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Effects of Subchronic Manganese Chloride Exposure on Tambaqui (*Colossoma macropomum*) Tissues: Oxidative Stress and Antioxidant Defenses

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Abstract This study aimed to evaluate oxidative stress parameters in juvenile tambaqui (*Colossoma macropomum*) exposed to 3.88 mg l^{-1} Mn^{2+} for 96 hours. Biomarkers of oxidative stress, such as thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST) activities, as well as content of reduced glutathione (GSH), were analyzed in gill, liver, brain, and kidney. The presence of Mn^{2+} in the water corresponded to increased levels of Mn^{2+} accumulation according to the following sequence: gill > kidney > brain > liver. There was a significant increase in TBARS levels (40 %) and SOD activity (80 %) in addition to a significant decrease in GSH content (41 %) in gills of fish exposed to waterborne Mn^{2+}. In hepatic tissue of the exposed animals, TBARS levels decreased significantly (35 %), whereas SOD (82 %) and GST activities (51 %) as well as GSH content (43 %) increased significantly. In brain of exposed juvenile fish, only significant decreases in SOD (32 %) and CAT activities (65 %) were observed. Moreover, the kidney of exposed fish showed a significant increase in TBARS levels (53 %) and a significant decrease in SOD activity (41 %) compared with the control. Thus, the changes in biomarkers of oxidative stress were different in the tissues, showing a specific toxicity of this metal to each organ.

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Manganese (Mn^{2+}), an essential trace metal, is found in all tissues of bacteria, plants, humans, and fish because it is required for normal amino acid, lipid, protein, and carbohydrate metabolism in vivo (Erikson et al. 2004). This metal is one of the most abundant elements and is widely used in industry (Gerber et al. 2002), pesticide formulations (Belpoggi et al. 2002), glass and ceramic production, and manufacture of dry cell (Srivastava et al. 1991; Mergler et al. 1994; Bader et al. 1999). It is also present at very high concentrations in formation water (produced water or oil field brine) from oil and gas extraction (Baldisserotto et al. 2012). Whereas Mn^{2+} deficiency is extremely rare, toxicity due to Mn^{2+} overexposure is more prevalent (Crossgrove and Zheng 2004). Mn^{2+} undergoes oxi-reduction reactions and may have negative physiological effects owing to oxidative stress induction (Huang et al. 2011).

Oxidative stress occurs due to either the overproduction of reactive oxygen species (ROS) or a decrease in cellular antioxidant levels. As a metal ion, Mn^{2+} is toxic because it enhances ROS formation and catecholamine oxidation by products (Prabhakaran et al. 2008; Falfushynska et al. 2011). ROS generated in tissues and subcellular compartments are efficiently scavenged by the antioxidant defense system, which is composed of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and nonenzymatic antioxidants, such as

reduced glutathione (GSH). These antioxidant defenses can protect cells from lipid peroxidation (LPO), protein oxidation, and DNA damage (Halliwell and Gutteridge 1999).

There are several studies on exposure to Mn^{2+} and other metals in different aquatic species. In general, these studies aimed to analyze mortality and metal bioaccumulation in tissues (Nath and Kumar 1987; Seymore et al. 2006; Crafford and Avenant-Oldewage 2011). Only a few investigations have evaluated possible oxidative damage involved in aquatic animals exposed to Mn^{2+} (Jena et al. 1998; Falfushynska et al. 2011).

Tambaqui (*C. macropomum*) is an abundant species in the Amazon basin and is very important to the local economy (Affonso et al. 2002). This species has great longevity and high tolerance to changes in dissolved oxygen levels and pH (Marcon and Wilhelm 1999; Milsom et al. 2002; Florindo et al. 2004). Such characteristics make of tambaqui a good model for the study of metals.

Experiments with metals and native fish have become essential to assess the risk of environmental contamination. Thus, the purpose of this study was to evaluate oxidative stress generated in several organs of tambaqui exposed to high waterborne Mn^{2+} levels for 96 hours.

Materials and Methods

Chemicals

All reagent-grade chemicals were purchased from Sigma (St. Louis, MO).

Fish

Juvenile tambaqui (100–300 g) were obtained from Fazenda Santo Antônio in Rio Preto da Eva, Amazonas, Brazil. Fish were transported to the Laboratory of Ecophysiology and Molecular Evolution, National Institute of Amazon, and maintained in aerated well water for at least 21 days and were fed commercial dry food pellets once a day. Water parameters were as follows: temperature 28 °C, pH 6.3, Ca^{2+} 11 μmol l^{-1}, Na^{+} 34 μmol l^{-1}, Cl^{-} 28 μmol l^{-1}, Mg^{2+} 0.8 μmol l^{-1}, K^{+} 15 μmol l^{-1}, dissolved organic matter 0.9 mg C l^{-1}, background Cu^{2+} 1.7 μg l^{-1}, and background Cd^{2+} 0.3 μg l^{-1}. The experimental protocol was approved by the Animal Health Committee of Federal University of Santa Maria, Rio Grande do Sul, Brazil.

Exposure to Mn

Stock solutions were prepared by dissolving manganese chloride ($MnCl_2$) in water and added it to the experimental aquarium after the acclimation period. Juvenile fish were randomly separated into two 72–l aquaria ($n = 10$ each aquarium), one exposed to 3.88 ± 0.239 mg l^{-1} Mn^{+2} (measured level) and another nonexposed to the metal (control) for 96 hours. The water was not renewed during the experimental period. Then the fish were placed in containers filled with water and ice for 5 minutes for anaesthetization, after which blood was sampled from the caudal vein with heparinized syringes; the fish were killed by section of the spinal cord. The gills, liver, brain, and kidneys were removed and immediately frozen in liquid nitrogen. The tissues were stored at −70 °C for measurement of oxidative stress parameters or at −20 °C for posterior digestion with concentrated nitric acid (HNO_3, 1 N; Merck). Mn concentrations in digested tissues and water samples were analyzed using graphite furnace–atomic absorption spectrophotometry (inductively coupled plasma–mass spectrometry (Elan DRCII Perkin Elmer SCIEX–Canada). Certified standards provided by the manufacturer were used throughout this study. Mn activity was calculated using the speciation program Visual MINTEQ version 3.0 (Gustafsson 2012).

