

## Research Article



# Effects of Heavy Metal Contamination on Blood Parameters, Egg Quality, and Histopathology of Layer Chickens influenced by Crude Oil Exploration

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### ABSTRACT

**Introduction:** Petrochemical pollution from oil spills, gas flaring, and effluents containing heavy metals is prevalent in the oil-producing regions of Delta State, Nigeria, posing systemic health risks to humans and animals. The present study assessed the effects of crude oil exploration on laying chickens across 21 poultry farms in seven zones of Delta State, Nigeria.

**Materials and methods:** Blood, egg, and tissue samples (liver and kidney) from 105 layers, comprising 15 chickens from each zone (Aniocha, Ijaw, Ika, Isoko, Itsekiri, Ukwani, and Urhobo), were analyzed for hematological, biochemical, and histological parameters.

**Results:** The current findings revealed that packed cell volume (PCV) was significantly higher in Urhobo (45.56%) and Ijaw (39.22%) compared to the normal range of PCV. Hemoglobin levels ranged from 12.77 g/dL (Aniocha) to 15.46 g/dL (Ijaw), while white blood cell counts varied from 6.37  $\mu$ L (Aniocha) to 8.40  $\mu$ L (Urhobo). Red blood cell counts were significantly lower than the normal range from 4.01  $\mu$ L (Isoko) to 5.10  $\mu$ L (Urhobo). Serum albumin levels peaked in Urhobo (5.27 g/dL), whereas lower values were observed in Isoko and Ijaw farms. Alanine aminotransferase was elevated in Urhobo (43.83 IU/L), Itsekiri (38.72 IU/L), and Ukwani (44.51 IU/L), exceeding physiological norms. Cadmium concentrations exceeded permissible limits across all zones, with the highest level in Urhobo (21.032 ppm). The current findings highlighted the presence of environmental toxicity associated with oil-related pollution, signifying disruptions in blood chemistry, egg quality, and organ function in poultry.

**Conclusion:** Elevated levels of hematological and biochemical parameters beyond physiological norms pose a threat to animal health, compromise food safety, and endanger public health, underscoring the critical need for environmental monitoring and remediation in Delta State, Nigeria.

## 1. Introduction

Crude oil extraction in Nigeria's Delta region leads to severe environmental and health impacts, primarily from heavy metal pollution in water, soil, and air. Efforts to mitigate the effects of crude oil spillage in the Niger Delta have included bioremediation techniques, such as the use of microorganisms to degrade hydrocarbons in contaminated soils, and phytoremediation using plants to absorb heavy metals<sup>1,2</sup>. Several studies have demonstrated the efficacy of bioaugmentation in reducing hydrocarbon levels in oil-polluted sites, improving soil quality for agricultural use<sup>3</sup>. However, these efforts are often limited by inadequate funding, inconsistent implementation, and insufficient monitoring of restored areas<sup>4</sup>. Activities such as oil well

operations, tank farm storage, waste dumping, gas flaring, and petrochemical effluent discharge elevate concentrations of heavy metals such as vanadium, cadmium, mercury, nickel, and lead in ecosystems<sup>5,6</sup>. Gas flaring, a persistent practice, emits sulfur dioxide and nitrogen oxides, contributing to acid rain that degrades soil fertility, reduces biodiversity, and damages economic crops<sup>7</sup>. Crude oil pollution causes extensive, multi-system damage to human health and animal welfare, disrupting fundamental biological processes, including growth pathways, and reproductive success<sup>8,9</sup>. Therefore, this environmental degradation disrupts agricultural systems to the region's socio-economic stability. For over five decades, crude oil

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refining companies have released a wide range of air pollutants, water, and solid hazardous wastes into the environment, which have become a major concern to human health, animal welfare, and husbandry in Nigeria<sup>10</sup>. Poultry production, a vital source of eggs and meat, supports nutritional security and economic livelihoods in Nigeria<sup>11,12</sup>. However, heavy metal pollution from crude oil activities compromises poultry health and productivity. Previous studies reported that chickens in contaminated environments exhibit decreased feed intake, lower hatchability rates, stunted growth, and compromised organ function<sup>13,14</sup>. The intersection of crude oil pollution, animal health, and human nutrition necessitates integrated solutions to protect poultry's role in food security without compromising ecosystem integrity. The present study investigated the impacts of crude oil contamination on sustainable egg production protocols, food safety measures and poultry health poultry farms operating in petroleum-affected regions in Niger Delta.

## 2. Materials and Methods

### 2.1. Ethical approval

The animals in the present study were handled and treated humanely, with all procedures conducted in accordance with the guidelines approved by the Animal Welfare and Ethics Committee of the Teaching and Research Farm, University of Ibadan, Nigeria (Approval No. AWE2018/0013478).

