

Risk Assessment of Polycyclic Aromatic Hydrocarbons PAHs in Soils and Tubers Crops Collected from Ikot Oborenyin, South-south Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author MEA designed the study, managed the experimental process and wrote the first draft. Author EVG managed the literature searches, performed laboratory analyses. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to investigate the levels, patterns sources and health risk of Polycyclic Aromatic Hydrocarbons (PAH) in soils and root crops of Ikot Oborenyin, Nigeria.

Study Design: Samples were collected from two sites; the study and control sites. At each site, a plot was chosen with a variety of tuber crops. *Manihot esculenta*, *Colocasia esculenta*, *Dioscorea rotundata* and *Dioscorea dumentorum*.

Methodology: Concentrations of 16 priority PAHs were measured using gas chromatography-Mass spectrometry. Health risk assessment was carried out using the toxicity equivalency factor method.

Results: Results revealed detectable levels of the 16 PAHs in soil and plant samples, with concentrations ranging from 19.8 µg/kg to 80.7 µg/kg. PAH levels in tuber crops were *Manihot esculenta* (12.3-74.8 µg/kg); *Colocasia esculenta* (26.2-110.4 µg/kg); *Dioscorea rotundata* (21.5-166.4 µg/kg) and *Dioscorea dumentorum* (19.3-80.7 µg/kg). Higher molecular weight PAHs were dominant in soils with a 4 ring > 3 ring > 5 ring > 6 ring > 2 ring gradient. Lower molecular weight

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PAHs were the most abundant in the crop samples, accounting for 56.8% to 69% of plant PAHs. Results show that combustion of liquid fuel was the primary source of PAHs. BaP_{eq} values calculated for soils and plants were higher than the allowable target values for soils and vegetables. **Conclusion:** The study revealed the potential for carcinogenic risks associated with soils and some food crops obtained from Ikot Oborenyin, Nigeria, consequently, remediation of the soils is suggested to prevent adverse effects.

Keywords: Polycyclic aromatic hydrocarbons; health risk assessment; soils; tuber crops; toxicity equivalency factor; Ikot Oborenyin.

1. INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) constitute a class of hazardous organic chemicals with three or more fused benzene rings in linear, angular and cluster arrangements [1]. They are resistant to degradation, can remain in the environment for long periods, and have the potential to cause adverse environmental effects. PAHs are mainly of anthropogenic origin and have no significant natural sources [2]. Some of them are susceptible to dispersion on a global scale because they move between the atmosphere and the earth's surface in repeated, temperature-driven cycles of deposition and volatilization. PAHs are truly multimedia contaminants which occur in all parts of the environment: Atmosphere, inland and sea waters, sediments, soils and vegetation.

PAHs in the environment are found primarily in soil, sediment and oily substances, as opposed to water or air [3]. They are introduced into soil from atmospheric deposition after local and long-range transport, which is supported by the presence of PAHs in soil of regions remote from any industrial activity [4].

Trace levels of PAHs are widely occurring in modern ecosystems. Since some PAH members are mutagenic, their presence in plant, is of increasing concern because PAHs may ultimately be transferred to food. Moreover, the presence of PAHs in soils has been correlated with highway traffic and cancer incidence [5,6]. The general population may be exposed to these compounds by inhalation of the compounds in tobacco smoke and contaminated air, as well as ingestion of contaminated food. Populations living in the vicinity of hazardous waste sites may be at greater risk of potential exposure to PAHs than the general population through inhalation, ingestion and direct contact with contaminated media [7].

Toxicity Equivalent Factor (TEF) method of risk assessment is used to evaluate the toxicity and assess the risks associated with a given compound. TEF is an estimate of the relative toxicity of a chemical compound to a reference chemical [8]. For a mixture of PAHs, the reference chemical is Benzo(a)pyrene (BaP). BaP is chosen because the toxicity of the chemical is well characterized. The toxicity equivalent factor of each PAH is an estimate of the relative toxicity of the PAH compound compared to BAP [9].

Several studies investigating uptake of PAH by plants in Nigeria and elsewhere have been reported [10,11,12,13], however no investigations had been conducted on the concentrations of PAH in soils and tuber crops of Ikot Oborenyin, Akwa Ibom state, Nigeria, and the health risks associated with them. This constitutes the aim of this Study. The study focused on the composition and pollution levels of PAH in arable soils of Ikot Oborenyin, Ikot-Abasi, Nigeria. The toxicity of PAHs on human health and the ecosystem were also investigated.

2. METHODOLOGY

2.1 Study Area and Sample Collection

The study was conducted in 2013 at Ikot Oboroenyin, a farming settlement in Edemeya, Ikot Abasi local government area of Akwa Ibom State, Nigeria. The major occupation of natives are fishing, farming and trading. Food crops cultivated in the area are sold at several markets across the state. The area which lies between latitude 4°36'39"N and longitude 7°35'17"E was used as a base for small scale crude oil refining. Agricultural farmland adjoining the oil-refining base were selected for the study. Reference farmlands which served as controls were located far away from the base. Fig. 1 is a map showing the study area with sampling sites.

