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Biochemical biomarkers in nile tilapias (*Oreochromis niloticus* Linnaeus, 1758) of different weights exposed to contaminants

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Abstract: Nile tilapias (*Oreochromis niloticus*) of three groups with different weights (juvenile, adults of lower and higher weight) were exposed to benzo[a]pyrene (0.5 mg.L^{-1}), cupper (0.5 mg.L^{-1}), cadmium (0.5 mg.L^{-1}) and diazinon (1.0 mg.L^{-1}), for 72 h. In order to determine how animals of the same species respond to such contaminants over weight gain, biochemical biomarkers (glutathione S-transferase, superoxide dismutase, catalase, glutathione peroxidase, acetylcholinesterase, carboxylesterase and lipid peroxidation levels) were analyzed in liver and in gills. In the juvenile group the catalase was induced by exposure to cupper in gills, while lipid peroxidation levels were low. In the same group the glutathione S-transferase was induced in gills, likewise glutathione peroxidase was induced by diazinon in liver. In gills of the group of adults of lower weight the low lipid peroxidation levels, in exposure to diazinon treatment, may be related to inhibition of carboxylesterase. In the group of adults of higher weight was not observed adverse effect of contaminants, except for exposure to diazinon in esterases. Thus, this study prove clear evidence that it is necessary to take into account in biomonitoring programs, the weight, the tissue and the development, when using tilapia as tested organism.

Keywords: biomarkers, contaminants, enzymes, tilapia, weights.

Biomarcadores bioquímicos em tilápias do nilo (*Oreochromis niloticus* Linnaeus, 1758) de diferentes pesos expostas a contaminantes

Resumo: Tilápias do Nilo (*Oreochromis niloticus*) de três grupos de peso diferentes (juvenis, adultos de menor e maior peso) foram expostas ao benzo[a]pireno (0,5 mg.L^{-1}), cobre (0,5 mg.L^{-1}), cádmio (0,5 mg.L^{-1}) e diazinon (1,0 mg.L^{-1}), por 72 h. A fim de determinar como os animais da mesma espécie respondem a tais contaminantes de acordo com o peso, foram analisados a glutationa-S-transferase, superoxido dismutase, catalase, glutationa peroxidase, acetilcolinesterase, carboxilesterase e níveis de peroxidação lipídica em fígado e brânquias. No grupo das juvenis a enzima catalase foi induzida pela exposição ao cobre nas brânquias, enquanto que os níveis de peroxidação lipídica foram baixos. Assim como a glutationa-S-transferase nas brânquias, a glutationa peroxidase no fígado também foram induzidas pela exposição ao diazinon nesse mesmo grupo. Nas brânquias do grupo dos adultos de menor peso, os níveis de peroxidação lipídica foram baixos para a exposição ao diazion, provavelmente pode estar relacionado com a inibição da carboxilesterase. No grupo dos adultos de maior peso não foi observado nenhum efeito adverso dos contaminantes, exceto para a exposição ao diazinon nas esterases. Desta forma, este estudo comprova que quando se utiliza tilápias nos biomonitoramentos é necessário considerar o peso, o tecido e a fase de desenvolvimento.

Palavras-chaves: biomarcadores, contaminantes, enzimas, tilápia, peso.

Introduction

With the acceleration of the industrial process, many chemicals are produced annually and released improperly in rivers, lakes and seas (Cajaraville et al., 2000). These compounds come from industrial effluents, agricultural, household and accidental spillage of waste chemicals. Among them are commonly found toxic metals, pesticides and polycyclic aromatic hydrocarbons (PAH), that are very harmful to both aquatic biota and to humans (Abdelkhalek et al., 2015; Firat et al., 2011; Lopez–Barea & Pueyo, 1998; Rashed, 2001).

This type of exposure can affect fish biochemistry (Üner et al., 2006), causing imbalance between the formation and elimination of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$) and hydroxyl radical (HO•) (Durmaz et al., 2006), which can oxidize molecules (Torres et al., 2002) causing DNA, proteins and lipids damage (Almeida et al., 2009).

In phase II of biotransformation, glutathione S-transferase (GST) is the family of enzymes most active, because it has multiple functions, among them the defense against oxidative damage and lipid peroxidation products (Bouraoui et al., 2009; Palanikumar et al., 2011).

On the other hand, the esterases are enzymes capable of hydrolyzing carboxylic ester bonds of a wide variety of substrates. The Acetylcholinesterase (AChE) is related to the rapid and effective hydrolysis of the neurotransmitter acetylcholine in inactive products such as acetic acid and choline in the nerve synapses (Fukuto, 1990; Pretto et al., 2010). And Carboxylesterase (CbE), that also presents multiple functions, hydrolyzes a series of esters of fatty acids and other toxic compounds (Barron et al., 1999; Wheelock et al., 2008).

