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Analysis of organochlorine and organophosphorus pesticide residues in blood samples of sheep and rabbits from villages of Jimeta-Yola, Adamawa State, Nigeria

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Blood samples of sheep and rabbits from five different villages of Jimeta-Yola, Adamawa State, Nigeria were collected for determination of organochlorine and organophosphorus pesticide residues. Preparation of blood samples was carried out using standard procedure of QuEChERS method of extraction. The concentrations of all the pesticides in blood samples of sheep and rabbits were determined using Gas Chromatography–Mass Spectrometry (GC-MS) Shimadzu (GCMS-QP2010), equipped with electron capture detector. Organochlorine and organophosphorus pesticide residues were significantly higher in the blood samples of sheep than in the blood samples of rabbits. According to the concentration and detection frequency, o,p'DDE, p,p'DDD, aldrin and dieldrin were the most dominant compounds among the organochlorine pesticide residues. The results of this study showed that highest concentration of o,p-DDE in blood samples of sheep was detected at Namtari village with a mean value of 0.034±0.001 mg/l. For o,p'-DDT, the highest concentration (0.014±0.001 mg/l) was observed at Wurojabbe, p,p'-DDD had the highest concentration at Doubeli with a value of 0.028±0.001 mg/l. Also, the highest concentration of aldrin in the blood samples of sheep was detected at Namtari with a value of 0.028±0.001 mg/l. For dieldrin, the highest concentration was recorded also at Namtari with a mean value of 0.036±0.001 mg/l. Despite the bans and restrictions on the use of some of these pesticides in Nigeria, the observed concentrations of the studied pesticides from these villages could explain either their persistence in the environment or continued use in the study area. Hence, routine monitoring of pesticide residues in these villages is necessary for prevention, control and reduction of environmental pollutions, so as to minimize health risks.

Key words: Animals blood, pesticides, extraction, clean-up, gas chromatography-mass spectrometry (GC-MS).

INTRODUCTION

Various scientists from different parts of the world have attempted to assess the level of pollution in different environmental media and at times related the results with plants and animals living in the environment. The need to preserve our environment is an important component of sustainable development of a society, with a view to maintaining ecological balance, and improving the quality of life and conditions that may affect human and animal

health (Milam et al., 2015).

There is a need for the growing demand for food productivity to meet the needs of the rising global population. This has led to advance agricultural technology and practices in which pesticides play an important role. Pesticides are generally used to increase the productivity of agricultural product (John et al., 2001). Pesticides have been widely used throughout the world since the middle of the 20th century. Pesticide plays a vital role in agricultural and animal production, though they contain substances with high toxic effects and persistence in the environment (Beyer and Biziuk, 2008). The toxicity of these pesticides goes beyond their biological activity and unfortunately includes toxicological actions toward animals (Imran et al., 2002). Pesticides generally have bioaccumulation ability; their persistence in the environment make them not only remain where they are applied but instead are found between the soil profiles and are absorbed in the soil several kilometers from where they were initially applied (Agarwal, 2009).

Organochlorine (OC) compounds are lipophilic and metabolized very little in sheep and rabbits. Therefore, an exposure of animal to this organochlorine (OC) compounds results in bioaccumulation and persistence in animal tissues (Falandysz et al., 2004). Pesticides containing organochlorine (OC) compounds undergo bioamplification through the food chain (Angulo et al., 1999; Borgå et al., 2001). Fagnani et al. (2011) defined organophosphorus (OP) pesticides as esters, amides or thiol derivatives of phosphoric acid. OP pesticides are easily hydrolyzed and therefore do not persist in the environment. However, their toxicity and the possibility of their accumulation especially fat-soluble OPs in animal blood, tissues, milk, and eggs pose risks for human health (Fagnani et al., 2011).

Pesticide residues in rabbits and sheep, generally accumulate by several ways. These are either applied to livestock through insecticide-impregnated ear tag, spray, self-treatment back rubber, dust bags injectable or spray of agricultural crops and fodder. The advantages of the use of pesticide on agricultural production range from protection of crops against insects to control of pests and diseases (Eskenazi et al., 2009). EU European Commission (2007) reported that animal blood is the most accessible body fluid for ascertaining the pesticide residue levels. The evaluation of serum levels of pesticides can be used as a biomarker of exposure for evaluating the health effects at certain levels (EU European Commission, 2007).

