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# Organochlorine Contaminants in Nile Tilapia, *Oreochromis niloticus* (Linnaeus 1758) in Densely Populated Areas of South-Western Kenya

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**Abstract:** Concentrations of OCP (Organochlorine Pesticide) residues were determined in cultured Nile tilapia (*Oreochromis niloticus*) fish in target stations within 1st order wetland of River Kuja, Kenya, between February-November, 2017. The aim was to assess the residue levels in wild and pond cultured fish within a heavily populated agricultural area of South Western Kenya. Biota samples were analysed for selected HCHs (Hexachlorocyclohexane) isomers, DDT (Dichlorodiphenyltrichloroethane) and metabolites and cyclodienes pesticide residues using GC-ECD (Gas Chromatography Equipped with Electron Capture Detector). Most of OCPs observed were BDL (Below Detection Limit) to detectable levels. Larger percentages (> 50%) of DDTs and HCHs were BDL and exhibited consistency, with significant ($p < 0.05$) differences in mean contamination levels in fish within the wetland, with average muscle tissue pesticides concentrate ranging between 0.229-2.541 μg/kg for Nile tilapia. Most dominant isomer in target species was Lindane (γ-HCH) (3.417 ± 0.983 μg/kg) and Endosulfans. Mean Endosulfan sulfate was 2.499 ± 0.071 μg/kg d.w. and most frequently detected, and Methoxychlor (2.235 ± 1.459 μg/kg), respectively. Mean Aldrin and Dieldrin was 2.028 and 0.574 μg/kg d.w. Concentration for DDT and its metabolites was 0.27-3.71 μg/kg for p, p'-DDE (DichlorodiphenylDichloroEthene), BDL-1.098 for p, p'-DDD (DichloroDiphenylDichloroEthane), and 0.105-3.518 μg/kg for p, p'-DDT with significant differences in mean values and ranges whose levels were below the WHO (World Health Organization) maximum acceptable thresholds of 0.2, 2, 20 and 5.0 μg/kg in fish and sea food. Interventions and monitoring need up-scaling in minimizing public health risks posed by consumption and exposure.

**Key words:** OCPs, *Oreochromis niloticus*, Lake Victoria Basin, residues, GC, pesticides.

## 1. Introduction

The need to produce a greater quality and quantity of food by pest control has resulted in the intensive use of pesticides [1, 2]. This has led to tremendous benefits in the area of agriculture, forestry, public health and domestic sphere and has also resulted in an economic boom [3]. This scenario has made pesticides use a tremendous tool in agricultural production to the extent that one-third of agricultural products are produced with application of various pesticides [4, 5]. Despite these overwhelming credits of pesticides use, there have been serious health implications to humans and the environment accrued from the use of pesticides [6]. These impacts range from potential risks to human health, both from occupational and non-occupational exposure to death of farm animals and alteration of the local fragile environment [7, 8]. Some of these POPs (Persistent Organic Pollutants) have become an integral part of Kenyan Society and are being used for diverse activities ranging from crop protection against insect pests, weeds, rodents, and fungal diseases to animal husbandry and in public

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health applications [9]. Other pesticide effects include: immunologic, carcinogenic, reproductive and neurological problems [10]. Because of these impacts, the use of most classes of pesticides has been banned in developed and some developing countries such as Kenya, especially the organochlorines [11]. In spite of this ban [12, 13], pesticides especially the organochlorines are still major pollutants [14, 15] in local water systems, due to the prevailing enforcement program [16, 17] on the usage of pesticides [18-20].

Previous studies have shown that increased anthropogenic activities and population growth within the Lake Victoria Basin catchment, approximately > 80 million persons [21], has led to pollution and degradation of its fragile aquatic ecosystem [22]. Food consumption has been identified as an important route of human exposure to pesticides with concentrations of pesticides in fish leading to several health concerns, particularly for high-risk population groups, such as pregnant women and children [23, 24]. Although the presence of trace levels of these pesticides in food is considered as an indication that contamination has occurred, the risk of adverse health effects also depends on their concentration, frequency of contact and duration of exposure [24]. Therefore, assessing health impacts of pesticide exposure involves the use of ecological risk assessment of species, populations, communities and ecosystems due to pesticide exposure [25, 26]. This assessment is necessary because of the tendency of pesticides to accumulate and persist in body tissues, leading to acute or chronic health effects [6, 27]. Since fishes such as *O. niloticus* are important sources of proteins and lipids for humans, so health of fishes such as histopathological changes in fish tissues is very important for human beings as well [28], and this health effect by pesticides on fish can be used as a biological indicator for pollution with pesticides in the aquatic environment.