To protect themselves, organisms also increase the expression of antioxidant enzymatic and non-enzymatic systems, in order to intercept the formed ROS. The main antioxidant enzymes that constitute this defense system (El-Gazzar et al., 2014) are: Superoxide Dismutase (SOD), a group of metalloenzymes that participates in the first line of defense against oxidative damage by catalyzing the reduction of the anion $O_2^{\bullet-}$ in H_2O_2 (Atli & Canli, 2010; Oruç & Usta, 2007) and it consists the main importance is the presence of SOD in aerobic organisms (Huggett et al., 1992). The Catalase (CAT) is an enzyme found in the peroxisomes of most cells and contains heme groups that facilitates the degradation of H_2O_2 to O_2 and H_2O, acting as oxidizing and reducing agent in reactions involving organic and inorganic substrates (Atli & Canli, 2007; Firat & Kargin, 2010). The decomposition of H_2O_2 prevents the formation of HO• that damages membranes (Huggett et al., 1992).

On the other hand, Glutathione Peroxidase (GPx) also catalyzes the reduction of H_2O_2 and lipid peroxides and involves both the Reduced Glutathione (GSH) and Oxidized Glutathione (GSSG) as electron donor for the reaction (Van Der Oost et al., 2003).

Another way to measure oxidative stress generated by contaminants is the formation of lipid peroxidation products (Oruç & Usta, 2007) that occurs through the attack of a free radical to an unsaturated fatty acid present in lipoprotein cell membranes resulting in the formation of a lipid hydroperoxide radical which may be metabolized generating alkanes, alkenes, aldehydes, among others (Lima & Abdalla, 2001).

Thus, it is important to diagnose the effect that contaminants generated in the biochemical parameters in fish. The species *Oreochromis niloticus*, Linnaeus, 1758 (Peciforme, Cichlidae), belongs to the family of cichlids, originally from the Nile River Basin, East Africa, and is spreaded over tropical Asia, Africa and South America (Chandrasekara & Pathiratne, 2007). It was introduced in Brazil in 1971 from East Africa (Rocha-e-Silva et al., 2004) because of similarities in climate, its abundance, rapid growth, maturation and intensive fish farming (Figueiredo-Fernandes et al., 2006; Fujimura & Okada, 2007; Linde-Arias et al., 2008). Tilapia is widely used as a test organism in bio-monitoring programs for water pollution in tropical regions (Chandrasekara & Pathiratne, 2007; Linde-Arias et al., 2008; Pathiratne et al., 2008; Peixoto et al., 2006).

Therefore, the different responses of aquatic animals against pollution, can be influenced by factors such as temperature, age, nutritional status, oxygen availability (Almeida et al., 2009), dose, species, route of exposure (Atli & Canli, 2010), among others. Factors that are not related to contamination can also cause an additional impact on enzyme systems and interfere in the response of biomarkers, when these conditions are not examined or controlled (Van Der Oost et al., 2003). For example, the Nile tilapia gender influences in the activities of SOD and GST (Figueiredo-Fernandes et al., 2006), the maturity of the gonads also reflected in enzyme activity (Lopes et al., 2001) and stage of development (Ahmad et al., 2004; Chandrasekara & Pathiratne, 2007; Hwang et al., 1995; Khan & Payne, 2002; Phillips et al., 2002).

So, it is important to take into account the weight of the animals and the stage of development to obtain better results in field studies and to understand how they biochemical systems behave to exposure of environmental pollutants. It is difficult to collect animals with strictly similar size and this can result in large

standard deviations which hampers the correct interpretation of data.

Thus, the main objective of this study was to analyze the responses of biochemical parameters of the stress generated by exposure to benzo[a]pyrene (B[a]P), diazinon, cadmium (Cd) and copper (Cu) from three groups of Nile tilapias (*Oreochromis niloticus)*, with different size and weight and examine if in a particular case of contamination is important to consider the weight of the fish and if they respond identically.

Material and methods

Experimental fish and exposure

Male Nile tilapias juvenile (9.60 ± 3.71 g and 6.30 ± 0.86 cm) and adult (lower weight: 68.83 ± 13.29 g and 13.77 ± 1.33 cm, higher weight: 147.56 ± 26.13 g and 17.08 ± 0.89 cm) were obtained by donation from fishing club "Clube dos Trabalhadores Rurais", located in Catanduva city, São Paulo state and also by Drª. Eliane Gonçalves de Freitas, of Department of Zoology and Botany of UNESP in Sao Jose do Rio Preto. The fish were taken to Biotério of the Institute of Biociencias, Letras e Ciencias Exatas (IBILCE - UNESP), acclimated for 15 days in water tanks of 500 L (average temperature of 28 °C with constant aeration), and fed daily with proper diet. This study was in accordance with Ethical Principles in Animals Research adopted by Brazilian College of Animal Experimentation (COBEA) and it was approved by the Committee of the UNESP(protocol n. 016/09 CEEA).

