PESTICIDES DISTRIBUTION IN SURFACE WATERS AND SEDIMENTS OF LOTIC AND LENTIC ECOSYSTEMS IN AGBEDE WETLANDS

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Abstract
An investigation on the availability and distribution of Lindane (HCHs) and Total organochlorine phosphate (TOCP) in the surface waters and sediments of selected water bodies in Agbede wetlands was carried out from December, 2012 to May, 2014 in order to cover seasonal trends in both matrixes. A Gas Chromatograph model Hewlett-Packard (HP9) 5890 Series II equipped with 63Ni Electron Capture Detector (ECD) of activity 15 mCi with an auto sampler was employed for the analysis of the water and sediment samples in the laboratory under standard condition. Spatial and temporal variations showed that the concentrations of TOCP closely followed that of Lindane in surface water and was a replica in the sediment matrix. Meanwhile, the values obtained for Lindane and TOCP in the sediment were slightly higher than (about 3 folds) that in the surface waters. The concentrations of pesticides in the surface waters and sediments of lotic and lentic ecosystems in Agbede wetlands were found to be far below the set limit of 0.01μgL⁻¹ by the Federal Ministry of Environment of Nigeria (FMEnv) and World Health Organisation (WHO) respectively. The low values recorded in this study suggested seldom application of pesticides particularly on a subsistence form and therefore not indicative of possibility of any serious health issues.

Key Words: Below Detection Limit, Concentrations, Ecological Zone, Lindane

Introduction
Water bodies located within farming communities in Nigeria are fast becoming polluted with pesticides, such pesticides include; Glyphosate, Paraquate, Lindane, amongst other Organochlorine phosphates (OCPs). Organochlorine pesticides are known to be very stable, resistant to natural breakdown, lipo-soluble compounds that are capable of bioaccumulation and biomagnifications in the fatty parts of biological organisms (Adeyemi et al., 2011; Ogbeide et al., 2015). The health effects of pesticides in man and animals include immune system malfunction, endocrine disruption, breast cancer, irritation, dizziness tremor, toxic and chronic convulsion (Ize-Iyamu et al., 2007; Ezemonye et al., 2009; Adebayjo

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et al., 2011; Adeyemi et al., 2011; Ogbeide et al., 2015).

Agbede wetlands is one of the most fertile alluvial flood plains in the Niger Delta ecological zone and famous for her Rice cultivation either in the subsistence form or semi-mechanised practice. Pesticides were first introduced to Agbede in the late 1960s by the then Agbede-Warrake farms established by the then Military Governor of Mid-Western Region (General Osaigbovo Ogbemudia) and were managed by the German company Rhabbo-Imes. The use of pesticides particularly herbicides were later re-introduced to the wetlands on a large scale semi-mechanised agriculture by Sparkling Dirisu farms between 1983 and 2000s. Since then the use of pesticides became flourished throughout Agbede agrozone and spread to neighboring communities. The brands of pesticides used in the farms included; premextra, glyphosate, paraquate, propan-2, 4-D amongst others for the cultivation of rice, maize and beans. While, the use of Gammalin-20 was popular among the local fishers in rivers and large ponds which became an annual or bi-annual event to harvest fishes depending on the approval by the community (Personal communication).

Pesticides especially organochlorine form an integral part of our society because of its diverse use in several activities including animal husbandry and public health applications (Zhou et al., 2006) and despite its benefits, there is a growing concern at local, regional and global levels about possible environmental contamination from the use of agrochemicals, disposal of outdated stocks, containers and packets (Doong et al., 2002; Sarkar et al., 2008; Upadhi and Wokoma, 2012).

In Nigeria, pesticide usage has soared in the past decades with at least 21 different documented types of organophosphates, organochlorines and carbamates insecticides available in the market to boost agricultural production and combat disease vectors of plants, animals and humans (Taiwo et al., 2012, Ogbeide et al., 2015). As such large quantities of these pesticides are released into the environment in the course of controlling agricultural pests, insect-borne diseases, and termites (Eqani et al. 2009).

Lindane is one of the Organochlorine and parameter understudied in this work and is a broad spectrum insecticide which has been used since 1949 for agricultural and non-agricultural purposes. The major agricultural purposes include seed and soil treatment and wood, and non-timber treatment (WHO, 1989; Ezemonye et al., 2008). Ezemonye et al. (2008) reported values of Lindane content in water from Below Detection Limit (BDL) to 1.37µgg$^{-1}$, BDL to 12.66µgg$^{-1}$ dry weight in sediment, and BDL to 16.67µgg$^{-1}$ in fish species (Chrysichthys furscatus) and BDL to 0.15µgg$^{-1}$ dry weight in Tillapia zilli. They observed that in the study the values were above the ecological bench mark (0.01µgL$^{-1}$) recommended by Federal Environmental protection Agency (FEPA) and World Health Organisation.

