Braz. J. Aquat. Sci. Technol., 2011, 15(2): 63-67

TOXICOLOGICAL ASSESSMENT USING *CLARIAS GARIEPINUS* AND CHARACTERIZATION OF AN EDIBLE OIL MILL WASTEWATER

ADAKOLE, J. A.

Department of Biological Sciences; Ahmadu Bello University, Zaria - Nigeria
drjaadakole@yahoo.com

ABSTRACT

Adakole, J. A. 2011. Toxicological assessment using *Clarias gariepinus* and characterization of an edible oil mill wastewater. Braz. J. Aquat. Sci. Technol. 15(2): 63-67. eISSN 1983-9057. Among the environmental problems in Nigeria is the lack of proper management of industrial waste-waters, which is capable of causing toxicity to aquatic organisms via leachate or direct discharge to the surrounding aquatic environment. In this study, characterization of an oil mill company wastewater revealed that concentration levels for some of the parameters (Total hardness -17726.15 ± 18.14 mg/l.$CaCO_3$, Electrical conductivity - 756.17 ± 21.43µS/cm, BOD - 87.39 ± 0.47mg/l, PO_4-P -2535.87 ± 30.10 mg/l, NO_3-N -102.67 ± 14.22 mg/l, and Sulphate - 16500.21 ± 65.47 mg/l) have potential toxic effects. The acute toxicity of the wastewater to fingerlings of *Clarias gariepinus* was determined in 96-hour static renewal bioassays. Symptoms of toxicity observed in the fish include various behavioral responses like rapid and erratic swimming, uncoordinated movement, vertical darting, with occasional agitated movement, swimming alternately on the lateral and ventral sides and rapid opercula movement. Prior to mortality recorded at some concentrations, there was negative thigmotropism with prolonged gaping of jaws. These observations as well as mortality records were dose – dependent. The toxic action of the oil mill company wastewater appeared to be a joint consequence of precipitation of mucus on the gills and stress. These changes may have lead to an overall reduction in the efficiency of the gill filament to aid diffusion of oxygen across the gill filament, resulting in death. The traces of blood observed around the gill covering, tips and base of tail fins and barbels on dead fishes and the reduced PVC in some concentration tanks suggest that the fish might have suffered from gill haemorrage. In the LC50 study, no death was recorded in the control. The mortality in the various test concentrations tanks varied between10% to 80%. The 96 hr LC50 obtained using the probit method was 55.3623ml/ with 95% confidence lower and upper limits of 44.9268ml/l and 67.3248ml/l respectively. The threshold for the oil mill wastewater is 98.4130ml/l.

Keywords: Edible oil company wastewater, Characterization, Clarias gariepinus, heamatological parameters, acute toxicity, LC50

INTRODUCTION

Wastewater discharged from edible oil industry contains a very concentrated amalgamation of organic and inorganic materials (Reddy et al., 2003). Soya bean oil for instance contains a significant quantity of organic phosphate in the form of phosphatides (Mhize et al,. 2000). For olive oil wastewater, the organic fraction contains a complex group of phenolic compounds which are responsible for several biological effects, including antibiosis (Rodriguez et al., 1998; Gonzalez et al., 1990), ovipositional deterrence (Girolami et al., 1996) and phytotoxicity (Capasso et al., 1992). Some nitrogenous compounds(especially amino acids), organic acids, sugars, tannins, pectins, carotenoids, oil residues and other substances(mainly sugars and polyalchohol) can serve as carbon and energy sources for the growth of various suitable organisms (Anas et al., 2000). The inorganic fraction of olive oil effluent contains chloride, sulfates and phosphoric salts among others (Piperidou et al., 2000).

A typical edible oil-processing plant could discharge large volumes of wastewater with high chemical oxygen demand, biological oxygen demand, electrical conductivity, TDS, TSS, grease, fats and oil (Mhize et al., 2000; Roux-van der merwe et al., 2005). Fish are intimately associated with the aqueous environment; physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish (Jawad et al., 2004; Adakole, 2005). The use of haematological techniques in fish studies is growing in importance for toxicological research, environmental monitoring and fish health conditions (Adakole & Balogun, 2005). Several investigators have reported possibilities that studies on fish blood frequently reveal conditions within the body of the fish long before there is any outward manifestation of disease (Van Vuren, 1986; Ladu & Ross, 1997; Oshode et al, 2008).