The exposure was held in 20 aquariums 40 cm x 60 cm x 50 cm with six divisions with a capacity of 17 L of water each. For each treatment and control groups of adults of lower and higher weight, it was used one aquarium with five individuals per group, so it was possible that each group was composed of five real replicas, however for the juvenile group, two aquariuns were used for control and each treatment, because the amount of tissue was insufficient for analysis, therefore, a pool sample was made.

The concentrations of diazinon (1.0 $mg.L^{-1}$), B[a]P (0.5 $mg.L^{-1}$), Cu (0.5 $mg.L^{-1}$) in the form of $CuCl_2$ and Cd (0.5 $mg.L^{-1}$) in the form of Cd $(C_2H_3O_2)_2.2\ (H_2O)$ were based on previous experiments performed in our laboratory, in which we observed that they were sufficient to induce biochemical responses significantly after three days of exposure. Thus, the fish were exposed for three days, after they were anesthetized with benzocaine (40 mg L^{-1}) and sacrificed for removal of tissues (liver and gills) in which biochemical parameters were evaluated. The tissue samples taken were frozen at -80 °C for later analysis.

Enzimatic activities

Preparation of tissues - The samples of liver and gills were weighed and homogenized (1:4, mass: volume) in solution buffer (20.0 $mmol.L^{-1}$ Tris; 1.0 $mmol.L^{-1}$ EDTA; 1.0 $mmol.L^{-1}$ DL-Dithiothreitol (DTT); 0.5 $mol.L^{-1}$ sucrose and 0.15 $mol.L^{-1}$ KCl at pH 7.5) containing 1.0 $mol.L^{-1}$ protease inhibitor Phenylmethanesulfon Fluoride (PMSF), then, centrifuged at 7426 g for 20 minutes at 4 °C.

The supernatant portion was collected and subjected to a second centrifugation for one hour at 50,000 g to obtain the microsomal and cytosolic fractions. In the cytosolic fraction the activity of the enzymes CAT, GPx, SOD and GST was analyzed.

Lipid peroxidation levels (LPO) - The samples of liver and gills were weighed and homogenized (1:3 mass: volume) in Tris solution, 0.1 $mol.L^{-1}$ pH 8.0. Then, it was added 300 µL of 2-thiobarbituric acid solution (TBA) 0.4 % diluted in HCl 0.2 $mol.L^{-1}$. The samples were incubated for 40 minutes at 90 °C. Then, the reaction of MDA with TBA formed a pink solution in the samples that were placed on ice to cool at -10 °C and added to each 1.0 mL of n-butyl alcohol. Soon after, they were centrifuged at 1123 g for three minutes and collected 700 µL of the supernatant for further analysis.

The analysis was done by detection in HPLC-UV at 532 nm of the pink color product formed in the reaction of malondialdehyde (MDA) and the thiobarbituric acid (TBA) (Almeida et al., 2003, 2004). 20 µL samples of MDA-TBA were injected and monitored at 535 nm for ten minutes.

The mobile phase consisted of potassium phosphate monobasic solution 50 $mmol.L^{-1}$ (pH 7.0) with 40 % methanol, isocratic pump (1 $mL.min^{-1}$). The column used was LC-18 (150 x 4.6 mm, 5 µm in diameter pore). To quantify the MDA formed, it was made a calibration curve, injected into the HPLC, with known concentrations of MDA derivatized with TBA, in which the data were expressed in nmol $TBAR.g^{-1}$ tissue.

AChE and CbE analysis - For the activities of AChE and CbE, the samples of liver and gills were weighed and homogenized 1:4 (mass: volume) in Tris solution 0.1 $mol.L^{-1}$ (pH 8.0). The homogenate was centrifuged for 30 minutes at 4 °C at 9168 g. The supernatant was collected and frozen at -80 °C. The activities of AChE and CbE were determined spectrophotometrically through the increase in absorbance of the sample at 412 nm in the presence of acetiltiocolina and 5,5 '-ditiobis-2-nitrobenzoic acid (DTNB) as substrates for AChE analysis and for CbE analysis, in the presence of substrate feniltioacetato (Ellman et al., 1961).

SOD analysis - The SOD activity was measured at 550 nm for one minute. The reaction contai-

ned potassium phosphate solution 50 mmol.L^{-1} (pH 7.8), 0.1 mmol.L^{-1} EDTA, cytochrome c, 0.2 U xanthine oxidase (XO), xanthine solution and sample. The cytochrome c and sample's SOD competed by superoxide generated by the system xanthine / XO, through the increase in absorbance (Mccord & Fridovich, 1969).

CAT analysis - The CAT activity was determined by the decomposition of hydrogen peroxide in spectrophotometer at 240 nm at 30 °C. The decrease in absorbance was made in bucket containing the reaction (Tris solution buffer 1.0 mol.L^{-1}, 5.0 mmol.L^{-1} EDTA (pH 8.0) and H_2O_2) and the sample of the enzymatic extract (Beutler, 1975).

