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Impact of Aluminum Phosphide on the Transferases in Liver and muscle of *Parophiocephalus obscurus*

Iniobong Reuben Inyang[1], Sylvester Chibueze Izah[2,*], Kesiena Desmond Okpogholor[1]

[1]Environmental Toxicology Research Unit, Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

[2]Department of Biological Sciences, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria.

Abstract

This study assessed the effect of aluminum phosphide on transferases in liver and muscle of *Parophiocephalus obscurus* (with mean weight of 42.20±1.5 gSD and mean length of 16.50± cmSD, respectively). The fish were obtained from a private fish farm in Yenagoa Metropolis, Nigeria, and the fish was allowed acclimatized to laboratory condition for 7 days, and then exposed to sublethal concentrations (0.00mg/L, 4.20mg/L, 6.30mg/L and 8.40mg/L) of aluminum phosphide for 14 days. Renewal bioassay was adopted in this study. At the end of the experimental period, the fish was dissected and the muscle and liver were collected, processed and analyzed for alanine aminotransferase and aspartate aminotransferase using colorimetric method. Results of the phosphatase at 0.00mg/L, 4.20mg/L, 6.30mg/L and 8.40mg/L were 94.50±6.44µ/L, 134.47±15.27 µ/L, 106.47±9.21 µ/L and 31.00±3.46 µ/L, respectively (liver), 107.50±9.24, 92.00±6.93 µ/L, 116.50±8.95 µ/L and 146.33±9.33 µ/L respectively (muscle) for aspartate aminotransferase; and 40.00±1.15µ/L, 26.50±3.18µ/L, 14.50±2.02µ/L and 9.80±1.44 µ/L, respectively (liver) and 17.00±1.75µ/L, 8.50±0.87µ/L, 21.00±2.89µ/L and 5.50±0.87 µ/L, respectively (muscle) for alanine aminotransferase. Statistically, there were significant variations ($p<0.05$) among the various concentration in the transferances. In addition, at some concentration, there was significant variations ($p<0.05$) between the level of the transferases in the muscle and liver. The significant alteration observed in the various concentrations is an indication that aluminum phosphide is lethal to fish. Therefore, caution should be exercise during the use of aluminum phosphide near biological system.

Corresponding author: Sylvester Chibueze Izah, Department of Biological Sciences, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria, Email: chivestizah@gmail.com

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Introduction

The contamination of the ecosystem is a major challenge to the environment itself and its associated biota [1, 2]. Environmental pollution is caused mostly by anthropogenic activities and to lesser effect by natural effects [3] especially in Nigeria. Contaminations associated with anthropogenic activities is significantly brought about by industrialization, unsustainable agricultural practices, population growth and urbanization [4, 5]. Basically toxins are materials/ substances that influence the physical, compound and natural qualities of biotic segments of the biological system just as the abiotic constituents, subsequently representing a danger to human wellbeing, and other creatures including plants, animals and microbes [3].

In the Niger Delta region of Nigeria, solid waste management is a major concern to environmentalist. Several wastes streams resulting from human activities are discharged into the environment (soil and surface water) where they cause an alteration or impact. The use of chemicals has also increased in the recent years. For instance, the use of pesticides has increased and its use depends on the target organism. Conventionally, rodenticides, herbicides, insecticides acaricides and fumigants are used to control rodents, weeds, insects, ticks and grain insects, respectively. The remains and empty cans of chemical substances such pesticides are careless discharged into the environment, and they can find their way to the aquatic organisms through runoff where they may impact on the aquatic organisms [6, 7, 8]. Basically, the constituents of the chemicals depend on the intended use.

Aluminum phosphide is a fumigant used in preserving grains [9] such as maize. Aluminum phosphide is toxic to both target and non-target organisms. Aluminum phosphide has been described as suicide poison with no effective treatment remedy when ingested [10]. Aluminum phosphide is known as "trebor" by local farmers in some parts of Nigeria [11]. Authors have reported that aluminum phosphide could be mistakenly grounded with maize during the processing of fish feed [11, 12]. The authors reported that Aluminum phosphide could cause an alteration in cyto-achitecture of the vital organs in animals. Probably due to its toxicity, there is a concern associated with its misuse [9]. The lethality of aluminum phosphide is related with the arrangement of phosphine gas when it gets in contact with dampness [9, 10, 11].

