

Research Article

Determination of Pesticide Residues in Different Fish Species from Dukku River in Birnin Kebbi, Kebbi State, Nigeria

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Abstract

The levels of pesticide residues (organophosphorus and organochlorine) were investigated in six different species of fish from the Dukku River using GC with ECD/FID. The results indicate the frequent presence of both OCs and Ops in the samples. Among the OCs analysed γ -HCH shows the highest detectable average value of 1.367 $\mu\text{g/g}$ followed by DDE 1.248 $\mu\text{g/g}$, α -HCH has 1.079 $\mu\text{g/g}$, DDT 1.050 $\mu\text{g/g}$ and the least α -endosulfan 0.026 $\mu\text{g/g}$. The detectable Ops showed that chlorpyrifos has the highest average value of 0.0842 $\mu\text{g/g}$, followed by methylparathion 0.043 $\mu\text{g/g}$ and the least phosphamid 0.016 $\mu\text{g/g}$. The total concentration of residues in the fish samples ranged between 3.738 $\mu\text{g/g}$ for the herbivorous Tilapia zilli to 8.152 $\mu\text{g/g}$ for Hepsetus odot a carnivore. The results generally showed that the carnivorous fish species have greater potential to accumulate the pesticide residues than the herbivorous fish. The detectable pesticide residues observed in this work are above the maximum permissible levels set by FAO/WHO indicating possible pollution of the water bodies.

Key words: Organochlorine, Organophosphorous, Dukku river, pesticide residues, Birnin Kebbi, fish.

Received: 14 December 2016

Accepted: 7 February 2017

Introduction

Fish accumulates toxic chemicals such as pesticides residues directly from water and diet, and contaminant residues may reach concentrations hundreds or thousands of times above those measured in the water, sediments and food (Godwin *et al.* 2003; Labonne *et al.* 2001; Osman *et al.* 2007).

Pesticides are used widely to improve agricultural production and also to prevent arthropod – borne diseases, pesticides have proved even more important in the protection of crops and crop products in order to feed the ever increasing human population, without the use of pesticides it is estimated that up to 50% of our agricultural food supply would be lost (Tschireley, 1979). The initial use of pesticides proved effective and popular because of long residual effect, low cost and low toxicity until 1960s when some studies showed their persistence in the ecosystem due to their long residual effect and resultant damage to the environment much more than it eased pest menace. The excessive and improper use of pesticides due to lack of appropriate knowledge about their applications can lead to untoward effects, contamination of the soil, surface and ground water resources (Khodadadi *et al.* 2010, Srivastava *et al.* 2010). Relevant poisoning in many countries, especially in developing countries is considered the second causes of mortalities after infectious diseases (Farshad, 2000).

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 foods stuffs which also produce side effects on man (Yazgan and Tanik, 2010). The side effects include short term ones like abdominal cramps, vertigo, headache, 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 the major health problems in the world

(Yazgan and Tanik, 2010; Ghasemi and Karam, 2009). Various studies also showed that farmers who work frequently with pesticides are prone to a higher risk of Parkinson disease (Hoek and Dawson, 2007; Bradman *et al.*, 2011).

Pesticides especially the organochlorine compounds are highly lipophilic in nature and therefore tend to accumulate in the fatty tissue of fish and mammals in general, leading to a decline in fish populations due to poor reproduction success and early fly mortality. Other adverse effect of pesticides include, increase of resistance populations, minor pests becoming major pests due to the elimination of their natural predators as a result of intense pesticide application (McEwen and Stephenson, 1979).

The ultimate effect of the pesticides is seen on the non-target organisms especially those inhabiting the aquatic environment. The aquatic environment is the ultimate sink for pollutants and any compounds produced on an industrial scale are likely to reach this environment sooner or later (Murty, 1986). Pesticide 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 (Aguilar *et al.* 1992). The presence of organophosphorous 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 fresh water 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 volume of water over the gills (Akueshi *et al.* 2003).

Fish are used extensively for environmental monitoring (Lanfrenchi *et al.* 2006), because they uptake contaminants directly from water and diet. Generally the ability of fish to metabolise OC is moderate, therefore, contaminant loading in fish is well reflective of the state of pollution in surrounding environments (Guo *et al.* 2008).

The determination of pesticide residues in fish may give indication of the extent of aquatic contamination and accumulation characteristics of these compounds in the tropical aquatic biota that will help in understanding the behavior and fate of these persistent chemicals (Kannan *et al.* 1995). This work, therefore, seeks to provide baseline information on levels of pesticide residue in the most common fishes from Dukku River that will assist in a scientific assessment of the impact of pesticides on Public Health and the ecosystem.

