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RESEARCH PAPER

Comparative studies on exposure of edible vegetables to spent engine oil and PAH components

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Abstract. The consequences of enhanced PAH deposition and accumulation in food crop and other biota can be traced to environmental pollution through human activities which has improved due to industrial revolution. The impact of this phenomena has been observed in the food chain as this toxicant accumulates within its system and therefore, it can be exposed to human being with detrimental effect. The study compared exposure of edible vegetables to PAH from spent engine oil and three purchased PAH component (benzo(a)pyrene, benzo(k)fluoranthene and benzo(ghi)perylene). PAH was extracted from soil and plant using soxhlet extraction method. The health risk review was done using risk assessment model. The unpolluted vegetables showed a higher growth performance when compared to the exposed vegetables pertaining to their bio-tolerance. However, unpolluted vegetable was significantly different ($P < 0.05$) from polluted vegetable. The result showed that Fluoranthene (Flu), benzo(a)Pyrene (B(a)P), Acenaphthene (Ace), Anthracene (Ant), Naphthalene (Nap) and Benzo(b)Fluoranthene obtained from spent engine oil polluted soil (SEOPS) were the most abundant in the soil. However, concentration of commercially purchased benzo(a)pyrene was observed to be higher in plants than (Benzo(k)fluoranthene and Benzo(ghi)perylene) utilized. Bioaccumulation factor total (BAFT) of commercially purchased B(a)P, B(k)F and B(ghi)P showed higher accumulation value (1.8, 1.5), compared to that of spent engine oil in edible vegetables. Analysis of the calculated assessing value (AV), Benzo(a)pyrene toxic equivalent quotient (BaPteq), food daily intake, and margin of exposure (MOE) showed potential risk concern when consumed, except for progressive lifetime cancer risk (PLCR). The PLCR poses relatively low health concern; nevertheless, prolonged exposure to these pollutants can affect humans as it possesses a high potential to bio-accumulate in edible vegetables.

Keywords: Margin of exposure (MOE); bioaccumulation factor; risk assessment; dietary intake

1. Introduction

Environmental pollution and toxicity orchestrated by prompt industrialization are progressively becoming terrifying to the general population. The repercussions may be observed in the environmental degradation which is experienced in the air, land, and water. The unrelenting organic pollutant such as polycyclic aromatic hydrocarbons (PAHs) is a hazard that has to be curbed as it has dented every part of the ecosystem. This PAHs are mostly obtained from human endeavors or inherent processes (Hahladakis et al., 2013; Nwogu et al., 2022). The increase in PAH

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discharge as a consequence of partial incineration from human activities allows its deposition in the soil, which has led to its accumulation in food crop and other biota, and is then transferred to humans through the food chain (Kim et al., 2013). Moreover, PAH contents have been detected in the water bodies of the environment, especially rivers within our local habitats. These water bodies serve as water sources to the locals occupying the area and it usually provides for activities, such as drinking, farming, etc. (Nwogu et al., 2022).

As PAH is deposited on soil surface, they become mobile due to its ability to bound to soil particles (Cachada et al., 2016). The mobility of PAH particle in the soil is dependent on the sorbet molecule of the soil and its pore size. This movement is made possible by PAH adsorption and soil conductivity (Abdel-Shafy & Mansour, 2016).

The PAH presence in the liquid located within the soil pores reduces soil aeration and water infiltration which has adverse effect on its productivity (Sakshi et al., 2019). PAHs adeptness to accumulate in food portend risk to different forms of life as their toxicity has the competence to alter the DNA of plants which can advance to genetic mutation, developmental malfunction, and cancer formation in both plant and animal (Čvančarová et al., 2015). The processes of PAHs assimilation in the plant is contingent as soil, plants, and PAH properties performs a major role through their interactions (Salehi-Lisar & Deljoo, 2015). Different strata of water body's pollution can cause plant contamination. PAHs accumulated in plants can be transferred to higher trophic level considering that is an essential part of the terrestrial ecosystems (Alghamdi & EL-Saeid, 2021). Plants can absorb PAHs and other organic pollutants through soil or air (Figure 1).

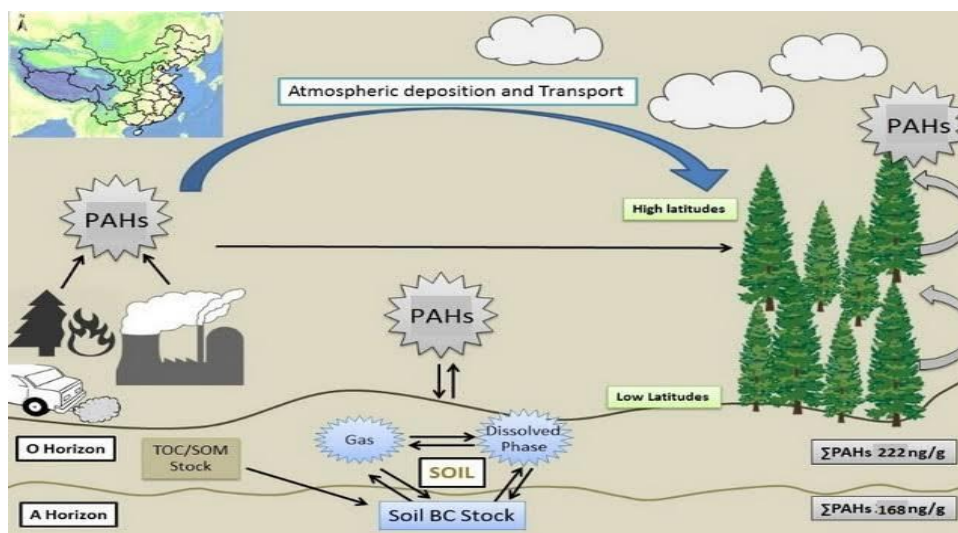


Figure 1. PAH sources and exposure route to edible vegetables (Chang et al., 2013).

