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Original Research Paper

Animal Science

HISTOPATHOLOGY OF AFRICAN CAT FISH EXPOSED TO DIESEL OIL

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ABSTRACT

The devastating consequences of oil spills are felt in the whole world as well as South-South region of Nigeria being one of the largest oil producing country in the world. Therefore, the effect of oil pollution on aquatic life has become a great concern to scientists, since much of the population is dependent upon marine and coastal ecosystems for food therefore this study was conducted to examine the lethal and sub-lethal toxicity effect of diesel oil on the hearth and gills tissue of Juvenile African catfish (Clarias gariepinus) using a renewable static bioassay.

A total number of 144 juvenile African catfish of average weight (425±0.02g) and length 35±0.2cm were used for both lethal and sub-lethal experiments Firstly, sixty fish were selected and exposed to five varying lethal concentration of diesel oil (1000 ml, 900 ml, 600ml,700 ml, 600 ml) and control (water) for 4 days. Secondly, other set of sixty fish were exposed to five varying sub-lethal concentration of diesel (100 ml, 200 ml, 300 ml, 400 ml, 500 ml) and control (water) for 14 days. Within few hours of fish exposure to lethal concentrations, all the fish died

For the fish exposed to sub lethal concentrations, no mortality was observed in treatment A (control) and treatment B (100 ml), indicating that the fishes are healthy. However, 50% mortalities were observed in treatment C (200 ml), 75% mortality in treatment D (300 ml) and 100% mortalities were observed in treatment E (400 ml) and F (500ml) respectively.

The histological investigations of the heart and gills tissue of the fish revealed great ulceration of heart tissue and totally erode gills villi was noticed in gill tissue of test organism. The results revealed that acute oil toxicity has severe effects on the fish mortality, heart and gill structure which may be deleterious for catfish populations.

Conclusively, this study has been able to establish the fact that, exposure of juvenile African catfish (Clarias gariepinus) to even low concentration (200ml) of diesel concentration can induce various toxicological effect and histological degradation which depend on the period of exposure and concentration of toxicant.

KEYWORDS : environment, histology, lethal, toxicity, diesel oil.

Introduction

Fish is known as important sources of high quality protein in human diet, providing 16% of the animal protein consumed by world's population (FAO, 1997), Fish is also rich in amino acids such as thiamine, lysine, minerals, and vitamins. In Nigeria, among the culturable fish species, African catfish (C*larias gariepinus*) is the most ecologically important, commercially valued and highly acceptable fish by both the fish farmers and consumers (Ita, 1980). They are found freely in Nigerian natural fresh water. Recently, it was discovered that most of the indigenous fishes are endanger not because of pouching but due to discharge of various pollutants into our natural fresh water bodies. Petroleum products is one of the pollutants that are most toxic to aquatic bodies (Pacheco and Santos, 2001a). Diesel fuel is refined petroleum product obtained from distillation of petroleum. It has an ignition temperature of 540^{0}C and is ignited by the heat of compression (Microsoft Encarta, 2009). Diesel is a complex mixture containing polycyclic aromatic hydrocarbons, which persist after a spill, pass readily from water into tissues, and are toxic to early life stages of fish (Schein et al., 2009). The contaminations of inland water bodies are mainly from petroleum truck accidents and principally from continuous leakages from underground bulk storage tank, thereby reaching ground water and later rivers (Tiburtius *et al*., 2005).Although, little research has been done on the effect of petroleum product on tropical fresh water organism (Akaishi *et al.,* 2004).Exposure to crude oil and derivatives can induce a variety of toxic symptoms in experimental organisms. Petroleum hydrocarbon can act as mediator in free radical generation in fish (Achubal and Osakwe, 2003). Other studies have also indicated that the exposure of fish to water soluble fraction of petroleum derivatives has effect on cortisol plasma concentration (Alkindi *et al.,* 1996 Pacheco and santos, 2001a,b), suggesting that these contamination might interfere in the fish stress response. Therefore, this study investigate the histopathological alterations in the heart and gills of African catfish (*Clarias gariepinus*) exposed to diesel oil.

Materials and Method

Experimental Procedures

The experiment was carried out at the fishery unit of teaching and research farm, LAUTECH Ogbomoso, Oyo state, Nigeria.

Test Organism

A total number of 120 healthy adult size Africa catfish (*Clarias gariepinus)* of average weight of 425±0.02g and average length of 35±0.2cm was procured from a reputable fish farm in Ogbomoso, Oyo state Nigeria and were transported in 60liters circular plastic tanks covered with net and wire mesh, which contained fresh water. The fish were acclimatized for two weeks prior the commencement of the experiment.

