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Sublethal Effects of Tannery Effluent on Some Hematological Indices and Growth of *Clarias gariepinus* (Teugels)

T. T. Gbem,[1] J. K. Balogun,[1] F. A. Lawal,[2] P. A. Annune,[3] J. Auta[1]

[1] Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria
[2] National Research Institute for Chemical Technology, Challawa Road, Kano, Nigeria
[3] Fisheries and Aquaculture Department, University of Agriculture, Makurdi, Nigeria

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Few studies exist on the effects of industrial effluent on Nigerian fish in particular and aquatic biota in general (Onwumere 1986; Gbem et al. 1997; Gbem 1998; Gbem et al. 2001). Hematological parameters act as physiological indicators to changing external environment (Gill and Pant 1981) as a result of their relationship with energetics (metabolic levels), respiration (hemoglobin levels) and defence mechanisms (leucocytes levels). These parameters provide an integrated measure of the health status of an organism which overtime manifest in changes in weight (growth).

This study therefore examined the effects of tannery effluent on blood parameters and growth of a common and economically important Nigerian fresh water fish, *Clarias gariepinus* (Teugels).

MATERIALS AND METHODS

Composite effluent was obtained in 50L capacity plastic cans from a modern commercial tannery in Kano between the hours of 6 – 11 am. The effluent was taken to the Department of Biological Sciences, Ahmadu Bello University, Zaria. Fish were obtained from rivulets around Ja'ma village near the Ahmadu Bello University Dam. Analysis of water form these rivulets showed them to be relatively unpolluted (see Table 1). Acclimation of fish was done in 160L capacity holding tanks at 25.5 ± 1^0C at prevailing natural photoperiods for 2wks. During this period of acclimation, fish were fed twice daily at 5% body weight with pelleted diet containing 35% crude protein level (CPL).

Based on the result of the 96h LC50 (Gbem, 1998), juvenile *Clarias gariepinus*, mean total weight 5.0 ± 0.2 g and mean standard length 9.05 ± 0.6 cm were exposed to 2 and 6% v/v composite tannery effluent for 8 wks. Each concentration was duplicated twice. There was a control for each experiment. A temperature range of 22–23^{0}C and the prevailing natural photoperiods were maintained. Each nominal concentration was renewed once every week including the control.

Blood was obtained by severation of the caudal region. Total erythrocytes count (TEC) and total leucocytes counts (TLC) were determined using an improved Neubauer counting chamber as described in Hesser (1960). Hemoglobin was estimated using the Sahli-Hellige method and readings recorded in g using the calibration charts prepared by Miale (1958). Hematocrit, (PCV), was done by the micro-Westegren method as

Correspondence to: T. T. Gbem

described in Blaxhall and Daisley (1973). The 'absolute values' made up of mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from the results of TEC, TLC, hemoglobin and PCV (Annune 1992).

To monitor growth, the guideline 210 of the OECD (1992) was followed. However, two exceptions were observed. First, instead of exposing 16 fish per tank as recommended, 10 fish were exposed to tanks of 0.6x0.4x0.3m. This was done to reduce social interactions (Lucas and Priede 1992) bound to occur in feeding. Second, measurements of growth were done for 56 d and not the recommended 28 d. This is because the experiment was designed to last for 56 d so as to observe the long-term effects of the toxicant on blood parameters. Normally, for growth studies, five concentration levels are recommended by the OECD guidelines. To accommodate this, two new concentrations, 1 and 4% v/v were introduced.

Fish under growth studies were fed at 5% body wt twice daily i.e. morning and evening with 35% crude protein level pelleted diet. Measurement of wt changes was done on a biweekly basis and percentage cumulative wt change determined.

For both hematological and growth studies, Statistical Analysis Systems (SAS), release 4.0 programme was used to run Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) to test for differences between different levels of treatment and to separate means respectively, where applicable.

RESULTS AND DISCUSSION

Results of hematological parameters are presented in Tables 2 and 3. The TEC in the control increased steadily from wk 2 to wk 8. A decrease with increased period of exposure in TEC was observed in the 2 and 6% v/v treated groups. This decrease was however more pronounced in the 6% tannery treated fish. There was significant difference ($p < 0.05$) between the control, 2% treatments and those of 6% groups up to wk 4. The TLC in the control fish and 6% treated fish increased steadily from wk 2 to 8. There was a decrease of TLC in fish exposed to 2% tannery effluent from 4.7×10^6 mm^{-3} in wk 2 to 0.95×10^6 mm^{-3} in wk 8. There was significant difference ($P < 0.05$) between fish exposed to 2% tannery effluent on one hand, and those of the control and 6% tannery effluent on the other hand. Statistical separation of means by DMRT showed the means of 6% and control groups to be higher than 2% treated group. Hemoglobin significantly increased ($p< 0.05$) in the 2 and 6% treated fish. DMRT showed the means of 6>2%>control. Hematocrit values increased in the control and fish exposed to 2% tannery effluent throughout the duration of exposure. An increase from 40 in wk 2 to 42 in wk 4 in values of PCV was recorded in 6% tannery treated group. This value however 'stabilized' back to 40.16 in wk 8. The 6% and 2% effluent treated haematocrits were significantly higher ($p < 0.05$) than those of the control. DMRT showed the means of 6 = 2% > control for wk 6 and 8.

