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Full Length Research Paper

Direct toxic assessment of treated fertilizer effluents to *Oreochromis niloticus*, *Clarias gariepinus* and catfish hybrid (*Heterobranchus bidorsalis* ♂ x *Clarias gariepinus* ♀)

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Acute static bioassay was employed to assess the toxicity of various ranges of effluent from the National Fertilizer Company of Nigeria (NAFCON) plant to three fish species: *Oreochromis niloticus, Clarias gariepinus* and hybrid (*Heterobranchus bidorsalis* ♀ x *C. gariepinus* ♂) from the coastal estuaries of the Niger Delta area, Nigeria. The lethal concentration values at 24, 48 and 72 h were 72.05, 30.81 and 15.26% for *O. niloticus* and 26.18, 10.32 and 19.84% for the hybrid, respectively. No mortality was recorded for *C. gariepinus*. The median lethal time for *O. niloticus* at 70% and hybrid at 50% of the different samples was 18.14 and 6.02hrs, respectively. Ammonia appeared to be the major toxic component. The safe concentrations of the effluents ranged between 1.53% and 77.21% for *O. niloticus*, and 3.15 and 5.50 % for the hybrid. Although the ranges of treated effluents discharged from the plant met set standards and can be classified as non-toxic, yet they caused mortalities to exposed species. This underscores the merit of direct toxicity assessment of effluents over the traditional physicochemical method which does not adequately protect the environment.

Key words: Toxicity assessment, fertilizer effluents, *Oreochromis niloticus, Clarias gariepinus*, catfish hybrid.

INTRODUCTION

Direct toxicity assessment (DTA) has been introduced in the regulation of effluent discharges into surface waters in preference to the traditional physicochemical parameters in determining the quality of receiving waters that do not adequately protect the aquatic environment (USEPA 1993; EA, 1996, 1997). In the traditional method, concentration limits are set for some physicochemical characteristics of the effluents and then attempts are made to extrapolate to real life effects based often on limited or inadequate data (Rowe et al., 1983). Exposure of live organisms to these treated effluents deemed to have satisfied set standards before discharge have produced various types and degrees of physiological changes in fish and death in some cases (Lawani and Alawode, 1987; Kemdirim, 1999; Subhashini and Padmini, 2000). Lawani and Alawode (1987) demonstrated that *Clarias gariepinus* exposed to effluents from paper mill had higher burdens of heavy metals (Hg. Pb, Cd) than the unexposed. Dehydroabietic acid, a major component of wood industry effluents interfered with cellular energetics in rainbow trout hepatocytes (Rissanen et al., 2003). In River Rido, Nigeria, Kemdirim (1999) recorded significant reduction in chlorophyll due to the effect of treated effluents from Kaduna Petroleum Refinery, Nigeria. Davids et al. (2002) also observed significant haematological changes in two tilapine species, *Sarotherodon melanotheron* and *Tilapia guineensis*, from adjourning rivers receiving treated

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effluents from the National Fertilizer Company, NAFCON and a nearby petrochemical company. Spinal curvature as well as delayed and less frequent spawning was recorded in flag fish exposed to refinery effluents (Rowe et al., 1983).

In DTA, live organisms are used to assess the toxicity of discharged aqueous effluents. DTA is usually the test done on effluent before Toxicity Identification Evaluation (TIE), by which the major source of toxicity in the effluent is determined. Use of DTA as a diagnostic tool is less cumbersome, more reliable in protecting the environment and more cost effective depending on the scale, than the traditional method. This method has been used in the management of effluents in the United States (USEPA, 1993), China (Wang, 2001), United Kingdom (EA, 1997). However, there is no report on the use of DTA to investigate the toxicity of effluents from the fertilizer complex, National Fertilizer Company of Nigeria (NAFCON) located at Onne, near Port Harcourt, Nigeria, in the estuaries of the Niger Delta area of Nigeria to fingerlings of three important aquaculture species, *Oreochromis niloticus, Clarias gariepinus* and hybrid catfish (*Heterobranchus bidorsalis* ♂ x *Clarias gariepinus* ♀*)*. This study was carried out to assess the toxicity of the various ranges of effluents from the outfall of the plant on the above mentioned species.

