ENZYME ACTIVITIES IN TISSUES OF NILE TILAPIA (*Oreochromis niloticus*), MULLET FISH (*Liza falcipinus*) AND CRAB (*Callinectes amnicola*) FROM OIL IMPACTED ABULOMA AND WOJI JETTIES, RIVERS STATE NIGERIA

ABSTRACT

This research was carried out to examine the distribution of enzymatic activities of some tissues (liver, muscles and gills) of selected aquatic organisms (Nile tilapia, Mullet fish and Crab) collected from some crude oil polluted rivers (Abuloma Jetty and Woji Jetty) and Ojimba-ama a non-oil polluted area which served as control. The aquatic organisms were obtained from rivers which are used as jetty for transportation of petroleum products and other industrial activities like welding, dredging, refuse dump, etc. Enzyme activities of fish and crab tissues (liver, muscles and gills) were measured for some biomarker enzymes such as catalase, rhodanase and glutathione S-transferase. Results were shown in means of triplicate values which were subjected to statistical analysis using analysis of variance (ANOVA). Enzyme activity in Nile tilapia across the three locations ranged from 44.39±0.01 µ/mgprotein to 171.45±0.01 µ/mgprotein with GST in gills having the lowest value and rhodanase in the liver recording the highest value. Enzyme activity in Mullet across the three locations ranged from 18.58±0.10 µ/mgprotein to 120.37±0.02 µ/mgprotein with catalase in muscles having the lowest value and rhodanase in the liver recording the highest value. Enzyme activity in Crab across the three locations ranged from 40.79±0.03 µ/mgprotein to 130.72±0.01 µ/mgprotein with GST in gills having the lowest value and rhodanase in the liver recording the highest value. Rhodanase showed the highest level of enzyme activity in all the tissues. Liver recorded the highest enzyme activity across all samples from the three locations which may be as a result of the liver being the principal detoxification organ for xenobiotic substances. From this study, there was an increase in the enzyme activities of the biomarkers across all the tissues which indicate a contamination from pollutants capable of causing oxidative stress in the organisms.

KEYWORDS: Biomarkers; Catalase; Gluthathione S-transferase; Rhodanase; Contamination.

1.0 INTRODUCTION

"The contamination of fresh water systems with a wide range of pollutants has become a matter of global concern. Many natural aquatic bodies have been extensively contaminated with heavy metals and hydrocarbons released from domestic, industrial and other anthropogenic activities. This may have serious effects on the ecological balance of the recipient environment" (Canli *et al.*, 1998). "Aquatic organisms as well as plants provides majority of the proteins present in food taken by humans. Seafood is a remarkably low-caloric food containing a high amount of protein that supports effective health condition" (Ayotunde *et al.,* 2012). "Fish are important bioindicator species and play an important role in the monitoring of water pollution because they respond with great sensitivity to changes in the aquatic environment" (Naigaga *et al.,* 2011). "The sudden death of fish can indicate heavy pollution, and the effects of exposure to sub-lethal levels of pollutants can be measured in terms of their biochemical, physiological or histological responses" (Mondon *et. al.,* 2001). "The binding of a toxic compound like a heavy metal with its receptor may induce cellular processes that have toxic or other adverse effects on the cell (deoxyribonucleic acid and nuclear protein)" (Flora *et al.,*2008). "In macro organisms, these processes subsequently affect organs, the organism itself or even the whole population. The acute, chronic and long term effects of chemical compounds on living systems can be studied by evaluating the biochemical and morphological changes in various organs, especially the liver of the fish" (Parasuraman, 2011). "The liver is the principal organ of metabolism and plays a role in many body processes, most especially in the detoxification of chemical compounds. Some of the enzymes involved in detoxification reactions in the liver can be used as biomarkers of exposure to toxic chemical pollutants. Biochemical markers are measurable responses of the exposure of an organism to toxic compounds. The biochemical markers measure effects of, or exposure to, toxic chemicals, type of toxicity, and the magnitude of their response often correlates with the level of pollution" (Weeks, 1995). "Biomarkers are defined as detectable biochemical and tissue-level changes that indicate altered physiology" (Smit *et al.* 2009). "A multivariate biomarker approach involving multiple biological and physiological measurements, as well as analytical chemistry where appropriate can also be used to assess the health of indicator species within ecosystems. Many pollutants mediate their toxicity through oxidative stress, resulting in changes in antioxidant defenses as well as damage to proteins, membrane lipids and DNA molecules. The result of such exposure leading to oxidative stress can impair cellular or biological function

