Studies on Heavy Metals Levels in Hepatic Tissues of Cat Fishes (*Clarias gariepinus*) from River Ibi, Donga and Gindin-Dorowa in Taraba State Nigeria, in Relation to key Enzyme of heme Biosynthetic Pathway

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**Abstract**

Fishes are excellent markers of the extent of pollution from heavy metals in aquatic environments given that they are found at various levels of the food chain. This study aimed to investigate the bioaccumulation of heavy metals (Zn, Pb, Cd, As, and Hg) as well as the activity of delta-aminolevulinic acid dehydratase (δ-ALA-D) in the livers of cat fishes (*Clarias gariepinus*) collected from three rivers (Donga, Ibi and Gindin-Dorowa) in Taraba State, Nigeria. The concentrations of heavy metals in the liver tissues were determined using an atomic absorption spectrophotometer in accordance with the method of AOAC (2019), while the δ-ALA-D activity was assayed using the method of Sassa (1982). Results revealed that only Zn and As were present in the liver samples from the three rivers. Pb was found only in the liver from Gindin-Dorowa at the concentration of 0.0012 mg/kg which is not significant (*P* < 0.05) when compared with other locations, while Hg and Cd were absent in all the liver samples. The liver sample from Gindin-Dorowa had the highest concentration of Zn (4.2500 mg/kg), followed by Ibi (3.2067 mg/kg), and Donga having the least (2.5500 mg/kg), which were all substantially (*P* < 0.05) different from one another. However, there was no significant (*P* > 0.05) difference in the As composition of liver from Donga (0.0013 mg/kg), Ibi (0.0012 mg/kg) and Gindin-Dorowa (0.0010 mg/kg). The result of δ-ALA-D activity showed that the highest enzymatic activity was found in the liver sample from Donga which has the least Zn and no Pb content, followed by Ibi sample. This validates the report that heavy metals impair δ-ALA-D activity. Nonetheless, the concentrations of all metals in fish livers from all regions do not exceed the acceptable limits set by international law, making them safe for human consumption and possibly not having a negative impact on public health. Since there is little or no industrial activity in the studied locations, these levels may be consequent to low anthropogenic inputs. The current situation should be safeguarded to prevent pollution of the river’s aquatic biota in the near future, and more appropriate steps should be made to guarantee higher fish quality and life in the rivers.

**Keywords:** heavy metals; bioaccumulation; delta-aminolevulinic acid dehydratase; Atomic Absorption Spectrophotometer; *Clarias gariepinus*; bioaccumulation

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Introduction

Heavy metal pollution of waterways has gained scientific attention consequent to its hazardous effects on living organisms [1–3]. The discharge of various treated and untreated liquid wastes to the water bodies, as well as rocks and soil that are immediately exposed to surface water, are some of the sources of heavy metals entering rivers and lakes [4]. Agrarian and industrial revolution increasing heavy metals are being added to water sources from various sources [5]. Their rise is as a result of human activities like burning fossil fuels, mining and smelting metal ores, farming, producing industrial waste, and inadvertently disposing of chemicals containing heavy metals [6]. As a consequence, heavy metal concentrations in the waterways have been rising over safe levels [7–9]. Given their persistence and potential for the absorption and biomagnification, the symptoms linked to heavy metal poisoning give rise to concern [10]. In addition, heavy metals are toxic and can damage soft tissues easily, even at very low concentrations [11]. Cobalt (Co), lead (Pb), mercury (Hg), arsenic (As), thallium (Tl), nickel (Ni), manganese (Mn), zinc (Zn), cadmium (Cd), and chromium (Cr) are a few of the more than ten (10) heavy metals that have a special significance in ecotoxicology due to their high tenacity [12]. Beyond a specific threshold, metal levels like Mn, Zn, and Cr are hazardous, whereas Pb, Ni, and Cd are dangerous even in trace amounts [13, 14]. When these heavy metal levels in drinking water exceed the advised limit for any individual element, toxicity is manifested. The WHO (2008) recommended limits for these heavy metals are as follows: for drinking water, 0.01 mg/L, 0.07 mg/L, 0.4 mg/L, 3.0 mg/L, 0.003 mg/L, and 0.05 mg/L, respectively; for Pb [15] and Ni [16], 2.0 mg/kg in fish. The concentrations of these heavy metals rise due to weathering of rocks and a variety of anthropogenic activities that are influenced by the seasons [17]. In addition to other negative consequences, exposure to excessive concentrations of these heavy metals can seriously harm the brain and kidneys, result in miscarriage in female pregnant patients, harm male reproductive systems, and possibly even result in death [18]. In order to evaluate the levels of metal contamination in lakes and rivers, fish have been recognized as one of the most important indicators [17, 19]. Heavy metals have been extensively researched in a variety of fish components, including the gills, liver, kidneys, and muscles [17, 20, 21]. To learn more about how these metals are transported to fish from the aquatic ecosystem, researchers have examined the movement factor of heavy metals in fish organs such as the gills, liver, muscles, and scales in relation to water and sediments [19–22]. Given ideal conditions, sediments serve a vital role in removing contaminants from aquatic environments by acting as important sinks for diverse pollutants such as pesticides and heavy metals [23]. Heavy metal concentrations have risen from fish organs and water to sediments, and they change with the seasons [24–25]. However, as water volume increases, the concentrations of heavy metals also drop down the river as a result of dilution [24]. As a result, drinking river water or consuming river fish that has accumulated heavy metals puts humans at risk for poisoning.

