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Siwela, Andrew H.

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Metal Accumulation and Antioxidant Enzyme Activity in *C. gariepinus*, Catfish, and *O. mossambicus*, Tilapia, Collected from Lower Mguza and Wright Dams, Zimbabwe

A. H. Siwela · C. B. Nyathi · Y. S. Naik

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Abstract The aim of this study was to measure antioxidant enzyme activities as biological indicators of pollution in tissues of two species of fish. Five *Clarius gariepinus* and three *Oreochromis mossambicus* were collected from Umguza Dam (polluted dam) whilst seven *C. gariepinus* and eight *O. mossambicus* were collected from Wright Dam (relatively pristine dam). Diphosphotriphosphodiaphorase and catalase activities were consistently lower (42 ± 2% and 78 ± 20%, respectively) in liver whilst malondialdehyde levels were two times higher in muscles of both species of fish collected from Umguza Dam. However, selenium-dependent glutathione peroxidase (Se-GPX) activity was elevated four-fold in liver and gills of *O. mossambicus* collected from Umguza Dam. Metal levels were two to five times higher in muscles of both species of fish collected from Umguza Dam. Fish from Umguza Dam seem to have responded to pollution by increasing Se-GPX specific activity in an effort to detoxify peroxides produced as a result of metal induced oxidative stress.

Keywords Antioxidant enzymes · Heavy metals · *O. mossambicus* · *C. gariepinus*

High levels of trace metals in freshwater may occur as a result of natural weathering of minerals in the sediments and bed rocks or as a result of anthropogenic activities such as mining, industrial, municipal and agricultural discharges (Winston 1991). Most trace metals are essential in small concentrations for normal metabolic processes in mammals including fish and humans. At abnormally high concentrations, metals can cause death in fish. Metals such as lead (Pb), cadmium (Cd), copper (Cu) mercury (Hg), silver (Hg) and cobalt (Co) have been shown to be extremely toxic when they bind to fish gills (Tao et al. 2000). Fish are exposed to metals through contaminated food and the water column in chronically contaminated aquatic ecosystems, the main routes of accumulation being through gills (Tao et al. 2000). Sublethal and chronic concentration of metals exerts their toxicity on fish by generating free radicals such as the hydroxyl radical (OH•), peroxyl-radical (RO2•) and superoxide (O2•−) and some non-radical ROS such as hydrogen peroxide (H2O2). These ROS can trigger oxidative damage to proteins, nucleic acids and lipids (Winston 1991). However, defensive antioxidant enzymes, which detoxify reactive oxygen species, are present in the liver, kidneys, gills and intestine (Buhler and Williams 1988).

Antioxidant enzymes have been used as biomarkers of pollution by metals and organic compounds that generate oxidative stress in molluscs (Cossu et al. 2000) whilst MDA levels have also been shown to be affected by oxidative stress (Rodrigues-Ariza et al. 1993). As antioxidant enzyme activities and MDA levels can be used as biomarkers of pollution, this study was undertaken to determine the relationship between concentration of metals, antioxidant enzyme activities and MDA levels in two species of fish collected from Mguza Dam (which receives domestic and industrial effluent from Bulawayo City sewage works) and from Wright Dam with no history of pollution.
Materials and Methods

Two species of fish, five Clarius gariepinus (catfish) and three Oreochromis mossambicus (tilapia) were collected from Umguza Dam (a polluted site) whilst seven C. gariepinus and eight O. mossambicus were collected from Wright Dam (a site with no history of pollution). These fish were chosen because they are benthic and pelagic feeders, respectively. The fish were periodically collected from gillnets cast over 24 h and the captured fish were killed on site and sexed. Only female fish were used in this study. The liver, gill, kidney and pectoral muscles were removed, snap frozen in liquid nitrogen and transported to the laboratory where they were stored at −80°C until used. Pectoral muscles from each fish species were thawed and 1 g digested on a hot plate using concentrated nitric acid and concentrated hydrochloric acid in a ratio of 3:1. The digestions were carried out for 6 h or until the samples were clear. Metal determination was then done in duplicate or triplicate with reagent blanks being assayed after every ten samples. In addition, a known concentration of the standard solution was also assayed after every ten samples to verify the analytical quality of the result since no certified standard reference material was available. For spike levels of 0.01 mg/kg, the recovery rate ranged from 81% to 110% depending on the metal assayed for using a Varian Atomic Absorption Spectrophotometer (Spectra AA 20 Plus, Victoria, Australia).