GPx analysis - The GPx was analyzed using the reaction (potassium phosphate solution 0.1 mol.L^{-1} and EDTA 5.0 mmol.L^{-1} at pH 7.0, glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate (NADPH), GSH and ultra-pure water). The activity was measured by the decrease of absorbance during the reduction of oxidized glutathione (GSSG), catalyzed by GR, in the presence of NADPH, monitored at 340 nm for one minute (Sies et al., 1979).

GST analysis - The GST activity was measured by the increase in absorbance at 340 nm for one minute in spectrophotometer. The reaction used 200 mmol.L^{-1} of 1-chloro-2 ,4-dinitrobenzene (CDNB) and 200 mmol.L^{-1} of GSH as substrates (Keen et al., 1976).

Protein determination - The estimation of protein concentration in the extracts was performed using bovine serum albumin (BSA) as standard and read at 595 nm in a spectrophotometer (Bradford, 1976).

Statistical analysis

The results of the analysis of biochemical biomarkers were made with Statistica 7.0 software. The enzimatic activities were first checked for normality Shapiro-Wilk's test and analyzed by ANOVA's test. It was applied the Levene's test to verify the homogeneity of the data. For homogeneous data, it was carried out Tukey's test for comparison between treatments in the same group and among the three control groups. For data that did not show homogeneity, and the normality test failed was applied the Kruskal-Wallis's test. The differences between treatments were accepted significant only the values of $p < 0.05$.

Results

As it can be observed, in groups of adults of lower and higher weight, the activity of AChE was inhibited by exposure to diazinon in gills and in liver, Tab. 1 and Tab. 2, respectively. In relation to treatment with B[a]P and Cd, occurred statistically significant decrease in the activity of AChE in gills of group of adults lower weight tilapias in compared to their controls, Tab. 1.

For exposure to diazinon in gills, the CbE enzyme activity was inhibited in adults of lower and higher weight.

The SOD activity in gills, Tab. 1, was inhibited in the group of juvenile tilapias to exposure to diazinon and Cd. The activity in group of adults of higher weight showed induction significant for exposure to diazinon and B[a]P.

In juvenile tilapias the activity of CAT was induced for exposure to Cu in gills, Tab. 1, and inhibited for exposure to B[a]P in liver, Tab. 2.

The GPx activity was significantly inhibited by exposure to B[a]P and Cu in gills, Tab. 1, of group of adults of lower weight. As well the activity in liver, Tab. 2, was also inhibited by all treatments compared to control of adult of higher weight.

For the group of juvenile tilapias, the MDA levels decreased significant only for exposure to Cd in gills, Tab. 1.

In the GST activity in the liver (Tab. 2) the exposure to B[a]P, Cu and Cd inhibited the enzyme activity in the group of juvenile tilapias. In adults of lower weight occurred an increase in activity for exposure to B[a]P in gills, Tab. 1. Comparing the two tissues, the gills had more answers than the liver.

There was significant difference between at least two of the three stages of development controls in the activity of AchE, CbE SOD, CAT, MDA levels and GST in gills, Tab. 1, and CAT, MDA levels and GST in liver, Tab. 2.

Discussion

As it is known, the esterases are inhibited by organophosphate and carbamate pesticides (Caldas, 2000), as well as by metals (Frasco et al., 2005). This was demonstrated in this study, the activity of AChE was inhibited by exposure to diazinon in group of adults of lower weight, in gills, and in adults of higher weight, in liver, but the juvenile group not showed significant difference. Many researchers have reported the inhibition of AChE in concentrations from 0.01 to 10.0 mg.L^{-1} of organophosphate (Chandrasekera & Pathiratne, 2005; Dembele et al., 2000; Durmaz et al., 2006; Lang et al., 1997).

According Banni et al. (2010), after 48 h and 72 h of exposure to B[a]P, AChE was inhibited and was not correlated to the enzymes of phase I and II, just as was observed in this study for the enzyme of phase II. They suggest that inhibition of AChE may be due to acute exposure of the PAH (daily 19 µg.L^{-1}) whereas the enzyme is considered highly sensitive to changes in biotic and abiotic.

On the other hand, Vieira et al. (2008) analyzed the effect of B[a]P in concentration of 0.016 mg.L^{-1} in the common goby fish Kroyer,

Tab. 1. Enzymatic activities measured in the gills of Nile tilapias (*Oreochromis niloticus*, Linnaeus, 1758).

