ACUTE TOXICITY OF A NIGERIAN ARMAMENT FACTORY EFFLUENT ON *OREOCHROMIS NILOTICUS* (LINNAEUS+)

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ABSTRACT

Effluents from a Nigerian armament factory were characterized using standard methods. The acute toxicity of this effluent on Oreochromis niloticus was determined in a 96h static bioassay under laboratory conditions, in 3%, 6%, 9%, 12% and 15% effluent concentrations. The effluent was generally acidic. Fourteen parameters had values above the Federal Environmental Protection Agency (FEPA) limit. The values for ammonia (x=3.0. mg/l), cyanide (x=2.27mg/l), sulphide (x=2.8 mg/l), and total alkalinity (x=403.0 mg/l) were consistently higher than FEPA approved limits in all batches of effluent investigated. Some heavy metals such as Nickel and chromium and also ammonia were consistently high in all the three batches of effluents analysed. The Effluent caused mortality to Oreochromis niloticus. This mortality was dose – dependent and occurred in all effluent concentration used. The 96h LC_{50} *was 4.16% while the lethal time (LT) was 21 hours for the 15% effluent concentration and 84 hours for the 6% effluent concentration. Hyperactivity and disorientation were observed in all fish before death and dead fish was observed to have oedematous gills and copious mucus deposition on the gills and body.*

KEY WORDS: Armament, Effluent, Factory, *Oreochromis niloticus*

INTRODUCTION

The need for self sufficiency in military hard-wares has informed nation, states to establish industries with responsibility to produce armaments. Nigeria has such factory, located in Kaduna. This factory produces assorted military ordinances for the nation. Effluent from this industry is discharged into the environment, where like other industrial effluents world-wide, has the potential to pollute rivers, vegetation and soil and pose great danger to the biotic component in these ecosystems. This capacity to pollute is because pollutants are mobile and easily leached through to underground waters (Ajibola and Uli 2004). Several studies have shown a positive correlation between industrial pollution and the health of aquatic organisms, (Miram and Desta 2002). Chhonkar *et al* (2000) reported that industrial effluents contain large quantities of toxic substances that have a number of adverse effects on the biota. Over 1500 of such substances, most of them toxic have been listed in our fresh water ecosystem (Odiete, 1999). Beetsh, (1982) analysed the pollutant load of Kaduna industrial discharges and reported abnormally high levels of heavy metals, biochemical oxygen demand and phenol in most of them.

Unfortunately, fishes live in close contact with their aquatic environment and are dependent on it. They are therefore affected by changes in it. The effect could be acute or chronic. Oladimeji (1986) has called for increased research on Kaduna industrial effluents. He noted that knowledge of their 96hLC50 is essential in recommending the permissible concentration that will not be toxic to fish. The bioassay technique is a useful tool for this assessment (Sprague, 1972). and the result can be useful in deciding how much treatment is required. It can also be used to test the effectiveness of the treatment method and expose any synergistic and antagonistic interactions among pollutants even when their quantity is low in the effluent.

The objective of this study is therefore to determine the chemical composition of effluent from the Defence Industries Corporation of Nigeria (DICON) and to determine the acute toxicity of different concentration of effluents from the industry on *Oreochromis niloticus*, which is a fresh water fish very common in river Kaduna.

METHODOLOGY

Effluent samples were collected from the effluent retention pond in the premises of DICON Kaduna in 2006. Samples were collected in clean 20L Jerry cans on daily basis and transported to the Laboratory in Nigeria Defence Academy, Kaduna. The contents were tightly capped to avoid the escape of volatile components. The tap water used for this experiment was collected directly from running tap in wide mouthed 300L plastic containers, aerated daily and allowed to stand for over two weeks to allow for dechlorination. The quality of the effluent was determined by chemical analysis. *Oreochromis niloticus* fingerlings, weighing between 8-10gm and length ranging from 6-7cm were collected from catches of local fishermen fishing in the unpolluted part of river Kaduna. They were transported to the laboratory in ice cooled containers. The fish was reared in dechlorinated tap water for 14 days prior to the commencement of the experiment (Sprague, 1972). During this period, the fish were fed to satiation twice daily on formulated food. Rearing water was changed daily while any uneaten food was removed using a scoop net to reduce the level of nitrogen contamination. Dissolved oxygen was measured with a dissolved oxygen meter, (Model Jenway 907) while water hardness was determined by EDTA titration (ASTM, 1980). The heavy metals present in the effluent were determined using the Atomic absorption spectrophotometer, AAS model DR 8890. The experiment was carried out in renewable static bioassay tanks. Feeding was stopped 24 hours to the commencement of the experiment.