MATERIALS AND METHODS

Fingerlings of *O. niloticus* (mean total length, TL, 3.3 ± 2.9 cm; mean weight, 1.2 ± 0.9 g), *C. gariepinus* (mean TL, 3.1 ± 2.1 cm; mean weight 1.0 ± 0.4 g) and hybrid (mean TL, 4.0 ± 2.2 cm; mean weight, 0.9 ± 0.4 g) were obtained and transported from African Regional Aquaculture Centre, Aluu, Port Harcourt, in oxygenated bags to the Department of Biological Sciences Laboratory, Rivers University of Science and Technology, Port Harcourt. They were acclimated separately in aquaria for two weeks. The fish were fed twice daily at 3% biomass on a 30% crude protein diet. Holding tanks were cleaned daily and water was changed every 24 h. Grab samples of effluents were collected from the outfall of the fertilizer complex on 3 occasions in jerry cans and chemical analysis of the same was done at the Analytical Laboratory, Research and Development, Nigerian National Petroleum Corporation, Port Harcourt, Nigeria, using laboratory methods described by APHA (1985).

Various concentrations of the respective effluents were prepared on a volume to volume (%) basis with dechlorinated tap water (Effiok, 1993). The concentrations for the exposure of the various fish species were: *O. niloticus,* 20, 30, 40 and 50%; *C. gariepinus*, 40, 60, 80 and 100%, and hybrid, 40, 50, 60 and 70%. Each of these had a control (0% effluents). All concentrations and the control had three replicates. The fish were not fed 24 h before and throughout the experimental period. The temperature, pH, ammonia, conductivity, urea, phosphate, dissolved oxygen and un-ionized ammonia of the control and test media were determined twice at 24 and 96 h during the experimental period (APHA, 1985). The fish were exposed to 20l each of the various concentrations of the effluents in 50l rectangular glass aquaria. The test began by random placement of 20 fingerlings of the respective fish species into the aquaria within an hour of the preparation of the test solutions of each effluent sample.

Opercular and tail beat frequencies and mortality were recorded at 6, 24, 48, 72 and 96 h. A fish was considered dead when there was lack of response to gentle prodding with a glass rod. The data obtained were subjected to ANOVA. Differences among means were determined with Duncan multiple range test. The lethal concentrations of the toxicant and lethal time (MLT) were calculated by probit method using SPSS version 10 on a PC. Safe concentrations of the effluents were obtained by multiplying the lethal concentration values by an application factor of 0.1 (EIFAC, 1983).

RESULTS

Effluents from the fertilizer complex had variable characteristics (Table 1). The qualities of the stock solutions imparts to test media some of their physicochemical parameters besides the influence of exposed fish (Table 2). Fish in the control for *O. niloticus*, hybrid and treatments for *O. niloticus* did not exhibit restless behaviors. However, *O. niloticus* and hybrid exposed to effluents concentrations above 30 and 50 ppm, respectively showed certain stressful behaviors like erratic swimming with inconsistent jumping, incessant gulping of air, loss of balance and eventually death. None of these behaviors were observed in *C. gariepinus,* in any of the effluents concentrations to which they were exposed. In *O. niloticus,* ANOVA showed that concentration of effluents and exposure time produced significant changes on the mortality ($p<0.001$), tail beat frequencies (TBF) ($p<0.001$) and opercular beat frequencies (OBF) ($p<0.001$). The interactions between the concentration of the effluents and time had significant effects on TBF ($p<0.001$, Figure 1), OBF ($p<0.001$, Figure 2) and mortality ($p<0.001$, Figure 3). The parameters studied in *C. gariepinus* responded as followed with time: (TBF, $p<0.001$; OBF, $p<0.001$) and concentration of exposure TBF ($p<0.001$) and OBF ($p<0.05$). Interactions between exposure time and concentration had significant effect on the TBF ($p<0.05$, Figure 4) and OBF ($p<0.05$, Figure 5). In the hybrid, the effects of time, concentration and interactions between time were significant at $p<0.001$ (Figures 6, 7 and 8). The responses of TBF, OBF, and the various concentrations and time of exposure are shown in Table 3.

Mortality per time curves for *O. niloticus* and hybrid indicate that mortality increased with time. However, the increment between 6 and 24 h was higher for hybrid than in *O. niloticus*. No mortality was recorded for *C. gariepinus* for almost 2 weeks. The effluents had variable toxicity to the fish which were related to the concentrations of exposure. The MLT (Table 4) and lethal concentration values (Table 5) appeared to have an inverse relationship with concentration and time, respectively.