To ensure consumers safety and commercialization, permissive residue levels (PRLs) have been determined for pesticide residues in products of plant and animal origin. Legislation in the European Union has established MRLs of 40 and 0.8 ng/g for the target OC pesticides dichloro-diphenyl-trichloroethane and endrin, respectively, in milk. The PRL for chlorpyrifos is ≤10 mg/g (Ullah et al., 2010). Most authorities of developing countries, however, maintain that they cannot afford to ban certain chemicals for reasons of cost, efficacy or both. As a result, most of these chemicals have been or continue to be used in large quantities in many countries, including sub-Saharan Africa (Ahmed, 1989). Several studies on soil and water pollution have been investigated in Egypt, where wide use of OC and OP pesticides in the 1960s and 1970s prompted the government to prohibit their use in 1980s (El-Sebae and Soliman, 1982; Soliman et al., 1997). However, OC and OP pesticides are still being used for agricultural practices in sub-Saharan Africa (Amr et al., 1995; Dogheim et al., 1990; California Department of Pesticide Regulation (CDPR), 2010b). Therefore, this study was conducted to assess the current status of OC and OP pesticide residue contamination in blood of sheep and rabbits in Yola, Adamawa State, Nigeria, particularly since the ban on the use of these pesticides in agriculture.

MATERIALS AND METHODS

Blood samples for pesticides analysis were collected severally from randomly selected sheep and rabbits from five different villages of Jimeta-Yola- Namtari, Ngurore, Jambutu, Doubeli and Wurojabbe. First, composite of the samples was made; threreafter 5 ml of blood samples was collected and transferred into residue free heparinized glass vials containing 200 USP units of heparin in 0.2 ml solution with the help of sterilized syringe. Blood samples were transported in dry ice to the laboratory and stored at -20°C until analyzed.

Extraction of pesticide residues in blood

Extraction was based on the method of California Department of Pesticide Regulation (CDPR) (2010b). With some modifications, 5 ml of blood sample was diluted with 25 ml distilled water; thereafter 2.0 ml of saturated brine solution was added and transferred to a separatory funnel and extracted with hexane by shaking the separatory funnel vigorously for 2 to 3 min, releasing the pressure intermittently. The layers were allowed to separate; afterwards, the extracts were also passed through anhydrous $MgSO_4$ and concentrated to about 1 to 2 ml using rotary vacuum evaporator.

Dispersive solid-phase extraction (dSPE) cleanup

From the extract obtained in blood, 1.0 ml was transferred to a 2-ml dSPE Cleanup tube that contains 150 mg of $MgSO_4$, 50 mg PSA sorbent and 50 mg C-18 sorbent and was shaken vigorously for 1 min. Then, the portions of the supernatant were transferred to the

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Table 1. Mean concentrations (mg/l) of some organochlorine pesticide residues in blood samples of sheep from some selected villages of Jimeta-Yola, Adamawa State, Nigeria.

Villages	o,p '-DDE	o,p '-DDT	p,p'-DDD	aldrin	dieldrin	Chlordane
Ngorore	0.023±0.001	0.009±0.001	0.013±0.001	0.016±0.001	0.021±0.001	<0.001
Namtari	0.034±0.001	0.013±0.001	0.024±0.001	0.028±0.001	0.036±0.001	<0.001
Wurojabbe	0.022±0.001	0.014±0.001	0.006±0.001	0.005±0.001	0.019±0.001	<0.001
Doubeli	0.016±0.001	0.008±0.001	0.028±0.001	0.002±0.001	0.007±0.001	<0.001
Jambutu	0.018±0.001	0.002±0.001	0.009±0.001	0.006±0.001	0.012±0.001	<0.001

Instrument detection limit (IDL): 0.001.

Table 2. Mean concentrations (mg/l) of some organochlorine pesticide residues in blood samples of rabbits from some selected villages of Jimeta-Yola, Adamawa State, Nigeria.