Olomukoro and Dirisu (2012), and Olomukoro et al. (2013) in their various studies on the benthic macroinvertebrates of lentic and lotic water bodies in Agbede wetlands, attributed the impairment and the low diversity of macrofauna to the application of Lindane in the water
bodies. They asserted that the purpose was to harvest fish for human consumption which has since become a common practice within the localities.

This pioneering work dealing with the investigation of pesticides distribution in the surface waters and sediments of lotic and lentic ecosystems in Agbede wetlands, was aimed at documenting their pesticides (Lindane and Total Organochlorine phosphate) status and to establish the basis for future studies within the wetlands and the catchment areas.

Materials and Methods

Study Area
The study area (06°16.3”E, 06°18.7”E) and latitude (06°52.2”N, 06°55.4”N) is part of Agbede wetlands located in Etsako West Local Government area of Edo State (Figure 1). The features of this locality have been described in detail by Olomukoro and Dirisu, 2014; and Dirisu and Olomukoro, 2015). The region is predominantly agrarian and all forms of human activities like washing, bathing, scattered settlements, fishing, mining of top soils and trading amongst others are observed here. Rainfall data between December, 2012 and May, 2014 as obtained from the Benin Meteorological Station during the sampling regime revealed that precipitation was lowest in January, 2013 (15.4mm) and December, 2012 (18.9mm), and highest in September, 2013 (564mm).

Sampling Locations
A total of seven sampling stations were designated for this study and included one Stream with three (3) stations and three Ponds (3) with four stations.

Station 1 is located on a stretch of Omodo Stream just by the confluence between Omodo and Egwavo Streams, accessible through Ayuele Secondary School while Station 2 is located at Odighie village by the bridge linking Agbede and Ama/Idegun towns which is over 987m from Station1. Station 3 is about 1.11km from station 2 and the last sampling point on the stream stretch which is located by the bridge at Egho village unto Rabho-Imes farms land. It is the major source of water for every form of activity by the various farm Camps. Nomadic activities are very high here and consequently there are always litters of cattle faecal matter onto the water and on the river banks. Station 4 is the first pond before Edion River when transiting to Auchi at Ogwedion. It is a major source of drinking water to cattle and a nestling ground to some birds’ species. It is fed by Edion River during the pick of wet seasons. Station 5 is the major but easily accessible Pond at Ukatosoma farm district. It is also a major fishery ground which is harvested bi-annually. Station 6 is about 830m away from station 5 while travelling toward Auchi town. It is a major source of drinking water to cattle herds within Ukatosoma farm district in Agbede. Station 7 is the second station established on the same pond described in station 6 above. The entire pond/burrow pit measures about 215m x 23m and located at less than 10m away from the high way.
Figure 1: Map of the Study Area
**Sample Collection**

Water samples for pesticides (n = 126) were collected in 1 L plastic bottles from each station and kept in cooler containing ice at temperature lower than 7°C. Sediment cores (n = 126) were equally collected at depth not greater than 5cm in black polyethylene bags using a light dredger improvised for very shallow wetlands and kept in separate cooler (Adopted from Ezemonye et al., 2008). Both samples were transported to the laboratory and further stored in the fridge at temperature of 5°C prior to analyses. Both samples were collected between December, 2012 and May, 2014.

**Pesticide Sample Extraction**

Pesticide residues in water samples were extracted using the procedure described by Osibanjo and Adeyeye (1997). N-hexane and dichloromethane (DCM) (90:10) were used to prepare a solvent mix used for the extraction. DCM was selected as the extraction solvent, as it was easily evaporated, facilitating the solvent-exchange step. Sixty (60 ml) of the solvent mix was introduced into a 1L separatory funnel containing 250 ml of water sample. The mixture was shaken vigorously for 3mins, with periodic venting and was allowed to stand for about 10 minutes for complete separation. The bottom aqueous layer was drained into the original sample bottle, while the organic layer was filtered into a 250 ml conical flask. This process was repeated thrice. Anhydrous sodium sulfate was used to dehydrate the samples. The sample was then concentrated to 10 mL using a rotary evaporator in water bath at 35-40 °C (USEPA, 2004). Pesticide residues in sediment samples were extracted based on the methods described by USEPA (2004); Ogbeide et al., (2015). Samples were air-dried, and then passed through 2 mm sieve. A solvent extraction mix of acetone and hexane (50:50) was prepared. Fifteen grams (15g) of well-mixed and finely grounded sample was measured into a solvent rinsed beaker. Twenty (20) ml of the solvent extraction mixture was added to the samples to form a surrogate mixture spiked with 1ml of the surrogate mix. The sample was placed in the sonicator and sonicated for about 20 – 25 minutes at about 50°C. Three grams (3g) of anhydrous sodium sulphate was added to the sample until a clear extract was developed. The extract solvent was then poured into a round bottom flask. This procedure was repeated once more with an additional 50ml of solvent mix, sonicate and allowed to settle in the beaker and decanted into the same round bottom flask. The extract was dissolved with hexane and re-concentrated to 1 to 3ml (USEPA, 2004).