In recent times, researchers have begun to estimate the risk posed to humans via the consumption of contaminated food products such as fish. Several studies including Refs. [29-31] and [32] have reported potential human health risk from the consumption of contaminated food [28]. The 1st order catchment area of Kuja River is a highland zone with high potential agricultural production and is densely populated (2,862 persons per $km^2$) [33]. Besides this, it supports pond aquaculture, and is capable of contributing to increased production of fish and improvement of household livelihoods. River systems are under increasing threats from solid waste dumping and their use as recipients of diffuse and point waste discharges. This may undermine their potential as sources of water for aquaculture. However, there are only a few studies in this region which have documented pesticide residues in cultured fish species. Therefore, this study was conducted in order to assess pesticide residue levels in cultured fish, especially Nile tilapia, *O. niloticus*, in heavily populated area of South Western Kenya.

## 2. Methodology

### *2.1 Study Area and Sample Collection*

This study was conducted in upstream Kuja River drainage basin in heavily populated areas of Kisii and Nyamira, and some randomly selected sites of adjacent Kericho and South Nyanza (Fig. 1) counties. The location of the area on which the study was carried out, lies within 34°40′0″ E-34°60′5″ E longitude and 0°24′04″ S-0°56′0″ S latitude. The areas were selected as suitable study sites because of the large human settlements [34] with intensive agro-chemical industry activities within their immediate vicinities.

This area has undergone rapid ecological changes resulting from agricultural practices that have led to massive deforestation, wetlands encroachment, degradation and water pollution [35, 36] within the vicinity of Lake Victoria basin. Fish samples were picked from stratified then randomly selected fish culture sites, and at specific selected points of streams and rivers in the counties that form part of the upper River Kuja catchment system in the Lake Victoria Basin, whose wetlands have intensively been

encroached and degraded by a rapidly rising human population [21, 37]. A total of 120 fish samples of Tilapia, *O. niloticus*, were randomly selected for this study. Sampling was done in eight sites (Fig. 1) during the period of between February and November 2017, involving randomly selected aquaculture farms, streams and rivers.

### *2.2 Extraction of Pesticide Residues in Fish Samples*

A simple, rapid SPE (Solid Phase Extraction) method was used to determine total lipids in each fish tissue based on methods described by Randall, et al. [38] and then lipids normalized concentrations were obtained using the ratio between pesticide concentration in tissue and lipid fraction in the tissue. The frozen composite edible portions of tissue samples for each species of Tilapia (*O. niloticus*) were used for extraction based on methods described by Steinwandter [39] and Osoro, et al. [40]. Twenty-five grams (25 g) of the sample was inserted into a homogenizer cup, and 100 mL of acetone was added. The sample was homogenized for 20 min at 100 rpm and further mixed with 5 g of anhydrous sodium sulphate. Extraction was done using a Soxhlet extraction for approximately 20-25 min using dichloromethane and *n*-hexane mixture. The resulting extract was dissolved with hexane and re-concentrated to 1 mL and eluted [41].