Villages	o,p '-DDE	o,p '-DDT	p,p'-DDD	aldrin	dieldrin	Chlordane
Ngorore	0.012±0.001	0.003±0.001	0.006±0.001	0.002±0.001	0.004±0.001	<0.001
Namtari	0.008±0.001	0.001±0.001	0.011±0.001	0.004±0.001	0.008±0.001	<0.001
Wurojabbe	0.002±0.001	<0.001	0.001±0.001	0.002±0.001	0.004±0.001	<0.001
Doubeli	<0.001	0.001±0.001	0.003±0.001	0.001±0.001	0.006±0.001	<0.001
Jambutu	0.003±0.001	0.002±0.001	0.001±0.001	0.002±0.001	0.008±0.001	<0.001

Instrument detection limit (IDL): 0.001.

liquid chromatography–mass spectrometry (LC-MS) certified vial for gas chromatography–mass spectrometry (GC-MS) analysis.

Determination of pesticide residues

The Shimadzu Japan GC/MS (GCMS – QP2010), equipped with electron capture detector was used for the chromatographic separation and was achieved using a HP-5MS 5% Phenyl Methyl Siloxane Column. The oven was programmed as follows: initial temperature 60°C for 0.5 min, then 20°C/min to 300°C for 9 min, with a final run time of 21.5 min and a constant column flow rate of 1 mL/min. The detection of pesticides were performed using the GC-ion trap MS with optional MSn mode. The scanning mode offered enhances selectivity over either full scanned or selected ion monitoring (SIM). In SIM, at the elution time of each pesticide, the ratio of the intensity of matrix ions increase exponentially versus that of the pesticide ions as the concentration of the pesticide approach the detection limit, decreasing the accuracy at lower levels. The GC-ion trap MS was operated in MSn mode and tandem MS function was performed by injecting ions into the ion trap and destabilizing matrix ions, isolating only the pesticide ions. The retention time, peak area and peak height of the samples were compared with those of the standards for quantization.

RESULTS

The mean concentrations of some organochlorine pesticide residues in blood samples of sheep from different villages of Jimeta-Yola, Adamawa State are presented in Table 1. The concentrations of o,p'-DDE range from 0.016±0.001 to 0.034±0.001 mg/l; o,p'-DDT from 0.002±0.001 to 0.014±0.001 mg/l; p,p'-DDD from 0.009±0.001 to 0.028±0.001 mg/l. For aldrin, the concentrations range from 0.002±0.001 to 0.028±0.001 mg/l, while the concentrations of dieldrin range from 0.007±0.001 to 0.036 mg/l, whereas the concentration of chlordane is less than the detection limit. For rabbits, the concentrations of organochlorine pesticide residues in blood samples of rabbits from some selected villages of Jimeta-Yola, Adamawa State, are as presented in Table 2. The concentrations of o,p'-DDE range from 0.002±0.001 to 0.012±0.001 mg/l; o,p'-DDT from 0.001±0.001 to 0.003±0.001 mg/l; and p,p'DDD from 0.001±0.001 to 0.011±0.001 mg/l. Then, for aldrin, the concentrations ranged from 0.001±0.001 to 0.004±0.001 mg/l, whereas the concentrations of dieldrin ranged from 0.004±0.001 to 0.008±0.001 mg/l.

The mean concentrations of some organophosphorus pesticide residues in blood samples of sheep from selected villages of Jimeta-Yola, Adamawa State are presented in Figure 1. The concentrations of dichlorvos ranges from 0.008±0.001 to 0.032±0.001 mg/l; Malathion from 0.005±0.001 to 0.028±0.001 mg/l; Chlorpyrifos from 0.003±0.001 to 0.019±0.001 mg/l; Parathion from 0.004±0.001 to 0.017±0.001 mg/l; and Phosphamidon from 0.013±0.001 to 0.023±0.001 mg/l; whereas the concentration of Ethion was not detected in the entire blood samples analyzed. For blood samples in rabbits as presented in Figure 2, the mean concentrations of Dichlorvos ranges from 0.001±0.001 to 0.012±0.001 mg/l; Malathion from 0.001±0.001 to 0.009±0.001 mg/l;

