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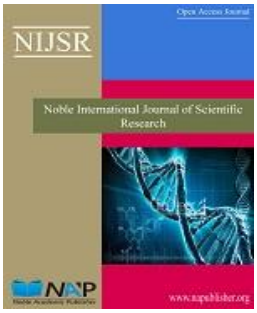

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EFFECT OF PHENOL ON THE KIDNEY AND LIVER BIOCHEMICAL AND METABOLITES OF CLARIAS GARIEPINUS

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ABSTRACT: Phenolic compound is among the toxic compound found in produced water. In addition, phenol has several industrial applications. This study assessed the biochemical and metabolites characteristics in liver and kidney of *Clarias gariepinus* with mean weight and length of 78.05±0.40g and 14.07 ±0.29cm, respectively, exposed to phenol. The fish was acclimatized to laboratory condition for 7 days, and then exposed to sublethal concentration (viz:0.00, 0.10, 0.20, 0.30ppm) of phenol in renewable bioassays for 14 days. The metabolites including alkaline phosphatase, alanine amino transferase and aspartate amino transferase, protein and albumin were analyzed in the liver and kidney of the fish sample. Results of the metabolites in the kidney and liver of were in the range of 3.00±0.23µ/L - 5.00±0.35µ/L and 20.50±1.85µ/L - 30.50±2.83µ/L, respectively (alanine amino transferase), 39.37±5.02µ/L - 45.57±4.045µ/L, and 48.00±2.04µ/L -165.00±8.43µ/L, respectively (aspartate amino transferase), 46.47±3.49µ/L - 75.00±5.48µ/L and 8.50±0.64µ/L - 63.53±3.493µ/L, respectively (alkaline phosphatase), 26.00±1.76g/L - 34.17±3.32g/L and 20.50±2.94g/L - 27.50±4.04g/L, respectively (total protein), and 5.49±0.35g/L - 6.70±0.45g/L and 5.80±0.46g/L - 7.00±0.17g/L, respectively (albumin). Statistically, there was significant difference ($p<0.05$) among the various concentrations for most of the metabolites in the different organs (liver and kidney). The variations observed among the different concentrations suggest that phenol is toxic to fish metabolites. Hence, there is the need to adequately treated effluents containing phenolic compounds before being discharged into the aquatic ecosystem.

Keywords: Ecotoxicology, Fish Toxicity, Metabolites, Phenol, Surface Water.

1. INTRODUCTION

Phenols are aromatic and organic compounds found in diverse variety of biotic and abiotic factors, and at certain conditions they could cause contamination [1]. Phenol in domestic and industrial effluents are the major route through which it enters the aquatic ecosystem, where they induce varying level of toxicity on aquatic organisms depending on the concentration, and age and species of the organisms exposed.

Produced water is among the industrial effluents that contain phenolic compounds. This produced water emanates from crude oil extraction. Due to the toxicity of phenol based compounds, different countries set a limit for their presence in water meant for domestic use. For instance, Department of Petroleum Resources, Nigeria, The Department of Petroleum Resources (DPR) [2] limits for phenol is 0.5mg/l in treated petroleum refinery (fuel oil/gasoline/lube oil category) and petrochemical refinery waste water (carbon black and polypropylene) and 0.1mg/l in petrochemical waste water containing linear alkyl benzene. The agency also set phenolic compounds limit of 0.02mg/l as highest desirable level and 0.002mg/l as desirable maximum permissible level in water meant for domestic use.

The effects of toxic substances have been widely assessed using different fish species. This is because fish has been recognized to possess bioaccumulation and bio magnification potentials; hence they are used to assess the health status of an aquatic ecosystem [3]. Fish has the tendency to absorb toxic substances through their body parts (gills, skin, intestine and mucous membranes) [1]. When this toxicant enters the blood they can cause an alteration in the metabolic and biochemical processes including histological, haematological, electrolytes, metabolites, morphological and behavioral response and death [4-13]. Different toxic substances have varying mode of action. Studies have indicated that the mechanism

of action of phenol and some of its derivatives include genotoxicity [1, 14, 15], carcinogenesis, mutagenesis [1, 16, 17], endocrine disruption [15, 18], metabolic disruption [1, 19], liver dysfunction, and reduction in growth rate and bioaccumulation [15].