### *2.3 GC (Gas Chromatographic) Analysis*

OCPs (Organochlorine Pesticides Residues) in fish samples were analysed using GC-ECD (Gas Chromatography Equipped with Electron Capture Detector). The injector and detector temperatures were maintained at 300 °C. Helium gas was used as the carrier gas and nitrogen as make-up gas at a constant

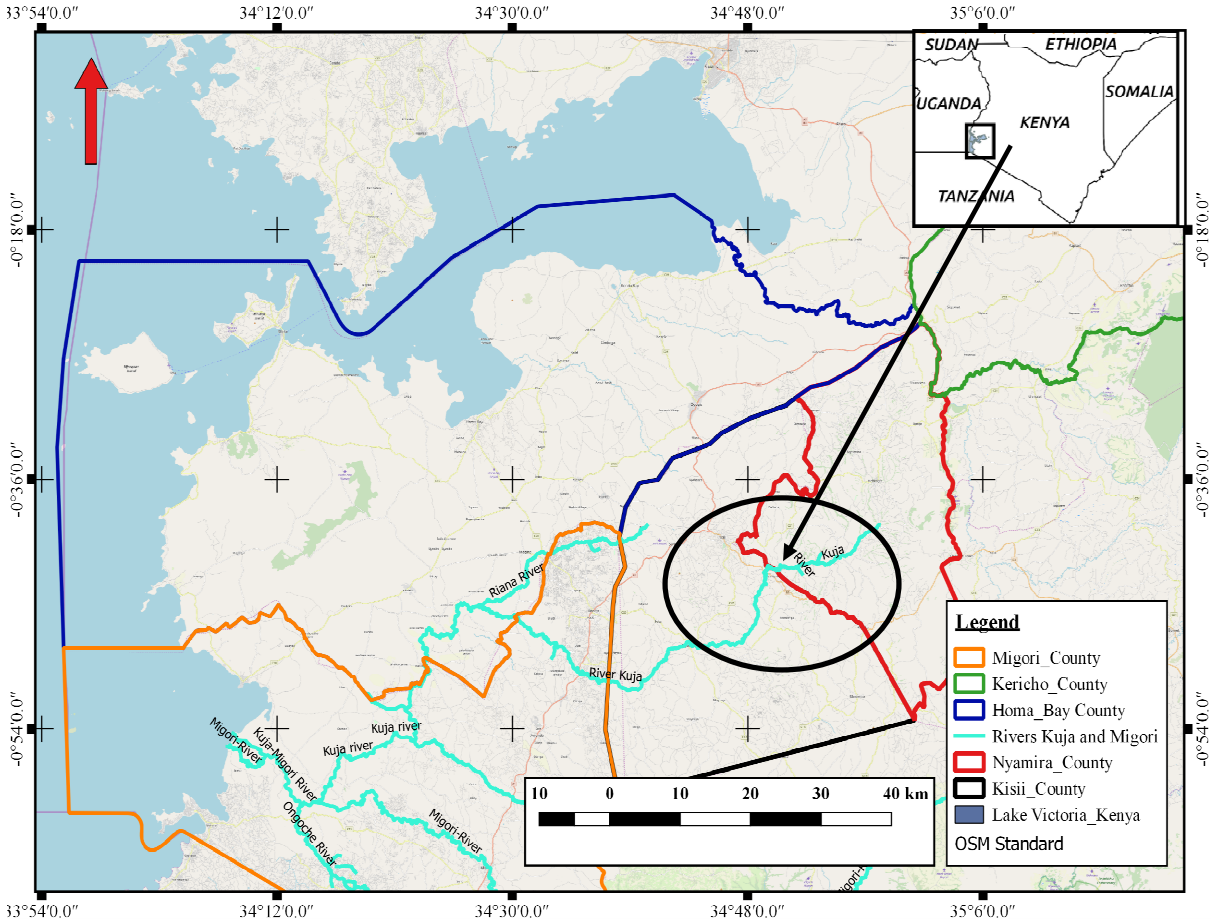


**Fig. 1 A Map showing sampling sites in the upstream River Kuja drainage basin, Kenya.**

flow rate of 1 mL/min. The injection volume was 1 µL with a pulsed splitless injection mode. The column used was HP (Hewlett Packard) capillary column with the following dimensions: 30 m long, internal diameter of 0.25 mm and film thickness of 0.25 µm.

*2.4 Data Analysis*

Statistical analyses were performed using GraphPad Prism version 5.03 (GraphPad Software Inc., California, USA). Data are presented as mean ± SEM (Standard Error of Mean). Statistical differences between wet and dry seasons were determined using Paired *t*-test. Further, statistical comparisons among and between levels of various organochlorines were performed using Repeated Measures ANOVA (Analysis of Variance) with Tukey's Post-test.

