TISSUE ANALYSIS OF *Clarias gariepinus* JUVENILES INJECTED WITH DIFFERENT CONCENTRATIONS OF CRUDE OIL

NWAMBA, Helen Ogochukwu
Department of Applied Biology and Biotechnology, Enugu State University of Technology, Enugu, Nigeria. **Email:** nwambahelen@yahoo.com **Phone:** +234 8036731557

ABSTRACT

Aspects of tissue analysis of Clarias gariepinus juveniles (90.60 ± 0.24g) were studied following injection of different concentrations of Bonny light crude oil (BLCO). Experimental fish were injected at concentrations: 0.00, 25.00, 50.00 and 75.00 µlg^{-1} to test fish responses during 4 days toxicity and 42 days recovery periods. A control experiment was left with fish not injected with BLCO. A 38% crude protein diet was fed to the fish at 3% body weight per day (p.w.d^{-1}) during the toxicity and 5% during the recovery period. The protein value (PV) increased as the BLCO concentration increased up to 75 µlg^{-1} at recovery phase and vis-versa at toxicity phase. This could be due to immune response mechanism which eventually dropped at recovery phase. The starch value (SV) and unsaponification values (USPV) were not affected, the saponification value (SPV) only increased between 25.00 and 0.0 µlg^{-1} and declined at 75 µlg^{-1}. The decrease in values of SPV in treated fish tissues has implication on energy metabolism. Of the four tissues values (PV, SV, SPV and USPV) of fish tested, only the USPV was not significantly different (P > 0.05) among the fish injected with the different concentrations of BLCO. The 0 and 25 µlg^{-1} has the same effect on mortality rate. Similar mortality values of the fish recorded with the 25 µlg^{-1} BLCO and the control suggests that this oil dosage was probably inadequate to alter the mortality rates of C. gariepinus juveniles. The survival rates increased with decreasing concentration.

Keywords: Tissue Analysis, Bonny Light Crude Oil, Mortality, Survival

INTRODUCTION

Oil spills constitute one of the most important sources of environmental problems in Nigeria's petroleum industry. Most of the Nigerian aquatic environments have witnessed a number of oil spillages in recent time. Nearly 3000 cases of oil spill accidents that occurred between 1976 and 1990 caused the release of about 2.40 million barrels of crude oil, resulting in various forms of environmental degradation, deprivation and spoilage (Akingbade, 1991). Oil producing communities in Nigeria have suffered most due to the oil spill menace. Akingbade (1991) recorded varying levels of petroleum hydrocarbons in the body organs of fishes, frogs and snails in areas where oil spills are prevalent. Working on rivers, lakes and reservoirs with continuous input of oil pollutants, Brown *et al*. (1991) recorded the presence of aromatic hydrocarbons including benzene in both water and fish tissues.

The degree of exposure of marine organisms to oil is often assessed by measuring their body burden of petroleum related aromatic compounds (ACs) because ACs are potentially harmful to animals. Fish and marine mammals extensively metabolize most ACs in their livers and predominantly excrete them into bile. The pollution of water sources due to xenobites may play a major role in decline of aquatic animals. Increasing awareness of the adverse effects of

anthropogenic activities and pollution on aquatic environment has focused interest on health of fish populations and possibilities to utilize the health parameters for assessment of the quality of aquatic environment (Henry *et al.,* 2004). The African catfish of the genus *Clarias* is a highly esteemed group of fishes in tropical African and it commands high market value. Its hardy nature and possession of accessory air breathing organs enable it tolerate adverse aquatic conditions (Reed *et al.,* 1967). The high demand for the African catfish (*C. gariepinus*) due to its rich protein profile, fast growth, good size, taste and flavour informed the need to investigate the health parameters and survival of this choice fish species in polluted water.

Against this background, the tissue analysis of *C. gariepinus* adults treated with different concentrations of Bonny light crude oil was investigated. The aim was to inject graded concentrations of the oil pollutant into the fish and assess its impact on the tissue quality of the fish and on the fish survival. The criteria for assessment were: protein value, starch value, saponification and unsaponification values of tissues of *C. gariepinus* juveniles.