Enzyme Group		Juvenile	Adults of lower weight	Adults of higher weight
AChE	Control	0.0895 ± 0.057	0.1709 ± 0.038	0.0660 ± 0.053[b]
	Diazinon	0.0730 ± 0.019	0.0250 ± 0.008[*]	0.0219 ± 0.008
	B[a]p	0.0967 ± 0.025	0.0912 ± 0.036[*]	0.0888 ± 0.030
	Cu	0.0557 ± 0.023	0.1251 ± 0.039	0.0885 ± 0.041
	Cd	0.0564 ± 0.015	0.0964 ± 0.037[*]	0.0606 ± 0.024
CbE	Control	0.1897 ± 0.078[b]	0.3896 ± 0.108	0.1785 ± 0.057[b]
	Diazinon	0.1361 ± 0.028	0.1094 ± 0.022[*]	0.0798 ± 0.016[*]
	B[a]p	0.1816 ± 0.055	0.2080 ± 0.037	0.1676 ± 0.039
	Cu	0.1422 ± 0.055	0.2501 ± 0.025	0.1539 ± 0.047
	Cd	0.1207 ± 0.044	0.1925 ± 0.057	0.1226 ± 0.037
SOD	Control	0.6927 ± 0.086	0.6641 ± 0.089	0.5656 ± 0.054[a]
	Diazinon	0.5869 ± 0.063[*]	0.6030 ± 0.122	0.6716 ± 0.065[*]
	B[a]p	0.6011 ± 0.046	0.5880 ± 0.101	0.6938 ± 0.063[*]
	Cu	0.6075 ± 0.087	0.6148 ± 0.109	0.6598 ± 0.069
	Cd	0.5285 ± 0.062[*]	0.6744 ± 0.110	0.5546 ± 0.114
CAT	Control	12.7703 ± 1.048	5.8989 ± 1.490[a]	6.6958 ± 0.632
	Diazinon	13.8341 ± 0.531	5.1449 ± 0.466	6.8068 ± 1.180
	B[a]p	12.8294 ± 0.741	5.8995 ± 1.512	6.6870 ± 0.622
	Cu	14.4545 ± 0.866[*]	6.0016 ± 1.731	5.9804 ± 1.590
	Cd	13.5220 ± 0.889	6.3512 ± 0.612	6.3527 ± 1.423
GPx	Control	0.0532 ± 0.010	0.0561 ± 0.005	0.0510 ± 0.011
	Diazinon	0.0566 ± 0.008	0.0537 ± 0.005	0.0638 ± 0.007
	B[a]p	0.0533 ± 0.006	0.0363 ± 0.005[*]	0.0573 ± 0.009
	Cu	0.0613 ± 0.016	0.0380 ± 0.010[*]	0.0618 ± 0.016
	Cd	0.0617 ± 0.008	0.0637 ± 0.010	0.0473 ± 0.005
MDA	Control	1.3163 ± 0.443	1.5752 ± 0.330	0.8067 ± 0.336[b]
	Diazinon	1.0998 ± 0.195	0.9702 ± 0.242	0.5175 ± 0.107
	B[a]p	0.9280 ± 0.229	1.4087 ± 0.228	0.7210 ± 0.170
	Cu	0.8354 ± 0.123	1.5024 ± 0.463	0.8076 ± 0.215
	Cd	0.6971 ± 0.202[*]	1.3908 ± 0.481	0.9940 ± 0.216
GST	Control	0.1815 ± 0.019	0.0972 ± 0.028[a]	0.1217 ± 0.006
	Diazinon	0.2263 ± 0.040	0.1242 ± 0.028	0.1208 ± 0.019
	B[a]p	0.2085 ± 0.029	0.2140 ± 0.043[*]	0.2532 ± 0.038
	Cu	0.2348 ± 0.030	0.0935 ± 0.011	0.0853 ± 0.010
	Cd	0.2075 ± 0.039	0.1004 ± 0.014	0.0854 ± 0.014

Activity expressed in U/mg protein (AChE, CbE, GST, CAT, GPx and SOD) the values are expressed in means ± standart deviation. (*) Significant difference from control. (a) Significant difference from control of juvenile group. (b) Significant difference from control of adults with lower weight group, $p<0.05$. B[a]p = benzo[a]pyrene; Cu = cupper; Cd = cadmium; AChE = acetylcholinesterase; CbE = carboxylesterase; SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase; MDA = lipid peroxidation levels; GST = glutathione S-transferase. For each treatment was used five individuals per group.

Tab. 2. Enzymatic activities measured in the liver of Nile tilapias (*Oreochromis niloticus*, Linnaeus, 1758).