**Clean Up of Extracts**

In order to remove any interfering substances co-extracted with the pesticide residues each extract (water and sediment) was cleaned up using a Florisil Solid Phase Extraction (SPE) method (USEPA, 2004). Activated florisil (1.5g) was packed into a column that had been plunged with glass wool. The column was further packed with 1 g and 0.5g pre-activated florisil adsorbent and sodium sulphate (Na₂SO₄) respectively. 10ml of n-hexane was used to condition the column prior to the clean-up. The extracts of the samples, blanks and spiked samples were transferred into the florisil column using a Pasteur pipette and waited until it was eluted. The eluate was collected into a 50 ml conical flask. The process was repeated twice. The column was further rinsed
with 10 ml n hexane. The eluate was concentrated to dryness on a rotary evaporator and recovered into 1ml ethyl acetate. The 1ml dried extracts were transferred quantitatively into 2 ml glass GC vials using a Pasteur pipette for analysis. Recovery of the column was determined using 0.01 mgkg\(^{-1}\) mixed standard solution of pesticides.

**Pesticide Analysis**

A Gas Chromatograph model Hewlett-Packard (HP9) 5890 Series II equipped with 63Ni Electron Capture Detector (ECD) of activity 15 mCi with an auto sampler was employed for the analysis of Lindane and Total organochlorine pesticide. The chromatographic separation was done using a VF-5ms of 30 mm capillary column with 0.25 mm internal diameter and 0.25 \(\mu\)m film thicknesses and equipped with 1 m retention gap (0.53 mm, deactivated). The GC conditions were as follows: The oven temperature programme: Initial temperature was set at 60\(^{\circ}\)C for 2min and ramped at 25\(^{\circ}\)C/min to 300\(^{\circ}\)C for 5mins and allowed to stay for 15 min giving a total run time of 58min. The injector setting is a pulsed spit less mode with a temperature of 250\(^{\circ}\)C at a standard pressure. The injection volume was 1.5ml. The detector temperature was 320\(^{\circ}\)C (held for 5 minutes), Helium was used as a carrier gas while Nitrogen gas (\(N_2\)) was used as the makeup gas, maintained at a constant flow rate of 29ml/min. The efficiency of the analytical method (the extraction and clean-up methods) was determined by recoveries of an internal standard. In doing this, one homogenized sample for each matrix was spiked with a 50\(\mu\)L of 100ng/ml of internal standard solution of Organochlorine/organophosphate pesticides and extracted under the same conditions as the analytes. To check for cross contamination and interferences, a blank sample was analyzed in each batch of analysis. Peak identifications were conducted by comparing the retention time of standards and those obtained from the extracts. Concentrations were calculated using a four-point calibration curve.

**Data Analysis**

Pesticides data obtained were analysed for one – way analysis of variance (ANOVA) using SPSS (20.0) and graphs were plotted with the aid of Microsoft office Excel 2007 for window 7.

**Results**

**Pesticides Studies in Surface Waters and Sediments**

Pesticide study was carried out to evaluate the concentrations of Lindane (HCH) and Total Organochlorine Phosphate (TOCP) in the surface waters and sediments of Agbede wetlands. The results are presented thus.

**Pesticides in Surface Water**

The results of the spatial and temporal variations in the levels of Lindane and total Organochlorine phosphate (TOCP) in the surface water bodies of Agbede wetlands are presented in Table 1 and Fig. 2 respectively.

Mean Lindane concentrations in the surface waters (lotic and lentic systems) ranged from below detection limit (BDL) at station 6 to a high of 4.40 \(x\) 10\(^{-3}\)\(\mu\)gL\(^{-1}\) at station 5 (same lentic environment). The spatial and temporal variations were between BDL in virtually all the stations but at different months (February to April, 2013, July to November, 2013 and January to April, 2014) and 0.0002\(\mu\)gL\(^{-1}\) as the maximum concentration. The concentrations statistically proved no significant differences \((P>0.05)\).
F=0.234) among the stations which did not differ both in time and space across the ecological types. Lindane content was mostly not detectable in February, March, April, July, August, September, October and November of 2013. Others were January, February, March and April of 2014 respectively (Table 1 and Fig. 2).

