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ORIGINAL ARTICLE

Assessment of the effects of Pesticides associated with the sugar industry and in Public health on Aquatic organisms: A case study of the A2 farmlands in Chiredzi, in the South Eastern part of Zimbabwe

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ABSTRACT
A study was carried out to assess the levels and effects of atrazine, dimethoate and dichlorodiphenyltrichloroethane on freshwater fish (Oreochromis mossambicus). The effects of the pesticides on biochemical endpoints of fish collected from a dam in Chiredzi, Zimbabwe was investigated. Water and fish samples were screened for atrazine, dimethoate and dichlorodiphenyltrichloroethane, pesticides commonly used in the region. Levels of dichlorodiphenyltrichloroethane were 131.3 µg/l and 171.7 µg/kg while atrazine was detected in concentrations of 6.15 µg/l and 142.0 µg/kg in water and fish tissues respectively. The observed results showed atrazine and dichlorodiphenyltrichloroethane levels above the limits permissible by the World Health Organization, in water. Trace levels of dimethoate were detected in fish and water samples. The activities of superoxide dismutase, catalase, glutathione-S-transferase and glutathione peroxidase from the fish liver and white muscle extracts were also assessed. The activities of all the enzymes were activated in all the fish sample extracts from the study area. The enhanced activities of the studied antioxidant enzyme system were attributed to exposure to pollutants in the water body. Alterations of the biochemical integrity of fish indicate negative effects of the studied pesticides on the wellbeing of fish and undoubtably on other aquatic biota as well.
Key words: Pesticides, biomarkers, water pollution, fish, antioxidant enzymes

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INTRODUCTION

Sugarcane is mainly grown in the South Eastern part of Zimbabwe covering over 40 000 hectares [1]. Insecticides and herbicides are extensively used in this region to control weeds and insect pests that affect the quality of sugarcane. Large quantities of these agrochemicals are applied in the sugarcane fields individually and as mixture combinations at different stages of cultivation as well as post-harvest. The annual quantities of herbicides applied in the sugarcane plantations in the South Eastern areas of Zimbabwe are close to 1, 237, 728 litres [2]. Monocultural practices associated with sugarcane plantations are only viable with the support of intense pesticidal use [3]. Unfortunately a very small percentage of applied pesticides carryout their intended function with the majority of chemical pesticides finding their way in the environment where that affect non-target organisms. The deleterious effects of pesticides in the environment include disruptions of predator-prey relationships and loss of biodiversity [4].

Chiredzi apart from being an area associated with sugar plantations it is a malaria prone region [5]. The main methods of controlling malaria in the Chiredzi region is the indoor residual spraying of DDT in households and the distribution of pesticide treated bed nets [6]. Most of the pesticides used by man in agriculture find their way to aquatic reservoirs, which serve as the ultimate sinks, for these and any other pollutants released by man in the environment through his day to day activities. Aquatic pollutants

impact on life differently at organism, population and ecosystem level affecting organ function, reproductive status, population size and species survival [4]. The conventional methods for monitoring water pollution only provide information on the presence and quantities of pollutants in aquatic environments but do not provide information on the effects of the pollutant on habitants of the aquatic environments [7]. In biological monitoring however, aquatic organisms are exposed to environmental pollutants and markers in these organisms used to determine the toxic effects of the water pollutants. These markers referred to as biomarkers include biochemical, cellular and chemical endpoints such as serum enzymes and serum biochemical constituents are used to assess environmental toxic effects of pollutants [7]. Chemical analysis complimented by biological approaches provide information on the well being of aquatic ecosystems [7]. Living organisms have metabolic systems which detoxify xenobiotics such as pesticides and hence protect themselves from the toxic effects of the chemical pollutants. Biological systems transform xenobiotics into forms that are less toxic and are either excreted or stored in the organisms. Different organs, in particular the liver play important roles in the detoxication of xenobiotics in living organisms [8]. Enzymes on the other the hand are vital in metabolic processes and alterations of certain enzymes can be used as indicators of exposure to foreign chemicals [9]. In the present study antioxidant enzymes in fish were used to assess the toxic effects of pesticides associated with sugarcane farming as well as pesticides used in public health on aquatic life.