Enzyme Group		Juvenile	Adults of lower weight	Adults of higher weight
AChE	Control	0.1239 ± 0.050	0.0957 ± 0.009	0.1220 ± 0.036
	Diazinon	0.0661 ± 0.046	0.0235 ± 0.009	0.0160 ± 0.007*
	B[a]p	0.0509 ± 0.022	0.1383 ± 0.058	0.1428 ± 0.057
	Cu	0.0578 ± 0.033	0.1567 ± 0.049	0.1525 ± 0.086
	Cd	0.0813 ± 0.061	0.1309 ± 0.053	0.1509 ± 0.042
CbE	Control	1.0471 ± 0.404	1.4584 ± 1.129	1.5925 ± 0.454
	Diazinon	0.8437 ± 0.383	1.5566 ± 0.384	1.0841 ± 0.089
	B[a]p	0.6180 ± 0.213	1.6509 ± 0.487	1.2931 ± 0.557
	Cu	0.6303 ± 0.197	1.6754 ± 0.478	0.9616 ± 0.089
	Cd	0.7260 ± 0.253	1.7943 ± 0.252	1.8194 ± 0.676
SOD	Control	0.5669 ± 0.101	0.6246 ± 0.108	0.5740 ± 0.082
	Diazinon	0.5265 ± 0.094	0.6847 ± 0.054	0.6266 ± 0.109
	B[a]p	0.6027 ± 0.086	0.6060 ± 0.085	0.5006 ± 0.047
	Cu	0.6399 ± 0.100	0.6380 ± 0.104	0.6236 ± 0.053
	Cd	0.6146 ± 0.133	0.6402 ± 0.102	0.6610 ± 0.077
CAT	Control	74.8480 ± 8.723	48.7240 ± 14.790[a]	59.7166 ± 14.862
	Diazinon	103.7178 ± 21.682	61.1508 ± 14.390	54.1569 ± 2.783
	B[a]p	36.9742 ± 4.438*	56.8969 ± 6.820	60.2223 ± 7.505
	Cu	42.7550 ± 5.816	58.7311 ± 6.298	59.6421 ± 12.327
	Cd	56.8530 ± 7.297	64.2304 ± 7.971	61.8824 ± 9.946
GPx	Control	0.0510 ± 0.009	0.0759 ± 0.036	0.0621 ± 0.011
	Diazinon	0.0671 ± 0.015	0.0511 ± 0.011	0.0466 ± 0.010*
	B[a]p	0.0351 ± 0.009	0.0441 ± 0.017	0.0422 ± 0.005*
	Cu	0.0346 ± 0.013	0.0497 ± 0.011	0.0377 ± 0.007*
	Cd	0.0348 ± 0.007	0.0650 ± 0.023	0.0368 ± 0.004*
MDA	Control	0.2897 ± 0.217[b]	2.5857 ± 1.272	1.4331 ± 0.152
	Diazinon	0.1902 ± 0.114	2.1627 ± 0.241	1.3610 ± 0.364
	B[a]p	0.1798 ± 0.053	2.3528 ± 0.600	0.5764 ± 0.227
	Cu	0.0883 ± 0.032	1.6548 ± 0.314	1.4329 ± 0.315
	Cd	0.1309 ± 0.076	2.3116 ± 0.389	0.5738 ± 0.703
GST	Control	3.0901 ± 0.498	1.1782 ± 0.659[a]	1.9342 ± 0.687
	Diazinon	2.8830 ± 0.749	1.4464 ± 0.461	1.8682 ± 0.310
	B[a]p	1.8502 ± 0.279*	1.0806 ± 0.546	1.7281 ± 0.208
	Cu	1.6822 ± 0.340*	1.5504 ± 0.329	1.6543 ± 0.533
	Cd	1.9727 ± 0.087*	1.7272 ± 0.381	1.4367 ± 0.344

Activity expressed in U/mg protein (AChE, CbE, GST, CAT, GPx and SOD) the values are expressed in means ± standart deviation. (*) Significant difference from control. (a) Significant difference from control of juvenile group. (b) Significant difference from control of adults with lower weight group, $p<0.05$. B[a]p = benzo[a]pyrene; Cu = cupper; Cd = cadmium; AChE = acetylcholinesterase; CbE = carboxylesterase; SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase; MDA = lipid peroxidation levels; GST = glutathione S-transferase. For each treatment was used five individuals per group.

1838 (Pomatoschistus microps), after 96 h and observed the damage to fish caused by the inhibition of AChE activity and induction of GST in accordance with the results obtained for the group of adult of lower weight. The results obtained in adults of lower weight in gills of the present study corroborates this findings.

In relation to CbE, the data were very similar to those of AChE in respect to gills that were more responsive than the liver in the conditions laid down, probably because the gills were in direct contact with contaminated water. Trídico et al. (2010) reported that the activity of CbE in Nile tilapias was inhibited in gills two days after exposure to diazinon at a concentration of 0.5 $mg.L^{-1}$, in accordance with the data presented in this research, indicating that the esterases in the gills are more sensitive than in the liver.

It is possible that the concentration used and/or time of exposure has not been sufficient to cause effects in liver, indicating the need for new studies under different experimental conditions. According to Barron et al. (1999), CbE levels found in liver of rainbow trout Walbaum, 1792 (*Oncorhynchus mykiss*), were higher than in other tissues, as observed in this study. This may justify a higher sensitivity in gills to contaminants.

In respect to the parameters of oxidative stress, the SOD was inhibited in the group of juvenile tilapias, to diazinon and Cd exposure and in the group of adults of higher weight the enzimatic activity was increased to diazinon and B[a]P exposure in gills. According Oruç & Usta (2007), the increase or decrease in the antioxidant system under stress, depends on the intensity and duration of exposure, and susceptibility of the species that are being subjected to this condition. Peters & Livingstone (1996) observed in embryos of the species of fish *Scophthalmus maximus*, Linnaeus, 1758, with 11 days of life, that the SOD activity decreased after Cd exposure, during embryo development and indicated that there was no need to detoxify the radical $O_2^{\bullet-}$.