Materials and Methods

Study Area: Dukku River Basin is located in Birnin Kebbi, North West of Nigeria. The river is the biggest and largest river in Kebbi State, running through several local government areas, towns and villages. From Argungu through Zauro, Birnin Kebbi, Kalgo, Bunza and Yauri. The people along this river route are predominantly farmers,

dealing in crop production and animal rearing. Dukku River received a wide variety of waste from agricultural land. This waste generated contaminates the water bodies with a variety of pesticides acting as point sources. Commercial fishing activity is also predominantly done in this river. Most farmers within the Dukku Basin used synthetic chemical pesticides to control pest on crops and animals whose residues ultimately entered into the river.

Sampling

Six fish species (*Heterotis niloticus*, *Channa obscura*, *Hepsetus odoe*, *Tilapia zilli*, *Clarias gariepinus* and *chrysichthys nigrodigitatus* [Table 1] from the Dukku River were selected for the study due to difference in their feeding habits. The fish samples of uniform size were bought directly from the fishermen when still alive. Samplings were done bi-monthly over a period of one year from June, 2014 to July, 2015. The samples were immediately wrapped in aluminum foil appropriately labeled, transported to the laboratory on ice and kept at -10°C in a freezer prior to extraction.

Sample Extraction

The extraction of pesticide residues from the fish samples was performed by solid dispersion method as described by the U.S Food and Drug Administration (FDA, 1994) and Akerblom, (1995). 200g fish sample (fresh weight) of each fish species were homogenized separately in a stainless steel blender. 25g of the homogenized fish sample of each species were ground separately with 35g of anhydrous sodium sulphate in a mortar to a free – flowing powder. The powder was extracted in a flask by shaking successively with ethyl acetate (20ml). The combined extract was filtered through a glass cotton wool and the solvent evaporated at 40°C with rotary evaporator to near dryness.

The concentrate was recovered with (3 x 2ml) portion of cyclohexane. The organic phase was then subjected to clean-up.

Clean-Up of Sample Extracts

Conditioned 8-ml C-18 solid phase extraction (SPE) cartridge was used for the sample clean-up (Zhou *et al.* 2000). The SPE cartridge system was conditioned by washing with

5ml distilled water, 5 ml ethyl acetate followed by 5ml methanol. Each fish extract was percolated through the cartridge with a flow rate of approximately 5ml/min under vacuum pump. The pesticides trapped in the cartridges were eluted with 6ml (2 x 3ml) ethyl acetate. The sample extract was concentrated using the rotary evaporator to 2ml for GC analysis.

Analysis and Quantification of Pesticide

Fish samples were analysed in a gas chromatograph [Shimadzu GC/MS – 17A) equipped with ^{63}Ni ECD and FID. The chromatographic separation was achieved by using a 35% diphenyl, 65% dimethyl polysil-oxane column. The oven were programmed as follows: initial temperature 40°C 1.5min, to 150°C, 15.0 min, 5°C/min to 200°C, 7.5 min, 25°C/min to 250°C with final hold time of 12 min and a constant column flow rate of 1ml/min. The detection of pesticide residues were performed using the GC – ion trap MS with optical 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 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, decrease the accuracy at lower levels. The GC – ion trap MS were operated 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 quantification. Percentage recoveries in spiked samples were between 65 – 100% hence the result of the study were not corrected for recoveries since all were within the normal acceptable range of 65 – 120% (Hill, 2000; FAO/WHO, 2007).

Pesticide Standard Used

All pesticide standards (OCs and Ops) used were purchased from Sigma Aldrich through BSC Lagos Nigeria.

Table 1: Characteristics of Fish Species Sampled from Dukku River

Code	Species	Common Names	Feeding Habit
DK-HN	<i>Heterotis niloticus</i>	African arowana	carnivorous
DK-CO	<i>Channa obscura</i>	snake head	carnivorous
DK-HO	<i>Hepsetus odoe</i>	African pike	carnivorous
DK-TZ	<i>Tilapia zilli</i>	Tilapia	herbivorous
DK-CG	<i>Clarias gariepinus</i>	Mud fish	carnivorous
DK-CN	<i>Chrysichthys nigrodigitatus</i>	cat fish	carnivorous

Results and Discussions

Dukku River is surrounded by farm lands. A large amount of agrochemicals are used there by farmer most of them having little or no knowledge about these chemicals that will ultimately enter into the river through running waters and subterranean canals and thus contaminating the water body and then taken up by biota.

