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Changes in Liver Enzyme Activities in African Catfish (*Clarias gariepinus*) Exposed to Crude Oil

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Abstract

The biochemical assessment of the effect of crude oil polluted water on some liver enzymes of African catfish (*Clarias gariepinus*) was carried out. A total of 30 active (mobile) juvenile catfish of average weight of 75.33 ± 3.0g were divided into six groups of five catfish and held for 30 hour in five different crude oil contaminated aqueous solutions (0.1%, 0.25%, 0.5%, 0.75%, 1.0%). Catfish in the control group were held in borehole water. The results obtained revealed a significant reduction ($p<0.05$) in the weight of catfish held in the crude oil polluted water at the end of the 30 hour experimental period. However, no significant difference ($p>0.05$) was obtained in the initial and final weights of control catfish. In comparison to the control, the results showed a significant increase ($p<0.05$) in the LDH activity and a significant decrease ($p<0.05$) in the AST and ALT activity of the liver of catfish as the concentration of crude oil increased. The data obtained may be interpreted as a possible adverse effect of crude oil on juvenile catfish as manifested by changes in liver enzymes.

Introduction

Crude oil became a widely available commodity worldwide about a century ago. However in Nigeria, the presence of substantial deposits of this "black gold" was detected only in the 1950s (Akpofure et al. 2000). Each year, according to the United State Environmental Protection Agency, an average of 14 million gallons of oil from more than 10,000 accidental spills is discharged into freshwater and saltwater environments worldwide particularly through the leakage of pipes carrying oil and from underground reserves.

Wetlands are important part of the Nigerian riverine, estuarine and coastal ecosystems. Oil is refined, stored or transported through these areas; and some wetlands are therefore subjected to occasional spills. Oil may spill directly into wetlands from pipelines or washed into wetlands from adjacent rivers or lakes. Leaks and spills of fuel oil, gasoline and other petroleum products pollute the environment in many ways. Oil spills pollute ground water, on which many people depend as a source of drinking water. Spills seep into streams, lakes and reservoirs, which are drinking water sources as well as habitats of fish, birds and other wildlife (Piatt et al. 1990). Oil spills can also come from large petroleum refineries, tank

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farms, tankers and pipeline. Thus, there is great public interest in oil spills and response activities that affect wetlands (Akpofure et al. 2000).

Whenever oil spills, spreading takes place almost immediately. The gaseous and liquid components evaporate, some get dissolved in water while the others may become oxidized or undergo bacterial degradation (Akpofure et al. 2000). The products eventually sink to the bottom and smother benthic plants and animals. The soil around the polluted area may become contaminated and adversely affect terrestrial animals and plants (Akpofure et al. 2000). The devastating consequences of oil spills have been felt in the Niger Delta region of Nigeria, from the aquatic to the aerial and terrestrial environment including human safety and health.

The effect of oil pollution on aquatic life is the concern of many scientists, since much of the world's population is dependent upon marine and coastal ecosystems for food, and this dependency increases as the demand for food increases. Research has shown a variety of adverse effects on marine organisms following exposure to crude oil (Suchanek 1993). The present study assesses the effect of crude oil on the liver of African catfish in terms of biochemical changes.

Materials and Methods

Collection of crude oil and preparation of various concentrations

Bonny light crude oil was obtained from the Department of Petroleum Resources (DPR), Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Nigeria and diluted with borehole water to obtain concentrations of 0.1%, 0.25%, 0.5%, 0.75% and 1% by volume.

Experimental fish

Juvenile catfish (*Clarias gariepinus*) of both sexes with a mean weight of 75.00 ± 3.0g (n=30) were obtained from a commercial fish pond at Unity Road in Ilorin, Kwara State, Nigeria. The weight was taken just before the fish were exposed to crude oil. The fishes were divided into six groups of five fish and kept in 30L plastic aquaria. Control fish were held in borehole water. Five other groups were exposed to the five concentrations of crude oil. All fish were fed *ad libitum* with commercial fish meal. The experiment lasted for 30 hours after an acclimation period of 5 days in borehole water and the weights of the catfish were taken at the end of the experiment.