Nevertheless, plants can take up PAHs through root or shoot (Li & Ceng, 2014). According to studies, the roots are the primary channel for PAH intake as they are the first point of contact in soil and plant interaction processes (Zhang et al., 2017). This is transferred via transpiration stream flux (Chen et al., 2015). Experimental analysis by Dupuy et al. (2016) and Alves et al. (2017) utilizing fluorescence microscopy demonstrated transport mechanism of PAH in the roots and shoots of different plants. Polycyclic aromatic hydrocarbons are a hazardous substance that should be of concern due to the adverse health effects of its exposure on humans. These effects are facilitated by the PAH concentration, exposure route and period, individual state of health, and PAH toxic potential (Pandey et al., 2017).

The aforementioned parameters are crucial in determining the impact of the exposure as it can comfortably elicit its toxic potentials on the affected individual. A survey carried out in a study in manufacturing domain of Lagos and Ogun state, Nigeria reported increased PAH pollution as

response to severe health challenges of people residing within the area (Alani et al., 2013). However, PAHs plant absorption processes is immensely influenced by the chemical structure of the toxicant (Figure 2). PAH can enter the human body either through dietary (edible plants and food) or non-dietary source (inhalation and dermal interaction). Studies have shown that dietary source is one of the major route humans are exposed to PAH. It has been observed that 70% exposure of PAH for non-smokers is associated with food intake. It has been reported that dietary exposure to PAHs may lead to several forms of cancer, increased level of DNA adduct, mutation, and reproductive effects (Kim et al., 2013). Therefore, it is pertinent to compare the degree of spent engine oil and PAH contamination in edible crops (fluted pumpkin and green), and the application of various health evaluation models to appraise its consequences on humans from daily consumption of edible crops. PAH content of the plant samples will be determined through Gas Chromatography, while the progressive life risk cancer, margin of exposure, food intake, and bioaccumulation factor will be utilized for the health risk consideration.

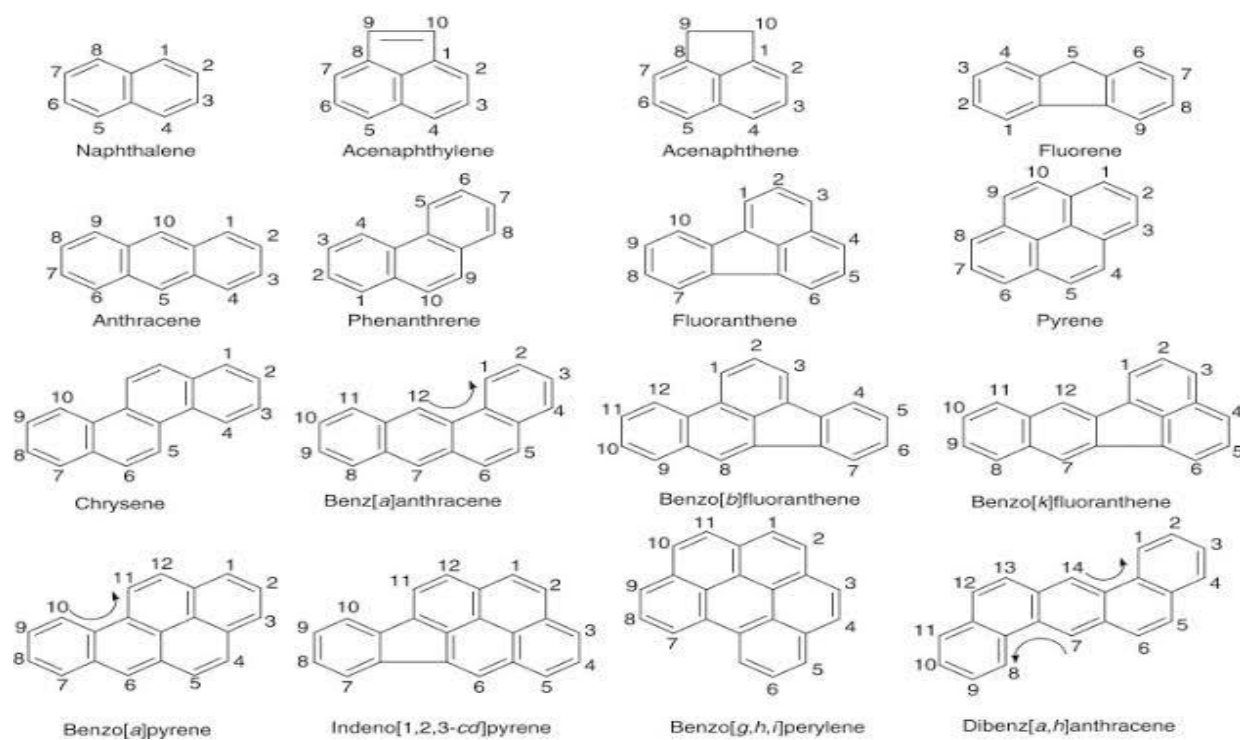


Figure 2. Different types of PAH (Abdel-Shafy & Mansour, 2016)

2. Experimental procedure

2.1. Seed and soil sample

The seedlings of the selected crop plants were purchased from a local market in Ihiagwa, Owerri West Local Government Area, Imo State, while the soil sample was obtained from the garden of the International Institute of Tropical Agriculture (IITA) in Ibadan, Oyo State, Nigeria.

