GROWTH PERFORMANCE AND SELECTED BIOCHEMICAL INDICES ASSESSMENT IN THE SERUM AND LIVER OF *CLARIAS GARIEPINUS* JUVENILES EXPOSED TO VARYING CONCENTRATIONS OF WATER SOLUBLE FRACTION OF CRUDE OIL

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ABSTRACT. *The effect of varying concentrations (0, 0.25, 0.05 and 1.0% v/v) of water soluble fraction (WSF) of crude oil on the growth performance and biochemical changes in the serum and liver of 144 Clarias gariepinus Juveniles stocked into 12 glass aquaria at the rate of 12 fish per tank in triplicate for 90 days was assessed. The growth performance indices of the fish juvenile over the 90 days of toxic exposure revealed that the mean weight gained, specific growth rate, and total feed intake of the fish specimen decreases with increase concentration of the WSF of crude oil. However, 10% and 20% mortality were recorded in the fish exposed to 0.5% and 1.0% WSF of crude oil respectively. The activity of ALT and AST in the serum of the fish were significantly higher ($p<0.05$) with the increased concentration of WSF of crude oil while the ALT and AST activity of the liver of the fish juveniles irrespective of the added concentration of WSF of crude oil showed no significant differences ($p>0.05$). The study concluded that WSF of crude oil had adverse effects on the growth performance, blood serum and liver activities in C. gariepinus juveniles.*

KEYWORDS. Crude oil, Growth performance, Serum, Enzymes, Fish, Pollution

INTRODUCTION

The potential utility of biomarkers for monitoring both environmental quality and health of organism inhabiting polluted ecosystems has been receiving increasing attention (Gauthier *et al.*, 2004, Yusuf *et al.*, 2017).

Niger River which is one of the coastal waters in Nigeria is an important ecosystem that serves as home to 36 families and nearly 250 species of fish, of which 20 are endemic (WWF, 2006). Crude oil spillages have been reported to contribute to the decimating of rich fisheries of the Nigerian coastal waters (Adeola, 1996). Wake (2005) associated reduction in

diversity and abundance of aquatic fauna with oil-laden refinery effluents. There were also strong evidences that local fisheries are affected by migrating fish avoiding oiled areas (Powell, 1987). Ezenwa and Ayinla (1994) noted that fish recruitment for the rivers in the Delta Area of Nigeria has been drastically reduced and this factor accounts for the poor catch of fish by the artisanal and pelagic fishermen, thereby leading to low food supply and income.

Generally, crude oil has been reported to be primarily toxic to aquatic organisms due to the presence of Polycyclic Aromatic Hydrocarbons (PAHs) (Heintz *et al.,* 1999). Baden, (1982) reported significantly increase in oxygen consume soluble fraction (WSF). Mortality and oxidative stress were observed in tilapia fingerlings exposed to the WSF of diesel fuel (Dede and Kaglo, 2001) and growth and feed utilization were significantly reduced in exposed *Heterobrachus bidorsalis* (Ofojekwu and Onah, 2002). Drastic changes in liver enzyme activities of the catfish, *Clarias gariepinus* were also reported following exposure to crude oil (Sunmonu and Oloyede, 2006).

However, the viscosity of oil in aquatic environment coupled with the presence of hydrocarbons in oil poses a carcinogenic threat to fish, birds and mammals (Mason, 1996). Oil spillage over time can affect species populations through the destruction of more sensitive juvenile stages (Horsefall and Spiff, 1998) and is capable of sublethal stress effects, mutation and changes in behavioural patterns in individual organisms.

Aquatic environments are made up of complex interactions between plants and animal species and their physical environment. Harm to the physical environment will often lead to physiological responses (such as unpleasant smell and taste of the toxicant, lower food intake, incessant jumping, gulping of air, restlessness, loss of equilibrium, increased opercular activities, surface to bottom movement, sudden quick movement, and resting at the bottom) which can result to death of one or more species in a food chain leading to damage for other species further up the chain (Sunmonu, 2006; Ezemoye and Ogbomida, 2010).

Fish which is a cheap source of animal protein play important role in the human diet. Therefore, the vulnerability of the *Clarias gariepinus* juvenile to the toxic effects of water soluble fractions of crude oil and its refined products in order to prevent the decimating rich fisheries in the Nigerian Coastal waters needs to be evaluated, hence this study.