There are several major environmental issues in Nigeria; among which is lack of proper management of industrial wastewaters. Wastes and wastewaters are commonly disposed of in uncontrolled and unlined landfills or indiscriminately channeled into nearby water bodies irrespective of their quality; posing serious risks to ecosystems and human health. In Nigeria, fish is regarded as one of the commonest source of proteins, thus contamination of water bodies where fish resides deserves a great attention. In this study, *Clarias gariepinus* was exposed to raw edible oil wastewater obtained from an edible oil company in Zaria – Nigeria; and the LC_{50}, effects on blood parameters and behavioral response were investigated. We also examined and characterized some physiochemical parameters of the effluent.

MATERIALS AND METHODS

The physical and chemical properties of the dilution and waste waters were determined in accordance with standard methods (APHA, 1998). Standard physical and chemical parameters, including; Temperature, TS, TDS, TSS, free CO_2, dissolved oxygen, pH, total hardness, total alkalinity, electrical conductivity, BOD, PO_4-P, NO_3-N, sulphate and fats & oil were determined.

Fingerlings of *Clarias gariepinus* obtained commercially (from Kaduna state fisheries, Kaduna, Nigeria) were utilized for this study. The fish is available and important in aquaculture in Nigeria. The care and use of the fishes were in accordance with international guidelines on the use of fishes for research (American Fisheries Society, 2004). They were acclimatized and maintained in plastic tank (500 L capacity) containing de-chlorinated tap water at 25 ± 2 °C for 14 days, during which they were fed with commercial fish pellet until average weight of 4.05 ± 0.58 g was reached. A photoperiod of 14-hour light and 10-hour darkness was maintained using fluorescence tube lamps during the acclimation and experimental periods. The water was kept oxygen saturated with aeration during acclimatization. Mortality during the period of acclimatization was less than 1%.

The bioassay test were carried out in 11 glass tanks, each of size 30.5 x 30.5 by 92.5cm, into which approximate volumes of oil company wastewater and dilution water were taken and mixed, to give a final volume of 25.00L. The control contains no toxicant. After a pilot study, the nominal concentrations of effluent/ wastewater used were: 0.0ml/L (0.0L wastewater and 25.0L dilution water), 20.00ml/L, 40.00ml/L, 60.00ml/L, 80.00ml/L and 100.00ml/L. The mixture of toxicant and dilution water (dechlorinated Ahmadu Bello University municipal water) was allowed to stand for 30 minutes before the introduction of the test fishes. Each tank contained 10 fish. Each assay was replicated simultaneously and repeated once to determine reproducibility. The test solution (mixture of effluent and dilution water) was renewed after each 48hours during each series of test.

The behavior and general conditions of the fish were observed before, during and after each bioassay. Mortality was recorded 15, 30 minutes; 1, 2, 4, 8, 24, 36, 48, 72 and 96 hours during exposure. A fish was considered dead when there was lack of opercular movement when prodded with a glass probe. The haematological examination was conducted on the fish that survived the 96 hour acute exposure in the various test tanks and control tank.

Two fish from each tank containing surviving fish were sacrificed immediately after the 96 hour exposure. Blood samples were collected at termination of acute exposure and at the start of experiments from the caudal vein of fish with 1 mL sterile syringe and needle and mixed in a 5 mL heparinized disposable bottles. Total erythrocyte and leukocyte count, packed cell volume and hemoglobin estimation were carried out in accordance to Blaxhall & Daisley (1973) while derived hematological values (MCV, MCH and MCHC) were calculated according to Jain (1986).

The SPSS 13.0 ® was used for the probit and other statistical analysis. Results are expressed as mean ± SE.

