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SOME TOXIC EFFECTS OF PETROLEUM PRODUCTS ON NIGERIAN FAVOURITE DELICACY (FRESH FISH)

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ABSTRACTS

Background: Fresh fish is a Nigerian delicacy and commonly sourced from ponds, streams, rivers and oceans. Petrol and diesel are common byproducts of petroleum. These products get to end users through many channels including long distance transportation to filling stations, dispensing to motor vehicles, jerry-cans/other containers. Accidental spillages and outright pouring into fish ponds, streams, rivers and oceans are possible. African catfish thrive in hash environment but, may indeed be a potential store house of toxicity in man from these petroleum products. **Aim/Objective:** Aim of this project is therefore to assess some toxicological effects of petrol and diesel on fish. **Methodology:** A total of 80 six-weeks old African catfish of average weight of 8.0grams were purchased from the fishery Department of Nnamdi Azikiwe University, Awka, Nigeria. They were allowed two weeks acclimatization in the Laboratory before the commencement of the experiment. Acute toxicity study to determine the LD_{50} was done based on Lorke's 1983, method. Chronic toxicity was done with none lethal doses of either petrol or diesel for a 14 week period. The animals were divided into 5 groups (A-E) consisting of 6 animals per group. The appropriate dose based on bi-weekly weight was daily orally administered with cannulation. They were allowed free access to feeds and water. Water in their plastic aquarium was changed daily. They were housed in our standard Laboratory environment and allowed 12 hours each of light and darkness. At the end of the 13week and beginning of the 14^{th} week, the animals were finally weighed and sacrificed. Blood samples were collected into EDTA and plain bottles. Sampling was done via cardiac puncture and free blood flow from cut-tails. Harvested organs were stored in fixatives for future use while blood samples were sent to the Laboratory to determine Packed cell volume (PCV), Full blood count, Urea, Creatinine, electrolytes, alanine transaminase, aspartate transaminase, alkaline transaminase enzymes and bilirubin. Raw data were collated and analyzed using SPSS version 20 to determine mean, analysis of mean variance while student t-test was used to determine P-value. P-value greater than or equal to 0.05 were adjudged as insignificant. **Results:** LD_{50} for petrol was 129.6mg/kg while that of diesel was 149.7mg/kg intraperitoneaelly in African catfish. There was retardation of growth in the high dose of petrol or diesel treated groups unlike control. There was however no significant differences in weight and length in treated groups compared to control. The PCV, total white cells and Lymphocytes were significantly elevated in dose dependent fashion for either petrol or diesel compared to the negative control. All liver enzymes and electrolytes were all significantly elevated in dose dependent fashion compared to the negative control. **Conclusion:** This work can be concluded to have demonstrated a dose dependent liver, renal and haemotoxicological effects of petrol and diesel on African catfish and may indirectly affect humans when the fish is eaten. **Recommendation:** It can be recommended that fish ponds should be sited far away from filling stations and mechanic workshops while our roads should be properly maintained to reduce vehicular accidents and incidents of fall-tankers.

KEYWORDS: Petroleum, Cat-fish, Toxicity, Fish-ponds, Nigerian delicacy; Fresh-fish.

INTRODUCTION

Petroleum products are materials derived from crude oil (petroleum) as it is processed in oil refineries. They are complex mixture of hydrocarbons and additives which could produce free radicals (Walther, 2005).

Petroleum products include refined crude oil, processed crude petroleum, fuel oil, treated crude oil residuum, casing head gasoline, natural gas gasoline, naphtha, distillate gasoline, kerosene, wax, asphalt, bitumen, tar blended gasoline, lubricating oil and petrol (Walther, 2005).

The petroleum industry in Nigeria is the largest on the African continent (Watts 2004). The petroleum sector is indispensible in Nigeria, as government revenues and foreign exchange still heavily rely on this sector (Watts, 2004).

In Nigeria, petroleum product spills apart from those occurring during refining of crude oil, may also occur during transfers at the jetty, accidents involving tankers, dispensing of products to vehicles as well as vehicular and generator repairs (Kadafa, 2012).

