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Organophosphorus Pesticide Residues in Different Tissues of Fish Samples from Alau Dam, Borno State, Nigeria

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Abstract: Levels of some Organophosphorus pesticides (Dichlorvos, Diazinon, Chlorpyrifos and Fenitrothion) residues were determined in the flesh, liver, stomach and gills of four commercially species (*Clariasgariepinus,Hetrotisniloticus, Oreochromisniloticus* and *Tilapia zilli*) fromAlau Dam,Konduga Local Government Area, Borno State. Fish samples of uniform size were collected and transported to the laboratory on the same day and later dissected to remove the flesh, liver, stomach and gills of each species of fish and store using 4% formalin pending extraction and analysis. The extraction, cleanup and de-fattening of the fishes organs were carried out using standard procedures. Pesticide residues in the fish samples were determined using GC/MS SHIMADZU (GC-17A) equipped with electron capture detector (ECD). The concentrations of these pesticides were found to be higher in the liverof*Oreochromisniloticus,* while the flesh of *Hetrotisniloticus*shows the lowest values. The concentrations of all pesticides were observed to be higher than the European Union (EU) set maximum residue limits (MRLs) and the Acceptable Daily Intake value (ADI) and this could be an important process of transferring pesticides to humans. The results also indicate presence and usage of these pesticides in the study environment and also demonstrate the pollution of Alau Dam with pesticide residues.Generally the levels of these organophosphorus pesticide residues suggested that the dams have been polluted due to human activities such as farming activities. Therefore, adequate measures should be taken to reduce the levels these pesticides so as to preserve the aquatic life in Alau Dam.

Key words: Organophosphorus · Pesticide · Tissues · Alau Dam · Fish · Extraction · Cleanup · De-Fattening.

INTRODUCTION

The increasing population necessitates more agricultural products and foodstuffs that consequently need more pesticide usage to destroy any pests [1, 2]. The pesticide compounds include Organophosphorus, Organochlorine, Carbamate and Pyrethroid derivatives [3]. They have some side effects on living organisms such as organophosphorus inhibits cholinesterase activity and it makes central nervous system (CNS) functional disturbances. Organochlorine accumulates in living organism bodies and also in food chain. Carbamate derivatives cause genetic mutations and CNS functional disturbances [3].

Pesticides are used widely to improve agricultural production and also to prevent arthropod-borne diseases. But they are used improperly due to the lake of appropriate knowledge about their applications and untoward effects. The excessive usage is harmful to ecosystem and they contaminate soil, surface and underground water resources [4, 5]. Relevant poisoning in many countries, especially in developing countries is considered the second causes of mortalities after infectious diseases [6].

Pesticides cause untoward effects on man in two ways. Firstly, they have direct effects on the health of persons who use them; and secondly, their remnants accumulate in foodstuffs which also produce side effects on man [7]. The side effects include short term ones like abdominal cramps, vertigo, headaches, diplopia, nausea, ocular disturbances and dermatopathies. Long term adverse effects include increased likelihood of respiratory failures, depression, nervous defects, prostate cancer, leukemia and infertility. These problems are considered

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the major health problems in the world [7-9]. The various investigations conducted on farmers in terms of their health status demonstrated that pesticides may increase the likelihood of Parkinson disease [8, 10].

Environmental pollution by pesticides has been identified as one of the major environmental impacts from agriculture [11]. Parent compounds as well as metabolites of pesticides have been identified in air [12], water [13] and soil [14]. The list of pesticide related compounds which have been identified in the environment and proved to be carcinogenic is growing as new methods of detection have been developed and sensitivities and specificities of assays have been improved.

