



## Research Article

# Hematological Disturbances and Tissue Residue Levels in Local Chickens Exposed to Organophosphate Pesticides in Alau-Dam And Fariya Communities, Maiduguri, Borno State, Nigeria

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## ABSTRACT

The indiscriminate use of pesticides in agricultural and peri-domestic environments poses a significant risk of residue accumulation in food animals, with potential public health implications. This study investigated the presence and concentrations of selected pesticide residues in the liver, crop, and muscle tissues of local chickens from Alau-Dam and Fariya communities, using chickens as sentinel animals, with a control group included for comparison. Tissue samples were analyzed for organophosphate and Pyrethroid pesticides using Gas Chromatography–Mass Spectrometry (GC–MS), and detected concentrations were compared with WHO/FAO Codex maximum residue limits (MRLs). Pesticide residues were detected in all study groups, with Chlorpyrifos, Dichlorvos, and Cypermethrin being the most frequently identified compounds. In chickens from Alau-Dam, 6 out of 15 samples tested positive, of which five exceeded the MRL, including muscle samples with Chlorpyrifos (0.789 µg/g) and dichlorvos (0.676 µg/g), representing concentrations  $\geq 10$  times the Codex limits. Samples from Fariya community showed notably high contamination, particularly Chlorpyrifos in liver tissue (0.247 µg/g), exceeding the MRL by over 20-fold, while other detected residues were generally below regulatory limits. Although considered a control, 26.7% of samples from the control group contained residues exceeding MRLs, with Dichlorvos (1.325 µg/g) and Cypermethrin (0.196 µg/g) detected mainly in liver tissues. Hematological assessments revealed alterations in red and white blood cell parameters among exposed chickens with  $P < 0.05$  from the statistical analysis, suggesting physiological responses consistent with pesticide exposure. Overall, the detection of pesticide residues at concentrations exceeding international safety limits, particularly in edible tissues, underscores a potential food safety and public health concern for communities consuming locally reared chickens. Continuous monitoring, enforcement of pesticide regulations, and public awareness on safe pesticide use are strongly recommended.

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## INTRODUCTION

Pesticides are used on vegetables, fruits, wheat, rice, olives, canola pressed into oil and on non-food crops such as cotton, grass and flowers to curtail pest infestation. Pesticides applied to food crops on the field can deposit potentially harmful residues (Botwe *et al.*, 2011). Following application of pesticides to crops, they may interact with the plant surfaces, be exposed to the environmental factors such as wind and sun and may be washed off during rainfall (Tudi *et al.*, 2021). The pesticide may be absorbed by the plant surface via their waxy cuticle as well as the root surfaces which can enter the plant transport system (systemic) or stay on the surface of the plant (contact). The pesticides that get into the plant tissues may be biotransformed or sequestered in the tissues to form the pesticide residue (Keikotlhaile & Spanoghe, 2011).

Consumption of food/feed contaminated by pesticide residue is the major source of peracute, acute, sub-acute, sub-chronic and chronic exposure to pesticides toxicity. It is estimated that dietary pesticide exposure is five times higher than exposure through other routes which include inhalation and contact (Albedair & Alturiqi, 2021). Associated health risks following exposure to pesticide residues range from acute symptoms which can manifest in form of nausea, coughing, headache, abdominal disorder, diarrhea and vomiting to chronic symptoms like endocrine disruption, reproduction and immune systems malfunctioning and development of some forms of cancers (Johnson, 2014).

Although, rinsing has been found to have significant effect on reducing residue concentration of pesticides compounds on vegetable crops up to 50-77% as reported by (Kumari, 2008) but the extent at which the residual concentration can be reduced following rinsing depends on some factors which includes; The chemical properties of the pesticides, the nature of the vegetable crop involved, the duration or length of time of the pesticides compound on the vegetable crop and the formulation in which the pesticides was applied (Elkins, 1989).

