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Assessment of Diagnostic Enzymes as Indices of Heavy Metal Pollution in Tilapia Fish

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***Abstract*—**Diagnostic enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined as indices of heavy metal pollution in *Tilapia guinensis*. Three different sets of fishes treated with lead (Pb), iron (Fe) and copper (Cu) were used for the study while a fourth group with no heavy metal served as a control. Fishes in each of the groups were exposed to 2.65mg/l of Pb, 0.85mg/l of Fe and 0.35 mg/l of Cu in aerated aquaria for 96 hours. Tissue fractionation of the liver tissues was carried out and the three diagnostic enzymes (AST, ALT, and ALP) were estimated. Serum levels of the same diagnostic enzymes were also measured. The mean values of the serum enzyme activity for ALP in each experimental group were 19.5±1.62, 29.67±2.17 and 1.15±0.27 IU/L for Pb, Fe and Cu groups compared with 9.99±1.34 IU/L enzyme activity in the control. This result showed that Pb and Fe caused increased release of the enzyme into the blood circulation indicating increased tissue damage while Cu caused a reduction in the serum level as compared with the level in the control group. The mean values of enzyme activity obtained in the liver were 102.14±6.12, 140.17±2.06 and 168.23±3.52 IU/L for Pb, Fe and Cu groups, respectively compared to 91.20±9.42 IU/L enzyme activity for the control group. The serum and liver AST and ALT activities obtained in Pb, Fe, Cu and control groups are reported. It was generally noted that the presence of the heavy metal caused liver tissues damage and consequent increased level of the diagnostic enzymes in the serum.

***Keywords*—**Diagnostic enzymes, enzyme activity, heavy metals, tissues investigations.

I. Introduction

MODERN industrial technologies use heavy metals both in the elemental and combined forms [1]. In recent years, serious concerns have been voiced about the deteriorating state of fresh water bodies with respect to trace metal pollution [2]. It is recognized that in freshwater systems, trace metals have high pollution potential that could be measured through the use of fish [3].

The results of researches with fishes indicate that metal distributions in fishes are both species-specific and site-specific. Heavy metals have also been shown to have the potentials to be toxic to living organisms if present at a level above a threshold, which varies between taxa, probably even at the specific level [3].

Increased oil and gas and other industrial activities in the Niger Delta region, Nigeria have also resulted in increased pollution of aquatic ecosystems with heavy metal toxicants. Heavy metals released into the environment have caused unexpected damages to our environment and natural resources as well as aquatic biota [4], [5]. Man – induced environmental damage resulting from these activities has created many problems in recent times [6]. The sea has been recognized as the ultimate repository of terrestrial matter which includes a vast array of manmade chemicals and industrial effluents. Industrial effluents have been shown to be a complex mixture with toxic agents which include toxic by-products [7]. Some organochlorines, suspended solids, dissolved and particulate organic matter as well as heavy metals have been identified in effluents by de Kock and co-workers [8]. Many effluents have been shown to be hot, of extreme pH value and normally contain high levels of dissolved salts [9]. Metcalf and Eddy [10] showed in their research that the most common means of treated wastewater disposal is discharge and dilution into ambient waters. There is also land application where the wastewater seeps into the ground and recharges underlying groundwater aquifers. The Qua Iboe river estuary, where an aspect of the present research was conducted hosts the Mobil Producing Nigeria (MPN) exploration and production (E&P) facilities. Its produced water is dumped at the Douglas creek which ultimately empties into the Atlantic Ocean. It is expected that the fishes that inhabit this aspect of the ecosystem would have taken up and bioaccumulated some of the heavy metal toxicants in the effluent into their tissues. The type and concentration of these heavy metals are investigated in this study.

In this research, tilapia *guniensis*, a common species in the study area was used to access the heavy metal toxicological integrity of the study area. A simulation study was also designed to limit this assessment to the effect of three heavy metals (copper, lead and iron) on the activity of three diagnostic enzymes (AST, ALT, ALP) in tilapia fish.

In Nigeria like other countries of the world, the level of pollution of water bodies with heavy metal is probably no longer within safe limits for aquatic life found in this ecosystem. The Qua Iboe River estuary (QIRE) is not an exception. Also, estimation of diagnostic enzyme activity was undertaken as an index of heavy metal pollution in static tests. It was designed to simulate aquatic ecosystem like the QIRE polluted with heavy metals toxicants.