In fish, the metabolites and biochemical parameters provide useful information about disruption of metabolic processes due to the effect of toxicants. According to Moraes, et al. [1], responses in the biochemical parameters are very sensitive and provide early information about the toxicants status. Sannadurgappa, et al. [20] reported that *Cyprinus carpio* exposed to sublethal concentration of phenol had decreased protein, carbohydrate and lipids content in the tissues of liver, gills, muscle, brain and intestine. The authors further reported that increased exposure enhanced decline of organic matter. Sannadurgappa, et al. [21] reported 35.0 mg/l in *Oreochromis mossambicus* exposed to phenol as acute toxicity. The authors further reported that on exposure to sublethal concentrations (2.3 and 3.5 mg/l) for 30 days, there a decline in protein, total carbohydrate, and total lipids in the tissues of liver, gill, and muscle of the *Oreochromis mossambicus*. In addition, the biochemical variations were dose and duration dependant [21]. Gad and Saad [15] studied *Oreochromis niloticus* exposed to sublethal concentrations of phenolic compound (0.7, 1.4 and 2.8 mg/L) for 16 weeks, and reported a decline in growth performance, serum tri-iodothyronine and thyroxin hormones, an increase in genotoxic potential, and bioaccumulation of phenol in the tissues (liver, muscles and gills). Moustafa, et al. [22] studied the effect of phenol, on Nile tilapia (*Oreochromis niloticus*), and reported erosion of fins and tail, pale gills and liver using macroscopic approach, and desquamated and hyperplastic gill lamellae, neuronal degeneration, hyperplasia of epidermis and Zenker's necrosis of muscles using microscopic technique. The author further reported residual phenol in the fish muscles with a concentration of 0.07, 0.25 and 1.15 ppm at 4, 8 and 12 weeks, respectively. In fish, phenol contamination could lead to metabolic dysfunctioning which could lead to growth, reproduction and disease resistance problems [1]. Therefore, this present study aimed at assessing the effects of phenol on the kidney and liver enzymes, total protein and albumin of *Clarias gariepinus.*

2. MATERIAL AND METHODS

2.1. Fish Sources and Acclimatization

Thirty (30) African cat fish (*Clarias gariepinus*) with mean weight and length of 78.05±0.4g and 14.07 ±0.29cm, respectively was purchased from a fish farm at Okaka, Yenagoa, Bayelsa State, Nigeria. The fish were transported to the Department of Biological sciences laboratory, Niger Delta University, Bayelsa State. The fish were allowed to acclimatize to laboratory condition for 7 days and fed once during the period with normal fish diet.

2.2. Experimental Set-Up

Three sub-lethal concentrations (0.10ppm, 0.20ppm and 0.30ppm) of phenol was made based on the preliminary test assessment by Inyang, et al. [23]. The concentrations were made by pipetting of the phenol from stock solution into aquarium, and then 25 L borehole water was added. The experiment lasted for 14 days and during the period, the toxicant was renewed. The characteristics of the physiochemical quality of the water used were determined following American Public Health Association (APHA) [24] standard and the values were 26.00 – 26.18^{o}C (Temperature), 6.15-6.32 (pH), 11.36-18.09mg^{-1} (alkalinity), 96.46-125.00µs/cm (conductivity), 3.98 – 6.20mg^{-1} (dissolve oxygen) and 0.16 -0.25NTU (turbidity).

2.3. Metabolites Evaluation

At the end of the experiment, the fish were dissected and the liver and kidney were collected. Approximately 0.5g of each organ was grounded with clean pestle and mortar and physiological saline was added for stabilization [9, 10]. The samples were then centrifuged for 15 minutes at 3000rpm and the supernatant were carefully collected for analysis. The aspartate amino transferase and alanine amino transferase was analyzed based on the colorimetric method of Reitman and Frankel [25], alkaline phosphatase was determined using the scheme of using Kind and King [26] method, total protein was determined using the scheme of Lowry, et al. [27] and albumin was evaluated following the method previously described by Henry, et al. [28].