MATERIAL AND METHODS

Fish and water samples were collected from a dam in the sugarcane plantations and from a reference dam situated in the National University of Science Technology (NUST) grounds.

Fish samples used for pesticide residue analysis were extracted using the quick, easy, cheap, efficient, rugged and safe (QuEChERS) method as described by [10]. The extracts were analyzed using a gas chromatography using an electron capture detector (ECD). The GC-ECD was equipped with a programmable temperature vaporization injector used in splitless mode. Hydrogen was the carrier gas at a constant flow rate of 30 ml/min. The column injection temperature was set at 70 °C and the ECD temperature was set at 300 °C. Injection volume was 2 µl and the oven temperature programme; 70 °C for 1 minute, ramped to 160 °C at 25 °C/min, followed by a 20 °C/min ramp to 300 °C and then held for 2 minutes . For the method validation, recoveries were prepared using fish from the reference NUST dam and distilled water samples as representative matrices. The matrices were spiked with the different pesticides to achieve 1 mg/kg of each.

For samples used for enzymatic assays, protein was determined using the method by [11] using bovine serum albumin as a standard. Superoxide dismutase was determined using the method of [12]. The method uses xanthine and xanthine oxidase to generate an oxygen free radical which complexes with nitroblue tetrazolium forming red formazan which is detected using an ultra violet spectrophotometer at 560 nm. Catalase activity was measured using the method of [13]. Hydrogen peroxide was the substrate and a decrease in absorbance was followed for 30 seconds at 240 nm using a UV-visible spectrophotometer. The method of [14] was followed to determine glutathione S-transferase using chloro-2,4-dinitrobenzene (CDNB) as a substrate. The rate of formation of a CDNB-glutathione conjugate measured at 340 nm using a UV-visible spectrophotometer. Glutathione peroxidase activity was determined following the method of [15]. Change in absorbance was followed at 340 nm using a UV-visible spectrophotometer.

RESULTS

Recoveries at 1 mg/kg pesticide spike levels ranged from 83- 103% on average (Table 1).

Table 1. Recovery percentages of pesticides spiked in sample at a concentration of 1 mg/kg.

	DDT	DDE	DDD	Atrazine	Dimethoate
Water	87	87	86	103	86
Fish	84	83	84	96	85

Values represent the average of 3 determinations and these are expressed as mean ± SD.

Pesticide screening in water

Significant levels of DDT and its metabolite dichlorodiphenyldichloroethane (DDD) were observed in water samples at concentrations of 131.30 ppb and 114 ppb respectively (Figure 1). Dimethoate and atrazine on the other hand were detected in low concentrations of 4.21 ppb and 6.15 ppb respectively (Figure 1).

