

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

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7 **ABSTRACT**

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

25 **1. INTRODUCTION**

26 Petroleum hydrocarbons are organic substances that are obtained from crude oil, as a
27 result of oil exploration and exploitation activities. Hydrocarbons from petroleum
28 pollution are widely distributed over the environment (air, soil and water) over the
29 world from oil spillage, purified petroleum products, gas flaring and have directly or
30 indirectly led to environment health risks. Petroleum hydrocarbon, as a pollutant,

31 consists of complex mixtures of paraffinic alicyclic and aromatic hydrocarbons and a
32 smaller proportion of non-hydrocarbon compounds such as naphthenic acid, phenols,
33 thiols, heterocyclic nitrogen and sulphur compounds [1].

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

42 The roles played by the oil and gas industries in improving the quality of life in Nigeria
43 cannot be overemphasized. Alongside the good things that brought an enhancement in
44 the standard of living was the emergence of deleterious substances in the environment
45 following oil and gas activities. The industrial pollutants, including CO₂ from exhausts of
46 automobiles and other pollutants such as heavy metals, constitute an important source of
47 environmental pollution. In oil drilling operations, crude oil, corrosive acid wastes, toxic
48 chemicals and other harmful industrial wastes are intermittently released into the
49 environment (air, soil and water). Sulphur and other toxic gases, generated by oil
50 companies, are usually released into the atmosphere. These, together with injected
51 particulates and unburned hydrocarbons, undergo series of chemical reactions in the
52 presence of sunlight, resulting in dense characteristic smog [4].

53 The effect of environmental pollution is enormous. Pollutants, when absorbed by living
54 organisms, cause the release of reactive intermediates which induce changes in tissues
55 of these organisms [4]. Several biochemical parameters have been studied to
56 explain/understand the organism's attempt to cope with the effects of the pollution.

57 Egbema is an area in the Niger Delta where oil and gas activities have gone on for over
58 fifty years and organisms grown in the area are exposed to the pollution in the
59 environment. The pollutants include spilled crude oil and or its refined products,

60 effluents with traces of heavy metals, particulates and toxicants from gas flaring and
61 green house gases. The Energy Solution Conference (2004) estimated that the Niger
62 Delta region has about 123 gas flaring sites [5]. About 45.8 billion kilowatts of heat is
63 released into the atmosphere from 1.8 billion cubic feet of gas burnt daily in the Niger
64 Delta region, leading to temperatures that render large areas non-habitable [5]. These
65 pollutants (crude oil and their products) are considered recalcitrant to (natural)
66 biodegradation, and persist in the ecosystem due to their hydrophobicity and low
67 volatility [6].

68 The oil and gas activities in the Niger Delta have led also to the emergence of undesirable
69 changes in the physical, chemical and biological characteristics of the land, water and air.
70 The changes affect the ecosystem adversely [6,7,8]. Animals and plants growing in such
71 environments have, over the years, consumed large doses of harmful pollutants. These
72 pollutants and any products of their degradation (no matter how small) can be
73 carcinogenic, mutagenic, and are potent immunotoxicants [9]. Mbaise on the other hand,
74 quite distant from Egbema, has no oil well or gas flaring site and as such may not have
75 experienced petroleum hydrocarbon pollution.

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

89 **2.0 MATERIALS AND METHODS**

90 2.1. Collection of experimental animals.

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

95 2.2. Preparation of blood and liver tissues

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

103 2.3 .Chemicals and Kits

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

108 Glucose oxidase kit was supplied by Randox Laboratories, USA. 1.5 milliliters of serum
109 was used for the determination. Serum glucose concentration was determined using the
110 glucose oxidase method as described by Trinder [10]. One milliliter of serum and liver
111 homogenate of each fowl was used for the estimation of lipid peroxidation. Lipid
112 peroxidation was estimated spectrophotometrically by assessing the amount of MDA
113 produced during lipid peroxidation. The MDA produced was assessed using the method
114 described by Varshney and Kale [11]. One milliliter of serum was used protein carbonyl
115 determination. Serum protein carbonyl was determined using the method described by

116 Shacter [12]. Histological study of liver sections was carried out using standard
117 procedure as described by Okoro [13].