Pesticides differ in their mode of action, uptake by the body, metabolism and elimination from the body and toxicity potential. Because of these differences some pesticides show acute short term effects, while others tend to accumulate in the body and with time demonstrate sublethal adverse health effects. Many of these compounds also persist in the environment and bioaccumulate in the animal and human tissues [15]. The degradation and transformation of the nonpersistent chemicals in the environment are dependent on their physicochemical properties, the environment in which they reside and the threshold levels of these chemicals in the environment. Degradation and transformation processes do not always result in decreased activity or dilution of the parent compound, for the degraded or transformed products are at times more toxic, resulting in biomagnification of the toxicity of the parent compound.

Pesticides can enter surface and ground waters through runoff from treated soils, leaching processes, aerial drift and inappropriate disposal methods. The developing countries, which still use organochlorine pesticides, have found that water bodies (lakes and rivers) have been contaminated with the environmentally persistent pesticide residues [16]. The presence of organophosphorus in sediment samples suggests that these environmentally persistent compounds have been resident in the water bodies for a long time and may have contaminated the aquatic flora and fauna. The most striking effect of some of the pesticides in water is their ability to concentrate in the fatty tissue of successively higher components of the aquatic food chain.

The frequent presence of pesticides and their high toxicity along with considerable bioaccumulation in freshwater fishes make them toxicants that should be given due consideration in aquatic toxicology. Fish accumulate xenobiotic chemicals, especially those with poor water solubility because of the very intimate contact with the medium that carries the chemicals in solution or suspension and also because fish have to extract oxygen from the medium by passing enormous volumes of water over the gills. Fish kill or injury due to pesticides contamination is considered the primary cause of reducing fish populations and other animals including humans through the food chain [17]. Behavioral avoidance of contaminants may be an additional cause of reduced fish populations [17].

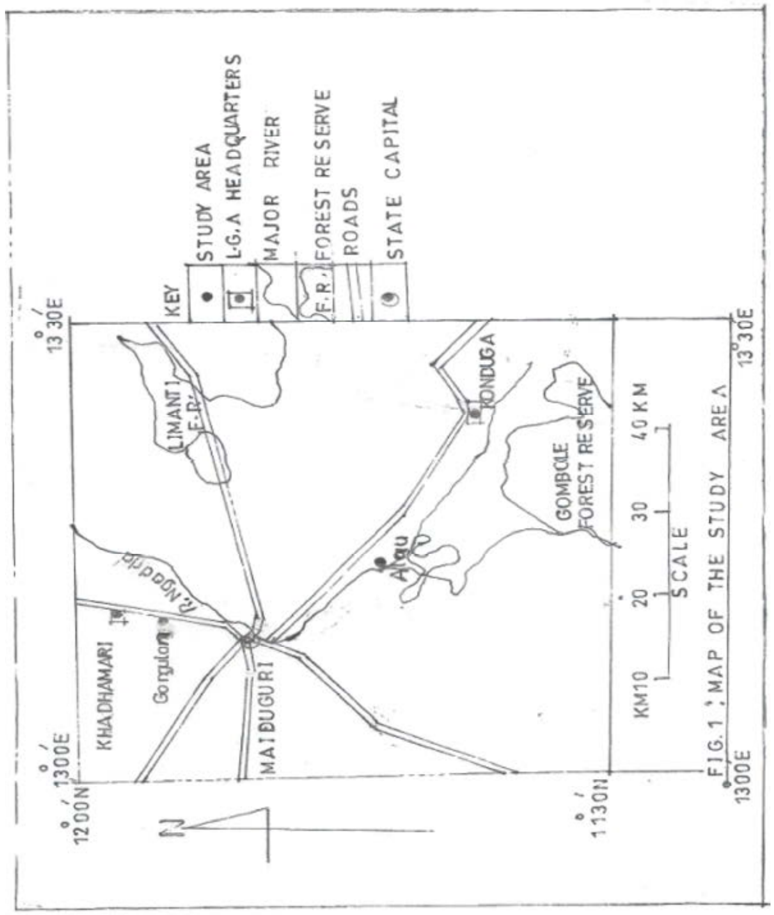
The Alau Dam area is primarily an agriculture area with intense pesticide usage.

