 1.2 $\mu g\ g^{-1}$) and NIST SRM 1566b (oyster tissue, 0.0367 ± 0.0043 $\mu g\ g^{-1}$). Recovery tests were also performed for tissue digests and water samples. After every ten measurements, two standard Th solutions were analyzed to check the slope of the calibration curve. If the slope difference was higher than 5%, the calibration curve was prepared again using all of the standards. The precision of the ICP-MS measurements was considered acceptable up to 2%.

### 2.3. Biochemical parameters

Muscle glycogen levels were determined according to Bidinotto et al. (1998) after KOH and ethanol addition for hydrolysis and precipitation of glycogen, respectively. For protein analysis, tissues were warmed at 100 °C with KOH and centrifuged at 1000*g* for 10 min. The supernatant was used to determine the total protein level according to Lowry et al. (1951). For lactate and glucose level determination, tissue samples were homogenized by adding 20% trichloroacetic acid using a motor-driven Teflon® pestle and centrifuged at 1000*g* for 10 min for flocculation of the proteins. The completely deproteinated supernatant was used for lactate determination using the method described by Harrower and Brown (1972) and glucose was measured according to Duboie et al. (1956).

### 2.4. Parameters of oxidative stress

The gills and the liver and muscle tissues were homogenized in a 1.15% ($\frac{w}{n}$) KCl solution containing 1 mM PMSF. The homogenates were centrifuged at 600*g* for 10 min to eliminate nuclei and cell debris, and the supernatant fraction was frozen at −70 °C for further measurements. The supernatants were used for the analysis of CAT, GST, SOD, and lipid peroxidation (LPO). Catalase activity was determined with the method described by Boveris and Chance (1973), in which the disappearance of $H_2O_2$ is followed spectrophotometrically at 240 nm. The results are reported as pmol mg protein$^{-1}$.

Glutathione-S-transferase activity towards CDNB (1-chloro-2,4-dinitrobenzene) was determined spectrophotometrically at 340 nm using the method described by Habig et al. (1974). Activity was calculated from the changes in absorbance at 340 nm using an extinction coefficient of 9.6 mmol cm$^{-1}$. One unit of GST activity was defined as the amount of enzyme catalyzing the conjugation of 1 μmol of CDNB with GSH per minute at 25 °C.

Total SOD (CuZnSOD + MnSOD) activity was determined in the gills and liver as the inhibition rate of autocatalytic adenochrome generation at 480 nm in a reaction medium containing 1 mM epinephrine and 50 mM glycine–NaOH (pH 10.5). A unit of SOD is defined as the amount of enzyme that inhibits by 50% the speed of detector (epinephrine) reduction. Enzyme activity was expressed

in unit mg protein$^{-1}$ using the method described by Misra and Fridovich (1972).

Lipid peroxidation was measured with thiobarbituric acid reactive substances (TBARS) using the method described by Buege and Aust (1978). The decomposition of lipid hydroperoxides produces low-molecular weight products, including malondialdehyde, which can be assessed by the TBARs assay. In this method, absorbance measurements at 535 nm were used to measure the reaction between thiobarbituric acid and the LPO products, resulting in the formation of a chromogen (Schiff's base). The results are reported as nmol mg protein$^{-1}$. The protein content of the homogenate was measured using the method described in Lowry et al. (1951) using bovine serum albumin as the standard.

### 2.5. Water parameters

Water alkalinity ($32.5 \pm 0.5$ mg L$^{-1}$ $CaCO_3$) was determined weekly by the sulfuric acid ($H_2SO_4$) method (Greenberg et al., 1976). Measurements of dissolved oxygen ($5.96 \pm 0.05$ mg L$^{-1}$) (YSI model Y5512 oxygen meter) and water pH ($7.30 \pm 0.01$) (Quimix 400A pH meter) were performed daily. Water hardness ($25.9 \pm 0.4$ mg L$^{-1}$ $CaCO_3$) was determined by the EDTA titrimetric method, and total ammonia nitrogen ($0.22 \pm 0.01$ mg L$^{-1}$) ($NH_3 + NH_4^+$) was determined by the direct nesslerization method (Greenberg et al., 1976). Waterborne Th levels (and in the tissues) were measured weekly by ICP-MS (inductively coupled plasma mass spectrometry).