Since, the advent of crude oil exploration in the country in 1956, spillages of crude and refined petroleum products have been commonplace, raising concerns regarding their polluting effects in aquatic ecosystems (Kadafa, 2012).

African catfish (*Clarias gariepinus*) is a species of catfish of the family Clariidae, the air-breathing catfish (Froese, 2014). They are found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems (Froese, 2014).

The rearing of the *Clarias gariepinus* in Africa started in the early 1970s in Central and Western Africa, as it was realized to be very suitable specie for aquaculture. It grows fast and feeds on a large variety of agricultural byproducts, hardy and tolerates adverse water quality conditions, reproduces easily even in captivity, it can be raised in high densities, resulting in high net yields.

In most countries, it fetches a higher price than tilapia as it can be sold live at the market. *Clarias gariepinus* was introduced all over the world in the early 1980s for aquaculture purposes and now found in countries far outside its natural habitat, such as Brazil, Vietnam, Indonesia, and India (Hoek, 2003).

Clarias gariepinus is an omnivore, large, eel-like fish, usually of dark gray or black coloration on the back and fading to a white belly (Froese, 2014).

It has an average adult length of 1–1.5 meters (3–5 ft). It reaches a maximum length of 1.7meters and can weigh up to 60 kg (Froese, 2014).

The fish has a slender body, flat bony head and broad terminal mouth with four pairs of barbells, only the pectoral fins have spines (Sunmonu, 2007).

It also has a large accessory air-breathing organ composed of modified gill arches that enables it adapt to adverse aquatic conditions (Sunmonu, 2007).

It is a nocturnal fish like many catfishes. It feeds on living, as well as dead, animal matter (Anoop *et al.*, 2009).

It can swallow a relatively large prey because of its large mouth and can survive in shallow mud for long period of time between rainy and dry seasons (Anoop *et al*, 2009).

Clarias gariepinus sometimes produces loud croaking sounds, not unlike the voice of the crow. Spawning mostly takes place at night in the shallow, inundated areas of the rivers, lakes or streams (McKee, 2017). Courtship and mating takes place in shallow waters between isolated pairs of males and females (McKee, 2017).

Courtship is preceded by highly aggressive encounters between males. During mating, the male lies in a U-shape curved around the head of the female, held for several seconds (McKee, 2017).

A batch of milt and eggs are released followed by a vigorous swish of the female's tail to distribute the eggs over a wide area. The pair usually rest after mating (from seconds up to several minutes) and later continue mating (McKee, 2017).

Parental care for ensuring the survival of the catfish offspring is absent except by the careful choice of a suitable site. Development of eggs and larvae is rapid, and the larvae are capable of swimming within 48–72 hours after fertilization (McKee, 2017).

Crude oil and its refined petroleum products contains several organic and inorganic substances including atoms of oxygen, sulphur, nitrogen as well as metals such as iron, vanadium, chromium and nickel and (Sunmonu, 2007). Some of these organic and inorganic substances contain toxic components of crude oil such as polycyclic aromatic hydrocarbons and water soluble fractions (Sunmonu, 2007).

Crude oil and its products can cause damage to aquatic ecosystem through the depletion of oxygen content of water body (Umar et al., 2017).

Crude oil and refined petroleum products concentrations of 0.01 ml/L are known to accelerate the death of fish in aquatic ecosystems (Ubong *et al.*, 2015; Umar et al., 2017). Oxygen is insoluble in crude oil and therefore does not easily pass through even when a thin of oil is present on water (Umar *et al.*, 2017).

Crude oil can therefore limit the amount of oxygen that enters into a body of water from the atmosphere (Umar *et al.*, 2017).

Crude oil and its products may also contain water soluble fractions that are toxic either directly or through the metabolic pathways to aquatic organisms including fish (Ubong *et al.*, 2015).

Today in Nigeria fish ponds abound. Though bulk of our consumed fish still comes from rivers and oceans, it is not uncommon to find segregated fish traps/ponds close to the expressways (eg Asaba end of Niger bridge).

Many tankers conveying petroleum products commonly fall along the ways and spill their contents into the rivers/ponds.