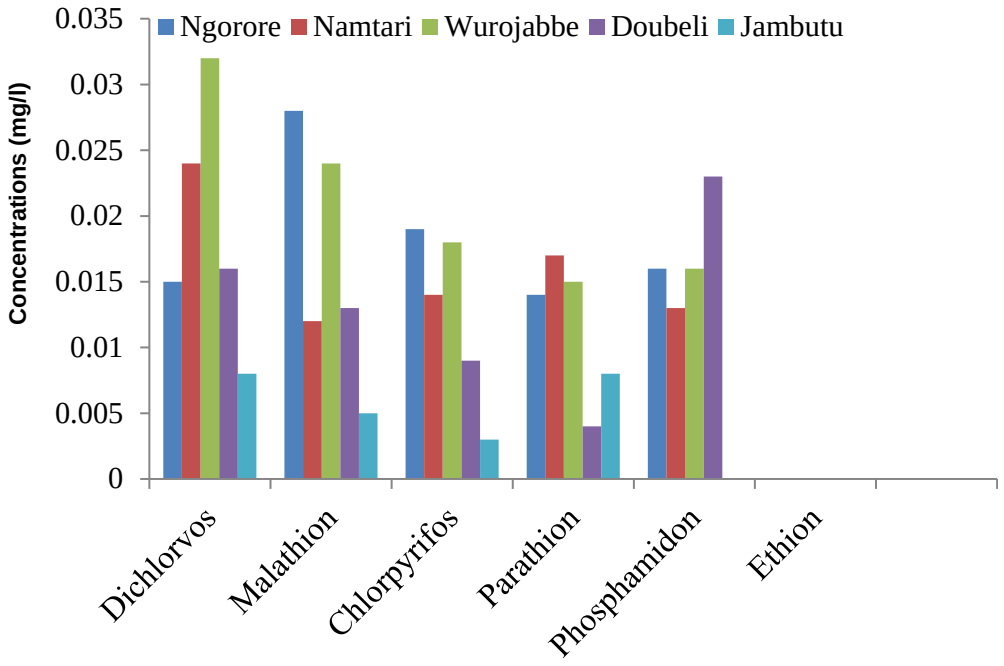

Figure 1. Mean concentrations of some organophosphorus pesticide residues in blood samples of sheep from some selected villages of Jimeta-Yola, Adamawa State, Nigeria.

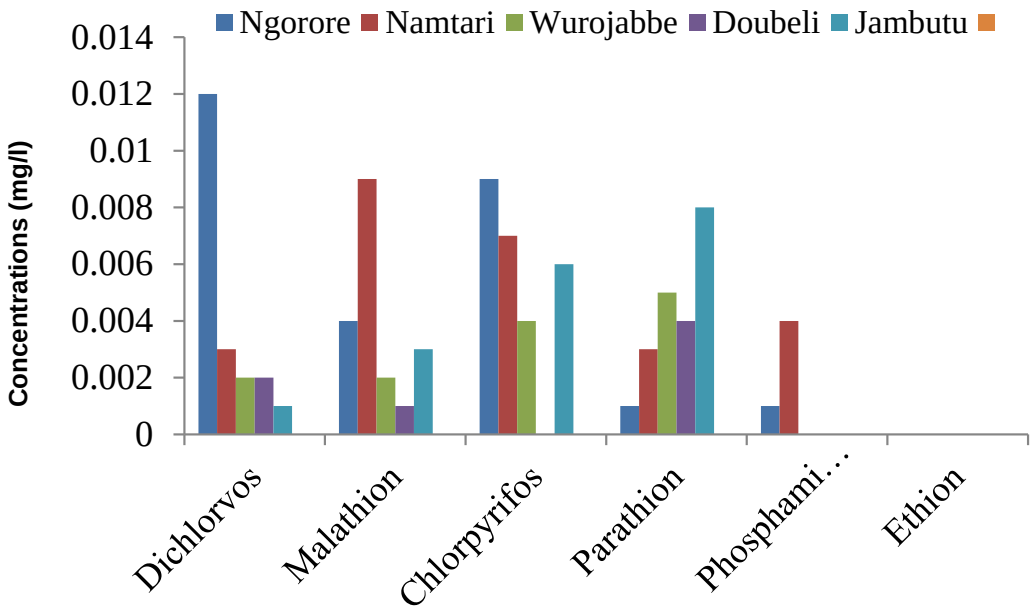

Figure 2. Mean concentrations of some organophosphorus pesticide residues in blood samples of rabbits from some selected villages of Jimeta-Yola, Adamawa State, Nigeria.