118 **2.4 Statistical Analysis**

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

123 **3. RESULTS**

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

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

133 Figure 4 presents the results of serum protein carbonyl concentration in the serum of the
134 native fowls from both environments. There was a significant ($P < 0.05$) difference in the
135 serum protein carbonyl concentration of fowls from the two environments. Native fowls
136 from Egbema showed a higher serum protein carbonyl concentration than those from
137 Mbaise.

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

141 4. DISCUSSION

142 Glucose concentration in the blood can be used as an index for oxidative stress [14]. High
143 glucose concentration in the blood might be as a result of insulin deficiency or resistance.
144 Hyperglycemia can also be due to the presence of non effective insulin, possibly due to
145 oxidative modification of proteins in association with the pancreatic beta cells. Infact,
146 chronic oxidative stress is the cause of glucose toxicity in the pancreatic islets in diabetes
147 [15]. The mean blood glucose concentration in fowls from Egbema did not differ
148 significantly from those of fowls from Mbaise, Silbergeld [14] reported that blood
149 glucose could be a sensitive indicator of environmental stress in fish. So far, there is no
150 report of any study on blood glucose concentration in terrestrial animals. In this study,
151 however, blood glucose concentration did not respond to established PHC pollution in the
152 NDA. Therefore blood glucose concentration might not be an indicator of PHC pollution
153 in the native fowl.

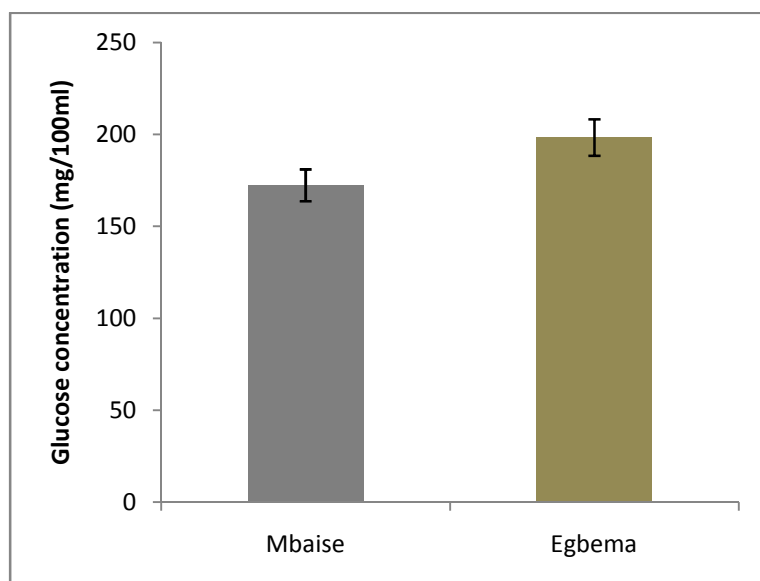


Figure 1: Blood glucose concentrations in the (twelve) fowls from Mbaise and Egbema