Soil sample was obtained with a hand trowel at the depth of 0-15cm. Seedlings of selected crop plants (*Telfairia occidentalis* and *Amaranthus hybridus*) were planted in a nursery bed (1 × 3m²) of sandy loamy soil. The seedling was resettling to different concentrations of spent engine oil made up of 100 ml, 200 ml, 300 ml, and PAH components of 20 mg/l each made up of benzo[a]pyrene, benzo[k]fluoranthene and benzo[ghi]perylene in a pierced plastic bucket. Seedlings of equal height were obtained for the study. All experimental analysis was in triplicate within a controlled environment at the International Institute of Tropical Agriculture (IITA) in

Ibadan, Oyo State, Nigeria. The experimental plant was thereafter set up on a bench top in the screen house. The screen house maintained a relative humidity and temperature of $22 \pm 0.24\%$ and $75.76 \pm 4.07^\circ\text{C}$ respectively.

Growth performance parameters: Growth parameters indicating the performance of the plant samples were determined weekly from the moment of transplant (Odjegba & Atebe, 2007). In this case, the values for each plant in a pot were determined and totaled for the two plants species in the bucket. Thereafter, the mean for the buckets were calculated. The leaves number on the *Telfairia occidentalis* and *Amaranthus hybridus* were counted weekly for each plant pot, and the average leaves number were obtained. Plant height was assessed using a meter rule. It was measured from plant base to tip. Leaf area was measured using meter rule through the utilization of paper graph sheet. Then, the area of the leaf was obtained using the formula: Leaf area = length \times width. On each day of the measurement, one plant from each of the replicate was carefully uprooted from the plastic buckets. The root areas were thoroughly washed in distilled water to remove the soil particles that can add to the weight of the plant. After that, the plant was weighed using electronic top weighing balance (model Mettler PM 34) to determine the fresh matter weight. After weighing, the plants were labelled accordingly and then dried-up at standard temperature within 14 days period and then reweighed.

2.2. Plant sample determination of PAH

The pulverized plant tissues were freeze-dried and extraction was done using an acetone and Hexane mixture (vol/vol = 1 : 1) for one hour, followed by an hour of ultrasonic extraction. The extraction process was repeated thrice. A rotary evaporated was used in solvent evaporation and transferred to 2 ml hexane. About 2 g column silica gel using 11 μL , 1 : 1 (v/v) extraction of hexane and dichloromethane for cleanup process. Samples were evaporated and transferred finally to 2 ml of ethanol. PAH was analyzed using GC equipped with flame ionization detector with UV detector wavelength calibrated at 254 nm. The mobile phase was pure methanol at 1.0 ml/min and the temperature at 30°C (Adekunle et al., 2018).

Bioaccumulation factor: Bioaccumulation factor (BAF) was utilized in determining the quantity of PAH taken up by the plants. The plant ability to accumulate PAH with respect to the initial concentration in the soil is known as the BAF (Ghosh & Singh, 2005). This factor helps to evaluate the bioaccumulation effect of PAH on each edible crop plants taken up from the soil. PAH bioavailability in soil can be assessed using the Equation (1).

$$\text{BAF} = \frac{\text{PAH concentration in plant tissue}}{\text{Initial concentration of PAH in substrate (soil)}} \quad (1)$$

The amount of PAH taken up by plant from soil can be ascertained through the BAF (i.e. PAH concentration that is bioavailable to plant).

2.3. Health risk assessment of PAH through plants consumption

Estimation of the health hazards related to polycyclic aromatic hydrocarbons, through the consumption of the PAH contaminated crop plants fluted pumpkin and green. A deterministic model proposed by USEPA was employed to evaluate the latent human health risks posed by PAH. In this method, benzo(a)pyrene has been utilized as an appropriate indicator for the existence of carcinogenic PAH and its effect in diet. Toxic equivalent factors (TEFs) was applied to calculate the total carcinogenic health risk linked to PAH obtained from cancer influences and individual

PAH compounds comparative to the cancer influence of benzo(a)pyrene equivalent concentration (BaP_{eq}) for each PAH (Okereke et al., 2016). The progressive lifetime cancer risk (PLCR) was exploited in the direction of evaluating non-carcinogenic health risks. Benzo(a)pyrene equivalent was calculated using the formula in Equation (2) and (3).

$$B(a)P_{eq}C = \sum (TEF \times Conc) \quad (2)$$

Where B(a)P_{eq}C = Benzo(a)pyrene equivalent concentration, TEF = Toxic equivalent factor.

$$AV = \frac{\left(\frac{RL}{SF}\right) \times BW}{CR} \quad (3)$$

Where AV = Assessing value, RL = Extreme tolerable risk level (0.000001), SF = slope factor (0.0073 µg/kg/d-adult/0.0061-children), BW = Body weight (adult = 60 kg/ children = 35 kg), and CR = Consumption rate (g/day).

Food daily intake (FDI): The food daily consumption of vegetable incremental poisoned with PAH was obtained through multiplications of individual concentration of PAHs in each plant by the consumed weight of plant by individual. Total food consumption of B(a)P_{eq} was obtained by multiplication of each sample concentration with the intake rate (IR) of 0.5 kg (Fang et al., 2014). The food exposure dose level daily (ED) daily was obtained with the Equation 4 (Halek et al., 2008).

$$ED = FDI B(a)P_{eq} = B(a)P_{eq} \times IR \quad (4)$$

Where: ED = Exposure dose, FDI B(a)P_{eq} = Food daily intake of Benzo(a)pyrene equivalent, B(a)P_{eq} = Benzo(a)pyrene equivalent, and IR = Intake rate.

