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# Effect of Chronic Exposure to Petroleum Hydrocarbon Pollution on Oxidative Stress Parameters and Histology of Liver Tissues of Native Fowl (*Gallus domesticus)*

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author LAN designed the study, performed the experiment and wrote the first draft of the manuscript. Author GOCO supervised the experiment, assisted in manuscript preparation. Both authors performed the statistical analysis. All authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

The effect of chronic exposure to petroleum hydrocarbon (PHC) pollution on the concentrations of glucose, serum and liver malondialdehyde (MDA), protein carbonyl and the histology of liver tissues of the native fowl *(Gallus domesticus)* from Egbema in the Niger Delta Area (NDA) was studied. Identical fowls from an unpolluted area of Mbaise served as the control. Results showed no significant difference ($P<0.05$) in the mean glucose concentration obtained for fowls from both environments. The values obtained for serum and liver MDA and protein carbonyl for the test and control fowls were found to be significantly ($P<0.05$) different. There were elevated concentrations of MDA, protein carbonyl in the serum as well as MDA from liver homogenates of fowls from Egbema when compared to those of fowls from Mbaise. Histological changes were also observed in the liver sections of fowls from Egbema as against none in the liver sections of fowls from Mbaise. These changes were characteristically necrotic and inflammatory. Thus, the findings from this study show, in clear terms, that PHC pollution (crude oil and gas flaring) markedly affected the Egbema environment and induced changes in tissues of the native fowl whose nativity and ancestry are from there.

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## 1. INTRODUCTION

Petroleum hydrocarbons are organic substances that are obtained from crude oil, as a result of oil exploration and exploitation activities. Hydrocarbons from petroleum pollution are widely distributed over the environment (air, soil and water) all over the world from oil spillage, purified petroleum products, gas flaring and have directly or indirectly led to environmental health risks. Petroleum hydrocarbon, as a pollutant, consists of complex mixtures of paraffinic alicyclic and aromatic hydrocarbons and a smaller proportion of non-hydrocarbon compounds such as naphthenic acid, phenols, thiols, heterocyclic nitrogen and sulphur compounds [1].

Nigeria is a major world producer of crude oil. Pollution of the environment due to oil spillage has steadily increased as a result of oil activities. Crude oil production and export have brought tremendous financial benefits to the country. Unfortunately, the oil industry has also brought serious pollution problems, resulting from numerous oil spillages, especially in the Niger Delta Area where the bulk of the oil exploration and exploitation are concentrated. In the Niger Delta Areas alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing over 2.8 million barrels of crude oil into the environment [2,3].

The roles played by the oil and gas industries in improving the quality of life in Nigeria cannot be overemphasized. Alongside the good things that brought an enhancement in the standard of living was the emergence of deleterious substances in the environment following oil and gas activities. The industrial pollutants, including $CO_2$ from exhausts of automobiles and other pollutants such as heavy metals, constitute an important source of environmental pollution. In oil drilling operations, crude oil, corrosive acid wastes, toxic chemicals and other harmful industrial wastes are intermittently released into the environment (air, soil and water). Sulphur and other toxic gases, generated by oil companies, are usually released into the atmosphere. These, together with injected particulates and unburned hydrocarbons, undergo series of chemical reactions in the presence of sunlight, resulting in dense characteristic smog [4].

The effect of environmental pollution is enormous. Pollutants, when absorbed by living organisms, cause the release of reactive intermediates which induce changes in tissues of these organisms [5]. Several biochemical parameters have been studied to explain/understand the organism's attempt to cope with the effects of the pollution.

Egbema is an area in the Niger Delta where oil and gas activities have gone on for over fifty years and organisms grown in the area are exposed to the pollution in the environment. The pollutants include spilled crude oil and or its refined products, effluents with traces of heavy metals, particulates and toxicants from gas flaring and green house gases. The Energy Solution Conference (2004) estimated that the Nigeria Delta region has about 123 gas flaring sites [6]. About 45.8 billion kilowatts of heat is released into the atmosphere from 1.8 billion cubic feet of gas burnt daily in the Niger Delta region, leading to temperatures that render large areas non-habitable [7]. These pollutants (crude oil and their products) are considered recalcitrant to (natural) biodegradation and persist in the ecosystem due to their hydrophobicity and low volatility [8].