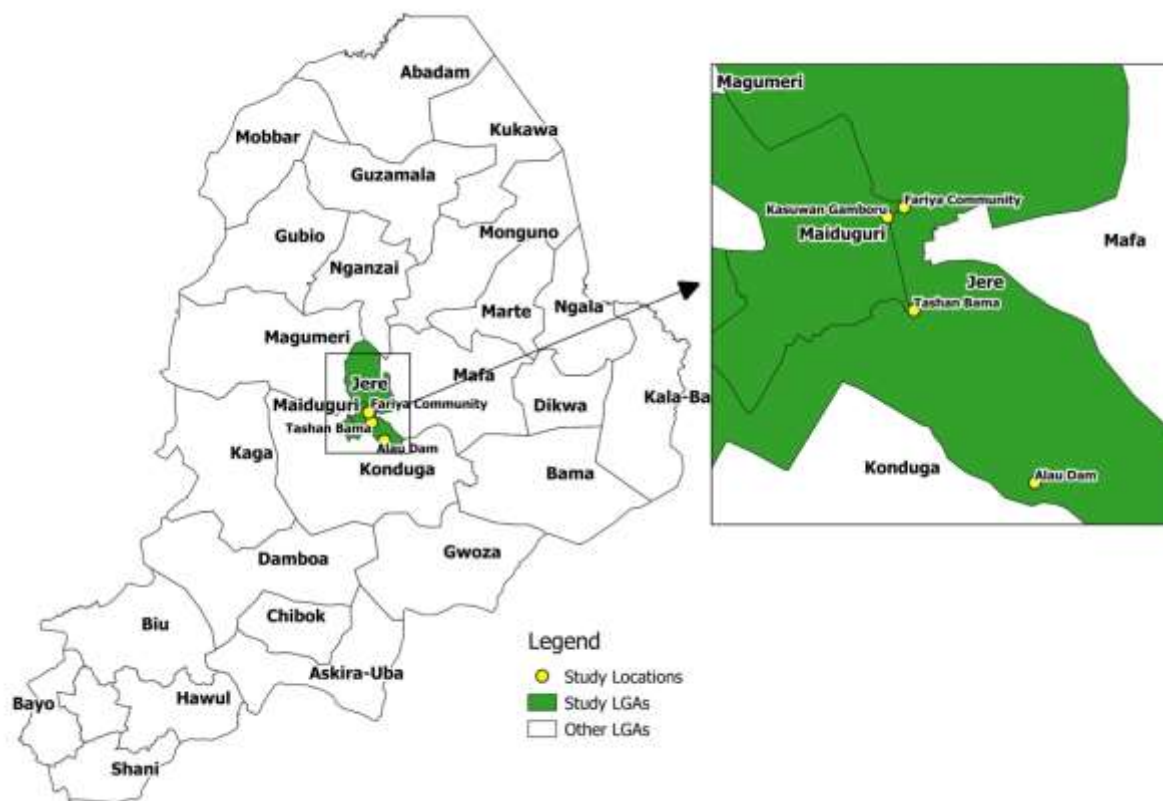
Vegetable crops are important components of the human and animal diet, since they provide essential nutrients and antioxidants that are required for most of the body physiological functions. High vegetables intake has been

encouraged not only to prevent consequences due to vitamin deficiency, but also to reduce the incidence of major diseases such as cancer, cardiovascular diseases and obesity. There has been increased use of pesticides in vegetable and crop production because they have rapid action; decrease toxins produced by pests and are less labor intensive than other pest control methods (Jeyanthi & Kombairaju, 2005). However, the use of pesticides during production often leads to the presence of pesticide residues in vegetables and other food crop after harvest (Shrestha *et al.*, 2010).

Exposure to organophosphates and other chemicals remains a considerable threat to numerous avian species. Birds, as essential elements of ecosystems, are abundant and responsive to both direct and indirect environmental influences, rendering them exceptional indicators for the early identification of environmental problems and dangers (Mitra *et al.*, 2011). Local chickens in vegetable farming communities, as well as other species of shorebirds, grassland, farmland, and migratory birds, are directly affected by lethal pesticide doses or suffer from secondary poisoning through consumption of pesticide-killed insects (Mitra *et al.*, 2020). This study was designed to examine the effect of organophosphate pesticide exposure on hematological parameters and tissue residual concentration in local hens sampled from vegetable farming communities of alau-dam and fariya in Maiduguri metropolis, Borno State, Nigeria.