Chlorpyrifos from 0.004±0.001 to 0.009±0.001 mg/l; Parathion from 0.001±0.001 to 0.008±0.001 mg/l; and Phosphamidon from 0.001±0.001 to 0.004±0.001 mg/l. Similarly, Ethion was not detected in all the blood samples analyzed.

Comparison of the total mean concentrations of some organochlorine pesticide residues in the blood samples

Figure 3 shows comparison of the concentrations of

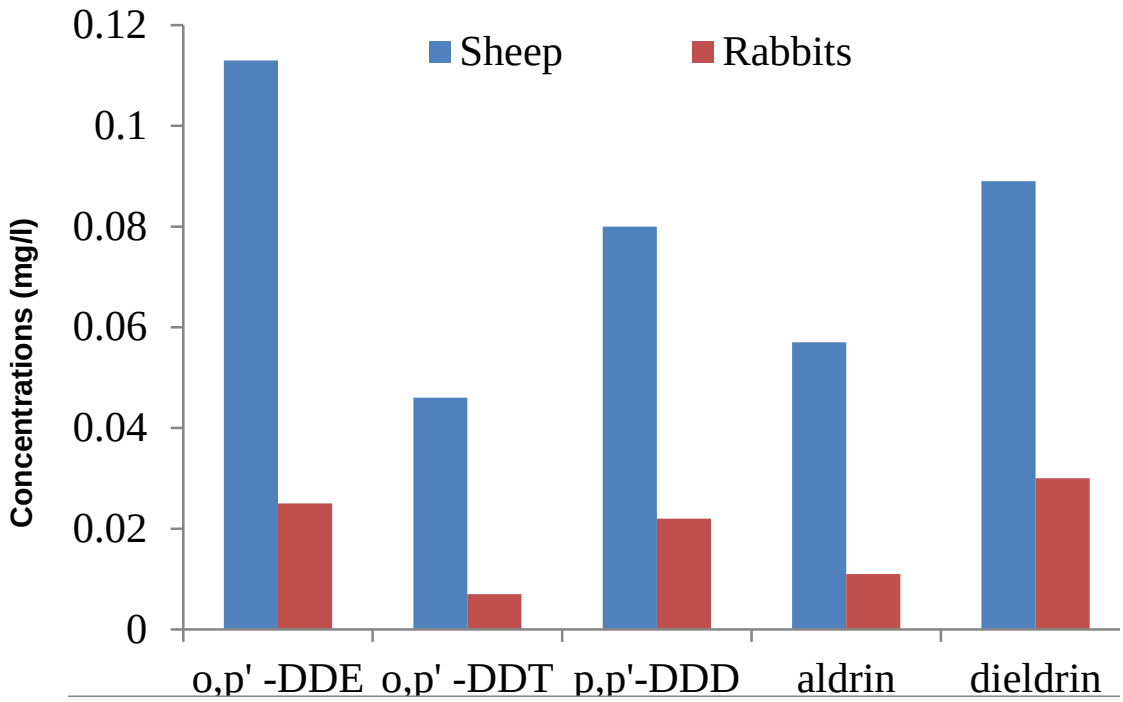

Figure 3. Comparison of the total mean concentrations (mg/l) of some organochlorine pesticide residues between blood samples of sheep and rabbits from selected villages of Jimeta-yola, Adamawa State, Nigeria.

some organochlorine pesticide residues between blood samples of sheep and rabbits. The mean concentrations of o,p'-DDE in the blood samples of sheep was 0.113 mg/l, while in the blood samples of rabbits a total concentrations of 0.025 mg/l was recorded. The mean concentrations of o,p'-DDT in blood samples of sheep was 0.046 mg/l, while 0.007 mg/l was observed in rabbits. Similarly, the mean concentration of p,p'-DDD in sheep was 0.08±0.001 mg/l while 0.022 mg/l was recorded as total concentration in blood samples of rabbits. Also, the mean concentration of aldrin in blood samples of sheep was 0.057 mg/l while total concentration in blood samples of rabbits was 0.011 mg/l. For dieldrin, the mean concentration of 0.089 mg/l in blood samples of sheep was recorded, while 0.03 mg/l in blood samples of rabbits was recorded.