The oil and gas activities in the Niger Delta have led also to the emergence of undesirable changes in the physical, chemical and biological characteristics of the land, water and air. The changes affect the ecosystem adversely. Animals and plants growing in such environments have, over the years, consumed large doses of harmful pollutants. These pollutants and any products of their degradation (no matter how small) can be carcinogenic, mutagenic, and are potent immune toxicants [9]. Mbaise on the other hand, quite distant from Egbema, has no oil well or gas flaring site and as such may not have experienced petroleum hydrocarbon pollution.

The native fowl (*Gallus domesticus)* was chosen as the experimental species since it has alife span of several years. In exceptional cases, it can live for 10–15 years. It is free-living and grazes in search of food (and water) in the environment. It feeds on insects, earthworm and other worms, larvae, tender leaves of seedlings, grass and other substances in the environment. Its free-living/feeding habit permits it to ingest pollutants as part of the ecosystem food chain (in their free form or localised in its food). The experimental fowls had their nativity in Egbema in the Niger Delta Area and their ancestors had existed in that PHC-polluted area for over fifty years. Control fowls were obtained from Mbaise that has neither oil well nor gas flaring sites is located far away from Egbema. The aim of this study was to investigate the effect of chronic exposure to petroleum hydrocarbon pollution on lipid per oxidation, glucose concentration and protein carbonyl content of the blood and histology of the liver tissue of the native fowl reared in an environment known to be polluted with petroleum hydrocarbon [9].

## 2. MATERIALS AND METHODS

### 2.1 Collection of Experimental Animals

The native fowls (*Gallus domesticus)* used for this study were reared and have their ancestary in Egbema for test and Mbaise for control respectively. Altogether, twelve (12) apparently healthy male fowls (6-9 months old) from each sample area were identified and used.

### 2.2 Preparation of Blood and Liver Tissues

The native fowls were allowed to acclimatize in the laboratory for 24 hours and then sacrificed. Blood was obtained by puncture of the neck artery. Blood sample was collected from each fowl and allowed to stand for 2 hours for clotting to take place. The serum was separated and collected by centrifugation and used for the determination of glucose, MDA and protein carbonyl concentrations. Each fowl was then dissected, the liver collected and stored in the refrigerator at 4ºC until needed for histological study.

### 2.3 Chemicals and Kits

Thiobarbituric acid (TBA), Trichloro acetic acid (TCA), 2, 4-dinitrophenyhydrazine (DNPH and Sodium dodecyl sulphate were bought from Sigma-Aldrich Chemical Company St. Louis, Mo, USA. Other chemicals were from varied local sources and of analytical grade.

Glucose oxidase kit was supplied by Randox Laboratories, USA. 1.5 milliliters of serum was used for the determinatiom. Serum glucose concentration was determined using the glucose oxidase method as described by Trinder [10]. One milliliter of serum and liver homogenate of

each fowl was used for the estimation of lipid per oxidation. Lipid peroxidation was estimated spectrophotometrically by assessing the amount of MDA produced during lipid per oxidation. The MDA produced was assessed using the method described by Varshney and Kale [11]. One milliliter of serum was used protein carbonyl determination. Serum protein carbonyl was determined using the method described by Shacter [12]. Histological study of liver sections was carried out using standard procedure as described by Okoro [13].

## 2.4 Statistical Analysis

Each reading was taken in triplicates. All data were expressed as means ± SD and analyzed for statistical significance by one-way Analysis of Variance (ANOVA) with the aid of a computer-based statistical packed (Graph pad Prism 5.3). Values were considered significant at $P<0.05$.

## 3. RESULTS

The results of glucose concentration in the blood of native fowls from Mbaise and Egbema in the Niger Delta Area is presented in Fig. 1. The figure shows that there was no significant ($P<0.05$) difference in glucose concentration in the blood of fowls from the two environments.