An intriguing possibility is the accumulation of the potential toxic effects of petroleum products in the "die-hard" African catfish but, later transferred to man with devastating/deteriorating effects when they are consumed.

AIM/OBJECTIVE

Aim of this project is therefore to assess some toxicological effects of petrol and diesel on cat-fish.

MATERIAL AND METHODS

A total of 80 six-weeks old African catfish of average weight of 8.0grams were purchased from the fishery Department of Nnamdi Azikiwe University, Awka, Nigeria. They were allowed two weeks acclimatization in the Laboratory before the commencement of the experiment. Acute toxicity study to determine the LD_{50} was done based on Lorke's 1983, method.

Chronic toxicity was done with none lethal doses of either petrol or diesel for a 14 week period. The animals were divided into 5 groups (A-E) consisting of 6 animals per group. The appropriate dose based on bi-weekly weight was daily orally administered with cannulation. They were allowed free access to feeds and water. Water in their plastic aquarium was changed daily. They were housed in our standard Laboratory environment and allowed 12 hours each of light and darkness.

At the end of the 13week and beginning of the 14th week, the animals were finally weighed and sacrificed. Blood samples were collected into EDTA and plain bottles. Sampling was done via cardiac puncture and free blood flow from cut-tails. Harvested organs were stored in fixatives for future use while blood samples were sent to the Laboratory to determine Packed cell volume (PCV), Full blood count, Urea, Creatinine, electrolytes, alanine transaminase, aspartate transaminase, alkaline transaminase enzymes and bilirubin.

Statistical Analysis

Raw data were collated and analyzed using SPSS version 20 to determine mean, analysis of mean variance while student t-test was used to determine P-value. P-value greater than or equal to 0.05 were adjudged as insignificant.

RESULTS

Acute Toxicity: (Necessary to deduce none lethal doses)

Table 1: Acute toxicity ($LD_{50)}$ of petrol on *Clarias gariepinus* (Phase one).

Group	Dose of petrol (mg/kg)	Number of death
A	10	0
B	100	0
C	1000	3

Group A: Dose of 10mg/kg of petrol, **Group B**: Dose of 100mg/kg of petrol, Group C: Dose of 1000mg/kg of petrol

Table 2: Acute toxicity ($LD_{50)}$ of petrol on *Clarias gariepinus* (Phase two).

Group	Dose of petrol (mg/kg)	Number of death
A	120	0
B	140	1
C	160	1
D	180	1

Group A: Dose of 120mg/kg of petrol, **Group B**: Dose of 140mg/kg of petrol, **Group C**: Dose of 160mg/kg of petrol, **Group D**: Dose of 180 mg/kg of petrol.

Table 3: Acute toxicity (LD_{50}) of diesel on *Clarias gariepinus* (Phase one).

Group	Dose of Diesel (mg/kg)	Number of death
A	10	0
B	100	0
C	1000	3

Group A: Dose of 10mg/kg of diesel, **Group B**: Dose of 100mg/kg of diesel, Group C: Dose of 1000mg/kg of diesel.

Table 4: Acute toxicity (LD_{50}) of diesel on *Clarias gariepinus* (Phase two).

Group	Dose of diesel (mg/kg)	Number of death
A	120	0
B	140	0
C	160	1
D	180	1

Group A: Dose of 120mg/kg of diesel, **Group B**: Dose of 140mg/kg of diesel, **Group C**: Dose of 160mg/kg of diesel, **Group D**: Dose of 180 mg/kg of diesel.

Summary:- LD_{50} for petrol was 129.6mg/kg while that of diesel was 149.7mg/kg intraperitoneaelly in African catfish.

Results on Chronic Toxicity

There was retardation of growth in the high dose of petrol or diesel treated groups unlike control. There was however no significant differences in weight and length in treated groups compared to control. The PCV, total white cells and Lymphocytes were significantly elevated in dose dependent fashion for either petrol or diesel compared to the negative control. All liver enzymes and electrolytes were all significantly elevated in dose dependent fashion compared to the negative control.

Table 5: Comparative effect of diesel and petrol on length and weight of the *Clarias gariepinus* (African catfish).