DISCUSSION

Behavioural responses of fish to most toxicants and differences in reaction times have been observed to be

Table 1. Physicochemical characteristics of effluents from the NAFCON plant used for the bioassay.

Parameter	Fertilizer plant effluents samples			FEPA[#] Limit (FEPA, 1991)
	Sample 1	Sample 2	Sample 3	
	O. niloticus	Hybrid	*C.gariepinus*	
Temp. at collection (ºC)	31	30	30	35
Temp. when analysed (ºC)	27	26	27	-
pH	7.70	8.40	6.50	6-9
Appearance	Slightly turbid	Colloidal	Slightly turbid	Clear
Odour	Free	Mildly pungent	Nil	Free
Oil (ppm)	Nil	Nil	Nil	-
NH_3	5.90	9.12	0.00	0.10
Conductivity (Ohms/cm)	202.80	253.50	74.99	400
BOD at 20ºC (ppm)	1.36	0.25	1.11	50
Dissolved oxygen (ppm)	3.50	3.10	2.00	5
TDS (ppm)	92.18	115.30	340.90	2000 max.
Urea (ppm)	158.00	192.10	137.00	5
Phosphate (ppm)	25.03	351.30	389.70	100
Zinc (ppm)	0.60	0.50	0.90	-
Iron (ppm)	0.40	0.40	0.80	-

[#]Federal Environmental Protection Agency.

Table 2. Physicochemical characteristics of bioassay medium for *O.niloticus*, *C. gariepinus* and hybrid.

Parameter	Concentration (%)				
O. niloticus	0	20	30	40	50
Temperature (°C)	25	26	26	26	26
pH	6.60	7.72	7.92	8.07	8.12
Ammonia (ppm)	0.00	4.52	7.39	8.38	10.71
Conductivity (µS/cm)	30	89	109	125	40.00
Urea	181.7	217.98	136.24	281	95.00
Phosphate (ppm)	3.05	97.55	68.59	116.0	134.00
Dissolved Oxygen (ppm)	1.74	1.96	1.54	19.8	1.68
UIA (%)	0.23	2.94	4.55	6.25	7.14
C. gariepinus	00	40	60	80	100
Temperature (°C)	24	25	25	27	26.00
pH	6.60	7.00	7.05	70.4	7.05
Ammonia (ppm)	0.00	0.00	0.00	0.00	0.00
Conductivity (µS/cm)	28	42	250	231	425.00
Urea	9.42	115.0	113.0	123.0	141.00
Phosphate (ppm)	1.74	91.34	120.0	994.3	215.00
Dissolved Oxygen (ppm)	2.04	1.26	1.46	1.42	1.34
UIA (%)	0	0.60	0.64	0.63	0.64
Hybrid	00	40	50	60	70
Temperature (°C)	25	25	26	25	27
pH	6.63	7.50	7.62	7.83	8.20
Ammonia (ppm)	0.00	2.73	3.48	4.97	11.82
Conductivity (ohms/cm)	30	95.23	108.20	134.23	408.30
Urea	22.7	86.28	199.81	322.0	408.30
Phosphate (ppm)	3.56	42.20	66.30	11.8	150.7
Dissolved Oxygen (ppm)	2.03	1.75	1.97	1.99	1.86
UIA (%)	0.25	1.79	2.33	3.70	8.33

UIA: un-ionized ammonia.

Table 3. Changes in the tail beat frequency (TBF/min), opercular beat frequency (OBF/min.) and cummulative mortality (%) of *O. niloticus*, hybrid and *C. gariepinus* exposed to treated ammoniacal fertilizer effluents

