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Analysis of Organochlorine Residues in River Benue at Makurdi using Gas Chromatography coupled with Electron Capture Detector

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ABSTRACT
Organochlorine pesticides (OCPs) concentrations along the Benue River at NASME and at Benue Brewery were studied during both wet and dry seasons. A total of 12 water samples were collected and determinations were made. Gas chromatography/electron capture detector was used for OCPs analysis. In these analyses, aldrin, α-BHC, β-BHC, δ-BHC, chlorothalonil, dieldrin, endosulphan I, endosulphan II, endrin, endosulphan sulphate, heptachlor B, heptachlor epoxide, lambda cyhalothrin, lindane, permethrin, P'P-DDT, P,P' DDD were widely distributed at the various locations. α- BHC has the least average concentration of 0.00643 ppm while permethrin has the highest average concentration (2.2506 ppm).The results portrayed by these research suggests some intolerable levels of pesticides concentrations at some locations.

Keywords: Electron capture detector, Gas Chromatography, Liquid-liquid extraction, Nigeria, Organochlorine, River Benue

INTRODUCTION

Among various environmental pollutants organochlorine pesticides (OCPs) raised anxiety in the society due to their toxic nature, carcinogenicity and long existence in the environment. Generally, OCPs show strong hydrophobic nature, which results in their accumulation in fatty tissues, and as consequences they become carcinogenic and endocrine disruptors in mammals (Gonzalez *et al*. 2005, Martinez *et al*., 2002). Most developed countries have banned their use since the 1970s, in favour of the more modern and readily degradable pesticide formulations. However, OCPs are still extensively used in developing regions because of their low cost and effectiveness. They enter the food chain through contaminated water, fish and shellfish (Nowell *et al.,* 1999; Carvalho *et al.,* 2002; MacIntosh *et al.,* 1996; Scheyer *et al.,* 2005; Connor *et al.,* 2005; Ferrante *et al.,* 2007). OCPs show low polarity, high thermal stability, and volatility. As a consequence, the analytical protocols proposed for their determinations are traditionally based on gas chromatography (GC) mainly coupled to detection techniques such as electron capture detector and mass spectrometry (MS) (Mangani, *et al.,* 2000, Yenisoy-Karakasu, 2006, Guardia-Rubio *et al.,* 2007). The OCPs are broad spectrum insecticides, and are the most widely used in many countries including Nigeria for agricultural purposes and control of mosquitoes (Bouman, 2004; Blaso *et al.,* 2005).

Environmental contamination by OCPs in water bodies have been a great concern, since most of these pesticide compounds are very persistent, bioaccumulative and their toxicity can pose harmful effects to humans and ecosystems, because these compounds are lipophilic and have low chemical and biological degradation rates (Barakat *et al*., 2002). A potential pathway for adverse effects of pesticides is through hydrologic systems, which supply water for both humans and natural ecosystems. Water is one of the primary ways that pesticides are transported from an application area to other locations in the environment. Pesticide contamination of groundwater is especially acute in rural agricultural areas where over 95 percent of the population rely upon groundwater for drinking. The organochlorine contamination pathways to water bodies are likely to be nonpoint sources via runoff, atmospheric deposition, and leaching due to agricultural applications, vector pest control and improper waste disposal methods (Carvalho *et al.,* 1996; Galindo *et al.,* 1999).

Pesticide residues reach the aquatic environment through direct runoff, leaching, and careless disposal of empty containers, equipment washing etc. (Milindis, 1994). Surface water contamination may have ecotoxicological effects for aquatic flora and fauna as well as for

human health if used for public consumption (Leonard, 1988; Miyamoto *et.al*, 1990). The aim of this study was to quantify and determine the levels of OCPs present in River Benue.

MATERIALS AND METHODS

Analytical Standards and Reagents

All chemicals and reagents used in this study were of high purity quality and were of residue grade. Dichloromethane and normal hexane were purchased from Sigma Aldrich while anhydrous sodium sulphate (Na_2SO_4) and silica gel (60 – 100 mesh ASTM) were purchased from Merck, Germany. The individual reference pesticide standards (ISO 9001Certified) used for GC analysis of the organochlorines was purchased from Restek Corporation, USA.