### 2.2. Study area

Twenty-one poultry farm sites in local government areas of Delta State, Nigeria, were selected due to the presence of crude oil extraction and refining activities in these areas. Farm clusters in seven different zones were grouped according to geo-locations, including Aniocha, Ijaw, Ika, Isoko, Itsekiri, Ukwani, and Urhobo, respectively.

### 2.3. Blood sampling and haematological analysis

The study utilized a total of 105, over 36 weeks old, Isa Brown layers, randomly selected through a stratified sampling approach across seven agricultural zones in Delta State. From each zone, three farms were included, with five chickens sampled per farm. Blood samples (5 mL each) were collected from three-layer chickens per farm following an overnight fast. Using sterile 24-gauge needles attached to 5 mL syringes, blood was drawn and immediately divided. The 2.5 mL was transferred to pre-labeled EDTA-coated vacutainers (gently inverted to prevent clotting), while the remaining 2.5 mL was aliquoted into plain test tubes for serum separation. The non-anticoagulated samples were allowed to clot at room temperature, then centrifuged at  $3000 \times g$  for 15 minutes to separate serum from cellular components. The serum was decanted into a clean, labelled tube for serum analysis, and hemolyzed blood samples were discarded. The tubes containing blood were taken for serum biochemical and haematological analysis in the laboratory, using routine clinical laboratory procedures. Haematological Parameters such as packed cell volume (PCV, %), red blood cell count (RBC,  $\times 10^6/\mu\text{L}$ ), white blood

cell counts (WBC,  $\times 10^3/\mu\text{L}$ ), mean corpuscular volume (MCV, fL), Haemoglobin (Hb, g/dL), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin count (MCHC, %), and Leucocyte count were determined. Serum indices were total protein (TP), Albumin (Alb), cholesterol, Globulin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Uric acid.

### 2.4. Histological analysis

Following euthanasia by exsanguination, representative tissue sections (0.5-1 g) were aseptically collected from the central hepatic lobe and renal cortex/medulla. Samples were immediately fixed in 10% neutral buffered formalin for 24 hours to ensure optimal histopathological preservation<sup>15,16</sup>. The tissues were dehydrated through a series of ethanol solutions (70%, 80%, 90%, and 100%), followed by clearing in xylene to eliminate ethanol. Tissues were then embedded in molten paraffin wax and allowed to harden in molds<sup>17</sup>. The paraffin-embedded tissues were cut into 4-5  $\mu\text{m}$  sections using a microtome and placed on glass slides. Sections were subsequently stained with hematoxylin for 5 to 10 minutes and eosin for 2 to 5 minutes to highlight cellular details. After staining, the slides were dehydrated in graded ethanol, cleared in xylene, and sealed with a cover slip using dibutylphthalate polystyrene xylene (DPX) mounting medium. The prepared slides were examined under a light microscope (Zeiss Axioscope, Germany) at  $\times 400$  magnification to detect signs of inflammation and necrosis. A scoring system, as described by Jones<sup>18</sup> was used to assess the extent of inflammatory cell infiltration.

### 2.5. Statistical analysis

The experimental data were analyzed using one-way analysis of variance (ANOVA), with post-hoc pairwise comparisons performed using Fisher's least significant difference (LSD) test at  $p \leq 0.05$  significance level, implemented in SAS version 9.2.

## 3. Results and Discussion

### 3.1. Haematological indices

Table 1 presents the haematological indices of layer chickens across crude oil-affected zones in Delta State, Nigeria. The PCV varied significantly ( $p < 0.05$ ), with the highest value in Urhobo (45.56%), followed by Ijaw (39.22%), Itsekiri (38.11%), Ukwani (37.56%), and Ika (37.78%). These values exceeded the normal range (31.50-36.70%) reported by Jones<sup>18</sup>, while Aniocha (31.67%) remained within the normal range. These elevated PCV levels suggested dehydration, possibly due to reduced water intake resulting from heavy metal contamination<sup>19,20</sup>. Decreased PCV in different chicken breeds (FUNNAB Alfa, Ross308, and native chickens) exposed to crude oil flaring, contrasting with the present findings, suggesting that exposure effects may vary with duration or intensity<sup>21</sup>. High PCV may indicate compensatory responses to hypoxia or chronic stress, as dehydration increases blood viscosity<sup>21</sup>. Hemoglobin levels

varied significantly across zones ( $p < 0.05$ ), ranging from 10.22 g/dL (Isoko) to 15.46 g/dL (Ijaw). Six study sites exceeded the normal reference range (10.7-12.20 g/dL). Isoko (10.22 g/dL) was the only zone that did not differ from normal levels. Elevated Hb may signal dehydration or adaptation to low oxygen availability due to environmental pollutants<sup>22</sup>. Studies comparing medicated and non-medicated broilers under humid tropical conditions indicated that environmental pollution, rather than dietary