The progressive life time cancer risk (PLCR): This is the likelihood of obtaining cancer due to exposure to carcinogenic substance in dietary intake. This deals with a progressive cancer disease in exposed population when compared to unexposed population. The PLCR was estimated via Xia et al. (2010) process. The Equation 5 is utilized:

$$PLCR = \frac{ED \times EF \times EDB[a]P_{eq} \times SF \times CF}{BW \times AT} \quad (5)$$

Where PLCR = is measured in µg/kg body wt/d, ED = exposure duration = life expectancy (70 yrs), EF = exposure frequency (365 days/yr), EDB[a]P_{eq} = exposure dose for B[a]P, SF = oral slope factor of benzo[a]pyrene (0.0073 µg/kg/d-adult/0.0061-children), CF = conversion factor, BW = body weight (adult = 60 kg/ children = 35 kg), and AT = average life span (70 yrs)(Tongo et al., 2017).

Margin of exposure: This was evaluated to determine the genotoxic and carcinogenic levels of the PAH that can be of issue to community health. This is risk evaluation model permitted by the Joint Expert Committee on Food Additives (JECFA), formed by FAO/WHO and European Food Safety Authority (EFSA) (Wu et al., 2015).

$$\text{MOE} = \frac{\text{SDL} \times \text{BW}}{\text{DDI}} \quad (5)$$

Where MOE = Margin of exposure, SDL = Standard dose level, BW = Body weight (60 kg for adult/35 kg for children), EED = Estimated exposure dose.

2.4. Statistical analysis:

Data collection for all parameters was subjected to analysis of variance for establishment of significant difference among means using Dunnet multiple comparison.

3. Result and discussion

3.1. Growth performance *Amaranthus hybridus* on polluted and unpolluted soil sample

The growth performance of *A.hybridus* on the polluted and unpolluted soil sample after the fourth week of transplanting was shown in Table 1. The results showed that B(ghi)P had the highest plant growth performance (15.43 cm, 8.83, 6.55 cm³, 0.51 g, and 0.06 g) in all the growth parameters when compared to other polluted plant samples. This was followed by SEOPS A. In view of, all the polluted soil sample, SEOPS C with the highest pollutant concentration had the least growth performance (9.37 cm, 4.17, 1.63 cm³, 0.10 g, and 0.02 g). This was followed by B(a)P even though it had a lesser pollutant concentration. Furthermore, the data obtained shows that there was a decrease in *A.hybridus* growth performance with an increase in pollutant concentrations after a period of four weeks.

Table 1. Growth performance of *A.hybridus* at weeks four after transplanting

Parameters	UPPS	B(a)P	B(k)F	B(ghi)P	SEOPS A	SEOPS B	SEOPS C
Plant height (cm)	18.12±1.64 ^a	11.97±0.50	14.33±0.64	15.43±1.48	14.83±1.16	12.50±1.31	9.37±0.22
Leaves number	12.67±1.51 ^a	6.67±1.21	8.83±1.17	10.50±1.52	9.33±1.21	6.67±1.21	4.17±0.98
Lear area (cm ³)	9.48±1.20 ^a	2.70±0.56	4.90±0.80	6.55±0.89	5.93±0.67	3.52±0.68	1.63±0.47
Fresh weight (g)	1.08±0.33 ^a	0.27±0.10	0.33±0.01	0.51±0.02	0.39±0.15	0.21±0.05	0.10±0.03
Dry weight (g)	0.18±0.01 ^a	0.03±0.01	0.03±0.01	0.06±0.01	0.05±0.01	0.03±0.02	0.02±0.01

Means labeled with the letter "a" are not significantly different from the control level mean.

LEGEND: ±Standard deviation; UPPS: Unpolluted plant sample, B(a)P: Benzo(a)pyrene polluted sample, B(k)F: Benzo(k)fluoranthrene polluted sample, B(ghi)P: Benzo(ghi)perylene polluted sample, SEOPS A: 100 ml spent engine oil polluted samples, SEOPS B: 200 ml spent engine oil polluted sample, SEOPS C: 300 ml spent engine oil polluted sample.

3.2. Growth performance *Telfairia occidentalis* on polluted and unpolluted soil sample

The growth activities of *T.occidentalis* at week four after transplanting is shown in Table 2. It was observed that at four weeks, SEOPS A had the highest plant height and dry weight (62.75 cm and 3.21 g) when compared to other polluted samples. However, in B(ghi)P, the leaf area and fresh weight were higher (18.0 cm³ and 13.02 g) when compared to other polluted samples. In addition, SEOPS C had the lowest performance even though there was a gradual increase in its growth performance. There was a significant difference (P<0.05) between the various toxin and unpolluted samples at the end of four weeks.

Table 2. Growth performance of *T.occidentalis* at week four after transplanting

Parameters	UPPS	B(a)P	B(k)F	B(ghi)P	SEOPS A	SEOP B	SEOPS C
Plant height (cm)	107.05±8.19 ^a	41.85±5.23	51.68±6.04	58.88±2.52	62.75±8.40	50.48±6.37	39.27±4.44
Number of Leaves	31.50±3.62 ^a	14.00±3.10	17.00±1.89	19.17±2.14	19.00±3.90	13.83±2.79	12.83±2.14
Leaf Area (cm ²)	23.98±3.29 ^a	14.30±1.36	15.18±1.28	18.00±1.45	17.27±1.49	14.88±1.52	13.5±1.28
Fresh Weight (g)	20.85±3.39 ^a	10.09±1.08	12.05±1.38	13.02±1.59	12.33±1.48	10.79±1.32	9.75±0.93
Dry Weight (g)	5.755±0.52 ^a	2.85±0.76	3.12±0.27	3.11±0.76	3.21±0.89	2.59±0.23	2.14±0.14

Means labeled with the letter “a” are not significantly different from the control level mean. LEGEND: ±Standard deviation; UPPS: Unpolluted plant sample, B(a)P: Benzo(a)pyrene polluted sample, B(k)F: Benzo(k)fluoranthrene polluted sample, B(ghi)P: Benzo(ghi)perylene polluted sample, SEOPS A: 100 ml spent engine oil polluted samples, SEOPS B: 200 ml spent engine oil polluted Sample, SEOPS C: 300 ml spent engine oil polluted sample.