## 3. Results

HCHs (Hexachlorocyclohexanes) concentration range detection in wet and dry seasons was BDL (Below Detection Limit)-(5.86 ± 0.554) µg/kg in sampling sites 4 and 7 (Fig. 2), except for sites 2 & 3. Lindane (γ-HCH) exhibited highest residue level in muscle tissue and was detected in over 75% sampled sites, followed by α-HCH and β-HCH with > 65% detection levels in Nile tilapia (*O. niloticus*) fish. The lowest pesticide detection level was by α-HCH with < 50% frequency score while in wet season concentrations ranged between BDL-3.52 µg/kg d.w., whereas in dry season residue level was between BDL-2.34 µg/kg indicating that those HCH compounds residue encountered in fish samples in the wet period contained higher residue concentrations. During the dry period, in sites 2, 3, 4 and 5, β-HCH and γ-HCH pesticides recorded BDL means. Highest residue levels of pesticide compounds in fish muscle were detected in sites 3, 4, 7 and 8 (2.72 ± 0.309; 3.12 ± 0.647; 3.52 ± 0.983; 1.85 ± 0.481 µg/kg) during the dry period (Fig. 2) while in wet period levels ranged between BDL-(2.54 ± 0.832) µg/kg. Those of α-HCH isomer mean residue levels recorded the highest mean concentration at 1.31 ± 0.301 µg/kg and the lowest by β-HCH at 1.71 ± 0.572 µg/kg (stations 3 and 4).

Statistical analysis on residue level mean results was carried out by Repeated Measures ANOVA and Paired *t*-test, with $p < 0.05$ as level of statistical significance. Mean contents of HCH isomers in Nile tilapia (*O. niloticus*) fish muscle tissue in dry season were not significantly different, at ($p > 0.05$) level of significance. Multiple comparison procedures indicated significant variance within each sampling period for sites 2, 6 and 7. The overall mean for lindane (γ-HCH) in *O. niloticus* was 3.52 ± 0.983 µg/kg (wet) and 2.54 ± 0.825 µg/kg (dry) seasons, and

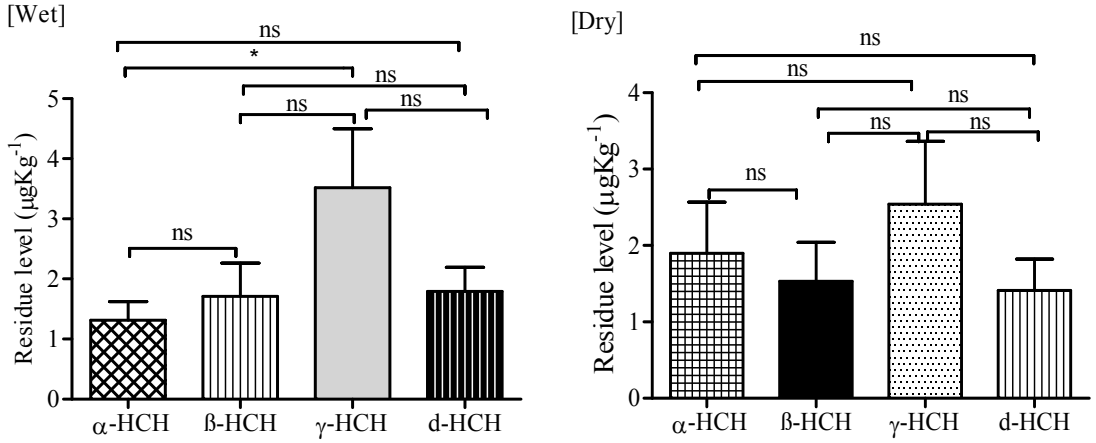


**Fig. 2 (a) wet and (b) dry: A comparison of the mean (± SEM) concentrations of HCH isomers in Nile tilapia (*O. niloticus*) fish samples during wet and dry seasons.**