ACUTE TOXICITY

The 96 hr static bioassay was conducted in the laboratory (Sprague 1973, APHA 1985). This was carried out in 107 liter glass aquaria with each treatment replicated. The following dilutions 3%, 6%, 9%, 12% and 15% plus a control (blank) were made using well aerated and dechlorinated tap water:. This effluent concentration was chosen after a range finding assay revealed that 2% had very little effect while concentration above15% had drastic effect on the fish. Thirty minutes was allowed for the effluent to be uniformly distributed in the experimental tanks (Gebre-Mariam and Desta 2002). Thereafter ten fingerlings from different holding tanks were scooped out and randomly introduced into the experimental aquaria. This procedure eliminated bias and allowed for the synchronization of time. The experiment lasted for 96 hrs and observations were made at 15, 30, 60 minutes 2, 4, 8, 24, 14, 24, 30 hours, 2, 3, 4 days (Sprague, 1972). Effluents were renewed on daily basis by gently transferring the fingerlings from one aquaria into the other containing fresh effluent of the same concentration. Fish were fed to satiation twice daily, the experiment was conducted under ambient light and temperature regime. (Gebre-Mariam, 1994). A fish was considered dead when it did not respond to gentle prodding.

DATA ANALYSIS

The percentage mortality, concentration for 50% mortality and probit kill were determined. The median lethal concentration and the median lethal time were also calculated using the log-probit method by graphical interpolation (APHAA W.W.A & APCF, 1992). The 95% confidence limits and slope of the probit line(s) were obtained from the probit analysis (Olomukoro and Igbinosum, 2004).

RESULT

The result of the analysis of variance indicated that there was no significant difference between the means of the weight and the means of the length of fish used for the experiment at 95% confidence limit. The result of the physiochemical analysis of the three batches of effluent used for this work is presented in Table I. Concentrations of 45% of the measured parameters deviated from the Federal Environmental Protection Agency, (FEPA) approved limits for industrial waste water. Generally the effluent was acidic (P^H 5.9) with a mean temperature value of 28.3 ^{o}c, which is below the FEPA set limits. Although total solid (x=813.3mg/l) and oil and grease (0.02mg/l) were within FEPA acceptable limits, the values for alkalinity in all batches of effluent used were higher than FEPA set limit by two folds. Similarly, the mean values for ammonia (x = 3.03mg/l) and sulphide (x = 2.8mg/l) were higher than FEPA approved limits in all the batches analyzed. Two major toxicants in most industrial effluents, phenol and cyanides had mean values of 2.69mg/l and 2.27mg/l respectively and this could be considered abnormally high. Other parameters with elevated values include total hardness (x=548.67mg/l) magnesium (x10.2mg/l) and iron (x=28.3mg/l). The values for heavy metals like lead (x=0.43mg/l) and cadmium (x=0.03mg/l) were below FEPA limit, while others like chromium (x=1.30mg/l) and nickel (x=1.30mg/l) did not meet FEPA standard.

Fish behaviours changed immediately the fish were introduced into the test concentrations, when compared with the control. These included agitated swimming and breaking the surface of the water to gulp air at frequent intervals. The fish turned on flanks and swarm in circular motions. All these were later followed by sluggish movement, period of quiescence and death. The intensity of these reactions were dose- dependent, and was noted to be more intense in the higher concentrations of 6%, 9%, 12% and 15%.

The effluent caused mortality to the test organism in all effluent concentrations used except in the control (Table 2). After 72 hours of exposure all effluent concentrations recorded mortality to the test organism. At the end of the 96 hours exposure period, 100%, 90%, 65%, 55% and 25% mortalities were recorded in 15, 12, 9, 6 and 3% effluent concentration (Table 2). This result showed that the rate of mortality was also dose-dependent. The lethal time (LT) which was time for 50% of the test organism to die revealed that mortality time was lower for higher effluent concentrations. This was 21hours for 15% effluent concentration and 84 hours for 6%. The LC_{50} which is the concentration that theoretically will kill 50% of the test organism was 4.16% (Fig. 1) The Table of the threshold values is presented in Table 3. The graph of threshold value determination (Fig.2) showed that a dilution of 3.71% was theoretically safe for fish to survive. Examination of dead fish revealed copious accumulation of mucus on the body and gill surfaces of the dead fish. In addition, there was conspicuous reddening of the tail fin of dead fish in the higher concentrations of 9%, 12% and 15%.