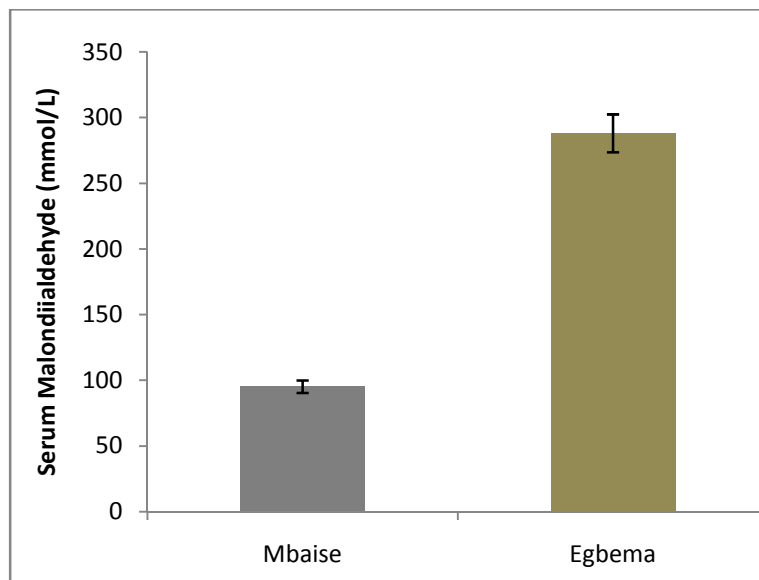


Figure 2: MDA concentrations in serum of (twelve) fowls from Mbaise and Egbema

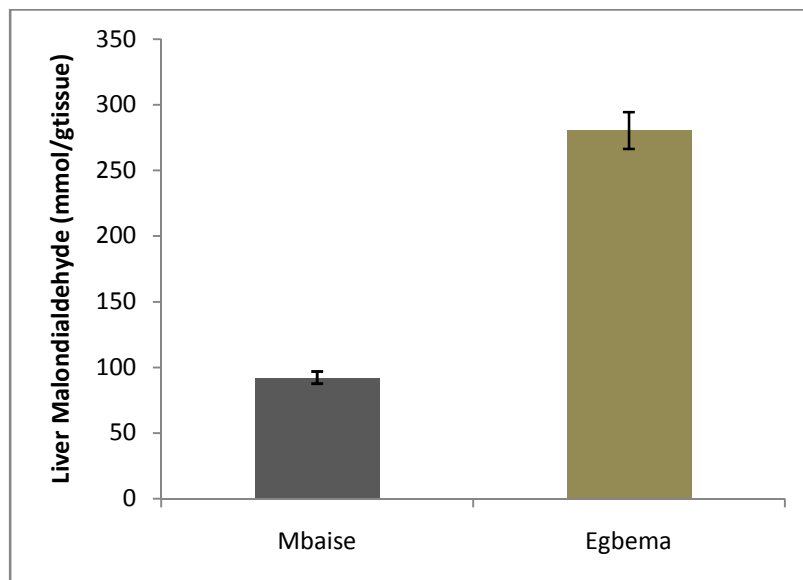


Figure 3: Liver MDA concentrations in (twelve) fowls from Mbaise and Egbema

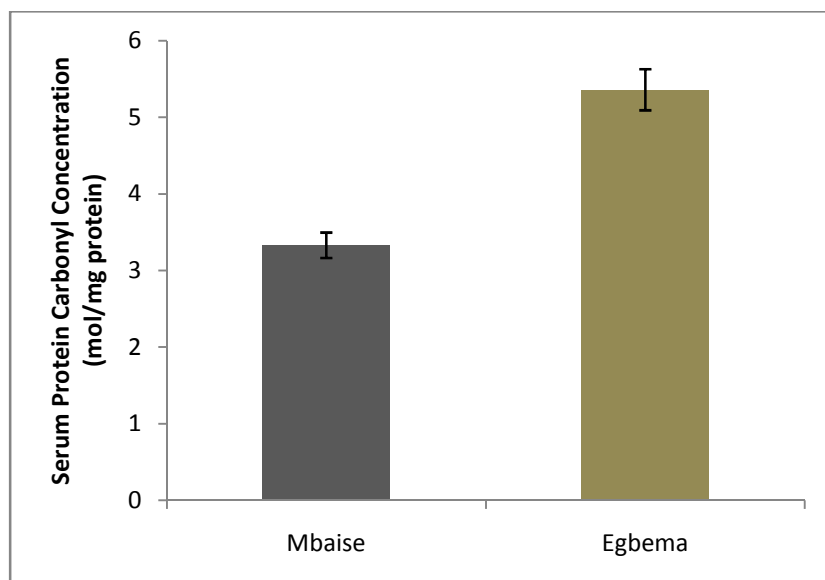


Fig. 4: Serum protein carbonyl concentrations of (twelve) fowls from Mbaise and Egbema.

