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Contributions to the environment of priority Polynuclear Aromatic Hydrocarbons from the Coal Camp Mechanic Village (CCMV) in Enugu, Nigeria.

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#### **ABSTRACT**

The Coal Camp Mechanic Village(CCMV) in Enugu,Nigeria generates automobile oily wastes which could contain the Polynuclear Aromatic Hydrocarbons (PAHs) and distribute them to the proximal segments of the environment. The current study investigated the presence and levels of the PAHs in soils of the CCMV, as well as in water and sediments of the nearby Okpete Stream. Samples were collected using standard methods and PAHs detected with a Gas Chromatograph interfaced with Flame Ionization Detector. Mean concentrations of the PAHs [except 1,2-Benzanthracene and Benzo(k) fluoranthene] exceeded the World Health Organization's permissible limit of  $0.2\mu g/L$  for drinking water. Two Principal Components (PCs) formed the extraction solution, with a cumulative percentage variability contribution of 96.6% in the original 16 variables; with PC 1 most highly correlated with Fluorene/1,2,5,6-Dibenzanthracene (0.979) and PC 2 with Acenaphthene (0.982). There was significant spatial heterogeneity in mean levels of the PAHs in water samples (Sig. F=0.013) at P<0.05, and Indeno (1,2,3-cd) pyrene (16.13429  $\mu g/L$ ) and Fluoranthene (92.30784  $\mu g/L$ ) contributed the difference most. Results raise public health alert as PAHs are known carcinogens. Recycling of waste/used oils is recommended for environmental sustainability.

Keywords: Polynuclear Aromatic Hydrocarbons, Mechanic Village, Carcinogenicity, Bioaccumulation, Enugu

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#### INTRODUCTION

A mechanic village is a cluster of automobile workshops that usually occupy several acres of land mapped out for automobile mechanics as against citywide automobile workshop practice. They are sited out of urban centers and kilometers away from urban residential areas. According to Nwachukwu *et al.* [1], the hazard of environmental pollution through disposing used automobile oil on the ground in many mechanic villages in developing countries such as Nigeria is of great concern to public health and ecology [2-4].

In Nigeria for example, more than 80% of spent engine and transmission oil could be wrongly disposed on the ground by mechanics, and these used engine oil contains a number of additives, impurities, and residues resulting from the combustion process. Some of these are poisonous or carcinogenic like the Polychlorinated Biphenyls (PCB) and Polynuclear Aromatic Hydrocarbons (PAHs).

Polynuclear Aromatic Hydrocarbons (PAHs), also known as polycyclic aromatic hydrocarbons or poly-aromatic hydrocarbons, are a large group of organic chemicals containing two or more fused aromatic rings of carbon and hydrogen atoms and also pollutants that are detrimental to the environment especially when they exceed threshold limits [5]. They are organic and widespread pollutants in the environment that are normally formed in combustion processes of carbonaceous materials at moderate or high temperatures and are found in soil, sediments, water and air [6, 7]. The major source of PAHs into the environment is anthropogenic which includes combustion from industrial and vehicle emissions, waste incineration and deposition of wet or dry atmospheric particulates [8], processing of coal, crude oil and natural gas, incomplete combustion of coal, oil, gas, garbage and other organic substances [9]. PAHs can also be introduced in the environment from natural sources, including biosynthetic activity, natural fire and volcanic eruptions.

The presence of PAHs in the environment is increasingly becoming an issue of global concern, especially in relation to their toxic nature to living organisms at excessive concentrations in soils, sediments and water, which are considered very important ecological matrices in the environment. Several laboratory studies have established the carcinogenicity of certain PAHs in at least, laboratory animals, even as researchers have reported increased incidences of skin, lung, bladder, liver, and stomach cancers in animals [9-12]. Also, animal studies show that PAH can also affect the hematopoietic and immune systems, and can produce reproductive, neurologic, and developmental effects [13, 14, 4, 5].

