

Histopathological Alterations of *Ptychadena mascariensis* Exposed to Sub Lethal Concentrations of Crude Oil

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Abstract: Study on the impact of sub-lethal concentrations (0mg/L, 0.3gm/L, 0.75mg/L, 1.5mg/L, 2.25mg/L and 3.0mg/L) of water-soluble fractions (WSFs) of crude oil on the toxic effects of WSF of crude oil on the histology of the skin, liver and heart of the frog *Ptychadena mascariensis* were carried out under laboratory conditions in the University of Port Harcourt, during a 12-week period of exposure in a renewal static bioassay system. At the end of the study, the skin, liver and heart of control frog retained their normal structural pattern. However, the cells of the skin, liver and heart frogs exposed to crude oil showed marked alterations. The skin showed massive fragmentation and dermal degradation. The liver exposed to WSF of crude oil showed mild to severe necrosis/degeneration of the hepatocytes, liver cells in disarray and distortion of cells, vacuolation, and hepatocellular foci. The tissues of the heart showed gradual increase in interstitial space and myocyte separation. The result of this study has shown that crude oil (WSF) is indeed toxic to frogs. The effect on *P. mascariensis* also indicated that this aquatic animal can serve as bio-indicator of crude oil contaminated fresh water bodies.

Keywords: Amphibians, bio-indicator, cardiomyocytes, hepatocytes, Water soluble fractions.

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1. Introduction

Amphibian populations are in decline in many areas of the world. In cities and the countryside, in rainforests and wetlands, countless areas which previously hosted a range of healthy amphibian populations now have fewer - and even no - frogs, toads, and salamanders. The Global Amphibian Assessment (GAA), collaboration among more than 500 scientists from 60 countries to monitor the status of amphibians worldwide in their publication in 2004, listed 32 percent of amphibian species worldwide as threatened with extinction (GAA, 2004). Numerous physical and chemical causes have been postulated and in some instances, interaction of multiple causes has been implicated. A number of hypotheses have been proposed as underlying causes of the worldwide decline of amphibian populations. These include habitat alteration and habitat destruction (Nafagha, 1999), predation (Lefcort and Blaustein, 1995), competition from exotic non indigenous species (Jennings and Hayes, 1985), parasites (Sessions and Ruth, 1990), disease (Carey and Bryant, 1998), ultraviolet radiation (Blaustein et al., 2003), climate change (Corn et al., 1989) and environmental contamination (Blaustein et al., 1994).

Frogs are excellent indicators of the quality of the overall environment due to the sensitivity of their skin and eggs to both aquatic and terrestrial agents as they can readily absorb substances from their environment, making them very sensitive to perturbations in ecosystems. For this reason, their aquatic larval stages are increasingly viewed as bio-indicators of health of aquatic systems (Hartwell and Olivier, 1998). In amphibian larvae no barriers are provided by scales, feathers or fur covering. The thinness of their skin makes them highly permeable to aquatic pollutants. In larval stages of amphibians, uptake of crude oil particles from the environment occurs principally through external and internal gills as well as cutaneous respiratory surfaces and gastro intestinal ducts. The primary routes of exposure of crude oil to larval amphibians are principally through direct uptake from water and diets (Grillitsch and Chorance 1995).

The health risk to the biological systems associated with the presence of crude oil spill in their environment is determined by the exposure conditions and the biological system itself namely by its constitution and condition, determining capacity and susceptibility. Exposure involves the crude oil itself as well as its concentration, form, route, frequency and duration of exposure. Only the bioavailable portion of a substance present in the environment i.e. the amount that finally interacts with the organism is of toxicological relevance. The water soluble fraction (WSF) of crude oil is that small fraction of oil containing components fully or sparingly soluble in water (Kavanu, 1964). Water soluble fractions constitute of dispersed particulate oil, dissolved hydrocarbon and soluble contaminants such as metallic ions (Kauss and Hutchinson, 1975). Tissue changes in organs (liver, lungs, kidney, heart, gastrointestinal tract) of test

organisms provide histological information on the nature of toxicants organisms are exposed to in their environments. Given the global amphibian population decline and paucity of information on the ecotoxicology of crude oil on tissues of amphibians provided the impetus for this study.