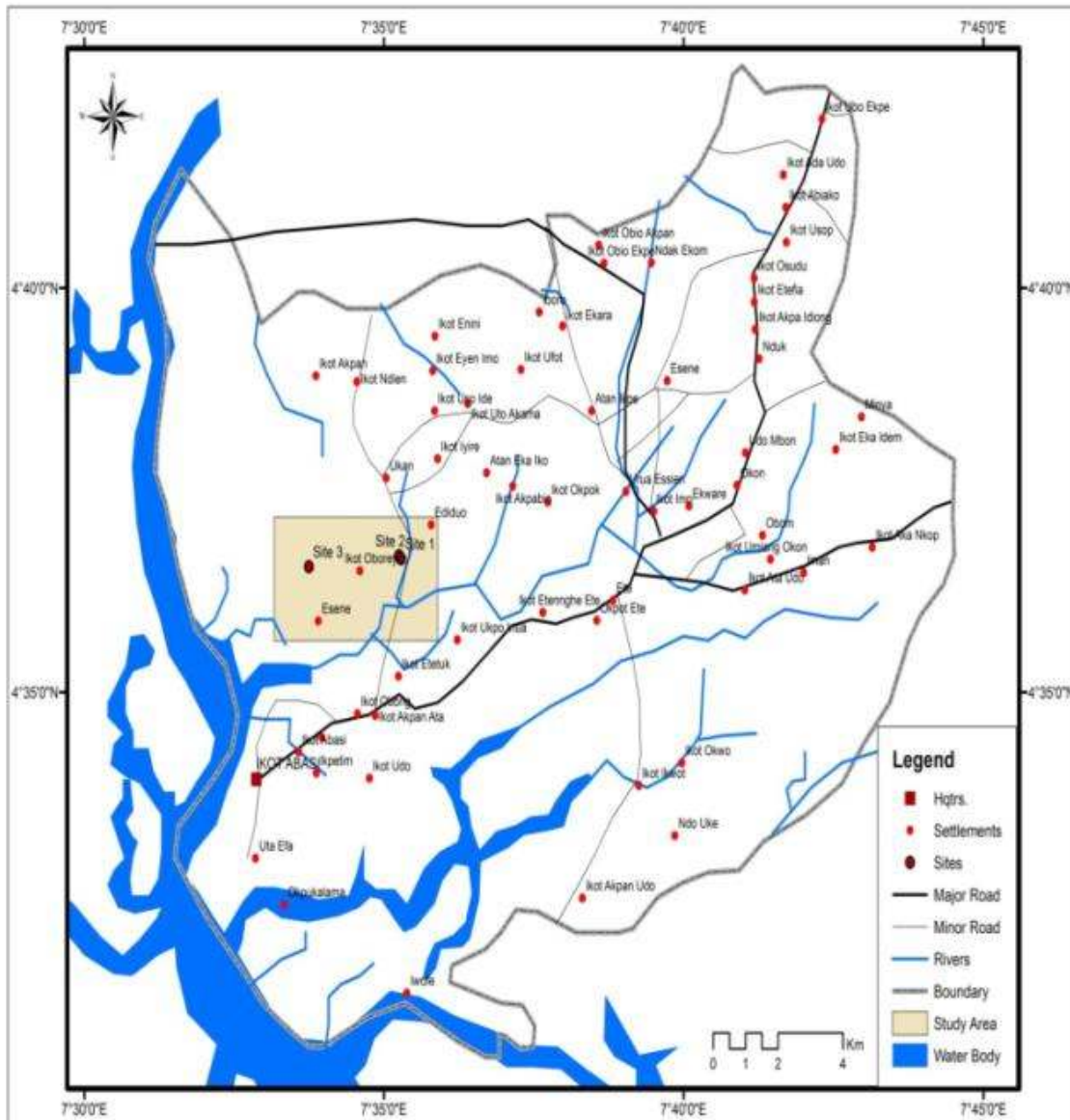


Fig. 1. Map of study area showing the sampling sites

2.2 Collection and Preservation of Samples

2.2.1 Soil samples

Soils were sampled as described by [14]. The samples were collected from two sites. The study site is close to the crude oil polluted site (less than 0.4 km) while the control site is located in a less contaminated area, approximately 4 km from the crude oil site. At each site a plot was chosen with a variety of tuber crops. About 800 g of fresh soil samples were collected at a depth of 0-20 cm. Samples were collected from four

locations (200 g each) at each site and thoroughly mixed as a composite bulk soil sample.

2.2.2 Tuber crops

Four root crops including *Manihot esculenta*, *Colocasia esculenta*, *Dioscorea rotundata* and *Dioscorea dumentorum* were sampled from each of the two sites. For each type of crop, several stands were collected as sub samples and transported to the laboratory for further treatment. At the laboratory the vegetable samples were thoroughly washed with running

water to remove dirt, chopped into pieces and dried to a free flow texture with anhydrous sodium sulphate.

2.3 Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Food Crops and Soil Samples

Extraction of samples were done as described by [15]. The samples were extracted with a sonicator via the addition of methylene chloride and acetone. The extract was diluted with methylene chloride and concentrated. Four (4) ml of cyclohexane was added to the concentrated extract and adjusted to about 2 ml. Silica gel was pre-activated by heating for 2 hours at 130°C. Slurry of 10 g of activated silica gel was prepared in methylene chloride and placed into chromatographic column. The column was tapped gently to settle the silica gel and elute the methylene chloride. About 2 cm of anhydrous sodium sulphate was added to the top of the silica bed. The column was pre-eluted with 40 ml pentane and the eluate discarded. Before sodium sulphate layer was exposed to air, 2 ml cyclohexane extract was transferred into the column, using an additional 2 ml cyclohexane to complete the transfer. About 25 ml of pentane was added with continuous elution of the column and the fraction discarded. Twenty-five (25) ml of a mixture of 40% methylene chloride and 60 ml pentane (ratio of 2:3) was added and the fraction was collected and extracted to 1 ml.