Water Parameters

Water samples were collected from each aquarium to determine water-quality parameters at the beginning and at the end of the experiment. Water alkalinity (10.83 ± 0.48 mg l^{-1} $CaCO_3$) was determined by the sulfuric acid method (Eaton et al. 2005). Measurements of dissolved oxygen (YSI model Y5512 oxygen meter) and water pH (7.1 ± 0.04) (Quimix 400A pH meter) were performed daily. Water hardness (13.22 ± 0.66 mg l^{-1} $CaCO_3$) was determined by the ethylene diamine tetraacetic acid titrimetric method, and total ammonia ($NH_3 + NH_4^+$, final value 1.23 ± 0.05 mg l^{-1}) was determined by the direct nesslerization method (Eaton et al. 2005).

Oxidative Stress Parameters

The tissues were homogenized as described previously by Azambuja et al. (2011). The homogenates were centrifuged at 1000 × *g* for 10 minutes at 4 °C to discard nuclei and cell debris, and the supernatant fraction obtained was frozen at –70 °C for analyses of oxidative stress parameters.

Lipid peroxidation (LPO) was measured by TBARS assay (Buege and Aust 1978). Results were expressed as nmol mg $protein^{-1}$. Commercially available malonaldehyde was used as a standard. Protein content was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Total SOD activity was based on the inhibition rate of autocatalytic adenocrome generation at 480 nm in a reaction medium containing epinephrine and glycine/NaOH

(pH 10.2). The enzyme activity was expressed as USOD mg protein^{-1}. One SOD unit was defined as the amount of enzyme needed for 50 % inhibition of adenochrome formation as described by Misra and Fridovich (1972). CAT activity was evaluated according to the decrease in the 240 nm absorption in a reaction medium consisting of phosphate buffer (pH 7.4) and hydrogen peroxide (H_2O_2), thereby determining the pseudo–first order reaction constant ($k^{'}$) of the decrease in H_2O_2 absorption. This was reported as nmol mg protein^{-1} (Boveris and Chance 1973). GST activity, expressed as μmol min^{-1} mg protein^{-1}, was determined according to Habig et al. (1974). The assay was performed using potassium phosphate buffer (pH 6.5) with reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene. Activity was calculated from the changes in absorbance at 340 nm (ε_{340} nm = 9.6 mM^{-1} cm^{-1}). One unit of GST activity was defined as the amount of enzyme catalyzing the conjugation of CDNB with GSH/min at 25 °C. Tissue sulfhydryl groups, an indirect measure of GSH, were evaluated at 412 nm after reaction with 5,5'-dithiobis-(2-nitrobenzoic acid). Proteins were eliminated through the addition of perchloric acid. The final product formed is the yellow 2-nitro-5-mercapto-benzoic acid. The results were reported as nmol protein^{-1} using ε_{412} nm = 13.6 mM^{-1} cm^{-1} (Ellman 1959).

Statistical Analysis

The results are expressed as the means ± SEs. Levene's test was performed to evaluate the homogeneity of variances. Unpaired Student *t* test was used for comparison of means. All analyses were executed by using GraphPad Instat software (San Diego, CA). Differences were considered significant at $p < 0.05$.

Results

The presence of Mn^{2+} in the water corresponded to increased levels of Mn^{2+} in the gill, brain, and kidney. The percentage of accumulation in liver was not significant. Mn^{2+} accumulation in the tissues occurred in the following sequence: gill > kidney > brain > liver (Fig. 1). Gills of tambaqui exposed to waterborne Mn^{2+} exhibited a significant increase in thiobarbituric acid reactive substances (TBARS) levels (40 %) in addition to a significant increase in SOD activity (80 %) and a significant decrease in GSH content (40 %). GST activity was unaffected, whereas CAT activity could not be detected (Fig. 2). Hepatic TBARS levels of the fish exposed to waterborne Mn^{2+} was decreased (35 %) compared with the control. This tissue also showed a significant increase in SOD (82 %) and GST activities (51 %), as well as GSH content (43 %), whereas no change in CAT activity was observed in animals exposed to this metal (Fig. 3). In brain, SOD and CAT activities were significantly decreased (32 and 65 %, respectively) in the group exposed to Mn^{2+} compared with control fish. Nonetheless, GST activity and TBARS levels were unaffected (Fig. 4). Moreover, TBARS levels increased significantly (53 %) in kidney of tambaqui exposed to waterborne Mn^{2+}. SOD activity was significantly decreased (41 %) in renal tissue of these animals, whereas no change in CAT activity was observed (Fig. 5).

Discussion

Because fish constitute an important link in the food chain, their contamination by toxic metals causes a direct threat not only to the entire aquatic environment but also to humans (Obasohan 2008). Toxicity of Mn^{2+} in fish, despite its highly variable levels in water (Linnik 2000) and dependence on complexation (Liccione and Maines 1988), has scarcely been studied (Falfushynska et al. 2011). In the present study, the calculated Mn speciation by Visual Minteq 3.0 showed that 97 % of total Mn existed mainly as the free ionic species, Mn^{2+}.