Results from this study have been shown in Tables 1 and 2 which is related to the concentration of OC and OP residues in the fish samples respectively. Endosulfan, a broad spectrum contact insecticide and acaricide was detected in 5 of the samples with the highest concentration of (0.035µg/g) found in *Clarias gariepinus* and the minimum concentration of (0.012µg/g) found in *Channa obscura*.

Heptachlor is another OC detected in the tissue of 3 of the samples with the maximum concentration of (0.061µg/g) found in *Clarias gariepinus* and a minimum of (0.11µg/g) concentration found in *Tilapia zilli*. The use of heptachlor was banned in many advanced countries with the effect from September 20, 1996.

Aldrin known for its effectiveness against termites was detected in the samples with the maximum concentration of (0.035µg/g) found in *Chrysichthys negrodigitatus* and a minimum concentration of (0.021µg/g) in *Tilapia zilli*.

Dieldrin another OC effective against termites was found to have a maximum concentration of (0.051µg/kg) in *Chrysichthys negrodigitatus* and a minimum of (0.011µg/g) in *Clarias gariepinus*.

DDT was detected in 5 of the samples with the maximum concentration of (1.067µg/g) in *Hepsetus odoe* and a minimum concentration of (1.025µg/g) in *Clarias gariepinus*. DDT was detected in most of the samples perhaps due to its persistence nature. Since DDT is known to undergo metabolic conversion and dehydrochlorination, presence of metabolites of DDT (DDE) encountered in this study might be due to such metabolic processes.

DDE a metabolite of DDT was detected in all the samples analysed with a maximum concentration of (1.511µg/g) found in *Hepsetus odoe* and a minimum concentration of (1.111µg/g) in *Tilapia zilli*. The high concentration of DDE along with DDT in this study may be due to a combination of past and present usage of these pesticides in both agriculture and public health programs in the catchment area.

Table 2: Levels of Organochlorine Pesticide Residues in Fish Tissue Sample of Dukku River. Concentration (µg/g)

Sample	α-endosulfan	Heptachor	Aldrin	Diadrin	DDT	DDE	α-HCH	γ-HCH	Methoxychlor	Endrin
DK-HN	0.031	ND	0.022	ND	1.051	1.112	1.020	1.211	ND	0.222
DK-CO	0.012	ND	0.033	ND	1.042	1.212	1.110	0.661	0.521	ND
DK-HO	0.022	0.033	ND	0.033	1.067	1.511	1.201	1.901	0.876	0.557
DK-TZ	ND	0.011	0.021	ND	ND	1.111	1.011	1.012	ND	
ND										
DK-CG	0.032	0.061	ND	0.011	1.025	1.222	1.051	0.712	0.725	0.255
DK-CN	0.035	ND	0.035	0.051	1.066	1.320	ND	1.705	0.975	ND
Average Value	0.026	0.035	0.028	0.032	1.050	1.248	1.079	1.367	0.774	0.345
Min. residue	0.012	0.011	0.021	0.011	1.025	1.111	1.011	1.012	0.521	0.222
Max. residue	0.035	0.061	0.035	0.051	1.067	1.511	1.201	1.901	0.975	0.557

ND = Not Detected.

Table 3: Levels of Organophosphate Pesticide Residues in the Fish Tissue Samples from Dukku River concentration (µg/g)

Sample	Dimethoate	Phosphamid	Parathion	Methylparathion	Chlorpyrifos	Diazinon		
Profenofos	Malathion							
DK-HN	0.012	0.010	0.032	0.051	0.0951	0.211	0.022	0.061
DK-CO	ND	ND	0.013	0.049	0.887	ND	ND	0.036
DK-HO	ND	0.022	ND	ND	0.912	ND	0.017	ND
DK-TZ	ND	ND	0.010	ND	0.551	ND	0.011	
DK-CG	0.022	ND	ND	0.011	ND	0.021	ND	ND
DK-CN	0.017	ND	0.039	0.061	0.911	ND	ND	0.022
Average Value	0.017	0.016	0.024	0.043	0.842	0.116	0.017	0.040
Min. residue	0.012	0.010	0.010	0.011	0.551	0.021	0.011	0.022
Max. residue	0.022	0.022	0.039	0.061	0.951	0.211	0.022	0.061

ND = Not Detected.