Routine exposure to chemical contaminants in workplace is a cause for concern over potential health risks to workers. In Nigeria for example, reports on occupational exposure and related health risks are almost non-existent, which reflects the scarce availability of survey data and criteria for determining whether an unsafe exposure has occurred. The current study was a contribution to close this gap in knowledge of source and migration of the PAHs in our environment.



#### **MATERIALS AND METHODS**

## **Study Area**

The study was carried out within and around the Coal Camp Mechanic Village (CCMV) in Ogbete, Enugu North Local Government Area of Enugu State, Nigeria. The CCMV, measuring about  $0.41 \mathrm{km}^2$  covers approximately 101 acres of land along the Agbani Road in Enugu, near the Nigeria Police Detective College; within latitude of  $6^{\circ}25'\mathrm{N}$  and longitude of  $7^{\circ}28\mathrm{E}$  (Figure 1) and altitude of 235 meters. About 177meters from the mechanic village is the Okpete stream, which flows from Ngwo through Jamboree to Mbanugo and Uwani. The CCMV is a major trading hub for motor spare parts and the fabrication of metals. The location provides a dramatic example of anthropogenic impacts on the environment. The automobile mechanic site was set up in the year 1981 for the purpose of repairing and maintaining automobile vehicles used in the transportation of humans, animal and other goods. The quantity of wastes generated in the mechanic village is projected to have increased from 1,197,000 in 1991 to 1,990,400 in 2007.

Two distinct seasons exist in the area, viz, the dry and rainy seasons; with the rainy season lasting from March to October, with peak of rainfall in July and a short dry spell in August popularly known as "August break". Annual rainfall ranges from 2259mm to 2500mm; mean temperature is 27°C, while relative humidity is about 70-80%. The topography of the location is relatively level ground that is strongly degraded towards the proximal Okpete stream. Its landscape has been sculpted by erosion, forming deep gullies with, elevations ranging between 25.5 and 15.1 meters in the north-west and central section and to about 45 meters on the east and south. The geology of the area consists of plain sandy-loam soil, which is about 0.05-2.0 mm in size.



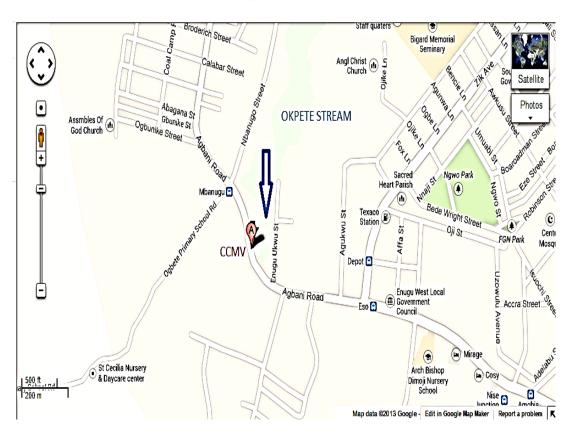


Figure 1. Map of the study area showing the Coal Camp Mechanic Village (CCMV) and Okpete stream

### **Field Sampling**

Three replicate samples were randomly collected each from soil (MVS 1-MVS 3), surface water (MVW 1-MVW 3) and sediments (MVSed 1-MVSed 3) around the mechanic village. Soil samples were collected from the depths of 0-30 cm with stainless hand-held auger while sediment samples were collected with a 50X50cm Eckman Grab and transferred in labeled aluminium foils to the laboratory. Water samples were collected with labeled 1liter amber glass bottles fitted with screw caps and lined with foils and transferred to the laboratory in iced-chest.

## **Laboratory Analysis**

The analytical procedures were in keeping with standard methods of APHA [15] and Fetzer [16]. The GC parameters used included helium as carrier gas, air and hydrogen as fuel gases, nitrogen as back-up gas, detector temperature of 35°C, in-let temperature of 25°C, initial oven temperature of 5°C, final oven temperature of 300°C, hydrogen flow rate of 30mL/mins., air flow rate of 300mL/mins, nitrogen flow rate of 30mL/mins, and helium flow rate of 30mL/mins.