The maximum allowed concentration of Mn^{2+} in Brazilian waters is 0.1 mg L^{-1} (Conselho Nacional do Meio Ambiente-CONAMA 2005), whereas this metal is present at 6.44 mg L^{-1} in the formation water from Urucu Reserve, Amazon (Baldisserotto et al. 2012). Therefore, the concentration used in the study is in between the maximum acceptable concentration and the concentration present in oilfield process water. Manganese is not distributed homogeneously throughout the organs in tambaqui. Most of the assessments on Mn^{2+} bioaccumulation are in accord with the measurement of trace metals in

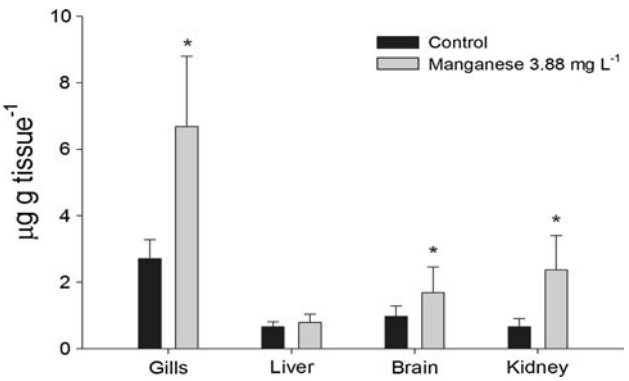

Fig. 1 Mn^{2+} levels in gill, liver, brain, and kidney of *C. macropomum* exposed to 3.88 mg L^{-1} waterborne Mn for 96 hours. *Significantly different from control by unpaired Student *t* test ($p < 0.05$)

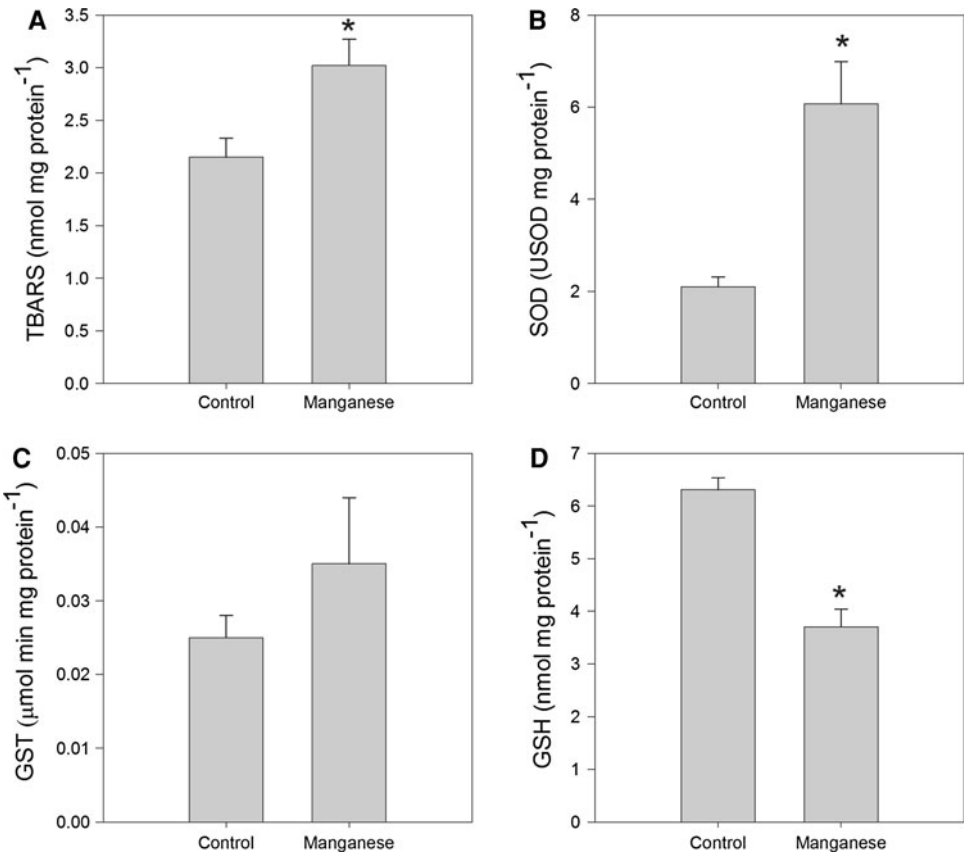

Fig. 2 TBARS levels (**a**), SOD (**b**), GST activities (**c**), and GSH content (**d**) in gill of tambaqui exposed to 3.88 mg l^{-1} Mn^{2+} for 96 hours. Data are reported as means ± SEs ($n = 10$). *Significantly different from control by unpaired Student t test ($p < 0.05$)

certain habitats rather than in a controlled exposure setting (Bharti and Banerjee, 2011; Alhashemi et al. 2012). Despite the lack of correlation between the data on bioaccumulation and oxidative stress parameters, the greater accumulation of the metal in tambaqui gills is in accordance with a preceding work with *Esox lucius* and *Abramis brama* (Rajkowska and Protasowicki 2012).

Fish gills represent a thin and extensive surface (≤90 % of total body surface) in intimate contact with water. They carry out three main functions: gas exchange, ion regulation, and excretion of metabolic waste products. Due to constant contact with the external environment, gills are the first target of waterborne pollutants (Perry and Laurent 1993) and are susceptible to damage caused by heavy metals. Metals induce oxidative stress by the overproduction of ROS; thus, a strong antioxidant defense is essential to neutralize the impact of these species (Ahmad et al. 2000; Kochhann et al. 2009). The increase in SOD activity observed in gills of tambaqui exposed to Mn^{2+} could represent a tissue response to compensate for the increased LPO.