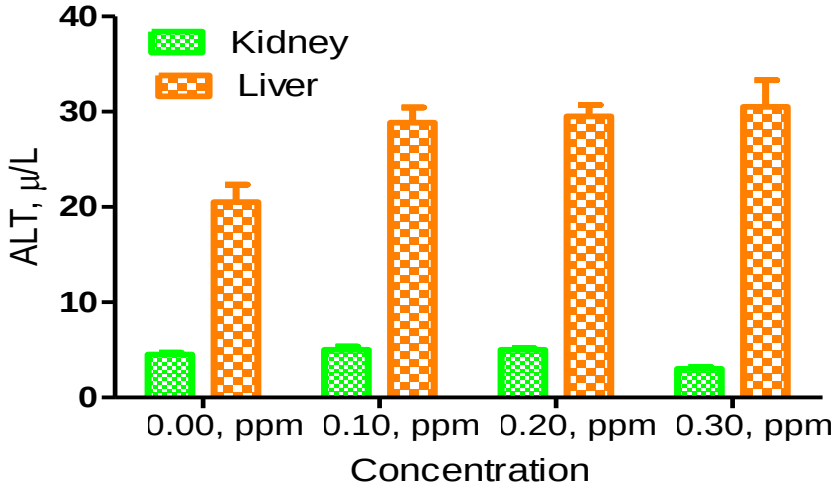
2.4. Statistical Analysis

SPSS version 20 was used for statistical analysis. One-way analysis of variance was carried out at p=0.05, and Duncan multiple range tests was used for mean separation. The charts (expressed as mean ± Standard error) were plotted using GraphPad prism 5.

3. RESULTS AND DISCUSSION

The concentration of alanine amino transferase in kidney and liver of *Clarias gariepinus* exposed to sub-lethal concentration of phenol is presented in Figure 1. At the 0.00, 0.10, 0.20 and 0.30 ppm the concentration was 4.50±0.23µ/L, 5.00±0.35µ/L, 5.00±0.17µ/L and 3.00±0.23µ/L, respectively, being significantly different ($p<0.05$) across the concentration for kidney), and 20.50±1.85µ/L, 28.83±1.62µ/L, 29.50±1.21µ/L and 30.50±2.83µ/L, respectively, being significantly different ($p<0.05$). Duncan multiple comparison showed that 0.00ppm and 0.30ppm concentration were the source of the observed variation in both liver and kidney, respectively. Generally, the concentration of alanine amino transferase in the liver were far higher than the level in the kidney.

Figure 1. Concentration of alanine amino transferase in kidney and liver of *Clarias gariepinus* exposed to sublethal concentration of phenol



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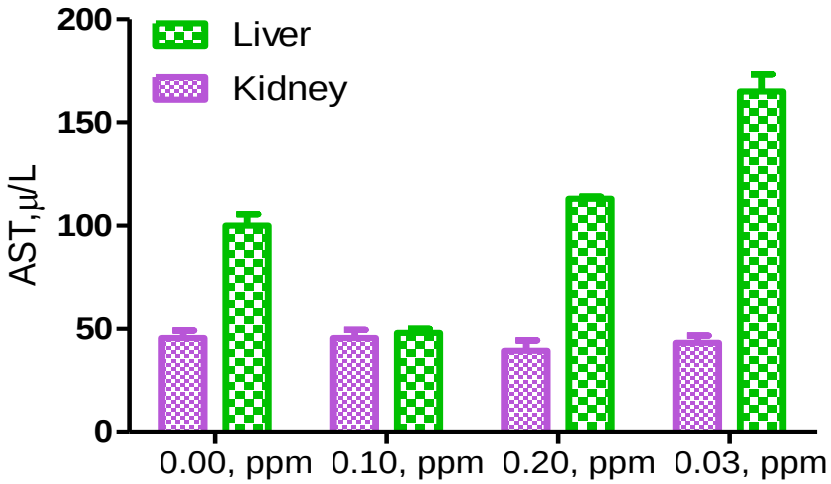
The level of aspartate amino transferase in kidney and liver of *Clarias gariepinus* exposed to sublethal concentration of phenol is presented in Figure 2. At the 0.00, 0.10, 0.20 and 0.30 ppm the level was 45.50±3.75µ/L, 45.57±4.045µ/L, 39.37±5.02µ/L and 43.17±3.64µ/L, respectively, being not significantly different ($p>0.05$) across the concentration (for kidney), and 100.00±5.54µ/L, 48.00±2.04µ/L, 113.00±1.15µ/L and 165.00±8.43µ/L, respectively, being significantly different ($p<0.05$). Generally, the concentration of aspartate amino transferase was higher in the liver than the kidney.