Sample extraction procedure involved weighing out 5g each of soil and sediment samples into a beaker and adding 10mL of analytical grade hexane to the samples. The mixture was shaken for 5minutes and filtered; and filtrates used for GC analysis. For water samples, 50mL of a sample was measured into 1 liter separating funnel, a drop of concentrated H<sub>2</sub>SO<sub>4</sub> was added to the sample in the separating funnel to release the hydrocarbon components and 5ml of solvent (analytical grade N-hexane) was subsequently added. Samples were vigorously shaken for 5mins and allowed to stand for another 20mins. Layers were formed that separated the extract (the top layer) from the lower layer which was discarded and the extract collected for GC analysis in a glass vial.

A column chromatography was set up using silica gel and a glass wool. Extracts were passed through the column to clean and remove biogenics, and then collected for GC analysis.GC was calibrated using commercially prepared external standards having 16 components of PAHs with concentration of 1000 ppm per component.GC parameters were set and a PAH extract was loaded using a micro-syringe to prompt the GC to run for a period of about 41minutes.

#### STATISTICAL ANALYSIS

Both univariate, bivariate and multivariate analyses were applied in the analysis of data. The descriptive statistics was used to compute means, standard errors, ranges, minimum and maximum levels of the PAHs. The factor analysis procedure, using the Principal Components Analysis (PCA) method of extraction for data reduction was used to remove redundant variables from the data file and to replace the entire file with a smaller number of uncorrelated factors. Factor rotation was achieved with the varimax method and the magnitude of the eigenvalues and 75% (0.75) rule for variance contribution were used for factor selection. Variation plots were used to represent mean levels of the PAHs spatially. The test of homogeneity in mean variance of levels of the PAHs across the sampling locations was conducted with the One Way Analysis of Variance (ANOVA) and post-hoc structure of group means detected with means plots at the 95% confidence interval.

## **RESULTS**

#### Descriptive variations in levels of the PAHs

In these environmental segments, the concentrations of Naphthalene (range=127.8720  $\mu$ g/L), Acenaphthene (range=194.6131 $\mu$ g/L), Acenaphthylene (range=148.4033 $\mu$ g/L), Fluoranthene (range=799.2927 $\mu$ g/L), Indeno (1,2,3-c,d)Pyrene (range=287.4523 $\mu$ L) and 1,2,5,6-Dibenzanthracene (range=102.1150 $\mu$ g/L) varied widely. Naphthalene, Acenaphthene and Acenaphthylene varied from negligible to 127.8720 (78.7649  $\pm$  19.7988), 194.6131 (5.6229  $\pm$  0.2898), and 148.4033 (0.4745  $\pm$  0.2376)  $\mu$ g/L respectively (Table 1). Fluorene varied from 70.7655-92.0001 (80.4890  $\pm$  2.7022)  $\mu$ g/L, Phenanthrene varied from 76.7499-82.4202 (79.5957  $\pm$  0.6665)  $\mu$ g/L and Anthracene varied from 78.6651-115.5235 (89.2676  $\pm$  5.1303)  $\mu$ g/L. Fluoranthene, Pyrene and 1,2-Benzanthracene varied from 90.7078-890.0005 (356.1985  $\pm$ 



0.0132), 72.7211-78.1113 (74.7369  $\pm 0.7925)$  and 0.0000-0.0072 (0.0047  $\pm$  0.0012)  $\mu g/L$  respectively.