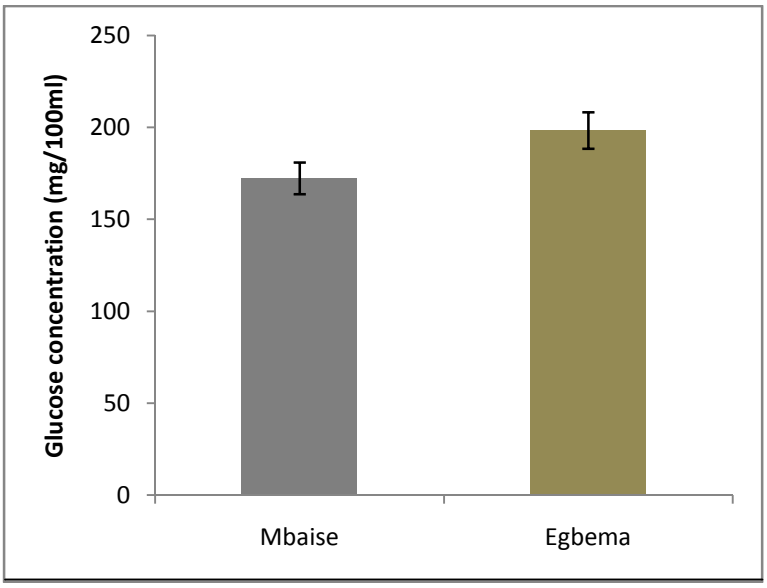


**Fig. 1. Blood glucose concentrations in the (n=12) fowls from Mbaise and Egbema**

Figs. 2 and 3 present the results of serum and liver MDA concentrations of native fowls from Mbaise and Egbema respectively. There were significant ($P<0.05$) differences in the serum and liver MDA concentrations from native fowls from Mbaise and Egbema. Native fowls from Egbem had higher MDA concentrations in their serum and liver tissues than those from Mbaise.

Fig. 4 presents the results of serum protein carbonyl concentration in the serum of the native fowls from both environments. There was a significant ($P<0.05$) difference in the serum

protein carbonyl concentration of fowls from the two environments. Native fowls from Egbema showed a higher serum protein carbonyl concentration than those from Mbaise.

Figs. 5 and 6 present the results of the histology of the liver tissues of the native fowls from Mbaise and Egbema. There were marked differences in the liver architecture of fowls from Egbema from those from Mbaise.