Sampling: Two sampling stations were selected at Makurdi Local Government Area namely: Benue Brewery (Latitude 7° 46´ N and Longitude 8° 32´ E) and Nigerian Army School of Military Engineering, NASME (Latitude 7° 46´ N and Longitude 8° 27´ E). The locations selected along River Benue are agricultural areas where there are different kinds of agricultural activities, mainly rice farming, cassava, yam, fruits and vegetables gardens. Samples were collected at Benue Brewery and NASME, Makurdi to ascertain if the industrial and agricultural activities have any effect(s) on pesticides prevalence. Samples from the river water with depths ranging from 1 to 1.5 m were collected directly into 1.0 L plastic bottles in each sampling site. The bottles were filled to the top with as little remaining air as possible, and sealed tightly. All samples were properly labelled with details of the source and sampling date, and stored at 4 °C until liquid-liquid extraction.

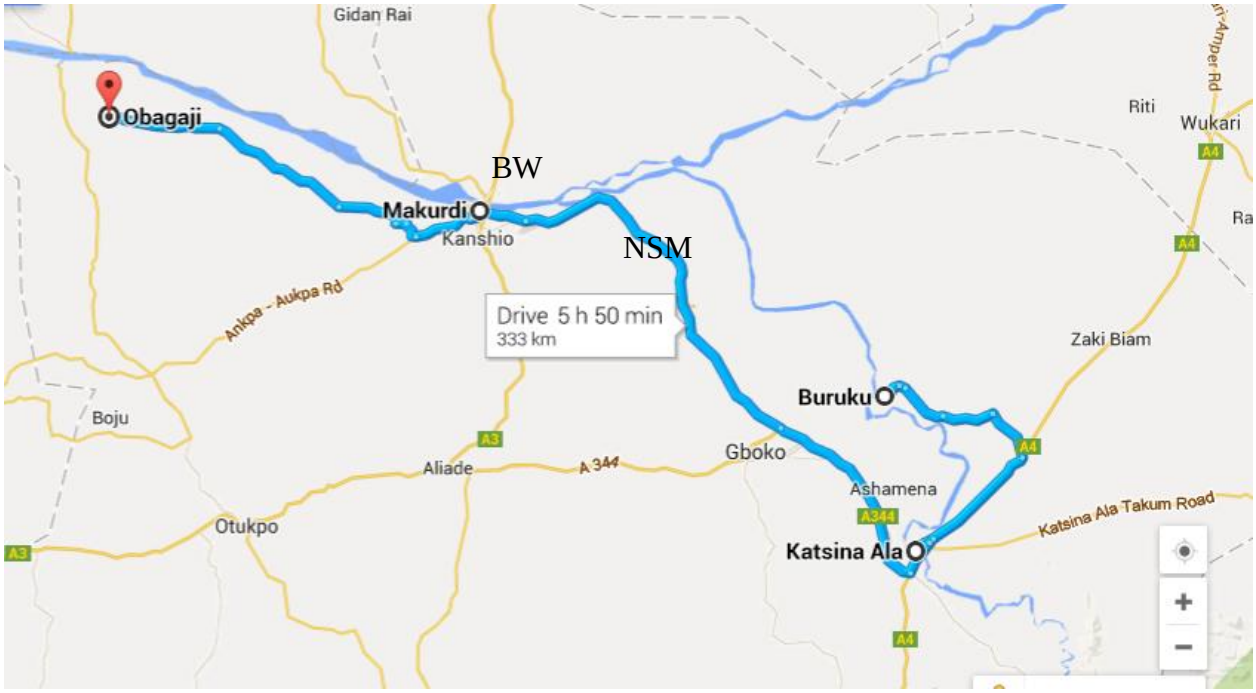

Fig. 1: Map of Benue Showing River Benue and the sampling sites

Source: adapted and modified from Google maps, 2014.