II. Materials and Method

A. Fish Samples Used for the Tests

1. Preliminary Assessment

Samples of *Tilapia* sp. were fished from three sampling stations: Douglas Creek, Mouth of Douglas Creek / Qua Iboe

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River and mouth of Qua Iboe River / Atlantic Ocean. The same species of fish was also fished from a fourth station, which was a control point (a location with no tidal influence from the Atlantic Ocean). The geographical coordinates of the four (4) sampling locations along the Qua Iboe River Estuary are given in Table I. For convenience, sampling stations II and III are referred to as Qua Iboe and Atlantic respectively.

TABLE I
GEOGRAPHICAL COORDINATES OF SAMPLING STATIONS

S/N	Sample Station	Description of location	GPS Coordinates
1	I	Douglas Creek	N 04° 33' 182" E 008° 00' 152"
2	II	Douglas creek/ Qua Iboe River mouth	N 04° 33' 107" E 007° 59' 366"
3	III	Atlantic Ocean/ Mouth of Qua Iboe River	N 04° 32' 712" E 007° 59' 358"
4	IV (Control)	Qua Iboe River at Eket-Ikot Abasi bridge head	N 04° 38' 371" E 007° 54' 891"

The fishing was done using the dragnet method [11]. Having established that the fishes accumulated substantial amounts of the heavy metal toxicants in their tissues, there was the need to further investigate damage to the fish tissues through toxicity studies and determination of activities of diagnostic enzymes by using same species of fishes from static tests.

2. Toxicity Studies to Determine the LC_{50} of *Tilapia guinensis*

Aerated aquaria used in this toxicity studies were similar to those described by [12], [13]. All tests were static and were conducted in accordance with the recommendations of Spraque [14]. In each experiment, a control (distilled water), and graded concentrations of the test heavy metal were used. In each test, 10 fish samples were exposed in an aerated tank containing 20L of test solutions. Observations for mortality were made hourly at regular intervals. Dead fish samples were removed at each observation. A total of 50 fish samples were used at each round of test (40 fish for test and 10 for control). This was repeated for each of the heavy metals. Fig. 1 shows a photograph of Tilapia fish used for the toxicity study.

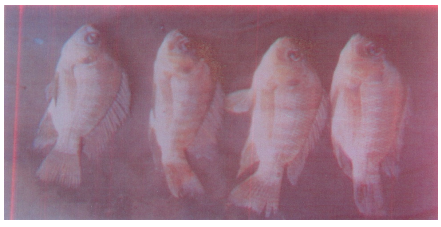

Fig. 1 Tilapia Fish Used for the Study

B. Enzyme Analysis

Enzyme analysis was carried out in liver and serum. The analyses focused on the liver's detoxification system and enzyme activity. The enzymes analyzed in the two tissues include alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The IFCC [15] methods for the measurement of catalytic concentration of the respective enzymes activities were employed. Survivors of the LC_{50} static tests were used for the enzyme studies. Blood was collected from the caudal vessels with a syringe into sample tubes that were not treated with anti-coagulant. After centrifugation, the serum was separated and frozen on dry ice and stored until needed for analysis.

III. RESULTS AND DISCUSSION

A. Heavy Metals Concentration in Tissues of Tilapia Fish from Qua Iboe River Estuary

Table II shows the heavy metals concentration in the tissues of tilapia fish obtained from the four (4) sampling locations in the study area.

TABLE II
HEAVY METAL CONTENT (MG/KG) OF FISH (DRY SEASON)