Figure 4 shows comparison of the concentration of organophosphorus pesticide residues between blood samples of sheep and rabbits. The concentration of dichlorvos in blood samples of sheep was 0.095 mg/l, while in rabbits, 0.02 mg/l was recorded. Malathion concentration of 0.082 mg/l was recorded in blood samples of sheep and 0.019 mg/l in rabbits. The mean concentration of chlorpyrifos in blood samples of sheep is 0.063 mg/l, whereas in rabbits, a concentration of 0.026 mg/l was recorded. Also, the total mean concentration of parathion in blood samples of sheep was 0.058 mg/l, while that of blood samples of rabbits was 0.021 mg/l. In addition, the total mean concentration of phosphamidon in blood samples of sheep was 0.068 mg/l, and that of blood samples of rabbits was 0.005 mg/l.

DISCUSSION

Organochlorine pesticide residues (o,p'-DDE, O,p'-DDT, p,p'-DDD, aldrin, diedrin and chlordane) in the blood samples

In blood samples of sheep, the highest concentration of o, p-DDE in blood samples of sheep was detected at Namtari village with a value of 0.034 ± 0.001 mg/l, while the lowest concentration was detected at Doubeli with a value of 0.016 ± 0.01 mg/l. For o,p'-DDT, the highest concentration (0.014±0.001 mg/l) was observed at Wurojabbe, whereas Jambutu village showed the lowest concentration (0.002±0.001 mg/l). Also, p,p'-DDD had the highest concentration at Doubeli with a value of 0.028±0.001 mg/l and the lowest concentration was detected at Wurojabbe with a value of 0.006±0.001 mg/l. Also, the highest concentration of aldrin in the blood samples of sheep was detected at Namtari with a value of 0.028±0.001 mg/l and the lowest concentration was recorded at Jambutu with a value of 0.002±0.001 mg/l. For dieldrin, the highest concentration was also recorded at Namtari with a mean value of 0.036±0.001 mg/l, while the lowest concentration was recorded at Doubeli village with a mean value of 0.007±0.001 mg/l. Whereas,