MATERIALS & METHODS

Water Soluble Fraction (WSF) Preparation

Fifteen (15) litres of Excravos-grade crude oil were sourced from the Department of Petroleum Resources, Warri, Delta State, Nigeria. Volumes of WSF of crude oil used for this experiment were prepared according to the method of Afolabi *et al.* (1985) under dim illumination. 200 ml of Crude oil was added to 800 ml of distilled water in a capacity borosilicate screw-capped conical flask. The mixture was shaken for 24 hrs using a Gallenkamp orbital stirrer and was allowed to stand for at least 3 hours after which it was poured into a glass-stoppered separating funnel. The mixture was further allowed to settle over night to allow the oil droplets in the mixture to settle in the upper layer leaving the clear WSF in the lower part of the separating funnel. The pure WSF was then siphoned into a dark coloured screw-capped Winchester bottle until needed for the experiment.

Fish Stocking and Exposure to WSF of Crude oil

Two hundred (200) six-week old juvenile *Clarias gariepinus* which were obtained from BODMET fish farm, Ile-Ife, Osun State, Nigeria were acclimatized to laboratory conditions in Fish Culture laboratory, Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria for 14-days and fed *ad libitum* with 2 mm Durantee floating feed. After acclimatization, 144 fish with a mean weight of 43.34 ±0.78 g were randomly selected and stocked in 12 glass aquaria (30 cm x 60 cm x 45 cm), each containing 30 litres of water at the rate of 12 fish per tank. WSF at 0% (control), 0.25%, 0.5%, and 1% concentrations were introduced into each tank in triplicates. The fish were fed at 3% of the body weight twice daily (Adewolu and Adeoti, 2010) for 90 days.

Data Collection

The initial weight of the fish were taken before the commencement of the experiment and biweekly subsequently. On-biweekly weighing days, crushed ice was added into water to reduce the fish activity before being removed for weighing after a couple of minutes. The ration allotted to the fish was adjusted based on the new weight gain by the fish after each weighing. The aggregate weight of the test fish per tank was used to calculate the fish growth performance indices as described by Sveier *et al.* (2000):

Mean weight gain (MWG) = $(W_f - W_i)$ n;
Where; W_i = initial body weight W_f = final body weight n=number of fish.
Specific Growth rate (SGR) = $(\text{Log } W_f - \text{Log } W_i)$ t x 100
Food Conversion ratio (FCR) = Feed Intake(g) / Weight gain
Total Food Intake (TFI) = Total weight of the fish/ Total number of fish in the tank
Survival rate= Number of fish at the end of experiment / number of fish stocked x 100

Serum and Liver Biochemical Assays

After 90 days of the experiment, blood sample for serum analysis was obtained through the caudal puncture from 5 randomly selected fish from each treatment using a disposable hypodermic syringe and needle. The blood sample was expressed into plain specimen bottle and was allowed to clot, then centrifuge at 3500 rpm for 15 mins to separate blood cells from the serum. This was then collected into another plain bottle and stored at -4ºC in a Thermocool deep freezer for the evaluation of Aspartate transaminase and alanine transaminase. The blood serum of the fish was analyzed for the activity of AST and ALT spectrophotometrically according to Reitman and Frankel (1957) and Schmidt and Schmidt (1963).

One (1) gram of the excised liver of the test fish was homogenized in a 10 ml Tris buffer solution using rotor of a Tri-R Stir-R electronic homogenizer. The homogenate samples were then assayed using spectrophotometer for the levels of Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Lactate Dehydrogenase (LDH) following the methods of Reitman and Frankel (1957), Schmidt and Schmidt (1963) and Weisshaar et al. (1995) respectively.