2.4 Chromatographic Conditions

The analysis was carried out using Agilent 6890 GC coupled with Agilent 5973 mass spectrometer. The mass spectrometer was operated with an electron impact ionization of 70 eV, an electron multiplier voltage of 1288 V, and an ion source of 230°C. The Column system was 30 m in length with a 32 mm bore diameter and a 0.25 film diameter. The Oven temperature was set at an initial temperature of 65°C and was allowed to run for 30 min. to reach a final temperature of 235°C. The Inlet temperature was 275°C, with a pressure of 14.8 Psi and a total flow rate of 2 ml/min.

2.5 Preparation of Polyaromatic Hydrocarbon Standard Mixture

About 5, 10, 20, 30, 40 and 50 µl of 1 mg/ml PAH stocks standard solution were added into separate vials. About 10, 20, 40, 60, 80 and 100

µl of 500 µg/ml surrogate standard (pyrene d₁₀) were added in each vial and make up the final volume to 1.0 ml with methylene chloride. The concentrations of PAH standards were 5, 10, 20, 30, 40 and 50 µg/ml or mg/L respectively. The concentrations of the surrogate standards in the PAH standard were 5, 10, 20, 30, 40 and 50 µg/ml or mg/L respectively.

2.6 Preparation of Surrogate QC Standard

Two 2 ml GC vial was rinsed with dichloromethane methylene chloride was used to rinse micro-syringe; 60 µl of 500 µg/ml surrogate standard (pyrene d₁₀) was measured into the vial. The final volume was made up to 1.0 ml mark with methylene chloride. This gave a concentration of 30 µg/ml.

2.7 Quality Control

Samples, blanks and spiked blanks were analysed with no interferences detected. Recovery efficiency was analysed with samples spiked with known amounts of PAH standards. Recovery of 16 PAHs ranged from 82% to 95% in the samples.

2.8 Health Risk Assessment

Health risk associated with the PAHs in food and soils were evaluated using the toxicity equivalency factor (TEF) method [8]. TEF for each PAH was an estimate of the relative toxicity of the PAH compounds compared to BaP. The total equivalent concentration was expressed as BaP equivalent (BaP_{eq}) BaP_{eq} for individual PAH was estimated using the equation

$$BaP_{eq} = \sum C_n \times TEF_n$$

where; C_n is the concentration of individual PAH sample, and TEF_n is the toxic equivalency factor of the individual PAH in the sample matrix.

2.9 Statistical Analyses

Data collected were subjected to statistical tests of significance using Students t-test at p = 0.05 to assess pairs results in the test and the control soils. Correlation analyses were performed to determine the relationship between pairs of PAHs in soil and plants at p = 0.05. All statistical analyses were done by SPSS software for windows.

3. RESULTS AND DISCUSSION

3.1 PAHs Contents of the Soils

The concentration of the 16 PAHs of test and control soils are shown in Fig. 2 as mean values of six samples. There were detectable and measurable amounts of Naphthalene (Nap), Acenaphthylene (Acy), Acenaphthene (Ace), Fluorene (Flu), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Fluo), Pyrene (Pyr), Benzo(a)anthracene (BaA), Chrysene (Chr), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Dibenzo(a,h)anthracene (DBA), Benzo(g,h,i)perylene (BghiP) and Indeno(1,2,3-d) pyrene (IND). The concentration of PAH ranged from 19.8 µg/kg to 80.7 µg/kg with a mean value of 47.9 µg/kg, in the test soils, while the values in the control soils were between 14.8 µg/kg and 53.2 µg/kg. Total PAH for test and control soils were 766.5 µg/kg and 319.2 µg/kg respectively. The individual PAHs were higher ($p < 0.05$) in the test soils than the control. The amount of PAHs were influenced by the site. Soils from the test site showed higher concentration because of contamination from nearby oil refining activities. The test site was situated close to a small scale oil refining base while the control was devoid of such activities. Therefore the source of PAHs in the area was due to the inputs oil combustion from the oil base.

3.2 Relative Proportion of PAHs

The relative proportion of individual PAHs in the soil was used to determine the possible sources (Fig. 3). Specifically Chr, Pyr, Flua, and BbF were the dominant components of the soil, with the order Chr>Pyr>Flu>BbF. Similar results were reported concerning soil samples from Liaohu [16], China [6] and Tiangn [14]. The trend of PAH was however different from that observed by [17]. The higher molecular weight (HMW) PAHs appeared dominant in the soils with 4 to 6 ring PAHs having percentage values of 65.7% while the lower molecular weight (LMW) PAHs with 2 to 3 ring had percentage values of 34.3%. Specifically, the four ring PAHs (36 %) were the main contributors to the total PAHs with a 4 ring> 3 ring>5 ring >6 ring> 2 ring gradient. The result was consistent with the typical pattern of relative concentrations of PAHs observed in soils from other polluted areas [18,19].