Total Organochlorine phosphate mean concentrations in the surface waters, ranged from $9.40 \times 10^{-3}$ to $1.30 \times 10^{-3} \mu g L^{-1}$ at station 1 (lotic system). Seasonal variations ranged between BDL and a high of $0.0003 \mu g L^{-1}$. These concentrations did not follow any definite seasonal pattern of fluctuations. The TOCP contents were mostly detectable during the wet seasons of 2013 and 2014 respectively. The mean concentrations did not differ significantly either ($P>0.05$, F=0.405) amongst the stations (Table 2 and Fig. 3).

![Figure 2: Lindane Residue Distribution in the Surface Waters of Agbede Wetlands](image)

![Figure 3: Total Organochlorine Phosphate Residue Distribution in the Surface Waters of Agbede Wetlands](image)
Fig. 4: Lindane Residue Distribution in the Sediments of Agbede Wetlands

Figure 5: Total Organochlorine Phosphate Residue Distribution in the Sediments of Agbede Wetlands
Table 1: Summary of the mean levels of Lindane and Total Organochlorine Phosphate in Surface Water of Agbede Wetlands from December, 2012 to May, 2014

| Parameter   | Unit | Lotic Systems | Lentic Systems | FMEnv. Limit | p-Value |
|-------------|------|---------------|----------------|--------------|---------|
|             |      | Station 1     | Station 2      | Station 3    | Station 4 | Station 5 | Station 6 | Station 7 |             |         |
| Lindane     | µg/l | Min-Max       | Min-Max        | Min-Max      | Min-Max   | Min-Max   | Min-Max   | Min-Max   | ±SD        | Min-Max   |
|             |      | ±0.0027 ±0.0016 | ±0.0041 ±0.0017 | ±0.0012 ±0.0012 | ±0.0028 ±0.0016 | ±0.0041 ±0.0007 | ±0.0028 ±0.0008 | ±0.0017 ±0.0012 | ±0.0012   | 0.00      |
| Total OCP  | µg/l | Min-Max       | Min-Max        | Min-Max      | Min-Max   | Min-Max   | Min-Max   | Min-Max   | ±SD        | Min-Max   |
|             |      | ±0.0047 ±0.0013 | ±0.0102 ±0.0063 | ±0.0074 ±0.0023 | ±0.0074 ±0.0013 | ±0.0079 ±0.0024 | ±0.0081 ±0.0018 | ±0.0036 ±0.0006 | ±0.0006   | 0.00      |

Table 2: Summary of the mean levels of Lindane and Total Organochlorine Phosphate in the Sediments of Agbede Wetlands from December, 2012 to May, 2014

| Parameter   | Unit | Lotic Systems | Lentic Systems | p-Value |
|-------------|------|---------------|----------------|---------|
|             |      | Station 1     | Station 2      | Station 3 | Station 4 | Station 5 | Station 6 | Station 7 |             |         |
| Lindane     | µgg  | (Min-Max)     | (Min-Max)      | (Min-Max) | (Min-Max) | (Min-Max) | (Min-Max) | (Min-Max) | ±SD        | Min-Max   |
|             |      | ±0.0027 ±0.0016 | ±0.0041 ±0.0017 | ±0.0012 ±0.0012 | ±0.0028 ±0.0016 | ±0.0041 ±0.0007 | ±0.0028 ±0.0008 | ±0.0017 ±0.0012 | ±0.0012   | 0.00      |
| Total OCP  | µgg  | (Min-Max)     | (Min-Max)      | (Min-Max) | (Min-Max) | (Min-Max) | (Min-Max) | (Min-Max) | ±SD        | Min-Max   |
|             |      | ±0.0047 ±0.0013 | ±0.0102 ±0.0063 | ±0.0074 ±0.0023 | ±0.0074 ±0.0013 | ±0.0079 ±0.0024 | ±0.0081 ±0.0018 | ±0.0036 ±0.0006 | ±0.0006   | 0.00      |
**Pesticides in Sediments**