MATERIALS AND METHODS

Fish: One hundred and twenty (120) juveniles of C. *gariepinus* (90.60 ± 0.24) were purchased from a private fish farm (Aqua Fish Farm Limited) in Iporoto, Inyiamagu, Ikwo Local Government Area Ebonyi State, Nigeria. The fish were transported in five plastic containers (25 litres capacity) to the fisheries laboratory of Enugu State University of Science and Technology, Enugu. Water temperature during transportation was maintained at 28 ± 0.40°C by introduction of ice cubes preserved in a portable cooler.

The fish were acclimated for 14 days on a 38 % crude protein maintenance diet (Table 1) at 3% body weight daily ($bw.d^{-1}$). Subsequently, the fish were randomly stocked in 12 plastic bowls (25L) containing 24L of dechlorinated tap water at 10 fish per container. The fish were then starved for 24 hours prior to the commencement of the treatment.

BLCO Injection: Injection of fish with graded concentrations of BLCO was carried out via the dorsal anterior musculature of the fish. In all cases, injection was done intramuscularly with the aid of a 2.50 ml hypodermal needle just below the dorsal fin. The crude oil doses applied were: 25 μlg^{-1} (T_1), 50 μlg^{-1} (T_2) and 75 μlg^{-1} (T_3); while the control fish were left without crude oil injection 0.00 μlg^{-1} (T_4). The fish were then fed a 38 % crude protein maintenance diet (Table 1) at 3% bw.d"[1] for 96 hours (4 days) toxicity period and later at 5% $bw.d^{-1}$ for 42 days recovery period. Water temperature (27 ± 1.20°C) and pH (6.60 ± 0.20) were recorded with the aid of maximum - minimum thermometer and a pH meter (Model pH -1-2010L), respectively.

Table 1: Gross composition of the experimental diet fed to *Clarias gariepinus* juveniles stocked in crude oil polluted water

Feed ingredient	% Composition
Yellow maize	9.29
Soyabean meal	54.84
Fish meal	16.65
Blood meal	10.97
Palm oil	5.00
Salt	0.25
Vitamin premix[1]	0.60
Mineral premix[2]	2.40
Total	100.00
Nutrients	
Crude protein	37.58
Ether extract	5.18
Ash	10.48
Dry matter	11.80
Nitrogen-free extract	34.46
Total	100.00

(1) Vitamin premix provided the following constituents diluted in cellulose (mg/kg o diet): thiamine, 10; riboflavin, 20; pyridoxine, 10; folacin, 5; pantothenic acid, 40; choline chloride, 3,000; niacin, 150; vitamin B12 0.06; retinyl acetate (500,000 IU/g), 6; menadione-Na-bisulphate 80; inositol, 400; biotin, vitamin C, 200; alphatocopherol, 200; cholecalcipherol 1,000,000 IU/g. (2) Contained as g/kg of mineral premix: $FeSO_4.7H_2O$, 5; $MgSO_4.7H_2O$, 132; K_2SO_4, 329.90; KI, 0.15; NaCl, 45; Na_2SO_4, 88; $AlCl_3$, 0.15; $CoCl_2.6H_2O$, 0.50; $CuSO_4.5H_2O$, 0.50; $NaSeO_3$, 0.11; $MnSO_4.H_2O$, 0.70; and Cellulose, 380.97.

Tissue Analyses: The fish tissue analysis was obtained from each treated plastic container and control after 96 hours toxicity (4 days) and 42 days recovery periods. In all cases, fish samples from each plastic container (T_1, T_2, T_3 and T_4) were sacrificed decapitated and the visceral organs removed.