## METHODOLOGY

### Study Location



*Source: modified Google map from BOGIS (bogis.bornostate.gov.ng) using GIS*

**Fig 1:** Map of Borno state showing the sample collection points within the study area.

### Ethical Approval

Ethical clearance was obtained from the Animal Used and Ethics Committee (AUEC) community of the Faculty of Veterinary Medicine, University of Maiduguri with AUP. Number **AUP-R007/2023**.

### Haematological Analysis

Blood samples collected were submitted to Side laboratory at the Veterinary teaching hospital, University of Maiduguri for full blood count (FBC) and differential leucocyte count (DLC)

### Tissue Samples collection Transportaion and Analysis

Five local chickens each was randomly obtained from the 2 vegetable farming communities and another 5 from Gamboru kasuwa and Tashan Bama markets to formed 3 groups consisting of 5 local chicken each ( $n=5$ ), there were 8 males and 7 female local among the 15 birds. the birds were

keep in the poultry pen at the animal house of the Department of Veterinary Physiology and Biochemistry, faculty of veterinary Medicine and were allowed to acclimatized for 5 days. The birds were fed with Vital feed<sup>®</sup> Finisher with water given to the *ad-libitum*. On the 6<sup>th</sup> day, blood (1.5 mL) samples were aseptically collected from each of the 15 birds from the wing vein using a 2 ml syringe and needle and immediately transferred into plain blood sample bottle for haematological analysis. The birds were humanely sacrificed following euthanasia using ketamin/Xylazine combination at the dose of 80/10 mg/kg body weight respectively (Wharton *et al.*, 2024). Tissues of the crop, liver and muscles weighing 20g from each in the respective groups were immediately harvested. The samples were then stored in a refrigerator at

4°C and later transferred to the Analytical Chemistry laboratory of the Department of Chemistry, Yobe state University using coolers with ice packs. (Naz *et al.*, 2014). Instruments used One micro litre of the reconstituted sample was injected into the GC machine for analysis

- i. Agilent GC 7890B, coupled with MSD 5977A was used and Helium as a carrier gas @ Analytical Lab, Dept of Chemistry, Yobe State University, Damaturu.
- ii. The column temperature programme was set at 150°C for 1 Min, ramp at 25°C/min to 230°C, hold for 2 min; ramp at 20°C/min to 240°C, hold for 1min; ramp at 20°C to 260°C, and hold for 3°C to 280°C and hold for 5min: ramp at 2°C/min to 285°C, and hold for 10 Min
- iii. Injection port temperature and transfer line were set at 240°C and 250°C respectively.
- iv. The injection was made in the splitless mode with purge on after 0.75min. (Liangliang, *et. al.*, 2019).

#### **Sample Extraction**

Sample Extraction was conducted according to (Liang-liang *et al.*, 2023); recovery studies were performed by spiking blank samples at three concentration levels, and the mean recovery ranged from 70–120% as QuEChERS method was employed, demonstrating satisfactory extraction efficiency. The LOD and LOQ were determined based on a signal-to-noise ratio of 3:1 and 10:1, respectively, and were found to be 0.1–0.5 µg/ml (LOD) and 0.3–5.0 µg/ml (LOQ).