### 2.6. Statistical analysis

Data are reported as mean ± SEM (*N*). Homogeneity of variances among the groups was tested with the Levene test. Data presented as the homogeneous variances and comparisons among different treatments were made by one-way ANOVA and the Dunnet test. Analysis was performed using the software Statistica (version 5.1), and the minimum significance level was set at $p < 0.05$. The linear relationships between different waterborne Th concentrations and Th bioaccumulation in the tissues, between some oxidative stress parameters and Th bioaccumulation in the tissues, and between the studied oxidative stress parameters were calculated with Sigma Plot 8.0.

## 3. Results

Exposure to different Th concentrations did not significantly alter the survival (100% for all treatments), weight gain (overall mean $0.82 \pm 0.5$ g) or specific growth rate (overall mean $3.12 \pm 0.8\%$ d$^{-1}$). The increase in Th concentration in the water corresponded to a progressive increase of Th levels in the gills and kidney (Fig. 1B and G, respectively). However, this relationship was not found in bile, brain, liver, muscle, or skin (Fig. 1A, C, D, E, and F, respectively). The control group showed higher muscle and skin percentages of Th accumulation, followed by the kidney and liver, with very small percentages of accumulation in the brain, bile and gills. In fish exposed to waterborne Th muscle, skin and gills accumulated the highest percentages of Th in all of the tested concentrations. The percentage of accumulation was insignificant in the other organs. The highest increase in the percentage of Th accumulation occurred in the gills, with an increase of 5.6% in the gills exposed to $25.3 \pm 3.2$ μg L$^{-1}$, 9.8% with $80.6 \pm 12.0$ μg L$^{-1}$, 12.6% with $242.4 \pm 35.6$ μg L$^{-1}$ and 30.4% with $747.2 \pm 59.1$ μg L$^{-1}$ Th (Fig. 2).

At the end of 30 d, muscle glycogen was significantly higher (89%) in silver catfish exposed to 25.3 μg L$^{-1}$ Th than in the control fish, whereas glucose levels were significantly lower (28%). However, silver catfish maintained at 242.4 and 747.2 μg L$^{-1}$ Th presented muscle glucose levels significantly higher (44% and 37%, respectively) than the control fish. The values of muscular lactate and ammonia were significantly lower (22–35%) and higher (40–62%), respectively, in silver catfish submitted to the highest waterborne Th concentrations (80.6–747.2 μg L$^{-1}$ Th) than in the controls. On the other hand, muscle protein levels were significantly higher (31–54%) in those exposed to the lowest waterborne Th concentrations (25.3–242.4 μg L$^{-1}$ Th) than in the control fish (Table 1).

### 3.1. Oxidative stress

The gills of juveniles exposed to 242.4 and 747.2 μg L$^{-1}$ Th showed significantly higher LPO levels (100% and 142%) (Fig. 3A). In addition, there was a significant positive relationship between Th accumulation in the gills and lipoperoxidation ($r^2 = 0.9046$, $p < 0.05$) (Fig. 4A). The GST activity in the gills of fish exposed to 747.2 μg L$^{-1}$ Th was significantly lower (84%) than in the controls (Fig. 3B). In addition, there was a significant negative relationship between Th accumulation and GST ($r^2 = 0.9789$, $p < 0.05$) (Fig. 4B). The gills of silver catfish exposed to the highest waterborne Th levels (242 and 747.2 μg L$^{-1}$ Th) presented significantly lower SOD activity than gills of control fish (42–43%) (Fig. 3C). Catalase levels were not detectable in this tissue.

The liver of silver catfish exposed to 25.3 and 80.6 μg L$^{-1}$ Th showed significantly higher LPO levels (200% and 190%, respectively) compared to the control fish (Fig. 5A). The GST activity in the liver of fish exposed to all tested waterborne Th did not show any significant difference from the control group (Fig. 5B). Specimens exposed to 25.3 and 80.6 μg L$^{-1}$ Th showed significantly lower CAT activity (69%) when compared to the control group (Fig. 5C). The SOD activity in the liver of fish exposed to 25.3 μg L$^{-1}$ Th also showed significantly lower (53%) values than that from the control fish (Fig. 5D). The muscle of silver catfish exposed to different waterborne Th levels did not show any significant change in the oxidative stress parameters compared to the control group. The SOD activity of muscle was below detection limits.