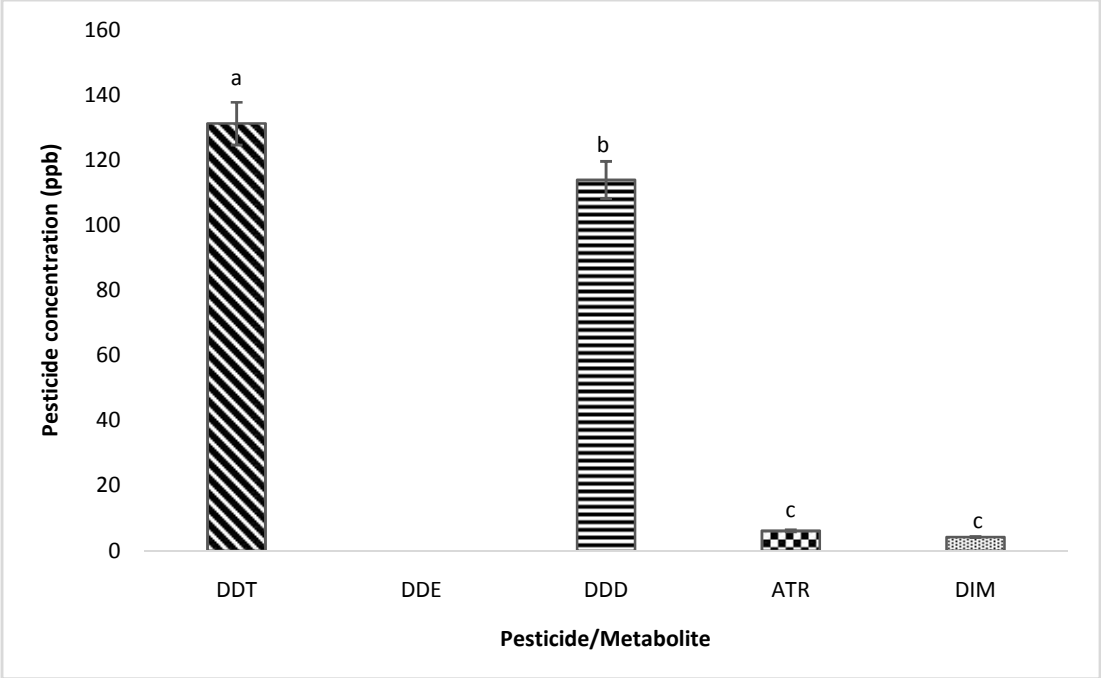

Figure 1. Pesticide levels in water from a Hippo Valley A2 farmland. Values were compiled from means ± SD of duplicate samples. Identical alphabetical letters above the bars represent statistical significant differences among samples while different alphabetical letters show no significant differences among the samples.

Pesticide screening in fish tissue

Fish samples contained high levels of DDT and atrazine in concentrations of 171.7 µg/kg and 142.0 µg/kg/ respectively (Figure 2). Dimethoate levels of 1.3 µg/kg were also detected. High levels of DDT, namely DDE and DDD, were also detected at average concentrations of 230.0 µg/kg and 182.0 µg/kg respectively.

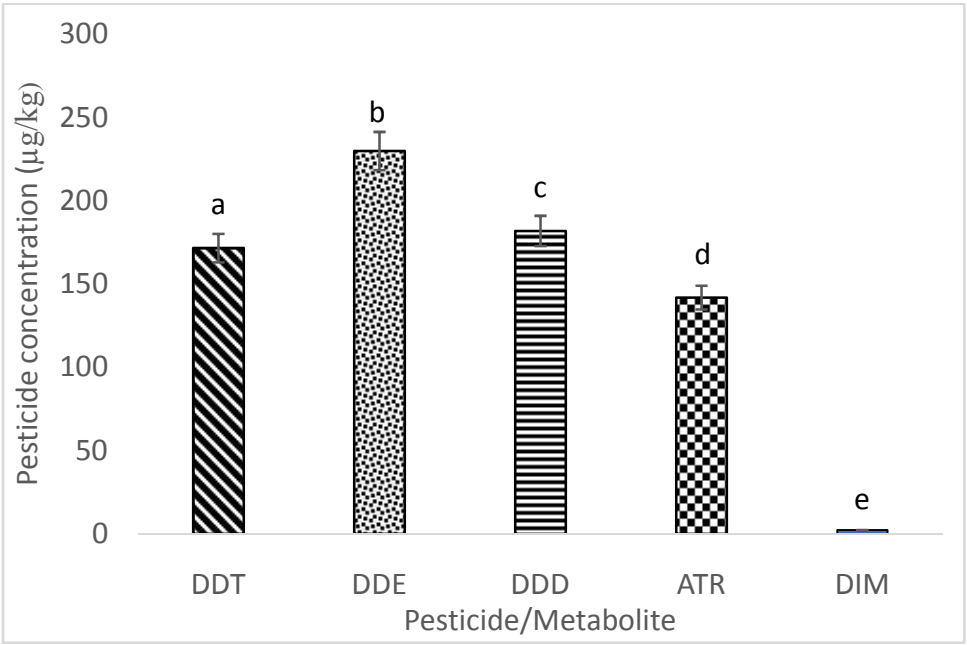

Figure 2. Pesticide residue levels in fish sampled from a Hippo Valley A2 farmland. Values were compiled from means ± SD of duplicate samples. Identical alphabetical letters above the bars represent statistical significant differences among samples while different alphabetical letters show no significant differences among the samples.

