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Comparative Profiling and Bioaccumulation of PAHs in Soil and *Talinum triangulare* Harvested from Charcoal Production Areas in Southern Nigeria

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ABSTRACT: This study investigated the comparative profiling and bioaccumulation of PAHs in soil and *Talinum triangulare* harvested from charcoal production areas in Southern Nigeria. *T. triangulare* was cultivated in three sites (1, 2 and 3) in a charcoal production environment for seven weeks. Plant and soil samples were collected and prepared for PAHs analysis using GC–MS. Results showed that PAHs were most abundant in the soil of site 1. The 3–rings PAHs were predominant in sites 1 and 2. Contamination classification of PAHs showed that all three experimental sites were heavily polluted with PAHs. The results also indicated that sites 1 and 2 had higher percentage of Low molecular weight PAHs (LMW PAHs) while heavy molecular weight PAHs (HMW PAHs) were higher in site 3. The ratios of LMW PAHs / HMW PAHs and that of fluoranthene / pyrene in all experimental sites denoted petrogenic origin of PAHs. However, values obtained for phenanthrene / anthracene indicated pyrolytic origin of PAHs. The proportion of the seven carcinogenic PAHs was similar in sites 1, 2 and 3 while the biological concentration factor showed the naphthalene and fluoranthene were concentrated by roots of *Talinum triangulare* in site 2 while Indenol[1,2,3-cd] pyrene in site 3.

Keywords: Polycyclic aromatic hydrocarbon (PAHs), *Talinum triangualare*, Pollution, Biological accumulation, Charcoal.

Introduction

Environmental pollution has been on the increase over the past decades due to emission of large number of pollutants into the environment by energy consumption of human activities. Polycyclic aromatic hydrocarbons (PAHs) are chemical pollutants of environmental importance (Nasr *et al*., 2010). They are class of ubiquitous environmental organic pollutants that are produced via partial combustion and pyrolysis of organic matter, or during the preparations of food products (Guo *et al*., 2013; Ruipeng *et al*., 2018). The 16 priority PAHs are sub – divided into three groups: Low molecular weight PAHs (LMW PAHs –Nap, Acy, Flu, Phe and Ant); Medium molecular weight PAHs (MMW PAHs – Flt, Pyr, BaA and Chr) and High molecular weight PAHs (HMW PAHs – BbF, BkF, BaP, Ind, DahA and BghiP) (Kim *et al*., 2013; Rongjie *et al*., 2020). Polycyclic aromatic hydrocarbon are hydrophobic organic pollutants (Osioma and Iniaghe, 2022) and are known to be mutagens, genotoxic and carcinogenic (Davi *et al*., 2016).

Charcoal is produced by heating wood in an airtight ovens (Chidumayo and Gumbo, 2013) and its usage has been linked to one of the principal sources of PAHs in soil (Xu *et al*., 2006; Zhang and Chen, 2017; Rongjie *et al*., 2020); and soil exposed to PAHs such as that used for the production of charcoal could be of public health concern and food safety. The presence of PAHs in different food products including grilled plantain, fish and vegetables was reported by Patel *et al*. (2020) and Tango *et al*. (2017).

Talinum triangulare (family: *Portulacaeae*) commonly called water leaf is cultivated and mainly consumed in the South-South areas of Nigeria (Catherine *et al*., 2017). Water leaf is recognised as a crop that is important for

its palatable and nutrient values. Its protective effect against cancer (Santhakumar *et al*., 2018), asthma (Billa *et al*., 2017), phytoextraction potential (Osioma *et al*., 2018) and heavy metal accumulating capacity (Oguh *et al*., 2019) have been reported. However, the bioaccumulation of PAHs by *Talinum triangulare* has not been investigated. Hence, this study was undertaken to comparatively profile and evaluate the bioaccumulation of PAHs in *Talinum triangulare* cultivated in charcoal production environment.