The other PAHs varied as follows: Chrysene 27.7667-33.0099 (30.7935  $\pm$  0.6753) µg/L, Benzo(b) fluoranthene 5.2215-9.8382 (7.6795  $\pm$  0.5056) µg/L, Benzo(a) pyrene 10.6115-18.4223 (13.2985  $\pm$ 1.1186) µg/L, Indeno(1,2,3-c,d) Pyrene 15.5511-303.0034 (111.5404  $\pm$ 47.6008) µg/L, 1,2,5,6 Dibenzanthracene 0.0000-102.1150 (33.8360  $\pm$  16.9183)µg/L, 1,12 Benzoperylene 5.6770-44.6605 (18.7878  $\pm$  6.0433)µg/L. However, Benzo(k)fluoranthene was undetected in the sample matrices.

## Principal Components Analysis (PCA)

The PAHs concentrations measured in soils, sediment and waters in the vicinity of the CCMV which were subjected to the PCA analysis produced communalities that were all high. The first 2 PCs formed the extraction solution, with a cumulative percentage variability contribution of about 96.6% in the original 16 variables (Table 2). This reduces the complexity of the data set by using these components, with only about 3.4% loss of information.

The rotation maintained the cumulative percentage of variation explained by the extracted components, with the values more evenly spread (Table 3). PC 1 alone contributed about 63.8% variation, while PC 2 contributed about 32.8% variation. The scree plot represents the eigenvalue of each component in the initial solution. The extracted components are on the steep slope, while the components on the shallow slope contributed little (8.1%) to the solution. The last big drop occurred between the 2<sup>nd</sup> and 3<sup>rd</sup> components (Figure 2).

Table 1. Descriptive statistics of levels (ppt) of the PAHs in soils, sediments and waters of the CCMV, Enugu.

Parameters	Minimum	Maximum	Range	Mean	SE
Naphthalene	0.0000	127.8720	127.8720	78.7649	19.7988
Acenaphthene	0.0000	194.6131	194.6131	5.6229	0.2898
Acenaphthylene	0.0000	148.4033	148.4033	0.4745	0.2376
Fluorene	70.7655	92.0001	21.2346	80.4890	2.7022
Phenanthrene	76.7499	82.4202	5.6703	79.5957	0.6665
Anthracene	78.6651	115.5235	36.8583	89.2676	5.1303
Fluoranthene	90.7078	890.0005	799.2927	356.1985	0.0132
Pyrene	72.7211	78.1113	5.3902	74.7369	0.7925
1, 2-Benzanthracene	0.0000	0.0072	0.0072	0.0047	0.0012
Chrysene	27.7667	33.0099	5.2432	30.7935	0.6753
Benzo(b)fluoranthene	5.2215	9.8382	4.6167	7.6795	0.5056
Benzo(k)fluoranthene	ND	ND	0.0000	ND	-
Benzo(a)pyrene	10.6115	18.4223	7.8108	13.2985	1.1186
Indeno(1,2,3-cd)pyrene	15.5511	303.0034	287.4523	111.5404	47.600
1,2,5,6-Dibenzanthracen	e 0.0000	102.1150	102.1150	33.8360	16.918
1,12-Benzoperylene	5.6770	44.6605	38.9835	18.7878	6.0433

SE = standard error of means, ND = not detected



The first PC was most highly correlated with Fluorene/1,2,5,6-Dibenzanthracene (0.979) (Table 4), and also had high loadings for Naphthalene (0.850), Acenaphthylene (0.921), Phenanthrene (0.932), Anthracene (0.949), Fluoranthene (0.933), Pyrene (0.974), 1,2-Benzanthracene (-0.792), and Benzo(a) pyrene (0.778). The second component was most highly correlated with Acenaphthene (0.982) and also had high loading for Naphthalene (0.510), 1,2-Benzanthracene (-0.591), Chrysene (0.969), Benzo(b) fluoranthene (0.954), Benzo(k) fluoranthene (0.894) and Benzo(a) pyrene (0.580).