Sampling Station	Heavy Metals	Fish			∑x	Mean ± SD
		1	2	3		
Douglas Creek 1	Cu	2.80	3.30	2.90	9.00	3.00 ± 0.27
	Pb	<0.20	<0.20	<0.20	-	-
	Ni	<0.10	<0.10	<0.10	-	-
	V	<0.20	<0.20	<0.20	-	-
	Zn	39.50	49.50	41.00	120.00	40.00 ± 0.87
	Fe	45.60	46.20	46.20	138.00	46.00 ± 0.35
Qua Iboe 2	Cu	<0.05	<0.05	<0.05	-	-
	Pb	<1.00	<1.00	<1.00	-	-
	Ni	1.95	2.03	2.02	6.00	2.00 ± 0.04
	V	<0.001	<0.001	<0.001	-	-
	Zn	95.70	95.40	95.40	286.5	95.50 ± 0.17
	Fe	38.10	39.30	39.60	117.0	39.00 ± 0.79
Atlantic 3	Cu	<0.05	<0.05	<0.05	-	-
	Pb	<1.00	<1.00	<1.00	-	-
	Ni	3.20	2.90	2.90	9.00	3.00 ± 0.17
	V	<0.001	<0.001	<0.001	-	-
	Zn	43.00	42.80	43.20	129.00	43.00 ± 0.20
	Fe	391.20	388.70	390.10	1,170.00	390.00 ± 1.25
Control 4	Cu	<0.05	<0.05	<0.05	-	-
	Pb	<1.00	<1.00	<1.00	-	-
	Ni	1.90	2.07	2.03	6.00	2.00 ± 0.09
	V	<0.001	<0.001	<0.001	-	-
	Zn	105.50	106.00	106.50	318.00	106.00 ± 0.50
	Fe	633.40	632.50	633.10	1,899.00	633.00± 0.46

The heavy metal content of fish is shown in Table II for all the sampling locations (Douglas creek, Qua Iboe, Atlantic and control). Copper was detected in fish tissues from the Douglas creek sampling site. Lead and vanadium were not detected in tilapia fish from any of the four sampling locations. Nickel was detected in fish samples from Qua Iboe (2, 00 ± 0.04 mg/kg), Atlantic (3, 00 ± 0.17) and control (2.00 ± 0.09 mg/kg). Zinc and iron were however present in varying concentrations in tilapia fish from all the four sampling locations. The least value for zinc (40.00 ± 0.87 mg/kg was obtained for the Douglas creek sampling site whereas the highest value obtained at the control site was 106. 00 ± 0.50 mg/kg. Whereas the lowest value for iron was 39. 00 ± 0.79 mg/kg (Qua Iboe station) it was 633. 00 ± 0.46 mg/kg in fish samples from the control location.

B. Toxicity Studies

The LC_{50} obtained for the toxicity study for Pb, Fe and Cu in the simulated study were 2.65, 0.85 and 0.35mg/L,

respectively as shown in Figs. 2–4.

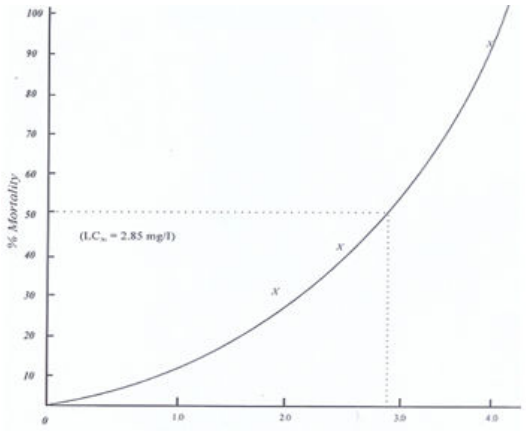

Fig. 2 Toxicity curve for lead (Pb)

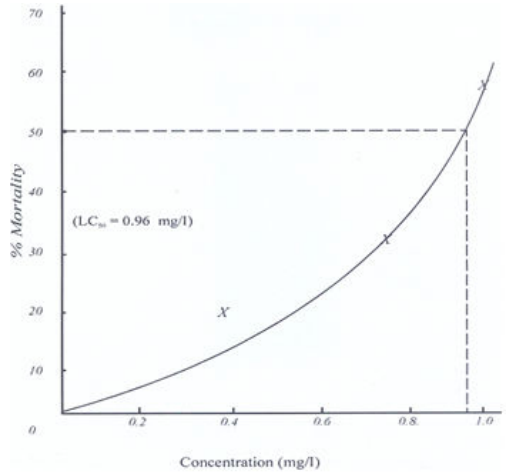

Fig. 3 Toxicity curve for iron (Fe)