## 4. Discussion

Silver catfish juveniles exposed to the highest waterborne Th concentrations showed the highest levels of Th bioaccumulation in the skin and gills (231 and 251 μg g$^{-1}$, respectively). Additionally, the skin, gills and muscle showed the highest body percentages of Th accumulation, which is similar to results previously observed in this species after 15 d of exposure to waterborne Th (Correa et al., 2008). The increase of gill, kidney and liver metal accumulation with the augment of waterborne cadmium levels was also observed in the African catfish, *Clarias gariepinus*, after 21 d of exposure, but the highest accumulation levels were in the kidney, followed by the gills and liver (Asagba et al., 2008). The major organ of accumulation for copper was the liver, and for zinc was the gill (McGeer et al., 2000). Therefore, accumulation in the tissues changes with the metal tested. Even though the highest waterborne Th level (747 μg L$^{-1}$ Th) is 22% higher in the present experiment than in the earlier study by Correa et al. (609 μg L$^{-1}$ Th) (2008), Th accumulation in the skin and gills after 30 d was 20% and 53% lower (Correa et al., 2008) than after 15 d. However, in general the exposure of silver catfish to waterborne Th for 30 d led to higher Th accumulation in the tissues (except gills and brain) than after 15 d of exposure. Our results are in agreement with Correa et al. (2008) in that the skin is an important organ of Th accumulation, but it remains to be seen if the higher accumulation of this element after 30 d compared to 15 d is due to its adsorption on the skin or because more Th was transferred from