#### **Sample Cleanup by silica/Alumina Column Chromatography**

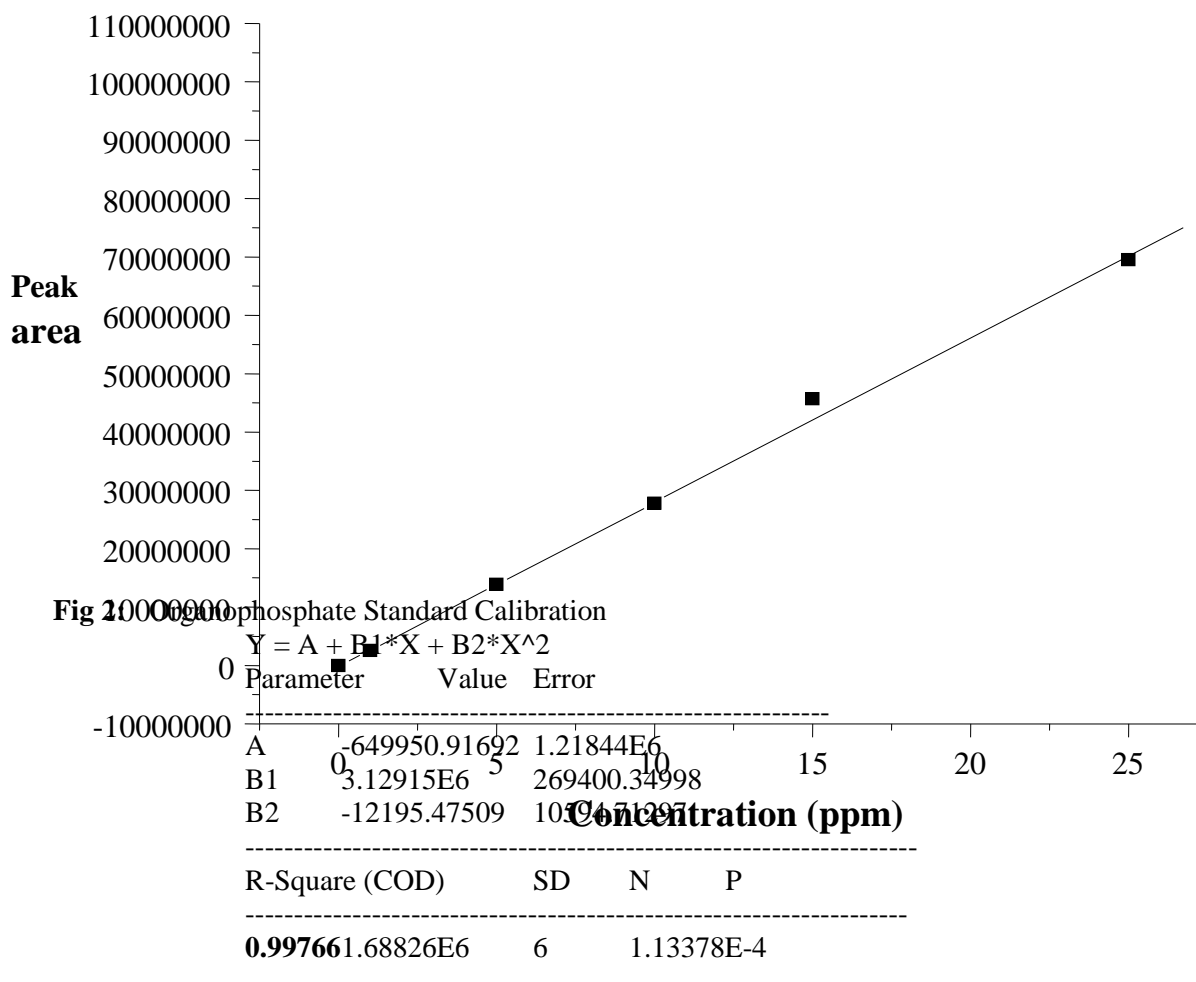
Sample cleaning was conducted according to (Barranco, 2003);

#### **Instrument analysis**

#### **Preparation of calibration standards and extracted calibration.**

Working standard solutions were prepared by diluting the stock solutions to 10µg/ml in ethyl acetate. Appropriate aliquots were taken and further diluted with ethyl acetate to give a series of calibration standard solutions with concentration of 10ng/ml, 20ng/ml, 50ng/ml, 75ng/ml, and 100ng/ml. Extracted calibration standards were prepared by spiking blank water samples (1L) with the working standard solution, prior to extraction, give concentration of 10ng/L, 20ng/L, 75ng/L and 100ng/L. after extraction and reconstitution (1L to 1ml) these equate to concentration of 10 ng/ml, 20ng/ml, 50ng/ml, 75ng/ and 100ng/ml (Hercegova *et al.*, 2010).

Quality control were assured throughout the analysis of the solvent blanks, All reagent used were analytical grade and each sample was analyzed in triplicates where the mean concentration was then calculated to obtained the average, this was done to all the samples that indicated positive for residue concentration. Recalibration curve was plotted to check their correlation coefficient which was 0.997 as shown in figure 3.2, which was an acceptable level of accuracy.