Materials and methods

Study area: The study area is located in Amukpe, Delta State. The site is used by Natives for charcoal production through pyrolysis for commercial purposes.
Cultivation of Talinum triangulare: *Talinum triangulare* was cultivated in three different sites (80 meters) apart around the charcoal production sites. The sites were designated as sites 1, 2 and 3. *Talinum triangulare* was cultivated for a period of seven weeks before harvest.
Collection of soil sample: Soil samples were collected from three sites around the charcoal production area. Three (3) soil samples were collected from each sampling site at depth of 0-15 cm using a soil auger and at each sampling point, approximately 0.50 kg of soil was collected, packed in pre-cleaned and well labelled polyethylene bags and taken to the laboratory where they were air-dried, mechanically ground with agate mortar and pestle and sieved with a 2 mm mesh size.
Collection of plant sample: *Talinum triangulare* (water leaf) was also collected from the various soil sampling sites using a stainless steel sampler (this helps in uprooting the plant from the soil) and was authenticated in the Herbarium unit (No. UPH/P/136), Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State Nigeria. The plant samples were also packed in pre-cleaned and well-labelled polyethylene bags and taken to the laboratory where they were washed with tap water and distilled water (to remove any adhered soil). They were then separated into the shoots and roots, dried in an electro-thermal oven at 105 °C, pulverized, and stored in a desiccator. The dried samples were size-reduced using agate mortar and pestle and sieved with 2 mm mesh size.
Samples extraction and analysis of PAHs: The sample extraction and analysis of PAHs was carried out as previously described by Tesi *et al*. (2022). Briefly, 10 g of the samples were weighed into an extraction thimble, and extraction was carried out for 10 h using hexane/dichloromethane mixture. The extract was concentrated using a rotary evaporator, cleaned-up on a column packed with acidified silica gel, Florisil, anhydrous Na_2SO_4 and copper powder. Elution was carried out with 40 mL of hexane/dichloromethane. The eluent was collected and evaporated to near dryness under a stream of nitrogen gas and kept in a vial for chemical analysis.
Instrumental analysis: The PAHs concentrations in the samples were quantified using Agilent 6890 with 5973 MSD GC–MS (Agilent Technologies, Wellington, DE, USA). The GC had a 30 m length DB-5 capaillary column with 0.25 µm film thickness (J & W Scientific, Folsom, CA). The mobile phase was ultrapure helium gas with linear velocity of 1 mL min^{-1}. The splitless mode was adopted for injection of 2.0 µL of the sample. The initial column temperature was fixed at 85 ºC with3 min held on time, subsequently stepped up to 200 ºC at 35 ºC min^{-1}. The injector, quadrupole and mass detector source were programmed at temperature of 250 ºC, 150 ºC and 230 ºC respectively.
Evaluation of biological accumulation coefficient: The biological accumulation coefficient (BAC) was calculated as a ratio of the concentration of PAHs level in shoots to that in soil (Yoon *et al.*, 2006):

$$BAC = \frac{[PAHs]_{shoot}}{[PAHs_{soil}}$$

Evaluation of biological concentration factor: The biological concentration factor (BCF) was calculated as a ratio of the concentration of PAHs level in plant roots to soil (Salah and Barrington, 2006):

$$BCF = \frac{[PAHs]_{root}}{[PAHs_{soil}}$$

Ratios for PAHs profiling: The following ratios were employed in the interpretation of source evaluation of PAHs.

- Phenanthrene / anthracene (values < 10 indicates pyrolytic source while values > 10 indicates petrogenic source (Qiu *et al*., 2009)
- Fluoranthene / pyrene (values < 1 indicates petrogenic origin while values > 1 indicates pyrolytic origin) (Qiu *et al*., 2009)
- LMW PAHs / HMW PAHs (values < 1 denotes combustion of fossil fuels or woods while values > 1 denotes petrogenic source of PAHs. (Yakan *et al*., 2017)
- 7 carcinogenic PAHs are BaA, Chr, BbF,BkF, BaP, IndP and DahA

Statistical analysis: All samples were prepared in triplicate determinations and results are expressed as mean ± standard deviation**.** Simple percentage and ratios were used in profiling PAHs phytoremediation quotients.