Fish have been generally used to survey the lethality of chemicals in the aquatic environment. Therefore, this study assessed the impact of aluminum phosphide on the transferases in liver and muscle of *Parophiocephalus obscurus*.

Materials and Methods

Experimental Stock and Acclimatization

Twenty-four grown-up *Parophiocephalus obscurus* with mean weight of 42.20±1.5 gSD and mean length of 16.50± cmSD, were prelevated from a private fish ranch in Yenagoa, Bayelsa State, Nigeria. The tests fish were transported to the Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, where the bioassay were done. The fish were allowed to adapt to research facility condition in a rectangular aquarium for 7 days. During the period, the fish were nourished with remains of digestive organs of other fish once a day.

General Bioassay Technique

Sublethal grouping of aluminum phosphide was made dependently on the range discovering test by Inyang [13]. The levels of the aluminum phosphide utilized for the bioassay were made by pipetting 0.40, 0.60 and 0.80 mL of the first grouping of aluminum phosphide (33% w/w) and afterward made up to 30 L with borehole water in aquarium to make a convergence of 4.20 mg/L, 6.30 mg/L and 8.40 mg/L. Every concentration contains 3 fish. A control (without toxicant) was likewise set-up. The toxicant was reestablished day by day for 14 days. The qualities of the water utilized for the bioassay were determined using APHA [14] method and the accompanying qualities were recorded: temperature 24.00 – 24.17°C, pH 6.17 - 6.34, conductivity 98.49 – 132.08µ/cm, alkalinity 10.30 – 16.07 mg/l, dissolved oxygen 4.36 – 7.17mg/l and turbidity 0.15 – 0.48 NTU.

Sample Collection and Analysis

At the end of the experiment period (14 days), the fish were dissected in order to obtain the liver and muscle. Approximately 0.5 g of each part were pounded in a neat pestle and mortar and physiological saline was included for adjustment. The samples were centrifuged for 15 minutes at 3000 rpm and the supernatant was

analyzed for aspartate amino transferase and alanine amino transferase using colorimetric technique previously described by Reitman and Frankel [15]

Statistical Analysis

SPSS version 20 was used for statistical analysis. The data acquired were expressed as mean ± standard error, and Single factorial was carried out at p=0.05 to show significant difference across the various concentrations, and Waller-Duncan tests statistics were used to discern the source of the observed difference. In addition, at various concentrations, the level of the transferases in the muscle and liver was compared using t-test. The charts showing the Standard error bar was plotted using GraphPad prism 5.

Results and Discussion

Figure 1 shows the concentration of Aspartate aminotransferase in liver and muscle of *Parophiocephalus obscurus* exposed to sublethal concentrations of Aluminum phosphide for 14 days. In the liver, the concentrations of aspartate aminotransferase at 0.00 mg/L, 4.20 mg/L, 6.30 mg/L and 8.40 mg/L were 94.50±6.44µ/L, 134.47±15.27 µ/L, 106.47±9.21 µ/L and 31.00±3.46 µ/L, respectively. There were significant variations ($p<0.05$) across the concentrations. Multiple comparisons showed no significant variation between 0.00 mg/L and 6.30 mg/L, and between 4.20 mg/L and 6.30 mg/L. While in the muscle the concentrations of Aspartate aminotransferase were 107.50±9.24, 92.00±6.93 µ/L, 116.50±8.95 µ/L and 146.33±9.33 µ/L at 0.00 mg/L, 4.20 mg/L, 6.30 mg/L and 8.40 mg/L, respectively. Statistically, significant difference ($p<0.05$) was noticed across the concentration. Pairwise comparison reviewed no significant variations between 0.00 mg/L, 4.20 mg/L and 6.30 mg/L, and between 6.30 mg/L and 8.40 mg/L.

On comparative analysis, the levels of Aspartate aminotransferase in the liver and muscle were significantly different ($p<0.05$) at 0.00 mg/L and 8.40 mg/L, and not significantly different ($p>0.05$) at 0.420 mg/L and 6.30 mg/L (Table 1). The levels of the Aspartate aminotransferase across the various concentration of the toxicant significantly differ in the muscle and liver of *Parophiocephalus obscurus.* This suggests the effects of Aluminum phosphide on the liver and muscle aspartate aminotransferase.