### Data Analysis

Data collected to determine the differences in mean concentration was subjected to analysis of variance and *P-values* less than 0.05 was considered statistically significant. Results obtained (Mean± SD) is presented in table while absolute residue concentration detected in tissue samples using GC-MS was also presented in tables.

**RESULTS****Table 1:** Full blood count (FBC) of domestic chickens obtained from Alau, and Fariya Communities compared with the control group

Sampling locations	PCV (%)	Hb(g/dL)	RBC( $10^{12}/L$ )	WBC
<b>Control</b>	30.00 $\pm$ 3.674	10.260 $\pm$ 1.130	2.424 $\pm$ 0.564 <sup>a</sup>	5.620 $\pm$ 1.956
<b>Alau</b>	30.20 $\pm$ 6.260	9.880 $\pm$ 2.317	1.678 $\pm$ 0.891 <sup>ab</sup>	4.440 $\pm$ 0.766
<b>Fariya</b>	35.40 $\pm$ 7.436	11.120 $\pm$ 2.423	1.114 $\pm$ 0.069 <sup>c</sup>	3.660 $\pm$ 0.433

*Values with different superscript within columns are significantly different ( $P < 0.05$ )*

Table 1: Values are presented in mean  $\pm$  SD. Means with different superscripts within a column differ significantly ( $P < 0.05$ ), this is as evident in the result of the RBC across the groups with steady decrease from the control, Alau and fariya community samples.

**Table 2:** Differential leucocytes count (DLC) of domestic chickens obtained from Alau and Fariya Communities compared with the control group

Sampling locations	Heterophils ( $10^3/\mu l$ )	Lymphocytes ( $10^3/\mu l$ )	Monocytes ( $10^3/\mu l$ )	Eosinophils ( $10^3/\mu l$ )	Basophils ( $10^3/\mu l$ )
<b>Control</b>	1628.8 $\pm$ 698.9 <sup>a</sup>	3568.6 $\pm$ 1130.9	305.6 $\pm$ 117.6	117.4 $\pm$ 67.7 <sup>a</sup>	0 $\pm$ 0
<b>Alau</b>	1012.4 $\pm$ 334.0 <sup>ab</sup>	3377.6 $\pm$ 473.2	295.8 $\pm$ 53.6	94.2 $\pm$ 87.7 <sup>ab</sup>	0 $\pm$ 0
<b>Fariya</b>	499.6 $\pm$ 92.0 <sup>c</sup>	3016.2 $\pm$ 247.2	204.2 $\pm$ 77.3	0.0 $\pm$ 0.0 <sup>c</sup>	0 $\pm$ 0

*Values with same superscript within columns are significantly different ( $P < 0.05$ )*

Table 2: Values are presented as mean  $\pm$  SD. Means within the same column bearing different superscript letters differ significantly ( $P < 0.05$ ). Heterophil counts were significantly reduced in samples from Alau and Fariya compared with the control group. Similarly, eosinophil counts were lower in Alau samples, while a complete absence of eosinophils was observed in Fariya samples.

**Table 3:** Residue concentration ( $\mu\text{g/g}$ ) in liver, crop, and muscles of local chickens from Alau-Dam Community

Pesticides Type			Pesticides concentrations (µg/g) in liver, crop, and muscles of local chickens from Alau-Dam														
S/N	Component	MRL	Liver					Crop					Muscle				
0		µg/g	AL1	AL2	AL3	AL4	AL5	AC1	AC2	AC3	AC4	AC5	AM1	AM2	AM3	AM4	AM5
		WHO															
1	Chlorpyrifos	0.01	0.023*	ND	0.009	ND	ND	ND	ND	0.020*	ND	ND	0.012*	ND	ND	0.789**	ND
2	Cyhalothrin	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	Cypermethrin	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.252*	ND
4	Dichlorvos	0.05	0.099*	ND	0.001	ND	ND	ND	ND	0.063*	ND	ND	0.059*	ND	ND	0.676**	0.372*
5	Dimethoate	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	Imidacloprid	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	LambdaCyhalothrin	0.01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	Pyrazophos	—															

\*Pesticide residue greater than MRL set by WHO/FAO CODEX joint food standard programme 21<sup>st</sup> Edition

AL= Alau liver sample

AC= Alau crop sample

AM= Alau muscle sample

ND: Not detected

\*: Residue detected greater than MRL

\*\*: Significant Residue concentration  $\geq 10\text{X}$  the MRL

Values with No asterisk – Below Maximum Residue Limit (MRL)

Table above showed a significant cypermethrin and dichlorvos residue concentration from local chicken's muscles samples collected from local Alau dam farming community.