Table 2. Extraction Sums of Squared Loadings in levels of the PAHs in soils, sediments and waters of the CCMV, Enugu

Components	Total	% of Variance	Cumulative %
1	10.351	64.696	64.696
2	5.096	31.850	96.546

Table 3. Rotation Sums of Squared Loadings in levels of the PAHs in soils, sediments and waters of the CCMV, Enugu

Components	Total	% of Variance	Cumulative %
1	10.201	63.758	63.758
2	5.246	32.788	96.546

Table 4. Rotated Component Matrix in levels of the PAHs in soils, sediments and waters of the CCMV, Enugu

	Components		
PAHs	1	2	
Naphthalene	0.850	0.510	
Acenaphthene		0.982	
Acenaphthylene	0.921		
Fluorene	0.979		
Phenanthrene	0.932		
Anthracene	0.949		
Fluoranthene	0.933		
Pyrene	0.974		
1,2-Benzanthracene	-0.792	-0.591	
Chrysene		0.969	
Benzo(b) fluoranthen	е	0.954	
Benzo(k) fluoranthene	е	0.894	
Benzo(a) pyrene	0.778	0.580	
Indeno(1,2,3-c,d) pyre	ene 0.932		
1,2,5,6-Dibenzanthrac	ene 0.979		
1,12-Benzoperylene	0.958		



The normality plot in rotated space reveals that the extracted components were skewed in distribution patterns (Figure 3). Fluorene, 1,2,5,6-Dibenzanthracene, Pyrene, Anthracene, 1,12-Benzoperylene, Indeno (1,2,3-cd) pyrene, Fluoranthene and Acenaphthylene were more closely associated than with the other PAHs. However, Benzo(a) pyrene, Naphthalene and Phenanthrene also appeared to be more closely related than with the others, even as Chrysene and Benzo(b) fluoranthene were also more closely related.

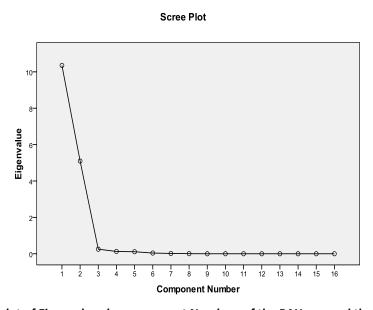


Figure 2. Scree plot of Eigenvalues by component Numbers of the PAHs around the CCMV, Enugu

## Component Plot in Rotated Space

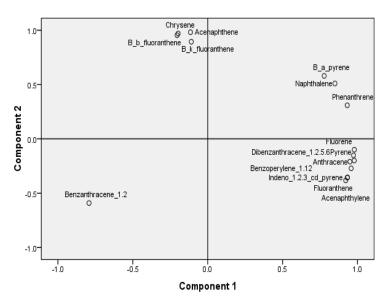


Figure 3. Component Plot in Rotated Space of the PAHs detected around the CCMV, Enugu

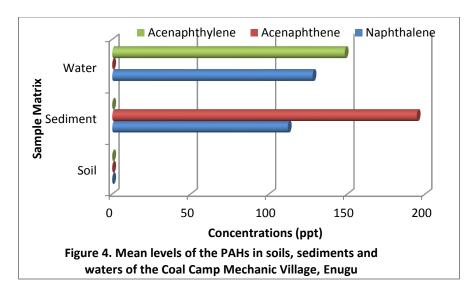


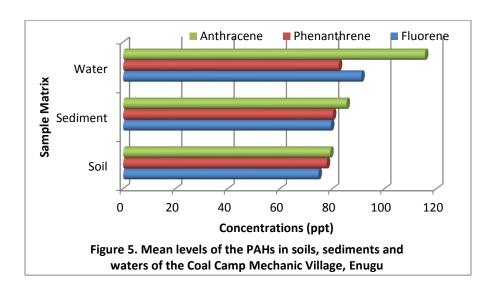
## **Spatial Distributions of the PAHs**