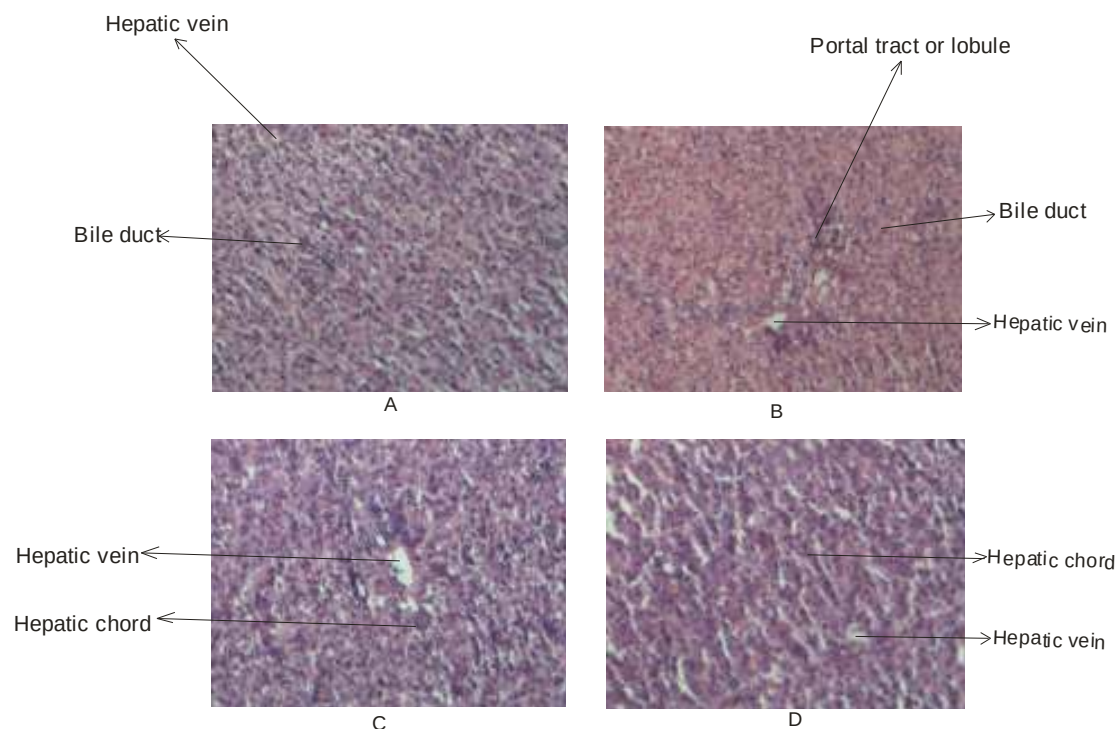


Figure 5: Light microphotographs of stained sections of formalin-fixed liver tissues of *Gallus domesticus* from Mbaise (Magnification x 10x10x10).

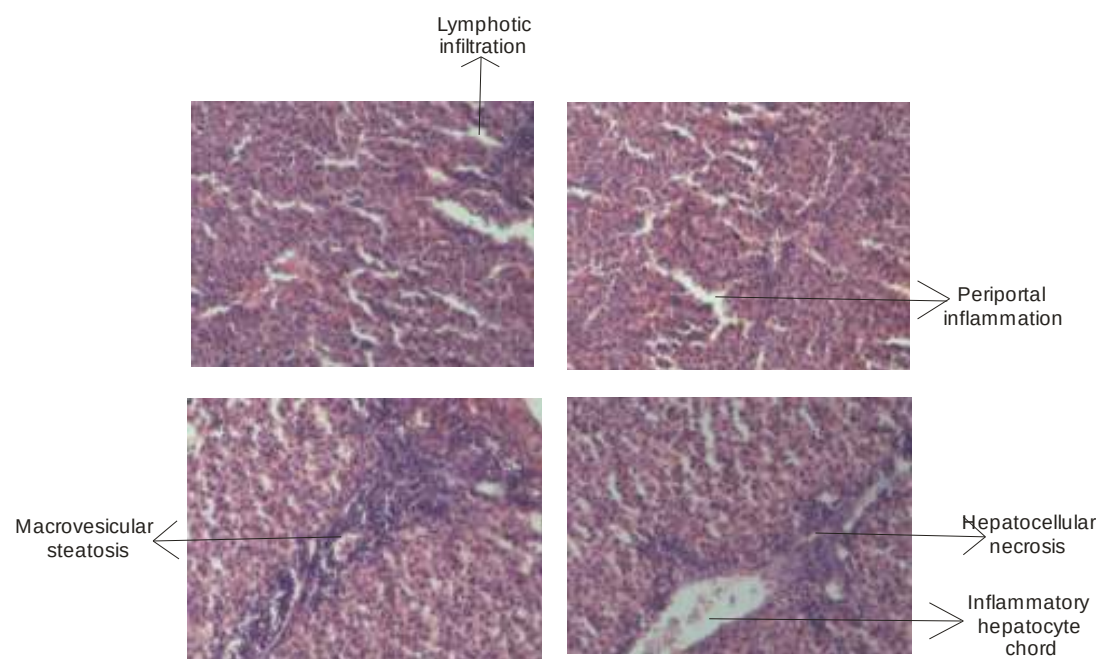


Figure 6: Light microphotographs of stained sections of formalin-fixed liver tissues of *Gallus domesticus* from Egbema (Magnification x 10x10x10)