factors, likely drives Hb variations in Delta State. The lower Hb levels observed in Isoko (10.22 g/dL) could reflect either reduced crude oil exposure or potential genetic resilience in local poultry populations. Alternatively, these differences may stem from farm-specific management practices or comparatively lower heavy metal contamination in this zone<sup>22</sup>. The WBC values ranged from  $6.37 \times 10^3/\mu\text{L}$  (Aniocha) to  $8.40 \times 10^3/\mu\text{L}$  (Urhobo), which was significantly different ( $p < 0.05$ ) from the normal range.

**Table 1.** Haematological indices of layer chickens from different zones of petroleum-affected regions in Niger Delta, Nigeria in 2021

Parameters	Zones							SEM	Standard range
	Urhobo	Isoko	Ijaw	Itsekiri	Ukwani	Aniocha	Ika		
PCV (%)	45.56 <sup>a</sup>	32.34 <sup>c</sup>	39.22 <sup>b</sup>	38.11 <sup>b</sup>	37.56 <sup>b</sup>	31.67 <sup>c</sup>	37.78 <sup>b</sup>	0.68	31.5-36.70
Hb (g/dL)	13.12 <sup>b</sup>	10.22 <sup>c</sup>	15.46 <sup>a</sup>	12.67 <sup>b</sup>	12.54 <sup>b</sup>	12.77 <sup>b</sup>	13.30 <sup>b</sup>	2.23	10.7-12.20
Platelets ( $\times 10^3/\text{mm}^3$ )	90.22 <sup>a</sup>	84.56 <sup>a</sup>	95.11 <sup>a</sup>	69.33 <sup>b</sup>	85.56 <sup>b</sup>	65.56 <sup>c</sup>	21.56 <sup>d</sup>	16.19	8.22-8.88
WBC ( $\times 10^3/\mu\text{L}$ )	8.40 <sup>a</sup>	7.40 <sup>bc</sup>	7.79 <sup>b</sup>	6.84 <sup>cd</sup>	7.43 <sup>bc</sup>	6.37 <sup>d</sup>	7.82 <sup>b</sup>	0.10	4.90-9.70
MCH (pg)	30.01 <sup>a</sup>	26.47 <sup>c</sup>	30.04 <sup>a</sup>	29.90 <sup>a</sup>	28.76 <sup>ab</sup>	27.96 <sup>b</sup>	28.54 <sup>ab</sup>	0.24	31.9-40.9
MCHC (%)	34.40 <sup>a</sup>	33.30 <sup>a</sup>	33.70 <sup>a</sup>	36.90 <sup>a</sup>	33.70 <sup>a</sup>	33.60 <sup>a</sup>	33.20 <sup>a</sup>	47.83	30.00-34.40
MCV (fl)	90.03 <sup>b</sup>	79.41 <sup>c</sup>	90.12 <sup>b</sup>	94.33 <sup>a</sup>	89.76 <sup>b</sup>	80.78 <sup>c</sup>	79.36 <sup>c</sup>	0.82	102.0-129.0
RBC ( $\times 10^6/\mu\text{L}$ )	5.10 <sup>a</sup>	4.01 <sup>b</sup>	4.37 <sup>b</sup>	4.46 <sup>b</sup>	4.86 <sup>a</sup>	4.15 <sup>b</sup>	4.22 <sup>b</sup>	0.07	10.30-12.90

Standard range: Jones<sup>18</sup>; <sup>abcd</sup> means that within the rows with the same superscript letters are not significantly different ( $p > 0.05$ ). Hb: Haemoglobin, PCV: Packed cell volume, MCHC: Mean cell haemoglobin count, MCV: Mean cell volume, MCH: Mean cell haemoglobin, SEM: Standard Error of mean.

Increased WBC suggested immune activation from toxicants as reported for grower rabbits fed shea butter nut meal in lowland climatic conditions in Nigeria<sup>23</sup>. Reports of dietary effects on WBC changes may stem from crude oil-related stress as observed in the seven zones of the study. These values aligned with those for broiler chickens fed rice husk supplemented with oyster mushrooms and biozyme in South West Nigeria, where crude oil exploration is not as high as that found in Delta State<sup>24</sup>. Elevated MCHC in Urhobo and Itsekiri indicated red cell dehydration, and values fell within the range as reported for broiler chickens and Wistar rats<sup>25,26</sup>. Stable MCH values across all zones indicated normal red cell function; these values remained intact, reflecting a positive impact on digestibility indices, as reported in broiler chickens<sup>19,27</sup>. The value obtained for MCV, ranging from 79.36 to 94.33 fL, was within normal ranges, suggesting no microcytic anemia. The RBC counts ranged from 4.01 to  $5.10 \times 10^6/\mu\text{L}$  were significantly ( $p < 0.05$ ) lower than normal ( $10.30-12.90 \times 10^6/\mu\text{L}$ ), indicating anemia, likely due to chronic toxicity as reported by Alabi et al.<sup>21</sup>