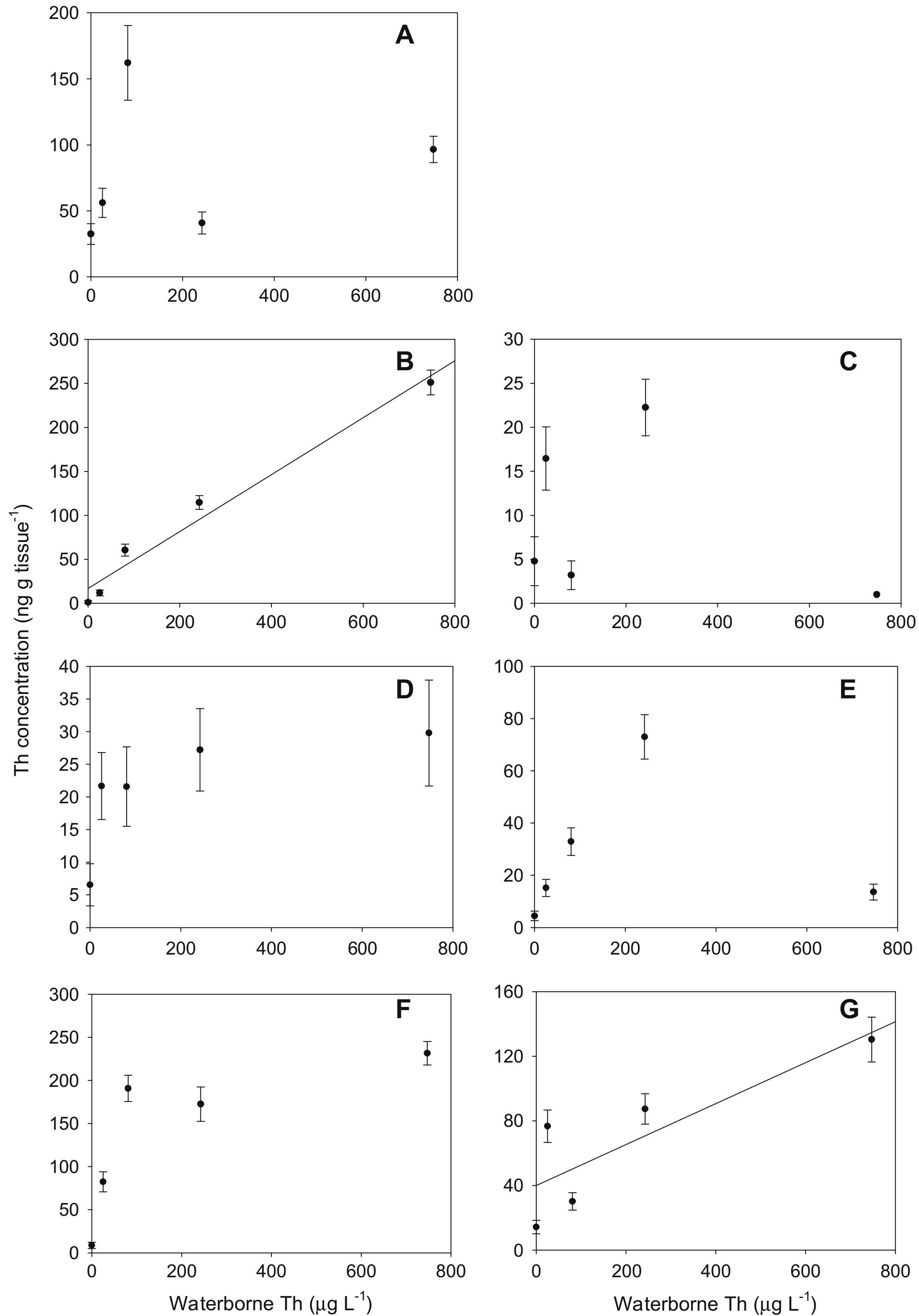


**Fig. 1.** Thorium accumulation in bile (A), gills (B), brain (C), liver (D), muscle (E), skin (F), and kidney (G) of silver catfish exposed to different waterborne Th levels for 30 d. The samples for Th accumulation at 747 μg $L^{-1}$ in (C) were lost. (B) $y = 17.04 + 0.32x$ ($r^2 = 0.97$); (G) $y = 39.96 + 0.12x$ ($r^2 = 0.71$), where $y$ = Th concentration (ng g $tissue^{-1}$) and $x$ = waterborne Th (μg $L^{-1}$). A, C, D, E and F – no significant relationship.

the gills to the blood and finally accumulated in the skin. Rainbow trout also presented a gradual increase of total Th burden in the tissues over 27 d of exposure to waterborne Th (Poston, 1982). Fish collected in rivers contaminated by wastewaters of uranium and iron ore mines presented Th accumulation in the muscle and bone (Pyle and Clulow, 1998; Carvalho et al., 2007).

In fish, oxidative stress has been documented in both field and laboratory exposure studies. The trace metals may increase the

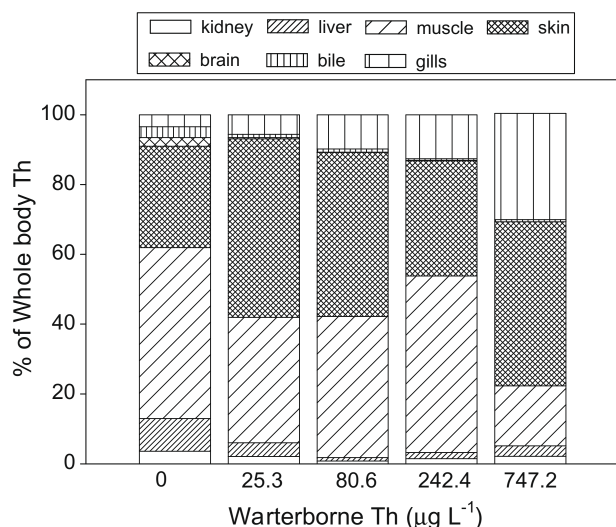


**Fig. 2.** Partitioning of total Th of silver catfish exposed to different Th levels for 30 d, expressed as the relative contribution of each tissue compartment.

intracellular development of ROS through the Fenton/Haber–Weiss reaction, increasing the prooxidants or decreasing the antioxidants (Almeida et al., 2002; Winston, 1991). The analysis of the parameters related to oxidative damage in silver catfish exposed to waterborne Th revealed that there were changes in the activity of prooxidants (lipoperoxidation) and antioxidants (SOD, CAT, and GST). The LPO, an important process in cellular damage, is mediated through free radical metabolites, which affect and alter the cellular antioxidant system. Antioxidants are active scavengers of free radicals involved in the lipid peroxidation process. Superoxide dismutase and CAT protect organisms from oxidative damage by removing partially reduced oxygen species (DiGiulio et al., 1989). The superoxide anion ($O_2^{\cdot -}$) is reduced to $H_2O_2$ by SOD, and $H_2O_2$ is converted to water and oxygen by CAT. Thus, the induction of SOD and CAT activities indicates the involvement of ($O_2^{\cdot -}$) and $H_2O_2$ (Wilhelm Filho, 1996, 2007). GST is involved in the detoxification of many xenobiotics, and plays an important role in protecting tissue from oxidative stress. A critical role for GST is defense against oxidative damage and peroxidative products of DNA and lipids (Fournier et al., 1992).