which can lead to disease. Biomarkers of oxidative stress, such as changes in antioxidant enzyme activity or in degree of accumulation of damaged molecules, can offer an early warning sign for exposure to redox-active xenobiotics" (Van der Oost et al., 2003). "These oxidative stress parameters have been associated with various disease pathologies and organism longevity in a number of species, thereby establishing ecological relevance in these cases. A large number of biomarkers of oxidative stress have been used in fish studies and these include both the antioxidant defense mechanisms possessed by the cell, enzymatic and molecular, as well as oxidative damage products" (Van der Oost *et al.,* 2003). The antioxidant defense system includes antioxidant enzymes such as catalase (EC 1.11.1.6), Glutathione S-transferases (GSTs: EC. 2.5.1.18), etc, and detoxifying enzyme namely: Rhodanese (thiosulphate: cyanide sulphur transferase, EC 2.8.1.1).

2.0 MATERIALS AND METHODS

2.1 Study Area

Nile tilapia, Mullet fish and Crab samples were obtained from Abuloma Jetty, Woji Jetty and Ojimba-ama rivers (control site). Abuloma is located in Port Harcourt city in Rivers State and linked to several communities (Okujagu-ama, Ojimba-ama, Kalio-ama, Okuru-ama and so on) by rivers. The shores of Abuloma Jetty river is being utilized by different companies that carry out construction and maintenance of various sea vessels, tugboats, barges and also transportation of crude oil products. However, these activities are done on the river leading to contamination of the water body as a result of careless discharge of crude oil during transportation, washing away of metallic substances during welding, dumping of refuse into the river and so on. Woji being the second study area is a community in Obio-Akpor L.G.A in Rivers State which also has rivers linking to other communities like Azuabie, Oginigba, etc. The Abuloma and Woji Jetty have similar industrial activities going on daily. Ojimba-ama is a community in Okrika L.G.A Rivers State which served as control site in this study where less industrial activities compared to Abuloma and Woji are carried out but dredging activities has been recently done on the river.

2.2 Samples

Fresh samples of *Oreochromis niloticus* (Nile Tilapia), *Liza falcipinus* (Mullet) and *Callinectes amnicola* (Crab) were collected from Abuloma Jetty, Woji Jetty and Ojimba-ama (control) river in Port Harcourt, Obio-Akpor and Okrika Local Government Areas of Rivers state, Nigeria respectively. At each site, three individual fish and crabs samples were collected, weighed, measured, placed on a dissection board and excised to remove the liver, muscles and gills. About 50g of each tissue type was collected. Samples were kept on ice before being transferred to a deep freezer (-4^{o}C) and kept for further analysis. Glutathione S-transferase (GST), catalase and rhodanese enzymes were isolated from the liver, muscles and gills of the fish by homogenization of each tissue (with specific buffers for each enzyme) and centrifugation. Serial dilutions of the crude enzymes were then assayed for residual enzymatic activities in triplicates using various standard methods.

2.3 Evaluation of Enzymatic Activity

2.3.1 Gluthatione S-transferase (GST)

GST activity was determined by the method described by Habig *et al*. (1974), using 0.1 M phosphate buffer with pH 6.5. This follows the increase in absorbance at 340 nm due to the formation of the conjugate 1-chloro-2,4-dinitrobenzene used as substrate at the presence of reduced glutathione.

2.3.2 Catalase

Catalase activity was determined by the method described by Xu *et al*. (1997). This method was based on the first order reaction of catalase with H_2O_2. The enzyme sample was added to the H_2O_2 phosphate buffer solution and the absorbance of H_2O_2 at 240 nm was measured every 5 seconds for 60 seconds by a spectrophotometer.

2.3.3 Rhodanase

Rhodanese (a detoxifying enzyme) activity was measured according to the method of Lee *et al*. (1981), as modified by a previously described method using a 50 mM borate buffer of pH 9. The absorbance was read at 460 nm against a reagent blank containing no enzyme sample.

2.4 Statistical Analysis

Data obtained were subjected to Analysis of variance (ANOVA) using the statistical package SPSS (version 21) and test of significance was done at 95 % confidence level.