### 3.2. Serum biochemical indices

Table 2 demonstrates that heavy metal contamination in poultry drinking water induces hepatorenal degeneration, presenting critical health risks to poultry populations. Serum ALT and AST activities are reliable indicators of tissue damage, especially in the liver, which serves as the primary site for the metabolism of heavy metals<sup>28</sup>. Elevated ALT levels were associated with necrotic lesions in internal organs, while AST alterations reflected more widespread tissue damage<sup>28</sup>. The AST value in laying chicken varied across zones. Isoko (83.58 U/L), Ika (37.13 U/L), Urhobo (122.72 U/L), Ijaw (108.65 U/L), Itsekiri (106.58 U/L), Ukwani (123.71 U/L), and Aniocha (95.11 U/L). These levels

were generally within normal ranges, except for Ika, which showed notably lower ( $p < 0.05$ ) AST activity. Serum ALT levels exhibited both normal and elevated concentrations, indicating variable degrees of tissue damage. Unlike previous studies, which reported significant increases in ALT and AST in rats exposed to HgCl<sub>2</sub>, the present study found no consistent elevation ( $p < 0.05$ ) in enzyme levels across all zones<sup>29</sup>. However, elevated ALT levels among layer chickens in seven zones in the present study suggested necrotic lesions, particularly in the liver<sup>30</sup>. The observed AST values in layer chickens across the seven zones of Delta State, were primarily within normal ranges and this contrast with reports on mercury-induced enzyme elevation in other species<sup>29</sup>, demonstrating significant elevations in AST levels in fish (*Labeo rohita*) exposed to sublethal concentrations of mercury, while attributing these increases to hepatocellular damage and leakage of cytoplasmic enzymes into the bloodstream due to mercury's oxidative stress and disruption of cell membrane integrity<sup>30</sup>. This discrepancy may reflect species-specific responses or differences in exposure levels. Mercury exposure increases AST activity and causes vascular degeneration in the kidneys, as well as liver necrosis, in chickens<sup>31</sup>; yet, such severe histopathological changes were not uniformly observed in the current findings. Hepatic degeneration occurs through heavy metal bioaccumulation, where HgCl<sub>2</sub>-mediated DNA damage triggers characteristic peri-portal fatty infiltration and necrosis<sup>32</sup>. These findings suggested that while heavy metal toxicity affected serum biochemical parameters, the extent of damage varied by region and exposure intensity. While most AST values remained within normal ranges, elevated ALT levels in some chickens indicate liver damage, reinforcing ALT's utility as a biomarker for hepatic dysfunction<sup>28,33</sup>. The current results contrast with prior reports of significant enzyme elevation due to mercury exposure, suggesting poultry may exhibit unique resilience

or differing metabolic responses<sup>28</sup>.

**Table 2.** Serum biochemical indices of laying chickens in petroleum-affected regions in Niger Delta, Nigeria during 2021

Parameters	Urhobo	Isoko	Ijaw	Itsekiri	Ukwani	Aniocha	Ika	SEM	Standard rage
Total protein (g/dL)	13.82a	12.28ab	14.38a	10.72b	12.281ab	12.523ab	12.943ab	0.300	5.20-6.90
Albumin (g/dL)	5.27a	0.87c	3.48b	1.56c	3.45b	1.64c	1.16c	0.26	2.10-3.45
Direct Bilirubin (mg/dL)	0.80a	0.13c	0.82a	0.23c	0.52b	0.25c	0.17c	0.04	0.1-0.3
Indirect Bilirubin (mg/dL)	1.09a	0.17c	1.09a	0.31c	0.67b	0.45bc	0.22c	0.06	0.2-0.8
Cholesterol (mg/dL)	104.82a	86.68a	89.32a	91.90a	103.98a	94.11a	90.82a	1.744	52.0-148.0
Uric acid (mg/dL)	4.98b	4.84b	4.29b	4.53b	3.96b	4.24b	9.54a	0.81	2.47-8.08
ALP (IU/L)	35.11a	21.11c	36.00a	12.67c	18.00b	12.67c	12.00c	5.05	24.50-44.40
AST (IU/L)	122.72a	83.58b	108.65ab	106.58ab	123.71a	95.11b	37.13c	0.926	88.0-208.0
ALT (IU/L)	43.83a	29.85b	29.17b	38.72ab	44.51a	34.48ab	13.26c	1.81	9.50-37.20

Standard range reference: Jones<sup>18</sup>; abc means that the values within the rows with the same superscript letters are not significantly different ( $p > 0.05$ ). AST: Aspartase amino transferase, ALT: Alanine amino transferase, ALP: Alkaline Phosphatase, SEM: Standard Error of mean.