Parameter	Exposure time (h)				
	6	**24**	**48**	**72**	**96**
Oreochromis niloticus					
TBF/min	107.80±19.81[d]	105.13±19.52[d]	101.67±16.98[c]	73.27±7.64[b]	54.44±4.73[a]
OBF/min	76.33±16.64[b]	84.80±48.96[c]	115.73±24.26[e]	93.13±16.31[d]	57.44±5.04[a]
Mortality	10.67±8.08[a]	27.33±10.29[b]	54.00±17.07[c]	66.67±28.87[d]	75.67±22.83[e]
	Concentration (%)				
	0.0	**40.0**	**50.0**	**60.0**	**70.0**
TBF/min	61.00±1.20[a]	74.33±6.62[b]	92.67±14.77[c]	109.75±17.02[d]	130.92±18.75[e]
OBF/min	44.27±1.99[a]	79.00±9.64[a]	91.53±15.62[c]	103.33±15.94[d]	133.75±10.42[e]
Mortality		56.00±22.76[b]	54.00±22.76[a]	55.00±17.92[ab]	67.08±16.31[c]
Hybrid					
	Exposure time (h)				
	6	**24**	**48**	**72**	**96**
TBF/min	69.97±3.46[a]	62.30±3.93[b]	50.33±7.73[c]	45.63±10.54[c]	47.92±13.99[b]
OBF/min	118.02±16.51[b]	130.89±19.72[c]	144.32±22.71[d]	128.23±13.19[c]	91.05±0.99[a]
Mortality	27.33±10.29[a]	51.33±18.34[b]	69.67±21.56[c]	70.83±24.99[c]	50.00±31.62[b]
	Concentration (%)				
	0.0	**20.0**	**30.0**	**40.0**	**50.0**
TBF/min	69.86±2.45[d]	51.50±1.04[b]	55.17±10.10[c]	54.96±8.99[c]	48.00±8.27[a]
OBF/min	89.54±1.87[a]	115.67±10.22[b]	127.25±10.10[c]	143.43±9.98[d]	184.13±9.05[e]
Mortality		66.33±17.54[b]	62.92±15.50[a]	74.17±15.12[c]	81.67±13.15[d]
Clarias gariepinus					
	Exposure time (h)				
	6	**24**	**48**	**72**	**96**
TBF/min	49.53±8.51[a]	50.13±6.20[a]	53.53±7.91[ab]	57.40±6.34[b]	56.20±8.12[b]
OBF/min.	132.0±3.15[a]	129.60±4.03[ab]	134.80b±3.61[c]	137.07±3.12[cd]	140.20±5.30[d]
	Concentration (%)				
	0.0	**40.0**	**60.0**	**80.0**	**100.0**
TBF/min	54.67±3.17[a]	49.27±7.16[b]	37.13±3.03[a]	55.80±4.22[c]	69.93±2.98[d]
OBF/min.	137.60±3.11[b]	136.47±4.64[b]	134.53±3.17[ab]	133.80±5.99[ab]	131.27±3.99[ab]

Means in the same column with same superscript are not significantly different ($p>0.05$).

Table 4. Median Lethal Times, MLT_{5O} (hrs) and associated 95% confidence limits for *O. niloticus, C. gariepinus* and hybrid exposed to treated ammoniacal fertilizer plant effluents.

Fish Species		Concentration (%)			
O. niloticus		**40**	**50**	**60**	**70**
	MLT_{50}	41.88 (37.82-45.98)	43.95 (39.77-48.16)	31.46 (39.77-48.16)	18.14 (11.91-23.24)
	MLT_{90}	74.43 (67.99-83.32)	78.22 (71.59-87.33)	73.44 (65.43-84.93)	61.44 (53.23-74.12)
C. gariepinus	No data				
Hybrid		**20**	**30**	**40**	**50**
	MLT_{50}	18.14 (11.91-23.24)	23.60 (17.16-29.06)	14.02 (8.16-18.65)	6.02 (0.98-9.28)
	MLT_{90}	61.44 (53.23-74.12)	73.09 (64.27-86.09)	50.04 (42.78-61.74)	25.63 (20.88-33.37)

due to the effect of chemicals, their concentrations, species, size and specific environmental conditions (FAO, 1981). The responses recorded for the fishes in this study are similar to those reported by other authors

Table 5. Lethal concentrations, LC_{50} and LC_{90} (%) and associated 95% confidence intervals (C.I.) of the different ranges of treated ammoniacal plant effluents for fingerlings of *O. niloticus*, *C. gariepinus* and hybrid.