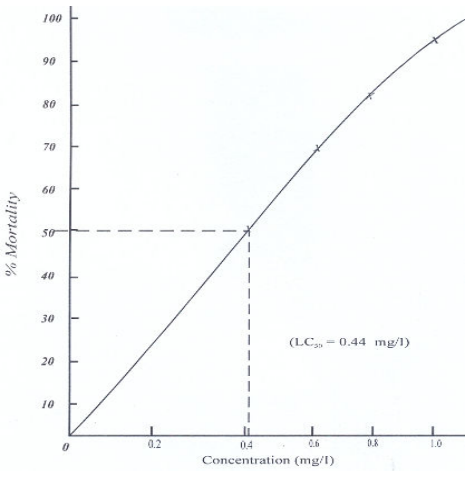

Fig. 4 Toxicity curve for copper (Cu)

C. Enzyme Studies

The results obtained for the serum and liver levels of enzymes under study are presented in Table II.

1) Activity of Aspartate Aminotransferase (AST) in Serum of Tilapia Fish Exposed to Heavy Metals for 96hr

The serum level of AST was measured in fish exposed to 2.65mg/L of lead, 0.85mg/L of iron and 0.35mg/L of copper. The heavy metals caused induction of the enzyme and hence enhanced serum activity as shown in Table II. Mean values of enzyme activity obtained were 14.67 ± 2.65 IU/L, 40.41 ± 11.29 IU/L and 20.52 ± 8.11 IU/L for the lead, iron and copper groups, respectively. The control group (with no heavy metal exposure) had a serum mean level of 15.28 ± 6.10 IU/L (Table II).

2) Activity of Alanine Aminotransferase (ALT) in Serum of Tilapia Fish Exposed to Heavy Metal for 96hr

Measurement of (ALT) activity in serum of fish exposed to 2.56mg/L of lead, 0.85mg/L of Iron and 0.35mg/L of copper gave mean serum levels of 51.93 ± 2.60 IU/L, 15.04 ± 3.06 IU/L and 46.87 ± 4.46 IU/L, respectively (Table II). The control group had 16.37 ± 0.79 IU/L as its activity.

3) Activity of Alkaline Phosphatase (ALP) in Serum of Tilapia Fish Exposed to Heavy Metal for 96hr

An increase in serum level of ALP in fish exposed to heavy metals (2.65mg/L of lead, 0.85mg /L of iron and 0.35mg /L of copper) gave the result shown in Table II. The mean values for each group are shown to be 19.56 ± 1.62 IU/L for lead group, 29.67 ± 2.17 IU/ L for the iron group and 1.15 ± 0.27 IU/L for the copper group. The control group was 9.99 ± 1.34 IU/L. From the result, it was obvious that whereas lead and iron caused increased release of the enzyme into the blood circulation (increased tissue damage), copper rather caused a reduction in the serum level as compared to the level in the control group.

4) Activity of Alkaline Phosphatase in Liver of Fish Exposed to Heavy Metals for 96hr

The fish samples were exposed to 2.65, 0.85 and 0.35mg/L of lead, iron and copper, respectively. The values of 102.14 ± 6.12, 140.17 ± 2.06 and 168.23 ± 3.52 IU/L were obtained for the enzyme activities produced by the lead, iron and copper groups while that of control group was 91.20 ± 9.42 (Table II).

TABLE II
MEAN VALUES OF ENZYME ACTIVITY (AST, ALT, ALP) (IU/L) AND TOTAL PROTEIN (G/L) IN SERUM AND LIVER OF FISH EXPOSED TO HEAVY METAL FOR 96 HRS

Enzyme	Tissue	Groups			
		Pb group	Fe group	Cu group	control group
AST	Serum	14.67 ± 2.65	40.41 ± 11.29	20.52 ± 8.11	15.28 ± 6.10
ALT	Serum	51.93 ± 2.60	15.04 ± 3.06	46.87 ± 4.46	16.37 ± 0.79
ALP	Serum	19.56 ± 1.62	29.67 ± 2.17	1.15 ± 0.27	9.99 ± 1.34
AST	Liver	6.67 ± 0.33	11.52 ± 0.37	14.47 ± 0.12	4.30 ± 0.11
ALT	Liver	18.72 ± 1.02	28.51 ± 3.21	21.82 ± 1.10	16.35 ± 0.50
ALP	Liver	102.14 ± 6.12	140.17 ± 2.06	168.23 ± 3.52	91.20 ± 9.42
TOTAL PROTEIN	Liver	32.05 ± 0.51	28.33 ± 0.46	29.79 ± 0.48	26.50 ± 0.27
	Serum	50.42 ± 3.05	46.16 ± 1.26	93.63 ± 6.28	39.30 ± 2.09