RESULTS

Growth Performance and Feed Utilization

The bi-weekly growth performance of the *C. gariepinus* juveniles exposed to varying concentrations of WSF of crude oil is shown in Figure 1. The fish in the control treatment had the better growth performance. The growth of the fish juvenile exposed to varying concentration of WSF of crude oil was observed to be retarding at the sixth week of the experiment, although a sudden rise was observed in the growth of the fish specimens exposed to 0.05% WSF of crude oil at the tenth week, but was however later retarded in the subsequent weeks. The growth performance indices of the fish juvenile over 90 days of toxic exposure, as shown in Table 1, revealed that the mean weight gained, specific growth rate, and total feed intake of the fish specimens which ranged between 4.80 ±1.06 g (1.00% WSF) to 62.62 ±2.25 g (0% WSF (Control)); 0.05 ±2.25% (1.00% WSF) to 0.45 ±2.90 (0% WSF), and 13.77 (1.00% WSF) to 16.37 (0% WSF) respectively were statistically different ($p<0.05$) and was also observed to decrease with increased concentration of the WSF of crude oil.

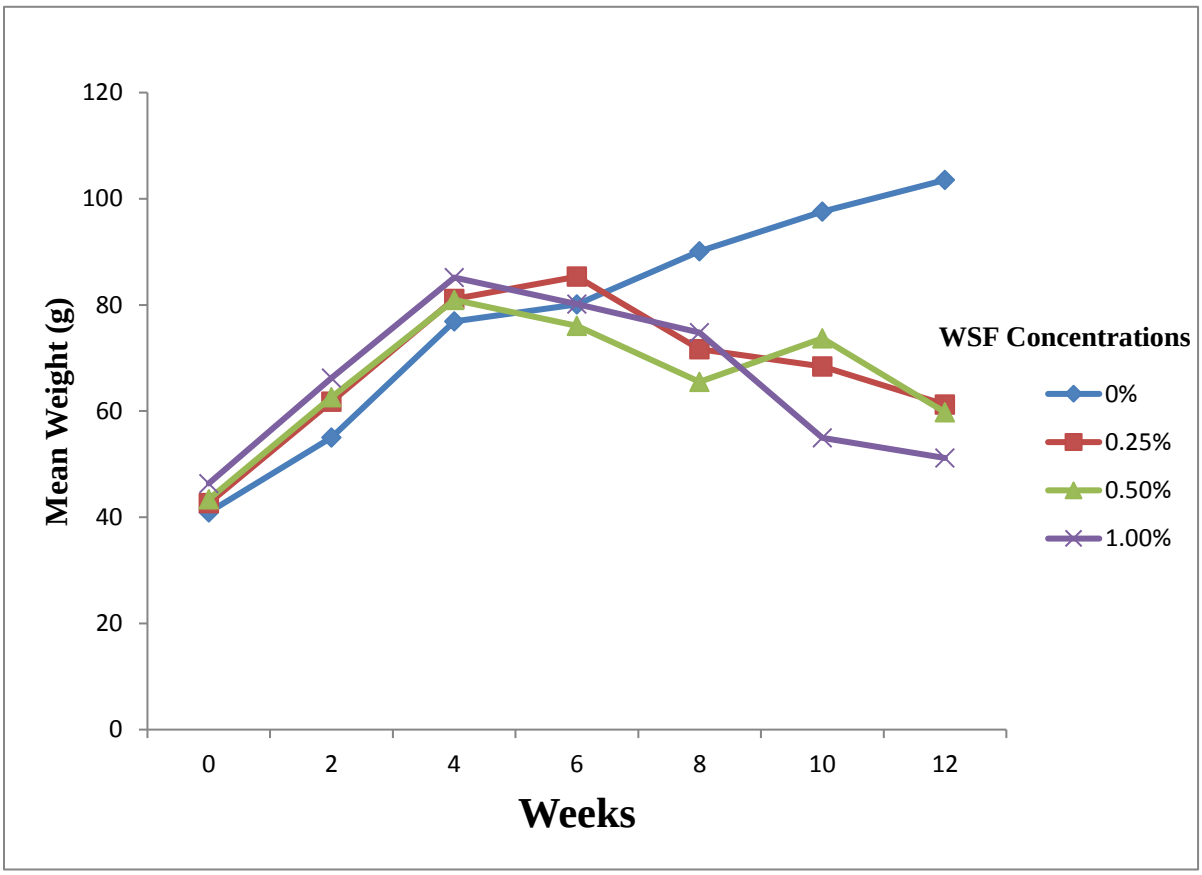

Figure 1: The bi-weekly growth performance of the *C. gariepinus* juveniles exposed to varying concentrations of WSF of crude oil.

The FCR of the fish exposed to WSF of crude oil were 3 or more folds than the FCR of the fish in control treatment.