HCH is used against sucking and biting pest and as smoke for control of pest in grain stores. It is used as dust to control various soil pests such as flea beetles and mushroom flies. HCH is a mixture of eight isomers of which five are found in crude product. Only the gamma isomer or lindane has powerful insecticidal properties. It is very effective against a variety of insects, including domestic insects and mosquitoes. Lindane appears in the list of pesticide for restricted use. Only the α , γ - isomers of HCH were detected in the fish sample with concentrations of (1.011 – 1.201 $\mu\text{g/g}$) and (1.012 – 1.701 $\mu\text{g/g}$) respectively. The maximum concentration of γ -HCH compared to α -HCH might be because γ -HCH is more resistant to biological and chemical degradation under aerobic conditions (Elbeit *et al.* 1981).

Methoxychlor was detected in 4 of the samples with maximum concentration of (0.975 $\mu\text{g/g}$) in *Chrysichthys nigrodigitatus* and a minimum concentration of (0.521 $\mu\text{g/g}$) in *Channa obscura*.

Endrin was found in 3 samples with a maximum concentration of (0.557 $\mu\text{g/g}$) in *Hepsetus odoe* and a minimum concentration of (0.222 $\mu\text{g/g}$) in *Heterotis niloticus*.

Table 3 shows the concentration levels of the various organophosphorus (Ops) pesticide residues found in the samples.

Dimethoate is a systemic and contact insecticide and acaricide, effective against red spider mites and thrips on most agricultural and horticultural crops. Dimethoate was detected in 3 of the samples with a maximum concentration of (0.22 $\mu\text{g/g}$) found in *Clarias gariepinus* and a minimum concentration of (0.012 $\mu\text{g/g}$) found in *Heterotis niloticus*. Phosphamid was detected in 2 of the samples, (0.022 $\mu\text{g/g}$) was the maximum concentration found in *Hepsetus odoe* and (0.010 $\mu\text{g/g}$) was the minimum concentration detected in *Heterotis niloticus*.

Parathion was detected in 4 samples with a maximum concentration of (0.039 $\mu\text{g/g}$) in *Chrysichthys nigrodigitatus* and a minimum concentration of (0.010 $\mu\text{g/g}$) detected in *Tilapia zilli*.

Methylparathion is rapidly absorbed into the blood stream through all normal routes of exposure in water. Methylparathion is subjected to photolysis, with a half-life of 8 days during the summer and 36 days in winter (Kaiser and Tola, 1980). It was detected in 4 samples with (0.061 $\mu\text{g/g}$) concentration found in *Chrysichthys nigrodigitatus* and a minimum of (0.011 $\mu\text{g/g}$) detected in *Clarias gariepinus*.

Chlorpyrifos is moderately toxic to humans. It is a suspected neuroteratogen (Kidd and James, 1991). Chlorpyrifos has become one of the most widely applied insecticide in homes, restaurants against cockroaches, termites etc. It was detected in all the samples with the maximum concentration of (0.951 $\mu\text{g/g}$) found in *Chrysichthys nigrodigitatus* and a minimum concentration of (0.551 $\mu\text{g/g}$) in *Tilapia zilli*.

Diazinon was detected in 2 samples with a maximum concentration of (0.211 $\mu\text{g/g}$) found in *Heterotis niloticus* and a minimum concentration of (0.021 $\mu\text{g/g}$) found in *Clarias gariepinus*.

Profenofos is another OPs detected in 3 samples with a maximum concentration of (0.022 $\mu\text{g/g}$) in *Heterotis niloticus* and a minimum concentration of (0.11 $\mu\text{g/g}$) in *Tilapia zilli*.

Malathion an important and widely used contact insecticide and acaricide for the control of aphids, red spider mites, leaf hoppers and thrips on a wide range of vegetable and other crops, was detected in 3 samples with a maximum concentration of (0.061 $\mu\text{g/g}$) in *Heterotis niloticus* and a minimum concentration of (0.022 $\mu\text{g/g}$) in *Chrysichthys nigrodigitatus*

The OCs residues in this study showed higher concentration and frequency than the OPs (Tables 1 & 2). The OCs 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 environmentally persistence bioaccumulation and biomagnification through the food chains. The OPs are less persistent and are readily deactivated and degraded by micro-organisms and therefore accumulate less in the tissue (Essumang *et al.* 2009).