Fish Species	Concentration	24 h	48 h	72 h	96 h
O. niloticus					
	LC_{50}	72.05 (63.82-98.59)	30.81 (-48.36-42.86)	15.26 (-62.58-29.54)	*
	Safe concentration	7.21	3.08	1.53	
	LC_{90}	138.40 (106.96-63.21)	114.14 (87.27-14.43)	51.58 (45.12-65.19)	*
	Safe concentration	13.84	11.41	5.16	
C. gariepinus	No data				
Hybrid					
	LC_{50}	26.18 (19.33-30.48)	10.32 (-5.04-6.99)	19.84 (16.85-22.60)	*
	Safe concentration	2.62	1.03	1.98	
	LC_{90}	55.00 (47.38-2.29)	40.82 (35.51-51.45)	31.52 (28.89-36.42)	*
	Safe concentration	5.50	4.08	3.15	

*Percent responding at doses is the same hence the statistics could not be reported.

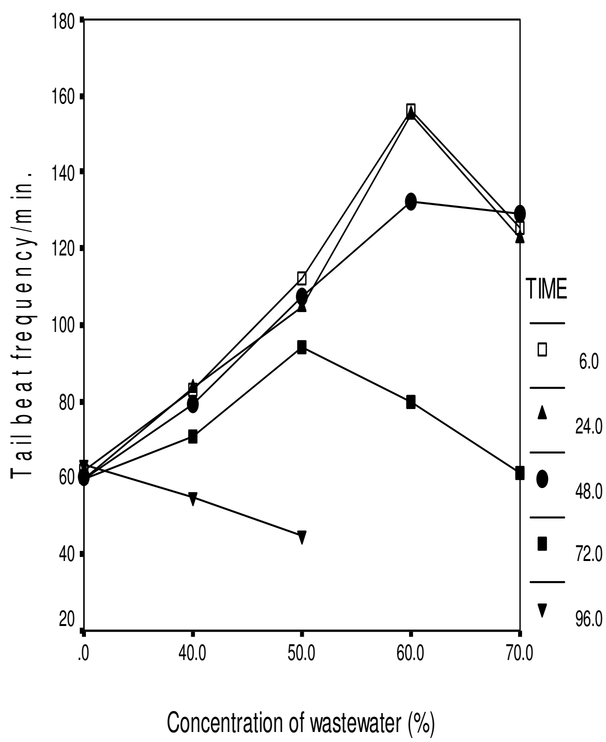

Figure 1. Effect of interactions between concentration of effluent and exposure time (h) on estimated values of tail beat frequency of *Oreochromis niloticus.*

for clariids under various stress conditions (Annune and Ajike, 1999; Onusiriuka and Ufodike, 2000; Nwanna et al*.,* 2000). Besch (1975) identified four main phases in the responses of fish to toxicants. The contact phase (brief period of high excitability), exertion (visible avoidance characterized by fast swimming, leaping and

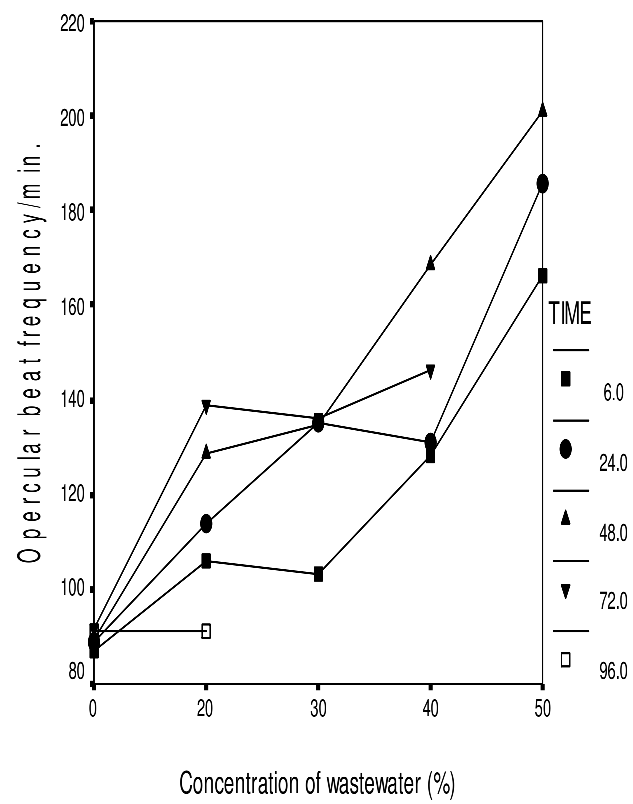

Figure 2. Effect of interactions between concentration of effluent and exposure time (h) on estimated values of opercular beat frequency of *Oreochromis niloticus*.