Pesticides are extensively used in the area for vegetables, cereals and fruit productions as well the control of vector-borne diseases for public health. Economic activities in the Dam also include fishing. Fish from the Alau Dam serves as the major source of income for most of the inhabitants. Fish in the Dam also constitutes an important source of protein for the inhabitants in and around the Metropolis. Agricultural activities have impacted negatively on the Dam water drains from these activities empty into the Dam. Bioaccumulation and bioconcentration of pesticides in the fish species are capable of reaching toxic levels in the fish even when exposure is low.

MATERIALS AND METHODS

Study Area: Alau Dam is located in Konduga, Borno State, North Eastern Nigeria (Map1). The Dam is nine meter high with a square reservoir area of about 50 km^2. The maximum storage capacity is 112 million meter cube. Alau Dam received water from River Yedzram and River Gombole which meet at a confluent at Sambisha and flow as River Ngada into Alau Dam. Alau Dam received a wide variety of waste from agricultural land. This waste generated contaminates Alau Dam with a variety of pesticides acting as point sources. This Lake is also use for commercial fishing. Alau Dam received a wide variety of waste from agricultural activity within this area. Most farmers within the Alau dam area used synthetic chemical pesticides to control pests on vegetables including a number of highly persistent organophosphoruspesticides.

Sample Collection: Fish samples (*Clariasgariepinus, Hetrotisniloticus, Oreochromisniloticus and Tilapia zilli)*were caught using gill nets from Alau Dam, Konduga Local Government Area, Borno State, Nigeria. Fish samples of uniform size were collected in order to avoid the possible error due to size differences. The fish were labeled with an dentification number.



Map 1: Map of Study Area

Samples of fishes were transported to the laboratory on the same day, identified by an expert in the department of Fisheries, University of Maiduguri and later dissected to remove the flesh, liver, stomach and gills of each species of fish and stored using 4% formalin, pending extraction and analysis.

Extraction of Pesticides from Fish Samples: The fish samples (20g) were weighed into a 150 ml conical flask followed by the addition of 20 g and 5 g of anhydrous sodium sulfate and sodium hydrogen carbonate, respectively. 100 ml of 1:1 (v/v) ethyl acetate/ dichloromethane mixture were transferred into the 20g fish samples and thoroughly mixed by shaking the conical flask while corked. 20g of anhydrous sodium sulfate were then added to the content of the conical flask followed by 20g of sodium hydrogen carbonate. The conical flask were corked tightly and the mixture shaken thoroughly for 10 min. The content was allowed to stand for 3h. The organic layer were decanted into a 200ml round bottom flask and evaporated using the rotary evaporator at 40°C. The pesticide in the rotary flask were dissolved and collected with 2 ml of ethyl acetate and transferred into a 2 ml vial and ready for the clean-up.

Silica Gel Clean-up of Sample Extracts: Ten gram (10g) portion of deactivated silica gel were weighed and transferred into a 10 mm glass chromatographic column followed by addition of 3 g of anhydrous sodium sulfate. 10 ml of the 1:1 (v/v) ethyl acetate/dichloromethane mixture were used to wet and rinse the column. The extract residue that is water and fish in 2 ml ethyl acetate were transferred into the column and the extract vial rinsed (three times) with 2 ml ethyl acetate. The column were eluted with 80 ml portion of ethyl acetate/dichloromethane at a rate of 5 ml/min into a conical flask as fraction one. The column were eluted again with 50 ml portion of ethyl acetate/dichloromethane for the second elution and added to the first extract. All the fractions of each sample were concentrated to dryness using a rotary evaporator at 40°C. Each residue were dissolved and collected in 2 ml ethyl acetate for gas chromatograph analysis.