The fish gills are a multifunctional organ performing vital functions such as respiration, osmoregulation, acid–base balance, and nitrogenous waste excretion (Evans et al., 2005). This study showed an increase in the lipoperoxidation of silver catfish gills exposed to 242.4 and 747.2 μg $L^{-1}$ Th, validating the results obtained in the bioaccumulation analysis in this tissue. The increase in LPO could be attributed to the fact that the epithelial membrane is the first site of contact of trace metals at the gill surface. The peroxidative damage to gill membranes may result from oxidative deterioration of polyunsaturated fatty acids, thereby impacting the solute and water transport and osmoregulatory functions of gills (Evans, 1987). Metals induce oxidative stress by the production of ROS, so a strong antioxidant defense is essential to neutralize the impact of ROS (Ahmad et al., 2000). In this study, the SOD activity of the gills in juveniles exposed to 242.4 and 747.2 μg $L^{-1}$ Th decreased compared to control fish. This result was similar to that found in the gills of the freshwater fish *Channa punctata* exposed to a mixture of four toxic metals (Cu, Cd, Fe, and Ni) for 15 and 30 d (Pandey et al., 2008). The GST activity in the gills of silver catfish exposed to 747.2 μg $L^{-1}$ Th decreased, which suggests compensation for the increase in the oxidant levels because there was an increase in TBARS levels. This result was similar to GST levels found in silver catfish juveniles exposed to 609 μg $L^{-1}$ waterborne Th for 15 d (Correa et al., 2008). In addition, in this study there was a correlation between the LPO levels and the GST activity in the gills, validating the oxidative damage.

The liver of fish exposed to 25.3 and 80.6 μg $L^{-1}$ Th showed a decrease in the CAT activity. A decrease in the CAT activity was also found in the liver of Nile tilapia (*Oreochromis niloticus*) maintained in a polluted environment (Bainy et al., 1996) and in silver catfish exposed to 25.3 μg $L^{-1}$ Th for 15 d (Correa et al., 2008). The SOD–CAT system provides the first defense against oxygen toxicity. In the present study, a decrease in SOD and CAT activity in the liver of silver catfish exposed to 25.3 μg $L^{-1}$ Th was found. Moreover, an increase in the liver LPO in silver catfish exposed to 25.3 and 80.6 μg $L^{-1}$ Th was detected. Probably the lower SOD and CAT activities in the liver of silver catfish exposed to these Th levels are a response to the high LPO, demonstrated by the higher TBARS levels. A similar result was also observed in zebrafish (*Danio rerio*) exposed to different uranium levels (Barillet et al., 2007). An increase in LPO levels and a decrease in antioxidant enzymes' expression was found in the liver of rats that received thorium nitrate intraperitoneally for 30 d (Kumar et al., 2008). GST levels in the liver of silver catfish were not activated by exposure to waterborne Th.

The quantitative differences in the antioxidant defenses found in fish species are variable (Wilhelm Filho and Marcon, 1996) suggesting the possibility of a correlation between the antioxidant enzymes' activity with physiological aspects of the fish (Winston, 1991). In the muscle of silver catfish exposed to waterborne Th, there were no differences in the parameters evaluated, which could be due to the fact that this organ might be less affected by oxidative damage.