### 3.3. Histological findings

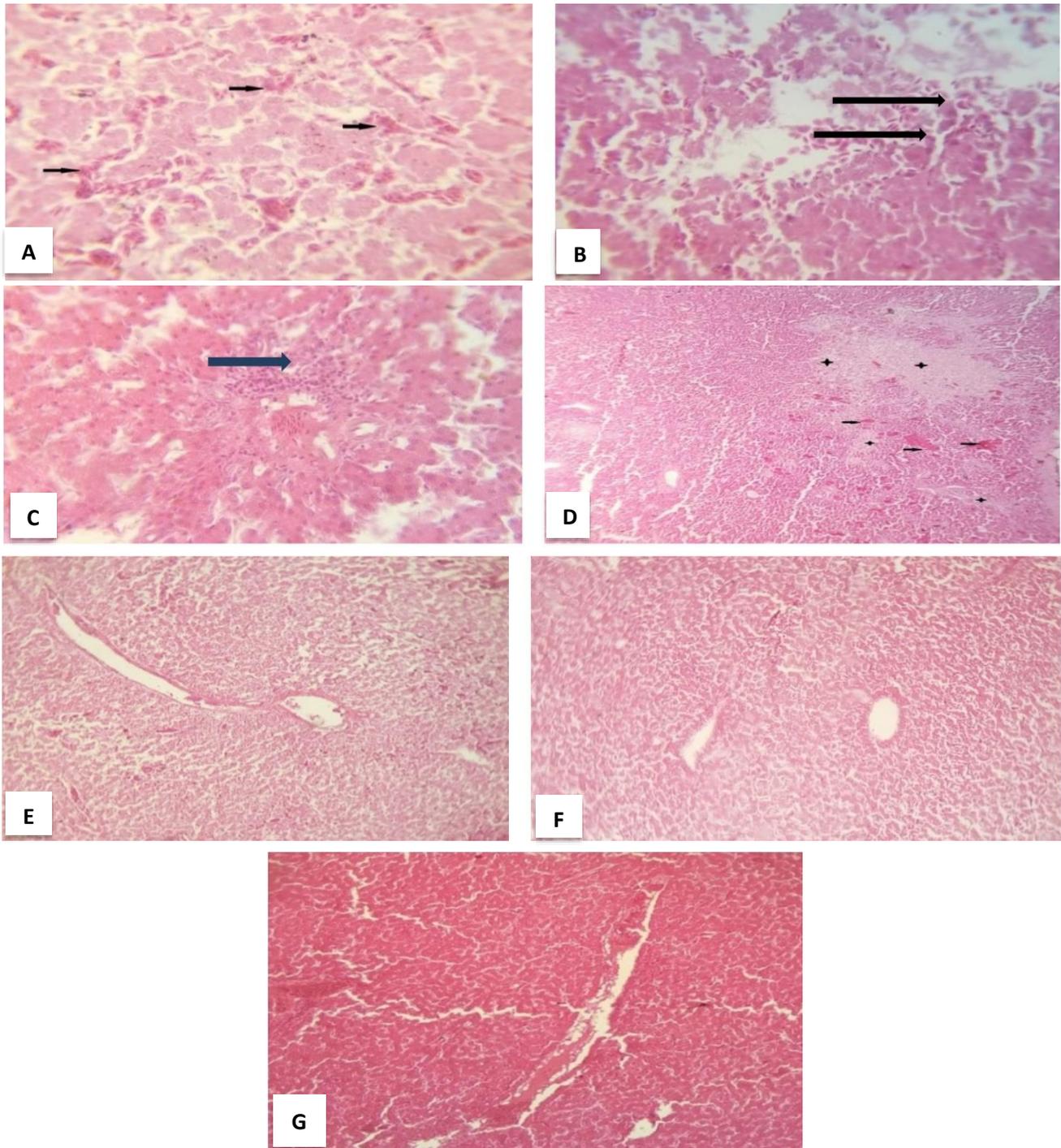
Crude oil exploration in Delta State, Nigeria, has led to environmental contamination, with heavy metals posing significant risks to wildlife and livestock, including poultry<sup>34</sup>. Layer chickens essential for egg production are especially prone to accumulating heavy metals such as lead, cadmium, and mercury, which disrupt organ function<sup>35</sup>. Table 3 shows the histopathological effects of heavy metal contamination, resulting from crude oil exploration on the liver and kidney of layer chickens in Delta State, Nigeria, where samples from seven ethnic regions, Urhobo, Isoko, Ijaw, Itsekiri, Ukwani, Aniocha, and Ika revealed varying degrees of hepatic and renal lesions. In Figure 1, layer chickens exhibited mild to moderate hepatic necrosis, with specific regions showing hemorrhagic lesions, hyperplasia of Kupffer cells, and periportal macrophage infiltration. Hepatic necrosis and Kupffer cell hyperplasia suggest