No mortality was recorded in the control (0% WSF) culture tank and in the fish exposed to 0.25% WSF of crude oil. However, 10% and 20% mortality were recorded when the fish juveniles were exposed to 0.50% and 1.00% WSF of crude oil respectively (Table 1).

Enzyme Activities

The summary of assayed hepatic enzyme activities in the blood serum of *C. gariepinus* juveniles exposed to WSF of crude oil and the control is shown in Table 2. ALT activity in the serum of the fish juveniles exposed to 1.00% WSF (30.98 ±1.52 U/L) was significantly higher ($p<0.05$) than in the fish juveniles within the control culture tank and those exposed to lower concentrations of crude oil. Generally, the trend showed that the higher the concentration of the WSF of crude oil, the higher the serum ALT concentration. Analysis also showed that exposure of the fish juveniles to WSF of crude oil significantly ($p<0.05$) increased the hepatic ALT concentration (Table 2).

Table 1: Mean Growth Performance* of *C. gariepinus* Juveniles Exposed to Different Concentrations of WSF of Crude Oil for 90 days

Growth Performance Indices	Concentration of WSF			
	0% (Control)	**0.25%**	**0.50%**	**1.00%**
Initial mean weight (g)	40.93^{a} ±0.52	42.65^{a} ±0.32	43.41^{a} ±0.63	46.37^{a} ±0.92
Final mean weight (g)	103.53^{a} ±5.28	61.26^{b} ±3.12	59.81^{b} ±2.26	51.17^{b} ±3.58
Mean weight gain (g)	62.61^{a} ±2.25	18.61^{b} ±2.44	16.40^{b} ±3.49	4.80^{c} ±1.06
Mean Daily Weight Gain (g)	0.70^{a} ±0.30	0.21^{b} ±0.17	0.18^{b} ±0.15	0.05^{c} ±0.40
Specific Growth Rate (%)	0.45^{a} ±0.96	0.18^{b} ±0.72	0.15^{b} ±0.45	0.05^{c} ±0.25
Total Feed Intake (TFI) (g)	16.37	14.16	13.86	13.77
Feed Conversion Ratio (FCR)	0.26^{a} ±0.08	0.76^{b} ±0.37	0.85^{b} ±0.60	2.87^{c} ±1.41
% Mortality	0	0	10	20

*Values with different superscripts are significantly different at $p<0.05$

Table 2: Serum* ALT and AST Levels in *C. gariepinus* Juveniles Exposed to Different Concentrations of WSF of Crude Oil

WSF Concentration	Enzyme Activity (U/L)	
	Alanine Transaminase (ALT)	Aspartate Transaminase (AST)
0%	9.60^{c} ±0.30	448.00^{c} ±14.07
0.25%	9.67^{c} ±1.02	497.45^{bc}±11.74
0.50%	17.51^{b} ±0.57	537.09^{b} ±20.16
1.00%	30.98^{a} ±1.52	612.20^{a} ±18.77

*Values with different superscripts are significantly different at $p<0.05$

Exposure of *C. gariepinus* juveniles to WSF of crude oil significantly ($p<0.05$) increased the activity of AST (Table 2). The results showed that AST activity in the serum of fish juveniles exposed to 1% WSF of crude oil (612.20 ±18.77 U/L) was significantly higher ($p<0.05$) than in the control and test fish exposed to 0.25% and 0.50 % of WSF of crude oil.

As shown in Table 3, the ALT activity in the liver of fish juveniles irrespective of the added concentration of WSF of crude oil showed no significant difference ($p>0.05$). Nonetheless, ALT activity in the liver of fish juveniles exposed to 1.00% WSF (70.50 ±1.40 U/L) was correspondingly lower than in 0.50% WSF (81.25 ±5.80 U/L).

Similarly, AST activity in the liver was lowest in the fish juvenile exposed to 1.00% WSF of crude oil (399.13 ±17.18 U/L) (Table 3) and the enzyme activity was also observed to decrease in the liver of the fish with higher concentrations of WSF of crude oil exposure, though not significantly.

