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Evaluation of weight changes, condition factor and Organosomatic indices of *Clarias gariepinus* exposed to sub-lethal concentrations of an Oilfield wastewater

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ABSTRACT Twenty-eight adult *Clarias gariepinus* (mean weight 205 ± 12.89g SD; Mean length; 31.13 ± 3.82cm SD) were exposed to various concentrations in quadruplicates (0. 10, 20, 30, 40, 50 and 60%) of an oilfield wastewater and were investigated for weight changes (at a weekly interval for four weeks), condition factor (K) and organosomatic indices which include liver (hepatosomatic index), spleen (spleenosomatic index), kidney (renatosomatic index) and heart (cardiosomatic index). The result showed a significant decrease ($p \leq 0.05$) in weight of fish as the concentration increased. Results of condition factor showed a significant decrease ($p \leq 0.05$) in the initial (K1) and final (K2) in exposed *C. gariepinus.* Amongst the Organosomatic indices used to assess stress in *C. gariepinus*, some significant increases ($p \leq 0.05$) were observed in liver (hepatosomatic index), spleen (spleenosomatic index) and kidney (renatosomatic index) when compared to the control. However, the effect in the heart (cardiosomatic index) was similar to the control as the values fluctuated around control values. The indices measured in this (except cardiosomatic index) work can be used to measure long term effect of exposing *Clarias gariepinus* to oilfield wastewater.

Keywords: Clarias gariepinus, oilfield wastewater, Weight changes, Organosomatic indices

Introduction

Organosomatic indices can be described as the ratio of organs to body weight (Ronald and Bruce, 1990). Measured organ mass in relation to body mass can be directly linked to toxic effects of chemicals on target organs (Carlson and Zelikoff, 2008). They can also be used as indices of change in nutritional and energy status (Maxwell and Dutta, 2005). Commonly used organosomatic indices in various stress-related studies include hepatosomatic indices (HSI), viscerosomatic indices (VSI), spleenosomatic indices (SSI), cardiosomatic indices (CSI) and renatosomatic indices (RSI). Singh and Canario (2004) observed that hepatosomatic index is one of the most investigated biomarkers due to the important role of the liver in detoxification of pollutants, while Dogan and Cao (2011), observed that organosomatic index can be used as a bioindicator for endocrine disruption in fish exposed to chemicals. Recently, organosomatic indices and fish condition factor have been used to determine the sub-lethal effects of pollutants during clinical diagnosis of physiology (Yi *et al.,* 2007*;* Ozer *et al.,* 2008; Mlambo *et al.,*2009; Ariweriokuma *et al.,* 2011). Scientists have reported a decrease in the weight of liver in organisms exposed to various toxicants such as propoxur and heavy metals (Istitoris *et al.,* 2001), carbofuran (Soufy *et al.,* 2007) and cypermethrin (Ariweriokuma *et al.,* 2011). Decrease in the weight of liver suggests a decrease in the production of endoplasmic reticulum for protein synthesis in liver tissue under toxicant exposure (Bennet and Wolke, 2004). Liver reduction could also be as a result of decreased lipid storage (Gabriel *et al.,* 2010).

Several authors have reported a decrease (Arellano *et al.,* 1999; Anderson *et al.,* 1998; Azmat *et al.,* 2007) or increase (Mcmaster *et al.,*1991) in condition factor when organisms were exposed to different toxicants. According to Mcmaster *et al.* (1991), the increased condition factor observed in white sucker (*Astostomes commessomi*) suggests a disruption in metabolic capability and altered energy allocation. The decreased in condition factor, however is believed to be due to the impairment of olfactory systems which might have affected feeding, resulting in alterations of metabolic activities and energy allocation of the fish systems (Ariweriokuma *et al.,* 2011).

This study was undertaken to ascertain the toxicity of sub-lethal concentrations of oilfield wastewater on *Clarias gariepinus,* by determining the effects on its weight, condition factor and organosomatic indices.