chronic exposure to metals such as cadmium, which disrupts hepatocyte function<sup>36</sup>. Renal lesions ranged from mild (40%) to marked (60%) as illustrated in Table 3, with interstitial hemorrhages, vascular congestion, and nephrosis observed in Figure 2. These observations showed multiple foci of interstitial hemorrhagic lesions, marked vascular congestion, and sparse heterophil and lymphocyte aggregates, including lobular necrosis, dilated sinusoidal spaces, and nephrosis across all five regions, indicative of significant renal impairment. Similarly, renal interstitial hemorrhages and nephrosis correlate with lead and mercury accumulation, impairing tubular integrity<sup>37</sup>. The higher prevalence of lesions in Urhobo, Isoko, Ijaw, Itsekiri, and Ukwani likely reflects proximity to oil exploration sites, consistent with studies linking crude oil pollution to organ damage in livestock<sup>38,39</sup>.

**Table 3.** The histology of Internal organs of laying chickens reared in petroleum-affected regions in Niger Delta, Nigeria during 2021

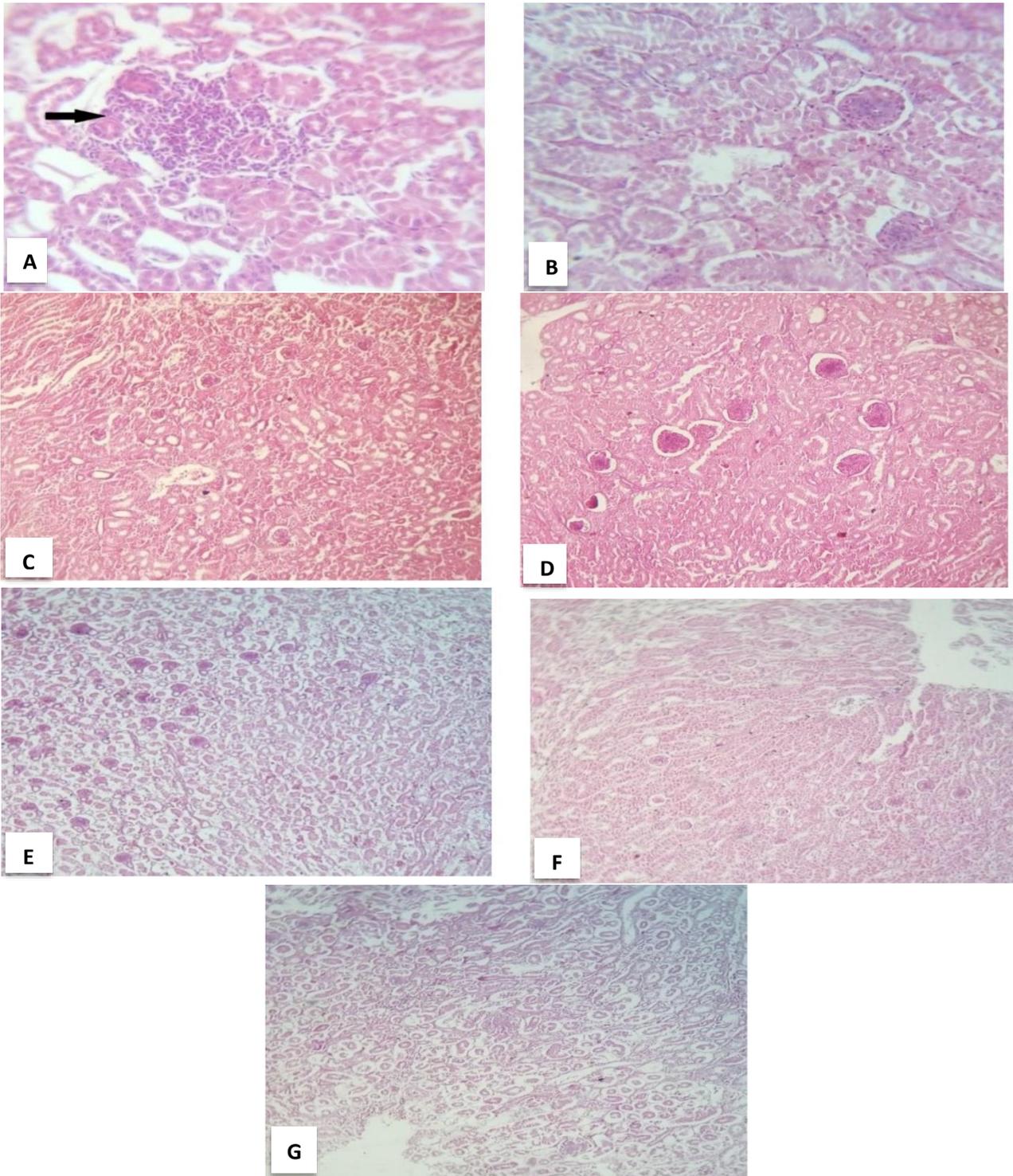
Parameters (%)	Zones						
	Urhobo	Isoko	Ijaw	Itsekiri	Ukwani	Aniocha	Ika
Liver necrosis/Lesion							
Moderate/Mild	100 (5/5)	60 (3/5)	60 (3/5)	20 (1/5)	20 (1/5)	NVL	NVL
Severe	0 (0/5)	20 (1/5)	0 (0/5)	20 (1/5)	0(0/5)	0 (0/5)	0 (0/5)
Kidney necrosis/Lesion							
Moderate/Mild	40 (2/5)	60 (3/5)	0 (0/5)	0 (0/5)	NVL	NVL	NVL
Severe	0 (0/5)	0 (0/5)	0 (0/5)	0 (0/5)	0(0/5)	0(0/5)	0(0/5)