The experimental fish were fed with commercially prepared fish feed or pellets twice daily during the acclimatization. The states of the fish were monitored times daily and to observe the morphological changes in fish exposed to diesel oil. The water was changed every two days and the water was well aerated.

Collection of Diesel Oil

Diesel oil was purchased from reputable filling station in Ogbomosho, Oyo state, Nigeria. The samples were collected in 25litres keg.

Lethal and Sub Lethal Tests

After acclimatization period, the fishes were randomly selected for lethal and sub lethal tests.

Lethal Test (Acute Test)

Five varying concentration, 600ml, 700ml, 800ml, 900ml and 1000ml of diesel oil respectively were used for this test. Thirty (30) fishes were separated into five plastics tank of 60liters containing 20liters of water and the diesel concentration. Six fishes were placed in each tank containing various concentration of diesel oil and

20liters of water. The treatments were replicated twice, the behavioral and mortalities of the test organism were monitored for 3days, burrowing the method of Adewoye and Fawole, 2002b (96 hours)

Sub-Lethal Test (Chronic Test)

In this test, five other varying concentration 100ml (B), 200ml (C), 300m (D), 400ml (E), and 500m (F) of diesel oil and control (A) were used for this test.

Histopathological Procedure

Fishes were randomly selected from the treatments, control included, fish from control was sacrificed. The abdominal cavities were opened; the targeted organs (Hearts and Gills) were excised, removed and kept in plastic container containing formalin. Thereafter, the organs were taken to the laboratory for analysis. The histopathological effect was observed under light microscope, using a camera-mounted microscope to document findings.

Data Collection

The data collected were mortality, number of survival, behavioral changes of fish during the period of exposure and the following were calculated; number of dead fishes and number of survival.

Results and Discussion

Results

Percentage mortality of juvenile African catfish (*Clarias gariepinus*) exposed to varying sub lethal concentration of diesel oil is presented in Table 1.

Table.1**,** below shows percentage mortality of juvenile African catfish (*Clarias gariepinus*) exposed to varying sub-lethal concentration of diesel oil. At each exposure time, abnormal behavior such as the restlessness, loss of equilibrium and erratic movement were observed as soon as the media started to act on the test organisms. The affected fish became very weak, gasping for air and died in increasing number at relatively small increase in diesel oil concentration. No mortalities was observed in treatment A (control) and treatment B (100 ml), indicating that the fishes are healthy. However, 50% mortalities were observed in treatment C (200 ml), 75% mortality in treatment D (300 ml) and 100% mortalities were observed in treatment E (400 ml) and F (500ml) respectively.

Table 1: Percentage Mortality of juvenile African catfish *(Clarias gariepinus)* Subjected to Varying Sub Lethal Concentration of Diesel Oil

Diesel Oil concentration (ml) and Treatment	No. of dead fishes after 14days in treatment	No. of dead fishes after 14days in replicate	Total mortality	% mortality
Control A	0	0	0	0.00
100 B	0	0	0	0.00
200 C	3	3	6	50.00
300 D	4	5	9	75.00
400 E	6	6	12	100.00
500 F	6	6	12	100.00

The section of the heart tissue of juvenile African catfish (Clarias gariepinus) exposed to ordinary water (control) and varying sub-lethal concentration of diesel oil were showed in Plate A-F.

In plate A, the heart tissue has a normal muscular arrangement, the nucleus and tissue fibers are well arranged. In Plate B, the heart tissue has a normal muscular arrangement; no significant damage is seen. In Plate C, no significant damage is seen in the heart tissue. In Plate D there is great ulceration of the muscle in this micrograph as enlargement of the heart muscles occurred. In Plates E and F Major histological abnormalities observed were ulceration of the muscle of the heart, congestion of lumen and cellular necrosis

Plate G - L Shows The section of the gill tissue juvenile African catfish (Clarias gariepinus) exposed to ordinary water (control) and varying sub-lethal concentration of diesel oil were shown in Plates G-L. In plate G (control), there is a normal cell of the gill with normal length of the gill sack and normal cartilage and muscle. Plate H (100 ml), in this gill tissue, the sack becomes thinner and larger than the control; the cartilage becomes enlarged as the condrocytes becomes reduced. In Plate I (200 ml), there is segmentation of the gill tissue and it becomes interlocking while it changed to pseudo epithelium. In plate J (300 ml), the gills are larger, but the villi of the gills cartilage increases than length of the villi of the gillsin control. In Plate K and (400 ml and 500ml) gills villi have been totally eroded, the cartilages were equally lysied, and the muscles were affected.