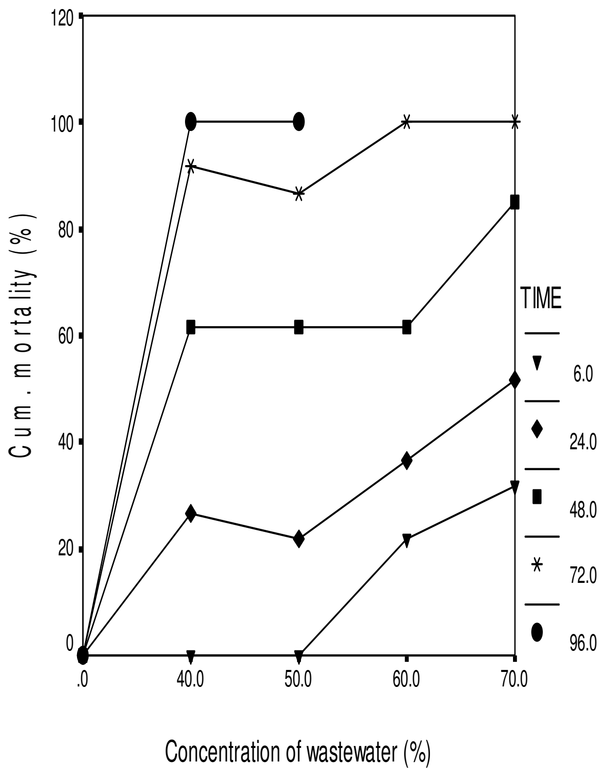

Figure 3. Effect of interactions between concentration of effluent and exposure time (h) on estimated values of cumulative mortality of *Oreochromis niloticus*.

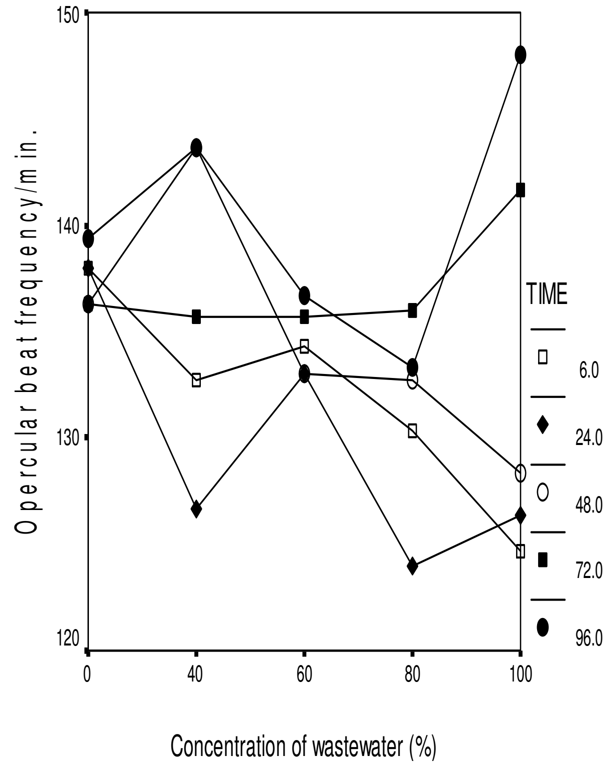

Figure 5. Effect of interactions between concentration of effluent and exposure time (h) on estimated values of opercular beat frequency of *Clarias gariepinus*.