RESULTS

The physicochemical parameters of the dilution water and edible oil wastewater are presented on Table 1. The oil company wastewater samples had foul smell and the concentrations of BOD - 87.39 ± 0.47mg/l, PO_4-P -2535.87 ± 30.10 mg/l, NO_3-N -102.67 ± 14.22 mg/l, and Sulphate - 16500.21 ± 65.47 mg/l were higher than the FEPA limits. Values of hardness - 17726.15 ± 18.14 mg/l.$CaCO_3$, alkalinity - 1262.42 ± 9.25 mg/l.$CaCO_3$ and electrical conductivity - 2756.17 ± 21.43μS/cm were high even though their limits are not stated by FEPA.

Behavioral response of *C. gariepinus* to the oil company wastewater include rapid and erratic swimming, uncoordinated movement, darting up and down with occasional jumpy movement, swimming alternately on the lateral and ventral sides and rapid opercula movement. Prior to mortality recorded at some concentrations, there was negative thigmotropism with prolonged gaping of jaws. These observations were dose-dependent. The skin of exposed fishes became progressively sloughed off with increasing concentration and mortality was directly proportional to the concentration of the wastewater. Fish mortality occurred in all aquaria except within the control (Table 2). The 96-hr LC_{50} obtained using the probit method was 55.3623 ml/l with 95% lower and higher confidence limits of 44.9268 ml/l and 67.3248 ml/l respectively. The threshold for the oil mill wastewater is 98.4130 ml/l for *C. gariepinus*.

The hematological changes observed are presented in Table 3. Erythrocytes, leukocytes and heamoglobin concentration increased initially and then decreased with increased concentration of the wastewater. There was no discernable trend in the concentrations of heamatocrit, MCV, MCH and MCHC

Table 1 - Physico-chemical characteristics of dilution water and edible oil company wastewater.

Parameters	Dilution water Mean ± SD	Wastewater Mean ± SD	FEPA (1991)
Temperature (°C)	24.75 ± 1.11	35.20 ± 3.44	< 40
Total solids (mg/l)	3.88 ± 0.36	1296.67 ± 8.18	
NS Total dissolved solids (mg/l)	3.67 ± 1.98	1296.67 ± 19.57	2000.00
Total suspended solids (mg/l)	0.21 ± 0.02	2.14 ± 0.32	15.00
Dissolved oxygen (mg/l)	6.76 ± 0.26	8.84 ± 1.32	NS
Free carbon dioxide (mg/l)	0.98 ± 0.76	-	NS
pH	7.14 ± 0.82	9.15 ± 0.55	5.5 – 9.5
Total hardness (mg/l.$CaCO_3$)	42.51 ± 1.90	17726.15 ± 18.14	NS
Total alkalinity (mg/l.$CaCO_3$)	5.18 ± 0.05	1262.42 ± 9.25	NS
Electrical conductivity(±S/cm)	92.10 ± 3.72	2756.17 ± 21.43	NS
Biological oxygen demand (mg/l)		87.39 ± 0.47	50.00
Phosphate-phosphorus (mg/l)		2535.87 ± 30.10	5.00
Nitrate-nitrogen (mg/l)		102.67 ± 14.22	20.00
Sulphate(mg/l)		16500.21 ± 65.47	500.00
Fats and oil		4.00 ± 0.12	NS

when compared with the control values. The heamatocrit decreased from 31.0% in the control to 28.0% in the highest concentration. The highest erythrocyte concentration (182 x 103 cells/mm3) was recorded in the 60.00ml/l concentration while the least concentration (116 x 103 cells/mm3) was observed in the 100ml/l concentration tank. All the test concentration tanks had higher values of leukocytes than in the control tank. The heamoglobin value varied between 9.10g/100ml and 9.80g/100ml. The mean MCV, MCH and MCHC values in the test fishes are 180.76 ± 21.14μm3, 58.085 ±16.00Pg/cell and 32.21 ± 0.78g/100ml respectively.

DISCUSSION

The dilution water quality varied but were assumed not have had any influence on fish mortality. The oil company wastewater samples had foul smell and the concentrations of BOD, PO4-P, NO3-N and sulphate were higher than the FEPA (1991) limits. Values of hardness, alkalinity and electrical conductivity were high even though their limits are not stated by FEPA.