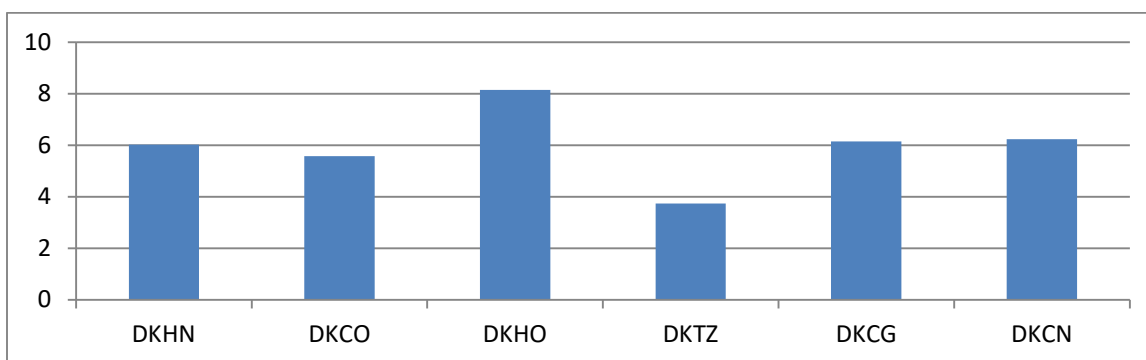


Figure 1 showing the total pesticide residues (organochlorines and organophosphorus) in the samples.

The total pesticide residues (OCs and Ops) in the fish species ranged from 3.738 µg/g to 8.152 µg/g with the highest level detected in DK – HO and lowest level in DK – TZ (Figure1). The sequence of pesticide detected in the fish samples was DK – HO > DK – CN > DK – CG > DK – HN > DK – CO > DK – TZ. The variation in concentration of pesticides detected in fish species could be attributed to difference in the metabolic characteristics of the fish species as well as their feeding habits. The herbivorous diet is dominated by phytoplanktons or benthic algae while the carnivorous feeds on insects, detritus and other herbivores (Fianko *et al.* 2013).

Residue levels in fish vary with species due to differences in biology (trophic level, habitat and reproductive season), detoxification capacity, and ecology (Wasswa and Kiremise, 2004). Again, the difference in residue levels may also be a reflection of different exposure regimes and innate individual differences in metabolism. The variation in concentration of residue in different fish species suggested a higher accumulation potential by those species. The diet and metabolic characteristics of the fish species affect the accumulation of pesticides in fish. Tilapia is primarily an herbivorous cichlidae, its diet is dominated by phytoplanktons (chlorophyllcea, cyanophyceae and englenophyceae) or benthic algae. The catfish represent the most common species in the aquatic ecosystem and feeds on insects (chiromidae) and detritus (Adeyemi *et al.* 2008).

Some investigations have been done on OC residues in fish (Tilapia) from Lake Bosomtwi in Ghana by Darko *et al.* (2008) who reported a mean concentration of DDT (3.64 ± 1.81 µg/g). This result is however higher than that observed in this studies.

Hardell *et al.* (2010) reported the rate of OC pesticides in muscle tissue of fishes and DDE was the predominant target compound. The results obtained in this study generally agree with their findings.

Among the Ops determined, chlorpyrifos shows the highest concentration and phosphamid the lowest. The concentration of Ops pesticide residues detected in this study fell above the WHO and FAO (Codex, 2009) set Maximum Residue Limits (MRLS) 0.04 µg/g for dimethoate, 0.30 µg/g for chlorpyrifos, 0.01 µg/g methylparathion and parathion, 0.02 for malathion. The concentration level of γ , α – HCH, DDT, DDE, endrin, endosulfan, Heplachlor, methoxychlor are below the extraneous residue limit of seafood set by codex Alimentarius Commission of FAO/WHO (2009)

Conclusion

Dukku River is very important to the Kebbi people in so many ways. It serves as a source of irrigation water for vast majority of farmers, drinking water for humans and animals as well as source of commercial fish farming. Unfortunately, it is bedeviled with multiple anthropogenic influences that impact negatively on its resources. The results obtained from this study indicate the presence of both OCs and OPs pesticides residues. The total pesticide residues in the fish samples range from 8.152 µg/g in DK – HO to 3.738 µg/g in DK - TZ.

The OCs showed higher detectable value than the Ops with the carnivorous fishes showing greater potentials to accumulate the residues than the herbivorous. Most of the detectable compounds are generally persistent, lipophilic and bioaccumulative both in environment and at each trophic level of the food chain. Many of the pesticide detected in this work are either banned in many countries or are in the list of restricted pesticide usage. Their presence in this study shows both recent and old usage which persist due to the environmentally persistent nature of some of the residues. The concentrations of most of the pesticides were observed to be higher than the set maximum residue limits (MRLs) and the Acceptable Daily Intake Value (ADI) by FAO/WHO. Generally, the levels of these pesticide residues suggest that the Dukku River has been polluted due to human activities such as farming activities.

Therefore, adequate measures should be taken to reduce the levels of these pesticides so as to preserve aquatic life and reduce potential risk for public health.

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