Gill filaments, liver and kidney from each fish were thawed and homogenized with 5 volumes of 50 mM potassium phosphate buffer (pH 7) and centrifuged at 9,000 g for 10 min at 4°C. The supernatant was aliquoted and stored at −80°C until required. The activity of CAT was determined spectrophotometrically according to the method described by Clairborne (1989) whilst DTD activity was measured according to the method described by Lind et al. (1990) modified and adapted for a 96 well microplate reader in our laboratory. The reaction mixture was in a final volume of 200 µl and a decrease in absorbance at 600 nm over 3 min at 30°C was recorded. Se-GPX activity was determined as described by Scholz et al. (1981) using 1.5 mM H₂O₂ as a substrate, after adapting the method for a 96 well microplate reader. Consumption of NADPH was monitored at 340 nm over 3 min at 30°C. The activity of superoxide dismutase (SOD) was measured according to the method of Sun et al. (1988) whilst lipid peroxidation was assessed by measuring MDA concentration according to the method of Draper and Hadley (1990). Protein was measured according to Lowry et al. (1951) using bovine serum albumin as the standard. All enzymatic assays were performed in either triplicate or quadruplicate using either a Perkin Elmer UV/VIS Spectrometer, Lambda 2 (Perkin Elmer Corporation, FRG) or a Spectra Max 96 well plate reader (Spectra Max 340, Molecular Devices Corporation, California, USA).

Results and Discussion

Table 1 shows levels of metals in both C. gariepinus and O. mossambicus pectoral muscles collected from Umguza and Wright Dams whilst Figs. 1 and 2 show antioxidant enzymes specific activities of C. gariepinus and O. mossambicus collected from both locations.

Hepatic DTD specific activities were significantly depressed (p < 0.01) in C. gariepinus and O. mossambicus collected from Umguza Dam when compared to those collected from Wright Dam (Figs. 1, 2). In C. gariepinus kidney, higher activity of DTD was observed in those fish collected from Umguza Dam compared to those collected from Wright Dam (p < 0.01). Fish from Umguza dam had a higher transition metal body burden compared to fish.

Table 1 A comparison of metal content (mg/kg wet weight) in C. gariepinus and O. mossambicus muscle collected from Lower Mguza and Wright Dams

<table>
<thead>
<tr>
<th>Metal</th>
<th>Lower Mguza Dam</th>
<th>Wright Dam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. gariepinus</td>
<td>O. mossambicus</td>
</tr>
<tr>
<td>Cd</td>
<td>0.10 ± 0.03ᵃ</td>
<td>0.12 ± 0.02ᵃ</td>
</tr>
<tr>
<td>Zn</td>
<td>1.18 ± 0.43ᵃ</td>
<td>1.23 ± 0.14ᵃ</td>
</tr>
<tr>
<td>Cu</td>
<td>0.13 ± 0.06ᵃ</td>
<td>0.13 ± 0.04ᵃ</td>
</tr>
<tr>
<td>Ni</td>
<td>0.16 ± 0.06ᵃ</td>
<td>0.09 ± 0.06ᵇ</td>
</tr>
<tr>
<td>Fe</td>
<td>3.35 ± 1.33ᵃ</td>
<td>4.81 ± 2.05ᵃ</td>
</tr>
<tr>
<td>Pb</td>
<td>0.31 ± 0.08ᵃ</td>
<td>0.43 ± 0.04ᵃ</td>
</tr>
</tbody>
</table>

ᵃ Metal concentrations with a common alphabetical superscript within rows for each fish species indicate that means are not significantly different, p > 0.05. Values are expressed as mean ± SD for sample, n
from Wright Dam (Table 1). Because of their ability to redox cycle, transition metals are pro-oxidants generating a mixture of $O_2^-$ and $H_2O_2$ which represent a major source of ROS (Imlay 2003). High pro-oxidants have been shown to decrease DTD activity compared to lower doses (Sturve et al. 2005). The primary metabolic role of DTD is the reduction of a broad range of substrates that undergo redox cycling. It has also been shown that DTD is involved in the inhibition of initiation and propagation in lipid peroxidation by maintaining membrane bound co-enzyme Q in its reduced antioxidant state as well as oxidation of proteins and DNA (Ernster and Dallner 1995). The decreased activity of DTD in the liver of fish may compromise the enzyme’s protective role against ROS production since the liver is the major organ for xenobiotic metabolism whilst the higher renal DTD activity observed in both fish species collected from Umguza may be indicative of the protective role of the enzyme in the kidney.

