ANALYSIS OF OXIDATIVE STRESS IN GILL AND LIVER OF OREOCHROMIS MOSSAMBICUS (PETERS) LIVING IN THE PALAMAN RIVER, CHIDAMBARAM, TAMILNADU, INDIA

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Abstract
In the aquatic environment, despite the presence of constitutive or enhanced antioxidant defence systems, increased levels of oxidative damage will occur in organisms exposed to contaminants that stimulate the production of reactive oxygen species. The increased ROS production and subsequent oxidative stress has been associated with a pollutant-mediated mechanism of toxicity in fish organs. The present study has been designed to understand the oxidative stress response modulation in fish gill and liver focussing on the various antioxidants that counter peroxidative damage in the fish Oreochromis mossambicus collected from Palaman river, Chidambaram, Tamilnadu at different seasons from January to December 2014.

Water samples were collected between 7.00 am and 9.00 am. The months were divided into different seasons such as Post Monsoon (January, February and March); Summer (April, May and June); Pre-Monsoon (July, August and September) and Monsoon (October, November and December). The average of monthly data was taken for representing the seasonal data. The analysis of the physical as well as chemical parameters of water such as water temperature, pH, dissolved oxygen, chloride, sulphate, nitrate and BOD using standard protocols. In general, the water quality parameters were within the permissible limit. The activities of antioxidant enzymes were analysed and the mean SOD and CAT activities were found to be significantly higher in liver (P < 0.05). However, the activity of gill CAT was found to be significantly lower (P < 0.05). The mean activities of G6PD in liver and gill were also studied. The G6PD enzyme values were significantly higher in the liver, whereas the activity of G6PD in the gill of fish was lower (P < 0.05). The values of GSH in liver of the fish were observed to be significantly higher (P < 0.05) whereas GSH levels in gill values were lower. The mean values of LPO in liver and gill of the O. mossambicus were found to be higher. Particularly in gill tissue, LPO level was found to be about 6 times higher.

Keywords: Palaman river, anti-oxidants, pollution.

INTRODUCTION
It is well-known that the aquatic environment is contaminated with many types of organic and non-organic pollutants (Sen and Kirikbakan, 2004) of municipal, industrial, agricultural and mining industry origin (Lenartova et al., 1997). Such pollutants affect the integrity of ecosystems and physiological fluctuations of animals (Sen and Kirikbakan, 2004) as well as of people as consumers (Perez – Lopez et al., 2002). Harmful effects on population are sometimes difficult to prove in wild animals because many of these effects tend to manifest themselves only after a rather long period of time (Van der Oost et al., 2003). Fish have been used as aquatic contamination indicators for many years. In the case of an environmental disaster, they are unable to leave the site affected (Gadzala et al., 2004), bioaccumulate toxic substances (Andrade et al., 2004) and because they are the last link in the food chain in the aquatic environment they may negatively influence the safety of food and raw materials of animal origin (fish and fish products) (Gadzala et al., 2004).

Understanding the reaction and response to the exposure to toxic substances in fish may be very important from the environmental point of view (Van der Oost et al., 2003) as freshwater ecosystems are under the pressure of complex mixtures of contaminants released in the environment due to various human activities. Hydrological changes, hydromorphological degradation and invasive species also can contribute to the set of stressing factors (Amado et al., 2004).
2006; Sureda et al., 2006; Dos Anjos et al., 2011). Oxidative stress in aquatic organisms, principally fish, has great importance for environmental and aquatic toxicology. Because oxidative stress is induced by many chemicals, including some pesticides, these contaminants may stimulate reactive oxygen species and alteration in antioxidant systems. Pro-oxidant factor actions in fish can be used to assess pollution of specific areas or worldwide marine pollution (Üner et al., 2006 and Slaninova et al., 2009). The increased ROS production and subsequent oxidative stress has been associated with a pollutant-mediated mechanism of toxicity in fish tissues. The use of biochemical or physiological measurements as indicators of toxicity is under constant development and has the advantage of delineating effects prior to the manifestation of diseases. Hence the present study was designed to understand the oxidative stress response modulation in fish gill and liver focussing on the various antioxidants that counter peroxidative damage in the fish Oreochromis mossambicus collected from Palaman river, Chidambaram, Tamilnadu.