In the 'absolute values' presented in Table 3, MCHC was approximately maintained throughout the period of exposure. There was no significant difference recorded between treatments. The MCV values of 6% effluent treated fish were significantly lower ($P < 0.05$) than those of the 2% treated fish and the control. DMRT showed the means of 2% effluent treated fish and the control fish to be significantly different from

those of the 6% treated fish. Though MCH increased in all the groups (both control and treated groups), the values were however not significantly different in any of the group.

Results of the growth studies are presented on Figure 1. Though wt increases were observed in the control, 1 and 2% tannery effluent treated groups, the first two were significantly higher ($p < 0.05$) than the 2% treated group. In the 4 and 6% tannery effluent groups, there was a slight wt gain in wk 2 and then, a trend of continuous decrease in 6% treated fish throughout the exposure period. The 4% treated group showed a slight recovery in wk 8.

The decrease in TEC recorded in this study is similar to findings by McLean (1973) who exposed fish to pulp mill effluent. These changes were attributed to the possibility of inhibition in erythrocyte production and also, a possible increase in the rate of erythrocyte destruction or haemodilution. Pulp mill effluent are known to contain several heavy metals (Martel et al. 1994) many of which are also present in tannery effluent (Lawal et al. 1986; *Lawal et al. 1997*). An increase in TLC and decrease in TEC of the 6% tannery effluent treated group over time of exposure does not however point to haemodilution as a factor in operation.

The 2% effluent treated group experienced a continuous decrease in both TEC and TLC throughout the study period (Table 2). In this treatment group, haemodilution could be advanced as a reason for changes in these parameters. Gbem, (1998) found a decrease in the TEC of *Clarias gariepinus* exposed to an acute toxic level of tannery effluent. Zambariborsch and Biu (1977) exposed the round goby *Gobus melanostomus* to hexachloran (lindane) and observed marked decrease in both the TEC and TLC within 96h. They advanced dyscrasia as being more likely the cause for this decrease and that this could be induced by the chemical interfering with haemopoiesis and/or alteration of cell membrane by hydrolysis of the acetylcholine in the body fluids.

The increase in PCV and hemoglobin values in the treated groups could be explained as having been caused by haemoconcentration since changes occurred in erythrocyte numbers. Changes in blood cell volume (MCV), could occur over an extended period of time depending on the oxygen conditions. Hypoxic conditions are known to cause increases in blood volume (Hurtado, 1964). This may also affect PCV as was shown by Huston and DeWilde (1969) in *Salvalinus fountanalis.* Significant changes ($p < 0.05$) in the values of MCV were observed in this study. Hematocrit values of the treated fish increased from 15 to 35%. Casillas and Smith (1977) found a range of between 10–20% for *Onchorhynchus mykiss*. It would appear plausible that a slightly hypoxic environment was created *in vitro* by (muscular) exertion during stress. Gbem (1998) observed gill damages in the 6% treated group, invariably impairing oxygen uptake.

A dose – dependent impairment of growth was observed in *C. gariepinus* exposed to tannery effluent with the highest concentration (i.e. 6%) exhibiting the most marked suppression of growth. The control fish exhibited the highest growth followed by 1 >2 > 4 > 6% v/v treated groups. Similar findings have been made when other freshwater teleosts were exposed to toxic effluents. Onwumere (1986) exposed *Oreochromis niloticus* to refinery effluent and found a dose-dependent impairment of growth.