Liquid-liquid Extraction (LLE):

Method earlier described by Helena *et al.*, (2002) was adopted with slight modification. Exactly 100 mL of dichloromethane was added to 100mL of unfiltered water sample placed in a 250 mL separating funnel and shaken for about 5 minutes to enhance thorough mixing and allowed to stand for 20 minutes on retort stand. Then the aqueous phase was drained into a conical flask and the extraction was repeated twice using fresh portions of solvent. The resulting three portions of the extracts were combined and evaporated to near dryness using rotary evaporator after which possible residual water was removed with anhydrous sodium sulphate. The extract was dissolved with 1.0 mL of acetone and transferred into the sample vial for GC analysis. A 1.0 µg/mL standard working solution was prepared using high QC solution. A 5-point calibration curve (0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL) was created by adding appropriate volume of this 1.0 µg/mL solution to blank. Internal standard solution was added to have a final concentration of 10 µg/mL.

Instrumental Determination:

An Agilent 7890 A Gas Chromatograph coupled with ECD equipped with Agilent 7693 Autosampler, fused HPSMS silica capillary column of 30m length, 0.32mm id and 0.25µm film thickness was used for the analysis. The oven temperature was programmed from an initial temperature of 150 ^{o}C (2 minutes hold) 220^{o}C at a rate of 5^{o}C per minute and was maintained at 220^{o}C for 2 minutes before raised to 240^{o}C at rate of 5^{o}C per minute and maintained at 240^{o}C for a minute and finally raised to 280^{o}C. The injection temperature was maintained at 250^{o}C, while the detector temperature was maintained at 300^{o}C. Nitrogen gas was used as carrier gas at flow rate 4.0L per minute.

Statistical analysis: Statistical analysis was carried out using the statistical package for social sciences (SPSS 16.0) programme

RESULTS AND DISCUSSION

Figs. 2 and 3 show the chromatograms of OCPS at Brewery in May and June, 2013 respectively while Figs. 4 and 5 show the chromatograms for OCPs in August, 2013 and February, 2014 respectively . It is clear from these chromatograms that the shapes of the peaks are sharp indicating a good separation. The retention times of each organochlorine pesticide were identified by running gas chromatograph for individual pesticides under the reported identical conditions of the experiments. The capacity factor and the separation factor were calculated. The chromatographic conditions were optimized by varying the temperatures of injection, column and detector.

Table 1: Retention time and Correlation coefficient of Calibration Curves

Pesticides	Retention time (min)	Regression equation	Correlation co-efficient (R^2)
α-BHC	10.2	97090x	0.734
β-BHC	10.3	95326x	0.754
δ-BHC	10.8	40727x	0.872
Chlorothalonil	11.1	95272x	0.866
Dieldrin	11.8	50727x	0.789
Endosulfan I	12.6	52181x	0.841
Heptachlor B	16.9	20727x	0.766
Heptachlor epoxide	17.1	45090x	0.820
Lambda cyhalothrin	17.8	20727x	0.766
P'P-DDT	17.9	36488x	0.820
Permethrin	20.5	22182x	0.721