MATERIALS AND METHODS

Study Area: Palaman river is a major waterbody flowing through Chidambaram. The major landmarks nearby the Palaman Canal’s banks in Chidambaram are Thillai Amman Temple, Anna Kulam, Kumaran kulam, Annamalai University Distance Education Center, Chidambaram Bus Stand, Ganapprakasan Kulam, Ammapettai Bus Stop, Chennai - Nagapattinam Highway and State Highway 212 (Fig.1).

Physico-chemical analysis: Water samples were collected between 7.00am and 9.00am. The months were divided into different seasons such as Post Monsoon (January, February and March); Summer (April, May and June); Pre-Monsoon (July, August and September) and Monsoon (October, November and December). The average of monthly data was taken for representing the seasonal data. The analysis of the physical as well as chemical parameters of water such as water temperature, pH, dissolved oxygen, chloride, sulphate, nitrate and BOD using standard protocols (APHA, 2008). Temperature and pH were recorded immediately at study site itself.

Preparation of liver and gill homogenates: Fish liver and gill tissues were rapidly removed and frozen in a dry ice-refrigerated container and kept until examination. Prior to the analysis, tissue samples were unfrozen. Then they were weighed, perfused with 1.15% ice-cold KCl, minced, and homogenized in 5 volumes (w/v) of the same solution, using a Heidolph 50110 R2R0 homogenizer. Antioxidant systems and LPO assays were performed on the supernatant preparation in a Sorvall RC-2B centrifugation of the homogenate at 14,000 rpm for 30 min at +4 °C (Gul et al., 2004). G6PD activity was analyzed with Beulter’s method (1984). CAT activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm (Beulter, 1984). SOD activity was measured according to the method described by Fridovich (1974). GST activity was measured by the method described by Mannervik and Guthenberg (1981). GSH levels were determined by measuring a highly coloured yellow anion formed by the reduction of DTNB [5, 5’-Dithiobis (2-nitrobenzoic acid)] with nonprotein sulphhydryl compounds of tissue samples (Beulter, 1984). The levels of GSH were calculated as μmol/mg protein. The LPO level in the tissue samples was expressed as malondialdehyde (MDA) (Okawa et al., 1979). Total protein contents were determined using the method by Lowry et al., (1951), using bovine serum albumin as a standard.

Statistical analysis: The data obtained were subjected to standard statistical analysis. Duncan’s multiple range test (Bruning and Kintz, 1968) was performed to determine whether the parameters altered significantly with different seasons. The significance of the results was ascertained at P < 0.05.

RESULTS

Physico-chemical characteristics of the water: Water temperature ranged from 25 ± 31°C. Maximum temperature noted in the summer season and minimum value recorded at monsoon season. pH did not show much variation in all the seasons. Low value of DO was recorded in the pre monsoon period as 3.02mg/L and maximum value of 5.6 mg/l in the monsoon period. The value of chlorides ranged from 1.6 – 3.8 mg/l. The minimum value recorded in the monsoon period and the maximum value of 3.8mg/l recorded at the pre monsoon period. Sulphates showed an increasing trend as post monsoon > summer > pre monsoon = monsoon. Similar trend was noted in nitrate as 0.12, 0.13, 0.14 mg/l for post monsoon, summer, pre monsoon and monsoon periods respectively (Table 1.). BOD values are also within permissible limit and recorded 1.0, 1.07, 0.7 and 1.20 for post monsoon, summer, pre monsoon and monsoon periods. In general, the water quality parameters were within the permissible limit.

Superoxide Dismutase (SOD): The mean activities of antioxidant enzymes are shown in Table 2. The activities of antioxidant enzymes were analysed and the mean SOD and CAT activities were found to be significantly higher in liver (P < 0.05). However, the activity of gill CAT was found to be significantly lowers (P < 0.05). The activity of SOD found to be reduced in gill as as 1.62 µ moles / mg protein in monsoon period. The value gradually increased as 2.65 µ moles / mg protein in post monsoon period, 4.58 µ moles / mg protein in summer season and 4.69 µ moles / mg protein in pre monsoon period (Table 2). But in liver...
the SOD activity showed an increased value of 6,590 µ moles / mg protein in monsoon season. The value decreased as 5,290, 4,890 and 4,590 µ moles / mg protein respectively for post monsoon, summer and pre monsoon seasons.