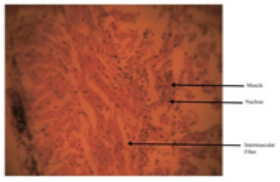

Plate A: Showing a section of the heart tissue of a juvenile African catfish (Clarias gariepinus) exposed to ordinary water (control) (Mg x 400)

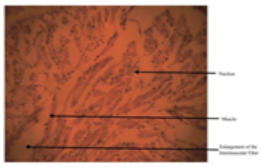

Plate B: Showing a section of the heart tissue of a juvenile African catfish (Clarias gariepinus) exposed to sub-lethal concentration (100ml) of diesel oil. (Mg x 400)

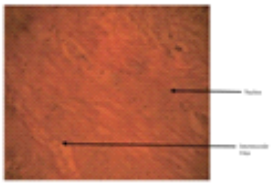

Plate C: Showing a section of the heart tissue of a juvenile African catfish (Clarias gariepinus) exposed to sub-lethal concentration (200 ml) of diesel oil. (Mg x 400)

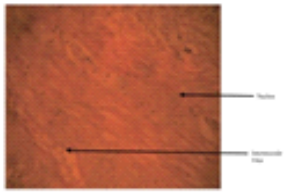

Plate D: Showing a section of the heart tissue of a juvenile African catfish (Clarias gariepinus) exposed to sub-lethal concentration (300 ml) of diesel oil. (Mg x 400)

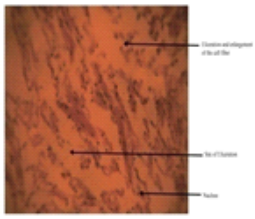

Plate E: Showing a section of the heart tissue of a juvenile African catfish (Clariasgariepinus)exposed to sub-lethal concentration (400 ml) of diesel oil.

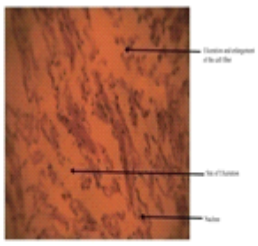

Plate F: Showing a section of the heart tissue of a juvenile African catfish clariasgariepinus) exposed to sub-lethal oncentration (500 ml) of diesel oil.

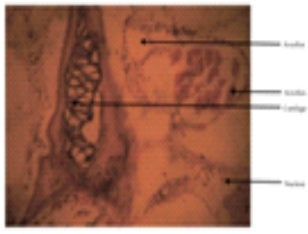

Plate G: Showing a section of the gill tissue of a juvenile African catfish (Clarias gariepinus) exposed to ordinary water (control) (Mg x 400).

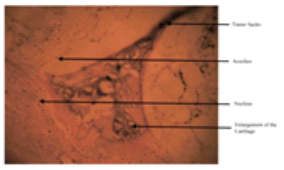

Plate H: Showing a section of the gill tissue of a juvenile African catfishClariasgariepinus) exposed to sub-lethal concentration (100 ml) of diesel oil. (Mg x 400).

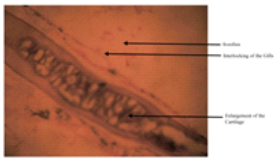

Plate I: Showing asection of the gill tissue of a juvenile African catfish (Clariasgariepinus) exposed to sub-lethal concentration (200 ml) of diesel oil. (Mg x 400).

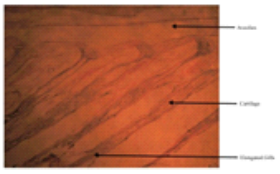

Plate J: Showing asection of the gill tissue of a juvenile African catfish (Clariasgariepinus) exposed to sub-lethal concentration (300 ml) of diesel oil. (Mg x 400)

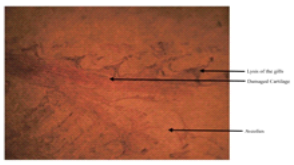

Plate K: Showing a section of the gill tissue of a juvenileAfrican catfish (Clariasgariepinus) exposed to sub-lethal concentration (400ml) of diesel oil.