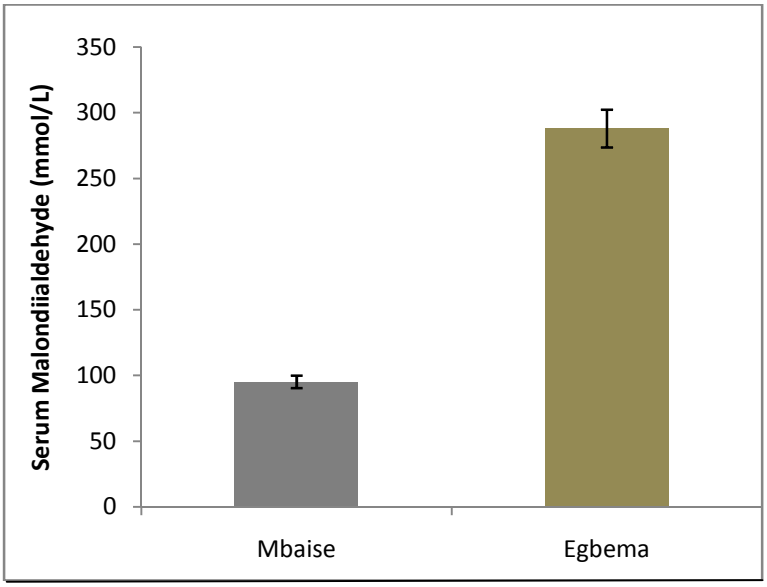


**Fig. 2. MDA concentrations in serum of (n=12) fowls from Mbaise and Egbema**

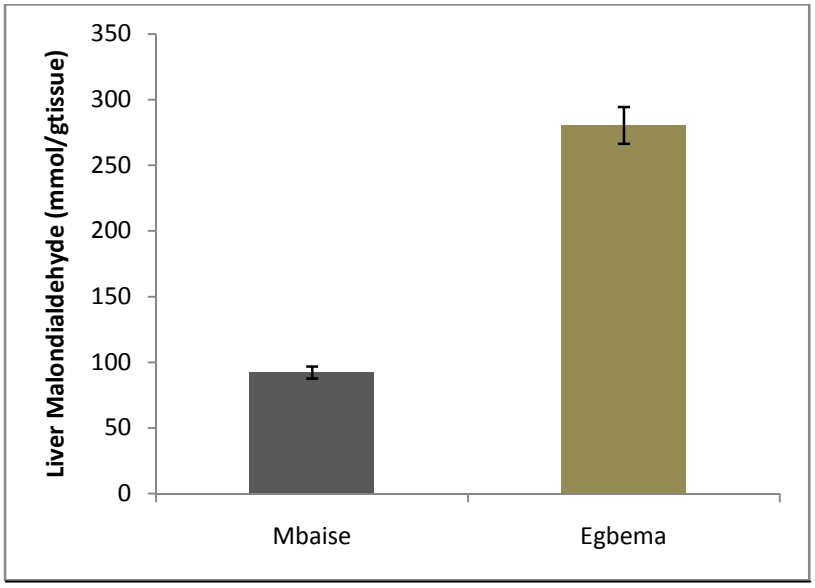


**Fig. 3. Liver MDA concentrations in (n=12) fowls from Mbaise and Egbema**

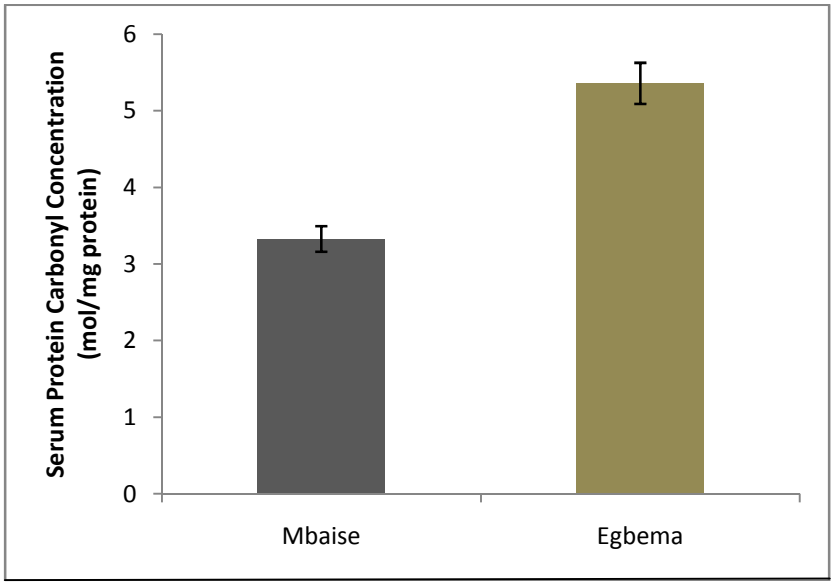


**Fig. 4. Serum protein carbonyl concentrations of (n=12) fowls from Mbaise and Egbema**

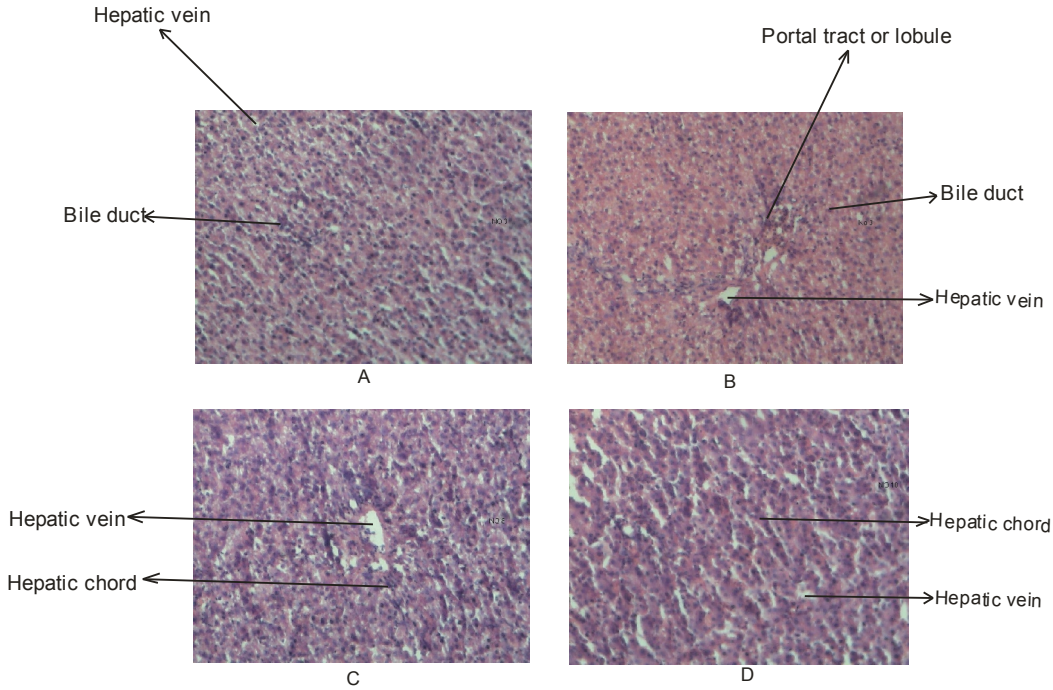


**Fig. 5. Light microphotographs of stained sections of formalin-fixed liver tissues of *Gallus domesticus* from Mbaise (Magnification x 10x10x10)**

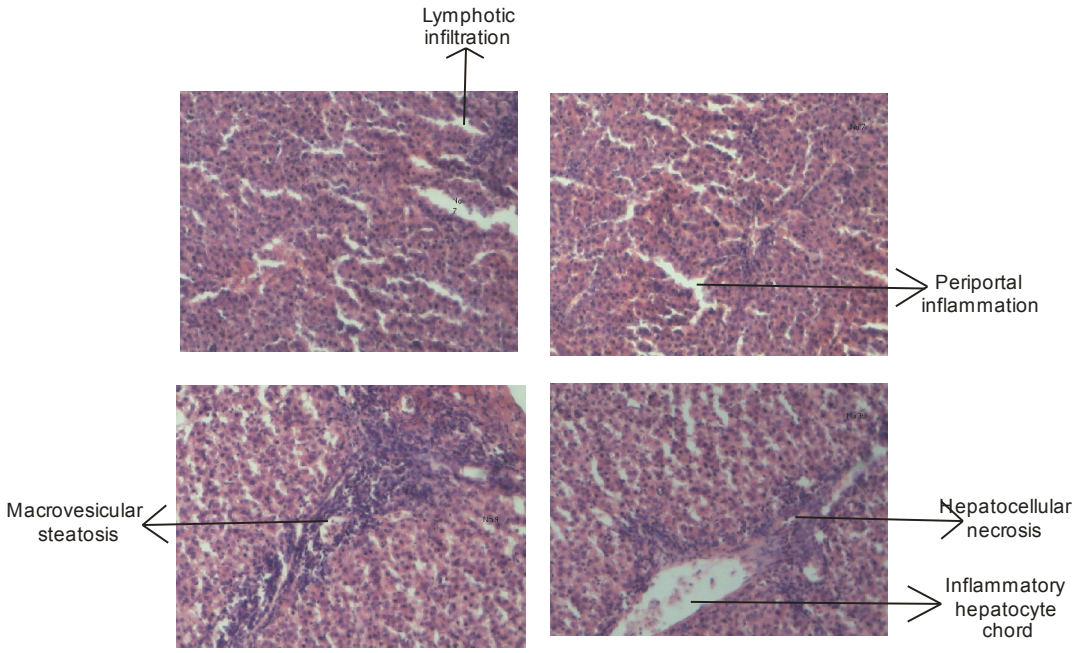


**Fig. 6. Light microphotographs of stained sections of formalin-fixed liver tissues of *Gallus domesticus* from Egbema (Magnification x 10x10x10)**

## 4. DISCUSSION

Glucose concentration in the blood can be used as an index for oxidative stress [14]. High glucose concentration in the blood might be as a result of insulin deficiency or resistance. Hyperglycemia can also be due to the presence of non effective insulin, possibly due to oxidative modification of proteins in association with the pancreatic beta cells. In fact, chronic oxidative stress is the cause of glucose toxicity in the pancreatic islets in diabetes [15]. The mean blood glucose concentration in fowls from Egbema did not differ significantly from those of fowls from Mbaise, Silbergeld [14] reported that blood glucose could be a sensitive indicator of environmental stress in fish. So far, there is no report of any study on blood glucose concentration in terrestrial animals. In this study, however, blood glucose concentration did not respond to established PHC pollution in the NDA. Therefore blood glucose concentration might not be an indicator of PHC pollution in the native fowl.