Materials and methods

Experimental Fish and Acclimation

Twenty-eight adults of *C. gariepinus* (8 weeks old - mean weight 205 ± 12.89g SD; mean length; 31.13 ± 3.82cm SD)were obtained from the African Regional Aquaculture Center (ARAC) at Aluu in Ikwerre Local government area of Rivers State, Nigeria. The fish were acclimatized individually in rectangular plastic aquaria containing borehole water (20L) for two weeks. The top of the aquaria were covered to control escape of fish. The water was changed daily and the aquaria were washed with a piece of foam without using any form of soap or detergent. The fish were fed twice daily (8.00 a.m. and 5.00p.m.) with a diet of 35% crude protein at 1% biomass Before exposure to the toxicant, individuals were weighed using a sensitive weighing balance (H12-Satorius model, Portugal).

Collection of Test toxicant (oilfield wastewater)

The test toxicant (oilfield wastewater) was collected from Ebocha oil centre in Ogba/Egbema Local Government Area of Rivers State, Nigeria (N05 27′ 40.45′ E006 41′ 52.14′) on three occasions. These represented different ranges of the discharge at the discharge point. The oilfield wastewater samples were transported to the laboratory immediately after collection.

Experimental Procedure

There were seven [0 (control), 10, 20, 30, 40, 50, and 60 % v/v] treatment levels each with four replicates. Fish were introduced individually into each of these concentrations contained in an aquarium and exposed for a period of 28 days at room temperature (30±2^{o}C). Fifteen litres of each prepared concentration was used and fish was fed as in the acclimation period. Each test solution was renewed weekly and the fish weighed. During the exposure period which lasted for 28 days, some water quality parameters namely temperature, pH, salinity, turbidity, conductivity, total dissolved solids (TDS), total suspended solutes (TSS), Chloride, dissolved oxygen (DO), biochemical oxygen demand (BOD), alkalinity and total hydrocarbon content (THC) were taken daily using the methods described by APHA (1998). At the end of the experiment the fish were sacrificed. Then, the liver, heart, spleen, and the kidney was carefully removed and weighed.

Organosomatic Indices

Organosomatic indices of the liver, heart, spleen and kidney were then calculated for the twenty-eight fish (Jenkins, 2004: Adams *et al.,* 1996) using the formula:

$$\textbf{Organosomatic indices} = \frac{\textbf{Weight of organ}}{\textbf{Weight of fish}} \times 100$$

Foulton's Condition Factor

Values of Foulton's condition factor (Fulton, 1902) were calculated using the formula:

$$\textbf{Foulton's condition factor (K)} = \frac{\textbf{Weight of fish}}{L^3} \times 100$$

Where; L = Length of fish.

Data Analysis

The data obtained from the tests were subjected to statistical analyses. T-tests were used to compare initial and final condition factors; regression analyses were applied to determine the change of weight of fish with time for each individual. The slopes obtained were compared by analysis of variance (ANOVA) and the means of each treatment was compared with the control Dunnett's procedure with a family error rate of 0.05.The mean gonadosomatic indices were compared by ANOVA and Dunnett's comparison with control. All analyses were performed using MINTAB for Windows version 16.

Results

The result of the physico-chemical properties of the constituted concentrations of the oilfield wastewater and their mean values are presented in Table 1. Generally, the values increased with increased concentration of the effluent: turbidity (1.5±0.05 – 3.5±0.0NTU), conductivity (183.33±28.87 – 13666.67±288.68µs/cm), TDS (48.33±2.89 – 7316.67±275.3ppm), chloride (9.33±1.16 - 3726±64.29ppm), BOD (0.82±0.03 – 1.73±0.02), ammonia (0.0±0.0 – 0.02±0.0ppm), nitrite (0.001±0.0 – 0.01±0.0ppm) and THC (0.0±0.00 - 11.81±3.12ppm). However, there was a decrease in DO (3.97±0.45 to 2.0±0.0ppm) with increased concentration of the effluent.
The result of effect of oilfield wastewater on the weight of *Clarias gariepinus* as presented on Fig. 1 showed that there was a generally a reduction in

weight of fish with increased duration of exposure to the toxicant The slopes of the regressions indicated an increasing rate of reduction in weight with increasing concentration of effluent with apparent distortion of the pattern by a dip at % concentration (Fig. 2). Analysis of variance gave a significant difference is slopes ($F_{6,21} = 7.30$, $p<0.001$), and Dunnett's test showed significantly higher negative slopes in all test concentrations in comparison with the control ($p<0.05$).