De-fattening of the Fish Sample Extracts: Fifty (50) ml of 1:1 (v/v) hexane/acetonitrile solution were added to 2 ml pesticide extracted from the fish samples in a 100 ml separator funnel. The separator funnel were shaken gently for 3 min while releasing the gas pressure. The separator funnel were allowed to stand for 20 min. to allow for phase separation of the organic solvents. The acetonitrile fractions containing the pesticides were collected into a 50 ml beaker while the fat containing hexane solvent phase were discarded. The acetonitrile solvent extract obtained were further cleaned-up using 25 ml of the pure hexane. The acetonitrile fraction were concentrated with rotary evaporator at 40 °C and the content of the flask dissolve and collected with 2 ml of ethyl acetate into a 2 ml vial. The vial containing the pesticides extracts were stored in the refrigerator at 4°C for GCMS analysis.

Determination of Pesticide Residues: The SHIMADZU GC/MS (GC – 17A), equipped with fluorescence detector were use for the chromatographic separation and were achieve by using a 35% diphenyl/65% dimethyl polysiloxane column. The oven were programmed as follows: initial temperature 40°C, 1.5 min, to 150°C, 15.0 min, 5°C/min to 200°C, 7.5 min, 25°C/min to 290°C with a final hold time of 12 min and a constant column flow rate of 1 ml/min. The detection of pesticides were perform using the GC-ion trap MS with optional MSn mode. The scanning mode offer enhances selectivity over either full scan or selected ion monitoring (SIM). In SIM at the elution time of each pesticide, the ration of the intensity of matrix ions increase exponentially versus that of the pesticide ions as the concentration of the pesticide approach the detection limit, decrease the accuracy at lower levels. The GC-ion trap MS were operate in MSn mode and perform tandem MS function 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 sample were compared with those of the standards for quantization.

Data Handling: Data collected were subjected to one-way analysis of variance (ANOVA) were used to assess whether pesticide residues varied significantly between fish samples and tissues, possibilities less than 0.05 ($p<0.05$) were considered statistically significant. All statistical calculations were performed with SPSS 9.0 for Windows.

RESULTS

The mean concentrations of some organophosphorus pesticides (Dichlorvos, Diazinon, Chlorpyrifos and Fenitrothion) in different organ of *Tilapia zilli*is are presented in Figure 1. The concentrations of the organophosphorus pesticides in the liver of Tilapia zilli ranged from 0.77 to 2.22µg/g; 0.55 to 1.76µg/g gills; 0.32 to 1.45µg/g stomach and 0.27 to 0.67µg/g flesh. The highest concentration of this pesticide was recorded significantly in the liver, while flesh recorded the lowest value, also most abundant organophosphorus pesticide residue recorded was chlorpyrifos with values ranging from 0.77 to 2.22, while diazinon recorded the lowest values ranging from 0.32 to 1.45. The order of tissues pesticide bioaccumulation are in the order of liver>gills>stomach>flesh. Figure 2 present the concentrations of some organophosphorus pesticide residues (Dichlorvos, Diazinon, Chlorpyrifos and Fenitrothion) in different organs of *Clariasgariepinus* from Alau Dam. The concentrations of these pesticides in the liver of *Clariasgariepinus*ranged from 1.45 to 2.98µg/g liver; 1.21 to 2.34µg/g gills; 0.87 to 1.87µg/g stomach and 1.06 to 1.32µg/g flesh. The highest concentrations of this pesticide were significantly recorded in liver, while flesh recorded the lowest values. The concentrations of some organophosphorus pesticide residues (Dichlorvos, Diazinon, Chlorpyrifos and Fenitrothion) in different organs of *Hetrotisniloticus*are as presented in Figure 3. The Concentrations of these pesticides in the liver of *Hetrotisniloticus*ranged from 0.56 to 1.66µg/g liver; 0.36 to 1.04µg/g gills; 0.21 to 0.76µg/g stomach and 0.11 to 0.34µg/g flesh. The highest concentration was recorded in liver, while flesh recorded the lowest value. Figure 4 present the concentrations of some pesticide residues in different organs of *Oreochromisniloticus* from Alau dam. The levels of this pesticide in the liver ranges from 2.43 to 3.54µg/g liver; 2.07 to 3.11µg/g gills; 1.45 to 2.87µg/g stomach and 1.22 to 1.89µg/g flesh. The highest concentration of 2.87µg/g was significantly recorded in liver, while flesh recorded the lowest value of 1.22µg/g. Figure 5 shows a comparison in the concentrations of some organophosphorus pesticide residues (dichlorvos, diazinon, chlorpyrifos and fenitrothion) in the liver of *Tilapia zilli, Clariasgariepinus, Hetrotisniloticus* and *Oreochromisniloticus*. Levels of dichlorvos ranged from 1.02 to 2.87µg/g; 0.56 to 2.43µg/g diazinon; 1.13 to