NVL: No visible lesion



**Figure 1.** Comparative photomicrographs of laying chickens' livers from petroleum-affected regions (A-G) in Niger Delta, Nigeria in 2021

**A:** Liver with arrows highlighting hepatic sinusoids congested with erythrocytes (Urhubo region), **B:** Hepatic congestion (Isoko region), **C:** Liver showing a focus of inflammation with lymphocytes aggregating (arrow; Itsekeri region), **D:** Liver with areas of chronic inflammation and fibrosis (stars). It shows congestion of the hepatic sinusoids (arrows; Ijaw region), **E:** Normal liver (Ukwani region), **F:** Normal liver (Aniocha/Oshimili region), **G:** Normal liver (Ika region). H&E.



**Figure 2.** Comparative photomicrographs of layer chickens' kidneys from petroleum-affected regions (A-G) in Niger Delta, Nigeria in 2021 **A:** Kidney with the arrow indicating a focus of tubulointerstitial inflammation with an aggregate of lymphocytes (Urhobo region), **B:** Normal kidney of layer chicken (Isoko region), **C:** Normal kidney of layer chicken (Itsekiri region), **D:** Normal kidney of layer chicken (Ijaw region), **E:** Normal kidney of layer chicken (Ukwani region), **F:** Normal kidney of layer chicken (Aniocha/Oshimili region), **G:** Normal kidney of layer chicken (Ika region). H&E.

Heavy metal concentrations in eggs from seven poultry farms across Delta State, Nigeria, indicated significant

variations in cadmium (Cd), vanadium (V), iron (Fe), nickel (Ni), and mercury (Hg) levels (Table 4).

**Table 4.** Concentrations (ppm) of heavy metals in layer chickens in table eggs in Delta State, Nigeria

Parameters	Zones							SEM	Permissible. Limits
	Urhobo	Isoko	Ijaw	Itsekiri	Ukwani	Aniocha	Ika		
Cadmium	21.032 <sup>a</sup>	12.066 <sup>cd</sup>	12.328 <sup>cd</sup>	14.728 <sup>b</sup>	12.628 <sup>cd</sup>	10.963 <sup>d</sup>	13.434 <sup>c</sup>	1.156	0.00-0.005
Vanadium	0.021 <sup>c</sup>	0.054 <sup>ab</sup>	0.056 <sup>ab</sup>	0.081 <sup>a</sup>	0.053 <sup>ab</sup>	0.070 <sup>a</sup>	0.006 <sup>bc</sup>	0.007	0.00
Iron	3815.7 <sup>ab</sup>	4594.4 <sup>a</sup>	4174.2 <sup>ab</sup>	3632.8 <sup>ab</sup>	3060.4 <sup>b</sup>	3615.7 <sup>ab</sup>	4032.4 <sup>ab</sup>	132.367	0.30
Nickel	0.035 <sup>a</sup>	0.019 <sup>b</sup>	0.016 <sup>b</sup>	0.013 <sup>b</sup>	0.011 <sup>b</sup>	0.016 <sup>b</sup>	0.016 <sup>b</sup>	0.002	0.00-1.12
Mercury	0.029 <sup>a</sup>	0.007 <sup>b</sup>	0.031 <sup>a</sup>	0.030 <sup>a</sup>	0.002 <sup>b</sup>	0.011 <sup>b</sup>	0.002 <sup>b</sup>	0.004	1.00-2.68

Permissible limits: WHO (2004)<sup>40</sup>; abc means within the same row with the same superscript letters, are not significantly ( $p > 0.05$ ) different, SEM: Standard Error of the mean.