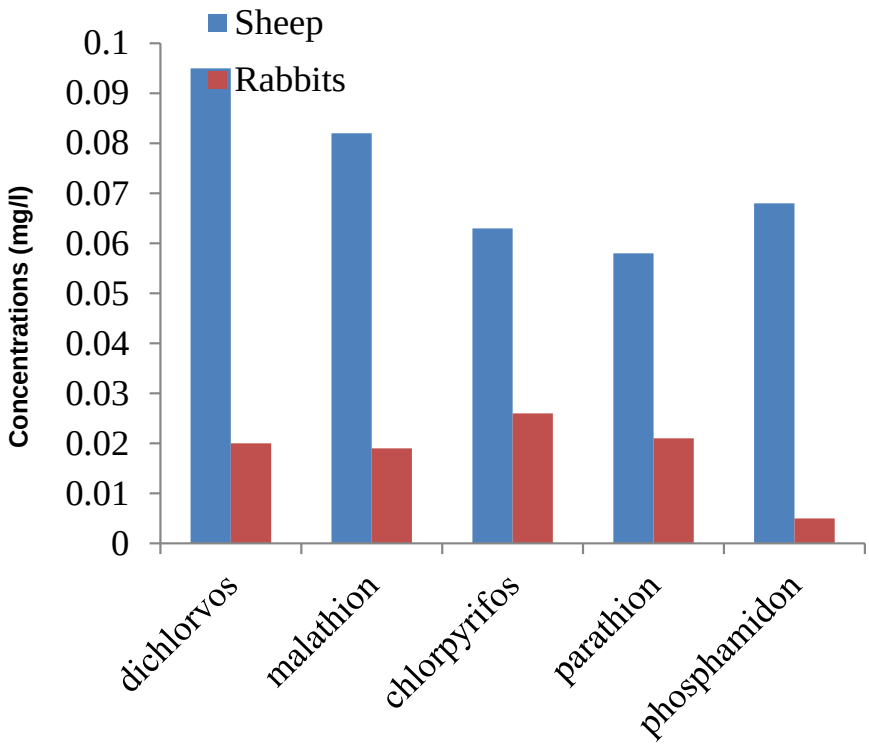

Figure 4. Comparison of the total mean concentration (mg/l) of some organophosphorus pesticide residues between blood samples of sheep and rabbits from selected villages of Jimeta-Yola, Adamawa State, Nigeria.

Chlordane was not detected in any of the blood samples analyzed, because either the animals have not been exposed to it or the chemicals are not available in these areas.

For blood samples of rabbits, the highest concentration of o,p-DDE in blood samples was observed at Namtari location with a concentration of 0.008±0.001 mg/l, while the lowest concentration was observed at Wurojabbe with a mean value of 0.002±0.001 mg/l, and also Doubeli location which showed less than detection limit. For o,p'-DDT, the highest concentration was recorded at Ngorore with a mean value of 0.003±0.001 mg/l, whereas the lowest concentration was observed at Namtari and Doubeli location with a mean value of 0.001±0.001 mg/g; meanwhile, less than the detection limit was observed at Wurojabbe. Also, for p,p'-DDD, the highest concentration was detected at Namtari with a value of 0.011±0.001 mg/l and the lowest concentration was observed at Wurojabbe and Jambutu with a mean value of 0.001±0.001 mg/l. Also, aldrin recorded the highest concentration at Namtari location with a value of 0.004±0.001 mg/l and the lowest concentration was detected at Doubeli with a mean value of 0.001±0.001 mg/l. For dieldrin, the highest concentration was detected at Namtari and Jambutu with a mean value of 0.008±0.001 mg/l, while the lowest concentration was detected at Ngorore and Wurojabbe with a total mean value of 0.004±0.001 mg/l. Whereas, Chlordane was not detected in any of the blood samples analyzed.

The total concentrations of dichlorodiphenyltrichloroethane (DDT) and its metabolites in the blood samples of sheep were in the range of 0.046±0.001 to 0.113±0.001 mg/l, while in blood samples of rabbits, it ranges from 0.007±0.001 to 0.025±0.001 mg/l. Based on the concentrations and detection frequency, o,p'DDE, p,p'DDD, aldrin and dieldrin were the most dominant compound among the organochlorine pesticide residues (OCPs). Similar results of OCPs residues in animal's meat have been reported in recent investigations. The concentrations of the degradation products, which are dichlorodiphenyldichloroethylene (DDE) (0.138 mg/l) and dichlorodiphenyldichloroethane (DDD) (0.102 mg/l) in all the blood samples from the villages both in sheep and rabbits, were more than that of the parent compound (0.053 mg/l), DDT, which indicates past usage of the DDT pesticide. DDT normally degrades under aerobic condition to DDE and under anaerobic condition to DDD, thus a higher DDE + DDD/ DDT ratio is an indication of past usage. Although, the use of DDT has been banned in Nigeria since 2008, it is still being used. DDT and its DDE and DDD metabolites persist in the environment and are known to bio accumulate in animal's fat and plant waxes (Akan et al., 2015). DDT, DDD and DDE have all been classified by the National

Agency for Food and Drug Administration and Control (NAFDAC) as probable human carcinogens. Also, long-term exposure to DDT, DDE or DDD induced liver cancer in animals (Agency for Toxic Substances and Disease Registry (ATSDR), 2002). The concentration of o,p'DDT (0.046 mg/l) and aldrin (0.057 mg/l), dieldrin (0.089 mg/l) and its metabolites were higher in the blood samples of sheep when compared with blood samples of rabbits as mentioned above. The high concentration of OC residue in the blood samples of sheep could be attributed to the high level of grazing of these animals in the vicinity. There is also an increase in agricultural activities which has enhanced the use of pesticides in crop and animal production. This is because sheep and other ruminants grazed freely on contaminated environment and drink water from ponds, stream, rivers and other possible contaminated water sources. Sheep in the process are exposed to high levels of organochlorine pesticides in the environment, in agreement with the report of Nwude et al. (2010).