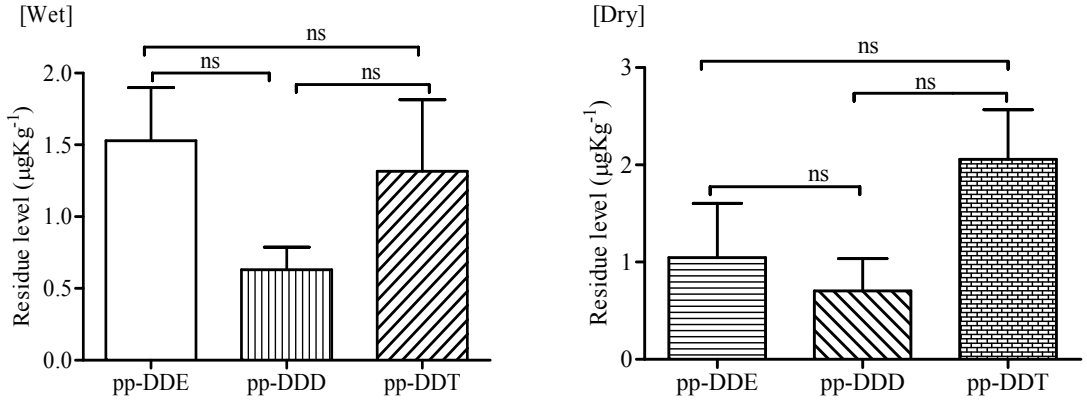


**Fig. 3 (a) wet and (b) dry: A comparison of the mean (± SEM) concentrations of DDT isomers in Nile tilapia (*O. niloticus)* fish samples during wet and dry seasons.**

the variations between lindane and alpha wet season was highly significant (ANOVA: $t = 3.588$; $p = 0.0089$; $p < 0.05$). Tukey's Multiple Comparison Test shows γ-HCH having a highly significant variation effect on α-HCH and β-HCH.

DDT (Dichlorodiphenyltrichloroethane) metabolite residue levels detected in fish (μg/kg) samples are shown in Fig. 3. Though DDT means were lower when compared to HCHs, their mean ranges in wet period were between 0.26-(3.76 ± 0.370), BDL-(1.09 ± 0.164), 0.15-(3.52 ± 0.499) μg/kg for *p*, *p*'-DDE, *p*, *p*'-DDD and *p*, *p*'-DDT respectively. In dry period, metabolite values were in range between BDL-4.39, BDL-2.79 and BDL-3.613 μg/kg d.w. for *p*, *p*'-DDE, *p*, *p*'-DDD and *p*, *p*'-DDT, respectively. The overall mean for DDTs is 1.53 ± 0.371, 0.63 ± 0.157 and 1.32 ± 0.498 μg/kg for fish sample residue levels in the wet season. In dry season, overall mean was 1.05 ± 0.556, 0.705 ± 0.331 and 2.06 ± 0.501 μg/kg in fish muscle tissue. Statistical analysis by Repeated Measures ANOVA and Tukey's post-test for both wet and dry season indicated that the mean contents of determined DDT metabolites were not significantly different, at $p > 0.05$ level of significance. For instance, in wet season, *p*, *p*'-DDD and *p*, *p*'-DDT metabolite values (ANOVA: $F = 1.680$; $p = 0.263$; $p > 0.05$) means had no significant difference during the dry period (ANOVA: $F = 0.287$; $p = 0.156$; $p > 0.05$) in target stations within the wetland.

Out of seventeen pesticides monitored during the dry season, site 8 showed the highest frequency (46%) of residues detected in *O. niloticus* samples. In the wet season, residue levels detected in site 4 had shown the highest frequency (56%) of pesticides detection. Overall, cyclodienes mean concentration levels during wet season ranged between 0.55-(2.03 ± 0.481) μg/kg in Heptachlor epoxide and Aldrin levels in sites 7, 2 and 4 respectively, whereas those detected during dry season were between (0.607 ± 0.272)-(2.34 ± 0.816) μg/kg in Endrin aldehyde and Endosulfan I respectively (Fig. 4).