3.3. The absorption of PAH components from spent engine oil (SEO) in plants samples

Concentrations of PAH components in *Amaranthus hybridus* and *Telfairia occidentalis* from spent engine oil polluted and unpolluted leaf samples is presented in Table 4. It was observed from the results that only acenaphthylene, fluoranthene, dibenzo(a, h)anthracene, and benzo(a)pyrene were detected in the unpolluted soil for *A.hybridus* and *T.occidentalis*. However, higher concentration values were seen in *A.hybridus* than in *T.occidentalis*. Of all the polluted leaf samples, it was observed that phenanthrene and benzo(k)fluoranthrene were not detected in *A.hybridus*, while benzo(ghi)perylene and benzo(b)fluoranthrene were not detected in *T.occidentalis*. In addition, the two to four ring PAH (ACY, NAP, ACE, ANT, FLO, FLU and B(b)F) were detected to have accumulated more than the five to six ring PAH (B(a)P, D(a,h)A, B(ghi)P, etc., in both *A.hybridus* and *T.occidentalis*. Although *T.occidentalis* showed higher accumulation concentrations when compared to *A.hybridus*. Furthermore, *A.hybridus* had a higher concentration of B(a)P (0.5460 mg/ml) in SEOPS A when compared to *T.occidentalis*. This was followed by ACE, ANT and ACY (0.5249 mg/ml, 0.4994 mg/ml, and 0.4311 mg/ml) in SEOPS A of *T.occidentalis*. However, the least accumulated concentration was NAP (0.0005 mg/ml) in SEOPS A of *T.occidentalis*.

3.4. The uptake of benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene by plants samples

The concentrations of the B[a]P, B[k]F, and B[ghi]P in *A. hybridus* and *T.doccidentalis* are presented in Table 5. Of all the three PAH components used *A.hybridus* showed higher concentrations (2.7164 mg/ml, 1.7381 mg/ml, and 1.0635 mg/ml), when compared to *T.occidentalis* (2.0815 mg/ml, 1.7376 mg/ml, and 0.9604 mg/ml). Furthermore, acenaphthylene,

Table 4. Concentrations of PAHs in *Amaranthus hybridus* and *Telfairia occidentalis* from spent engine oil polluted sample

PAH components	<i>A.hybridus</i>				<i>T.occidentalis</i>			
	UPSS	SEOPS A	SEOPS B	SEOPS C	UPSS	SEOPS A	SEOPS B	SEOPS C
Acy	0.0007 ±0.000	0.0331 ±0.008	0.0143 ±0.003	0.0698 ±0.023	0.0005 ±0.00	0.4311 ±0.022	0.0641 ±0.013	0.0081 ±0.006
Phen	-	-	-	-	-	0.1243 ±0.005	0.0930 ±0.035	0.1005 ±0.052
B(k)F	-	-	-	-	-	0.2038 ±0.008	0.1948 ±0.015	0.1457 ±0.023
B(ghi)P	-	0.0946 ±0.016	0.0724 ±0.017	0.1545 ±0.012	-	-	-	-
1-2Benz	-	0.0841 ±0.014	0.0421 ±0.015	0.0833 ±0.015	-	0.0825 ±0.009	0.1045 ±0.027	0.1152 ±0.017
Flu	0.0187 ±0.014	0.1981 ±0.055	0.2309 ±0.025	0.2582 ±0.014	0.0130 ±0.005	0.4195 ±0.005	0.3891 ±0.130	0.4360 ±0.054
D(ah)A	0.1105 ±0.032	0.2491 ±0.082	0.0311 ±0.021	0.0352 ±0.014	0.1071 ±0.002	-	0.0140 ±0.007	0.0359 ±0.020
B(a)P	0.0940 ±0.032	0.5460 ±0.092	0.3912 ±0.032	0.1802 ±0.015	0.0807 ±0.001	0.0308 ±0.013	0.0224 ±0.009	0.0309 ±0.009
Flo	-	0.1667 ±0.055	0.1210 ±0.022	0.1651 ±0.019	-	0.1343±0.007	0.1843 ±0.019	0.2017 ±0.110
Ace	-	0.2961 ±0.156	0.2629 ±0.021	0.2970 ±0.113	-	0.5249 ±0.017	0.4726 ±0.103	0.2469 ±0.215
Ant	-	0.1016 ±0.008	0.1904 ±0.024	0.2038 ±0.019	-	0.4994 ±0.037	ND	0.5001 ±0.120
Pyr	-	0.0577 ±0.018	0.0558 ±0.022	0.0549 ±0.020	-	0.0479 ±0.002	0.0621 ±0.029	0.0957 ±0.048
Nap	-	0.1441 ±0.101	0.1217 ±0.007	0.1172 ±0.010	-	0.0005 ±0.000	0.0032 ±0.001	0.0044 ±0.002
B(b)F	-	0.1890 ±0.010	0.1893 ±0.089	0.1872 ±0.019	-	-	-	-

Legend: Acy-acenaphthylene; Phen-phenanthrene; B(k)F-benzo(k)fluoranthene; B(ghi)P- benzo(ghi)perylene; 1,2 Ben- 1,2 benzanthrene; Flu-fluoranthene; D(ah)A-dibenzo(ah)anthracene; B(a)P-benzo(a)pyrene; Flo-Florene; Ace-acenaphthene; Ant-anthracene; Pyr-pyrene; Nap-Naphthalene; B(b)F-benzo(b)Fluoranthene; UPSS: Unpolluted plant sample; SEOPSS A: 100 ml spent engine oil polluted soil sample; SEOPSS B: 200 ml Spent engine oil polluted soil sample; SEOPSS C: 300ml Spent engine oil polluted soil sample. -: Not detected. All units are in mg/ml.