Reduced physical activities especially feeding was noticed in the 4 and 6% tannery effluent treated fish. This could have contributed to wt loss. Changes in carbohydrates

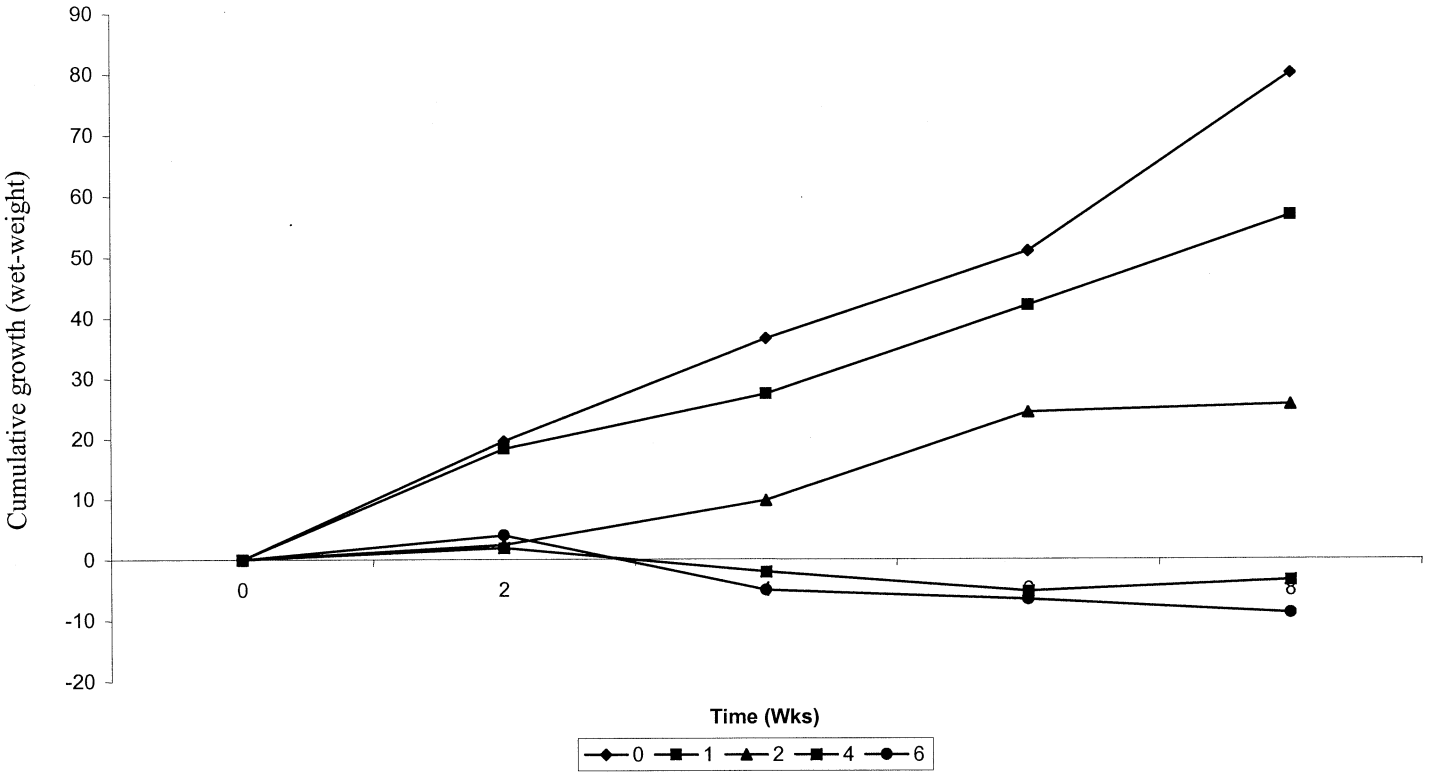

Figure 1. Percentage cumulative wet-weight changes in *Clarias gariepinus* exposed to tannery effluent

Table 1. Physico-chemical quality of reference site water

Parameter	Range	Mean * ± SD
Temperature (^{0} C)	22-23.6	22.3 ± 2.4
pH	7.6-8.0	7.8 ± 0.2
Dissolved Oxygen (mg^{-1})	7.9-8.6	8.04 ± 1.1
Conductivity (μscm^{-1})	118-123	120.0 ± 16
Hardness (mgl^{-1} $CaCo_3$)	126-133	129.0 ± 1
Cu		0.02
Pb		0.02
Zn		0.03
Cr		0.01

* Dry season averages for four months when fish were obtained

Table 2. Total erythrocyte and leucocyte counts (x 10^6 mm^3), hemoglobin (g/100ml), hematocrit (%) of juvenile *Clarias gariepinus* exposed to tannery effluent.