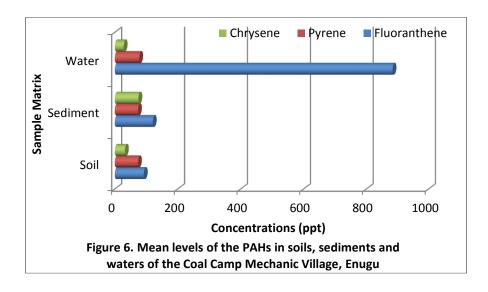
Naphthalene, Acenaphthene and Acenaphthylene were undetected in the soil matrix, while their mean maximum concentrations of 127.87199  $\mu g/L$ , 194.61311 $\mu g/kg$  and 148.40329 $\mu g/L$  were recorded in water, sediment, and water samples respectively (Figure 4). Mean minimum concentrations of Fluorene (74.66797 $\mu g/kg$ ), Phenanthrene (77.81493 $\mu g/kg$ ) and Anthracene (79.11273 $\mu g/kg$ ) were all recorded in the soil matrix, while their mean maximum concentrations of 91.04610, 82.42015 & 115.52345 $\mu g/L$  respectively were all recorded in the water matrix (Figure 5).

Mean minimum concentrations of Chrysene (28.32323µg/L), Pyrene (73.80593µg/kg) and Fluoranthene (92.30784µg/kg) were recorded in the water, soil and soil matrices, while their mean maximum concentrations of 75.14977µg/kg, 77.69405µg/L and 884.13261µg/L were recorded in the sediment, water and water matrices respectively (Figure 6). Mean minimum concentration of 1,12-Benzoperylene (6.97247µg/kg) was recorded in the soil matrix, while its mean maximum concentration of 42.30947µg/L was recorded in water (Figure 7). Mean minimum and maximum concentration of Indeno (1,2,3-cd)pyrene (16.13429µg/kg & 301.82729µg/L) were recorded in soil and water (Figure 7). Mean minimum level of 1,2-Benzanthracene (0.000035993µg/kg) was recorded in sediments, while its maximum level of 7107.79567µg/kg was recorded in soil samples (Figure 7). The mean minimum concentrations of Benzo(a) pyrene (11.63154μg/kg) and Benzo(b) fluoranthene (8.60070μg/L) were recorded in soil and water matrices respectively, while their maximum concentrations (18.36226µg/kg & 17.79562µg/kg) were all recorded in the sediments. However, 1,2,5,6-Dibenzanthracene was undetected in soil samples and the mean maximum concentration of 100.85433µg/L was recorded in water sample (Figure 8). The test of homogeneity in mean variance of concentrations of the PAHs revealed significant difference in the water samples (Sig. F=0.013) at P<0.05, even as there was statistical homogeneity in the sediment samples (Sig. F=0.983) across the sampling locations. The post-hoc analysis of variance (ANOVA) structure of group means that utilized the soil sample matrix as predictor variable revealed that in the water samples, the levels of Indeno (1,2,3-cd) pyrene (16.13429µg/kg) and Fluoranthene (92.30784µg/kg) contributed the observed heterogeneity most (Figure 9).