SOD is a key antioxidant enzyme in the metabolism of ROS because it removes superoxide anion ($O_2^{\bullet -}$) and prevents the formation of other ROS, such as hydroxyl radicals ($OH^{\bullet}$) (Enghild et al. 1999). $O_2^{\bullet -}$ is the first species in the cascade of univalent decrease of molecular oxygen and therefore is the first indicator of increased generation of ROS. Steady-state concentrations of $O_2^{\bullet -}$ are directly proportional to its rate of production and inversely proportional to the activity of scavenging enzymes, such as SOD (Ferreira et al. 2004). If there is an increase in SOD activity, there will be a decrease in $O_2^{\bullet -}$ and an increase in H_2O_2 production. H_2O_2 is removed by two enzymes: CAT and glutathione peroxidase (GPx). The latter uses GSH as a cofactor to remove the H_2O_2. The present study data also showed a decrease in GSH levels. Mn^{2+} toxicity is related to the depletion of GSH in different animal phyla, including aquatic animals (Madejczyk et al. 2009). The depletion of GSH can enhance Mn^{2+} toxicity, albeit to a lesser extent than that registered for Cu^{2+} (Maracine and Segner 1998; Bozocaarmutlu and Arinc Bozcaarmutlu and Arinc 2004).

There is no pattern of antioxidant behavior in gills of fish exposed to metals. Chromium (Cr) exposure (10 mg l^{-1} Cr^{3+} or Cr^{6+}) for 96 hours did not change GSSG and total GSH ratio, GST and glutathione reductase (GR) activities, and LPO levels in gills of *C. auratus*. However, Cr^{6+} treatment resulted in decrease of carbonyl proteins levels, whereas exposure to both concentrations led to a decrease in CAT activity (Kubrak et al. 2010). In turn, gills of *C. auratus gibelio* exposed to 1.7 mg l^{-1} Mn^{2+} for 14 days showed increased SOD activity in

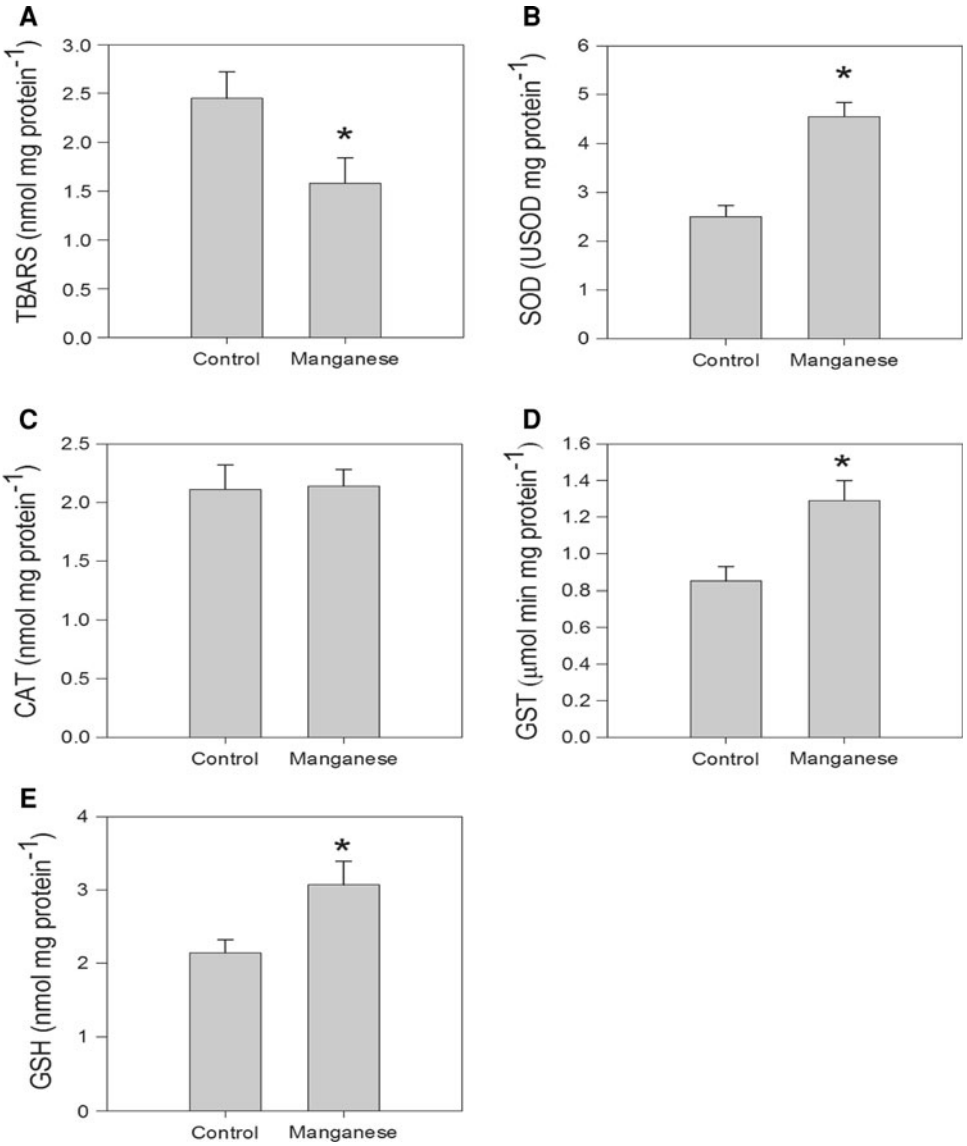

Fig. 3 TBARS levels (**a**), SOD (**b**), CAT (**c**), GST activities (**d**), and GSH content (**e**) in liver of tambaqui exposed to 3.88 mg l^{-1} Mn^{2+} for 96 hours. Data are reported as means ± SEs ($n = 10$). *Significantly different from control by unpaired Student t test ($p < 0.05$)

addition to decreases in LPO and GSH levels (Falfushynska et al. 2011). Moreover, exposure of *Channa punctatus* to different cadmium (Cd^{2+}) levels did not modify the amount of LPO and CAT activity in the gills, although Cd^{2+} induced a significant increase in the activity of the other enzymes, such as SOD, GPx, and GST as well as GSH content. Finally, Arabi and Alaeddini (2005) showed that supplementation of 5.5 mg l^{-1} Mn^{2+} reverted the deleterious effects of mercury (Hg^{2+}) and copper (Cu^{2+}) to *Oncorhynchus mykiss* exposed because its application inhibited LPO levels, decreased GST activity, and increased GSH content in the gill samples.