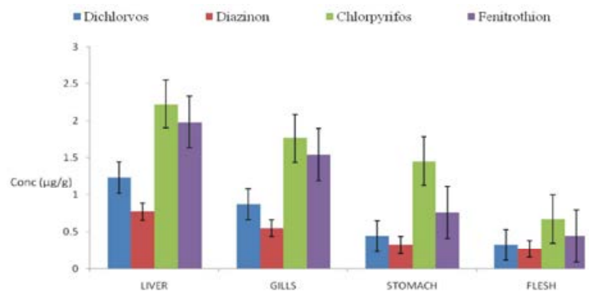


Fig. 1: Mean concentration and standard error of some oganophosphorus pesticide residues in different organs of tilapiazilli from alau dam.

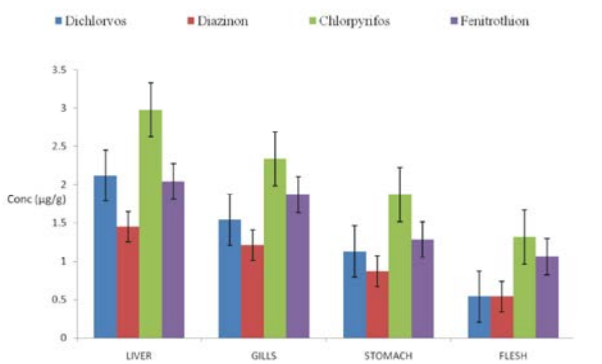


Fig. 2: Mean concentration and standard error of some oganophosphorus pesticide residues in different organs of Clarias Grariepinus from Alau Dam.

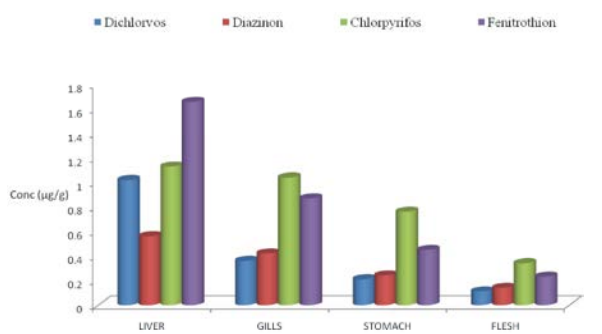


Fig. 3: Mean concentration and standard error of some oganophosphorus pesticide residues in different organs of Hetrotis niloticus from Alau Dam.

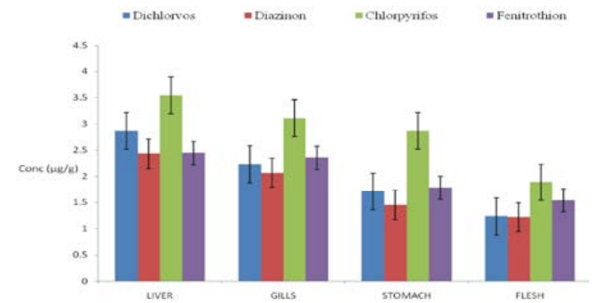


Fig. 4: Mean concentration and standard error of some oganophosphorus pesticide residues in different organs of Oreochromis niloticus from Alau Dam.