In conclusion, chronic Th exposure causes alterations in the oxidative parameters of silver catfish gills, which are correlated with the Th accumulation in this organ. The levels of GST decreased in the gills of fish exposed to 747.2 μg $L^{-1}$ Th and SOD activity decreased in silver catfish exposed to 242.4 and 747.2 μg $L^{-1}$ Th. In addition, the increase in the LPO in the gills exposed to 242.4 and 747.2 μg $L^{-1}$ Th suggests that higher oxidative damage occurred in the gills. However, in liver and muscle, these changes were observed mainly in the lowest waterborne Th level. In spite of liver and gills being targets organs, they have different morphophysiological characteristics and functions. The liver has a higher amount of mitochondria and consequently a higher level of enzymatic and non-enzymatic antioxidants that allows this organ to maintain its integrity. The reduction of catalase activity in silver

**Table 1**
Metabolic parameters of the muscle of silver catfish exposed to different waterborne Th levels for 30 d.

| Th (μg $L^{-1}$) | Glycogen (μmol $g^{-1}$) | Glucose (μmol $g^{-1}$) | Lactate (μmol $g^{-1}$) | Protein (mg $g^{-1}$) | Ammonia (μg $g^{-1}$) |
|---|---|---|---|---|---|
| 0 | 0.55 ± 0.029 | 0.43 ± 0.02 | 25.71 ± 1.29 | 193.4 ± 11.67 | 4.35 ± 0.30 |
| 25.3 ± 3.2 | 1.04 ± 0,27* | 0.31 ± 0.007* | 22.24 ± 1.15 | 253.80 ± 16.31* | 4.56 ± 0.16 |
| 80.6 ± 12.0 | 0.82 ± 0.063 | 0.37 ± 0.021 | 16.73 ± 0.61* | 297.96 ± 22.03* | 6.13 ± 0.14* |
| 242.4 ± 35.6 | 0.74 ± 0.057 | 0.62 ± 0.036* | 20.03 ± 0.71* | 254.76 ± 17.30* | 6.49 ± 0.27* |
| 747.2 ± 59.1 | 0.60 ± 0.052 | 0.59 ± 0.035* | 18.91 ± 0.84* | 220.82 ± 8.03 | 7.05 ± 0.38* |

Values expressed as mean ± SEM ($n$ = 7).
* Significantly different from the control group by one-way ANOVA and Dunnet test ($p$ < 0.05).