Table 5: Concentrations of PAHs in *Amaranthus hybridus* and *Telfairia occidentalis* from PAH polluted samples.

PAH components	A. hybridus				T. occidentalis			
	UPSS	B(a)P	B(k)F	B(ghi)P	UPSS	B(a)P	B(k)F	B(ghi)P
Acy	0.0007±0.000	0.2323±0.035	0.3108±0.102	0.3001±0.031	0.0005±0.00	0.2074±0.004	0.2437±0.006	0.1948±0.062
Phen	-	-	-	-	-	-	-	-
B(k)F	-	-	1.7381±0.89	-	-	-	1.7376±0.480	-
B(ghi)P	-	-	-	1.0635 ±0.026	-	-	-	0.9604±0.116
1-2Benz	-	-	-	-	-	-	-	-
Flu	0.0187±0.014	0.2218 ±0.123	0.2219±0.013	0.2111±0.030	0.0130±0.005	0.2174±0.003	0.2209±0.055	0.3110±0.143
D(ah)A	0.1105±0.032	0.1495 ±0.066	0.1217±0.013	0.1302±0.018	0.1071±0.002	0.1207±0.004	0.1081±0.010	0.0967±0.039
B(a)P	0.0940±0.032	2.7164 ±0.825	0.5497±0.151	0.1209±0.024	0.0807±0.001	2.0815±0.017	0.4291±0.113	0.2109±0.046
Flo	-	-	-	-	-	-	-	-
Ace	-	-	-	-	-	-	-	-
Ant	-	-	-	-	-	-	-	-
Pyr	-	-	-	-	-	-	-	-
Nap	-	-	-	-	-	-	-	-
B(b)F	-	-	-	-	-	-	-	-

LEGEND: Acy-acenaphthylene; Phen-phenanthrene; B(k)F-benzo(k)fluoranthene; B(ghi)P- benzo(ghi)perylene; 1,2 Ben- 1,2 benzanthrene; Flu-fluoranthene; D(ah)A-dibenzo(ah)anthracene; B(a)P-benzo(a)pyrene; Flo-Florene; Ace-acenaphthene; Ant-anthracene; Pyr-pyrene; Nap-Naphthalene; B(b)F-benzo(b)fluoranthene; UPSS- Unpolluted plant sample; PAH A- Benzo(a)pyrene; PAH B- Benzo(k)fluoranthene; PAH C- Benzo(ghi)perylene. - : not detected. All units are in mg/ml.

fluoranthene, dibenzo(a,h)anthracene, and benzo(a)pyrene, which were detected in the unexposed plants, were present at higher concentrations in the plant exposed to these three individual PAH components for both *A.hybridus* and *T.occidentalis*.

3.5. Bioaccumulation factor of three selected PAHs

The bioaccumulation of purchased B(a)P, B(k)F, and B(ghi)P and soil contaminated with spent engine oil in *A.hybridus* and *T.occidentalis* are presented in Table 6. The result obtained compares the bioaccumulation factor (BAF) values of purchased B(a)P, B(k)F, and B(ghi)P, as well as that from spent engine oil polluted soil. It was observed that the BAF values of the three purchase PAHs and that from SEO polluted soil were less than one. However, the individually purchased PAH had a higher BAF values when compared to PAH from SEOPS in both plants. The BAF_{Total} of the three PAH components were ≥ 1 both in *A.hybridus* and *T.occidentalis*. On the other hand, *A.hybridus* had a higher BAF_{Total} value (1.8) when compared to *T.occidentalis* (1.5). While the BAF_{Total} of the three PAH components from spent engine oil polluted soil was slightly greater than one in *A.hybridus* (1.3) and ≤ 1 (0.5) in *T.occidentalis*.

Table 3. Bioaccumulation factor of PAHs.

Pollutants	Parameters	<i>A.hybridus</i>	<i>T.occidentalis</i>
Purchase PAH	B(a)P	0.85	0.65
	B(k)F	0.55	0.55
	B(ghi)P	0.35	0.32
	BAF _{Total}	1.80	1.50
SEO	B(a)P	0.51	0.04
	B(k)F	-	0.49
	B(ghi)P	0.78	-
	BAF _{Total}	1.30	0.50

LEGEND: B(k)F: benzo(k)fluoranthene, B(ghi)P: benzo(ghi)perylene B(a)P-benzo(a)pyrene - : not detected. All units are in mg/ml.