These differences stemmed from environmental factors, feed quality, and management practices unique to each zone. Cadmium levels ranged from 10.963 ppm in Aniocha to 21.032 ppm in Urhobo, far exceeding the permissible limit of 0.00-0.005 ppm<sup>41</sup>. Urhobo farms recorded the highest Cd concentration, significantly different ( $p < 0.05$ ) from other zones. At the same time, Aniocha had the lowest Cd concentration. Elevated Cd across all zones suggested contamination from feed, soil, or water, possibly contributing to industrial activities and agricultural runoff prevalent in Delta State<sup>42</sup>. High Cd poses health risks, including kidney damage and bone disorders<sup>43</sup>. Although no permissible limit for vanadium in eggs is specified, variations may reflect soil composition or feed additives<sup>44</sup>. Iron levels were notably high, ranging from 3060.4 ppm in Ukwani to 4594.4 ppm in Isoko, exceeding the 0.30 ppm limit. Iron levels in the Isoko zone were highest, differing significantly from Ukwani ( $p < 0.05$ ). High Fe may result from soil content or feed supplementation to boost egg production<sup>45</sup>, but excessive intake can cause oxidative stress<sup>42</sup>. Nickel levels, ranging from 0.011 ppm in Ukwani to 0.035 ppm in Urhobo, were within the 0.00-1.12 ppm limit. Urhobo showed significantly higher Ni ( $p < 0.05$ ) compared to values obtained for broiler, layer, and local cockerel in Anambra State, Nigeria<sup>46</sup>, which could be possibly due to contaminated feed or water<sup>47</sup>. Mercury levels were low, from 0.002 ppm in Ukwani and Ika to 0.031 ppm in Ijaw, below the 1.00-2.68 ppm limit, with no significant differences ( $p > 0.05$ ), indicating minimal environmental Hg contamination<sup>48</sup>. High Cd and Fe levels, particularly in Urhobo, raise consumer safety concerns, likely tied to industrial activities. Accumulation of cadmium in the body negatively affects different organs, which include the central nervous system, brain, bones, kidneys, liver, lungs, and placenta<sup>48</sup>. Other negative impacts that have been reported include immunological effects, reproductive, hepatic, developmental toxicity, and haematological effects<sup>49</sup>. Farmers should ensure quality feed and monitor soil and water, while regulatory enforcement is critical to mitigate health risks, including Cd's carcinogenic potential<sup>48</sup>.

## 5. Conclusion

Crude oil exploration in Delta State, Nigeria significantly disrupts the haematological and biochemical profiles of layer chickens, indicating systemic toxicity. Elevated PCV, Hb, WBC, and MCHC, alongside reduced RBC counts, suggested dehydration, immune activation, and anaemia. High serum albumin, bilirubin, and uric acid levels reflected hepatic and renal stress. Histopathological findings revealed widespread liver and kidney damage, confirmed the harmful effects of heavy metal contamination. The present results underscore the urgent need for mitigation strategies, including water treatment and dietary interventions, to safeguard poultry health in oil-producing regions. Therefore, integrating epidemiological studies to evaluate the human health risks from consuming contaminated eggs and poultry products in these regions in Delta State, Nigeria would provide a holistic assessment of the public health implications, thereby aligning with the food safety concerns, which will enhance understanding of heavy metal toxicity in poultry and inform targeted policies to protect both animal and human health in oil-polluted regions.

## Declarations

### Competing interests

The authors declared no competing interests that influence the objectivity or integrity of this study.

### Authors' contributions

All authors contributed significantly to the study. Jerome U. Unukevwere, Unity D. Osayande and Olatunbosun Odu were involved in the conceptualization, design and data collection. Jerome U. Unukevwere and Osayande D. Unity were involved in the analysis, interpretation, and manuscript preparation. The authors reviewed and approved the final edition of the manuscript for publication.

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## Availability of data and materials

All data supporting this study are available upon request. Interested readers may contact the corresponding author via email or other communication platforms to access the data.

## Ethical considerations

The authors confirmed that this manuscript is an original submission, prepared exclusively for the Journal of World's Poultry Science and not under consideration elsewhere. The final manuscript was thoroughly checked for plagiarism, data fabrication, and duplication to ensure scientific integrity.

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