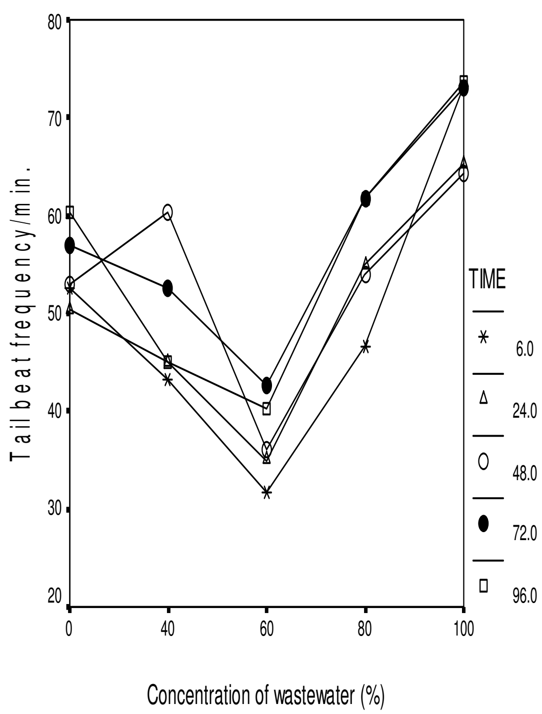

Figure 4. Effect of interactions between concentration of effluent and exposure time (h) on estimated values of tail beat frequency of *Clarias gariepinus*.

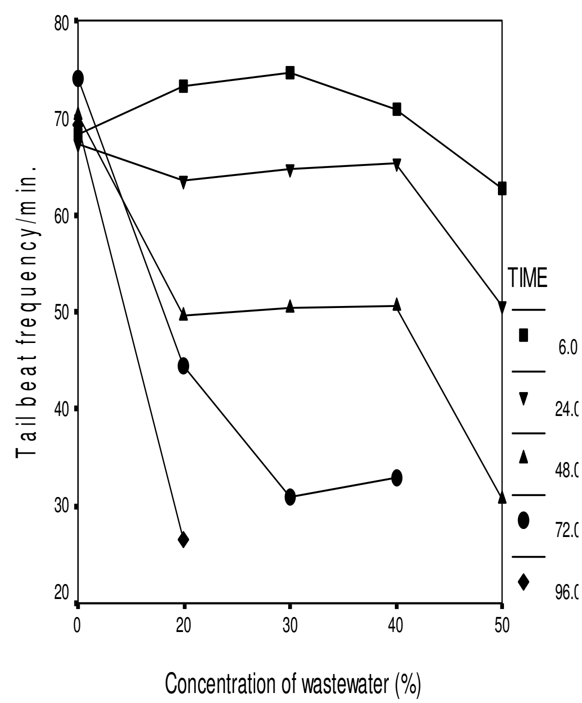

Figure 6. Effect of interactions between concentration of effluent and exposure time (h) on estimated values of tail beat frequency of hybrid.

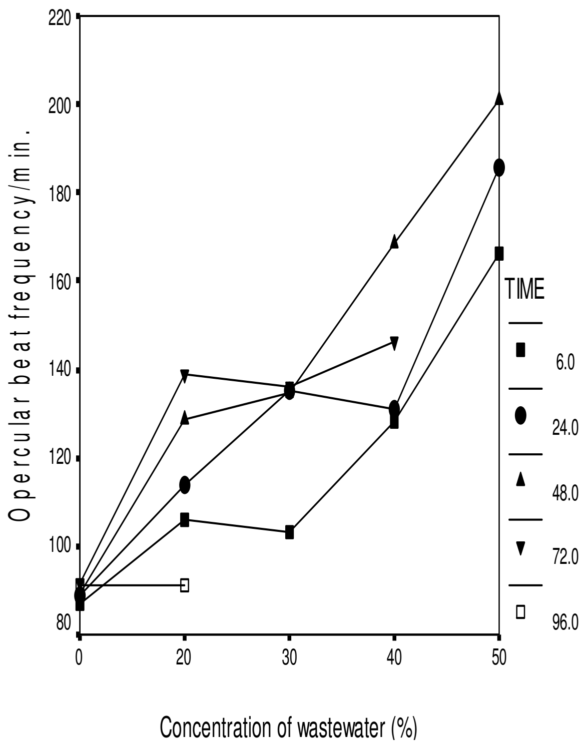

Figure 7. Effect of interaction between concentration of wastewater and exposure time (h) on estimated values of opercular beat frequency of hybrid.