Liver is the main organ of various key metabolic pathways and the most frequently studied tissue regarding oxidative stress. Our data showed that liver LPO levels were decreased in tambaqui exposed to Mn^{2+}. In turn, the activity of antioxidant enzymes, such as SOD and GST, and the content of nonenzymatic antioxidant GSH presented an opposite pattern, whereas CAT activity was unaffected. The increased formation of GSH in liver of tambaqui exposed to Mn^{2+} suggests a role in the defense of cells against oxidative stress. Furthermore, our study also showed that GST plays an important role in the detoxification of the end products of LPO.

Similar results were also shown in *C. auratus gibelio* exposed to 1.7 mg l^{-1} Mn^{2+} for 14 days. This species showed a decrease in levels of liver LPO associated with an increase in Mn-SOD activity compared with the respective control (Falfushynska et al. 2011). Moreover, Huang et al. (2011) also described a decrease in LPO levels in addition to an increase in GSH content in liver of rats exposed to Mn^{2+}. However, in opposition to our data, Casalino et al. (2004) reported an increase in LPO levels in liver of rats 24 hours after administration of 2.0 mg kg^{-1} Mn^{2+}.

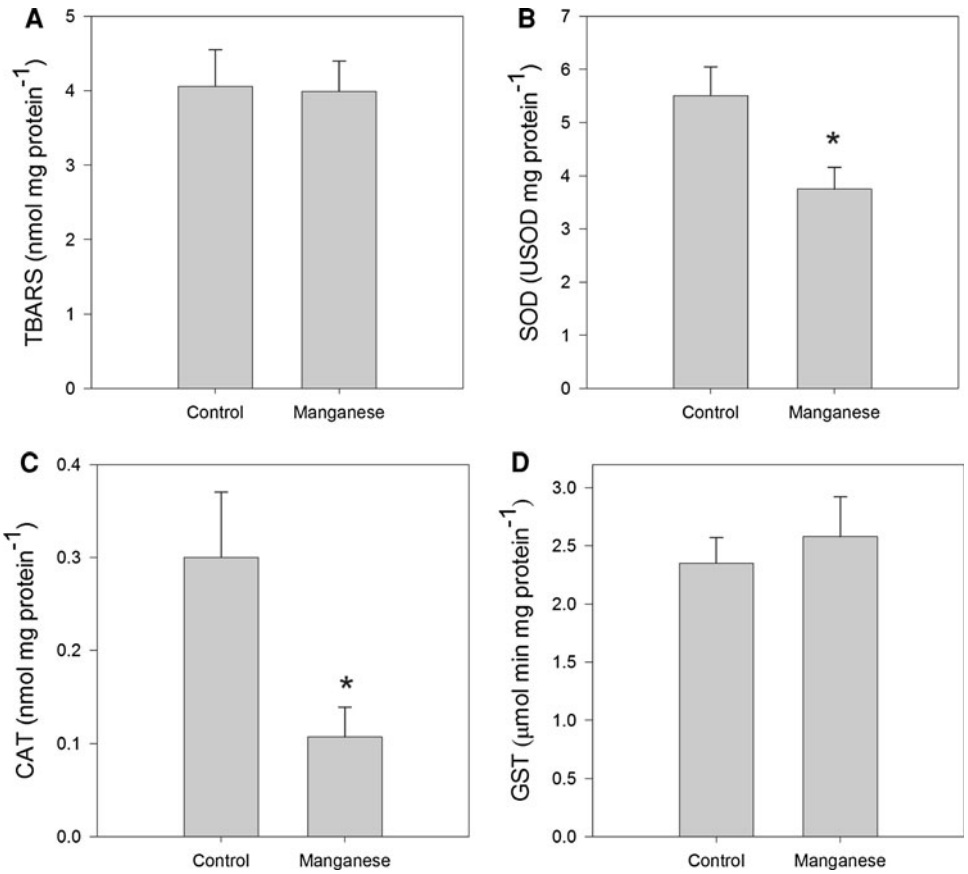

Fig. 4 TBARS levels (**a**), SOD (**b**), CAT (**c**), and GST activities (**d**) in brain of tambaqui exposed to 3.88 mg l^{-1} Mn^{2+} for 96 hours. Data are reported as means ± SEs ($n = 10$). *Significantly different from control by unpaired Student t test ($p < 0.05$)

Nevertheless, these investigators found an increase in GST activity in this organ, thus corroborating our findings in tambaqui. Induction of GST activity depends on the type of tissue and nature of the inducer. In another experiment, *C. punctatus* exposed to sublethal concentrations of Cd^{2+} for 24, 48, 72, and 96 hours presented increased levels of liver LPO and modulated activities of SOD, CAT, GPx, GR, and GST as well as GSH content (Dabas et al. 2011). Thus, the current results suggest that the increase in both types of antioxidants (enzymatic and nonenzymatic) in liver of tambaqui exposed to Mn^{2+} is compensating for the decrease in LPO levels.

The brain is very susceptible to oxidative damage by ROS as it contains high amounts of unsaturated lipids and uses approximately 20 % of total the body's oxygen demand (Stella and Lajtha 1987). Our data showed that LPO levels and GST activity remained unchanged, whilst SOD and CAT activities decreased in brain tissue of tambaqui exposed to Mn^{2+} compared with the respective control. This decrease observed in SOD and CAT activities indicates oxidative damage to organs in the presence of Mn^{2+}.