PAH diagnostic ratio is used as a tool for identifying and assessing pollution sources

[20,21]. The diagnostic ratios used in the present study were BaA / BaA + Chr and Flua / Flua + Pyr ratios. BaA / BaA + Chr ratio of 0.2 to 0.35 indicate mixed petrogenic and pyrogenic origin and > 0.35 indicate pyrogenic origin [22]. Values of Flua /Flua + Pyr ratios is used to distinguish between different combustion origins such as burning of liquid fossil fuels or coal wood or grass [6]. BaA/BaA+Chr ratio was 0.37 revealing that combustion was the primary source of PAHs [6]. To identify the specific combustion source Flua /Flua+Pyr ratio was calculated. The value was 0.49 (between 0.4 and 0.5) which pointed to combustion of liquid fossil fuel [6]. The results from the present study were similar to those observed by Jiao et al. [6] who reported that PAHs from the soils of northern Bohai and Yellow Seas China were due to automobile fuel consumption. The total PAHs for the test site was 766.5 µg/kg. This value far exceeded the recommended limits of 200 µg/kg prescribed for agricultural soils [7]. In comparison with other studies, the values were lower than those reported by Lang et al. [16] and Mielke et al. [23], with respective values between 675.4 to 1001.9 µg/kg and 647 to 40692 µg/kg, but higher than those reported by Zhao et al. [24] with values between 146 and 645 µg/kg. Generally the soils from Ikot Oborenjin were contaminated with PAH at levels above background values.

3.3 PAH Levels in Food Crops

The concentration of PAH in the food crops are shown in Fig. 4 (*Manihot esculenta*), Fig. 5 (*Colocasia esculenta*) Fig. 6 (*Dioscorea rotundata*) and Fig. 7 (*Dioscorea dumentorum*). All the PAHs were of measurable levels in the examined samples. For *Manihot esculenta*, the levels were between 12.3 and 74.8 µg/kg; for *Colocasia esculenta* the levels were between (26.2 and 110.4 µg/kg). Levels for *Dioscorea rotundata* and *Dioscorea dumentorum* were 21.5-166.4 µg/kg and 19.3-80.7 µg/kg respectively. The order of total PAHs were *Dioscorea rotundata*> *Colocasia esculenta*> *Dioscorea dumentorum* > *Manihot esculenta*. Naphthalene was the most abundant PAH in the crops with respective values of 74.8, 110.4, 166.4 and 80.7 for *Manihot esculenta*, *Colocasia esculenta*, *Dioscorea rotundata* and *Dioscorea dumentorum*. Total PAHs were 588.6 for *Manihot esculenta*, 873.2 for *Colocasia esculenta*, 932 for *Dioscorea rotundata* and 738.5 for *Dioscorea dumentorum*. The amount of PAH in the food crops depended on the growing site; concentrations were higher ($p < 0.05$) in the test

site than the controls, showing that the values were influenced by contamination. The results obtained in the present study were higher than those observed from vegetables grown in Tianjin, China, where the values ranged between 0.28 and 0.69 $\mu\text{g/g}$ [13] and Greece with values ranging between 0,025 and 0.29 $\mu\text{g/g}$ dry weight [11]. LMW PAHs of between 2-3 rings were the most abundant in the crop samples, accounting for 56.8% to 69% of plant PAHs. The PAH concentrations in the plants were generally higher than the concentrations in the soil.

Analysis of total PAH concentration in soil and plant samples showed low correlations (Fig. 8) which was suggestive of the fact that the crops could be absorbing PAHs from the air [25] and atmospheric deposition could have been more significant than root accumulation. It had been reported that the dominant transfer pathway of PAHs from environment to plants is by foliar uptake [14], therefore it is highly probable that atmospheric deposition rather than root up-take may be the mechanism of uptake of PAH by plants in this study.