Variables	Petrol		Diesel				
	High dose	Low dose	High dose	Low dose	Control	F Value	*P* value
Length (cm)	12.2±3.32	12.9±3.54	12.5±3.40	13.2±3.62	15.2±5.72	0.70	0.598
Weight (g)	20.6±10.1	24.4±11.6	22.5±11.4	25.9±11.8	45.4±31.8	1.09	0.391

Table 6: Comparative effect of petrol and diesel on some haematological parameters of the *Clarias gariepinus*

Variables	Petrol		Diesel				
	High dose	Low dose	High dose	Low dose	Control	F Value	*P* Value
Red blood cell count (mc/mcl)	1.73 ± 1.34	3.0 ± 0.11	2.41±1.19	3.14±0.10	3.13±1.54	33.8	<0.001
White blood cell count (c/mcl)	5363± 4156.5	6980± 84.9	6420± 3149.3	6370± 67.8	5018.3± 2460.5	73.7	<0.001
Platelet count (/mcl)	70.2±54.4	122.2±3.06	100.7±49.5	125.8±2.32	113.2±55.5	22.5	<0.001

Table 7: Comparative effect of petrol and diesel on biochemical parameters of *Clarias gariepinus*

Variables	Petrol		Diesel				
	High dose	Low dose	High dose	Low dose	Control	F Value	*P* Value
Serum ALT (iu / l)	24.8±19.2	28.5±1.33	26.9±13.3	24.1±1.59	6.77±3.32	129.2	<0.001
Serum AST (iu / l)	20.3±15.7	19.9±0.33	22.8±11.2	18.0±0.35	13.7±6.74	252.6	<0.001
Serum creatinine (mg/dl)	0.38±0.29	0.42±0.21	0.38±0.19	0.40±0.01	0.30±0.16	8.38	<0.001
Serum urea (mg/dl)	4.61±3.57	6.23±0.13	5.28±2.59	5.98±0.14	4.70±2.30	20.1	<0.001

SALT: Serum alanine aminotransferase, SAST: Serum aspartate aminotransferase, SCREAT: Serum creatnine, SUREA: Serum urea level.

DISCUSSION

Fresh fish is a Nigerian delicacy and commonly sourced from ponds, streams, rivers and oceans. The commonest fresh-fish is the Cat-fish. Cat fish is a die-hard animal and can survive in very harsh conditions.

Petrol and diesel are common byproducts of petroleum. These products get to end users through many channels including long distance transportation to filling stations, dispensing to motor vehicles, jerry-cans and other containers. Accidental spillages and outright pouring into fish ponds, streams, rivers and oceans are possible. African catfish thrive in hash environment but, may indeed be a potential store house of toxicity in man from these petroleum products (Petrol/diesel)(Arazu *et al.*, 2017)

Several recent studies have indicated aluminum as one of the aetiological agents of Alzheimer disease. Alzheimer's disease is now becoming one of the most common neurodegenerative diseases and global epidemiologically (Tom;jenovic, 2011).

Aluminum is very toxic to the brain and humans get over 90% of aluminum via diets especially, seafood (Shrimps, fish and snails). These animals get aluminum through contaminations or deliberate channeling of industrial waste into rivers/seas (Ranau *et al*., 2014).

Significant levels of total hydrocarbons were found in shellfishes in crude oil polluted stations in the Niger Delta area when compared with unpolluted sites. The spillage or discharge of refined petroleum products may damage the aquatic ecosystem in a number of ways which include direct kill of organisms through coating and asphyxiation (Utibe and Edafe, 2017).

Polycyclic Aromatic Hydrocarbons (PAHs) consist of two or more fused benzene (aromatic) rings. PAHs are considered trace contaminants of freshwater and marine sediments all over the world (ITOPF, 2011a). They are recognized as major contributors to environmental pollution affecting aquatic ecosystems under the influence of human activities. PAHs are released to the environment from a number of anthropogenic activities, for example petroleum product spills (ITOPF, 2011b). However, the combustion of fossil fuels from traffic, power generation, industrial processes such as smelters, refining, and a number of other anthropogenic activities also contribute to the build-up of PAHs in the environment. PAHs are also emitted from pyrogenic sources. Hence, incineration of garbage under non-optimal conditions (low temperatures), grass, and forest fires are other major sources of PAHs (ITOPF, 2011b).