Chtourou et al. (2010) described similar data because they verified a decrease in the antioxidant enzymes in cerebral cortex of rats that received Mn^{2+} in drinking water for 30 days. Mn^{2+} is an important cofactor for a variety of enzymes, including SOD (Hurley and Keen 1987). This metal scavenges $O_2^{\bullet -}$ and $OH^{\bullet}$ even when SOD activity is inhibited (Hussain and Ali 1999). However, the prooxidant effects of Mn^{2+} have been confirmed repeatedly in in vitro and in vivo studies (Ali et al. 1995; Zhang et al. 2004; Jiao et al. 2008). An in vitro analysis showed that 18.31 mg l^{-1} Mn^{2+} significantly inhibited CAT activity in brain of fish and lizards (Jena et al. 1998). Cr exposure (10 mg l^{-1} Cr^{3+} or Cr^{6+}) of *C. auratus* for 96 hours resulted in increased brain content of carbonyl protein and no changes in SOD, CAT, and GST activities in this tissue (Kubrak et al. 2010). The same investigators published another study in 2011, in which they evaluated the effects of various concentrations of cobalt (Co^{2+}) on brain of *C. auratus.* Exposure to 50 mg l^{-1} Co^{2+} for 96 hours did not affect LPO levels and GR activity; however, this induced a decrease in SOD, CAT, and glucose-6-phosphate dehydrogenase activities (Kubrak et al. 2011). These findings are in accordance with our data.

Finally, our results also showed that Mn^{+2} exposure of tambaqui may reflect the development of renal oxidative stress because it led to an increase in LPO levels associated with a decrease in SOD activity, although no change was observed in CAT activity. SOD, along with CAT, represents the first barrier against ROS and is essential to cell

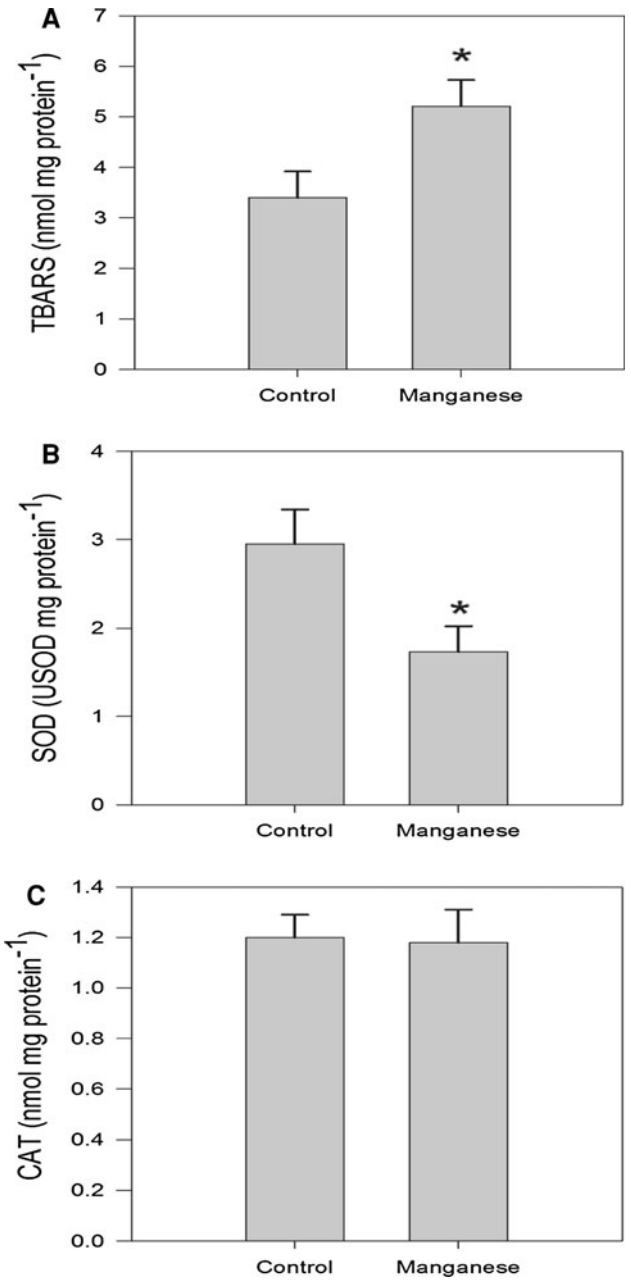

Fig. 5 TBARS levels (**a**), SOD (**b**), and CAT activities (**c**) in kidneys of tambaqui exposed to 3.88 mg.L^{-1} Mn^{2+} for 96 hours. Data are reported as mean ± SE ($n = 10$). *Significantly different from control by unpaired Student t test ($p < 0.05$)

survival (Remacle et al. 1992; Mates et al. 1999; Halliwell 2001). Travacio and Llesuy (1996) reported that different models of oxidative stress involve a biphasic response of antioxidant enzyme activities. At first, enzymatic activities are markedly decreased, but with time the activity levels increase, probably as a consequence of a new synthesis and/or enzymatic activation.

C. punctatus exposed to Cd^{2+} (6.7, 13.4, and 20.1 mg l^{-1}) for various time periods (24, 48, 72, and 96 hours) presented increased levels of LPO as well as SOD, GST, and GR activities, whereas CAT activity was decreased (Dabas et al. 2011). In turn, *C. auratus* exposed to various concentrations of Cr^{6+} for 96 hours showed increased renal hydroperoxide levels and SOD activity and no significant differences in CAT activity (Velma and Tchounwou 2010).

The results of the current research clearly show that there were changes in the balance of pro-oxidants and antioxidants in different organs of tambaqui. Such changes were more evident in liver and kidney. Furthermore, there was no correlation between the oxidative stress results and the bioaccumulation data. Present findings may contribute to the scarce literature regarding fish subchronic exposure to Mn^{2+}.