Results

The results obtained from the experiment are presented below:

Table 1: Concentration (mg kg^{-1}) of PAHs in soil samples from experimental sites

PAHs	Number of rings	Site 1	Site 2	Site 3
Naphthalene	2	0.00	0.03	0.176
Acenaphthylene	3	1.92	0.064	0.00
Acenaphthene	3	1.94	1.422	0.906
Fluorene	3	14.65	0.492	1.202
Phenanthrene	3	16.62	0.318	0.00
Antthracene	3	0.00	0.194	0.00
Fluoranthene	4	2.65	0.008	0.456
Pyrene	4	5.17	0.402	0.926
Chrysene	4	4.09	0.214	0.156
Benzo[a]anthracene	4	0.71	0.006	0.100
Benzo[b]fluoranthene	5	4.98	0.068	0.488
Benzo[k]fluoranthene	5	4.86	0.184	0.476
Benzo[a]pyrene	5	1.56	0.114	0.244
Indenol[1,2,3-c,d]pyrene	6	0.00	0.142	0.062
Dibenzo[a,h]anthracene	5	0.00	0.104	0.00
Benzo[g,h,i]pyrene	6	0.43	0.00	0.222
Σ16 PAHs		**59.58**	**3.762**	**5.414**
Σ2- rings		**0.00**	**0.064**	**0.176**
Σ3- rings		**35.13**	**2.49**	**2.11**
Σ4- rings		**12.62**	**0.63**	**1.64**
Σ5- rings		**11.4**	**0.47**	**1.21**
Σ6 – rings		**0.43**	**0.142**	**0.284**

Results in Table 1 shows the concentration of 16 detected PAH in soil samples from studied areas. PAHs are most abundant in Site 1 compared to Site 2 and Site 3. Overall, 3 - rings PAHs are highly present in the experimental sites. 2 –ring PAHs were not detected in Site 1.

The profile and ratios of concentrations of PAHs in soil samples from experimental areas were depicted in Table 2. Higher percentage of LWM PAHs (Naphthalene, Acenaphthlene, Aceenaphthene, Fluorene, Phenanthrene and Anthracene) was present in Site 1 and 2. MMW PAHs are found more in the Control Site. The table also showed that HWM PAHs and cPAHs are higher in Site 3 relatively compared to Sites 1 and 2. The ratio of LWM PAHs/HMW PAHs are higher in Site 2 while Flt/Pyr is higher in Site 1 and Phe/Ant higher in Site B.

Table 2: Profile and ratios of concentrations of polycyclic aromatic hydrocarbons in soil samples from charcoal production sites

	Site 1	Site 2	Site 3
LMW PAHs	35.13 (58.96%)	2.25 (66.99%)	2.284 (42.19 %)
MMW PAHs	12.62 (21.18%)	0.630 (16.75%)	1.638(30.26%)
HMW PAHs	11.83 (19.86%)	0.612 (16.27%)	1.492(27.56%)
Σ7cPAHs	16.20 (27.19%)	0.832(22.12%)	1.526(28.19%)
ΣPAHs	59.58	3.762	5.414
LMW PAHs / HMW PAHs	2.97	4.18	1.53
Flt / Pyr	0.51	0.02	0.49

Phe / Ant	0.00	1.64	0.00

PAHs *= Polycyclic Aromatic Hydrocarbons;* ***LMW PAHs*** *= Low Molecular Weight PAHs;*
MMW PAHs *= Medium Molecular Weight PAHs;* ***cPAHs*** *= Carcinogenic PAHs*
LMW / HMW *= Sum of lower molecular weight PAHs versus sum of higher molecular weight PAHs*
Flt / Pyr *= Fluoranthene versus Pyrene;* ***Phe / Ant*** *= Phenanthrene versus Anthracene.*

Concentrations of PAHs in shoot of *Talinum triangulare* is expressed in Table 3. 3-, 4- and 5- rings PAHs are higher in water leaf harvested from Site 1 while 2- and 6- rings PAHs were higher in *Talinum triangulare* from Site 2.

Table 3: Concentration (mg kg^{-1}) of PAHs in shoot of *Talinum triangulare* from charcoal production site