The results indicate that the edible oil wastewater is toxic; producing dose-responsive increases in mortality and deviation from the normal heamatological parameters of *C. gariepinus*. The data also shows that several parameters of the oil mill wastewater were above FEPA-limit set for discharge of wastewater into aquatic environment.

The behavioral response exhibited by *C. gariepinus* on acute exposure to edible oil company wastewater could be due to respiratory impairment and nervous system failure caused by the toxicant. Similar observations was made by Adakole (2005) who reported that hyperactivity of fish due to introduction to an unfavorable environment as the primary and principal sign of nervous system failure due to chemical poisoning which affect physiological and biochemical activities. Pal and Konar (1981) observed that disruption of the functioning of the nervous system of fish might be the cause of slow and lethargic swimming, erratic movement and loss of equilibrium. Similarly, Oshode et al, (2008) observed that exposure of *C. gariepinus* to acute concentrations of leachate form municipal solid waste landfill causes behavioural abnormalities,

Table 2 - Mortality record of *Clarias gariepinus* exposed to various concentrations of an edible oil mill wastewater for 96 hours. “Rep 1”: Replicate 1; “Rep 2”: Replicate 2; “Mean *”: Mean of replicates 1 and 2.

Tank	Conc.(ml/l)	Log conc.	Mortality at 96 hours			% mortality	Probit mortality
			Rep1	Rep.2	Mean*		
Control	0	0	-	-	-	-	-
1	20	1.3010	2	0	1	10.00	3.7184
2	40	16020	3	4	3.5	35.00	4.6147
3	60	1.7781	5	6	5.5	55.00	5.1257
4	80	1.9030	7	7	7	70.00	5.5244
5	100	2.0000	7	9	8	80.00	5.8416

Table 3 - Acute effects of an edible oil company wastewater on heamatological parameters of *Clarias gariepinus*.

	control	20.00ml/l	40.00ml/l	60.00ml/l	80.00ml/l	100.00ml/l
Heamatocrit (%)	31	30	31	29	28	28
Heamoglobin (g/100ml)	9.5	9.3	9.8	9.6	9.2	9.1
Erythrocytes (cells/mm^3)	140 x 10^3	142 x 10^3	161 x 10^3	182 x 10^3	169 x 10^3	1160x 10^3
Leukocytes (cells/mm^3)	13.5 x 10^3	19.8 x 10^3	20.1 x 10^3	18.5 x 10^3	16.1 x 10^3	15.0 x 10^3
MCV (μm^3)	221.42	211.27	192.54	159.34	165.68	175.00
MCH (Pg/cell)	67.85	65.54	60.86	52.74	54.43	56.87
MCHC (g/100ml)	30.64	31.00	31.61	33.10	32.85	32.0

which include; agitated swimming, loss of equilibrium, air gulping and death.

The onset of mortality and final mortality rates were dose-dependent. The oil mill wastewater's LC_{50} (55.3623ml/l) and threshold (98.4130ml/l) obtained for *C.gariepinus* is lower than the LC_{50} (70.7945ml/l) and threshold (128.00ml/l) reported by Adakole (2005) for *C. gariepinus* due to its exposure to metal-finishing company wastewater. Thus suggesting that edible oil mill wastewater is more toxic than metal-finishing wastewater. These results indicate that the fingerlings of *C. gariepinus* were susceptible to the edible oil company wastewater.