The condition factor (K) of *Clarias gariepinus* exposed to sub-lethal concentrations of oilfield wastewater showed consistent reductions between the initial (K1) and the final (K2) values (Fig. 3) in all test conditions. Whereas the reduction in in the condition factors were not significant in the controls and 10 % effluent, significant differences were obtained at the 20, 40 and 50 % wastewater effluent exposures (Table 2).

The exposure of *C. gariepinus* to varying concentration of oilfield wastewater resulted in insignificant difference in CSI with values ranging from 0.17 ± 0.08 to 0.33 ± 0.04 (Table 3). Similarly the values of HIS did not show significant differences between the control (2.29 ± 0.85) and other treatments except for 20 % exposure. On the other hand, there were significant differences in the SSI ($p<0.001$) and RSI ($p=0.001$). In comparison with the control, the SSI values were significantly lower at 10 % effluent exposure but significantly higher at 40 %. The RSI ranged from 1.37 ± 0.27 in the control to 3.10 ±0.5 at 20 % exposure which showed significantly higher value than the control.

The relationships between some physicochemical parameters and the regression coefficients the change of weight in the fish with exposure time in the different treatments are presented in Figure 4. All the parameters had positive regressions, except DO which had a negative relationship. However, the coefficients of determination of the regressions were generally low.