Table 2: Concentration of OCP (ppm) in the water samples

Sample Codes	Aldrin	α-BHC	β- BHC	σ BHC	chlorothalonil	Dieldrin	Endosulfan I	Endosulfan II	Endosulfan sulphate	Endrin	Heptachlor B	Heptachlor epoxide	Lambda cyhalothrin	lindane	permethrin	P'P-DDT	PP'-DDD
1A	0.0	_	0.01407	0.02655	0.01596	0.0	0.0	0.0	0.0	0.00033	0.00473	_	0.0	0.00029	0.16628	_	_
1B	_	_	_	_	0.01599	_	_	_	_	_	0.00449	_	0.10232	_	0.00166	_	_
1C	0.0	0.00421	0.01212	0.00272	0.01730	_	_	0.00007	0.02452	0.00136	0.00647	_	0.13795	0.00093	0.17168	0.01243	_
1D	0.01052	0.00588	0.03124	_	0.03699	0.01052	0.10628	0.06121	0.14909	0.33535	0.02695	0.15109	0.72852	0.00848	0.15176	0.01643	_
1E	_	_	0.01190	_	0.01592	0.0	0.00177	0.00174	0.04076	0.0	0.00567	_	0.12565	0.00035	0.17317	0.01297	_
1F	_	0.00522	0.01346	0.02889	0.01993	_	_	_		_	0.00959	_	0.10953	0.00350	_	_	0.00252
2A	_	_	_	_	_	_	_	_	0.02404	_	0.00173	_	_	0.0	0.01715	0.01313	_
2B	0.02384	0.01340	0.02581	0.06835	0.07562	0.01417	0.05515	0.05524	0.05623	0.09597	0.02196	0.00278	65.1730	0.0	0.03735	0.02799	0.02208
2C	_	_	0.01302	_	0.01661	_	_	_	_	_	0.00917	_	0.13145	0.0	0.02388	_	_
2D	_	0.00439	0.01323	0.02883	0.01903	-	-	-	0.02982	-	0.00947	-	0.10448	0.0	23.1098	0.01217	-
2E	0.02728	_	0.06734	0.04296	0.04021	0.03058	0.12670	-0.04770	0.06122	0.12357	0.01789	0.00831	114.640	0.0	0.90434	0.04123	0.03850
2F	0.01398	0.00548	0.02124	0.03042	0.02468	0.00389	0.00829	0.01905	0.03785	0.01803	0.01960	_	1.67859	0.0	0.0	0.02482	0.01170