Determination of pH

The pH of the various concentrations of crude oil polluted water was taken using with a Ken EIL pH meter at the start of the experiment before the fish were introduced and after harvesting.

Preparation of liver homogenate

At the end of the experimental period of 30 hours, the catfish were harvested from the aquaria and allowed to stay in a dissecting tray for about 5 minutes to reduce slime production. The catfish were then dissected and the liver was placed in a beaker containing 0.25M sucrose solution. Slices of the liver were weighed, chopped into small pieces and then

homogenized with a pre-cooled pestle in mortar in a bowl of ice chips. The homogenized tissue was further diluted with 0.25M sucrose solution to obtain a 1:5 dilution and stored in a deep freezer at -4°C.

Enzyme assay

The protein content of the liver homogenate was determined using the biuret method of Henry et al. (1974). Alanine transaminase (ALT) and aspartate transaminase (AST) were assayed using the procedure of Schmidt and Schmidt (1963) while the method of Wroblewski and La Due (1955) was employed in the assay of lactate dehydrogenase (LDH).

Statistical analysis

All data were analysed statistically using Analysis of Variance (ANOVA) by employing the method of Steel and Torrie (1960). Significant difference between the treatment means was determined at 5% confidence level using Duncan's Multiple Range Test (Duncan 1955).

Results

The initial and final weights of catfish cultured in various concentrations of crude oil polluted water for 30 hours are presented in Table 1. There was no significant difference ($p>0.05$) in the initial and final weights of the catfish held in the control treatment (borehole water). However, a significant reduction ($p<0.05$) in weight was observed in the catfish cultured in the crude oil treatments. The initial and final pH of various concentrations of crude oil polluted water is presented in Table 2. The values obtained at the end of the 30 hours culture period tend towards being neutral in all the experimental groups. Table 3 shows the specific activities of selected enzymes in the liver of catfish exposed to various concentrations of crude oil for 30 hours. There was no significant difference ($p>0.05$) in lactate dehydrogenase activity in the liver of catfish exposed to 0.1% crude oil and in the control. However, there was a progressive significant increase ($p<0.05$) in the LDH activity in the liver of catfish at higher crude oil concentrations. The specific activities of AST and ALT in the liver of catfish exposed to various concentrations of crude oil for 30 hours were significantly reduced ($p<0.05$) compared to that of control (Table 3).

Table 1. Initial and final weights of catfish exposed to various concentrations of crude oil for 30 hour

Concentrations of crude oil (%)	Initial weight (g)	Final weight (g)
Control	75.33±3.00[a]	75.32±3.01[a]
0.10	75.29±2.93[a]	66.28±2.03[a]
0.25	75.24±3.19[a]	60.02±2.11[c]
0.50	75.27±3.34[a]	59.70±2.04[c]
0.75	75.34±3.04[a]	57.10±2.27[c]
1.00	75.25±3.00[a]	50.23±1.60[d]

Values are means ± SEM for five fish
[a,b,c,d] Row and column values with different superscript letters are significantly different ($p<0.05$).

Table 2. Initial and final pH of the various concentrations of crude oil polluted water

Concentration of crude oil (%)	Initial pH	Final pH
Control	6.8	7.0
0.10	6.6	7.1
0.25	6.3	6.85
0.50	6.2	6.6
0.75	6.0	6.9
1.00	5.0	6.7

Table 3. Specific activities of selected enzymes in catfish liver after exposure to various concentrations of crude oil for 30h

Concentrations of crude oil	Specific activity (µmol/mg protein/min)		
	LDH	AST	ALT
Control	274.90±2.57[a]	279.13±2.48[a]	369.00±3.49[a]
0.10	276.09±3.79[a]	173.84±1.18[b]	259.44±3.35[b]
0.25	289.13±3.29[b]	103.36±1.1.3[c]	161.85±1.54[c]
0.50	302.96±4.10[c]	69.30±1.22[d]	123.90±1.20[d]
0.75	353.72±4.25[d]	54.34±0.75[e]	94.63±2.75[e]
1.00	377.25±3.85[e]	41.93±0.24[f]	63.89±1.02[f]

Values are means ± SEM for five fish
[a,b,c,d,e,f] Column values with different superscript letters are significantly different ($p<0.05$).