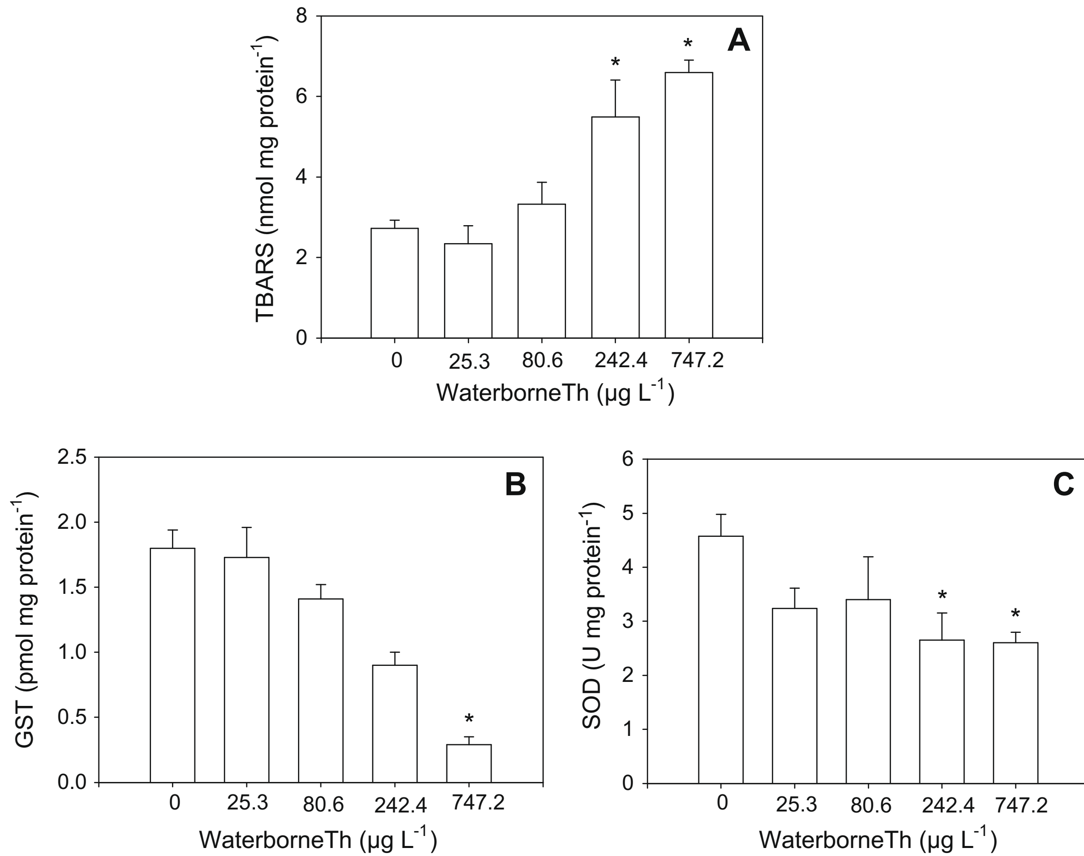


**Fig. 3.** TBARS determination (A), GST activity (B) and SOD activity (C) in gills of silver catfish exposed to different waterborne Th levels for 30 d. [a]Significantly different from the control by one-way ANOVA and Dunnet test ($p < 0.05$).

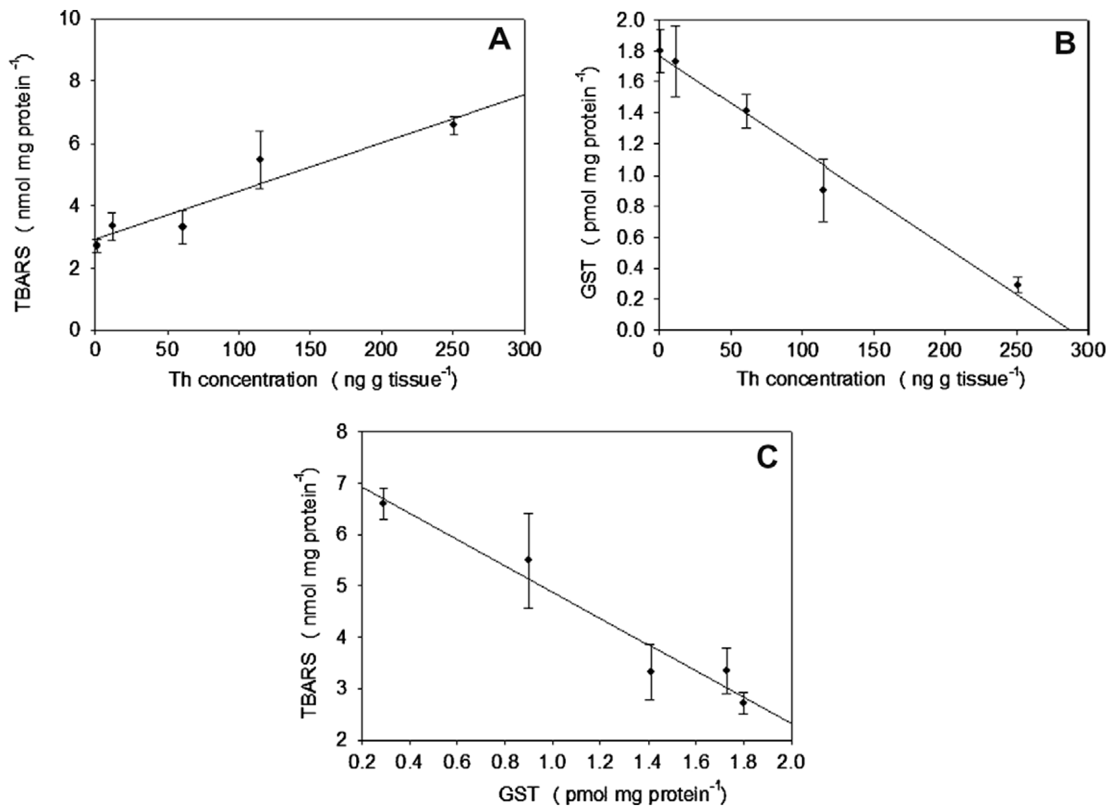


**Fig. 4.** Relationship between TBARS and Th (A), GST and Th (B), and TBARS and GST (C) in gills of silver catfish exposed to different waterborne Th levels for 30 d.