In the heme biosynthesis pathway, δ-ALA-D is an enzyme that catalyzes the condensation of two aminolevulinic acid (ALA) molecules into porphobilinogen, a precursor to heme [26]. The impairment of heme biosynthesis that may arise from δ-ALA-D inhibition [27] might lead to an accumulation of ALA, which may disrupt aerobic metabolism and also promote some prooxidant activities [28]. Elegant researches have also demonstrated that heavy metals such as lead inhibit the activity of mammalian δ-ALA-D in tissues, including blood liver, kidney, and bone marrow, resulting in accumulation of δ-ALA-D which can eventually lead to tissues toxicity and impairment of some aerobic metabolisms [29]. Because they have the ability to bioaccumulate in many body areas, heavy metals are lasting environmental pollutants that are particularly dangerous to people. Due to their solubility in water, the majority are exceedingly hazardous, and even at low concentrations, they may still cause injury [30]. Long-term exposure to heavy metals from various sources may result in chronic accumulation in fish livers that disrupt several biochemical processes and cause disorders of the heart, liver, kidney, and bones [31]. Ibi, Donga and Gindin-Dorowa rivers are three important rivers in Taraba State, Nigeria which are of economic value and liable to untreated agricultural, urban, and industrial effluents that include heavy metals. Since these rivers’ tributaries run through populous residential areas, towns, industrial, and agricultural locations, this could lead to bioaccumulation of heavy metals in people who drink the water and consume the fish from these rivers. However, there is little or no experimentally guided information as regard heavy metals concentration in the hepatic tissues vis-à-vis the activity of hepatic δ-ALA-D in fishes from the rivers of the three different locations. The present study is therefore expedient in order to determine whether the fish from the previously mentioned rivers is fit for human consumption.

Materials and methods

Reagents/Chemicals

Tris-HCl buffer pH 7.4, Sodium Dodecyl Sulphate, Distilled H2O, ALA, trichloro acetic acid and Erlich’s reagent were gotten from Sigma Chemical Co. in St. Louis, Missouri, USA. The remainder of the chemicals, all of which were of analytical grade, were purchased from well-known commercial vendors.

Collection of Fish Samples (Figure 1)

Adult catfishes (Clarias gariepinus) of about 2.0 kg each were caught from Ibi, Gindin-Dorowa and Donga Rivers in Taraba State with the service of local fishermen using a trawl net at the river and the fishes were used for the assessment of heavy metals and assay of hepatic δ-ALA-D activity.

Collection of Liver Tissues

The fishes were decapitated, liver was taken out, immediately chilled, and prepared for analysis by washing in 50 mM Tris-HCl buffer (pH 7.4).

Heavy Metals Analysis

Heavy metals analysis content of the liver tissues was determined according to the method of AOAC (2019).

Aashing

2 g of liver sample was placed in a platinum dish before being placed in a muffle furnace, which was heated to roughly 550 °C for 4–5 hours. Once the sample turned completely to ash, it was removed and allowed to cool in a desiccatar.