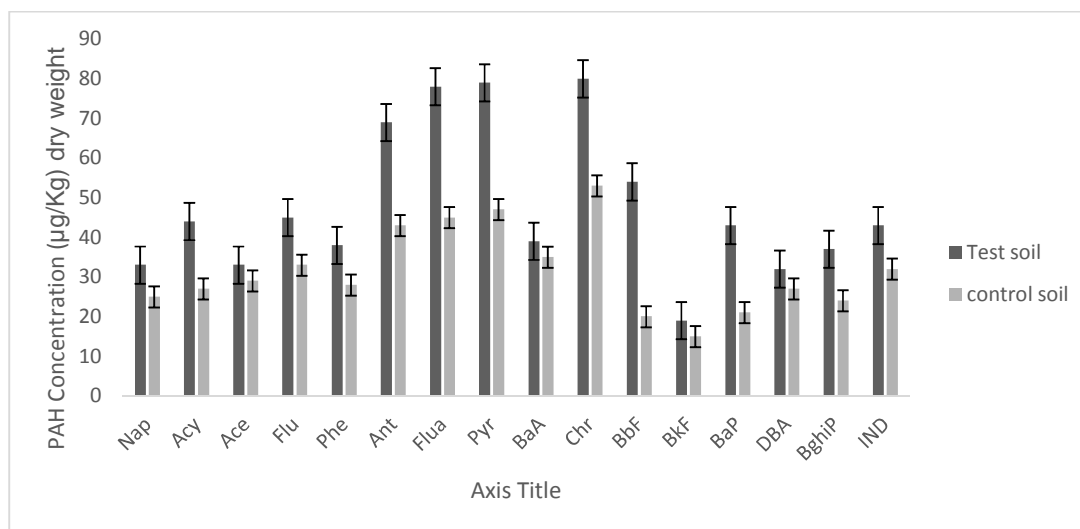


Fig. 2. PAHs concentration of the soils from Ikot Oboroenyin, IkotAbasi L.G.A

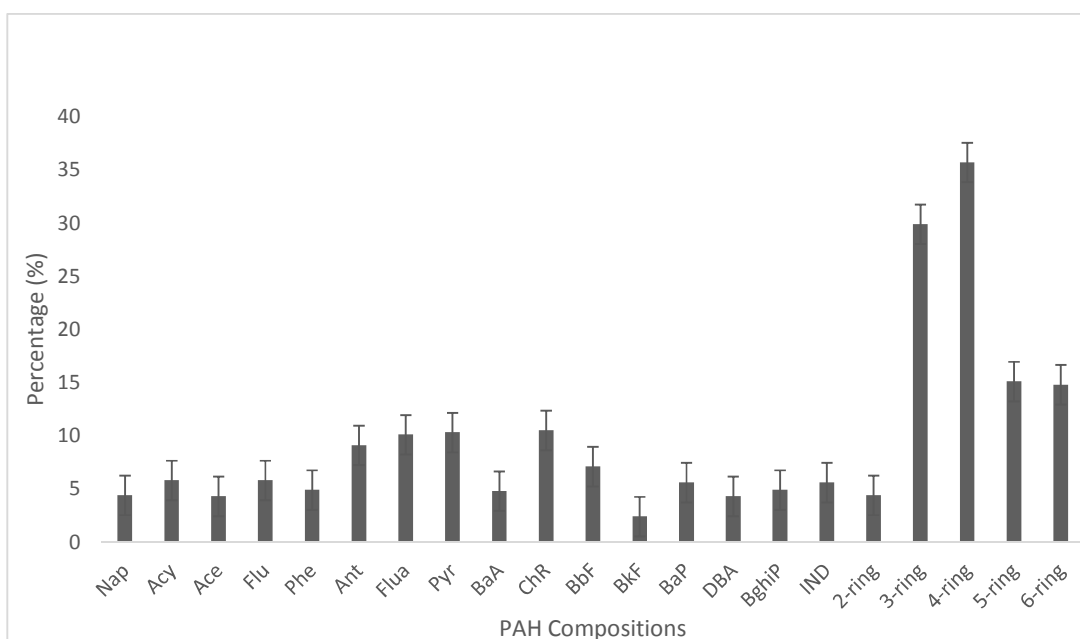


Fig. 3. Relative proportions of PAH components in soils

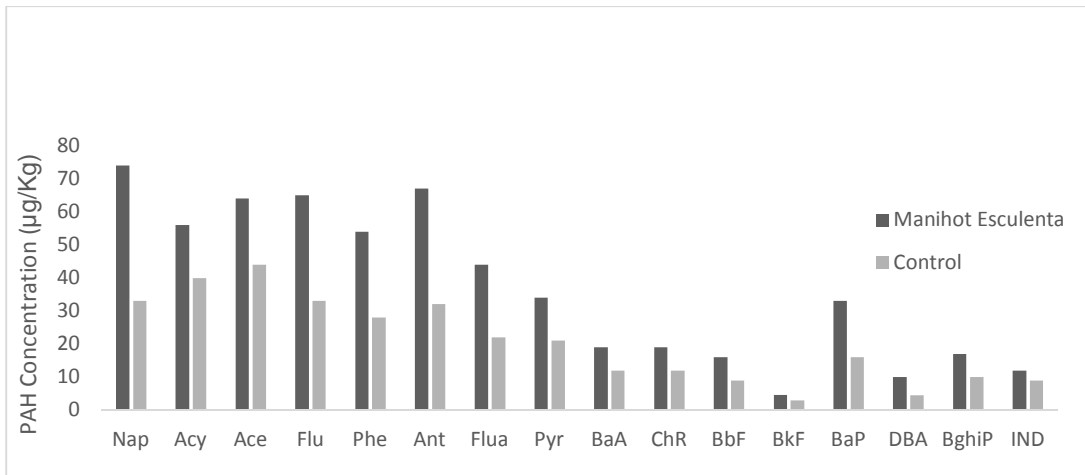


Fig. 4. Concentration of PAH in *Manihot esculenta*

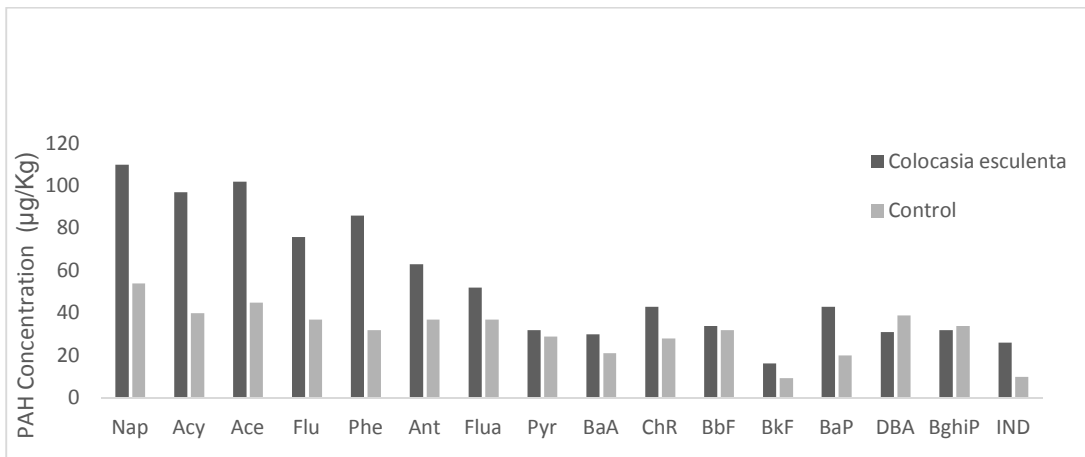


Fig. 5. Concentration of PAH in *Colocassia esculenta*

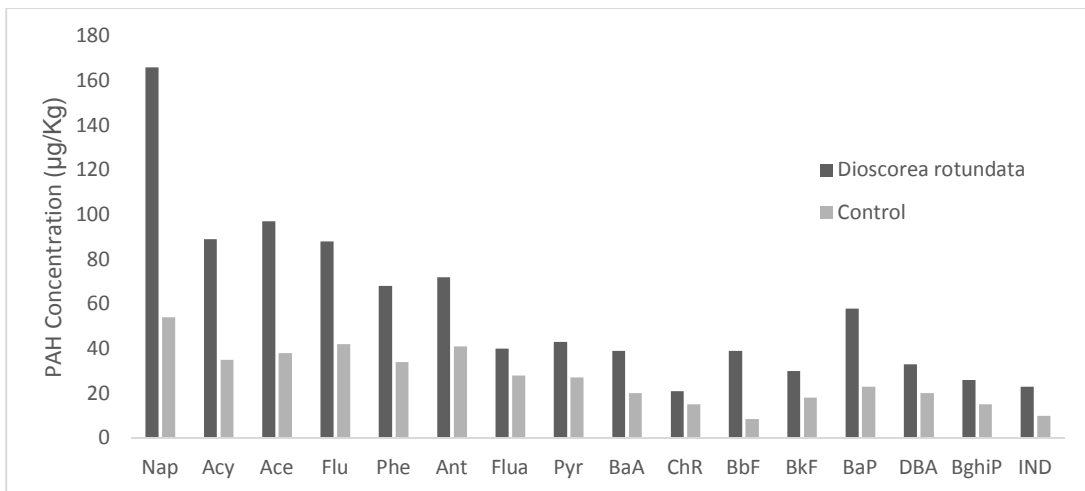


Fig. 6. Concentration of PAH in *Dioscorea rotundata*