Catalase (CAT): CAT activities were found to be higher in gill at pre monsoon period as 7.68 µ moles / mg protein. Low value of CAT recorded is gill was 2.52 µ moles / mg protein in monsoon period. The values gradually increased from the order monsoon < post monsoon < summer < pre monsoon (Table 2). In liver, the highest CAT activity recorded in the pre monsoon period as 162.11 µ moles / mg protein. Like in gill, the CAT activity was less in monsoon period as 129.50 µ moles / mg protein.

Glutathione S transference (GST): In gill, the GST value was increased in monsoon season (10.70 µ moles / mg protein / min). The value showed a decreasing trend as 7.15, 4.24 µ moles / mg protein / min at post monsoon and summer periods (Table 2). In liver, the GST value was lowest value of 0.006 µ mole / mg protein / min recorded during monsoon period in the liver. The value recorded during post monsoon and summer was 0.024, 0.049 and 0.072 and 0.076 µ g /g wet wt. of tissue for Monsoon, Post Monsoon, summer and Pre Monsoon seasons (Table 2). Similar trend in GSH was noted in liver also. The values were 0.024, 0.049, 0.072 and 0.076 µ g /g wet wt. of tissue for Monsoon, Post Monsoon, summer and Pre Monsoon seasons respectively (Table 2).

Glucose 6 Phosphate Dehydrogenase (G6PD): The G6PD in the gill showed a maximum value of 0.008 µ moles / mg protein / min. The minimum value recorded in monsoon period as 0.004 µ moles / mg protein / min. The value of G6PD in the gill was in the order Pre Monsoon > summer > Post Monsoon > Monsoon (Table 2). In liver, the lowest value of 0.006 µ moles / mg protein / min was recorded in Monsoon season. The value increased as 0.12, 0.028 and 0.031 µ moles / mg protein / min respectively at Post Monsoon, summer and Pre Monsoon seasons respectively (Table 2).

Reduced Glutathione (GSH): The GSH content of the gill was in the order 0.011 > 0.042 > 0.056 > 0.061 µ g /g wet wt. of tissue for Monsoon, Post Monsoon, summer and Pre Monsoon seasons (Table 2). Similar trend in GSH was noted in liver also. The values were 0.024, 0.049, 0.072 and 0.076 µ g /g wet wt. of tissue for Monsoon, Post Monsoon, summer and Pre Monsoon seasons (Table 2).

Lipid Peroxidation (LPO): The mean values of LPO in gill and of the O. mossambicus were found to be higher. Particularly in gill tissue, LPO level was found to be maximum as 20.81 µ g /g wet wt. of tissue at Pre Monsoon period. Low value of LPO was noted as 5.0 µ g /g wet wt. of tissue in the gill at Monsoon period. The order of increase in different seasons was Monsoon < Post Monsoon < summer < Pre Monsoon (Table 2).

Table 1. Seasonal variation of physico-chemical parameters of Palaman river from January to December 2014.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Post- Monsoon</th>
<th>Summer</th>
<th>Pre- Monsoon</th>
<th>Monsoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Temperature</td>
<td>28 ± 2.00</td>
<td>31 ± 2.00</td>
<td>30 ± 2.00</td>
<td>25 ± 2.00</td>
</tr>
<tr>
<td>pH</td>
<td>7.8 ± 1.00</td>
<td>8.2 ± 1.00</td>
<td>8.4 ± 1.00</td>
<td>8.1 ± 1.00</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>4.5 ± 1.20</td>
<td>3.8 ± 1.00</td>
<td>3.02 ± 1.20</td>
<td>5.6 ± 1.50</td>
</tr>
<tr>
<td>Chloride</td>
<td>2.6 ± 0.05</td>
<td>3.2 ± 0.02</td>
<td>3.8 ± 0.05</td>
<td>1.6 ± 0.06</td>
</tr>
<tr>
<td>Sulphate</td>
<td>2.5 ± 0.25</td>
<td>2.6 ± 0.24</td>
<td>2.8 ± 0.26</td>
<td>2.8 ± 0.20</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.12 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.13 ± 0.05</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>BOD</td>
<td>1.0 ± 0.00</td>
<td>1.07 ± 0.02</td>
<td>0.7 ± 0.01</td>
<td>1.20 ± 0.01</td>
</tr>
</tbody>
</table>

*Average Values ± SE based on DMRT; n=3.