5) Total Protein Concentration in Serum and Liver of Tilapia Fish Exposed to Heavy Metals for 96hr

Values of 32.05 ± 0.51, 28.33 ± 0.46, 29.79 ± 0.48 as well as 26.50 ± 0.27 (mg/l) of total protein were obtained for liver of fish exposed to 2.65, 0.85 and 0.35 mg/l of lead, iron, copper and control groups respectively. The values for the serum total protein of fish exposed to the same levels of heavy metals as in the liver were 50.42 ± 3.05, 46.16 ± 1.26, 93.63 ± 6.28 and 39.30 ± 2.09 mg/l for lead, iron, copper treated groups and the control group respectively.

Biochemical investigations are commonly used to assess the structural integrity of the liver. In clinical practice, a group of tests is usually performed. Among them is the serum transaminase (aminotransferase) activity as a measure of the integrity of the liver cells [16]. In the present study, the aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were investigated.

The activities in serum of the transaminases – aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have become widely used in liver diseases. AST is present mainly in the liver and the heart, although small amounts are present in several other tissues. ALT is also widely distributed, but its measurement in liver disease is rather more specific as its activity is relatively low in tissues other than the liver. The normal serum level in human for each is less than 40IU/L [16].

Alkaline phosphatase in serum is used as an index of cholestasis. The isoenzymes are widely distributed but are found particularly in the liver. The normal serum level for human is 20 – 85IU/L.

It was seen from the result of the control group that without exposure to heavy metals, the fish had some basal levels of the enzymes and hence activity. Table II shows the basal levels of the enzymes (control group) both in the liver and serum. It would also be seen that the levels were raised above the basal levels both in the liver and serum when the fish were exposed to heavy metals (Pb, Fe and Cu). For instance, ALT activity in serum was 16.30 ± 0.79 IU/L in the control group and 51.93 ± 2.60IU/L in the lead group while it was 46.87 ± 4.46 IU/L in the copper group. It was however 15.04 ± 3.06 IU/L in the iron treated group. AST activity was 15.28 ± 6.10IU/L in the control group, 40.41 ± 11.29 IU/L in the iron group and 14.67 ± 2.65 IU/L for the lead treated group.

In the liver, AST activity was raised in all the heavy metal treated groups. Variability between the groups was obvious in both liver and serum. It is also obvious that the production of enzyme was heavy metal dependent.

In the liver, AST activity was raised in all the heavy metal treated groups. Variability between the groups was evident in both liver and serum. It is also obvious that the production of enzyme was heavy metal dependent.

It is however worth noting that the serum levels of these enzymes are indicative of the degree of damage of the liver cells. Naturally, the serum levels of these diagnostic enzymes are very low but if there is necrosis of the liver tissues, more are released into the blood circulation thus raising the serum levels.

Total protein was also seen to be raised in the serum of fish. Synthesis of total protein by the liver tissues was also increased due to the heavy metal exposure. The production of the enzymes/protein is usually in response to the presence of the pollutant in an attempt to metabolize the pollutant into less toxic forms.

IV. Conclusion

From the results obtained, it can be concluded that aquatic resources from the Qua Iboe river estuary are contaminated with heavy metal pollutants. The pollutants resulted in histological lesions in fish tissues [12] and elicited increased diagnostic enzymes (AST, ALT, ALP) production as body defense mechanisms. Tissue damages were seen to result in the release of the diagnostic enzymes with consequent rise in the serum of the fish. Prolonged and continuous pollution of the Qua Iboe river estuary with these pollutants will lead to (i) death of some (non-tolerant) organisms and (ii) adaptation and subsequent presence of aquatic resources resistant to the pollutants. The contaminated aquatic resources (tilapia fish) are consumed not only by residents of Akwa Ibom State but also by Nigerians in general. Continuous consumption of these contaminated aquatic resources could pose health risks to consumers. Regular research to monitor levels of the heavy metal pollutants in Qua Iboe river estuary and in aquatic resources with time should therefore be encouraged.

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