**Table 4:** Residue concentration ( $\mu\text{g/g}$ ) in liver, crop, and muscles of local chickens from Fariya Community

Pesticides type			Pesticides Concentrations ( $\mu\text{g/g}$ ) in liver, crop, and muscles of local chickens														
S/No	Component	MRL ( $\mu\text{g/g}$ )	Liver					Crop					Muscle				
			FL1	FL2	FL3	FL4	FL5	FC1	FC2	FC3	FC4	FC5	FM1	FM2	FM3	FM4	FM5
1	Chlorpyrifos	0.01	0.006	ND	ND	0.247**	ND	ND	ND	ND	0.019	ND	ND	ND	ND	0.01	0.01
2	Cyhalothrin	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	Cypermethrin	0.05	ND	ND	ND	0.078	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	Dichlorvos	0.05	0.03	ND	ND	*0.209	0.127	ND	ND	ND	ND	ND	ND	ND	0.01	0.03	0.04
5	Dimethoate	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	Imidacloprid	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	lambda.Cyhalothrin	0.01	ND	ND	ND	ND	ND	ND	ND	ND	0.005	ND	ND	ND	ND	ND	ND

\*Pesticide residue greater than MRL set by WHO/FAO CODEX joint food standard programme 21<sup>st</sup> Edition

**FLS** = Fariya Liver sample

**FCS** = Fariya crop sample

**FMS** = Fariya Muscle sample

**ND**: Not detected

\*: Residue detected greater than MRL

\*\*: Significant Residue concentration >10X MRL

Values with No asterisk – Below Maximum Residue Limit (MRL)

Table 4 above showed one of the local chicken liver samples having residue concentration >10X the MRL set for Chlorpyrifos with two other liver samples having concentration that are greater than the MRL set by Codex



**Table 5:** Residue concentration ( $\mu\text{g/g}$ ) in liver, crop, and muscles of local chickens of local chicken from the control group

Pesticides Type			Pesticides Concentrations (µg/g) in liver, crop, and muscles of local chickens															
S/No.	Component	MRL (µg/g)	Liver					Crop					Muscle					
			CL1	CL2	CL3	CL4	CL5	CC1	CC2	CC3	CC4	CC5	CM1	CM2	CM3	C M4	C M5	
1	Chlorpyrifos	0.01	ND	ND	0.004	ND	ND	0.004	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	Cyhalothrin	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	Cypermethrin	0.05	ND	ND	ND	ND	*0.196	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	Dichlorvos	0.05	ND	ND	ND	ND	**1.325	0.001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	Dimethoate	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	Imidacloprid	0.05	ND	ND	ND	ND	0.046	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	lambda.Cyhalothrin	0.01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	Pyrazophos	—	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

\*Pesticide residue greater than MRL set by WHO/FAO CODEX joint food standard programme 21<sup>st</sup> Edition

CLS = Control liver sample      CCS = Control crop sample      CMS = control muscle sample

ND: Not detected

\*: Residue detected greater than MRL

\*\*: Significant Residue concentration  $\geq 10\text{X}$  the MRL

Values with No asterisk – Below Maximum Residue Limit (MRL)

Table 5 indicated high residue concentration that is greater than than 10 times the set MRL in one pf the liver sample analysed. There were also traces of residue detected for Chlorpyrifos, Cypermethrin and dichlorvos though lower the set MRL.