A similar result was also reported by Cao et al. (2010) when exposed for 80 days fish larvae of the species bastard-halibut (*Paralichthys olivaceus*, Temminck & Schlegel, 1846) with different concentrations of Cd. They noted that in concentration of 48 $\mu g.L^{-1}$ Cd the SOD activity was inhibited. On the other hand, when juvenile halibut were exposed to Cd (12, 24 and 48) $\mu g.L^{-1}$, the SOD was induced. Thus, the researchers suggested that changes in SOD activity, by exposure to diazinon and Cd, may be affected by several factors, including stage of development, as it was also evidenced in this work.

The CAT activity, in the group of juvenile tilapias, was significantly inhibited by exposure to B[a]P in liver, probably, this result indicates that this tissue was not affected by the action of ROS and did not cause oxidative stress. Palanikumar et al. (2011) observed opposite results when milk fish (Chanos chanos, Forsskål, 1775) were exposed to B[a]P, (0.001, 0.004, 0.007, 0.014 and 0.031) $mg.L^{-1}$ by 96 h, in which the CAT activity was induced in head, gills and muscles with increasing of concentrations, thus, suggested that the CAT was activated by conversion of H_2O_2 to H_2O. As well as in the group of juvenile to Cu exposure in gills, the activity was increased.

Maria & Bebianno (2011) also observed an increasing of the CAT activity in digestive glands of mussels (*Mytilus galloprovincialis*, Lamarck, 1819) after exposure for seven days to 10.0 $\mu g.L^{-1}$ B[a]P, suggesting that this result was a sign of that occurred oxidative stress generated by xenobiotic metabolite. Banni et al. (2010) also noted that after 24 h and 48 h of treatment with B[a]P in concentration of 75 nM, the CAT activity in digestive glands of *M. galloprovincialis* was induced, indicating that this effect was due to the metabolite of B[a]P.

Vieira et al. (2008) reported that PAH are capable of producing $O_2^{\bullet-}$ in fish species *P. microps* after 96 h of exposure to B[a]P at concentrations of (4, 8 and 16) $\mu g.L^{-1}$, which were catalyzed by SOD converting it to H_2O_2, which right after was degraded by the action of CAT and GPx. However, in this work was not possible to verify this hypothesis probably the B[a]P did not generate superoxide radicals in the Nile tilapias.

The reductions observed in the GPx activity in gills and livers may also indicate that other antioxidant enzymes that were not tested had been effective against the formation of ROS, compensating the decrease of antioxidant enzymes, such as GPx. This result was similar to the study proposed by Atli & Canli (2010) in which the low activity of GPx was compensated by higher CAT after exposure for 48 h by Cd (10 mM) in kidney of Nile tilapias.

For exposure to diazinon in liver, the GPx was inhibited in the group of adult of higher weight tilapias. This decrease in enzyme activity was also observed by Oruç & Usta (2007) in kidneys of colorful carp (*Cyprinus carpio*, Linnaeus, 1758) exposed to a concentration of 0.018 ppb of diazinon for 15 days, they proposed that the decreased activity, indicating the inefficiency of the tissue in neutralizing the damage caused by ROS. On the other hand, Durmaz et al. (2006) suggested that the CAT and the GPx can compete with each other for the same substrate that generated, in case, a reduction in GPx activity of the digestive tract of Nile tilapias after one and 30 days of exposure to diazinon (1 and 2 ppm).

In the group of adult of higher weight tilapias, the B[a]P, Cd and the Cu exposure also

caused reduction in GPx activity in liver. Almeida et al. (2009) observed a decrease of the GPx in liver of Nile tilapias after 96 h of exposure to Cd (0.75 mg L-1), Liu et al. (2006) also noted inhibition of activity, however, to liver goldfish (*Carassius auratus*, Linnaeus, 1758) after 40 days of treatment with Cu in concentrations of 0.005 and 0.05 mg.L^{-1}, indicated that the accumulation of H_2O_2 can impact the function of CAT and GPx.

In the present study, we could not observe the relationship among the changes in antioxidant enzymes with the lipid peroxidation levels in liver. It may be that other antioxidant enzymes not analyzed in this work have shown increased activity, which would cause a greater cell protection, causing decreased levels of MDA.

In gills of group of juvenile tilapias, the MDA levels in all treatments showed decrease, however, only to Cd exposure was significant. In the study performance by Almeida et al. (2009) it was observed a significant increase in the level of lipid peroxidation in liver of Nile tilapias exposed to Cd (0.75 mg.L^{-1}) for two days. As the present study showed the opposite result, this may indicate that the concentration of Cd used did not affect the groups.