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References

Affonso EG, Polez VLP, Corrêa CF, Mazon AF, Araújo MRR, Moraes G et al (2002) Blood parameters and metabolites in the teleost fish *Colossoma macropomum* exposed to sulfide or hypoxia. Comp Biochem Physiol C Toxicol Pharmacol 133:375–382

Ahmad I, Hamid T, Fatima M, Chand HS, Jain SK, Athar M et al (2000) Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch). Biochem Biophys Acta 1523:37–48

Alhashemi AH, Sekhavatjou MS, Kiabi BH, Karbassi AR (2012) Bioaccumulation of trace elements in water, sediment and six fish species from a freshwater wetland. Iran Microchem J 104:1–6

Ali SF, Duhart HM, Newport GD, Lipe GW, Slikker W (1995) Manganese-induced reactive oxygen species: comparison between Mn + 2 and Mn + 3. Neurodegeneration 4:329–334

Arabi M, Alaeddini MA (2005) Metal-ion-mediated oxidative stress in the gill homogenate of rainbow trout (*Oncorhynchus mykiss*): antioxidant potential of manganese, selenium, and albumin. Biol Trace Elem Res 108:155–168

Azambuja CR, Mattiazi J, Riffel AP, Finamor IA, Garcia LO, Heldwein CG et al (2011) Effect of the essential oil Lippia alba on oxidative stress parameters in silver catfish (*Rhamdia quelen*) subjected to transport. Aquaculture 319:156–161

Bader M, Dietz MC, Ihrig A, Triebig G (1999) Biomonitoring of manganese in blood, urine and axillary hair following low-dose exposure during the manufacture of dry cell batteries. Int Arch Occup Environ Health 72:521–527

Baldisserotto B, Garcia LO, Benaduce AP, Duarte RM, Nascimento TL, Gomes LC et al (2012) Sodium fluxes in tamoatá, *Hoplosternum litoralle*, exposed to formation water from Urucu reserve (Amazon, Brazil). Arch Environ Contam Toxicol 1:78–84

Belpoggi F, Soffritti M, Guarino M, Lambertini L, Cevolani D, Maltoni C (2002) Results of long-term experimental studies on the carcinogenicity of ethylene-bis-dithiocarbamate (Mancozeb) in rats. Ann N Y Acad Sci 982:123–136

Bharti S, Banerjee TK (2011) Bioaccumulation of metals in the edible catfish *Heteropneustes fossilis* (bloch) exposed to coal mine effluent generated at northern coalfield limited, Singrauli, India. Bull Environ Contam Toxicol 87:393–398

Boveris A, Chance B (1973) The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem J 134:707–716

Bozcaarmutlu A, Arinc E (2004) Inhibitory effects of divalent metal ions on liver microsomal 7-ethoxyresorufin O-deethylase (EROD) activity of leaping mullet. Mar Environ Res 58:521–524

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310

Casalino E, Sblano C, Landriscina V, Calzaretti G, Landriscina C (2004) Rat liver glutathione S-transferase activity stimulation following acute cadmium or manganese intoxication. Toxicology 200:29–38

Chtourou Y, Fetoui H, Sefi M, Trabelsi K, Barkallah M, Boudawara T et al (2010) Silymarin, a natural antioxidant, protects cerebral cortex against manganese-induced neurotoxicity in adult rats. Biometals 23:985–996

Conselho Nacional do Meio Ambiente-CONAMA (2005) Resolução CONAM n° 357. Available at: http://www.mma.gov.br/port/conama/res/res05/res35705.pdf. Accessed: March 16, 2012

Crafford D, Avenant-Oldewage A (2011) Uptake of selected metals in tissues and organs of *Clarias gariepinus* (sharptooth catfish) from the Vaal River System: chromium, copper, iron, manganese and zinc. Water S Afr 37:181–200

Crossgrove J, Zheng W (2004) Manganese toxicity upon overexposure. NMR Biomed 17:544–553

Dabas A, Nagpure NS, Kumar R, Kushwaha B, Kumar P, Lakra WS (2011) Assessment of tissue-specific effect of cadmium on antioxidant defense system and lipid peroxidation in freshwater murrel, *Channa punctatus*. Fish Physiol Biochem 38:469–482

Eaton AD, Clesceri LS, Rice EW, Greenberg AE (2005) Standard methods for the examination of water and wastewater (21st ed), Centennial edn. Monrovia, CA

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77

Enghild JJ, Thogersen IB, Oury TD, Valnickova Z, Hojrup P, Crapo JD (1999) The heparin-binding domain of extracellular superoxide dismutase is proteolytically processed intracellularly during biosynthesis. J Biol Chem 274:14818–14822

Erikson KM, Dobson AW, Dorman DC, Aschner M (2004) Manganese exposure and induced oxidative stress in the rat brain. Sci Total Environ 334–335:409–416

Falfushynska HI, Gnatyshyna LL, Stoliar OB, Nam YK (2011) Various responses to copper and manganese exposure of *Carassius auratus gibelio* from two populations. Comp Biochem Physiol C Toxicol Pharmacol 154:242–253

Ferreira SM, Lerner SF, Brunzini R, Evelson PA, Llesuy SF (2004) Oxidative stress markers in aqueous humor of glaucoma patients. Am J Ophthalmol 137:62–69

Florindo LH, Reid SG, Kalinin AL, Milsom WK, Rantin FT (2004) Cardiorespiratory reflexes and aquatic surface respiration in the neotropical fish tambaqui (*Colossoma macropomum*): acute responses to hypercarbia. J Comp Physiol B 174:319–328

Gerber GB, Léonard A, Hantson P (2002) Carcinogenicity, mutagenicity and teratogenicity of manganese compounds. Crit Rev Oncol Hematol 42:25–34