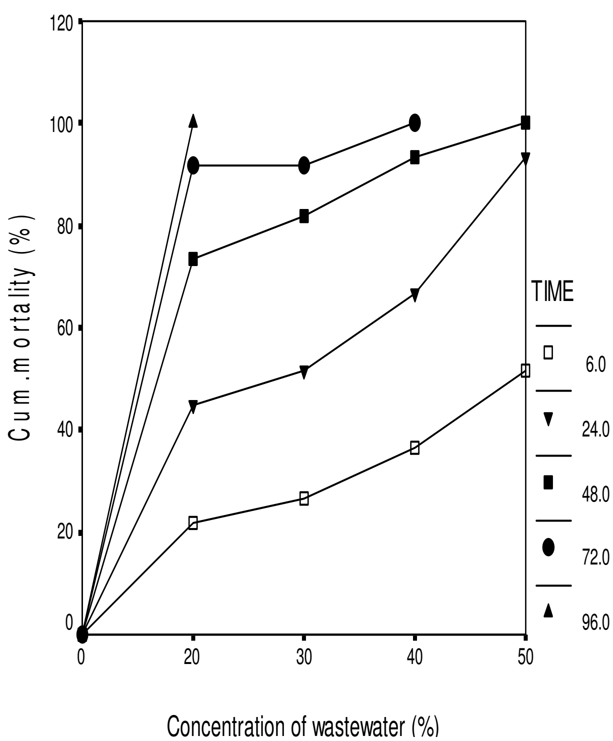

Figure 8. Effect of interaction between concentration of wastewater and exposure time (h) on estimated values of tail beat frequency of hybrid.

attempt to jump out of the toxicant), loss of equilibrium followed by the lethal (death) phase when opercular movement and responses to tactile stimuli cease completely.

Differences in mortality of *O. niloticus, C. gariepinus* and hybrid may be due to the differential toxicity of the stock solutions (Tables 1 and 2). Progressive increase in the free ammonia, un-ionized ammonia, pH, phosphate, urea and conductivity with increase in effluent concentration in the bioassay for *O. niloticus* and hybrid may imply increased toxicity with raised values of the various physicochemical parameters. Although the dissolved oxygen levels in the test solutions were very low, the critical (limiting) and lethal levels of oxygen vary markedly with fish species. Generally, tropical fish species have a high tolerance for low dissolved oxygen levels. Ammonia appeared to be the major source of toxicity in the effluents causing mortality to exposed fish. The stock solution as well as the test solutions to which *C. gariepinus* was exposed had no ammonia when compared with that for *O. niloticus* and hybrid. This agrees with the observations of Kuma and Krishnamoorthi (1983), who implicated ammonia as the main cause of the toxicity of effluents from fertilizer plants. Toxicity of ammonia may be influenced by a number of other environmental factors such as temperature, pH, dissolved carbon dioxide and oxygen and bacterial conversion of ammonia into nitrite (EIFAC, 1973). These factors may in themselves be toxic to fish or act synergistically with ammonia. The un-ionized form of ammonia is more toxic than the free form. It was thought to be lipophilic as it easily diffuses across respiratory membranes (Kormakik and Cameron, 1981), accounting for their toxic effects. Besides, Walker and Schenker (1970) have shown that it produced impairment of cerebral energy in fish. Most of the characteristics associated with ammonia toxicity were observed in the *O. niloticus* and hybrid before death occurred.

The inverse relationship between MLT and lethal concentrations, the effluents concentrations and length of exposure time for *O. niloticus* and hybrid may indicate that the survival time for both species declined with increase in effluents concentration and time of exposure. The relationship between the MLT_{50} and LC_{50} on one hand and effluents concentration on the other hand may best be explained by the degree to which effluents were treated before discharge, its components and the degree of biodegradation that they must have undergone during the experimental period.

There have been reported cases of fish kills following discharges of these effluents into the receiving water body, body; but data from this experiment are insufficient for a conclusive verdict on the role of the effluents in the reported mortalities. Similar fish mortalities have been reported in India following contamination with treated effluents from several industries (Das, 2003). Oladimeji and Onwumere (1987) showed that, treated refinery effluents, certified fit for discharge into the environment by traditional physicochemical method, at sub lethal concentrations produced hemorrhage of the fins, erosion of the caudal fins, reduced growth and gill damage in *O. niloticus*.

The ranges of the discharges from the fertilizer complex include those that may be toxic to the exposed species thus confirming the observation by USEPA (1996) that traditional physicochemical methods cannot protect the environment receiving treated effluents, hence the need to employ direct toxicity assessment to test effluents before discharge into the receiving water body.

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