3.6. Food intake of PAH in relation to health hazard *A.hybridus*

The food intake hazard in relation to PAH consumption of *A.hybridus* exposed to benzo(a)pyrene, benzo(k)fluoranthene, benzo(ghi)perylene, and different spent engine oil polluted soil are presented in Table 7. The result showed that benzo(a)pyrene had the highest food daily intake (FDI) value of 1.358 ng/kg when compared to benzo(a)pyrene, benzo(k)fluoranthene, and benzo(ghi)perylene of spent engine oil polluted soil. This was followed by benzo(a)pyrene from soil contaminated with spent engine oil. In benzo(a)pyrene toxic equivalent (B(a)P_{TEQ}), benzo(a)pyrene had the highest value when compared with benzo(k)fluoranthene and benzo(ghi)perylene. Furthermore, the value of benzo(a)pyrene value exceeded that of the assessing values of adult and children.

Moreover, the progressive lifetime cancer risk (PLCR) values from the three PAH components and that from spent engine oil polluted soil for children were higher in relation to that of adult. In addition, spent engine oil polluted soil benzo(ghi)perylene was observed to have the highest margin of exposure (MOE) value for both adult and children. However, the margin of exposure value for adult was higher than that of children except for benzo(k)fluoranthene from spent engine oil polluted soil which was not detected.

Table 7. Health risk associated with consumption of *A. hybridus* exposed to PAH components.

Pollutants	PAH components	Mean ±SD (mg/ml)	TEF	ED=FDI (Ci x IR) ng/kg	B(a)Pteq (Ci x TEFi) ng/kg	PLCR adult	PLCR children	MOE×10a adult	MOE×10a children
SEOPS	B(a)P	0.372±0.05	0.100	0.186	0.037	8.30x10 ⁻⁹	1.18x10 ⁻⁸	93.5	54.6
	B(k)F	-	0.030	-	-	-	-	-	-
	B(ghi)P	0.107±0.02	0.009	0.054	0.001	2.40x10 ⁻⁹	3.44x10 ⁻⁹	244.4	142.6
PAH	B(a)P	2.716±0.83	0.100	1.358	0.272	6.03x10 ⁻⁸	8.64x10 ⁻⁸	12.8	3.8x10 ⁻³
	B(k)F	1.738±0.89	0.030	0.869	0.050	3.86x10 ⁻⁸	5.53x10 ⁻⁸	9.7	5.6
	B(ghi)P	1.064±0.03	0.009	0.532	0.010	2.36x10 ⁻⁸	3.38x10 ⁻⁸	24.8	14.5
AV-Adult	0.084 ng/kg								
AV-Children	0.056 ng/kg								

Legend: SEO: Spent engine oil, PAH: Polycyclic aromatic hydrocarbons, B(a)Pteq: Benzo(a)pyrene toxic equivalent, B(a)P: Benzo(a)pyrene, B(k)F: Benzo(k)fluoranthrene, B(ghi)P: Benzo(ghi)perylene, TEF: Toxic equivalent factor, AV: Assessing value, PLCR: Progressive lifetime cancer risk, MOE: Margin of exposure, FDI: Food daily intake, -: Not detected.

3.7. Health hazards linked with food intake of PAH from *T. occidentalis*

Health hazards linked with food consumption of *T.occidentalis* exposed to benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene and spent engine oil polluted soil are presented in Table 8. The results showed that benzo(a)pyrene and benzo(k)fluoranthrene had the highest food daily intake (FDI) value (1.040 ng/kg and 0.869 ng/kg) when compared to that from spent engine oil. The benzo(a)pyrene toxic equivalent quotient showed that benzo(a)pyrene had the highest toxic equivalent quotient of 0.208 ng/kg which exceeded the assessing value for both adult (0.084 ng/kg) and children (0.056 ng/kg). The progressive lifetime cancer risk of PAH from spent engine oil polluted soil, benzo(a)pyrene, benzo(k)fluoranthrene, and benzo(ghi)perylene for children were higher when compared with the adult. Furthermore, the margin of exposure value was higher in benzo(a)pyrene from the spent engine oil polluted soil compared to the three different PAH components. However, the margin of exposure values of adult was higher than that of children, except for benzo(ghi)perylene, which was not detected.

Table 8. Health risk associated with consumption of *T.occidentalis* exposed to PAH components

Pollutants	PAH components	Mean ±SD (mg/ml)	TEF	ED=FDI (Ci x IR) ng/kg	B(a)Pteq (Ci x TEFi) ng/kg	PLCR adult	PLCR children	MOE×10a adult	MOE×10a children
SEOPS	B(a)P	0.028 ±0.02	0.1	0.014	0.0028	6.00 x 10 ⁻¹⁰	8.90 x 10 ⁻¹⁰	1242.9	725
	B(k)F	0.181 ±0.01	0.03	0.091	0.0054	4.00 x 10 ⁻⁹	5.79 x 10 ⁻⁹	92.3	53.8
	B(ghi)P	-	0.009	ND	-	-	-	-	-
PAH	B(a)P	2.082 ±0.01	0.1	1.040	0.208	4.62 x 10 ⁻⁸	6.62 x 10 ⁻⁸	16.7	9.8
	B(k)F	1.738 ±0.48	0.03	0.869	0.052	3.86 x 10 ⁻⁸	5.52 x 10 ⁻⁸	9.67	5.6
	B(ghi)P	0.960 ±0.12	0.009	0.482	0.009	2.14 x 10 ⁻⁸	3.07 x 10 ⁻⁸	27.3	15.9
AV-adult	0.084ng/kg								
AV-children	0.056ng/kg								

Legend: SEO: Spent engine oil, PAH: Polycyclic aromatic hydrocarbons, B(a)Pteq: Benzo(a)pyrene toxic equivalent, B(a)P: Benzo(a)pyrene, B(k)F: Benzo(k)fluoranthrene, B(ghi)P: Benzo(ghi)perylene, TEF: Toxic equivalent factor, AV: Assessing value, PLCR: Progressive lifetime cancer risk, MOE: Margin of exposure, FDI: Food daily intake.