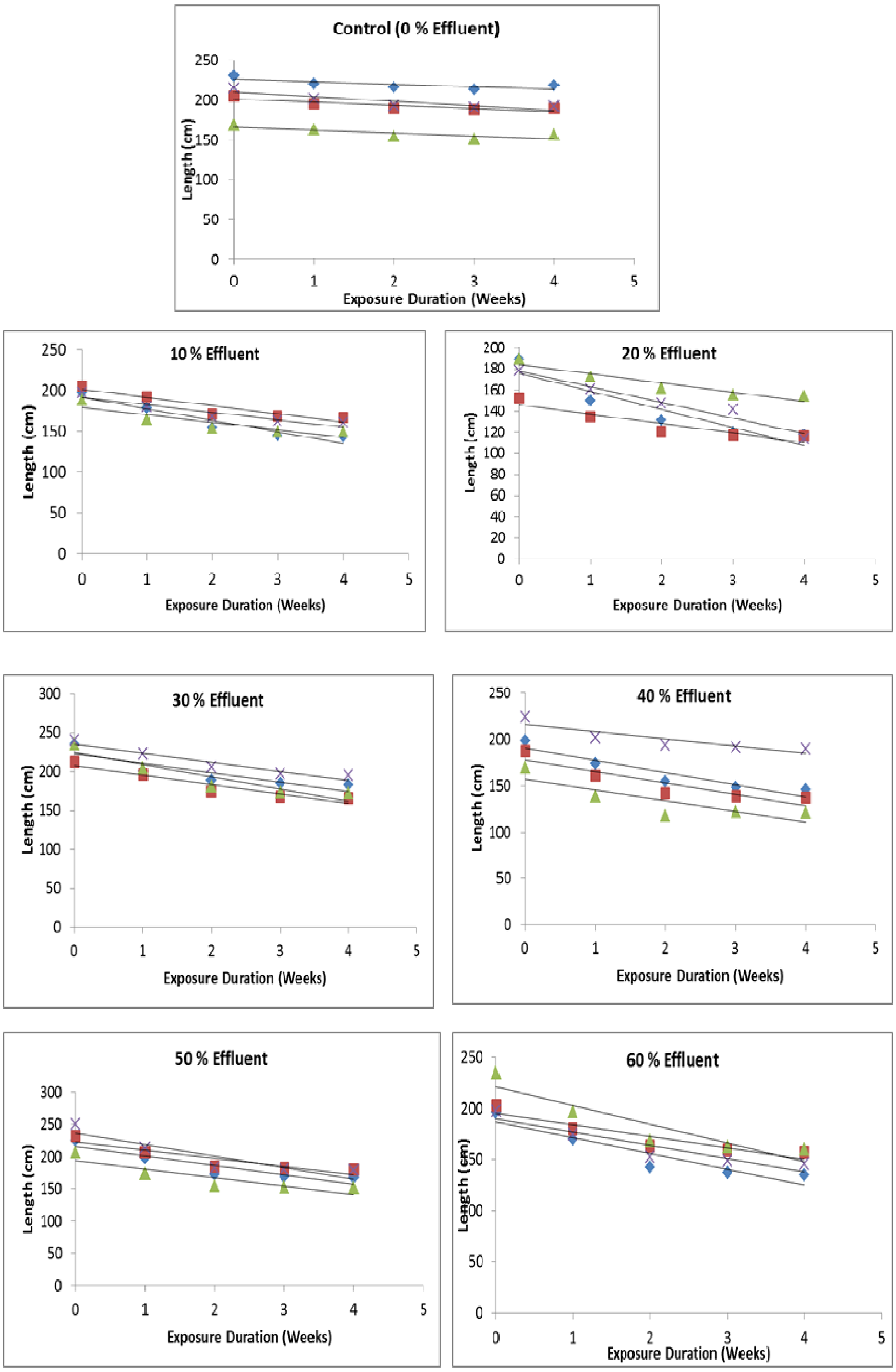

Fig. 1: Regressions of change in weight with time of C. gariepinus exposed to different concentrations of oilfield wastewater effluent over a period of four weeks.

Table 1: Mean (± SD) levels of physicochemical concentrations of the constituent concentrations of oilfield wastewater used in the analysis

Table 1: Mean (± SD) levels of physicochemical concentrations of the constituent concentrations of oilfield wastewater used in the analysis

Physicochemical	Concentration of Oilfield wastewater (%)						
properties	0	10	20	30	40	50	60
Temperature (^{o}C)	26.17±0.29	26.07±0.4	26.5±0.5	25.0±0.0	26.83±0.29	27.33±0.29	27.0±0.0
pH	7.1±0.1	7.47±0.31	7.67±0.12	7.23±0.25	7.47±0.31	8.0±0.0	8.0±0.0
Salinity (ppm)	0.0±0.0	47.67±2.52	517.33±28.31	1500±50	2443.33±309.25	3233.33±251.66	4556±51.07
Turbidity (NTU)	1.5±0.5	5.33±0.58	13.33±4.16	16±1.0	23.33±3.06	32.0±2.0	35±0.0
Conductivity (µs/cm)	183.33±28.87	3766.67±251.66	1060±121.66	2166.67±288.68	4216.67±225.46	8433.33±404.15	13666.67±288.68
TDS (ppm)	48.33±2.89	976.67±25.17	1620±158.75	2966.67±152.75	4783.33±256.58	6146.67±128.58	7316.67±275.38
Chloride (ppm)	9.33±1.16	1760±52.92	1926.67±110.15	2316.67±189.29	2726.67±253.25	3472.67±25.33	3726.67±64.29
DO (ppm)	3.97±0.451	3.4±0.2	3.3±0.1	2.43±0.15	2.33±0.12	2.3±0.0	2.2±0.0
BOD (ppm)	0.82±0.03	1.02±0.03	1.18±0.06	1.52±0.06	1.58±0.02	1.64±0.0	1.73±0.02
Alkalinity (ppm)	9.33±1.16	20.0±2.0	23.5±0.5	26.67±3.06	31.33±1.16	32±0.0	14±0.0
Ammonia (ppm)	0.0±0.0	0.02±0.015	0.01±0.0	0.01±0.001	0.02±0.002	0.02±0.0	0.02±0.0
Nitrite (ppm)	0.001±0.0	0.01±0.001	0.008±0.0	0.01±0.001	0.01±0.0	0.01±0.001	0.01±0.0
THC (ppm)	0.0±0.0	1.98±0.082	3.09±0.085	3.49±0.445	6.17±0.651	7.45±0.135	11.81±3.118
PAH (ppm)	0±0	0.0106±0.006	0.019±0.006	0.03133±0.008	0.03433±0.005	0.07567±0.021	0.07344±0.002
Iron (ppm)	0.00067±0.001	0.01167±0.005	0.04433±0.001	0.06667±0.003	0.081±0.000	0.0623±0.081	0.21333±0.006
Lead (ppm)	0±0	0.005±0.000	0.008±0.000	0.03433±0.044	0.012±0.000	0.022±0.000	0.025±0.000