Superoxide dismutase activity

Fish samples from the study area had enhanced levels of superoxide dismutase activity when compared to fish samples from the control dam. In both samples from the control and the study regions enzyme activities were higher in liver samples than in muscle tissue samples.

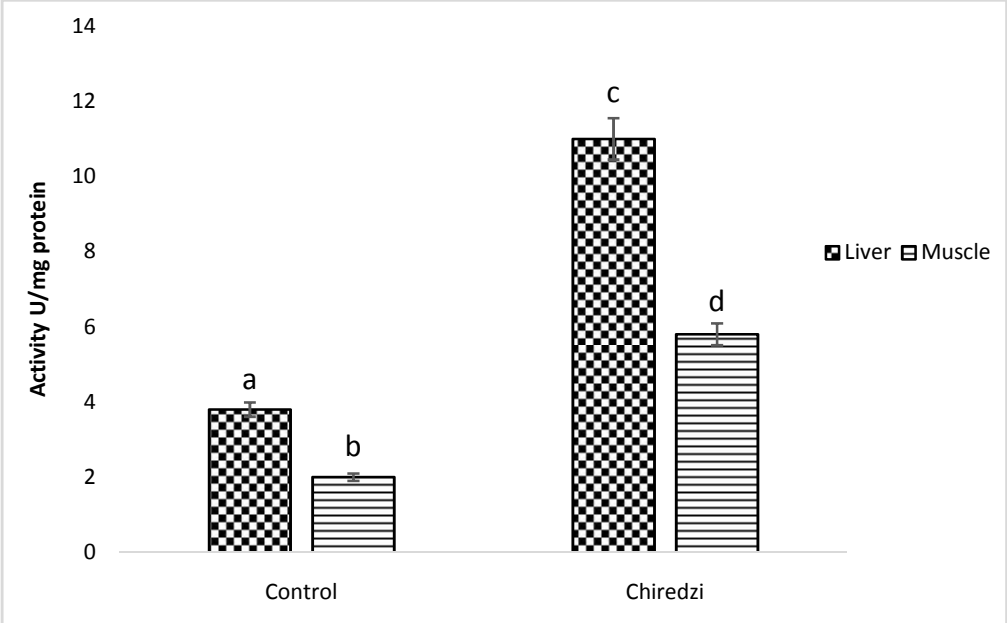

Figure 3. Superoxide dismutase activities in liver and white muscle samples of fish**.** Values were compiled from means ± SD of duplicate samples. Identical alphabetical letters above the bars represent statistical significant differences among samples while different alphabetical letters show no significant differences among the samples.

Catalase activity

Fish samples obtained from the study area had catalase activities that were significantly increased ($p<0.05$) when compared the enzyme activities of fish obtained from the control site (Figure 4). Catalase activities in liver samples were much higher when compared to the enzyme activities in muscle samples in both the controls and samples from the study area.