*Table 2. Activity of different antioxidant enzymes in the gill and liver of O. mossambicus

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Antioxidants</th>
<th>Post- Monsoon</th>
<th>Summer</th>
<th>Pre- Monsoon</th>
<th>Monsoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>SOD</td>
<td>2.65 ± 0.01</td>
<td>4.58 ± 0.02</td>
<td>4.69 ± 0.05*</td>
<td>1.62 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>CAT</td>
<td>5.25 ± 0.02</td>
<td>7.64 ± 0.01*</td>
<td>7.68 ± 0.02*</td>
<td>2.52 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>GST</td>
<td>7.15 ± 0.01</td>
<td>4.24 ± 0.01</td>
<td>4.32 ± 0.01*</td>
<td>10.70 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>G6PD</td>
<td>0.005 ± 0.00</td>
<td>0.006 ± 0.001</td>
<td>0.008 ± 0.001</td>
<td>0.004 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>GSH</td>
<td>0.042 ± 0.02*</td>
<td>0.056 ± 0.002*</td>
<td>0.061 ± 0.01*</td>
<td>0.011 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>LPO</td>
<td>16.02 ± 1.12*</td>
<td>20.0 ± 1.15</td>
<td>20.81 ± 1.16</td>
<td>5.0 ± 0.75</td>
</tr>
<tr>
<td>Liver</td>
<td>SOD</td>
<td>5.290 ± 1.10</td>
<td>4.890 ± 1.16</td>
<td>4.590 ± 1.05</td>
<td>6.590 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>CAT</td>
<td>136.7 ± 12.10</td>
<td>148.6 ± 14.12*</td>
<td>162.11 ± 10.75*</td>
<td>129.5 ± 10.16</td>
</tr>
<tr>
<td></td>
<td>GST</td>
<td>130.02 ± 7.64</td>
<td>236.12 ± 2.32*</td>
<td>239.15 ± 6.12*</td>
<td>120.2 ± 4.25</td>
</tr>
<tr>
<td></td>
<td>G6PD</td>
<td>0.012 ± 0.02</td>
<td>0.028 ± 0.01*</td>
<td>0.031 ± 0.06*</td>
<td>0.006 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>GSH</td>
<td>0.049 ± 0.25*</td>
<td>0.072 ± 0.28*</td>
<td>0.076 ± 0.27*</td>
<td>0.024 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>LPO</td>
<td>4.0 ± 0.25</td>
<td>5.0 ± 0.15</td>
<td>6.0 ± 0.50</td>
<td>3.0 ± 0.25</td>
</tr>
</tbody>
</table>

*SOD, CAT expressed as µ moles / mg protein; GST, G6PD in µ moles / mg protein / min; GSH, LPO in µ g /g wet wt. of tissue. Average Values ± SE based on DMRT; n=3; *p > 0.05
DISCUSSION

Any water body is a potential body medium for the production of aquatic organisms (Sikoki and Veen, 2004; Bronmark and Hansson, 2005). Therefore water quality affects a corresponding change in the relative composition and abundance of the organisms in that water (Hepper, 1988; Sunder raj, 1994). Water is unable to support the aquatic life, when there are no dissolved substances (Chatterjee, 1993) and the presence of different ratio may alter the quality of water. Water chemistry determines its suitability and deterioration of water quality due to continuous fluctuations in its physicochemical and biological parameters. Throughout the study period high temperature was recorded in summer and pre monsoon periods. However, WHO (1998) did not recommend any confirmed value for freshwater resources. The pH values ranged from 7.8 to 8.4 and the values were within permissible limit (Table 1). Under normal conditions, the pH value of water varies. Since phytoplankton use the carbon dioxide during day light for the production process through photosynthesis, and the pH of water increase in the day hours (Sunder raj, 1994). The narrow range of pH indicated stability as most of the aquatic organisms are adapted to an average pH and do not withstand abrupt changes (George, 1997). The relation of temperature with pH could be explained on the basis of the fact that solubility of minerals and other inorganic matter increases with increase in water temperature (Swingle, 1967). Dissolved Oxygen (DO) ranged from 3.02 to 5.6 mg/L. The highest DO recorded in the monsoon period. The chlorides are more in pre monsoon and the range is in between 3.8 – 1.6 mg/L. Sulphate level ranged from 2.8 – 2.5 mg/l. The amount of nitrate ranged from 0.14 to 0.12 mg/L. The lower concentration of nitrate may be due to biological destruction and self-purification properties of water bodies (Karna and Kulkarni, 2009). Non polluted waters are generally deficient in nitrate and phosphate levels. Biochemical Oxygen Demand (BOD) ranged from 0.7 – 1.07 mg/L. The water quality parameters analysed were within the permissible limit under investigation at all seasons.