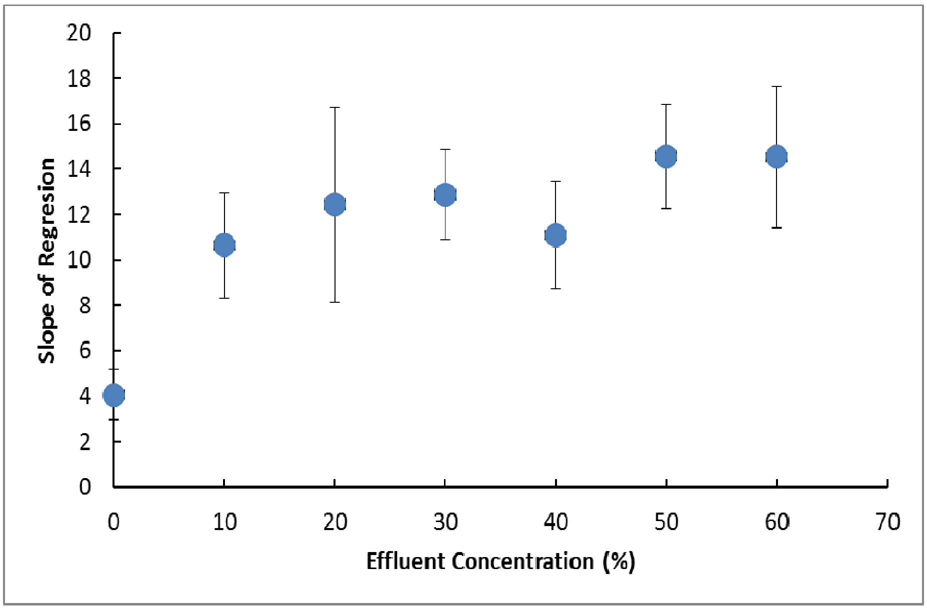

Fig. 2: Mean (±SD) of regression slopes on change of weight of C. gariepinus exposed to different concentrations of oilfield wastewater. Note that the slopes were negative

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Table 2: T-tests for differences in condition factors between initial and final condition factors of C.gariepinus exposed to different dilutions of oilfield effluent.

	df	Estimated Difference	95 % CI of for Difference	T-Value	P-Value	
Control (0 %)	5	0.058	(-0.346, 0.463)	0.37	0.727	
10 %	5	0.156	(-0.185, 0.498)	1.18	0.292	
20 %	5	0.2590	(0.0542, 0.4639)	3.25	0.023	
30 %	5	0.1396	(-0.1034, 0.3825)	1.48	0.200	
40 %	5	0.2056	(0.0394, 0.3719)	3.18	0.025	
50 %	5	0.1299	(0.0167, 0.2431)	2.95	0.032	
60 %	5	0.1908	(-0.0520, 0.4336)	2.02	0.099	