Basically, aspartate aminotransferase is an essential enzyme found in many cells of the body especially in the liver, muscle, and kidney. Typically, the aspartate aminotransferase tends to be low in normal condition, but when an organism is exposed to stressed condition and the liver and other cells that contain significant amount of aspartate aminotransferase is

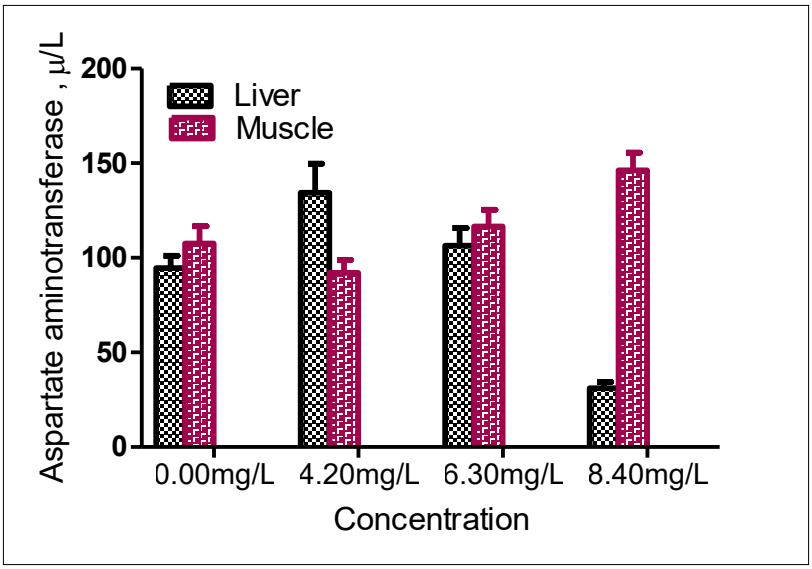

Figure 1. Concentration of Aspartate aminotransferase in liver and muscle of *Parophiocephalus obscurus* exposed to sublethal concentration of Aluminum phosphide for 14 days

Table 1. Comparative analysis of Aspartate aminotransferase (µ/L) level in liver and muscle of *Parophiocephalus obscurus* exposed to sublethal concentration of Aluminum phosphide for 14 days

Concentration, mg/L	Liver	Muscle	t-value	p-value
0.00	94.50±6.44	107.50±9.24	-1.143	0.000
4.20	134.47±15.27	92.00±6.93	2.532	0.064
6.30	106.47±9.21	116.50±8.95	-0.781	0.478
8.40	31.00±3.46	146.33±9.33	-11.584	0.000

Data were expressed as mean ± standard error

adversely affected, its level will be elevated. The trend observed in this study is in accordance with those previously reported: *Clarias gariepinus* were exposed to Fluazifop-p-Butyl [8] and phenol [7]; and *Clarias lazera* were exposed to dimethoate [16]. In this study, the aspartate aminotransferase was observed in both muscle and liver at high concentration in most of the concentrations. This suggests the effect of aspartate aminotransferase on the liver and muscle cells.

The concentrations of alanine aminotransferase in liver and muscle of *Parophiocephalus obscurus* exposed to sublethal concentration of Aluminum phosphide for 14 days are presented in Figure 2. At 0.00 mg/L, 4.20 mg/L, 6.30 mg/L and 8.40 mg/L the concentration of alanine aminotransferase in the liver were 40.00±1.15µ/L, 26.50±3.18µ/L, 14.50±2.02µ/L and 9.80±1.44 µ/L, respectively. Statistically, there were significant difference ($p<0.05$) across the concentrations. Mean separation showed that the significant variations that occurred were from 0.00 mg/L and 4.30 mg/L. At 0.00 mg/L, 4.20 mg/L, 6.30 mg/L and 8.40 mg/L the concentration of alanine aminotransferase in the muscle were 17.00±1.75µ /L, 8.50±0.87µ/L, 21.00±2.89µ/L and 5.50±0.87 µ/L. Pairwise comparison reviewed no significant variations between 0.00 mg/L and 6.30 mg/L, and between 4.20 mg/L and 8.40 mg/L. On comparative analysis, the concentrations of alanine aminotransferase in the liver and muscle were significant different ($p<0.05$) at 0.00 mg/L and 4.20 mg/L. In addition, there was no significant difference ($p>0.05$) at 0.630 mg/L and 8.40 mg/L (Table 2).