Results showed no statistical significant difference ($p = 0.571$; $p > 0.05$) between fish muscle mean residues obtained during the dry season but mean results observed during wet season between Endrin and Dieldrin were highly significant (ANOVA: $p = 0.003$; $p < 0.05$). The rest of the cyclodienes mean contents in dry season were not significantly different, at $p > 0.05$ level of significance (Fig. 4). However, Dieldrin which is a metabolite of Aldrin was detected at sites 3, 1 and 6 in lower levels, indicating a result of atmospheric deposition and oxidation activity in the environment [20]. The PCA (Principal Component Analyses) showed that high amounts of isomers and cyclodiene pesticides including γ-HCH and Endrin were obtained at station 1 (A) and 2 (B) which have effects of high anthropogenic and horticultural farming activities. Endosulfan I and II is characterised

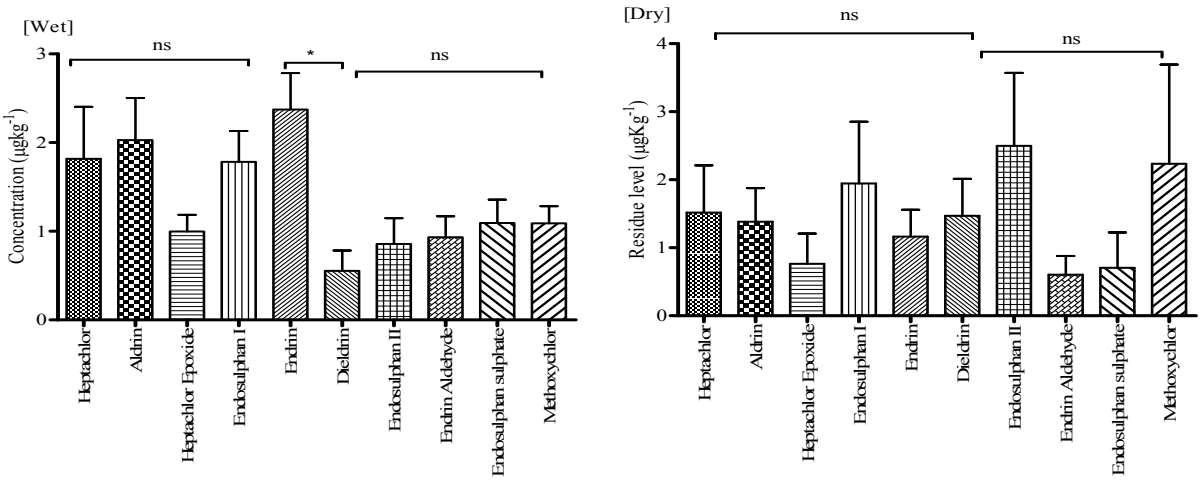


**Fig. 4 (a) wet and (b) dry: A comparison of the mean (± SEM) concentrations of HCH isomers in Nile tilapia (*O. niloticus*) fish samples during wet and dry seasons.**