Figure 2. Concentration of AST in kidney and liver of *Clarias gariepinus* exposed to sublethal concentration of phenol



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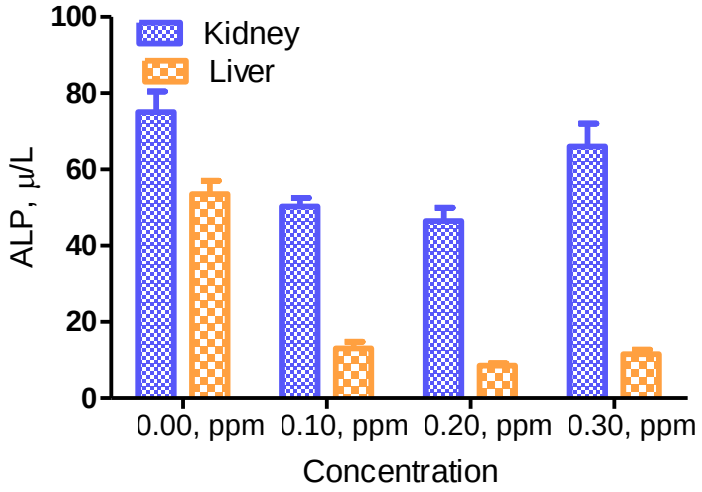
The level of alkaline phosphatase in kidney and liver of *Clarias gariepinus* exposed to sublethal concentration of phenol is presented in Figure 3. At 0.00, 0.10, 0.20 and 0.30 ppm concentration, alkaline phosphatase was 75.00±5.48µ/L, 50.30±3.33µ/L, 46.47±3.49µ/L and 66.00±6.00µ/L, respectively, being not significantly different ($p<0.05$) across the concentration (for kidney), and 63.53±3.493µ/L, 13.03±1.76µ/L, 8.50±0.64µ/L and 11.63±1.18µ/L, respectively, being significantly different ($p<0.05$). Multiple comparison showed that 0.30ppm concentration were the source of the observed significant variation in the liver Generally, the concentration of alkaline phosphatase was higher in the kidney compare to the level in the liver.

Figure 3. Concentration of alkaline phosphatase in kidney and liver of *Clarias gariepinus* exposed to sublethal concentration of phenol



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The values suggest that phenol causes an alteration in alanine transaminase, aspartate transaminase, alkaline phosphatase in the fish, suggesting kidney and liver damage. This indication that phenol caused disruption of the biochemical and physiological processes of liver and kidney of the fish. A decline in alkaline phosphatase may cause an alteration in biosynthesis and energy metabolism mechanisms in the fish. This suggests the effects of phenol on the metabolic processes in the exposed fish. Previous researchers have indicated that alteration in alanine and aspartate aminotransferases and tissular protein of fish exposed to phenol [1, 19, 21, 29].

Alanine transaminase and aspartate transaminase are actively involved in the catabolism of amino of amino acids [5]. A decline in the values suggests a decrease hepatocellular production and enzyme's activity. The alteration in values may due to the interruption in the secretion functions. Basically, an alteration in Alanine transaminase affects liver functions including energy production processes and amino acids metabolism [30].

According to Karthikeyan, et al. [31], Inyang, et al. [5], changes in alanine transaminase, aspartate transaminase and alkaline phosphatase shows that the toxicant affect the transportation processes of ions across the cell membrane, glutathionic metabolism as well as metabolism and regulation of amino acids. The changes could also cause instability in the metabolites in the kidney and liver due to the effect of the toxicants.

The level of total protein in kidney and liver of *Clarias gariepinus* exposed to sublethal concentration of phenol is presented in Figure 4. At 0.00, 0.10, 0.20 and 0.30 ppm concentration, total protein was 33.00±3.46g/L, 34.17±3.32g/L, 30.43±2.87g/L and 26.00±1.76g/L, respectively (for kidney), and 24.00±2.89g/L, 20.50±2.94g/L, 27.50±4.04g/L and 21.50±1.67g/L, respectively (for liver). Statistically, there was no significant difference ($p<0.05$) across the various concentration for both liver and kidney. Total protein level was lower in the liver compare to the kidney values.