The remaining (fish tissue) was thoroughly washed and used for chemical analysis. Fish tissues were analyzed for their proximate composition using the method of Windham (1996). Tissue analysis of fish done at 96 hours (4 days) for the toxicity phase and 42 days recovery phase were: protein value (PV), starch value (SV), saponification value (SPV) and unsaponification value (USPV) (Windham, 1996).

Mortality: Daily records of the fish mortality and survival were taken during the 96 hours (4 days) toxicity and the 42 days recovery periods. The data were used in calculation of mortality and survival rates, respectively.

Statistical Analysis: All the data obtained were subjected to analysis of variance (ANOVA) to determine if statistical difference existed among treatment means at 5% level of significance.

RESULTS

The tissue analysis for the nutritional value of *C. gariepinus* injected with different concentrations (25.00 — 75 μlg^{-1}.) of BLCO and control indicated that the protein value increased with increasing concentration during the toxicity phase of this study and decreased at recovery period with increasing concentration (Table 2). The SPV, SV and USPV of the fish were not affected by crude oil concentrations and duration of the study. Both the toxicity and recovery phases of the study recorded significant differences in the PV and USPV ($P<0.05$) as well as in the SPV ($P<0.05$) of the fish tissue as the crude oil concentrations in the fish increased from 25.00 to 75.00 μlg^{-1}. The mortality rate (MR) of the fish during the 4 days toxicity and 42 days recovery periods increased as the concentration of injected BLCO increased from 50.00 to 75.00 μlg^{-1} (Figure 1). The MR of the fish under the control treatment had the same effect as that of fish injected with 25µl. g^{-1}. The fish subjected to 75.00μlg^{-1} BLCO injection generally had higher percentage mortality (33.00%) at the recovery period than those injected with 50μlg^{-1} BLCO.

The survival rates (SR) of the fish obtained decreased with increasing concentrations of BLCO. Data obtained from the toxicity and recovery periods showed that there were no significant differences ($P<0.05$) between the SR values of the fish injected with 25 μlg^{-1} BLCO and those under the control study. The fish survived better when exposed to 50 μlg^{-1} BLCO than when injected with 75 μlg^{-1} BLCO (Figure 1).

DISCUSSION

The PV of the fish in (50.00 and 75.00 μlg^{-1} BLCO) increased at toxicity period. This implied that the interactive effect of the crude oil and the tissue protein probably resulted in increased protein concentrations in the fish tissue. Secondly, the organ responsible for protein metabolism was better disposed to synthesize protein at toxicity period to boost the immune mechanism. The SV values of the fish under the control, for both the toxicity and recovery periods did differ significantly from those treated with the crude oil injections. These results also implied that the differences between the metabolism of starch in the injected fish and that in the control fish were significant and that the SV and SPV of the fish were affected by the presence of crude oil in the fish body. The saponification value of the fish tissue increased only in the control fish. The result from both the toxicity and the recovery periods indicated that the reactions of the BLCO concentrations in the fish reduced saponification value of the treated fish. At 75.00 μlg^{-1} BLCO concentrations, the fish were less resistant than those injected with 25.00 μlg^{-1} BLCO, and hence the highest mortality was recorded with fish injected with 75.00 μlg^{-1} BLCO. Lee (1975) reported that mortality increased with the increase in the crude oil concentration in the fish. The similar MR values recorded with 25.00 μlg^{-1} BLCO and

Table 2: Tissues protein, starch, saponification and unsaponification values of *Clarias gariepinus* injected with different concentrations of Bonny light crude oil

Duration	4 Days			42 Days			Control
Crude oil concentration (µlg^{-1})	75	50	25	75	50	25	0
Tissue Protein value (IU/l)	52.95	43.55	30.50	8.10	14.10	18.90	19.60
Tissue Starch Value (IU/l)	0.20	0.21	0.15	0.18	0.16	0.10	0.20
Saponification value (IU/l)	20.19	20.20	20.17	19.80	21.20	21.10	39.20
Unsaponification Value (IU/l)	0.40	0.35	0.30	0.45	0.40	0.35	0.18