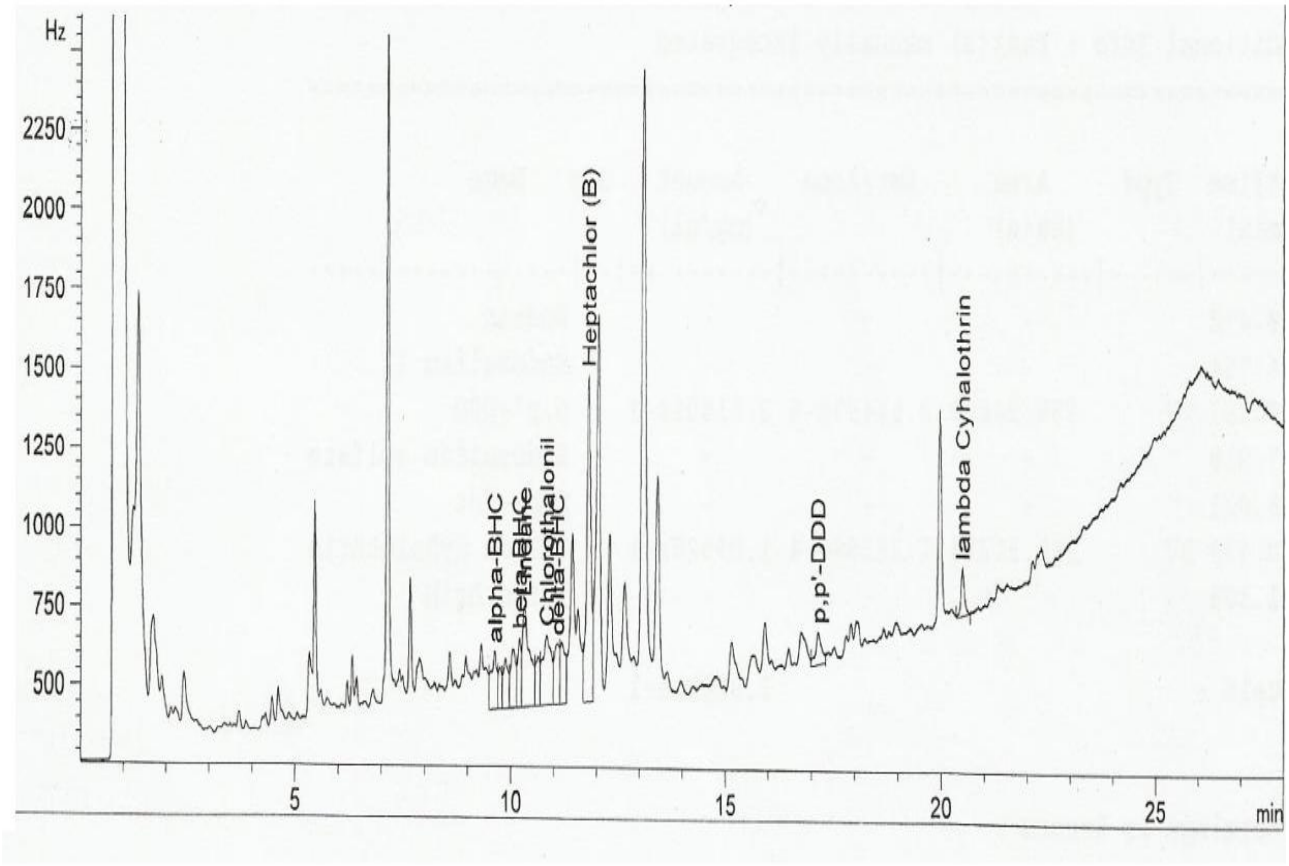

Fig. 2: GC-ECD Chromatogram of Organochlorine Pesticides at Benue Brewery May, 2013