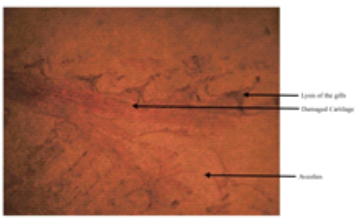

Plate L: Showing a section of the gill tissue of a juvenileAfrican catfish (Clariasgariepinus) exposed to sub-lethal concentration (500ml) of diesel oil.

Discussion

Hyperactivities observed in these studies are attributed probably due to the disturbances in the metabolic state, resulting in the depletion of energy. It is possible that animals which have higher metabolic activities could require higher level of oxygen and thus would embark on higher respiratory activities (Canli and Kergin, 1995). Lethargies and loss of equilibrium observed in these studies may due to depletion of energy in the body of exposed animal. Also, Lethargies and loss of equilibrium as recorded in this work is an indication of impairment abnormal carbohydrate metabolism and are possible results of hormonal impairment (Anderson *et al., 1988*). This study reveals the impairment of the carbohydrate metabolism, which results in the depletion of energy, causing Lethargies and loss of equilibrium and those organisms that cannot tolerate the toxicant enter into a state of comma and later died. It was also observed that, the rate of mortalities greatly increased in the concentration of diesel oil. This is the reflection of what Fryer (1977) reported as regarded all categories of toxicant, a threshold is reached at which there is no drastic survival of animal. Animal's lies within a tolerable zone only below the threshold, but above the tolerance zone are the zone of resistance. Histological observation in the heart tissues (Plate A), shows a normal muscular arrangement and the nucleus and tissue fiber are well arranged in untreated group, however in treated group (Plate B-D), major histological abnormalities observed were ulceration of the muscle of the heart, congestion of lumen and cellular necrosis, which showed a progressive architectural distortion varied concentration and period of exposure, this is in agreement with the submission of Srivastava (1994) who reported that teleost accumulated lead both directly from diet and indirectly from diet and directed from aqueous medium through an active food chain by the surface lamellae. The most generally encountered type degenerative changes were congested, cellular infiltration and focal necrosis. Cellular necrosis as observed in this work probably resulted from excessive work required by fish to get rid of the toxicants from its body during its process of detoxification. (Frieberg *et al.,* 1971) submitted that, fish are known to possess sequestering agent (metallothianein), the bioaccumulation of these trace elements in the heart tissue reaches a proportion in which the function of the hearth is impeded, this resulting in a progressive degeneration of the heart cells syncytial arrangement.

Therefore, necrosis became evident as the concentration increases and this may be due to the ability of fishes to regenerate new heart cells. It was also observed that the histopathological changes in the heart caused circulatory problems. This is evident and more pronounced as observed in the myofibrils. The damage done to the heart tissue of the African cat fish (*Clarias gariepinus*) held in (500 ml) of diesel concentration is generally related to important hepatic lesions such degenerative and necrotic processes, this observation was in line with submission of Chang *et al.,* (1998) and Pacheco and Santos (2002).

Histological observations in the gills (Plates E-I). The main sites of heavy metal uptake and accumulation of effluents are the gills and gastrointestinal tracts (Annume and Iyaniwura, 1993; Lovegrove and Eddey, 1982; Pantreath, 1973).

Diesel oil can be absorbed via skin,gill and mouth. Gills are the most important tissue for gas and ion exchange, acid-base regulation and nitrogenous excretion. Gills have direct contact with water current. The presence of any pollution in external environment has effect on these tissue organs. A negative effect on the gills serves as a stressor and may result in fish death and it also predisposes the fishes to disease.

Therefore, the histological changes observed in the heart and gills of the adult African catfish fish (*Clarias gariepinus*) in the present study indicate that the fish were responding to the direct and the effects of the contaminants more than other effect such as stress. Such information confirms that histopathological alterations are good biomarkers for both food and laboratory assessment, particularly in topics area that are naturally subjected to a multiplicity of environmental variation of depletion due to chemical contamination.

Conclusion

Conclusively, this study has been able to establish the fact that, exposure of juvenile African catfish (*Clarias gariepinus*) to even low concentration (200ml) of diesel concentration can induce various toxicological effect and histological degradation which depend on the period of exposure and concentration of toxicant. In view of the toxicity effect of this of this diesel oil, it can be inferred that, spillage of diesel oil can induce damages to the tissue and organs, which might make all the living entities in polluted environment vulnerable to disease, and eventually leads to death. In long run, therefore, contaminants as diesel oil may pose serious threat to health, survival and affect fish population.

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