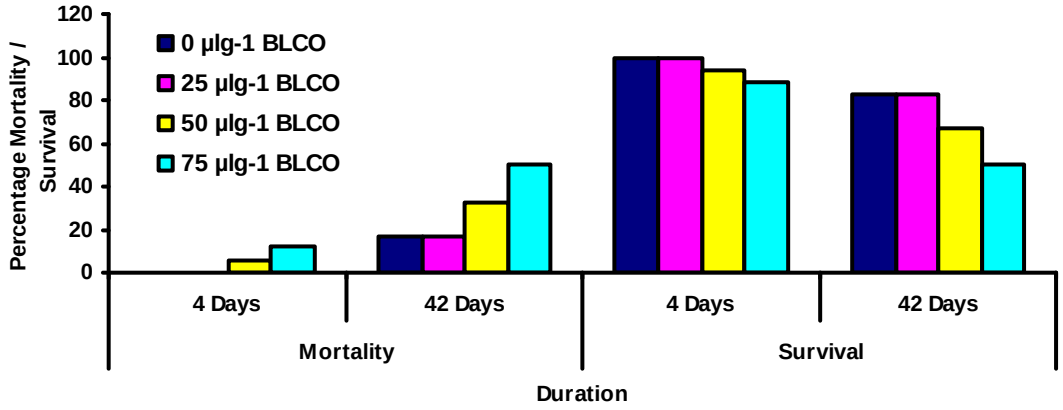

Figure 1: Percentage mortality and survival of *Clarias gariepinus* injected with different concentrations of Bonny light crude oil

the control signified that the 25.00 µlg^{-1} BLCO dosage was probably not enough to effect any change in the mortality rate of *C. gariepinus juveniles*. Mortality of *C. gariepinus juveniles* was directly proportional to BLCO concentrations. The *C. gariepinus* with 25.00 µlg^{-1} BLCO survived in the same manner as those under the control treatment. The least SR values recorded with the 75.00 µlg^{-1} BLCO injection at both periods of this study were consistent with the report of Bryan (1976). Survival of *C. gariepinus juveniles* was inversely proportional to BLCO concentrations.

REFERENCES

AKINGBADE T. (1991). *Nigeria: On the Trial of the Environment.* Triple Environmental Systems Associates Limited, Lagos, Nigeria.

BROWN, E. R., KCHI, E., SINCLAIR, T. F. and HAZADR, J. J. (1999). Water pollution and disease in fish (an epizootiological survey). *Journal of Environmental Pathology and Toxicology,* 2: 919 – 925.

BRYAN, G. W. (1976). Heavy metal contamination in the sea. Pages 185 – 302. *In:* JOHNSTON, R. (Ed.) *Marine Pollution*. Academic Press, London.

HENRY, P., MARA, R., COURCOT, L., LACOUTURE, D. and BERTHO, M. L. (2004). Heavy metals in four fish species from the French coast of the eastern English channel and southern Bight of the North Sea. *Environmental International,* 30: 675 – 683.

LEE, R. F. (1975). Fate of petroleum hydrocarbons in marine zooplankton. Pages 549 – 553. *In: Proceedings on Prevention and Control of Oil Pollution.* America Petroleum Institute, Washington DC, USA.

REED, W., BURCHAR, I., HOPSON, A. J., JONATHAN, J. and IBRAHIM, Y. (1967). *Fish and Fisheries of Northern Nigeria.* Government Press, London.

SEMEEKA-CYMERMAN, A. and KEMPERES, A. J. (2003). Biomonitoring of water pollution with *Elodea candensis*. A cast study of three small Polish rivers with different

levels of pollution. *Water, Air and Soil Pollution,* 145: 139 – 153.

WINDHAM, W. R. (1996). Animal feed. Chapter 4, Pages 1 – 38. *In:* CUNIFF, P. (ed.). *Official Methods of Analysis, Association of Official Analytical Chemists (AOAC),* 16th Edition, Volume 1, Gaithersburg, Maryland, USA.