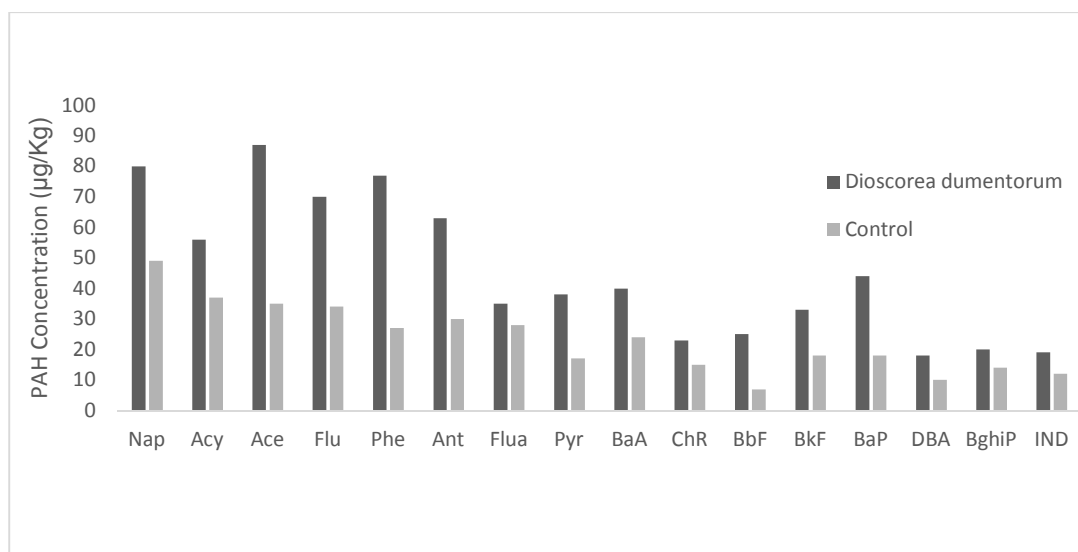


Fig. 7. Concentration of PAH in *Dioscorea dumentorum*

The lower molecular weight dominance in all the root crops were different from the PAH patterns in the soils, where the higher molecular weight PAHS were more dominant. Similar trends were observed in other studies [10,11,26]. The observed trend may be due to their greater water solubility, bioavailability and volatility [14] as a result, they are taken up more readily by the plant roots.