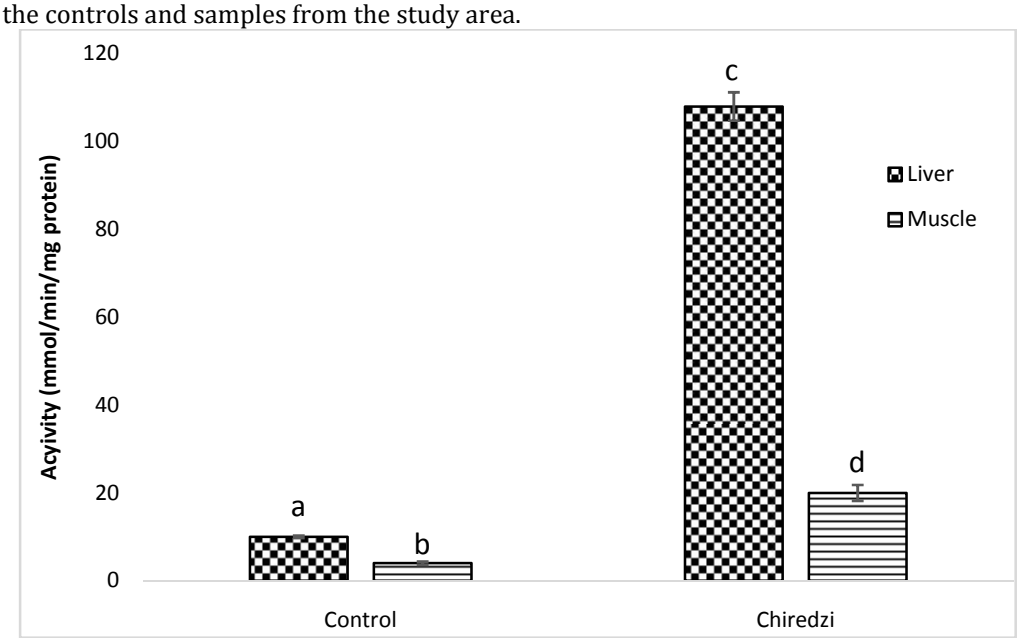

Figure 4. Catalase activities of fish liver and white muscle samples**.** Values were compiled from means ± SD of duplicate samples. Identical alphabetical letters above the bars represent statistical significant differences among samples while different alphabetical letters show no significant differences among the samples.

Glutathione peroxidase activity

Glutathione peroxidase activities were significantly higher ($p<0.05$) in fish samples from the study area when compared to GPx activities in fish samples from the control site (Figure 5).

There was also a significant difference ($p<0.05$) between enzyme activities of liver and muscle fish samples with the enzyme activities being higher in liver samples than in muscle samples.

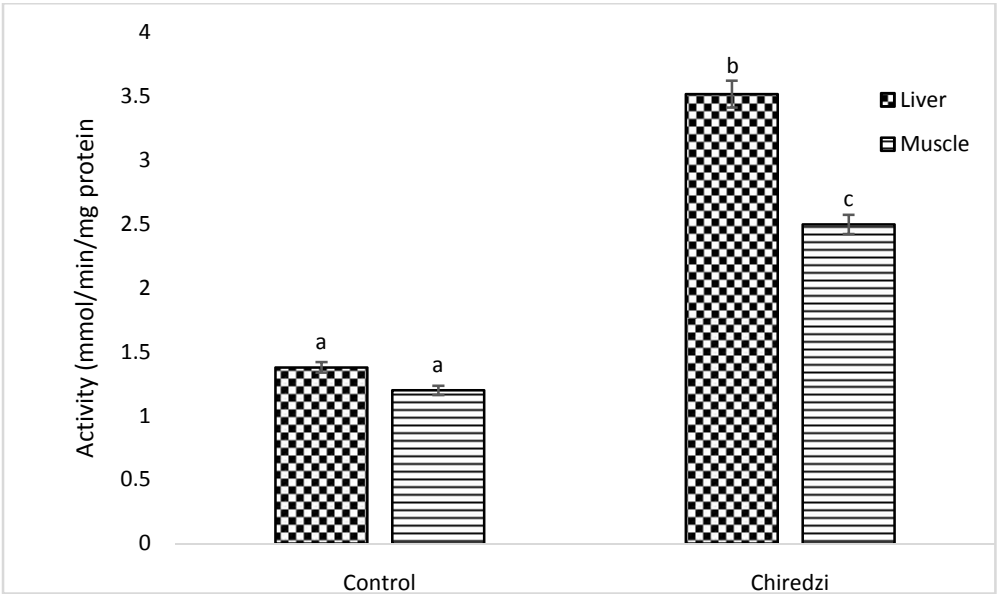

Figure 5. Glutathione peroxidase activities in liver and white muscle samples of fish**.** Values were compiled from means ± SD of duplicate samples. Identical alphabetical letters above the bars represent statistical significant differences among samples while different alphabetical letters show no significant differences among the samples.