2. Materials and Method

Adult frogs *Ptychadena mascariensis* were collected from unpolluted ponds from Mgbudiya village in Ahoada East LGA of Rivers state in Nigeria. They were collected using hand nets to prevent injury to animals during capture since they are active animals. The frogs were transferred to the large (475 L) glass aquaria in the Laboratory of the University of Port Harcourt. The aquaria were covered with wire mesh to prevent them climbing out since they were very active animals. Eggs clutches were collected from eight different populations of frogs soon after oviposition and moved to a smaller plastic (7.6 L) aquarium filled with dechlorinated tap water (~25 eggs per aquarium and 40–50 eggs per population) where the eggs were observed until hatching. Eggs hatched into tadpoles in 6 to 10 days. Tadpoles were maintained at 22–24°C with 12L: 12D fluorescent lighting. Clean water was maintained by pouring off old water every 2 days and replacing it. Adult frogs were fed *ad libitum* on a variety of live foods three times per week on insects (ants and flies) and earthworms. The tadpoles were fed with beans and corns seeds. Tadpoles were exposed to the toxicant eight weeks after hatching which is approximately half of the mean time required to metamorphose as previously observed in this species in the laboratory (Nafagha, 2007). Tadpoles were pooled into a single tank and then randomly assigned 18 5-L cubical plastic transparent tanks with 20 tadpoles per tank for each treatment including the controls filled with 1 cm of fluid. A static renewal bioassay was carried out with three replicates for 84 days (12 weeks). The tadpoles were introduced into the crude oil contaminated water from where they were fed with diet of corn and cowpea seeds. The water samples were analyzed as described in standard method for examination of water and wastewater (APHA). At the end of the experiment, the limbs were formed and they were now juvenile frogs Gosner stages 44–47 (Gosner, 1960).

The toxicant used was Bonny light crude oil, collected from SPDC flow station at Alakiri, Okrika. The water soluble fractions was prepared following the method of (Anderson et al., 1974) by adding 1 part of crude oil to 9 parts of fresh water. Range finding tests were done to determine the threshold concentrations (Nafagha 2007).

The frogs were sacrifice by decapitation after chloroform anaesthesia and fragments from the liver and heart were quickly removed. The frogs' skin was removed from the ventral section of the thigh. The frogs' skin, livers and hearts was prepared for sectioning or microtone and microphotography following the standard methods.

3. Results and Discussion

The skin of the frogs provides the most extensive interface between the external and internal environment. Microscopic examination of the slides of skin of the control frogs showed conformity to the natural architectural pattern of normal parallel arranged cells. There were no discernible changes in the structure of the histology of the skins of the frogs in the control to that of the frogs in low concentrations (0.3, 0.75 mg/L). However from 1.5 mg/L there was an observed gradual fragmentation of the skin cells and dermal degradation (See Fig 1a-c). Fragmentation and degradation of the skin is a precursor to lesions on the skin. Lesions on the skin of frogs are not particularly deadly on their own but the lesions predisposes the frogs to various dermal infections are very deadly (Berger *et al.*, 1998) The fungus *Batrachochytrium dendrobatidis* which attacks amphibians have been known to wipe out the populations of frogs in relatively pristine habitats in Austraulia, Costa Rica, New Zealand, some parts of Europe and Africa (Reed *et al.*, 2003).

It is also believed that environmental stressors inhibit the production of peptides in the frogs. Skin peptides vary between amphibian species and even geographically (Donnellan *et al.*, 2000). The peptides are believed to cause cell death by disruption of the cell membrane into peptide-coated vesicles (Rollins-Smith *et al.*, 2002). A range of peptides from amphibian skin are active against *B. dendrobatidis* in vitro in a concentration dependent manner. The authors suggest that there may not be enough peptides present on the skin to be effective, or that environmental factors that stress the frog may inhibit production or release of the peptides.