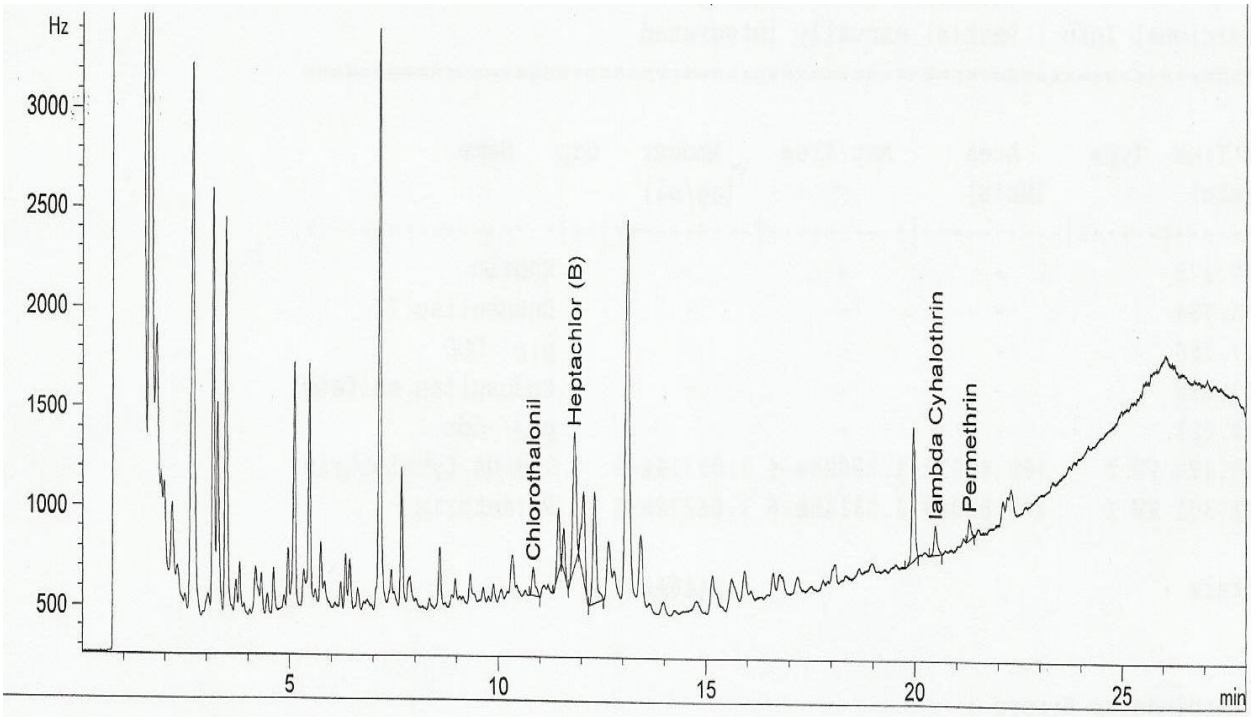

Fig. 3: GC-ECD Chromatogram of OCPs at Benue Brewery in June, 2013

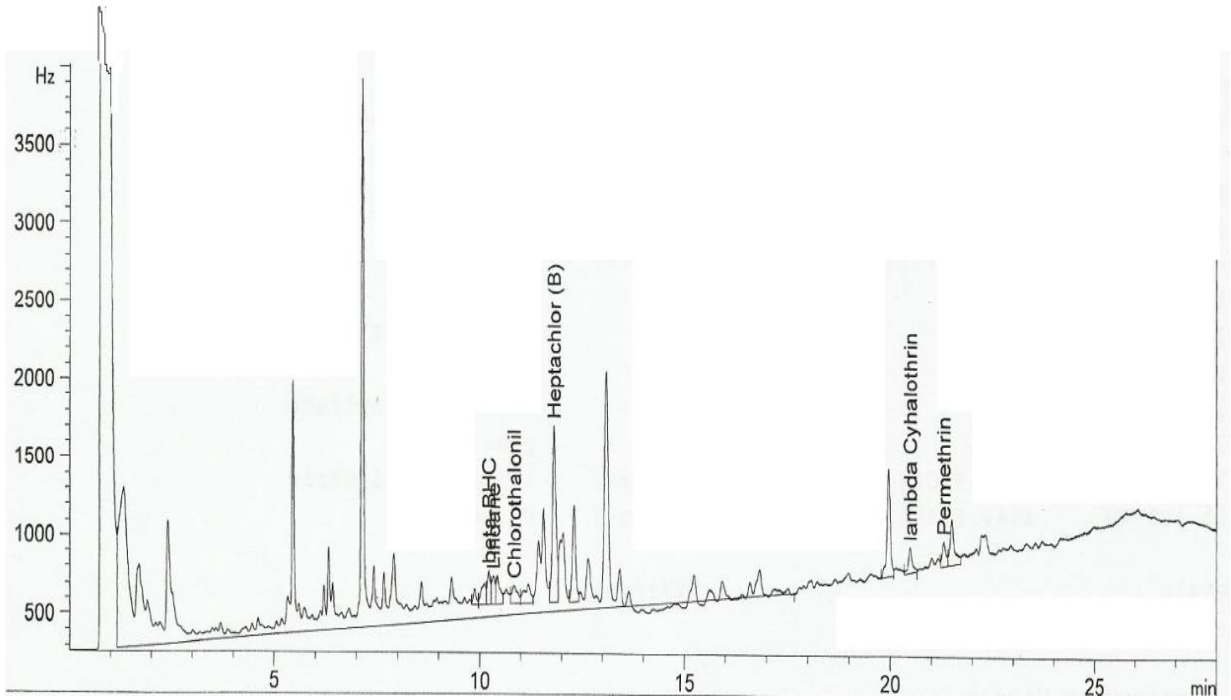

Fig. 4: GC-ECD Chromatogram of OCPs at NASME in August, 2013

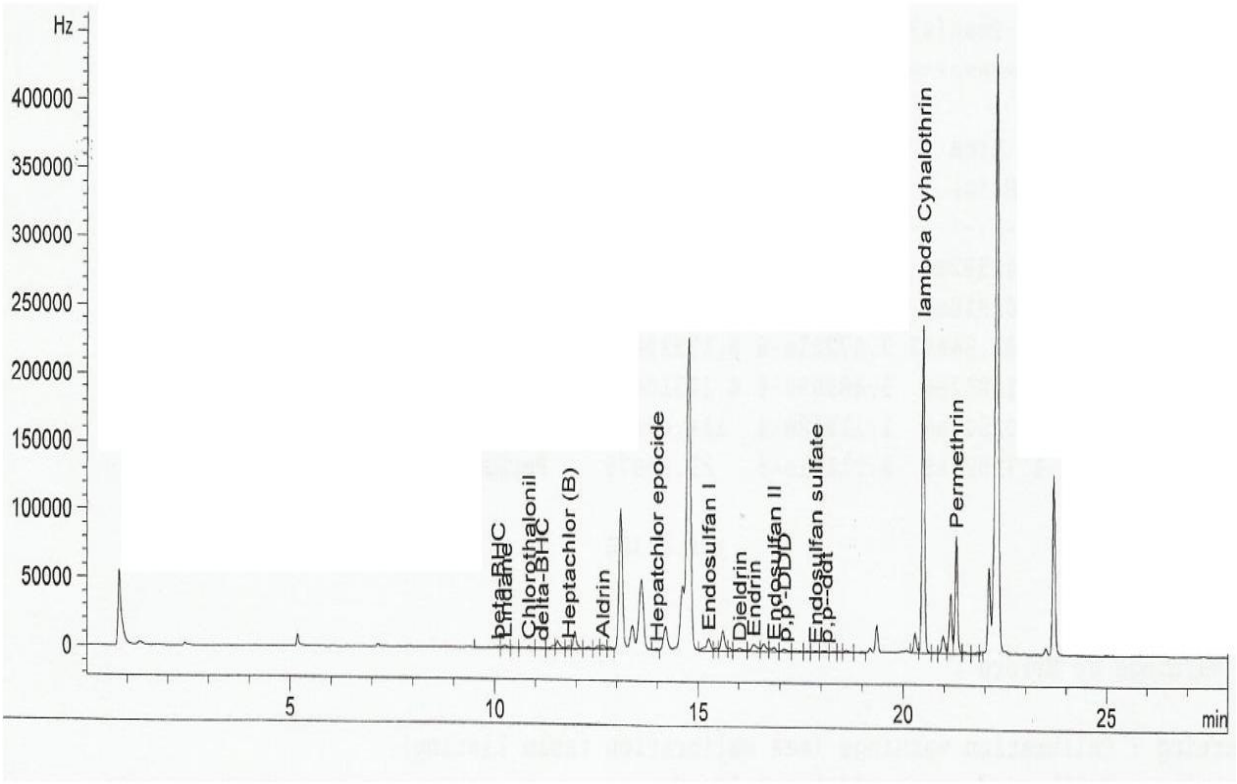

Fig. 5: GC-ECD Chromatogram of OCPs at NASME in February, 2014

A perusal of Table 2 indicates that analysis of organochlorine pesticides have been carried out for water samples collected from River Benue at NASME Barracks and Benue Brewery, Makurdi. The mean concentration of aldrin was 0.013 ppm. The maximum concentration of aldrin of 0.027 ppm was found at location code 2E while the minimum concentration of 0 ppm was detected at location codes 1A and 1C. Locations with concentrations below LOD are indicated with (-). α-BHC had a mean concentration of 0.006 ppm with the maximum an d minimum concentrations of 0.013 ppm (location code 2B) and 0.004 ppm (location code 1C) respectively. It has an MRL of 0.026 ppm which was above all the values of concentrations recorded for α-BHC across the sample locations. Idowu *et al*. (2013) reported concentration of α-BHC below LOD.