Discussion

The easiest index that can be used to establish the effect of crude oil on fish is the relative changes in the initial and final weights of catfish (Table 1). There was no significant difference ($p>0.05$) in the initial and final weights of the control fish, but there was a significant decrease ($p< 0.05$) in the weights of the fish in the crude oil treatments. Val and Almeid-Val (1999) suggested that the crude oil covers the water surface and so hinders the dissolution of oxygen, resulting in lower blood oxygen content as the fishes are starved of oxygen; ultimately affecting their growth. It is also possible that the fish feed was given an unpleasant taste and smell of crude oil, resulting in lower food intake. This is in line with the work of Hill et al. (2000), who reported that an effective weight loss can occur on modifications of diet and physical activities by virtue of decline in energy production. The energy input decreases leading to an imbalance between the energy input and energy output. Hence the body weights of experimental fish reduce.

The pH is a measure of hydrogen ion concentration of a solution. Compared with the control, the initial pH of the culture media tends to be decreasing (acidic) as the concentration of crude oil increases. This is probably due to deposition of carbonic acid or its metabolites into the medium. However, the final pH values for the different culture media tend towards neutral. This may be attributed to some secretions from the catfish into the water environment in their bid to survive. However, this assumption is not fully supported. Further investigation is needed to be carried out on this deduction.

At the end of the experimental period, all the experimental groups of catfish showed a significant increase ($p<0.05$) in LDH activity in comparison with the control. This is probably a reflection of an increase in anaerobic metabolism. As mentioned earlier, when crude oil spills into water, it forms a slick or coat over the surface of the water thereby preventing oxygen penetration into the water. As a result, oxygen in the water is depleted and there will come a time when the oxygen level will not be sufficient for the catfish (Val and Almeid-Val 1999). Thus, the catfish switch to anaerobic respiration for energy production to take care of metabolism. The increase in LDH activity across the experimental groups as observed in the present study is likely to be due to an anaerobic degradation of glucose leading to lactate accumulation. The accumulated lactate ionizes rapidly resulting in acidification in muscles and limits the period of vigorous activities.

It is well known that the transaminases are very active in the liver, hence they are marker enzymes and their activities can be detected in very small amount. The data obtained in the present study is in line with the findings of Mousa and Khattab (2003) where it was found that there was inhibition of AST and ALT activities in the liver of catfish after

intoxication with dietary ochratoxin. Abdel Tawwab et al. (2001) also observed a similar result in liver AST and ALT of Nile tilapia after exposure to mercury. These workers ascribed the reduction in enzyme activity to liver necrosis caused by the toxicants and a possible damage to the hepatocytes. The decrease in the activities of these enzymes could be attributed to inhibition of the enzyme or a reduction in the rate of synthesis of the enzyme in the liver. This observation is in line with the work of Karmen et al. (1995) where a similar trend of result was obtained.

Conclusion

1. The pH readings obtained showed that crude oil is acidic.
2. The significant reduction (30%) in the weight of catfish exposed to crude oil for 30 hours was due to the inability of catfish to feed due to the unpleasant taste and smell.
3. The high activity of LDH shows that crude oil interferes with oxygen supply to the catfish, leading to anaerobic degradation of glucose.
4. The reduced activity of AST and ALT, which are marker enzymes in the liver, indicates that crude oil damages the hepatocytes.

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