Lindane residue mean concentrations in the sediments were between 0.0012 µg g⁻¹ dw⁻¹ at station 3 (lotic system) and 0.0041 µg g⁻¹ dw⁻¹ at station 2 and 5 respectively (lotic and lentic systems). Temporal and spatial variations ranged from BDL to 0.0075 µg g⁻¹ dw⁻¹ at station 3 and 4 respectively in August, 2013. There was a high significant difference (P<0.001, F=0.000) amongst the tested means. Fluctuations across the sampled stations (Table 2 and Fig. 4) were observed. On the other hand, total Organochlorine phosphate (TOCP) had its mean concentrations ranging from a low of 0.0037 µg g⁻¹ dw⁻¹ at station 7 (lentic system) to a high of 0.0102 µg g⁻¹ dw⁻¹ at station 2 (lotic system). The minimum and maximum concentrations of TOCP in the sediments were between 0.0013 µg g⁻¹ dw⁻¹ at station 2 and 0.0055 µg g⁻¹ dw⁻¹ at station 3. A high significant different (P<0.001, F=0.000) was statistically observed for this amongst the means tested (Tables 2 and Fig. 5).

**Discussion**

This study found differential concentrations of Lindane and total Organochlorine phosphate in the surface waters and sediments of the lotic and lentic systems in Agbede wetlands. For about 3 decades now, the locals have a history of harvesting fishes from both the lotic and lentic ecosystems. During this study there was no application of pesticide(s) to the aquatic systems for the above mentioned purpose, this could be a confirmation of the gradual withdrawal from such unhealthy practice after several advocacies among the locals. However, pesticides usage is still popular amongst the farmers particularly for controlling weeds in their farms and homes. The concentrations of Lindane in the surface water were mostly not detectable (below detection limit, BDL) and when detected the levels were far below the bench mark of 0.01 µg L⁻¹ set by Federal Ministry of Environment of Nigeria (FMEnv 1991) and World Health Organisation (WHO) for surface water. Lindane contents in the lotic and lentic systems were not detectable between February and April, in 2013, July to November, 2013 as well as January to April, 2014. The varying low concentrations recorded in this study were found to be contrary to the work of Ezemonye et al. (2008) on Warri River with values between BDL and 1.37 µg L⁻¹.

On the other hand, Total Organochlorine Phosphate (TOCP) concentrations were equally very low in the water samples. The levels of TOCP in the stream (stations 1 to 3) and the ponds (stations 4 to 7) were comparably like that of Lindane in the surface waters. TOCP concentrations in this study were similar in trends to the study by Idowu et al. (2013) in South-Western Nigeria.

The concentrations of Lindane in sediments were found to be higher than what was obtained for the surface waters of Agbede wetlands. Lindane contents was detectable throughout the sampled months across most of the stations, thus with station 1 dominating the content concentration. There was no distinction regarding whether Lindane concentrations were higher in the lotic systems than in the lentic systems except that station 1 consistently recorded higher values between July, 2013 and May, 2014. These concentrations were much lower than the benchmark for surface waters (0.01 µg L⁻¹). Higher concentrations of Lindane content in sediments of Warri River was recorded by Ezemonye et al. (2008). Ogbeide et al. (2015) recorded higher concentrations of Lindane (HCHs) from rivers in Illushi flood plain, Ogbeide and Owan respectively with values ranging from
4.89 to 5.22 $\mu$g$^{-1}$. The low concentrations of Lindane in surface waters and sediments of Agbede wetlands could be attributed to the seldom application of its content particularly as it is occasionally used for harvesting of fishes in the ponds and/ or in running waters.

Total Organochlorine phosphate concentrations in the sediments were equally similar in trends to the Lindane contents in the same sediments. These concentrations were highly different from ($P<0.001$) one station to the other with values much lower than what was obtained from the river water and sediments from cocoa-producing areas of Ondo State Central Senatorial District (Idowu et al. 2013) and that of Ogbeide et al. (2015) for Owan, Ogbese and Illushi Rivers in Edo State, Nigeria. This study strongly suggests that organochlorine contaminations of water bodies in Agbede wetlands are sourced from the run-offs from farm lands near aquatic environments as in this case.

**Conclusion**

In all fairness, the contamination of surface waters and sediments of Agbede wetlands with pesticides has been within the sustainable livelihood, which demonstrates that pesticides values from both the waters and sediments were of extremely low concentrations when compared to the Federal Ministry of Environment set standard, implying low and seldom application of the products in the study area. However, it should be noted that their sources are either point or non-point, as run-offs are capable of transporting contents over long distances. This study seeks to recommend that a bi-annual monitoring programme should be jointly established by the State Ministries of Agriculture and Environment through collaboration with Hydrobiologists and Ecotoxicologists in the University systems in Edo State, with a view to monitoring the major pesticides levels in the various matrixes.

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