The correlation matrix of PAHs crops and soils is shown in Table 1. Correlation studies are used to investigate chemicals originating from similar sources [6]. There were statistically significant ($\alpha = 0.05$) Pearson's correlations between LMW PAH pairs such as; Acy/Nap ($r = 0.71$); Ace/Acy ($r = 0.73$); Flu/Nap ($r = 0.93$); Flu/Ace ($r = 0.86$) and Phe/Ace ($r = 0.88$) except for Ant. Generally there were negligible correlations between LMW and HMW PAH pairs such as Nap/BbF ($r = 0.02$); BbF/ Acy ($r = 0.001$); DBA/Acy ($r = 0.01$) IND/Ant ($r = 0.06$) and BghiP/ Acy ($r = 0.014$) amongst other PAH pairs. There were also statistically significant ($\alpha = 0.05$) Pearson's correlation between HMW PAH pairs such as IND/BbF ($r = 0.91$); IND/BghiP ($r = 0.88$); DBA/BbF ($r = 0.74$) and BghiP/BbF ($r = 0.84$) amongst other HMW PAH pairs. The results show that the LMW PAHs originated from similar sources which were different from sources of HMW PAHs [6]. This results also further corroborates the earlier assertion that the LMW PAHs had a different source of uptake by the plants, from the HMW PAHs. Generally, soils and root tubers in Ikot Oborenyin had detectable levels of PAHs

measured. The levels of PAHs in test soils were higher than control soils. By comparison, the concentrations of PAHs in the crops were higher than those in the soils. Results indicate different sources of PAH inputs for plants and soils.

3.4 Risk Assessment

Risk assessment was done to assess the risk associated with the PAHs. TEF values for the various PAHs had been calculated by USEPA, 2000 and the values are shown in Table 2. BaPeq which estimates the toxicity of PAHs were calculated by multiplying the concentrations of individual PAHs and summing up the values – The total BaPeq for the various plants are shown in Table 2. Bapeq values calculated for soils was 91.9 $\mu\text{g}/\text{kg}$ -BaPeq. In comparison with other studies the BaPeq were lower than India (650 $\mu\text{g}/\text{kg}$ BaPeq) [27], Terragona Spain (124 $\mu\text{g}/\text{kg}$ BaPeq) [28] and Shanghai, China (892 $\mu\text{g}/\text{kg}$ BaPeq) [29] The BaPeq values were however higher than the Dutch target value of 32.96 $\mu\text{g}/\text{kg}$. This indicated the potential for carcinogenic risks associated with the soils. BaPeq were also calculated for the crops studied. BaPeq were; 49.96 $\mu\text{g}/\text{kg}$ -BaPeq, 78.48 $\mu\text{g}/\text{kg}$ -BaPeq, 105 $\mu\text{g}/\text{kg}$ -BaP, and 75.2 $\mu\text{g}/\text{kg}$ -BaPeq for *Manihot esculenta*, *Colocasia esculenta*, *Dioscorea rotundata* and *Dioscorea dumentorum*, respectively. These values were all higher than the national limiting pollutants for BaP of 10 $\mu\text{g}/\text{kg}$, indicating possible risk associated with the consumption of the food crops [30].