Xue *et al*., 2019 also demonstrated the effects of dissolved supersaturation on fish exposed to petroleum products.

Our present study recorded four deaths of *Clarias gariepinus*. Petroleum products like diesel and petrol can cause blockage of atmospheric oxygen from dissolving in water thereby limiting the supply of oxygen to fish fingerlings resulting to incidence of excretory waste products (carbon dioxide, ammonia) in the ambient water environment. Increases in the free carbon dioxide concentration as the exposure period increases could be synonymous with decreases in dissolved oxygen concentration and this probably explained the mortality of *Clarias gariepinus* juveniles recorded during the experiment.

This study agreed with Nwamba *et al* (2001) experiment on *Heterobranchus bidorsalis* treated with 1.25ml/L crude oil. The mortality experienced in the petroleum fractions treatments (1.25ml/L) was in consonance with Lee's (1975) view that crude oil and its refined products concentration of 0.01ml/L accelerated the death of fish in aquatic environment.

One can confidently deduce the retardation of growth on the effect of metabolic activities arising from the toxic effect on both liver and kidneys. The red blood cells were decreased therefore causing anemia which might lead to growth retardation. The outcome of this study also revealed that there was statistically significant difference in the hematological and biochemical parameters of *Clarias gariepinus* exposed to various concentrations of the refined petroleum products used. There was significant difference in the mean red blood cell count (p<0.001), white blood cell count (p<0.001), platelet count (p<0.001), serum alanine aminotransferase (p<0.001), serum aspartate aminotransferase (p<0.001), serum creatinine (p=0.001) and serum urea (p<0.001) following exposure to different concentrations of refined petroleum product used.

The exposure of *Clarias gariepinus* to petrol and diesel in this study resulted to decrease in the red blood cell. The observed reduction in the counts of red blood cell suggests an anaemic condition in the *Clarias gariepinus.* The significant reduction in red blood cell counts may be attributed to cytotoxic effect and suppression of erythropoiesis caused by constituents of the petroleum product. This is in line with previous studies which showed that the erythroid colony-forming unit (CFU-e) was very susceptible to the cytotoxic effect of the crude oil derivative benzene (Sunmonu *et al*, 2007a). The red blood cell count which dropped significantly during the study shows that there was a reduction in the level of oxygen carried to the tissues. The major functions of white blood cells are to fight infection, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. A significant increase in white blood cell count was observed in this study. This might be suggestive of infection. Also ingestion of petrol and diesel by the fish may have induced an increase in the metabolic rate, with resultant increase in the generation of free radicals leading to cellular damage. However, this observation is in contrast with the findings of a previous study in which there was a reduction in total white blood cell count in mammal (goats) exposed to crude oil (Ekiz and Yalcintan, 2013). It was argued that the reduction in white blood cell count in goats may be as a result of stress induced by crude oil hydrocarbons. The result of this study further revealed that there was a significant decrease (p<0.001) in the platelet count compared to the control group. The significant reduction may be attributed to negative impact of the petroleum product on the thrombocytes of the *Clarias gariepinus.* In a related study, toxic components in crude oil induced anemia by causing bone marrow hypoplasia and interfered with platelet production in the animals, hence the reduction of platelets (Schwertz, *et al.*, 2010). Our study further demonstrated toxic effects on the liver and kidneys. Hepatocytes rely on antioxidant enzymes to protect them from the activity of reactive oxygen species which are products of xenobiotic transformation. Also kidney tissue damage might lead to anemia and hypoxia. Karakoc and Barlas, 2019 added to line of logic with their histopathologic demonstration of liver and kidneys damages of fish exposed to pollutants in the aquaculture.

CONCLUSION

This work can be concluded to have demonstrated a dose dependent liver, renal and haemotoxicological effects of petrol and diesel on African catfish and may indirectly affect humans when the fish is eaten.

Recommendation: It can be recommended that fish ponds should be sited far away from filling stations and mechanic workshops while our roads should be properly maintained to reduce vehicular accidents and incidents of fall-tankers.

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