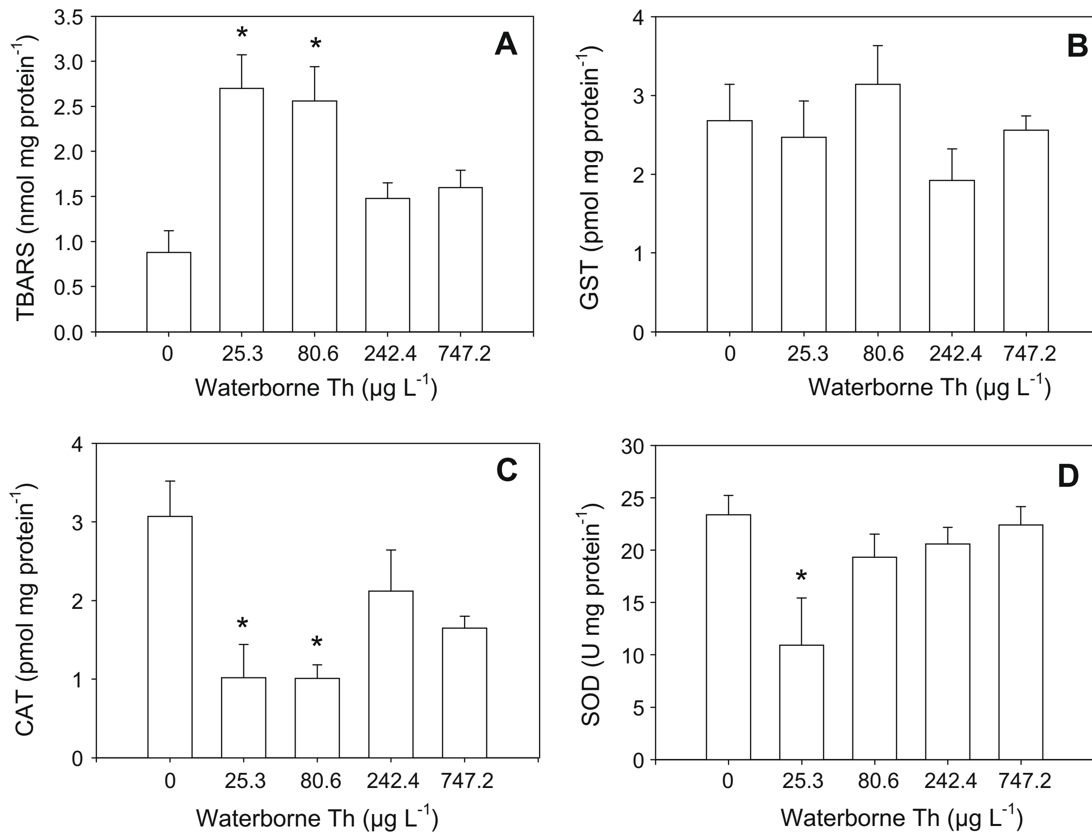


**Fig. 5.** TBARS determination (A), GST activity (B), CAT activity (C), and SOD activity (D) in liver of silver catfish exposed to different waterborne Th levels for 30 d. *Significantly different from control by one-way ANOVA and Dunnet test ($p < 0.05$).

catfish exposed to 25.3 and 80.6 $\mu g\,L^{-1}$ Th led to an increase in TBARS levels, which did not occur in fish kept at higher Th levels probably because the damage was so intense that was not expressed by the lipoperoxidation.

Metabolic intermediates in the muscle were altered by waterborne Th exposure, but beside this, no clear relationship was found. The increased levels of glycogen observed in silver catfish exposed to 25.3 $\mu g\,L^{-1}$ Th, taken together with glucose increase and lactate reduction in fish exposed to higher Th levels might indicate gluconeogenesis increase in the liver, probably using lactate as substrate. The gluconeogenesis increase would help to maintain glycogen levels, since these levels increased in silver catfish exposed to the lower Th levels and remained unaltered in those kept at higher Th levels. In addition, another effect of Th exposure in the muscle of silver catfish was the increase of protein levels. These results suggest that the fish exposed to Th may have a compensatory mechanism to deal with possible tissue protein lost by means of increasing protein synthesis. Similar effects were described after fish exposure to pesticides (Gill et al., 1991; Fonseca et al., 2008). However, the increase of ammonia levels is a clear indication of amino acid catabolism in this tissue. Thus, Th exposure clearly disrupt protein turnover in the muscle. The mechanisms underlying this differential pattern in Th toxicity in the different organs are unknown and further studies are needed to explain these differences.

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