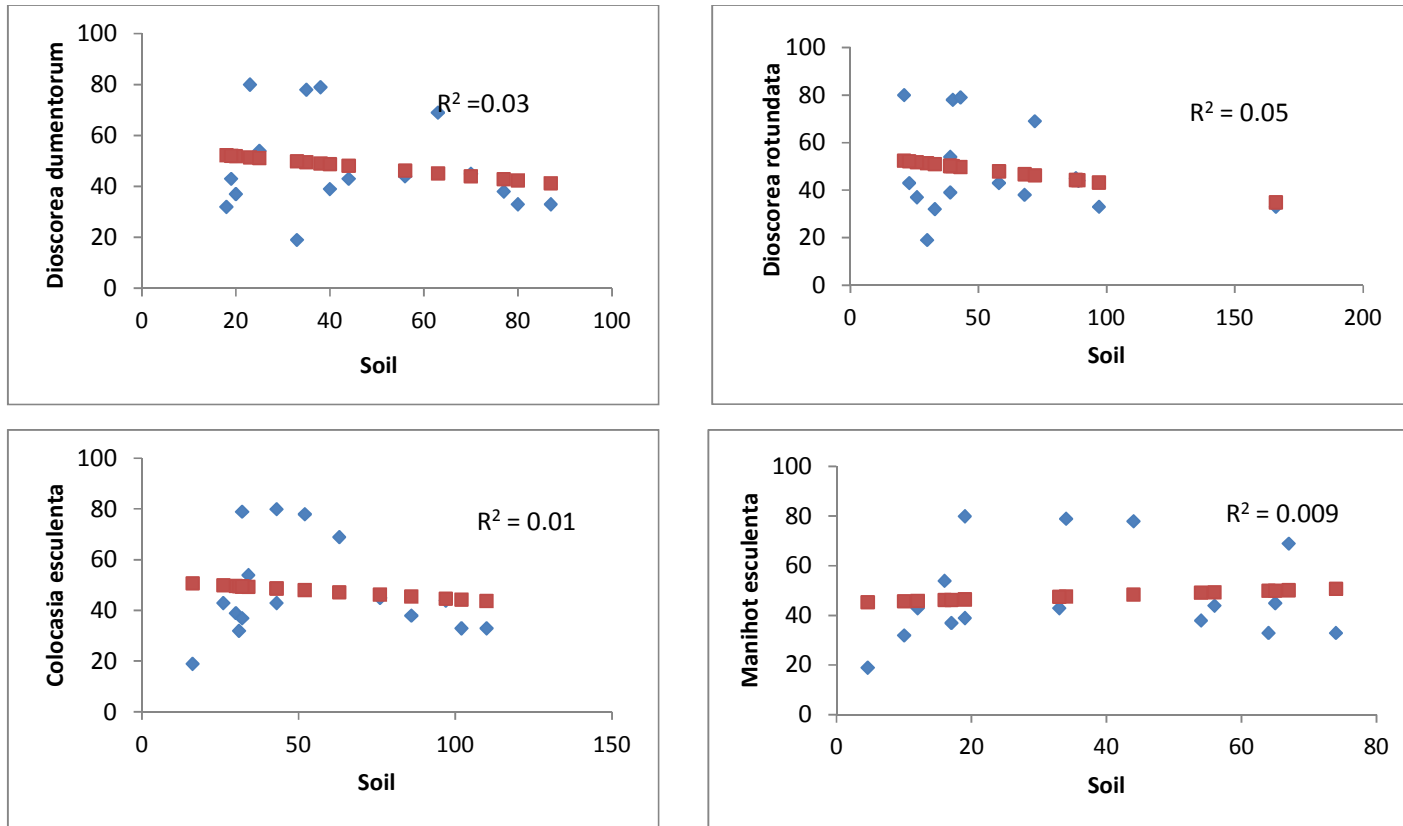


Fig. 8. Correlations plots of total PAH levels in soils and food crops of Ikot Oborenyin, Nigeria

Table 1. Correlation matrix of PAHs crops and soils collected from Ikot Oborenyin

	Nap	Acy	Ace	Flu	Phe	Ant	Flua	Pyr	BaA	Chr	BbF	BkF	BaP	DBA	BghiP	IND
Nap	1															
Acy	0.71	1														
Ace	0.68	0.723	1													
Flu	0.93	0.685	0.863	1												
Phe	0.36	0.553	0.888	0.569	1											
Ant	0.09	0.001	0.083	0.003	0.334	1										
Flua	0.38	0.133	0.57	0.611	0.444	0.047	1									
Pyr	0.3	0.323	0.66	0.548	0.621	0.209	0.724	1								
BaA	0.01	0.001	0.001	0.001	0.001	0.035	0.017	0.231	1							
ChR	0.38	0.115	0.451	0.572	0.288	0.009	0.939	0.734	0.102	1						
BbF	0.02	0.001	0.151	0.125	0.158	0.2	0.594	0.698	0.428	0.686	1					
BkF	0.14	0.02	0.137	0.132	0.122	0.004	0.094	0.006	0.813	0.012	0.084	1				
BaP	0.52	0.259	0.19	0.334	0.062	0.268	0.029	0.01	0.53	0.006	0.23	0.59	1			
DBA	0.1	0.212	0.011	0.014	0.001	0.129	0.214	0.202	0.396	0.296	0.74	0.16	0.517	1		
BghiP	0.04	0.014	0.086	0.133	0.052	0.03	0.687	0.465	0.193	0.783	0.84	4E-04	0.07	0.73	1	
IND	0.15	0.041	0.283	0.325	0.208	0.064	0.785	0.77	0.315	0.9	0.91	0.02	0.054	0.55	0.878	1

Pearson's correlation coefficient are shown at $\alpha = 0.05$ (two tailed). r values $+0.70$ indicates very strong positive correlations; 0.40 to $+0.69$ indicates strong positive correlations; 0.30 to 0.39 indicated moderate positive correlations and 0.01 to 0.19 indicates negligible correlation

Table 2. Toxicity Equivalency Factors (TEF) and BaP equivalent (BaP_{eq}) for individual PAHs in food crops and soils

	TEF (USEPA)	<i>M. esculenta</i>	<i>C. esculenta</i>	<i>D. domenturum</i>	<i>D. rotundata</i>	Soil
Nap	0.001	0.074	0.11	0.166	0.08	0.033
Acy	0.001	0.056	0.097	0.089	0.056	0.044
Ace	0.001	0.064	0.1	0.097	0.087	0.033
Flu	0.001	0.065	0.076	0.088	0.07	0.045
Phe	0.001	0.054	0.086	0.063	0.077	0.038
Ant	0.01	0.67	0.63	0.72	0.63	0.69
Flua	0.001	0.044	0.052	0.04	0.035	0.078
Pyr	0.001	0.034	0.032	0.043	0.038	0.079
BaA	0.1	1.9	3	3.9	4	3.9
ChR	0.001	0.019	0.043	0.021	0.023	0.08
BbF	0.1	1.6	3.4	3.9	2.5	5.4
BkF	0.1	0.46	1.62	3	3.3	1.9
BaP	1	33	43	58	44	43
DBA	1	10	31	33	18	32
BghiP	0.01	0.17	0.32	0.26	0.2	0.37
IND	0.1	1.2	2.6	2.3	1.9	4.3
BaP _{eq}		49.96	78.48	105.68	75.2	91.9
Σ16 PAHS		588.6	873.5	932.8	728.2	766.5

4. CONCLUSION

The levels of PAHs in soils exceeded the recommended ERL guidelines, Analysis of origin of PAH suggested the source to be combustion of liquid fuel. The high carcinogenic potency of PAH in soils is an indication of a high risk associated with samples. Consequently, remediation of the soils is suggested to prevent adverse effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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