TABLE 1: Physico- chemical Analysis of DICON effluent

	Batches of effluent collected				
Parameter	1st	2nd	3rd	Mean & Std. Dev.	Fepa Limit
Appearance	Greenish/ Yellow	Greenish/ Yellow	Greenish/ Yellow	-	-
Odour					
Temperature °C	25.500	30.700	28.700	28.3 ± 2.62	<40°C
P^H	6.500	5.300	5.800	5.86 ± 0.60	6-9*
Conductivity (mhos)	1130.000	1140.000	989.000	1086.33 ±84.44	NI
Total Alkalinity mg/l	420.000	390.000	399.000	403.00 ±15.39	200*
Total Solid mg/l	790.000	840.000	810.000	813.33 ±25.16	2030
Total Hardness mg/l	558.000	537.000	551.000	548.66 ±10.69	200*
Suspended Solid mg/l	280.000	24.100	34.100	112.73 ±144.94	30*
Total Dissolved solid	4500.000	3900.000	3441.000	3947.00 ±531.06	2000*
Nitrate mg/l	0.900	1.700	1.130	1.24 ±0.411	20
Chloride mg/l	18.000	14.500	16.800	16.43 ±1.77	60
Cadmium mg/l	0.030	0.020	0.040	0.03 ±0.01	1
Lead mg/l	0.44	0.72	0.21	0.43 ±0.05	1
Chromium mg/l	1.2	1.4	1.3	1.30 ±0.01	0.5
Nickel mg/l	1.4	1.25	1.35	1.30 ±0.15	<1*
Vanadium mg/l	0.010	0.010	0.010	0.01 ±0.00	<1*
Copper mg/l	3.410	7.100	4.9	5.13 ±2.1	<1*
Manganese mg/l	0.060	0.040	0.038	0.04 ±0.01	5
Magnesium mg/l	11.240	9.100	10.310	10.2 ±0.1069	5*
Ammonia NH_3-H/mg	2.900	3.100	3.100	3.03 ±0.11	0.20*
Sulphide mg/l	3.100	2.400	2.900	2.80 ±0.36	0.20*
Phosphorous (PO_4) mg/l	4.90	4.10	5.20	4.73 ±5.68	5
BOD at 20° C mg/l	10.000	0.010	2.010	0.01 ±0.00	10
Phenol mg/l	2.24	3.18	1.41	2.27 ±0.05	0.05*
Cyanide mg/l	2.410	2.750	2.900	2.6867 ±0.25	0.1*
Dissolved Oxygen mg/l	5.000	4.600	3.900	4.50 ±0.55	20
Oil and Grease mg/l	0.100	0.300	0.100	0.02 ±0.1	10
Zinc	6.08	8.49	6.91	7.16 ±12.24	1.0*
COD mg/l	22.100	4.1	23.100	29.67 ±4.05	40
Iron	25.500	30.700	28.700	28.30 ±2.62	20*

Note: NI – Not indicated
*** -** indicate chemical parameters with value higher than FEPA limit
+ Source: FEPA (1991)

TABLE 2: Cumulative Mortality of *Tilapia Noliticus* Exposed to Dicon Effluent during 96hrs Acute Toxicity Bioassay

Effluent/ Concentration Time	0% A	0% B	3% A	3% B	6% A	6% B	9% A	9% B	12% A	12% B	15% A	15% B
4hrs	0	0	0	0	0	0	0	0	0	0	1	0
12hrs	0	0	0	0	0	0	0	0	0	0	2	2
14hrs	0	0	0	0	0	0	0	0	1	1	3	3
18hrs	0	0	0	0	0	0	1	1	3	2	5	4
24hrs	0	0	0	0	1	1	3	2	4	3	7	5
48hrs	0	0	0	0	2	2	5	3	6	5	8	7
72hrs	0	0	1	2	4	3	6	5	7	7	9	8
96hrs	0	0	2	3	6	5	7	6	9	9	10	10
Total	0		5		11		13		18		20	
Mean mortality	0/10		2.5/10		5.5/10		6.5/10		9/10		10/10	
% mortality	0		25		55		65		90		100	