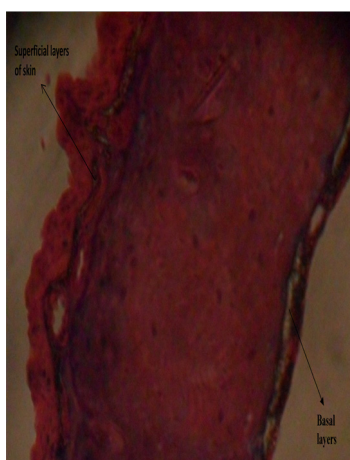


Fig 1: H & E X 400. Normal Skin. In control Treatment

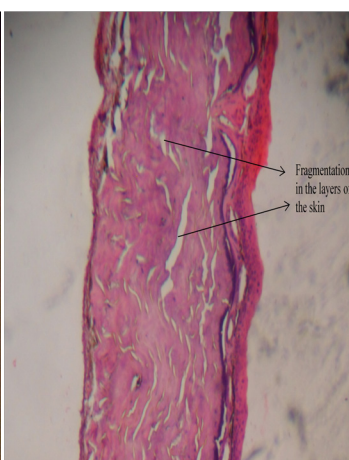


Fig 2: H & E X400 Skin. Treatment at 0.75mg/l crude oil at showing increasing spaces

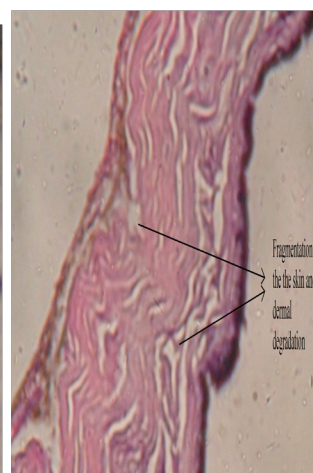


Fig 3: H & E 400. Skin. Treatment 3.0mg/L showing fragmentation of the skin.

There were marked cellular changes in the cells of the liver exposed to WSF of crude oil compared to those of the cells in the control. Evidence of the induced changes were observed such as inflammation of the liver cells, liver cells in disarray and distortion of cells, degenerative liver changes, vacuolation, and cell shrinking. An important observation was a clear cell foci which exhibited an altered staining pattern. Focal lesions are precursors to the development of hepatocellular neoplasm indicating a reduced capacity to metabolize xenobiotics. Hepatocellular foci of altered hepatocytes suggest an early stage in step wise formation of hepatic neoplasia and as such provide an excellent example of histopathological bio-indicators for contaminants exposures (Hinton *et al.*, 1992). Another important change in the liver was necrosis, which is a passive mode of cell death which shows that the capacity to maintain homeostasis was affected. This result is similar to those of (Vincent-Akpu, 2006) in which of liver tissues of *T. guineensis* exposed to drilling fluid (xp-07 and parateq) showed hepatic necrosis and pyknosis of the nucleus. Cloudy swelling, excessive bile secretion, focal necrosis, atrophy and vacuolization have been reported in the in liver of bullfrog (*Hoplobatrachus Occipitalis*) when they were exposed to sub lethal concentrations of cadmium (Ezemonye and Enuneku, 2011). The cellular degeneration in the liver may also be due to oxygen deficiency as a result of degeneration of the liver, heart and or vascular dilation and intravascular haemolysis with subsequent stasis of the blood (Păunescu *et al.*, 2005).