PAHs	**Number of rings**	**Site 1**	**Site 2**	**Site 3**
Naphthalene	2	0.11	0.116	0.00
Acenaphthylene	3	0.168	0.176	0.00
Acenaphthene	3	0.154	0.114	0.00
Fluorene	3	0.178	0.00	0.00
Phenanthrene	3	0.018	0.026	0.00
Anthracene	3	0.10	0.002	0.00
Fluoranthene	4	0.034	0.192	0.116
Pyrene	4	0.006	0.00	0.182
Chrysene	4	0.022	0.102	0.176
Benzo[a]anthracene	4	0.012	0.032	0.024
Benzo[b]fluoranthene	5	0.00	0.00	0.009
Benzo[k]fluoranthene	5	0.004	0.00	0.00
Benzo[a]pyrene	5	0.004	0.076	0.056
Indenol[1,2,3-c,d]pyrene	6	0.044	0.002	0.188
Dibenzo[a,h]anthracene	5	0.002	0.002	0.010
Benzo[g,h,i]perylene	6	0.006	0.00	0.004
Σ16 PAHs		**77.75**	**25.15**	**7.30**
Σ 2- rings		**1.00**	**2.05**	**0.35**
Σ 3- rings		**23.55**	**3.85**	**3.90**
Σ 4- rings		**23.45**	**5.25**	**1.00**
Σ5- rings		**28.50**	**6.75**	**1.65**
Σ6 – rings		**1.25**	**7.25**	**0.40**

Table 4 shows the concentration of 16 detected PAHs in the root of *Talinum triangulare* harvested from charcoal production sites. 4-rings PAHs are more pronounced in the roots of *Talinum triangulare* from Sites 2 and 3. The total PAHs concentrations recorded from the three sites were relatively closed. 2-rings PAHs appear to be in the lowest concentration from the three sites.

Table 4: Concentration (mg kg^{-1}) of PAHs in root of *Talinum triangulare* from charcoal production site

PAHs	**Number of rings**	**Site 1**	**Site 2**	**Site 3**
Naphthalene	2	0.06	0.034	0.00
Acenaphthylene	3	0.03	0.038	0.07

Acenaphthene	3	0.00	0.028	0.162
Fluorene	3	0.00	0.028	0.06
Phenanthrene	3	0.49	0.030	0.034
Anthracene	3	0.02	0.002	0.004
Fluoranthene	4	0.02	0.194	0.186
Pyrene	4	0.03	0.152	0.156
Chrysene	4	0.08	0.002	0.012
Benzo[a]pyrene	4	0.06	0.006	0.004
Benzo[b]fluoranthene	5	0.05	0.172	0.024
Benzo[k]fluoranthene	5	0.00	0.034	0.00
Benzo[a]pyrene	5	0.04	0.078	0.130
Indenol[1,2,3-c,d]pyrene	6	0.06	0.036	0.064
Dibenzo[a,h]anthracene	5	0.01	0.00	0.166
Benzo[g,h,i]perylene	6	0.04	0.088	0.00
Σ16 PAHs		**0.99**	**0.922**	**1.072**
Σ 2- rings		**0.06**	**0.034**	**0.00**
Σ 3- rings		**0.54**	**0.126**	**0.33**
Σ 4- rings		**0.19**	**0.354**	**0.358**
Σ5- rings		**0.10**	**0.284**	**0.154**
Σ6 – rings		**0.10**	**0.124**	**0.064**

The bioaccumulation coefficient of PAHs by *Talinum triangulare* from experimental site is shown in Table 5. From the results, accumulation of individual PAH by *Talinum traingulare* is less than 1 (< 1) except Nap, Acy and BaA in Site 2 and Chry in Site 3 which is greater than 1 (> 1).

Table 5: Bioaccumulation coefficient of polycyclic aromatic hydrocarbons by *Talinum triangulare* from experimental Sites

PAHs	**Number of rings**	**Site 1**	**Site 2**	**Site 3**
Nap	2	0.00	3.87	0.00
Acy	3	0.09	2.75	0.00
Ace	3	0.08	0.08	0.00
Flu	3	0.01	0.00	0.00
Phe	3	0.001	0.08	0.00
Ant	3	0.00	0.01	0.00
Flt	4	0.01	24.00	0.30
Pyr	4	0.001	0.00	0.20
Chry	4	0.03	0.48	1.13
BaA	4	0.002	5.33	0.24
BbF	5	0.001	0.00	0.00
BkF	5	0.001	0.00	0.00
BaP	5	0.003	0.67	0.23
IndP	6	0.00	0.01	3.03
DahA	5	0.00	0.02	0.00
BghiP	6	0.01	0.00	0.02

The biological concentration factors of PAHs by *Talinum triangulare* from experimental sites are shown in Table 6. Nap and Flt were concentrated by the roots of *Talinum triangulare* in Site 2 while IndP was concentrated by vegetables in Site 3. All other PAHs from the experimental sites according to Table 6 showed BCF values of less than 1 (BCF < 1).