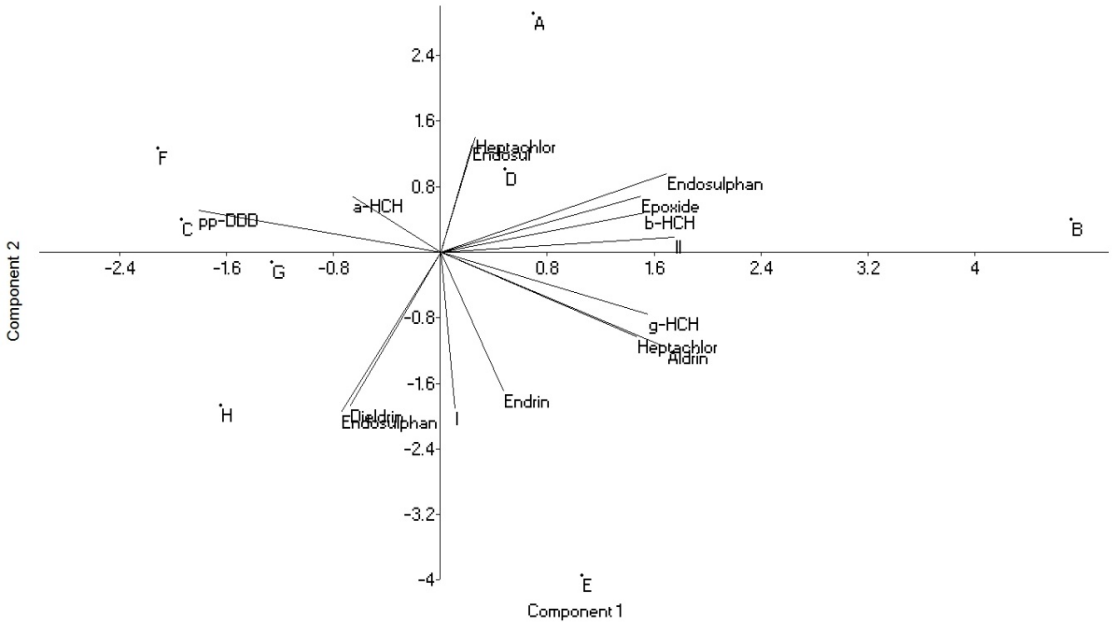


**Fig. 5 PCA, agglomerative, for sampling stations (1-8: A-F) and OCPs mean occurrence in 1st order River Kuja catchment, Kenya.**

stations 4 (D), 6 (F) and 8 (H) due to the fact that it is used primarily on food crops like tea, fruits, vegetables and on grains, hence its significant availability. It is also used as wood preservative in many construction activities in the study area.

## 4. Discussion

Results in this study are in congruent with studies done by Ezemonye, et al. [14] whereby OCPs in riverine *Tilapia zilli* from Ogbesse river, Nigeria, were investigated and mean residues levels were between 0.02-1.73 µg/kg with β-HCH isomer having the highest mean level (1.73 µg/kg) though pesticide residue level was noted to be highest in mud fish (2.3 µg/kg). It was also noted that mean levels were higher during the wet season. Although isomer contamination levels are slightly different from this study and that of Refs. [24] and [26], this study also noted that the

levels in *O. niloticus* fish investigated are found to be higher during wet season than in the dry season. This could probably be due to large amounts of runoffs experienced in wet season offloading organic contaminants into the water column in the catchment at higher rates than during the dry season. Secondly, due to topographical set-up of the local area, most fish ponds in study area were located down at river valleys, hence easily receiving rain water from nearby coffee, tea, banana, horticultural and similar agro-chemical establishments as run-offs. There was no statistical significant difference ($p > 0.05$) between metabolite levels both in dry and wet seasons [7, 14]. However, results in this study carried out by Paired *t*-test statistical analysis method indicated a highly statistical significant difference between γ-HCH isomer and all the other isomers during the dry period.

DDT residue levels uptake and presence in this study could be attributed to previous time of use for pest control and public health reasons locally [20, 32]. Due to lipophilic effects of some organochlorine compounds, they are likely to be carried far and wide from its epicenter after undergoing bioaccumulation in biota such as fish [27]. It is also assumed that some organic contaminants could enter the trophic level through feeds that are commercially available in the market and are used as part of fish culture management efforts. As similar results have been observed in other fresh water river basins, current study result variations present evidence of contamination of cultured fish locally, arising from various anthropogenic practices in upstream Kuja River catchment fish culture sites (Fig. 4), hence providing evidence of potential risk to environment and humans [18, 25, 37]. PCA indicates that sampling stations such as 2 (B) and 5 (G) that lack active human influence (Fig. 5) recorded low detection residue levels hence not showing heavy characterization.

## 5. Conclusion

Overally, although the commonly regulated pesticide residues (HCHs, DDT metabolites and Cyclodienes) in fish and fish products were found to be below the FAO/WHO maximum residue levels, there is indication of potential accumulation in farmed *O. niloticus* fish tissue samples analysed. Therefore regular monitoring of pesticide residues in the environment; and better agricultural practices, together with enforcement of existing pesticide use regulations is recommended to forestall environmental contamination and potential human health risks.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this research article.

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