β-BHC had an average concentration of 0.022 ppm, while its maximum concentration of 0.067 ppm was detected at location code 2E and the minimum concentration of 0.012 was detected at location code, 1E. In a similar report concentration of δ-BHC below LOD was detected. The mean concentration of δ- BHC is 0.033 ppm. The maximum concentration of δ- BHC 0.068 ppm was found at location code 2E while the minimum concentration of 0.003 ppm was detected at location codes 1A and 1C. The mean concentration of aldrin was 0.0189 ppm. The maximum concentration of δ-BHC 0.0238 ppm was found at location code 1A while the minimum concentration of 0.0 ppm was recorded at location codes 2B. Furthermore, he observed concentration of aldrin below LOD while Adeyemi *et al.* (2011) reported 0.417 ppm in water samples of Lagos Lagoon. Idowu *et al.* (2013) recorded concentration below LOD while Adeyemi *et al.*, (2011) detected dieldrin concentration of 0.032 ppm in water samples of Lagos Lagoon. The MRL for aldrin in drinking water is 0.000049 ppm. Chlorothalonil had a mean concentration of 0.031 ppm with the maximum and minimum concentrations of 0.076 ppm (location code 2B) and 0.003 ppm (location code 1C) respectively. Dieldrin had an average concentration of 0.020 ppm while its maximum concentration of 0.076 ppm was detected at location code 2E and the minimum concentration of 0 ppm was detected at location code, 1E. The mean concentration of endosulfan I is 0.050 ppm. The maximum concentration of endosulfan I 0.127 ppm was found at location code 2E while the minimum concentration of 0.00 ppm was detected at location codes 1A and 1C. Endosulfan II had a mean concentration of 0.013 ppm with the maximum and minimum concentrations of 0.612 ppm (location code 2B) and 0 ppm (location code 1C) respectively. Idowu *et al.* (2013) reported 1.924 ppm and 0.005 ppm for endosulfan I and endosulfan II which compares well with concentrations detected in this study. Their MRL of 62 ppm is above concentrations reported in this work. Endosulfan sulphate had an average concentration of 0.047 ppm while its maximum concentration of 0.149 ppm was detected at location code 2E and the minimum concentration of 0 ppm was detected at location code, 1E. Heptachlor epoxide had a mean concentration of 0.063 ppm with the maximum and minimum concentrations of 0.151 ppm (location code 1D) and 0.002 ppm (location code 2B) respectively. Adeyemi *et al.* (2011), reported 0.037 ppm for heptachlor epoxide which is in agreement with values reported in this study.

Heptachlor B had a mean concentration of 0.0119 ppm with the maximum and minimum concentrations of 0.027 ppm (location code 1D) and 0.002 ppm (location code 2A) respectively. Idowu *et al.*, 2013 on organochlorine pesticide residue levels in river water and sediment from cocoa-producing areas of Ondo State Central Senatorial district reported values of heptachlor B below LOD. The MRL of heptachlor B is 0.000079 ppm while that of heptachlor epoxide is 0.000039 ppm. Comparison of these regulatory limits with the values presented in Table 4 reveals that both heptachlor B and heptachlor epoxide exceeded the regulatory limits at all the locations where they were detected.

Lindane has a mean concentration of 0.002 ppm with the maximum and minimum concentrations of 0.009 ppm (location code 1D) and 0.00 ppm (location code 2A-2F) respectively. Lindane happened to be one of the most widely distributed of the OCPs studied. It had an MRL of 0.95 ppm. Comparison of the concentrations of lindane with the regulatory limit showed that the concentrations of lindane were still within the safety threshold as they are within 0.00 -0.029 ppm. Farshid, 2012 reported values 0.45-1.50 which are higher than concentrations detected in this study.

Permethrin had a mean concentration of 3.682 ppm with the maximum and minimum concentrations of 23.11 ppm (location code 2D) and 0 ppm (location code 1F) respectively. On the other hand PP-DDT had a mean concentration of 0.022 ppm with the maximum and minimum concentrations of 0.041 ppm (location code 2E) and 0.012 ppm (location code 2D) respectively. PP-DDD had a mean concentration of 0.038 ppm with the maximum and minim um concentrations of 0.003 ppm (location code 2F) and 0.00 ppm (location code 1F) respectively.

Comparison of results of all OCPs at a particular station were carried out using ANOVA at 5 % confidence ($p<0.05$) showed that within a

particular station and month and station, there were no significant differences with respect to any of the pesticides except for permethrin and lambda cyhalothrin at location codes 2D and 2E respectively.

CONCLUSION

The GC-ECD analysis in this research has revealed varying degrees of organochlorine pesticides in River Benue water at Makurdi. The reported concentrations of pesticides in River Benue water indicates that the river is polluted and the water is not fit for drinking, recreation purposes.

ACKNOWLEDGMENT:

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Key to codes:1=Brewery (BWR), 2=NASME (NSM) **A=May, B=June, C=August, D=September, E=February, F=March**

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