Table 6: Biological concentration factor (BCF) of polyaromatic hydrocarbons by *Talinum triangulare* from experimental sites

PAHs	**Number of rings**	**Site 1**	**Site 2**	**Site 3**

Nap	2	0.00	**1.13**	0.00
Acy	3	0.02	0.59	0.00
Ace	3	0.00	0.02	0.18
Flu	3	0.00	0.06	0.05
Phe	3	0.003	0.09	0.00
Ant	3	0.00	0.01	0.00
Flt	4	0.008	**24.00**	0.41
Pyr	4	0.005	0.40	0.17
Chry	4	0.02	0.01	0.08
BaA	4	0.09	**1.00**	0.04
BbF	5	0.01	2.53	0.05
BkF	5	0.00	0.19	0.00
BaP	5	0.03	0.70	0.53
IndP	6	0.00	0.25	**1.03**
DahA	5	0.00	0.00	0.00
BghiP	6	0.09	0.00	0.00

Discussion

Polycyclic aromatic hydrocarbons are regarded as potential recalcitrant compounds with adverse health implications when ingested by human. From the results of this research, PAHs are most abundant in the soil of site 1 as compared to the two other sites (2 and 3). Overall, 3- rings PAHs are highly present in sites 1 and 2. 2-rings PAHs were not detected in Site 1. However, employing the proposed contamination classification of PAHs by Maliszewska-Kordybach (1996) and Zhengyu *et al*. (2013), that is (non-contaminated: < 200 ng g^{-1}; weakly contaminated: 200 - 600 ng g^{-1}; contaminated: 600 - 1000 ng g^{-1}; heavily contaminated: > 1000 ng g^{-1}), all three sites investigated are heavily contaminated.

The profile and ratios of concentrations of PAHs in soil samples from experimental areas revealed that the sites (1 and 2) had higher percentages of LMW PAHs (Nap, Acy, Ace, Flu, Phe and Ant). MMW PAHs (Flt, Pyr, Chr and BaA) are found more in Site 3. From the profile and ratio analysis of PAHs, HMW PAHs (BbF, BkF, BaP, DahA, BghiP and Ind) were higher in Site 3 relatively compared to Sites 1 and 2. The values for ratios between LMW PAHs/HMW PAHs and that of Flt/Pyr in all experimental sites were higher than unity (> 1) which denotes petrogenic sources of PAHs (Qiu *et al*., 2009; Yakan *et al*., 2017). However, values for Phe/Ant ratio were less than 10 (< 10) which indicates pyrolytic sources of PAHs (Qiu *et al*., 2009). The proportion of carcinogenic PAHs (BaA, Chr, BbF, BkF, BaP, Ind and DaP) present in the three experimental sites was similar.

The concentration of PAH in shoot and root of *Talinum triangulare* is expressed in Tables 3 and 4. 3-, 4- and 5-rings PAHs are higher in *Talinum triangulare* harvested from sites 1 and 2 while 6- rings in Site 2 relatively compared to PAHs concentration of vegetable from Site 3. Also, 4- rings PAHs were more pronounced in the roots of *Talinum triangulare* from Sites 2 and 3 respectively.

The biological concentration factor of PAHs indicated that Nap and Flt were concentrated by roots of *Talinum triangulare* in site 2 while IndP was concentrated by vegetables in Site 3. All other PAHs from the experimental sites showed BCF values of less than 1 (BCF < 1). Bioaccumulation coefficient of individual PAH by *Talinum triangulare* is less than 1 (BAF < 1) except Nap, Acy and BaA in Site 2; Chry and IndP in Site 3 that are greater than 1(BAF >1).

Conclusively, the experimental sites were heavily polluted with PAHs as indicated by the contamination classification values. This may not be unconnected with the charcoal production activities. High bioaccumulation coefficient recorded by *Talinum triangualare* for Nap, Flt and BaA (Site 2) and IndP (Site 3) together with the biological concentration factor, present the vegetable as an accumulator for these compounds. However, of serious concern is the high level of PAHs in the soil of the charcoal production areas which could be dangerous to humans in that environment.

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