Digestion of Samples

2.00 g of ash samples (liver) was transferred into a kjeldal flask followed by addition of 25 mL of digestion acid (Aqua regia HCl: HNO3, 3: 1). The flask was swirled and heated gently at first until frothing stopped, then more strongly until cleared pale yellow solution was gotten. After allowing the mixture to cool, the digest was put into a volumetric flask measuring 100 ml, which was then filled with distilled water and then filtered using Whatman No. 1 filter paper. The filtrate was brought to the AAS (Bulk Scientific, VPG 20, La Vegas, USA), where each desired metal’s hollow cathode lamp was installed. The wavelength characteristics of each heavy metal were set for the determinations using the air acetylene integrated flame mode (all heavy metals). Standard for each metal was obtained by extrapolation from the calibration curve of standard [32].

Determination of δ-Aminolevulinic Acid Dehydratase Activity Preparation of tissue homogenate

The liver tissues were homogenized at 40 °C in a cold solution of 50 mM Tris-HCl buffer, pH 7.4, using a homogenizer. For the δ-ALA-D assay, a low-speed supernatant was produced by centrifuging the
homogenates at 4,000 rpm for 10 minutes.

**δ-Aminolevulinic Acid Dehydratase Assay**

By monitoring the rate at which the product porphobilinogen (PBG) forms, δ-ALA-D activity was assessed in accordance with Sassa’s [33] approach, with the exception that 84 mM potassium phosphate buffer, pH 6.4, and 2.4 mM ALA were utilized. At 37 °C, incubations were conducted for two hours. Using modified Ehrlich’s reagent at 555 nm and an Ehrlich-PBG salt with a molar absorption coefficient of 6.1 x 104 M⁻¹, the purple reaction products were identified.

**Statistical Analysis**

The mean ± standard deviation (SD) was used to express all values. The proper ANOVA was used to evaluate the data, and then, where necessary, Duncan’s multiple range tests.

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![Taraba State map](https://www.tmrjournals.com/atr)

**Figure 1** Taraba State map showing the three sampling location, River Ibi, Donga and Gindin Dorowa
Result

Heavy Metals Composition of Fish Liver Samples
The levels of heavy metals in the livers of *Clarias gariepinus* from the rivers; Donga, Ibi, and Gindin-Dorowa are displayed in Table 1. Zn and As were present in livers from the three rivers, Pb was found only in the liver from Gindin-Dorowa at the concentration of 0.0012 mg/kg which is not significant different when compared with Ibi and Donga with no Pb. Hg and Cd were found to be absent in all the liver samples from all locations. The Zn concentrations in the liver samples varied significantly (*P* < 0.05), with Gindin-Dorowa having the highest level (4.2500 mg/kg), followed by Ibi (3.2067 mg/kg), and Donga having the lowest level (2.5500 mg/kg). The As content of liver from Donga (0.0013 mg/kg), Ibi (0.0012 mg/kg), and Gindin-Dorowa (0.0010 mg/kg) did not differ significantly (*P* < 0.05).

δ-Aminolevulinic Acid Dehydratase Activity in Fish Liver Samples
The result of δ-ALA-D assay in the fish liver tissues reveals that the liver sample from Donga has the highest enzymatic activity which is markedly (*P* < 0.05) different when compared with Gindin-Dorowa and Ibi samples (Figure 2).

<table>
<thead>
<tr>
<th>Heavy Metals</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donga (mg/kg)</td>
</tr>
<tr>
<td>Lead</td>
<td>0.0000 ± 0.00000*a</td>
</tr>
<tr>
<td>Zinc</td>
<td>2.5500 ± 0.00000*b</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.0013 ± 0.00004*b</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.0000 ± 0.00000*b</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.0000 ± 0.00000*b</td>
</tr>
</tbody>
</table>

Results are presented as Mean ± SD, with *N* = 3. Means with identical letters in a row do not differ significantly (*P* < 0.05).

Figure 2 Delta-aminolevulinic acid dehydratase activity in the fish livers from the three rivers. Results are presented as Mean ± SD, means with identical letters do not differ significantly (*P* < 0.05).
Discussion

Fishes are effective markers for the extent of heavy metal contamination in aquatic systems, because they occupy various levels of the food chain [34]. The aquatic ecology is significantly affected by heavy metals.