In gills, it also was perceived the relation among the antioxidant enzymes tested and the levels of lipid peroxidation. In the group of juvenile tilapias, the CAT and the GPx were slightly induced by exposure to Cu and Cd, which probably caused reduction in the level of MDA. According to Bouraoui et al. (2009), Cu caused induction of CAT activity simultaneously with increased levels of lipid peroxidation in annelids *Hediste diversicolor*, O.F.Müller, 1776, after 36 h of exposure. Thus, they suggested that this antioxidant defense was inefficient. This result was opposite to present research, possibly, other enzymes not tested proved to be more efficient since the levels of MDA formed for exposure to Cu in the group of juvenile tilapias in gills were slightly reduced.

In the group of adults of lower weight tilapias, this relation can be done by esterase enzymes tested, such as AChE and CbE, which were activated by exposure to diazinon and that probably have caused a slight decrease in the level of MDA. This relation can be attributed to the effectiveness of the activity of AChE and CbE in gills for cell protection.

According to Oruç & Usta (2007) a decrease in AChE activity with an increase in lipid peroxidation after exposure for five days to diazinon in muscle of carp indicated the participation of ROS in cellular oxidative damage caused by the toxicity of diazinon. In the present study, this toxicity did not cause oxidative damage in gills of adult of lower weight tilapias. The same behavior was observed by Uner et al. (2006) that after 24 h exposure to 1 mg.L^{-1} of diazinon, there was a reduction in the levels of MDA in brain of Nile tilapias, indicating the protective effect of antioxidant systems.

Durmaz et al. (2006) also reported a decrease in MDA levels in alimentary tract of Nile tilapias after seven, 15 and 30 days of exposure to 1 ppm of diazinon, suggesting the probable decomposition of H_2O_2 by antioxidant enzymes SOD and CAT.

Palanikumar et al. (2011) suggested that high levels of lipid peroxidation in milk fish exposed to B[a]P were due a lack effectively remove of intermediate products such as H_2O_2 and $O_2^{\bullet-}$ by antioxidant enzymes. Nevertheless, in present research, untested enzymes of the antioxidant system, in tilapias of higher weight, probably have played a role in defense against ROS for this exhibition, relating with a slight reduction in the level of MDA formed.

These results indicated that the antioxidant system of juvenile tilapias was more efficient than the oxidant system of adults in the conditions tested in this work. Likewise, that the gills were more responsive than the liver.

In liver, the exposure to B[a]P, Cu and Cd inhibited the GST activity in the juvenile tilapias. These results are according to a research realized by Atli & Canli (2010) in which juvenile tilapias were exposed to 10 mM $CuCl_2.2H_2O$ for 48 h and it was observed a reduction in GST activity in liver and suggested that the excess Cu rapidly oxidizes GSH.

Pretto et al. (2011) reported the GST inhibition in gills of catfish (*Rhamdia quelen*, Quoy & Gaimard, 1824) after seven and 14 days of exposure to Cd (236 µg.L^{-1} and 414 µg.L^{-1}), due to the high production of radicals, caused by exposure to the metal. The same behavior was obtained in liver of group of juvenile tilapias of this study.

Cao et al. (2010) also observed reduction in enzyme activity during the metamorphosis of larvae of the species of fish *P. olivaceus* after 80 days of treatment with Cd at concentrations of 6, 12, 24 and 48 g.L^{-1}, and reported that this inhibition may be due to direct or indirect action of Cd on the enzyme or through the production of ROS that interacts with the enzyme or by the exhaustion of GSH.

In adult of lower and higher weight tilapias occurred a significant increase in GST activity for exposure to B[a]P in gills. However, only in group of lower weight, this increase in activity was significant. This same result was also observed by Vieira et al. (2008) in heads of fish species common goby after 96 h exposure to B[a]P (0.016 mg.L^{-1}). In this work they suggested that the conjugation by GSH is involved in the removal of B[a]P, indicating the high affinity receptor composed of aromatic hydrocarbons of GST.

Likewise Palanikumar et al. (2011) suggested, when they observed the GST induction after exposure to B[a]P (0.001, 0.004, 0.007, 0.014, 0.031) mg.L^{-1} for 96 h, in head, in gills and in muscle dorsal milk fish. Bouraoui et al. (2009) also noted an increase in GST activity after 36 h and 48 h of exposure to B[a]P (1 µM) in earthworms *H. diversicolor*.

In this study, the GST in gills of juvenile group showed attenuation of oxidative damage caused by exposure to B[a]P. Sayedd et al. (2003) reported that high values in GST activity are related to protection against ROS. On the other hand, the opposite result was observed in liver of juvenile tilapias. Thus, these data indicated that, for this enzyme, the liver was more responsive to the gills.

Conclusion

The data revealed that there is difference in enzymatic responses in relation the tissue analyzed, the stage of development and weight gain, so, it is important, in field studies, taking into account these factors to obtain more accurate results when using tilapias for biomonitoring environmental contamination.

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