The use of haematology as a diagnostic technique in this study is based on the premise that the parameters measured were not substantially influenced by the sampling procedure. Thus blood parameters measured in this study give insight into the functional and heamoestatic involvement of each parameter in the fish under various oil mill wastewater concentrations. Studies have shown that when the water quality is affected by toxicants, physiological changes will be observed in the values of one or more of the haematological parameters (Jawad, et al., 2004). Acute effects of oil mill wastewater on hematological parameters in *C. gariepinus* include increased level of erythrocyte number. This observation probaly indicate a compensatory erythropoiesis, which resulted in production of RBC to recompense the older ones that were rapidly destroyed due to decrease in blood's carrying capacity. The low erythrocyte levels preceding high values are in concert with earlier reports on fishes exposed to different toxicants (Alkahem, 1994; Mazon et al.2002, Adakole & Balogun, 2005)

Similarly, the initial increase and a subsequent gradual decrease of haematocrit in *C. gariepinus* due to its exposure oil mill wastewater are attributed to increased respiratory demand and a later diminishing of life processes respectively. An increase in fish osmoregulatory activity produces an increase in the blood oxygen carrying capacity; which in turn would bring about a significant change in the heamotocrit value (Jawad et al., 2004). Haemoglobin value in the highest concentration was 4.21% lower than in the control fish. The decreased haemoglobin in the test fish probably deprived *C. gariepinus* of a reserve of oxygen capacity thus restraining it from potentially higher arterial oxygen content. It has been observed that fish blood parameters such as haematocrit, haemoglobin concentration and RBC count are related to environmental factors such as water hardness, salinity, PO_4-P, NO_3-N among others (Ladu & Ross, 1997; Jawad et al., 2004). Additionally, the relationship between haemoglobin and oxygen shows adaptations not only to environmental conditions but also to metabolic requirements both of which govern oxygen availability and transport to the tissues (Wells, 1999).

An increase in MCHC is taken as an indication of shrunken erythrocytes (Adakole & Balogun, 2005). The values obtained in this study demonstrate a slight but insignificant shrinkage of erythrocytes caused by the oil mill wastewater. White blood cells (WBC) plays an important role in the immune system of living organisms. An unusually high WBC count indicates hypersplenism, inflammation, trauma and stress (Hechtman, 2011). The increase in leukocyte count observed during this study may be attributed to immune response of *C. gariepinus* to toxicants present in the oil mill wastewater. The potential for chemicals to cause damage to the immune system is of considerable public health significance (Oshode et al., 2008), as alterations in immune function can lead to increased incidence of hypersensitivity disorders, autoimmune and infectious diseases.

Several parameters of the wastewater are outside the safe limits recommended for its discharge. Studies have shown that when the water quality is affected by toxicants, physiological changes will be observed in the values of one or more of the haematological parameters (Van Vuren, 1986). Different rates of fish activity due to exposure to wastewater, demand different levels of metabolic activity. Such activity requires several physiological adjustments. These include heamatological parameters (Jawad et al., 2004), which play a significant role in the increase of blood supply to the muscles through variation. Heamatological parameters therefore can be of value in monitoring the effects of environmental changes on fish physiology.

REFERENCES

Adakole, J.A. 2005. Acute toxicity of a metal-finishing company waste water to *Clarias gariepinus* fingerlings. Nigeria. J. Aquat. Sci. 20(2): 69-71.

Adakole, J.A. & Balogun, J.K. 2005. Effects of acute concentrations of metal-finishing company waste water on haematological parameters of *Clarias gariepinus*. J. Trop. Biosci. 5(2): 12-16.

Alkahem, H. F. 1994. The toxicity of nickel and the effects of sublethal levels on haematological parameters and behaviour of the fish, *Oreochromis niloticus*. J. Univ. Kuwait Sci. 21: 243-252.

Anas, R.; Rosario. L.; Gerardo, A.C. & Antonio, G. 2000. Biomass production and detoxification of waters from the olive oil industry by strains of *Penicillium* isolated from waste water disposal ponds. Bioresources Technol. 74: 217 – 221.

APHA, 1998. American Public Health Association, American Water Works Association, Water Pollution Control Federation, 20th Edition. Standard Methods for the Examination of Water and Wastewater. New York, USA.

Blaxhall, P. C.& Daisley, K. W. 1973. Routine haematological methods for use with fish blood. J. Fish. Bio. 4: 711-718.

Capasso, R.; Cristinnzio, G.; Evidente, A. & Scognamiglio, F.F. 1992. Isolation, Spectroscopy and selective phytotoxic effects of polyphenols from vegetable wastewaters. Phytochemistry. 31: 4125– 4128.