Living systems encounter a variety of stresses during their continuous interaction with environment. Environmentally-induced stresses frequently activate the endogenous production of reactive oxygen species (ROS), most of which are generated as side products of tissue respiration. Hence, constant exposure to stressors may enhance ROS-mediated oxidative damage. Increased number of agricultural and industrial wastes enter aquatic environment and being taken up by aquatic organisms induce plural changes. Some of them directly enhance ROS formation whereas others act indirectly, for example, by binding with cellular thiols and reducing antioxidant potential. Fish are particularly threatened by water pollution. The use of sentinel species in biomonitoring needs to be discussed due to different level of their vulnerability by environmental toxicants. The activation of oxidative manifestations leads to the response of antioxidants, activation of expression of genes encoding antioxidant enzymes, elevation of the concentration of ROS scavengers. Nevertheless, there are considerable gaps in our knowledge on response to oxidative stress, particularly in the feral animals. Indeed, in field studies, wide spectrum of inter-site differences (higher, equal or lower activities of various antioxidant enzymes with tissue peculiarities and disbalance) have been observed in polluted compared to clean areas reflecting both mild stress conditions of the location or strong oxidative damage. Different models of the aquatic animal response, therefore, need to be analysed before conclusions can be drawn. In any case, the integrated approach with the appreciation of balance between prooxidant manifestations and antioxidant defence (enzymatic and nonenzymatic) in biological systems needs to be a control point to assess toxic effects under stressful environmental conditions. Aquatic environment is a sink for many environmental contaminants which can be absorbed by aquatic organisms leading to disturbing of antioxidant/prooxidant balance in fish (Lackner, 1998; Livingstone, 2001, Lushchak, 2011). That may cause oxidative stress, determined as a state when antioxidant defenses are overpowered by prooxidant forces (Livingstone, 2001, 1991). Moreover, dependent on the source of pollutant, steady-state ROS concentration can be enhanced transiently or chronically, disturbing cellular metabolism and its regulation and damaging cellular constituents (Lushchak, 2011). Synergistic or antagonistic effects of mixtures of pollutants are hardly interpreted and predicted exclusively from the chemical analyses; some contaminants are substantially accumulated in specific tissues without recorded toxic effects (Viarengo and Nott, 1993), while others demonstrate high toxicity even at low levels. These pollutants may promote the production of superoxide anion radicals by redox cycling, while transition metals such as iron catalyze the reaction of superoxide anion radicals and hydrogen peroxide to produce hydroxyl radicals through Fenton reactions (Winston and Di Gulio, 1991).

The SOD-CAT system provides the first defense against oxygen toxicity. SOD catalyzes the dismutation of the superoxide anion radical into water and hydrogen peroxide, which is detoxified by CAT activity. Usually a simultaneous induction response in the activities of SOD and CAT is observed when exposed to pollutants (Dimitrova et al., 1994). In the present study such a relationship was observed. The activities of SOD and CAT were found to be high in the liver tissue of fish due to pollution stress. The SOD activity was reported by several workers to be higher in fish from the polluted site (Rodrigueazariza et al., 1993), indicating a high production of superoxide anion radicals. The high levels of CAT in the liver tissue could be attributed to high production of peroxide radicals. Increased SOD and CAT activities in the liver may be a response to oxidative stress. As a contributory factor in water pollutant induced stress, the occurrence of high nitrite levels can be an important source of prooxidants for fish (Das et al., 2004), leading to the production of nitric radicals (nitrosative stress) as demonstrated in mammals by Lijima et al. (2003). In this study, SOD activity in the gill tissue was found to be higher whereas the CAT activity in gill tissue was found to be lower in fish collected from the polluted environment. CAT