The activities of CAT in gills of C. gariepinus from both dams were similar whilst the enzyme activity was significantly lower in liver and kidney ($p < 0.001$) of fish collected from Umguza Dam (Fig. 1). In contrast, similar CAT activity was observed in kidney of O. mossambicus collected from both locations whilst gill and hepatic CAT activities were significantly lower in O. mossambicus collected from Umguza Dam (Fig. 2). The decrease in hepatic CAT activity in fish from sites polluted by domestic and industrial wastes has been reported by other workers (Bainy et al. 1996) and fish exposed to organic chemicals (Sturve et al. 2005). Our results are in agreement with these studies but in contrast with those of Rodriguez-Ariza et al. (1993) who reported increased CAT activity in fish collected from polluted areas.

The specific activities of Se-GPX for both species of fish from both dams are shown in Figs. 1 and 2. Except in the kidneys of both fish species, significantly higher activity was observed in fish collected from Umguza Dam compared to those collected from Wright Dam. No significant differences in GPX activities were observed in either liver or kidney of O. mossambicus collected from Wright Dam (Fig. 2) whilst Se-GPX activity in gills was not detectable. In agreement with our results, Almar et al. (1998) reported significantly elevated Se-GPX activity in the liver of fresh water fish, Gobio gobio, collected from polluted water bodies although reduction of the enzyme activity has also been noted in fish liver and kidney (Bainy et al. 1996) as well as gills and digestive gland of mussels (Cossu et al. 2000) from a polluted site.

The specific activity of SOD was significantly higher ($p < 0.05$) in gill than liver of C. gariepinus collected from both Dams (Fig. 1). Statistically higher activity of SOD in gill compared to liver was noted in O. mossambicus collected from Umguza Dam ($p < 0.001$) but not in gills and
liver of those collected from Wright Dam. No significant differences in the activity of SOD was seen in gill or liver of *C. gariepinus* as a function of location. However, significantly depressed activity of SOD was seen in gills and liver of *O. mossambicus* collected from Umguzo Dam (*p* < 0.001).

Mean MDA levels in pectoral muscle of *C. gariepinus* collected from Umguzo and Wright Dams are shown in Fig. 3. MDA levels in pectoral fish muscles of both *C. gariepinus* and *O. mossambicus* collected from Umguzo were significantly higher than MDA levels in their counterparts collected from Wright Dam (*p* < 0.01). The higher levels of MDA as well as metals and the pronounced increase in Se-GPX in fish collected from Umguzo Dam suggests that these fish are under greater oxidative stress compared to those collected from Wright Dam. Our data on MDA, on one hand, is in agreement with the increased MDA levels found by others in short-term exposure studies of fish to metals and organic xenobiotics in laboratory studies. It was reported that acute exposure of carp to Cu$^{2+}$ resulted in increased Se-GPX activity and higher MDA levels in most tissues whilst superoxide dismutase and catalase activities were decreased (Winston 1991). Zn$^{2+}$ was also reported to increase liver Se-GPX (Radi andMarcovics 1988). Sanzheiz et al. (2007) found significantly elevated levels of thiobarbituric acid-reactive substances in fish collected from a polluted stream when compared to those from an unpolluted site. On the other hand, our data is in contrast to reports that molluscs (Cossu et al. 2000) and fish (Rodriguez-Ariza et al. 1993) from polluted areas display high GPX activities in parallel with low MDA suggesting adaptation by animals living in polluted areas. Overall, the increases in MDA levels and GPX activity and the decrease in CAT and DTD activities in livers of fish exposed to polluted areas suggest that these molecules can be used to discriminate between two differently polluted dams.

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**References**


![Fig. 3](image-url) A comparison of malondialdehyde concentration in female *O. mossambicus* and female *C. gariepinus* muscle collected from Lower Mguza Dam and Wright Dam. ***p < 0.001 significantly different from Lower Mguza Dam**