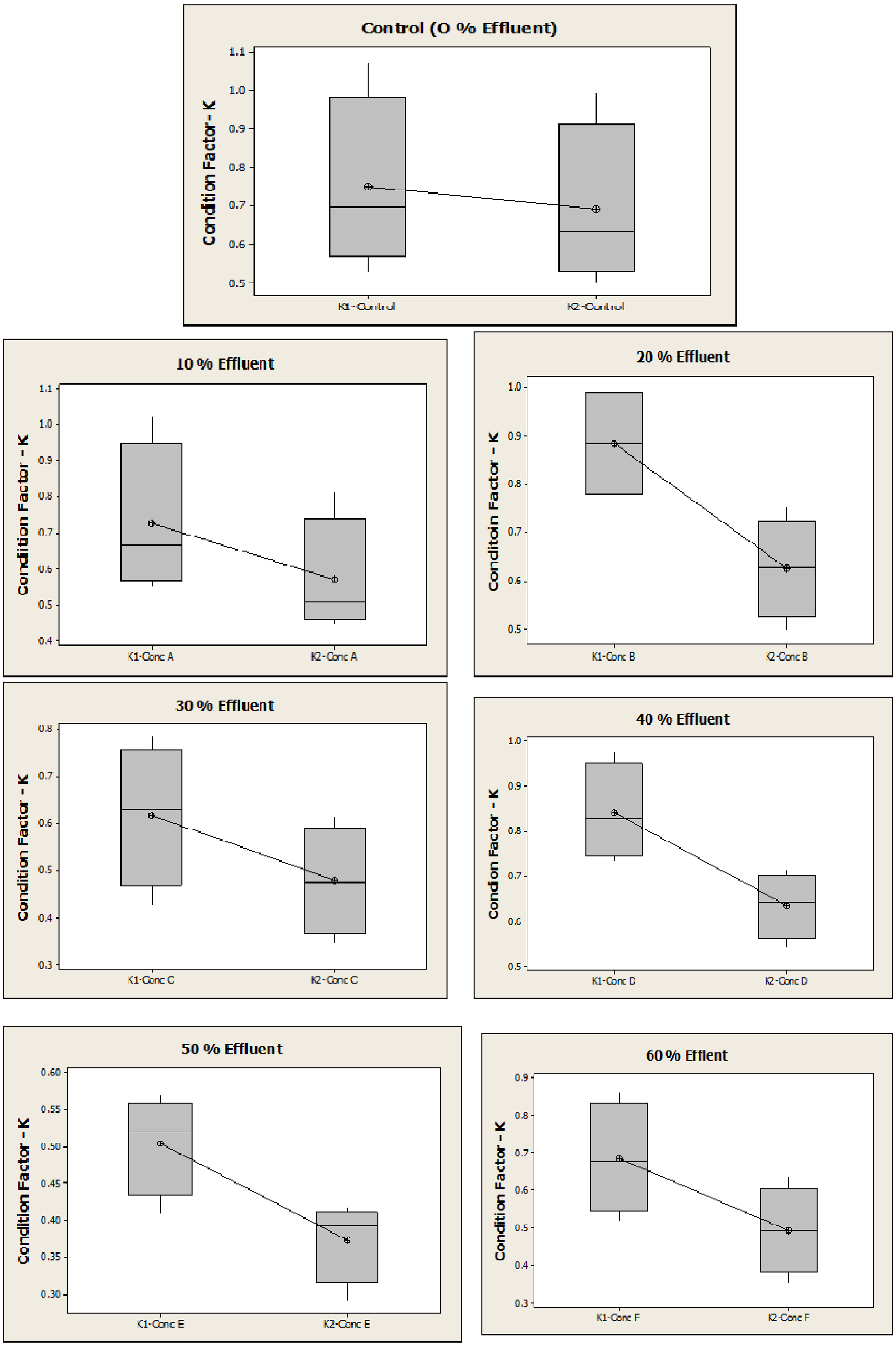

Fig. 3: Plots of initial (K1) and final (K2) condition factors of C. gariepinus exposed to different concentrations of oilfield wastewater effluent after 28 days of exposure.

Table 3: Organosomatic indices (mean ± SD) of *Clarias gariepinus* exposed to sub-lethal concentrations of oilfield wastewater for 28 days

Conc. Of oww(%)	Hepato-somatic Index (HIS)		Cardio-somatic Index (CSI)		Splenato-somatic Index (SSI)		Renato-somatic Index (RSI)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
	2.2947[A]	0.8566	0.21317[A]	0.04251	0.10737[A]	0.01536	1.3762[A]	0.2742
	2.7200[A]	0.7054	0.19008[A]	0.07645	**0.06481**	0.00457	1.7163[A]	0.2209
	4.0624	1.0203	0.26353[A]	0.11407	0.08086[A]	0.00457	**3.1047**	0.5177
	2.4892[A]	0.5026	0.17052[A]	0.08718	0.08329[A]	0.03055	1.8842[A]	0.3516
	3.2946[A]	0.9518	0.25804[A]	0.05552	**0.15522**	0.04164	1.7253[A]	0.6376
	3.3517[A]	1.1369	0.28472[A]	0.07864	0.11881[A]	0.00977	1.9331[A]	0.7172
	2.3737[A]	0.2575	0.33471[A]	0.04785	0.13450[A]	0.01079	1.6542[A]	0.2628
	$F = 2.48; p = 0.057$		$F = 2.31; p = 0.075$		$F = 8.96; p < 0.001$		$F = 5.74; p = 0.001$	

*Means with superscript 'A' in the column are not significantly different from the control (Dunnet's test P=0.05)