Glutathione S-transferase activity

Glutathione S-transferase activities in fish samples obtained from the study area were significantly higher than the GST activities of fish samples obtained from the reference site (Figure 6). Glutathione S-transferase activities in liver samples were much higher when compared to the enzyme activities in muscle samples in both the controls and samples from the study area.

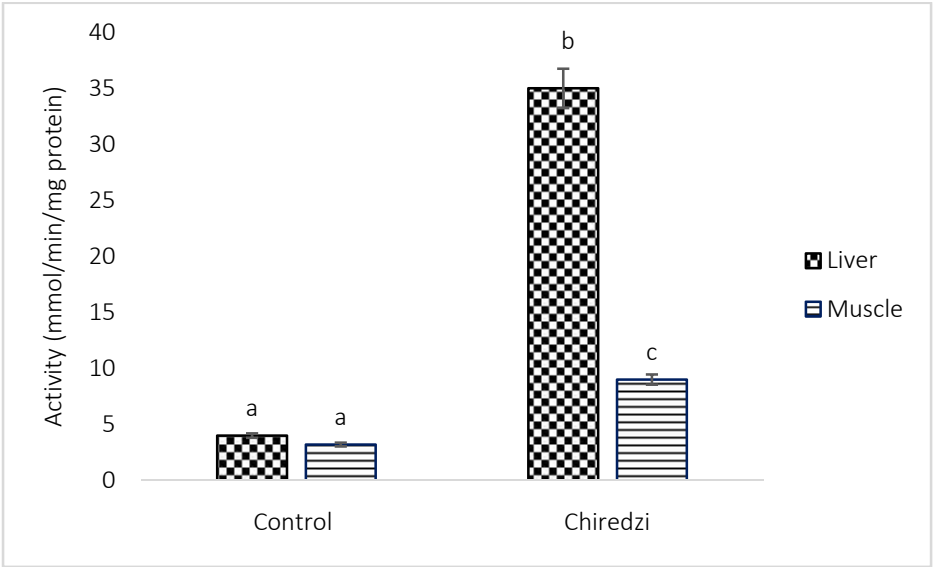

Figure 6. Glutathione S-transferase activities in liver and white muscle samples of fish. Values were compiled from means ± SD of duplicate samples. Identical alphabetical letters above the bars represent statistical significant differences among samples while different alphabetical letters show no significant differences among the samples.

DISCUSSION

Pesticide screening

All the fish and water samples analyzed contained DDT and its metabolites with higher values being obtained in fish tissue when compared to water samples (Figures 1 and 2). Low concentrations of dimethoate were found in both water and fish samples however, the quantities of this pesticide in water were about three time higher than in fish samples while significantly higher concentrations of atrazine (twenty-three-fold) were observed in fish samples when compared to levels found in water samples (Figures 1 and 2). The concentration of dimethoate observed in the Chiredzi water bodies was below the maximum acceptable concentration of 20 ppb as stipulated by the guidelines for Canadian drinking water quality [16]. The low levels of dimethoate in the fish tissue as compared to the water implies the efficiency of the fish's body defense system, in breaking down the pesticide into degradation products that can be excreted. Our results are supported by [17] who also reported the insignificant potential of dimethoate to bioaccumulate due to its hydrophilic nature.

Significantly higher levels of organochlorines than organophosphates were observed in fish and water obtained from the sugar estates. Levels of DDT and its metabolites in fish tissue were higher than in water samples. Organochlorines are lipid soluble and they tend to bioaccumulate in the fat deposits of biological systems. [18] reported that fish may concentrate certain pesticides in their body tissues and organs, particularly in the fatty areas. [19] also observed bioaccumulation of pesticide in fish muscles at levels above 500 times greater than in the water.