FEPA, (Federal Environmental Protection Agency). 1991. National interim guidelines and standards for industrial effluents, gaseous emissions and hazardous wastes management in Nigeria. Federal Environmental Protection Agency Decree,1988. (1988 No. 58). 238pp.

Girolami, V.; Vianello, A.; Stuparon, A.; Ragazzi, V.; Perez, R.; Delafuenate, G. & Falcon, M.A. 1996. Degradation of natural ligins and lignocelluloses substrates by soil inhabiting fungi imperfecti. FEMS. Microb. Ecol. 21: 213 – 219.

Gonzalez, D.M., Morenno, E., Sarmiento, J.Q. and Ramos-Cormenzana, A., 1990, Studies on antibacterial activity of wastewater from olive oil mill (Alpechin): Inhibitory activity phenolic and fatty acids. Chemosphere 20:423-432.

Hechtman, L. 2011. Clinical Naturopathic Medicine. Churchill Livingstone/Elsevier Australia Publishers. Sydney, Australia. 2nd edition. 1596p.

Jain, N.C. 1986. Schalm's Veterinary Haematology. Philadelphia: Lea and Febiger. pp 12-21.

Jawad, L.A.; Al-Mukhtar, M. A. & Ahmed, H. K. 2004. The relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha* (Family Clupeidae). Anim. Biodiv. Conserv. 27(2): 47-52.

Ladu, B.M.B. & Ross, L.G. 1997. The effect of method of immobilization on the Heamatological and tissue chemistry of rainbow trout, Oncorhynchus mykiss Walburn. J. Aquat. Sci. 12: 31-41.

Mazon, A.F.; Monteiro, E.A.S.; Pinheiro, G.H.D. & Fernadez, M.N. 2002. Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. Braz. J. Bio. 62(4a): 125-128.

Mhize, S.P.; Atkinson, B.W. & Bux, F. 2000. Evaluation of a laboratory scale biological process for the treatment of edible oil effluent. Water S.A. 26(4): 555-558.

Oshode, O.A.; Bakare, A.A.; Adeogun, A.O.; Efuntoye M.O. & Sowunmi, A. 2008. Ecotoxicological assessment using *Clarias gariepinus* and microbial characterization of leachate from municipal solid waste landfill. Int. J. Environ. Res. 2(4): 391-400.

Pal, A.K. & Konar, S.K. 1987. Long-term effects of organophosphorus insecticide, Methyl parathion on fish. J. Environ. Ecol. 3: 564-571.

Piperidou, C. I.; Chaidou, C. I.; Stalikas, C. D.; Soulti, K.; Pilidis, G. A. & Balis, C. 2000. Bioremediation of olive oil mill wastewater: Chemical alterations induced by *Azotobacter vinelandii*. J. Agr. Food Chem. 48: 1941– 1948.

Reddy, K.; Drysdale, G.D. & Bux, F. 2003. Evaluation of activated sludge treatment and settle-ability in remediation of edible oil effluent. Water S.A. 29(3): 245-255

Rodriguez, M.M.; Perez, J.; Ramos-Cormenzana, A.; Martinez, J. 1988. Effects of extracts obtained from olive mill wastewater on Bacillus megaterium ATCC 33085. J. Appl. Bacteriol. 64: 219 - 226.

Roux Van der Merwe, M.P.; Badenhorst, J. & Britz, T.J. 2005. Fungal treatment of an Edible-oil-containing industrial effluent. World J. Microb. Biot. 21 (6-7): 947-953.

Van Vuren, J.H.J. 1986. The effects of toxicants on the haematology of *Labeo umbratus* (Teleostei: Cyprinidae). Comp. Biochem. Physiol., 83(1):155-159.

Wells, G.M.G. 1999. Heamoglobin function in aquatic animals: Molecular adaptations to environmental challenge. Mar. Freshwater Res. 50: 933-936.

Submetido: Agosto/2010
Revisado: Janeiro/2011
Aceito: Janeiro/2011