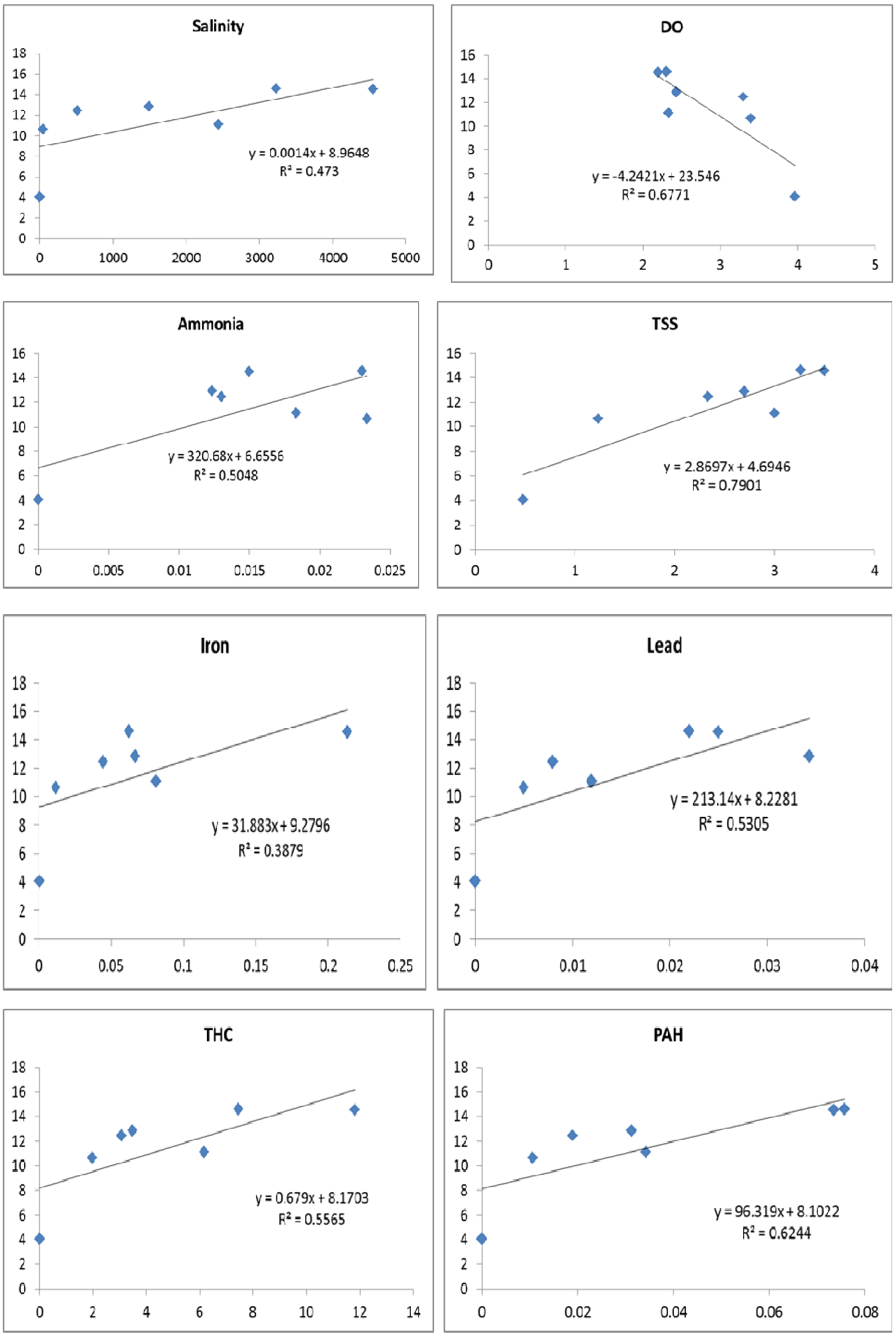

Fig. 4: Relationships between some physicochemical variables of exposure media and regression coefficients of fish weight vs exposure media

Discussion

Water quality parameters

Water quality parameters such as temperature, dissolved oxygen, free carbon (IV) oxide, pH, alkalinity and conductivity are important and affect fish health, growth and reproduction (Camus *et al.,* 1998; Adamu and Kori-Siakpere, 2011). However, Richards, (1977) reported that the main cause of mortality in aquarium fish was the inadequate maintenance of the water environment. The characteristics of the oilfield wastewater used in this study have been evaluated by Akani and Gabriel (2015); the values agree with that of Wemedo *et al*. (2012) but differ from that of Obire and Amusan (2003). Akani and Gabriel (2015) reported that all the physico-chemical properties except temperature, DO, BOD and THC fell above acceptable limits of the Federal Environmental Protection Agency (FEPA, 1991) of 35 ^{O}C, 5.0 ppm, 50.0 ppm and 48.0 ppm respectively. The low levels of DO indicate that the environment is stressed (Clerk, 1986*). C. gariepinus* has been shown to have a wide tolerance for temperature ranges, low BOD, DO and salinity (Ozmen *et al.,* 2006). The constituted concentration of the oilfield wastewater effluent in this study gave a range of values of the constituent variables which were correlated with the indices of change in weight in the fish to evaluate their roles in the observed patterns.