The concentrations of dimethoate observed in water in the current study were below the maximum allowable levels as stipulated by National Health and Medical Research Council of Australia [20] while the concentration of atrazine observed was higher than stipulated by Environmental Protection Agency [21]. The Environmental Protection Agency set the limit for atrazine in water at 2 ppb [23] while the National Health and Medical Research Council of Australia set the maximum acceptable value of dimethoate at 50 ppb [21]. The concentrations of atrazine observed in water in the current study were about three times more than the acceptable limit set by [21] implying that atrazine is probably affecting the health of aquatic biota residing in water bodies in the Chiredzi region. In the case of DDT, the maximum acceptable values set for DDT in both water and fish are 1.1 ppb [22], [21]. The levels of DDT observed in water samples from Chiredzi region in the current study were above a hundred fold when compared to the acceptable limits set by [22]. Although DDT is a pesticide of historical use that was stopped from being used for agricultural purposes years ago, the results show that it can still be detected in water and fish. Malaria is endemic in Chiredzi and DDT and other pesticides like deltamethrin are used in indoor residual spraying activities to control the Anopheles mosquitoes which are vectors of the disease. The presence of DDT and its metabolites in aquatic bodies could be possibly be due to improper disposal of DDT containers used in public health. Undoubtably heavy rains may wash and carry pesticide residues from the dumped containers to aquatic bodies. The concentrations of atrazine in fish from Hippo Valley were extremely high, exceeding the acceptable limit set by the European Commission 70 times [23]. This is a cause for concern as it shows that aquatic biota is exposed to these agrochemicals. [24] also highlights the toxicological effects of atrazine in a study that showed that concentrations of atrazine as low as 3 µg/l affect the health and behaviour of fish.

The concentrations of atrazine detected in water are then high enough to induce activities of some antioxidant enzymes.

Enzyme activity

Enhanced activities of antioxidant enzymes were observed in all fish samples obtained from Chiredzi suggesting that these aquatic organisms were oxidatively stressed [25] also observed elevated antioxidant enzyme activity in fish from a pesticide contaminated water body. Enzymes catalyze important metabolic processes in biological systems and alteration of enzymatic activity is now used as an indicator that the organism has been exposed to foreign chemicals [25]. Results from the current study show that higher alterations in enzyme activity were in the liver samples when compared to muscle tissue samples (Figures 3-4). This is supported by [26] who reported the liver as the main organ for xenobiotic metabolism. They reported the presence of most xenobiotic metabolizing enzymes and showed GST as representing up to 10% of the total liver cytostolic proteins [26].

The antioxidant enzymes; SOD, CAT and GPx are regarded as the first line of defense responsible for eliminating the ROS formed during the metabolism of pesticides in fish [27]. The elevated activities of SOD (Figure 3) suggests an adaptive response by the fish system to counteract the effects of superoxide anion radicals generated during normal metabolism of pesticides by the fish's system. Enhancement of activities of CAT and GPx (Figures 4 and 5) as expected was in response to elevated levels of hydrogen peroxide from the activity of superoxide dismutase. Our results are supported by [28] who also observed

enhanced activities of SOD and CAT in *Catla catla* fish exposed to methyl parathion an organophosphate. [29] also observed an elevation of the first line of defense against oxidative stress inducing pollutants in fish exposed to the herbicide atrazine. Superoxide dismutase and catalase activities were significantly increased in zebrafish exposed to atrazine. Alteration of the antioxidant enzyme system has been validated as a biochemical approach employable as early warning signal of exposure to chemical exposure [30]. Our results fit in with observations by other workers [31] which show the ability of aquatic organisms like fish to counter-balance the negative effects of environmental pollutants such as pesticides.

We can therefore, conclude from our results that human activities such as farming which is rampant in the Chiredzi region is polluting aquatic bodies in the region shown by pesticides detected in a dam positioned in the sugarcane plantation. Also public health practices carried out in Chiredzi region are indirectly contributing to pollution by pesticides of water bodies in the area. The agrochemicals undoubtably affect the wellbeing of aquatic organisms residing in the studied water reservoir reflected by the constant enhancement of their antioxidant enzyme systems which are indicators of oxidative- stress. Alterations of the biochemical nature in fish observed as alterations of antioxidant enzyme systems in contaminant-water exposed fish in the current study is an indicator of changes of a negative nature on the wellbeing of aquatic biota.

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COMPETING INTERESTS

The authors have declared that no competing interest exists.

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