3.1 RESULTS

Table 1: Enzyme activities (U/mgprotein) across tissues of Nile tilapia (*Oreochromis niloticus)*

TISSUES	ENZYME	ABULOMA JETTY	WOJI JETTY	OJIMBA-AMA
Liver	Catalase	89.25±0.04 [a]	115.21±0.01 [a]	97.36±0.01 [a]
	Rhodanase	140.30±0.07 [a]	171.45±0.01 [a]	120.76±0.01 [a]
	Gluthatione S-transferase (GST)	74.55±0.04 [a]	93.49±0.01 [a]	76.93±0.01 [a]
Muscles	Catalase	87.47±0.04 [a]	93.46±0.00 [a]	84.53±0.01 [a]
	Rhodanase	105.26±0.02 [a]	122.33±0.01 [a]	98.14±0.15 [a]
	Gluthatione S-transferase (GST)	61.88±0.04 [a]	82.20±0.02 [a]	69.55±0.02 [a]
Gills	Catalase	50.21±0.02 [a]	73.30±00.1 [a]	52.71±0.01 [a]
	Rhodanase	78.60±0.03 [a]	92.35±0.01 [a]	75.54±0.01 [a]
	Gluthatione S-transferase (GST)	48.40±0.01 [a]	67.44±0.03 [a]	44.39±0.01 [a]

Values are expressed as means ± standard error of mean (SEM) of three replicates. Mean values across row with the same superscript letter (a) are statistically significant while those without superscripts are statistically not significant when compared to each other ($p<0.05$).

Table 2: Enzyme activities (U/mgprotein) across tissues of Mullet Fish (*Liza falcipinus*)

TISSUES	ENZYME	ABULOMA JETTY	WOJI JETTY	OJIMBA-AMA
Liver	Catalase	65.85±0.06 [a]	51.77±0.02 [a]	77.12±0.01 [a]
	Rhodanase	120.37±0.02 [a]	94.52±0.00 [a]	102.63±0.00 [a]
	Gluthatione S-transferase (GST)	43.84±0.05 [a]	67.25±0.01 [a]	51.33±0.01 [a]
Muscles	Catalase	18.58±0.10 [a]	35.16±0.01 [a]	59.22±0.01 [a]
	Rhodanase	95.31±0.10 [a]	77.90±0.02 [a]	83.20±0.00 [a]
	Gluthatione S-transferase (GST)	36.31±0.06 [a]	53.28±0.01 [a]	41.78±0.02 [a]
Gills	Catalase	33.33±0.05 [a]	27.20±0.01 [a]	46.82±0.01 [a]
	Rhodanase	67.22±0.04 [a]	56.42±0.03 [a]	68.77±0.02 [a]
	Gluthatione S-transferase (GST)	28.67±0.01 [a]	33.83±0.01 [a]	24.64±0.01 [a]

Values are expressed as means ± standard error of mean (SEM) of three replicates. Mean values across row with the same superscript letter (a) are statistically significant while those without superscripts are statistically not significant when compared to each other ($p<0.05$).

Table 3: Enzyme activities (U/mgprotein) across tissues of Crab (*Callinectes amnicola)*

TISSUES	ENZYME	ABULOMA JETTY	WOJI JETTY	OJIMBA-AMA
Liver	Catalase	96.21±0.04 [a]	111.79±0.01 [a]	99.23±0.01 [a]
	Rhodanase	98.52±0.01 [a]	130.72±0.01 [a]	117.35±0.02 [a]
	Gluthatione S-transferase (GST)	55.42±0.12 [a]	72.53±0.02 [a]	84.65±0.01 [a]
Muscles	Catalase	58.09±0.03 [a]	94.49±0.04 [a]	78.63±0.02 [a]
	Rhodanase	83.63±0.01 [a]	110.23±0.01 [a]	93.41±0.00 [a]
	Gluthatione S-transferase (GST)	51.26±0.01 [a]	68.36±0.01 [a]	81.19±0.01 [a]
Gills	Catalase	44.27±0.01 [a]	76.46±0.01 [a]	65.53±0.01 [a]
	Rhodanase	64.71±0.01 [a]	86.82±0.00 [a]	74.58±0.02 [a]
	Gluthatione S-transferase (GST)	40.79±0.03 [a]	59.27±0.00 [a]	69.95±0.04 [a]

Values are expressed as means ± standard error of mean (SEM) of three replicates. Mean values across row with the same superscript letter (a) are statistically significant while those without superscripts are statistically not significant when compared to each other ($p<0.05$).