Results of heavy metals concentrations in the livers of Clarias gariepinus from river Donga, Ibi and Gindin-Dorowa are presented in Table 1. Pb was found only in the liver from Gindin-Dorowa at the concentration of 0.0012mg/kg which is not significantly (P < 0.05) different from Ibi and Donga with no Pb composition. These values are within the acceptable limits (0.02 mg/kg, 1 ppm, and 0.2 mg/kg) published by the European Union (EU) [35] and World Health Organization (WHO) [36]. Every organ and system in the body is susceptible to lead, which is a possible human carcinogen. High quantities of lead can have fatal effects on the kidney, brain, and other organs [37]. This result is different from 1.30 mg/kg and 0.78 mg/kg documented by Nwade et al. [38] and Tyokumbur and Unuma respectively [39]. It is also lower than the mean value 0.0291 mg/kg reported by Wangboje and Ikuhae [40].

Hg and Cd were found to be absent in the liver samples from all locations. The result is in discrepancy with mean values of 0.30, 0.0917 and 0.04 mg/kg of Cd documented by Enejie et al. [41], Wangboje and Ikuhae, [40] and Tyokumbur and Unuma [39] respectively and the permissible limit is 0.05 [36]. There was a variance in the Zn amounts in the liver samples and this is statistically significant (P < 0.05), with the highest level found in Gindin-Dorowa (4.2500 mg/kg) followed by Ibi (3.2067 mg/kg) and the least being Donga (2.5500 mg/kg). This value is lower than the permissible limits (40mg/kg respectively) reported by WHO [36]. Zn often accumulates in fish gills and causes structural damage that affects fish growth, development, and survival. Excess Zn also inhibits calcium uptake in fish, which can be fatal. Though, the As content of liver from Donga (0.0013 mg/kg), Ibi (0.0012 mg/kg), and Gindin-Dorowa (0.0010 mg/kg) did not differ considerably (P <0.05), the values are below the permissible limits (0.1mg/kg) reported by [42]. One of the primary threats to the public's health is As, a dangerous heavy metal. Routes of exposure to As include the workplace or tainted food and water. Due to damage to the capillary endothelium, a rise in vascular permeability causes vasodilatation and circulatory collapse [43].

In the heme biosynthesis pathway, the enzyme δ-ALA-D, also known as porphobilinogen synthase (PBGS, EC 4.2.1.24), catalyzes the asymmetric fusion of two aminolevulinic acid (ALA) molecules to generate porphobilinogen (PBG) [44]. All species share comparable biosynthetic processes for the pyrrole, which is a frequent precursor to tetrapyrrole pigments such as heme, chlorophyll, and cobalamin, coronins [45, 46]. Although PBGS exhibits great evolutionary heterogeneity in the use of metal ions for catalytic and allosteric activities, its sequence and structure are extremely substantially conserved [47, 48]. In humans and other animal species, δ-ALA-D is recognized as an excellent biomarker of Pb exposure and effect [49, 50]. As a sulphydryl enzyme [44, 51], studies have shown that several metals, including lead [52, 53], mercury [54, 55], and other substances that oxidize sulphydryl groups, have altered the activity of the enzyme δ-ALA-D, that has sulphydryls [56, 57]. δ-ALA-D activity assay in the fish livers shows that the highest enzymatic activity was found in the liver sample from Donga which has the least Zn and no Pb content, followed by Gindin-Dorowa (Figure 2). The variation in this concentration might be due to the effect of heavy metals present. This is in line with the report that elevated level of heavy metals decreases or inhibits the activities of δ-ALA-D [56, 57]. Moreover, the concentrations of fish liver heavy metals herein are similar to those reported in the fish muscles by Assulemen and Ale [58] from the studied areas.

Conclusion

Results revealed that only Zn and As were present in the liver samples from the three rivers, Pb was found only in the liver from Gindin-Dorowa, while Hg and Cd were absent in all the liver samples. In addition, the result of δ-ALA-D activity showed that the highest enzymatic activity was found in the liver sample from Donga which has the least Zn and no Pb content. However, the concentrations of all metals in fish livers from all locations do not transcend the international legislation permissible limits, thus safe for eating by humans and possibly not a concern to public health. Since there is little or no industrial activity in the studied locations, these limits may be due to low anthropogenic contributions.

Recommendation

The current situation should be safeguarded to prevent river contamination in the near future and more appropriate steps should be done to guarantee higher fish quality and aquatic life.

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