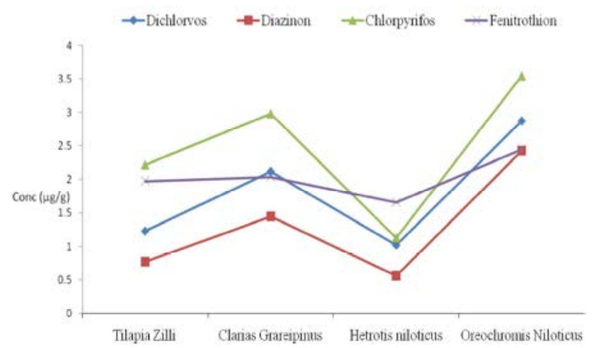


Fig. 5: Mean concentration and standard error of some oganophosphorus pesticide residues in the liver of four species of fish from alau dam

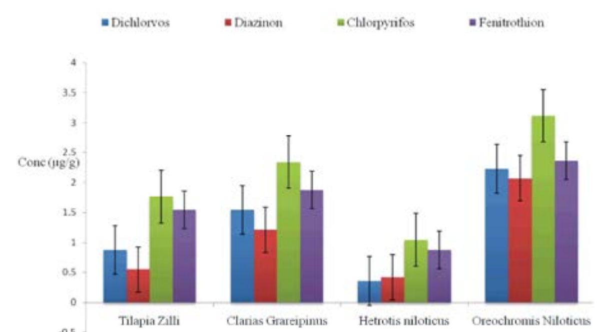


Fig. 6: Mean concentration and standard error of some oganophosphorus pesticide residues in the gills of four species of fish from alau dam

3.54µg/g chlorpyrifos and 1.66 to 2.45µg/g fenitrothion. The highest value was observed in *Oreochromisniloticus,* while *Hetrotisniloticus* recorded the lowest value. Figure 6 shows a comparison in the concentrations of organophosphorus pesticide residues in gills of *Tilapia zilli, Clariasgariepinus, Hetrotisniloticus* and *Oreochromisniloticus*. The concentration of dichlorvos ranged from 0.36 to 2.23µg/g; 0.42 to 2.07µg/g diazinon; 1.04 to3.11µg/g chlorpyrifos and 0.87 to 2.36 µg/g fenitrothion. Figure 7 present the comparison in the concentrations of some organophosphorus pesticide residues in stomach of *Tilapia zilli, Clariasgariepinus, Hetrotisniloticus* and *Oreochromisniloticus*. The concentration of dichlorvos ranged from 0.21 to 1.71µg/g; 0.24 to 1.45µg/g diazinon; 0.76 to 2.87µg/g chlorpyrifos and 0.45 to 1.78 µg/g fenitrothion. Figure 8 shows a

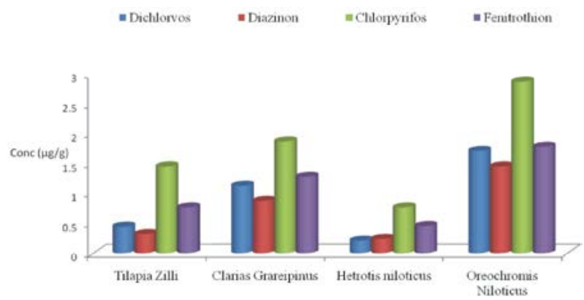


Fig. 7: Mean concentration and standard error of some oganophosphorus pesticide residues in the stomach of four species of fish from alau dam