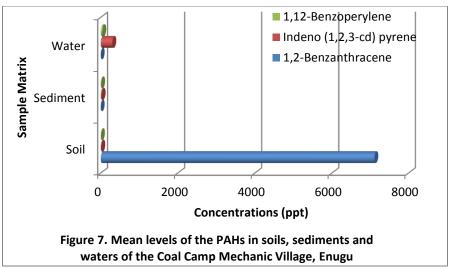


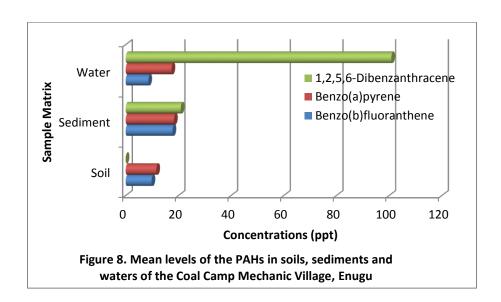














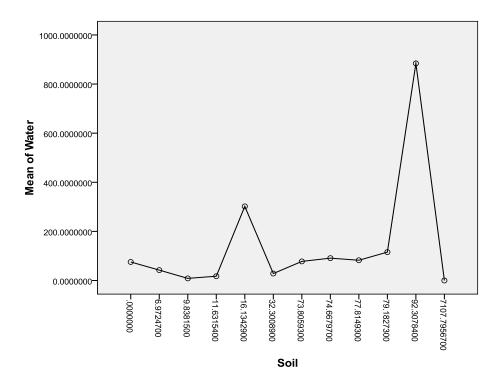


Figure 9. Means plot in levels of the PAHs between water and soil sample matrices of the CCMV, Enugu

#### DISCUSSION

Elevated levels of the Polynuclear Aromatic Hydrocarbons were generally detected in the soil, sediment and water samples around the Coal Camp Mechanic Village, except with Naphthalene, Acenaphthene, Acenaphthylene, Benzo(k) fluoranthene and 1,2,5,6-Dibenzanthracene in soil samples and with Benzo(k) fluoranthene in water samples where concentrations were undetected. This elevation could readily be fingerprinted to condemned/waste/used oil contamination of the environmental matrices from the mechanic village activities. The contributions of the PAHs in water and sediments of the adjoining water body must have been aided by runoffs after rainfalls. The possibility of contamination of proximal segments of the environment with surface pollutants has severally been identified by Narayanan [17], Kaufmann and Claveland [18], Ogbuagu *et al.* [19] and Okoli *et al.* [20], among other authors. Anyakora *et al.* [13] had also identified high concentrations of Benzo(a) pyrene in ground waters of the oil-producing Niger Delta region.

The maximum permissible limit of PAHs in drinking water, as stipulated by the World Health Organization (WHO), (besides that of benzo(a)pyrene that is 0.0001 mg/L, which corresponds to an excess life time cancer risk of  $10^{-5}$ ) is  $0.2 \mu \text{g/L}$  (=0.0002 mg/L) [21]. Thus, results obtained from the current research exceeded the WHO drinking water standards for PAHs and undoubtedly creates a great cause for public health concerns, especially as PAHs have been confirmed to be carcinogenic [9, 19], and are not only ingested by drinking contaminated waters, but are also further consumed when this water is used to prepare foods [22], thereby increasing the risk of accumulation in tissues of man and aquatic organisms such as plankton



[23]. Inevitably man suffers the greatest risk of bioaccumulation due to his position in the trophic chain; being a tertiary consumer in addition to his predisposition to other routes of entry into his body. Further, Ogbuagu *et al.* [19] stated that carcinogenicity is transgenic, as oncogenes (cancer prone genes) could be inherited by filial generations.

The current study also revealed high PCA variability contributions, especially of Acenaphthene, Fluorene and 1,2,5,6-Dibenzanthrene. This indicates that the presence and levels of these PAHs were of most environmental pollution significance than the others. The variability contribution was as high as 96.6%, leaving as much as 14 other PAH component contributing a meager 3.4% variability. The high communalities indicate that the extracted components represented the PCA variables well.

The observed marked spatial variations in concentration of the PAHs indicated that the sampling locations harboured significantly different levels of contaminations. The soil samples, which had the highest concentration of all the PAHs measured are located in closest proximity to the waste oil discharges, and this confirms that pollution inputs came from the mechanic village activities. The absence of Naphthalene, Acenaphthene, Acenaphthylene, and 1,2,5,6-Dibenzanthracene in soil matrix indicate that their presence in water and sediment samples was not anthropogenic, but rather petrogenic in origin and are closely related due to their molecular weights [6].