## DISCUSSION

The hematological parameters of the local chickens used as sentinel animals to determine tissue residue concentration had a Packed cell volume (PCV) between 24-40%, 28-43% and 26-36%, respectively for samples collected from Alau, Fariya community and the control group, respectively as seen in Table 1 above, this indicate an increased in the PCV with significant decrease in Red Blood Cell (RBC) across the groups. The average hemoglobin concentration of the three groups is 9.88, 10.2 and 10.26 g/dl for Alau, Fariya Community and the control group, respectively, while the average red blood cells count (RBC) for these groups are 1.46, 1.11 and 3.02 ( $\times 10^{12}/L$ ) for Alau, Fariya Community and the control group respectively. This finding is in agreement with the work of Adewumi *et al.* (2018) who reported on the “Effects of sub-lethal toxicity of Chlorpyrifos and DD-force pesticides on haematological parameters of *Clarias gariepinus*” where they observed consistent decreased in RBC with increased pesticides concentration across the treatment groups, (Adewumi, Ogunwale, Akingunsola, Falope, & Eniade, 2018). Equally, the White blood count (WBC) has an average of 4.44 and 3.66 ( $\times 10^{12}/mL$ ) across Alau Dam and Fariya community while the control group having an average of 5.8 ( $\times 10^{12}/mL$ ) Table 1. The differential leukocytes counts (DLC) indicate a slight raise across samples from Fariya community followed by that of Alau Dam samples with Hetrophilia and Eosiniphilia that are statiscally significant across the treatment groups as shown in Table 2 above, this correspond with the findings of (Kumar *et al.*, 2013) who studied the effect of pesticides on hematological parameters of fish in India where leucocytes declined in all the treated groups according to the level of exposure (Sinha *et al.*, 2022).

Tissue residue concentration detected in the liver, crops and muscle of chickens from Alau Dam community revealed 6 positive samples out of the 15 (6/15), among the 6 positive samples detected, result of 5 positive samples was found to be above the maximum residue level (MRL), while one out of five (1/5) of the muscle samples had a concentration of 0.789 $\mu$ g/g and 0.676  $\mu$ g/g of Chlorpyrifos and Dichlorvos, respectively, which is 10 times greater than MRL given by FAO/WHO CODEX (Commission, 1992). Dichlorvos,

Chlorpyrifos and Cypermethrin were the pesticides detected in the chicken samples analysed in this study as shown in Table 3, which is also similar to the findings obtained by (Akan *et al.*, 2013) who found Dichlorvos, Cypermethrin, Diazinon and Fanitrothion as the most common pesticides residue present in high concentration in the vegetables produced in this study area.

The residue concentrations of pesticides in chicken tissues from Fariya community revealed high levels of Chlorpyrifos in the liver (0.247 $\mu$ g/g), exceeding that of MRL by a factor of 24. Additionally, the analysis of a muscle sample from these groups of chicken revealed the presence of Dichlorvos and Chlorpyrifos that's below the MRL (Commission, 1992). Lambda-cyhalothrin was also found at the concentration of 0.005 $\mu$ g/g. that is also lower than MRL of 0.05 $\mu$ g/g, Table 4. This finding agrees with that of Kumar *et al.* (2011) who analyzed liver tissue samples from 254 Buffalos for Chlorpyrifos using high performance Liquid chromatography in India. 9.05% liver tissue sample was detected positive for pesticides residue out of which 0.75% were found to be above the MRL (Kumar *et al.*, 2011).

In Table 5 above, four out of the 15 samples (26.7%) were found to be positive and exceeded MRL for Dichlorvos and Cypermethrin which had the concentration of 1.325 and 0.196 $\mu$ g/g, respectively. The level of 1.325  $\mu$ g/g of Dichlorvos in the chicken liver tissue sample is quite alarming as the level of Dichlorvos is 26.6 times higher than the MRL of 0.05 $\mu$ g/g set for the pesticides. All the positive samples detected from the control group were from Liver and Crop as no residue was detected in the entire muscle sample analyzed from this group. The high tissue residue concentrations in local chickens, which exceed the CODEX-approved MRL, pose a significant public health concern. These elevated levels could also contribute to the development of chronic diseases that commonly affect the communities in the study area.

## AUTHOR CONTRIBUTION

UAM, MAS, and MMB concieved design, caaried out the analysis and write the first draft of the manuscript, BU, SIS, AYG and MAI, collected samples and prepare for transportation while ADT, EOA and YBM review the manuscript with valueble inputs.

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**CONFLICT OF INTEREST**

There is no any conflict of interest associated with this manuscript.

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