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being a primary antioxidant defense component, eliminates hydrogen peroxide (\(2\text{H}_2\text{O}_2\rightarrow2\text{H}_2\text{O}+\text{O}_2\)) a non-radical reactive oxygen species which can penetrate through all biological membranes and directly inactivate few enzymes and is considered as a sensitive biomarker of oxidative stress before major deleterious effects occur in fish (Gul et al., 2004; Sanchez et al., 2005), protecting animals from oxidative stress. The significant CAT inhibition in the gill samples could be attributed to a high production of superoxide anion radicals by the SOD enzyme, which has been reported to inhibit CAT activity in case of excess production (Kono and Fridovich, 1982). In addition, as previously reported by Dimitrova et al. (1994), the presence of heavy metals and their role in the decrease of CAT activity should also be considered (Dimitrova et al., 1994), since Ahmad et al. (2000) reported a CAT decrease due to a high concentration of copper (Ahmad et al., 2000). As a result, we thought that while SOD-CAT systems in the liver tissues from the polluted area may be a response to oxidative stress these systems in gill tissue may not respond to oxidative stress. Therefore, increased oxidative stress in the gill tissue may lead to oxidative damage.

Glutathione-S-transferases are a family of multifunctional enzymes that are involved in the detoxification of both xenobiotics as well as endogenous reactive compounds of cellular metabolism. GST was shown to catalyze essential steps in the biosynthesis of prostaglandins and leukotrienes (Skipsey et al., 1997). GST plays a critical role in mitigating oxidative stress in all life forms and GST activity also has been widely used as a biomarker to detect stress. As an antioxidant enzyme, a GST activity either has a significant increase or decrease with different patterns according to the exposed elements or exposure conditions. GST activity varied in different tissues and organs of aquatic animals (Farombi et al., 2007). The induction of GST in liver tissue observed suggesting an activation of the liver detoxification processes probably due to the presence of organic contaminants. However, the decrease in GST activity in gill tissue suggests a significant reduction in fish capacity to detoxify and rid themselves of chemicals. Although GST induction has been widely demonstrated following exposure to some organic contaminants (Stephensen et al., 2000), its inhibition has also been reported as a non-specific response to chemical challenge (Regoli et al., 2004). In this study, the higher hepatic glutathione concentration observed in \(O.\) mossambicus indicates an adaptive and protective role for GSH against oxidative stress induced by chemical contaminants. Similarly, high levels of GSH in catfish exposed to polluted sediment (Di Gulio et al., 1992). However, the decrease in GSH content observed in the gill tissues may be due to insufficient glutathione regeneration. Fish tend to adapt to oxidative conditions to which they are exposed. The increased G6PD activity in liver tissue demonstrates increased production of NADPH used in the detoxification process. This probably reflects an adaptation to oxidative conditions. However, low gill G6PD observed in \(O.\) mossambicus indicates that the low G6PD activity in the pollution may aggravate oxidative stress. Therefore, oxidative damage in gill tissue may be present. LPO activity is regarded as one of the best biomarkers for ecological risk assessment (Van der Oost et al., 2003); its importance lies in initiating the cell membrane dissolution process, leaving cells exposed to xenobiotic factors (Muriel, 1957). Many environmental pollutants and their metabolites have shown to exert toxic effects associated with oxidative stress, producing free radicals that initiate the LPO and cause damage to membrane proteins (Gutteridge, 1997). In this study, the measurement of LPO was provided as an indicator of pollution in liver and gill tissues, as indicative of oxidative stress. Hence, the determination of biomarkers on fish populations reflects whether they are subject to stress. The findings of the present investigation suggest that oxidative stress biomarkers, especially the estimation of antioxidant systems in fish, could provide a useful indicator of pollution in bodies of water. The induction of antioxidant systems (as observed in liver), as well as their inhibition (as observed in gill) should be considered a clear indication of the presence of pollution and environmental health degradation. The measurement of LPO, which has been described in several other studies as a biomarker of the effect of pollution, was also revealed to be a useful indicator of pollution load.

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