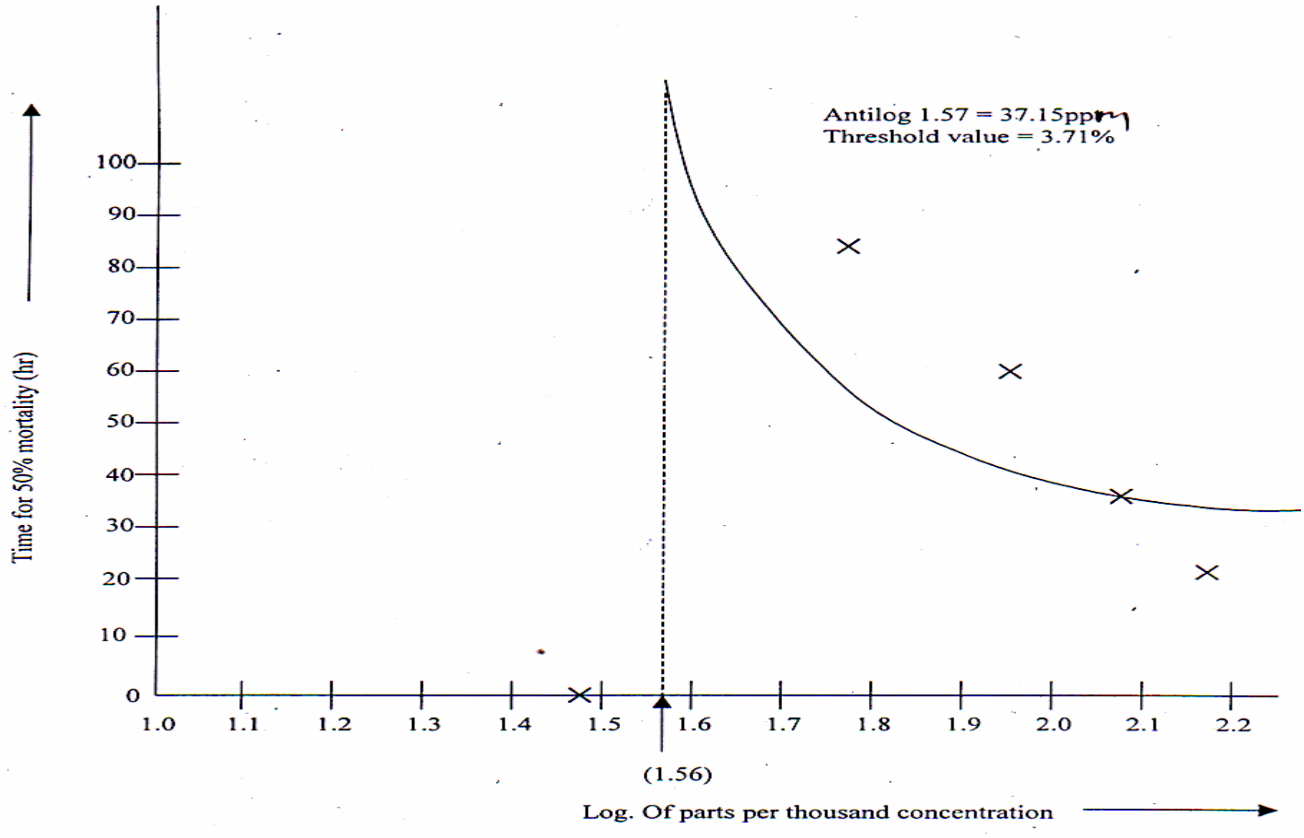

Fig 1. Graph of Threshold value Determination of DICON Effluent for *Tilapia niloticus*

Effluent Conc.	Antilog % Concentration	Mean mortality	Time for 50% mortality (hr)	% Mortality	Probit Kill
3% (30/1000)	1.4771	2.5/10	0	25	4.32
6% (60/1000)	1.7781	5.5/10	84	55	5.12
9% (90/1000)	1.9542	6.5/10	60	65	5.38
12% (120/1000)7	2.0791	9/10	36	90	6.28
15% (150/1000)	2.1760	10/10	21	10	0
0% Control	0	0/10	0	0	0