3.2 DISCUSSIONS

3.1: Enzyme activities across tissues of Nile tilapia (*Oreochromis niloticus)*

The results of the distribution of catalase, rhodanese and GST activities in the liver, gills, and muscle of Nile tilapia determined in this study are shown in Tables 1. Enzyme activities (µ/mgprotein) across the tissues/organs of Nile tilapia varied significantly ($p<0.05$) across the different locations, with liver having the highest level of enzymatic activity and rhodanese showing the highest significant activity ($p<0.05$) across body tissues. Other enzymes (such as catalase and GSTs) activities in the Nile tilapia tissues were also significant. The activities of rhodanese were higher than other enzymes in all of the tissues (muscle, gills and liver).

"The release of pollutants into the aquatic environment is known to cause detrimental effects to the environment and to the living organisms, giving a significant interest to the study of oxidative stress responses in aquatic organisms induced by toxicants" (Soares *et al.,* 2008). "Many pollutants can result in some degree of oxidative damage by generating free radicals and/or altering antioxidant enzyme systems which reactive oxygen species (ROS)" (Huang *et al.,* 2007). "Antioxidant defense enzymes have been proposed as biomarkers of contaminant or seasonally mediated oxidative stress in a variety of marine and freshwater organisms and their induction reflects a specific response to pollutants" (Borkovic *et al.,* 2005).

Catalase (CAT) is a well-known antioxidative enzyme and has been implicated in protection against H_2O_2. In this study, the activity of catalase was highest (115.21±0.01 µ/mg protein) in the liver from Woji, while the lowest catalase activity (50.21±0.02µ/mgprotein) was recorded in Abuloma gills, with a statistical significant difference ($p<0.05$) observed in Tilapia fish from these different locations. Therefore the observed increase in CAT activity may indicate an important role to protect cells against H_2O_2 production. The induction of catalase in the present study suggests that oxidative stress response still works well under the current conditions, and the increase of antioxidative enzymes may be a physiological adaptation for the elimination of ROS generation. "The elevated catalase activity could be associated with an adaptive mechanism by the organisms to rid itself of reactive oxygen species (ROS) resulting possibly from accumulation of PAH and heavy metals" (Livingstone *et al.,* 2000).

Rhodanase had its highest activity (171.45±0.01µ/mgprotein) in liver from Woji, while the lowest rhodanase activity (67.44±0.03µ/mgprotein) was recorded in Tilapia gills from Ojimba-

ama. The significant increase in rhodanase activity in the tissues of the Nile tilapia may be related to pollutants that increase ROS production resulting in oxidative stress.

GST activity, on the other hand reached its peak (93.49±0.01 µ/mg protein) in Tilapia liver from Woji and the lowest activity (44.39±0.01 µ/mg protein) in Ojimba-ama gills. The values of GST in the liver of Tilapia obtained in this study were found to be higher than values from studies on some freshwater fishes (Talas *et al.,* 2008; Wenju *et al.,* 2009; Gad and Yacoub, 2009). "Other authors also found that the activity of detoxification enzymes such as GST is increased in the presence of polycyclic aromatic hydrocarbon" (Vander Oost *et al.,* 2003). The increase in the activity of GST reported in the present study indicated the biotransformation pathway valid for petroleum products found in the river, a protective response in fish toward exposure to an oxidative stress inducing xenobiotics.

From the general analysis of Nile Tilapia, liver recorded the highest activity while gills had the lowest activity for all investigated enzymes. The pollutants affect various organs, particularly gills, which are exposed directly to the contaminants in the environment. However, most of the pollutants are transferred to the blood and transported to the liver, which is the principal detoxification organ for xenobiotic substances. Hepatic tissue is, therefore, the place where most of the pollutants accumulate and therefore where long-term damage is most likely to occur. These results suggest that the presence of heavy metals and PAHs, that have been reported to cause oxidative stress (Dimitrova *et al.,* 1994; Firat and Kargin, 2010), may be associated with the results found in the present study.

3.2: Enzyme activities across tissues of Mullet Fish (*Liza falcipinus*)

The results of the distribution of catalase, rhodanase and GST activities in the liver, gills, and muscle of Mullet fish determined in this study are shown in Tables 2. Enzyme activities (µ/mgprotein) across the tissues/organs of Mullet fish were significantly different ($p<0.05$) across the different locations, with rhodanase in liver from Abuloma (120.37±0.02µ/mgprotein) showing the highest significant activity ($p<0.05$) across body tissues. Other enzymes (such as catalase and GSTs) activities in the Mullet tissues were also significant. The activities of rhodanase were shown to be highest among the enzymes in all of the tissues.