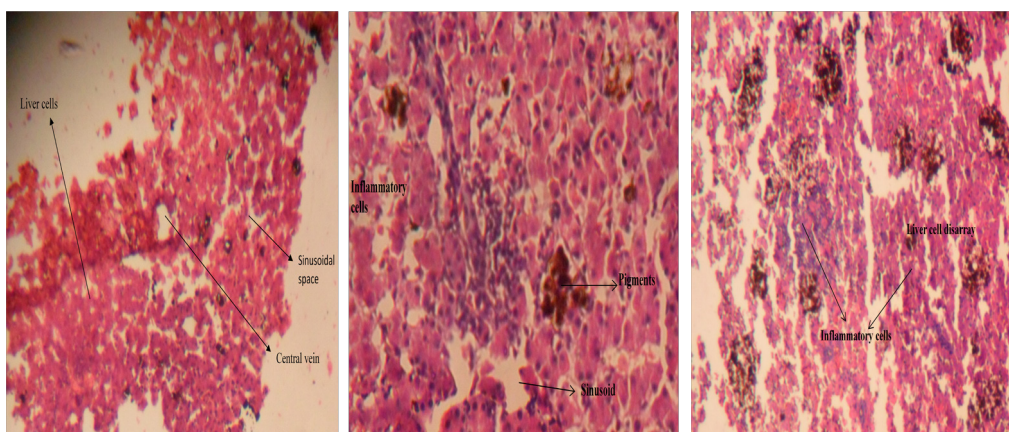


Fig 2a. H&E X400. Normal Liver at 0ml/g

Fig 2b. H & E X400. Liver at 0.75mg/L

Fig 2c. H & E X400. Liver at 3.0mg/L

In histological section, the normal heart section consists of sheets of cardiomyocytes, which are polyhedral. The heart cells of the frogs in the control group showed a normal structural pattern. The cells of the frogs exposed to crude oil showed a gradual fragmentation of the heart tissues. This fragmentation could lead to cardiac dysfunction and peripheral vascular defects, anaemia, and neuronal cell death. After passing through the blood vascular system of the frogs, the WSF of crude oil could have targeted the cardiac tissue, causing extensive damage. The myocardial cell degeneration and necrosis observed in the present study

could be attributed to the excessive calcium accumulation and/or increased catecholamine release, as previously reported by Lennard, and Huddart (1992). The study by **Incardona (2006)** demonstrated that the embryonic heart as the most important target tissue for PAH exposure using the zebrafish model system. They observed that edema and cardiovascular defects are common in fish embryos treated with PAHs. The blockade of cardiac conduction by AhR pathway components with antisense morpholino oligonucleotides have been demonstrated in PAHs treated fish. These studies suggest that transient changes in conduction may have subtle and perhaps irreversible effects during later stages of heart development.

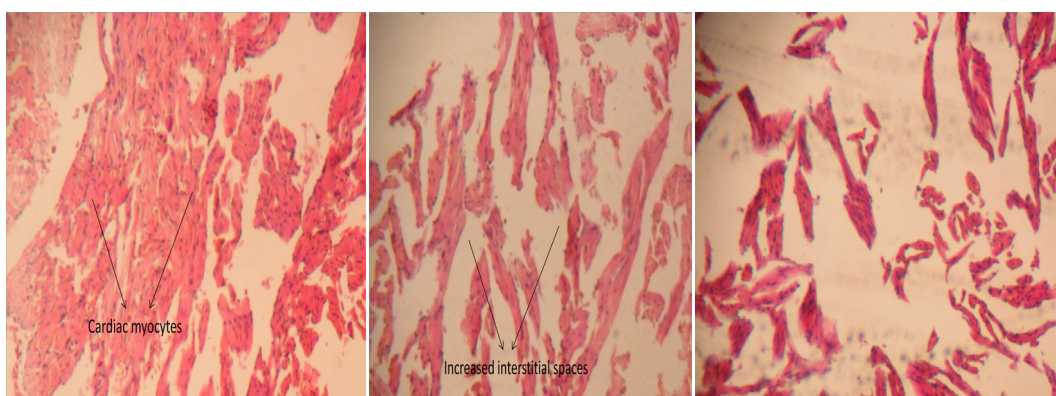


Fig 3a. H & E X400. Heart 0mg/L in WSF of crude oil.

Fig 3b. H & E X400. Heart in 0.75mg/L crude oil showed mild separation of cells.

Fig 3c. H & E X400. Heart in 3.0mg/L showed separation of the cells.

4. Conclusion

Histopathological alterations in frogs exposed to crude oil can be used as sensitive model to monitor aquatic pollution. The current study can be used as sensitive model to monitor aquatic pollution. The current study indicates that crude oil contamination definitively affect the frogs negatively. The use of histopathological responses as important biomarkers for the possible effects of crude oil on amphibians is an important tool for monitoring the health of these tetrapods in areas where they have the potential to be exposed.

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