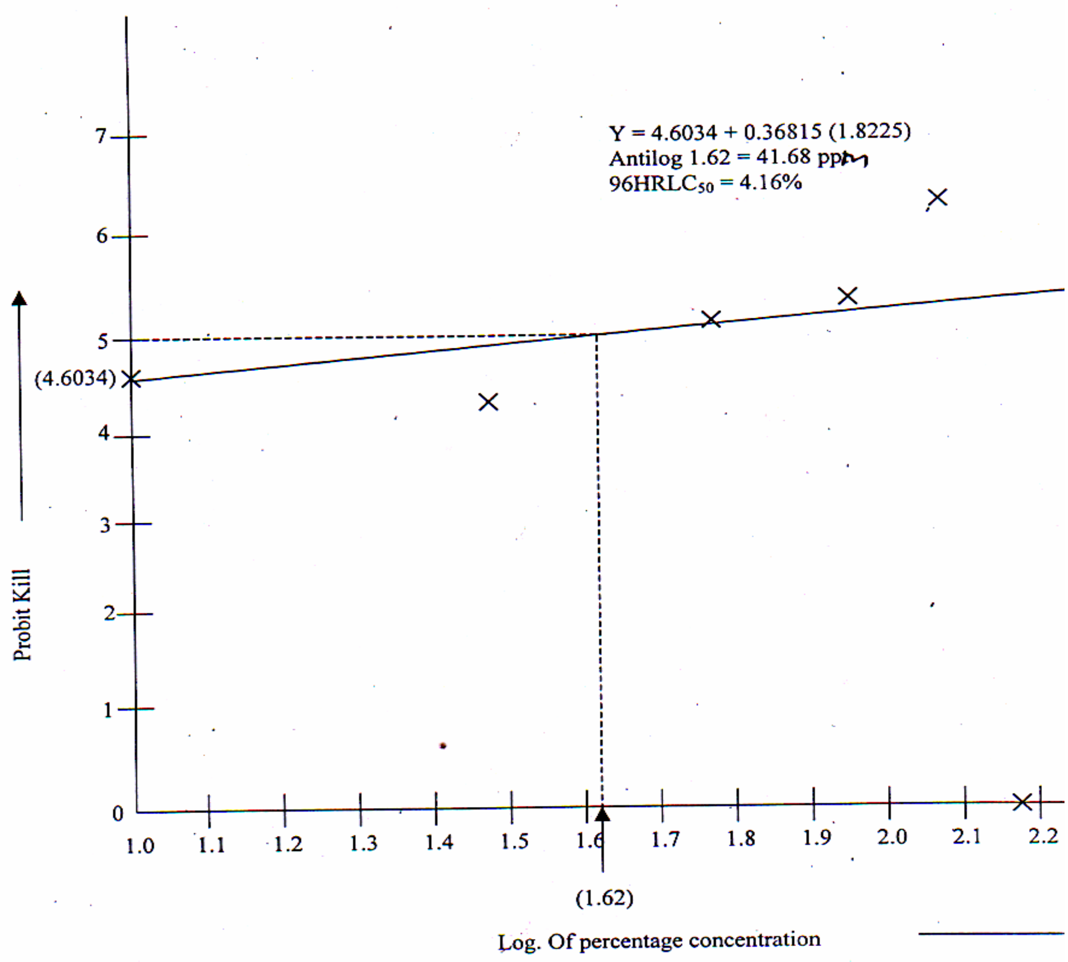

Fig 2: Graph of 96HRLC$_{50}$ of *Tilapia niloticus* exposed DICON effluent

DISCUSSION

The values obtained from the analysis of the tap water used for effluent dilution were within specifications for portable water, (FEPA, 1991). Therefore, the diluent water used could not have affected the result significantly. Also fish mortality was less than 5% during acclimation no fish mortality was observed after the 4th day of acclimatization. The impact of the effluent could not also have been due to dissolved oxygen tension because *Tilapia* is known to be tolerant to low oxygen levels. Ojuola and Onuoha (1987), and also Kutty and Mohammed (1992) have reported that Tilapia can survive in oxygen levels lower than 1ppm. The high value for total hardness (548.7mg/l) obtained can be attributed to the equally high values for alkalinity (403mg/l) and magnesium (10.2mg/l) in the effluent, both of which are known to contribute substantially to total hardness.