Figure 4. Concentration of total protein in kidney and liver of *Clarias gariepinus* exposed to sublethal concentration of phenol

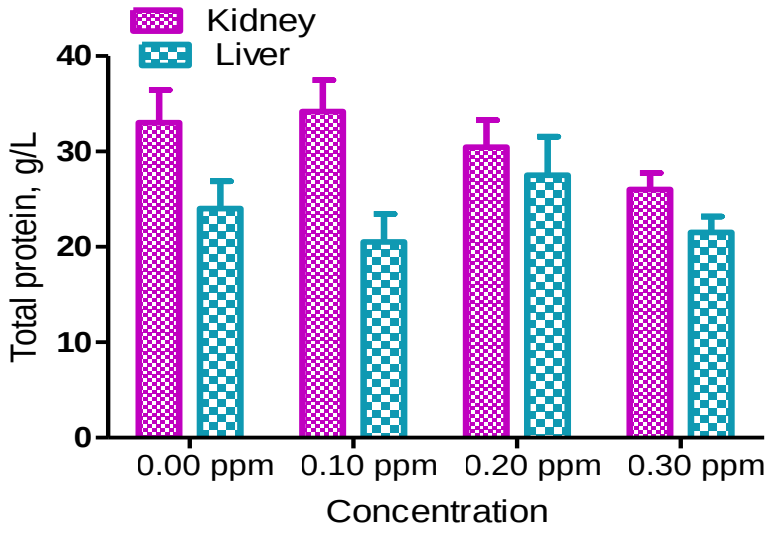

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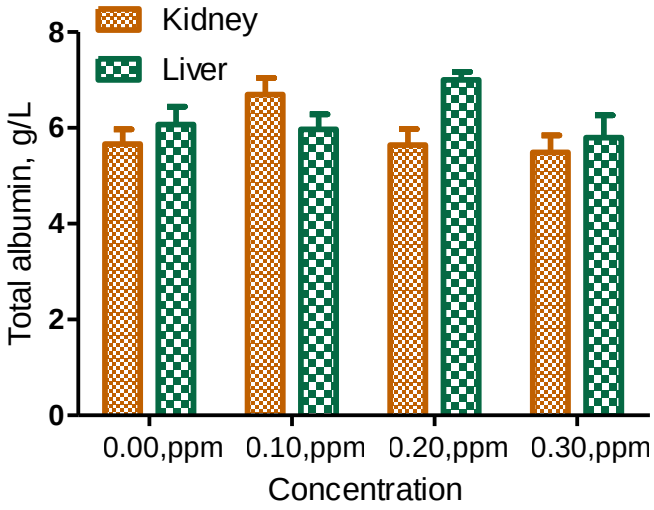
Figure 5 presents the concentration of total albumin in kidney and liver of *Clarias gariepinus* exposed to sublethal concentration of phenol. At 0.00, 0.10, 0.20 and 0.30 ppm concentration, total albumin was 5.66±0.30g/L, 6.70±0.45g/L, 5.64±0.34g/L and 5.49±0.35g/L, respectively (for kidney), and 6.67±0.38g/L, 5.97±0.32g/L, 7.00±0.17g/L and 5.80±0.46g/L, respectively (for liver). There was no significant difference ($p<0.05$) across the various concentration for both liver and kidney. Liver albumin was higher compare to the kidney values.

Figure 5. Concentration of total albumin in kidney and liver of *Clarias gariepinus* exposed to sublethal concentration of phenol



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Authors have reported that total protein in tissues play crucial role in several physiological processes including metabolism of nitrogenous substances used to enhance energy production due to low amount of carbohydrate [6, 32, 33]. According to Olusegun and Adedayo [34], Inyang, et al. [10], protein is among the major source of energy during stress condition. Albumin is also crucial during the transport of protein for steroid hormones and fatty acids from adipose tissue to muscles [6, 32, 33]. Therefore, an alteration could cause an inhibition in the transportation function of albumin. The findings of this study aligning with the work of Inyang, et al. [6] that reported significant variation in albumin and total protein in kidney and liver of *Parpohiocephalus obscurus* exposed to Lambda cyhalothrin. The author further reported that Lambda cyhalothrin causes an alteration in total protein and albumin in fish making them a useful indicator for assessing the health status of the aquatic ecosystem. Therefore, an alteration in the liver and kidney of the fish due to exposure to phenol is an indication of its toxicity.

4. CONCLUSION

Phenols and its derivatives major components of produced water that is often discharged into the aquatic ecosystem. Its certain concentration phenolic compounds are toxic to aquatic organisms including fish. This study investigated the effect of phenols on the metabolites of *Clarias gariepinus*. The study found that phenol altered aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total protein and albumin in both liver and kidney of *Clarias gariepinus*. Hence, there is the need to properly treat effluents containing phenolic compound before been discharged into the surface water.

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