In this study, the activity of catalase in the liver of Mullet fish from Ojimba-ama was the highest (77.12±0.01µ/mg protein) while the lowest catalase activity (27.20±0.01µ/mg protein) was recorded in Woji gills with a statistical significant difference ($p<0.05$) observed in Mullet fish from the different locations. The hepatic catalase activity of Mullet fish was greatly elevated compared to gills and muscles across all the locations. This may be due to the liver is the principal detoxification organ for xenobiotic substances.

Rhodanase showed the highest activity (120.37±0.02µ/mgprotein) in liver from Abuloma, while the lowest rhodanase activity (56.42±0.03µ/mgprotein) was recorded in Mullet gills from Woji. The significant increase in rhodanase activity in the tissues of the Mullet fish may be related to pollutants that increase ROS production resulting in oxidative stress.

GST had its highest activity (67.25±0.00µ/mg protein) in Mullet liver from Woji and the lowest activity (24.64±0.01 µ/mgprotein) in Ojimba-ama gills. The value of GST in the liver of mullet obtained in this study was found to be higher than values studied in some freshwater fishes (Talas *et al.*, 2008; Wenju *et al.*, 2009; Gad and Yacoub, 2009). From the general analysis of Mullet, liver recorded the highest activity while gills had the lowest activity for all investigated enzymes.

3.3: Enzyme activities across tissues of Crab (*Callinectes amnicola)*

The results of the distribution of catalase, rhodanase and GST activities in the liver, gills, and muscle of Crab determined in this study are shown in Tables 3. Enzyme activities (µ/mgprotein) across the tissues/organs of Crab were significantly different ($p<0.05$) across the different locations, with rhodanase in liver from Woji (130.72±0.01µ/mgprotein) showing the highest significant activity ($p<0.05$) across body tissues. Other enzymes (such as catalase and GSTs) activities in the Mullet tissues were also significant. This could be attributed to the high industry activities happening at the shoreline of the river which has introduced pollutants that has been absorbed into the tissues of the crab. The activities of rhodanase were higher than other enzymes in all of the tissues (muscle, gills and liver).

In this study, the activity of catalase was highest (111.79±0.01µ/mg protein) in the liver of Crab from Woji, while the lowest catalase activity (44.27±0.01µ/mg protein) was recorded in Abuloma gills, with a statistical significant difference ($p<0.05$) observed in crab from these different locations. The hepatic catalase activity of crab was greatly elevated compared to gills and muscles across all the locations. This may be due to the liver is the principal detoxification organ for xenobiotic substances.

Rhodanase had the highest activity (130.72±0.01 µ/mgprotein) in liver from Woji, while the lowest rhodanase activity (64.71±0.01µ/mgprotein) was recorded in crab gills from Abuloma. The hepatic rhodanase activity of crab was greatly elevated compared to gills and muscles across all the locations. This may be due to the liver is the principal detoxification organ for xenobiotic substances. The significant increase in rhodanase activity in the tissues of the crab may be related to pollutants that increase ROS production resulting in oxidative stress.

GST had its highest activity (84.65±0.01µ/mg protein) in crab liver from Ojimba-ama and the lowest activity (40.79±0.03µ/mg protein) in Abuloma gills. The values of GST in the liver of crab obtained in this study was found to be higher than values studies on some freshwater fishes (Talas *et al.,* 2008; Wenju *et al.,* 2009; Gad and Yacoub, 2009). From the general analysis of crab, liver recorded the highest activity while gills had the lowest activity for all investigated enzymes.

CONCLUSION

The main aim of this study is to reveal the distribution of enzymatic activities in tissues of selected aquatic organisms (Tilapia fish, Mullet fish and Crab) from Abuloma, Woji Jetty and Ojimba-ama rivers. Enzyme activities of fish and crab tissues (liver, muscles and gills) were measured for some biomarker enzymes such as catalase, rhodanase and gluthathione S-transferase. The pollutants affected various organs, particularly gills, which are exposed directly to the contaminants in the environment. There was an increase in the enzyme activities of the biomarkers across all the tissues which indicate a contamination from pollutants capable of causing oxidative stress in the organisms.

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