Organophosphorus pesticide residues (Dichlorvos, Malathion, Chlorpyrifos, Parathion, Phosphamidon and Ethion) in the blood samples

The highest concentration of dichlorvos in blood samples was detected at Wurojabbe with a mean level of 0.032±0.001 mg/l, while the lowest concentration was observed at Jambutu location with a mean value of 0.008±0.001 mg/l. For malathion, the highest concentration was recorded at Ngorore location with a mean value of 0.028±0.001 mg/l and the lowest concentration was observed at Jambutu location with a mean value of 0.005±0.001 mg/l. Also, chlorpyrifos had the highest concentration at Ngorore with a mean level of 0.019±0.001 mg/l, whereas the least concentration of chlorpyrifos was observed at Jambutu with a mean level of 0.003±0.001 mg/l. For parathion, the highest concentration was detected at point Namtari with a mean value of 0.017±0.001 mg/l, while the lowest concentration was recorded at Doubeli location with a mean level of 0.004±0.001 mg/l. The highest concentration of phosphamidon was recorded at Doubeli with a total mean value of 0.023±0.001 mg/l, while the least value was detected at Namtari village with a mean value of 0.013±0.001 mg/l, whereas less than the detection limit was observed at Jambutu location. Ethion was not observed in any of the blood samples analyzed.

The highest concentration of dichlorvos in the blood samples of rabbits was observed at Ngorore with a mean level of 0.012±0.001 mg/l, while Jambutu location showed the lowest concentration of 0.001±0.001 mg/l. Also, Malathion recorded the highest concentration at Namtari village with a mean value of 0.009±0.001 mg/l, while the lowest concentration was observed at Doubeli location with a total mean of 0.01±0.001 mg/l. Also, the highest concentration of chlorpyrifos was detected at Ngorore 0.009±0.001 mg/l, while the lowest concentration was recorded at Wurojabbe with a value of 0.004±0.001 mg/l and less than detection limit was observed at Doubeli. For parathion, the highest concentration of 0.008±0.001 mg/l was observed at Jambutu, with the lowest concentration of 0.001±0.001 mg/l at Ngorore. Phosphamidon recorded the highest concentration at Namtari location (0.004±0.001 mg/l), while the lowest concentration was observed at Ngorore with a value of 0.001±0.001 mg/l and less than the detection limit was observed at Wurojabbe, Doubeli and Jambutu. Also, Ethion was not detected in any of the blood samples analyzed.

Organophosphorus pesticides are widely used as agricultural insecticides and also have many uses in households for pest control. The mean concentrations of organophosphorus pesticide residues in the blood samples of sheep ranged from 0.003±0.001 to 0.032±0.001 mg/l, while that of blood samples of rabbits ranged from 0.001±0.001 to 0.012±0.001 mg/l. According to the concentrations and detection frequency, Dichlorvos (0.032±0.001 mg/l), Malathion (0.028.±0.001 mg/l) and Phosphamidon (0.023±0.001 mg/l) were the compounds with the highest organophosphorus pesticide residues in the blood samples of sheep. The high concentration of OP residue in the blood samples of sheep could be attributed to the high level of grazing of these animals in these areas. There is also an increase in agricultural activities which has enhanced the use of pesticides in crop and animal production. The maximum residue limit (MRL) is the maximum amount of the pesticide residue which is found in food substances that will not cause any health hazard (Yasmin and Souza, 2010).

Conclusion

The present study analyzed a wide variety of pesticides in animal blood samples from some selected villages of Jimeta-Yola. Generally, the residues in blood samples of both the sheep and rabbits were at very low concentrations because as soon as it enters into the body, most of the chemicals may get metabolized and the metabolites may accumulate to induce toxic effects. Despite the bans and restrictions on the use of some of these pesticides in Nigeria, the observed concentrations of the studied pesticides from the villages could explain either their persistence in the environment or continued usage in the study area. Hence, routine monitoring of pesticide and its usage in these villages is necessary for prevention, control and reduction of environmental pollution, so as to minimize health risks.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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