The levels of the alanine aminotransferase across the various concentrations of the toxicant (Aluminum phosphide) decreased in the muscle and liver of adult *Parophiocephalus obscurus* except the muscle at 6.30mg/L that showed significant elevation. This is an indication of the adverse effects of Aluminum phosphide on the concentration of aspartate aminotransferase in the liver and muscle *Parophiocephalus obscurus.* The trend observed in this study is in line with previously works on chemical toxicity to aquatic organisms [7, 8, 16]. Like Aspartate aminotransferase, alanine aminotransferase is an important parameter used in determining the working condition of the liver. Alanine aminotransferase is an important enzyme that is found in liver, muscle and kidney of organisms that contribute in the breakdown of proteins in the body of several organisms. In addition, the alanine aminotransferase helps the liver to filter toxic substances from blood, store some nutrients and bile production. Most of the alanine aminotransferase that is produced in the liver are stored there, and where the liver is damaged they will be released into the blood. Therefore, significant elevation in the alanine aminotransferase concentration of the liver and muscle of *Parophiocephalus obscurus* exposed to Aluminum phosphide suggests the effect of toxicity of the xenobiotics on the probe organisms. Based on the comparative assessment of the concentration of alanine aminotransferase in the liver and muscle of *Parophiocephalus obscurus,* it can be deduced that the liver accumulates more alanine aminotransferase than the muscle. This may be attributed to the role of liver alanine aminotransferase in breakdown of protein,

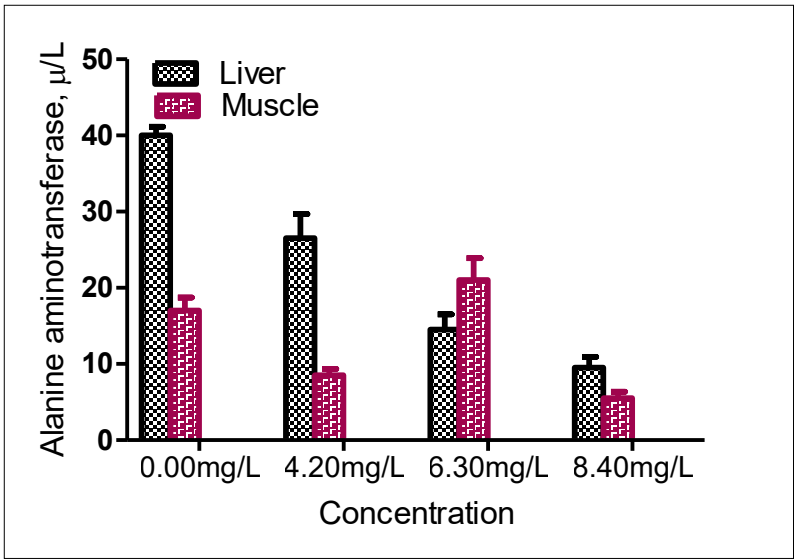

Figure 2. Concentration of Alanine aminotransferase in liver and muscle of *Parophiocephalus obscurus* exposed to sublethal concentration of Aluminum phosphide for 14 days

Table 2. Comparative analysis of Alanine aminotransferase (µ/L) level in liver and muscle of *Parophiocephalus obscurus* exposed to sublethal concentration of Aluminum phosphide for 14 days

Concentration, mg/L	Liver	Muscle	t-value	p-value
0.00	40.00±1.15	17.00±1.75	11.049	0.000
4.20	26.50±3.18	8.50±0.87	5.469	0.005
6.30	14.50±2.02	21.00±2.89	-1.848	0.139
8.40	9.80±1.44	5.50±0.87	2.376	0.070

Data were expressed as mean ± standard error

storage of nutrients and filtration of toxic substances.

Conclusion

This study evaluated the impact of aluminum phosphide on the transferases of muscles and liver of *Parophiocephalus obscurus*. The investigation showed that aluminum phosphide initiates critical alteration on the transferases (alanine aminotransferase and aspartate aminotransferase) in the liver and muscle of *Parophiocephalus obscurus*. The impacts >0.00 to 8.00 mg/L of aluminum phosphide could be detrimental to the exposed organisms. The impact could be enhanced if the concentration of the toxicant increased. Hence there is compulsory to be cautious during the use of aluminum phosphide near biological system.

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