The result of the bioassay text indicated that the fish was seriously perturbed by DICON effluent. The first target organ appeared to be the nervous system as was shown by the fish aberrant behaviour. Pal and Konar, (1987) found that the disruption of the functioning of the nervous systems of the fish by toxicants might be the cause of slow and lethargic swimming, erratic movements and equilibrium loss. Similar toxicant induced behavioural changes in fish has also been reported,(Omoregie and Ufodike, 1990). Svobodal, *et al* (2001), reported strong restlessness and loss of coordinated movement in fish exposed to a toxicant, diazone. The high level of ammonia (3.03mg/l) in the waste water may also have contributed to the slow movement of the fish because ammonia as low as 0.08mg/l have been reported to reduce swimming performance of coho salmon, (Wicks *et al* 2002).

The effluent inflicted high mortality to the test organisms. Improperly treated industrial liquid effluent are known to be harmful to the biota. Olomukoro and Igbinosun, (2002) reported that effluents from Nigerian breweries, Benin City was lethal to *Chironomus travalensis* at 30% concentration. Also 64% of fish fry die when exposed for 48 hour to tap water containing 20% effluent from Awassa textile factory, (Gebren- Mariam and Desta 2002). The mortality observed in this study could be generally attributed to those pollutants in the effluent with very high values. Concentrations of 45% of the measured physico – chemical parameters were above allowable limit for industrial waste water, (FEPA 1991). For instance, the mean value for phenol in this study was 2.27mg/l whereas phenol levels of 0.1 to 1.0mg/l is known to be toxic to fish depending on the chemistry of the phenol, (Otokunefor and Obiukwu 2005).

Other pollutants such as heavy metals are known to be acutely toxic to fish even in very small concentrations because they have capacity to lower the haemoglobin and haematocrit count in the exposed organisms, (Omoregie *et al* 1991). The implication is that the oxyphoretic capacity of the blood haematological variables will be compromised (Omoregire *et al* 1991). These observations have been collaborated by other authors, (Sikoki and Enajekop, 1991; Chinda *et al* 2005). Death could also be due to damage to liver cells of fish, because metals can be taken up by fish, (Onwumere and Oladimeji, 1991) and concentrated in the liver (Biney and Beeko 1991). Mortality results when the liver of these fish is damaged. Omoregie and Ufodike (1991) attributed death of *Oreochromis niloticus* to reduced liver cell vacuolation. Fish mortality increased with increase in effluent concentration, suggesting that the action of the effluent is dose dependent. Morphological examination of such dead fish revealed oedamatous gill filaments indicating that death was caused by damage to the respiratory system. The swollen gills also suffered histopathological damage and this has been reported for *Oreochromis niloticus*, (Omoregie and Ufodike 1991, Onwumere and Oladimeji 1991) and for *Clarias gariepinus* (Auta 2001, Okeke 2004). The effluent may habour a lot of bacteria which could cause the gills to swell after death. This is consistent with the report of Sniesko, (1970) who reported that fish killed by bacteria disease exhibited oedatomous gills.

The 96h LC_{50} for DICON effluent at the highest concentration used (15%) was very low. The implication of this is that DICON effluent is very toxic. The smaller the 96h LC_{50}, the more toxic is the effluent and any effluent with 96h LC_{50} less than 30% is considered very toxic (ASTM, 1980). The lethal time was equally shorter at higher effluent concentration. This suggests that the higher concentration penetrated the test organism faster to cause death, (Lloyd 1992).

Acute toxicity studies using whole effluents such as this is preferred because components of industrial effluents which have been judged “safe” because they were present in very low quantities may act synergistically with each other and become transformed into potentially toxic substances, (Olomukoro and Igbinosun 2004), and impose higher toxicity burden on the ecosystem than can be predicted from chemical analysis of the effluent, (ASTM, 1980). Also variations in the biotic factors in the environment may cause metals to undergo physical, chemical and biological transformations which can convert non-toxic compounds into toxic ones (Ajibola and Uli, 2000).

CONCLUSION

This study has reveled that treated effluents from DICON contain high concentrations of toxic pollutants. The research has equally shown that effluent concentration as low as 3% was acutely toxic to fish. There is therefore the need for a major improvement in the treatment of this effluent if the biota and the adjoining ecosystem which serve economic functions can be saved.

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