Hepatic lactate dehydrogenase (LDH) activity was lowest (294.05 ±72.48 U/L) in fish juveniles exposed to 0.25% WSF of crude oil was highest (705.72 ±28.54 U/L) in the fish exposed to 0.05% WSF. Analyses however showed that the activity of the enzyme in the fish exposed to 1.00% WSF of crude oil was not significantly different ($p<0.05$) from those of the fish in the control tank (Table 3).

Table 3: ALT, AST and LDH Levels* in the Liver of *C. gariepinus* Juveniles Exposed to Different Concentrations of WSF of Crude Oil

	Enzyme Activity (U/L)		
WSF Concentration	**Alanine Transaminase (ALT)**	**Aspartate Transaminase (AST)**	**Lactate Dehydrogenase (LDH)**
0%	42.75^{b} ±6.83	439.33^{a} ±35.57	478.73^{ab} ±87.58
0.25%	79.96^{a} ±6.96	476.40^{a} ±31.46	294.05^{b} ±72.48
0.50%	81.25^{a} ±5.80	432.29^{a} ±14.94	705.72^{a} ±28.54
1.00%	70.50^{a} ±1.40	399.13^{a} ±17.18	538.16^{ab} ±42.68

*Values with different superscripts are significantly different at $p<0.05$

DISCUSSION

Better growth performances were observed in the *C. gariepinus* juveniles within the control (0% WSF) compared to the test fishes exposed to varying concentrations of WSF of crude oil in this study. Poor growth performances in the exposed group could be attributed to the effects of the soluble fractions of crude Polycyclic Aromatic Hydrocarbon (PAH) and Total Petroleum Hydrocarbon (TPH) which probably affected the fish eco-physiology (Hodson *et al.*,1997). The high feed conversion ratio (FCR) observed in the exposed group indicated the inability of the exposed fish to convert feed consumed into required body protein (Sunmonu and Oloyede, 2006). Low mean weight gain recorded in the exposed fish might also be due to the immediate use up of protein content in feeds through oxidative stress rather than being utilized for new protein formation (Murty *et al.,* 1983).

However, relatively high mortality through possible disease contagion observed in test fish exposed to 0.5% and 1.0% WSF concentration of crude oil was due to the potency of environmental toxicants which increased disease susceptibility by interfering with immune, reproductive and developmental processes within aquatic animals (Couch and John, 1978). Excess mucus secretion observed in test fish was probably a neurological response to the irritation caused by test fish exposure to WSF of crude oil. Wang (1986) also reported that excessive mucus secretion could lead to the thickening of test fish skin or its gill epithelia which might result in fish mortality.

Exposure to varying concentrations of WSF of crude oil was found to induce some biochemical changes in the liver and blood enzymes in *C. gariepinus* juveniles. Alanine transaminase (ALT) activity was found to increase in the serum of the exposed test fish with the enzyme activity level increasing with succeeding concentrations of WSF of crude oil. Aspartate transaminase (AST) activity level in the serum of the exposed test fish especially those exposed to 0.5% and 1.0% WSF of crude oil were significantly higher ($p<0.05$) than those of the control fish (0% WSF). These observations corroborated the results of Lin *et al.*, 2002); Palanivelu *et al.*,2005); Abdel-Moneim *et al.,* (2008) and Wegwu and Omeodu (2010). According to the authors, hepatotoxicity of WSF of crude oil probably caused cell membrane damage where hepatic enzymes are emptied into the blood stream which probably resulted in the elevation of ALT/AST activity in the serum. However, the decreasing hepatic AST activity with increasing concentration of WSF of crude oil was attributed to biochemical changes due to the environmental stress resultant from exposure to the toxicants (Abdel-Moneim *et al.,* 2008)

Val and Almeid –Val (1999) reported that LDH activity increase in the liver was able to catalyse the conversion of pyruvate to lactate anaerobically. LDH activity recorded in this study was highest in the liver of *C. gariepinus* juveniles exposed to 0.5% and 1.0% WSF of crude oil. This may be due to low dissolved oxygen uptake which occurred in test fish under toxic exposure (Sunmonu and Oloyede, 2006) and could also be due to the presence of liver leisons which might have compromised its structural integrity (Ramesh *et al.,* 1993; Das *et al*., 2004 a,b)

CONCLUSION

The study concluded that WSF of crude oil had adverse effects on the growth performance, blood serum and liver activities in *Clarias gariepinus* juveniles.

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