Gustafsson JP (2012) Visual Minteq 3.0. Department of land and water resources engineering, Royal Institute of Technology, Stockholm

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. J Biol Chem 249:7130–7139

Halliwell B (2001) Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging 18:685–716

Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine. Int J Biochem Cell Biol 31:1454–1468

Huang P, Chen C, Wang H, Li G, Jing H, Han Y et al (2011) Manganese effects in the liver following subacute or subchronic manganese chloride exposure in rats. Ecotoxicol Environ Saf 74:615–622

Hurley LS, Keen CL (1987) Manganese. In: Underwood E, Mertz W (eds) Trace elements in human health and animal nutrition. Academic, New York, NY, pp 185–225

Hussain S, Ali SF (1999) Mangenese scavanges superoxide and hydroxyl radicals: an in vitro study in rats. Neurosci Lett 261:21–24

Jena BS, Nayak SB, Patnaik BK (1998) Age-related changes in catalase activity and its inhibition by manganese (II) chloride in the brain of two species of poikilothermic vertebrates. Arch Gerontol Geriatr 26:119–129

Jiao J, Qi YM, Fu JL, Zhou ZC (2008) Manganese-induced single strand breaks of mitochondrial DNA in vitro and in vivo. Environ Toxicol Pharmacol 26:123–127

Kochhann D, Pavanato MA, Llesuy SF, Correa LM, Riffel APK, Loro VL et al (2009) Bioaccumulation and oxidative stress parameters in silver catfish (*Rhamdia quelen*) exposed to different thorium concentrations. Chemosphere 77:384–391

Kubrak OI, Lushchak OV, Lushchak JV, Torous IM, Storey JM, Storey KB et al (2010) Chromium effects on free radical processes in goldfish tissues: comparison of Cr(III) and Cr(VI) exposures on oxidative stress markers, glutathione status and antioxidant enzymes. Comp Biochem Physiol C Toxicol Pharmacol 152:360–370

Kubrak OI, Husak VV, Rovenko BM, Storey JM, Storey KB, Lushchak VI (2011) Cobalt-induced oxidative stress in brain, liver and kidney of goldfish *Carassius auratus*. Chemosphere 85:983–989

Liccione JJ, Maines MD (1988) Selective vulnerability of glutathione metabolism and cellular defense-mechanisms in rat striatum to manganese. J Pharmacol Exp Ther 247:156–161

Linnik PN (2000) Heavy metals in surface waters of Ukraine: their content and forms of migration. Hydrobiol J 36:3–27

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Madejczyk MS, Boyer JL, Ballatori N (2009) Hepatic uptake and biliary excretion of manganese in the little skate, *Leucoraja erinacea*. Comp Biochem Physiol C Toxicol Pharmacol 149:566–571

Maracine M, Segner H (1998) Cytotoxicity of metals in isolated fish cells: importance of the cellular glutathione status. Comp Biochem Physiol A Mol Integr Physiol 120:83–88

Marcon JL, Wilhelm D (1999) Antioxidant processes of the wild tambaqui, *Colossoma macropomum* (Osteichthyes, Serrasalmidae) from the Amazon. Comp Biochem Physiol C Toxicol Pharmacol 123:257–263

Mates JM, Perez-Gomez C, De Castro IN (1999) Antioxidant enzymes and human diseases. Clin Biochem 32:595–603

Mergler D, Huel G, Bowler R, Iregren A, Belanger S, Baldwin M et al (1994) Nervous system dysfunction among workers with long-term exposure to manganese. Environ Res 64:151–180

Milsom WK, Reid SG, Rantin FT, Sundin L (2002) Extrabranchial chemoreceptors involved in respiratory reflexes in the neotropical fish *Colossoma macropomum* (the tambaqui). J Exp Biol 205:1765–1774

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Nath K, Kumar N (1987) Toxicity of manganese and its impact on some aspects of carbohydrate metabolism of a freshwater teleost, *Colisa fasciatus*. Sci Total Environ 67:257–262

Obasohan EE (2008) Heavy metals in the sediment of Ibiekuma stream in Ekpoma, Edo state, Nigeria. Afr J Gen Agric 4:107–112

Perry SF, Laurent P (1993) Environmental effects on fish gill structure and function. In: Rankin JC, Jensen FB (eds) Fish ecophysiology. Chapman and Hall, London, UK, pp 231–264

Prabhakaran K, Ghosh D, Chapman GD, Gunasekar PG (2008) Molecular mechanism of manganese exposure-induced dopaminergic toxicity. Brain Res Bull 76:361–367

Rajkowska M, Protasowicki M (2012) Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. Environ Monit Assess. doi:10.1007/s10661-012-2805-8

Remacle J, Michiels C, Raes M (1992) The importance of antioxidant enzymes in cellular aging and degeneration. EXperientia 62:99–108

Seymore T, Dupreez HH, Vanvuren JHJ (2006) Manganese, lead and strontium bioaccumulation in the tissues of the yellowfish, *Barbus-marequensis* from the lower Olifants River, Eastern Transvaal. Water S Afr 21:159–172

Srivastava AK, Gupta BN, Mathur N, Murty RC, Garg NChandra SV (1991) An investigation of metal concentrations in blood of industrial workers. Vet Hum Toxicol 33:280–282

Stella AMG, Lajtha A (1987) Macromolecular turnover in brain during aging. Gerontology 33:136–148

Travacio M, Llesuy S (1996) Antioxidant enzymes and their modification under oxidative stress conditions. Ciên Cult 48:9–13

Velma V, Tchounwou PB (2010) Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, *Carassius auratus*. Mutat Res 698:43–51

Zhang SR, Fu JL, Zhou ZC (2004) In vitro effect of manganese chloride exposure on reactive oxygen species generation and respiratory chain complexes activities of mitochondria isolated from rat brain. Toxicol In Vitro 18:71–77