154 The results obtained for lipid peroxidation showed that the mean serum and liver MDA
 155 concentrations were significantly different ($P < 0.05$) in fowls from both environments.
 156 The mean values of serum and liver MDA for fowls from Egbema were significantly
 157 ($P < 0.05$) higher (95.29 ± 0.05 mmol/L) and (288.14 ± 0.03 mmoles/g tissues) as against
 158 those for fowls from Mbaise (92.28 ± 0.06 mmol/L) and (280.20 ± 0.74 mmol/g tissues).
 159 This observation was not surprising as petroleum hydrocarbon pollution has been reported
 160 to induce oxidative stress in animals [16]. Reactive intermediates, when in excess, could
 161 mediate lipid peroxidation by causing changes in the permeability of cell membranes
 162 [17], as observed in fowls from Egbema as against those from Mbaise. These fowls from
 163 Egbema had to cope with the high concentration of reactive intermediates induced by the PHC
 164 pollution in their environment. The extent of lipid peroxidation process can be used to
 165 assess the progress of oxidative stress in the blood of native fowl, although it is a post
 166 mortem event in the development of the oxidative stress condition. This agrees with the
 167 reports of [16] who worked on oxidative stress in Nile tilapia (*Oreochromis niloticus*)
 168 and armored catfish (*Pterygoplichthys anisitsi*) exposed to diesel oil.

169 Fowls from Egbema gave a mean value for protein carbonyl concentration of 5.34 ± 1.02
170 moles/mg protein as against the value of 3.31 ± 1.01 moles/mg protein obtained for fowls
171 from Mbaise. These means were found to be significantly ($P < 0.05$) different. This
172 marked elevation of serum protein carbonyl concentration for fowls from Egbema is
173 attributable to high protein oxidation as a result of petroleum hydrocarbon pollution in
174 that environment.

175 Amino acids and their derivatives are used as biomarkers to assess oxidative protein
176 damage [18, 19]. Protein modifications, elicited by direct oxidative attack on lysine,
177 arginine, proline or threonine, by secondary reaction of cysteine, histidine or lysine
178 residues with reactive carbonyl compounds, can lead to the formation of protein carbonyl
179 derivatives [18]. The protein (thiol) moiety of cysteine is highly prone to oxidative attack
180 via several mechanisms such as inhibition of protein synthesis, bonding to sulphhydryl
181 groups, competition for sites with essential metabolites, replacement of essential atoms
182 etc leading to serious pathological effects [20]. Petroleum hydrocarbon-induced pollution
183 in Egbema significantly ($P < 0.05$) increased the mean concentration of protein carbonyl
184 (5.31 ± 1.02 moles/mg protein) in fowls reared in that environment when compared to the
185 value obtained for fowls from Mbaise (3.31 ± 1.01 moles/mg protein). Among the various
186 oxidative modifications of amino acids in proteins, it has been reported that protein
187 carbonyl concentration formation is one of the early markers of protein oxidation and it is
188 fairly stable in the living system [21].

189 The histology of the liver tissues revealed that there was a marked difference in the liver
190 architecture of fowls from Egbema when compared to that of fowls from Mbaise. Fowls
191 from Mbaise displayed normal architecture. The portal tracts contain hepatic artery,
192 hepatic vein and bile ducts. The hepatocyte chords were normal. There was no necro-
193 inflammation seen. However, in the liver tissues of fowls from Egbema, there was
194 slightly implicated inflammation with lymphocytic infiltration and hepatitis; liver tissues
195 displayed diffuse macrovesicular steatosis with progression interphase into steatohepatitis
196 with hepatocellular necrosis. This result corroborates with the reports of [22] who
197 highlighted histological changes in the liver, (kidneys, lungs and brain) of rats after

198 whole exposure to petrol vapour for seven days. Several authors [22, 23] implicated free
199 radicals in tissue injury. Histological changes in the liver tissues of fowls from PHC-
200 polluted area of Egbema were characterized by slight necrosis and tissue inflammation.

201 **5. CONCLUSION**

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

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214 **COMPETING INTEREST**

215 Authors have declared that no competing interest exist.

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