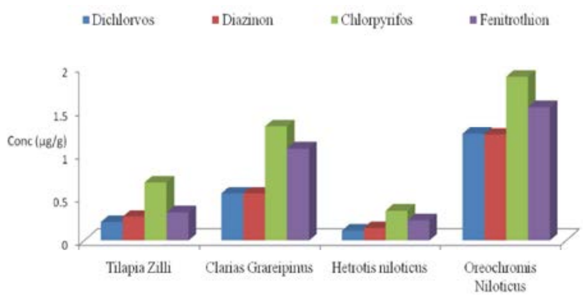


Fig. 8: Mean concentration and standard error of some oganophosphorus pesticide residues in the flesh of four species of fish from alau dam

comparison in the concentrations of some organophosphorus pesticide residues in stomach of *Tilapia zilli, Clariasgariepinus, Hetrotisniloticus* and *Oreochromisniloticus*. The concentration of dichlorvos ranged from 0.11 to 1.23µg/g; 0.14 to 1.22µg/g diazinon; 0.34 to 1.89µg/g chlorpyrifos and 0.23 to 1.54µg/g fenitrothion. The highest values of these pesticides were observed in stomach of *Oreochromisniloticus,* while *Hetrotisnitritius* recorded the lowest value.

DISCUSSION

The highest values of organophosphorus pesticide residues were detected in the liver of *Oreochro misniloticus,* while the least value was detected in the flesh of *Hetrotisniloticus*. The organophosphorus (Ops) pesticides are much more resistant to microbial degradation and have a propensity to concentrate in lipid rich tissues of aquatic organisms and most mammals. These properties lead directly to their most undesirable characteristics– the environmental persistence, bio-concentration and bio-magnification through the food chain. Dichlorvos, Diazinon, Chlorpyrifos and Fenitrothion which are readily deactivated and degraded by micro-organisms and therefore do accumulate [18]. Among the OPs determined, Chlorpyrifos shows the highest concentration followed by Fenitrothion, while Diazinon shows the lowest value. These pesticides are widely used as agricultural insecticides and also have many uses in households for pest control.The concentrations of organophosphorus pesticide residues (diazinon, chlorpyrifos and fenitrothion) detected in this study fell above the WHO and FAO [19] set maximum residue limits (MRLs) of 0.04µg/kg for diazinon, 0.30µg/kg for Chlorpyrifos and 0.01µg/kg for Fenitrothion. The Acceptable Daily Intake values (ADIs) of Diazinon, Chlorpyrifos, Fenitrothion for fish samples are 0.0002 µg/kg diazinon, 0.01 µg/kg Chlorpyrifos and 0.005µg/kg Fenitrothion respectively. The results obtained from the present study exceeded this Acceptable Daily Intake values and this may be attributed to the presence of this pesticide in the aquatic environment. The maximum residue limits (MRL) is the maximum amount of the pesticide residue which is found in food substances that will not cause any health effect or hazard [20]. These pesticides were significantly higher in the liver and gills of all the fishes studied than other organs, such high levels is due to the fact that fresh water fishes gills might be expected to be the primary rout for the uptake of water pollutants; while the liver serve as a storage organs for vast variety of nutrient. High accumulation of this pesticide in the gills and liver can also be as a result of detoxicating mechanisms and may originate from pesticides deposited in the sediments and food in the aquatic environment. However, the liver is the preferred organs for pesticides accumulation as could be deduced from the present study. Accumulation of pesticides in different species is the function of their respective membrane permeability and enzyme system, which is highly species specific and because of this fact pesticides accumulated in different organs in the fish as observed in the study.

CONCLUSION

Of all the pesticides study, Chlorpyrifos was the most abundant organophosphoruspesticides residue in the studied tissues of all the fish species. The concentrations of these pesticides were found to be higher in the liver*Oreochromisniloticus,* while the flesh of *Hetrotisniloticus*shows the lowest values. The concentrations of all the pesticides were observed to be higher than the European Union (EU) set maximum residue limits (MRLs) and the Acceptable Daily Intake value (ADI). The results also indicate presence and usage of these pesticides in the study environment and also demonstrate the pollution of Alau Dam with pesticide residues.Generally the levels of these organophosphorus pesticide residues suggested that the dams have been polluted due to human activities such as farming activities. Therefore, adequate measures should be taken to reduce the levels these pesticides so as to preserve the aquatic life in Alau Dam.

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