#### **SUMMARY AND CONCLUSION**

Results from this study indicate very high levels of the Polynuclear Aromatic Hydrocarbons in the soil, sediment and water matrices in the proximity of the Coal Camp Mechanic Village in Enugu, southeastern Nigeria. Levels severally exceeded the World Health Organization (WHO) maximum permissible limit for drinking water. The source of this pollutant could be fingerprinted to the activities of the mechanic village which generate waste/used oils and deposit them on bare grounds. The pollutants are subsequently transported through runoffs into the nearby surface water and repository sediments. Variability contributions of pollution appeared to be most associated with Acenaphthene, Fluorene and 1,2,5,6-Dibenzanthrene, and marked spatial variations was also observed across the environmental segments.

## RECOMMENDATION

The mechanic village waste oil discharges should be recycled for optimal resource utility and sustainable development. Regulatory agencies like the Federal Environmental Protection Agency (FEPA) should develop and enforce more stringent policies that will ensure the protection of soil and water resources and thus human health. However there is the need for further research on the presence of PAHs in aquatic organisms, including macrobenthos, plankton and microbial communities of the adjoining water body.



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#### **REFERENCES**

- [1] Nwachukwu MA, Alinnor J, Feng H. African J Environ Sci Technol 2012; 6(12): 464-475.
- [2] Boughton B, Horvath A. Environ Sci Technol 2004; 38(2):353-358.
- [3] Rahman MM, Siddiquee TA, Samdani S. Chem Eng Res Bull 2008; 12:24-27.
- [4] Ogbuagu DH, Ayoade AA. Food and Public Health 2012; 2(1): 50-54.
- [5] Ogungbuyi PI, Obasi RA, Abimbola AF. Int J EngTechnol 2013;3(6): 686-695.
- [6] Okoro D. African J Biotechnol 2007; 7(3): 282-285.
- [7] Unlu S, Sari E, Apak R, Balci N, Koldemir B. Global Adv Res J Environ Sci Toxicol 2013; 2(6): 144-151.
- [8] Wilson SC, Jones KC. Environ Pollut 1993; 312: 229-249.
- [9] Agency for Toxic substances and Disease Registry (ATSDR). Public Health Statement for Polycyclic Aromatic Hydrocarbons (PAHs).ATSDR Publication, Atlanta Georgia, 1995.
- [10] Phillips DH. Mutation Res 2002; 443: 139-147.
- [11] Luch A.The carcinogenic effects of polycyclic aromatic hydrocarbons. London ImpericalCollege Press.ISBN 1-860 94-4175, 2005.
- [12] United States Environment Protection Agency (EPA). Groundwater and Drinking water; Consumer Fact sheet on Benzo(a)pyrene US EPA, 2006.
- [13] Anyakora C, Ogbeche A, Coker H, Ukpo G,Ogoh C. Nigerian Quarterly J Hospital Med 2004; 14 (3-4): 288-293.
- [14] Masih A, Saini R, Taneja A. Int J Water 2008; 4(1-2): 136-147.
- [15] APHA (American Public Health Association). Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA/AWWA/WEF: Washington DC., 1998.
- [16] Fetzer JC. The chemistry and analysis of the large Polycyclic aromatic hydrocarbon. Polycyclic Aromatic Compounds. New York: Wiley,2000; 27(2): 243.
- [17] Narayanan P. Environmental Pollution: Principles, Analysis and Control. CBS Publishing and Distributors, New Delhi, 2007; pp.659.
- [18] Kaufmann R, Claveland C. (2008). Environmental Science .New York: McGraw-Hill, 2008; pp.552.
- [19] Ogbuagu DH,Okoli CG, Gilbert CL,Madu S. British J Environ Climate Change 2011; 1(3): 90-102.
- [20] Okoli CG,Ogbuagu DH, Gilbert CL, Madu S, Njoku-Tony RF. J Environ Prot 2011; 2: 848-854.
- [21] World Health Organization (WHO). Guidelines for Drinking Water Quality, Geneva, 1984.
- [22] Falco G, Domingo JL, Lobet JM, Teixido A, Casas C, Müller L. J Food Prot 2003; 66(12): 2325-2331.
- [23] Onyema IC. Res J Pharm Biol Chem Sci 2013; 4(3): 639-652.