Condition factor and Organosomatic indices

The condition factor is an organism-level response to factors such as nutritional status, pathogen effects and toxic chemical exposure causing greater-than normal and less-than normal weights (Andu and Kangur, 1996; Azmat *et al.,* 2007). The condition factor is used as an indicator of the well-being of the individual organism, because it integrates many levels of the organizational processes. There was an observed decrease in the initial and final condition factor of *C. gariepinus* exposed to sublethal concentrations of the oilfield wastewater. Several authors have reported a decrease (Anderson *et al.,* 1988; Arellano *et al.,* 1999; Azmat *et al.,* 2007) or increase (Mcmaster *et al.,* 1991) in condition factor when organisms were exposed to different toxicants. The decreased in condition factor, however is believed to be due to the impairment of olfactory systems which might have affected feeding, resulting in alterations of metabolic activities and energy allocation of the fish systems (Ariweriokuma *et al.,* 2011). This reason may be applicable in this study as there were regression analysis showed that some physicochemical parameters which are capable of interfering with olfactory systems had elevated values in the exposure media and regressed positively with reduction in weight changes with time. Arellano *et al.* (1999) exposed *Solea senegalensis* to cypermethrin and observed a decrease in condition factor; which may have been caused by a reduction in oxygen carrying proteins. On the other hand, Mcmaster *et al.* (1991), explained that an increased condition factor observed in white sucker (Astostomes *commessomi*) exposed to bio acute Kraft mill effluent suggests a disruption in metabolic capability and altered energy allocation.

Organosomatic indices which describes the ratio of organs to body weight (Ronald and Bruce, 1990), measures organ in relation to body mass and can be directly linked to toxic effects of chemical on target organ (Giullo and Hinton, 2008). They can also be used as indices of change in nutritional and energy status (Maxwell and Duta, 2005). Although significant difference was observed only between 20 % effluent exposure and control, there was a general increase in the liver (hepatosomatic index) of exposed fish in this study. This suggests that the liver cells were affected possibly causing an increase in the rate of production of endoplasmic reticulum for the synthesis of protein in the liver (Anderson *et al.*, 1998). The liver serves as an organ of detoxification; therefore in the presence of a stressor these qualities may have been compromised causing adverse effect on the fish (Gabriel, *et al*., 2009; Jerkins, 2004; Adams *et al*., 1996). Minimal changes were recorded in the heart (cardiosomatic index) as no significant difference was observed between any of the wastewater treatments and the control, which implies that the heart was not adversely affected by the oilfield wastewater. Gabriel *et al*., (2009) recorded similar findngs when *C. gariepinus* was intravenously injected with aqueous leaf extracts of *Lepidagathis alopcuroides.* Some significant alterations were also recorded in this study broadly showing enlargements of the spleen (spleenosomatic index) with 10 % exposure as an exception. Similar effects were observed on the kidney (renatosomatic index) of the exposed fish with consistently higher RSI in the exposure treatments in comparison with the control, and significantly so for the 20 % treatment. The spleen and kidney are are known to produce blood (haematopoietic) in the tissues of fish (Singh and Singh, 1998; Jerkins, 2004). Increase in their size suggests pathological response to combat the action of the oilfield wastewater from destroying the blood cells (Jerkins, 2004; Gabriel, *et al.,* 2009). Enlargement or swelling of the spleen is also considered to be indicative of disease or immune system problems (Goede and Barton, 1990).

Conclusion and Recommendation

This study has shown that exposure of *C. gariepinus* to sub-lethal concentrations of oilfield wastewater caused significant changes in weight, condition factor and organosomatic indices. These changes may be adversely disruptive for the survival of *C. gariepinus* in captivity, and can be used in monitoring the long-term effects of oilfield wastewater on *C. gariepinus*. It is therefore, advocated that oilfield wastewater be subjected to additional treatment for full conformance with effluent discharge standards before release into the environment to prevent ecotoxicological problems and consequently economic loss.

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