The results obtained for lipid per oxidation showed that the mean serum and liver MDA concentrations were significantly different ($P<0.05$) in fowls from both environments. The mean values of serum and liver MDA for fowls from Egbema were significantly ($P<0.05$) higher (95.29+0.05 mmol/L) and (288.14+0.03 mmoles/g tissues) as against those for fowls from Mbaise (92.28±0.06 mmol/L) and (280.20+0.74 mmol/g tissues). This observation was not suprising as petroleum hydrocarbon pollution has been reported to induce oxidative stress in animals [16]. Reactive intermediates, when in excess, could mediate lipid per oxidation by causing changes in the permeability of cell membranes [17], as observed in fowls from Egbema as against those from Mbaise. These fowls from Egbema had to cope with the high concentration of reactive intermediates induced by the PHC pollution in their environment. The extent of lipid per oxidation process can be used to assess the progress of oxidative stress in the blood of native fowl, although it is a post mortem event in the

development of the oxidative stress condition. This agrees with the reports of [16] who worked on oxidative stress in Nile tilaoia (*Oreochromis niloticus*) and armored catfish (*Pterygoplicthys anisitsi*) exposed to diesel oil.

Fowls from Egbema gave a mean value for protein carbonyl concentration of 5.34±1.02moles/mg protein as against the value of 3.31±1.01 moles/mg protein obtained for fowls from Mbaise. These means were found to be significantly ($P<0.05$) different. This marked elevation of serum protein carbonyl concentration for fowls from Egbema is attributable to high protein oxidation as a result of petroleum hydrocarbon pollution in that environment. Amino acids and their derivatives are used as biomarkers to assess oxidative protein damage [18,19]. Protein modifications, elicited by direct oxidative attack on lysine, arginine, proline or threonine, by secondary reaction of cysteine, histidine or lysine residues with reactive carbonyl compounds, can lead to the formation of protein carbonyl derivatives [18]. The protein (thiol) moiety of cysteine is highly prone to oxidative attack via several mechanisms such as inhibition of protein synthesis, bonding to sulphydryl groups, competition for sites with essential metabolites, replacement of essential atoms etc leading to serious pathological effects [20]. Petroleum hydrocarbon-induced pollution in Egbema significantly ($P<0.05$) increased the mean concentration of protein carbonyl (5.31±1.02 moles/mg protein) in fowls reared in that environment when compared to the value obtained for fowls from Mbaise (3.31±1.01 moles/mg protein).

Among the various oxidative modifications of amino acids in proteins, it has been reported that protein carbonyl concentration formation is one of the early markers of protein oxidation and it is fairly stable in the living system [21].

The histology of the liver tissues revealed that there was a marked difference in the liver architecture of fowls from Egbema when compared to that of fowls from Mbaise. Fowls from Mbaise displayed normal architecture. The portal tracts contain hepatic artery, hepatic vein and bile ducts. The hepatocyte chords were normal. There was no necro-inflammation seen. However, in the liver tissues of fowls from Egbema, there was slightly implicated inflammation with lymphocytic infilteration and hepatitis; liver tissues displayed diffuse macro vesicular steatosis with progression interphase into steatohepatitis with hepatocellular necrosis. This result corroborates with the reports of [22] who highlighted histological changes in the liver, (kidneys, lungs and brain) of rats after whole exposure to petrol vapour for seven days. Several authors [21,23] implicated free radicals in tissue injury. Histological changes in the liver tissues of fowls from PHC-polluted area of Egbema were characterized by slight necrosis and tissue inflammation.

## 5. CONCLUSION

It follows from this study that the mean values of the concentrations of (MDA) obtained for the serum and liver tissues and the serum protein carbonyl of native fowls from Egbema were significantly ($P<0.05$) higher than those of native fowls from Mbaise. Histological changes were observed in the liver sections of fowls from Egbema when compared to those of fowls from Mbaise .Chronic exposure to petroleum hydrocarbon pollution (crude oil and or gas flaring) has been shown to exert adverse environmental impact on the ecosystem in Egbema in the NDA. This contention is supported by the observed marked adverse changes in tissues of *Gallus domesticus,* employed as a biomarker in this study.

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## COMPETING INTEREST

Authors have declared that no competing interest exist.

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