EFFLUENT CONC.% V/V	DURATION OF EXPOSURE (WKS)			
	2	**4**	**6**	**8**
		Total Ervthrocytes		
Control (0)	251 ± 13^{a*}	262± 11^{a}	269 ± 18^{a}	280 ± 11^{a}
2	270 ± 17^{a}	268 ± 6^{a}	286 ± 11^{a}	266 ± 19^{a}
6	492 ± 39^{b}	320 ± 12^{b}	294 ± 4^{a}	161 ± 11^{b}
		Total Leucocytes		
Control (0)	11.9 ± 0.7^{a}	12 ± 0.7^{a}	18 ± 1.6^{a}	21 ± 2.1^{a}
2	4.7 ± 1.0^{b}	2.4 ± 0.2^{b}	1.12 ± 0.2^{b}	0.95 ± 0.1^{b}
6	16 ± 2^{a}	18.4 ± 1.7^{a}	24.6 ± 1.9^{b}	26 ± 1.3^{a}
		Hemoglobin		
Control (0)	7.7 ± 0.15^{a}	8.5 ± 0.25^{a}	9.2 ± 0.30^{a}	10.7 ± 0.46^{a}
2	10.0 ± 0.94^{b}	10.5 ± 0.22^{b}	12.6 ± 0.17^{b}	14.0 ± 0.26^{b}
6	13.3 ± 0.31^{b}	14.0 ± 0.41^{b}	13.9 ± 0.34^{b}	14.0 ± 0.23^{b}
		Hematocrit		
Control (0)	23.0 ± 1.6^{a}	28.3 ± 1.1^{a}	30.4 ± 1.1^{a}	35.3 ± 0.8^{a}
2	30.2 ± 1.2^{b}	35.5 ± 1.2^{b}	38.7 ± 1.3^{b}	42.8 ± 1.8^{b}
6	40.0 ± 1.2^{c}	42.0 ± 1.5^{c}	40.1 ± 1.9^{b}	40.12 ± 1.2^{b}

*Values with same letters in same columns are not significantly different
(Mean values ± S.E.M.) n=40

metabolism can occur in fish exposed to various stressful conditions. For example, the secretion of catecholamine and adrenocorticoid by fish in stressful conditions has been reported (Smart 1978). This leads to marked changes in carbohydrates reserves which according to Wedemeyer, et al. (1984) cause hyperglycemia. Hypoglycemic conditions in *O. niloticus* exposed to petroleum effluent have been reported by Omorigie et al. (1995). Fishes are noted to increase their metabolic activities and excretion of toxicant,

hence making more energy available for homeostatic maintenance than storage, which could be used for growth. Oladimeji and Ologunmeta (1987) observed a dose-dependent depletion of liver glycogen in *O. niloticus* exposed to Pb and attributed this to the inefficient absorption of soluble glucose from the intestine or breakdown of liver cells which store or synthesize glycogen. Gbem (1998) observed liver and gill damage in *C. gariepinus* exposed to tannery effluent. The changes in the liver cells could as well have led to a similar phenomenon. Gill damage could lead to reduced oxygen uptake leading to reduced oxygen transport by the erythrocytes. Reduced TEC as seen in exposed fish could actually compound this situation. This could lead to inefficient utilization of assimilated food or the inhibition of certain enzymes of the metabolic pathways.

Table 3. Mean corpuscular hemoglobin concentration MCHC (%), mean cell hemoglobin, MCH (μ.μg) and mean cell volume MCV (μ^3) of juvenile *Clarias gariepinus* exposed to tannery effluent.

EFFLUENT CONC.% V/V	DURATION OF EXPOSURE (WKS) 2	4	6	8
		MCHC		
Control (0)	33.4 ± 0.4[a*]	30.4 ± 0.9[a]	33.7 ± 0.5[a]	33.4 ± 0.8[a]
2	33.3 ± 0.2[a]	30.0 ± 0.4[a]	33.2 ± 0.8[a]	33.3 ± 0.7[a]
6	33.3 ± 0.3[a]	33.3 ± 0.3[a]	32.2 ± 0.6[a]	32.2 ± 0.8[a]
		MCH		
Control (0)	0.31 ± 0.1[a]	0.32 ± 0.1[a]	0.35 ± 0.0[a]	0.38 ± 0.0[a]
2	0.37 ± 0.1[a]	0.39 ± 0.1[a]	0.47 ± 0.1[a]	0.53 ± 0.1[a]
6	0.27 ± 0.1[a]	0.41 ± 0.1[a]	0.47 ± 0.1[a]	0.87 ± 0.2[a]
		MCV		
Control (0)	0.92 ± 0.1[a]	1.07 ± 0.2[a]	1.12 ± 0.4[a]	1.14 ± 0.4[a]
2	1.11 ± 0.1[a]	1.31 ± 0.2[a]	1.42 ± 0.3[a]	1.58 ± 0.5[a]
6	0.81 ± 0.2[b]	0.13 ± 0.1[b]	1.14 ± 0.2[b]	0.25 ± 0.0[b]

*Values with same letters in same column are not significantly different
(Mean values + S.E.M.) n=40

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