3.8. Discussion

3.8.1. Influence of spent engine oil and PAH on plants samples

The plant capacity to tolerate the stress caused by the pollutants was shown in its performance. In *amaranthus hybridus*, there was a significant difference in concentrations of the pollutants on growth parameters when compared to control, which indicates its detrimental effect to the plant's growth. Moreover, the concentration of SEOP C and PAH A appear to be more detrimental to the plant as they are significantly higher than other concentrations. However, the fresh weight of B(k)F, B(ghi)P, SEOPS A and the dry weight of B(ghi)P were not significantly different from the control within four weeks interval. Furthermore, the effects of the pollutants were visible in *Amaranthus hybridus* as it was not able to withstand the stress within the period of experiment as yellowing of leaves and stunted growth was observed. However, *Telfairar occidentalis* grown on contaminated soil with used engine oil and polycyclic aromatic hydrocarbon were also significantly different from the unpolluted plant sample except for dry weight of B(ghi)P, at fourth week. Effect of spent engine oil pollutant shows a trend of concentration dependent as SEOP C had the most toxic impact on the growth of both plants. The poor plant height and leave area recorded in both *A. hybridus* and *T. occidentalis* plant exposed to the pollutant could be attributed to interference on the moisture content of the soil which can lead to nutrient immobilization and poor mineral uptake. Consequently, reduced leave number obtained may be because of heavy metal toxicity and insufficient ventilation of the soil which can limit the transpiration and respiration by plant. Osuagwu et al. (2017) in his work observed the negative impact of spent engine oil on its proficiency to moderate the sprouting and seedling growth of *Z. mays*, *A. hypogea* and *V. unguiculata*. According to Wyszowski et al. (2004), the potential of hydrocarbons to daub the plants root with greasy substance contributes to the reduction in cell membrane absorbency, thereby unsettling the metabolic processes, and conferring toxicity to the cell. The pollutant was responsible for the fresh and dry weight level of the plant samples. Therefore, absorption of water and ions take place in the root, which has a direct contact with used engine oil and PAH polluted soil.

3.8.2. Bioaccumulation of spent engine oil and PAH pollutants on plant samples

Some plants have shown the ability to bio-accumulate some of these pollutants, which can be toxic to the food chain. This is because of PAH are carcinogenic, mutagenic, teratogenic, and have acute toxicity which can damage the endocrine system. Because of their high toxicity, the three different PAH components examined for the two plants samples revealed that *A. hybridus* accumulated more of the PAH components B(ghi)P and B(a)P than *T.occidentalis*. The latter accumulated more of B(k)P than B(a)P from spent engine oil. However, in soil polluted with purchased PAH, *A. hybridus* accumulated more of it as well. In general, *A.hybridus* bio-accumulates more PAH than *T.occidentalis* even though it could not withstand the toxicity exerted by PAH during the experimental period. This could be attributed to the plants characteristics as plants exudates released in the soil could trigger PAH metabolic transformation which could make them water-soluble using exo-enzyme. In addition, the potentials of the pollutant's concentration cannot be over-emphasized as the level of accumulation in the plants are concentration dependent. Pretorius et al. (2018) worked on the accumulation of metal, PAH, and alkyl PAH. They reported that an increase in PAH accumulation was observed in the roots of *E. purprea*. However, there is a possibility of Phyto-immobilization occurrence where restriction of absorbed pollutant

mobility occurs within the plant root, confining it to a specialized vacuole. This could explain the decrease PAH accumulation observed in *T. occidentalis*.

3.8.3. Health risk assessment of PAH through plant consumption

The increasing level of pollutants in the environment has led to the accumulation of toxic substance in the food chain, which has become a major concern as its effect is detrimental to human health. This assessing value determines the level of toxic chemical in edible substances which has the potential to cause distress in health (Wu et al., 2015). The toxicity level of the plant sample was evaluated using assessing value (AV) threshold which was harnessed to evaluate the health implication of PAH on individuals consuming this vegetable. The potency equivalent quotients of vegetables exposed to the three PAH components from purchased and spent engine oil were less than the assessing value except for purchased benzo(a)pyrene of both *A.hybridus* and *T.occidentalis*. This observation suggests that apart from benzo(a)pyrene, the other two PAH components of interest in this study possess low potential health risk. However, benzo(a)pyrene was higher when compared to the assessing value, indicating a significant potential health risk for consumers within the polluted area. The risk assessment of PAH especially benzo(a)pyrene in Rivers State, Nigeria, indicates that consumable vegetables in farmlands within an industrial cite area had toxic equivalent quotients higher than the assessing value, demonstrating significant health risk concern (Okereke et al., 2016). Ikue et al. (2016) reported on PAH risk assessment of catfish from a petroleum polluted site in Ogoni Land, Rivers State, which is consistent with the findings of this study.

Furthermore, the genotoxic and carcinogenic level of the vegetables exposed to PAH from both toxins was assessed through margin of exposure (MOE). This margin of exposure is the standard lower limit dose of probable exposure dose or concentration that could cause issues in public health. Margin of exposure was developed by Joint Committee on Food Additives (JECFA) and European Food Safety Authority (EFSA) in 2005 as a regulation for risk assessment of genotoxic and carcinogenic compounds (EFSA, 2005). PAH from toxicant-exposed vegetables were all below the 10,000 limit proposed by EFSA from both spent engine oil and purchased PAH components, showing that the exposure margin is of high health risk concern for consumers of this vegetables contaminated with PAH toxicant. This supports the study conducted by Martorell et al. (2010) on edible vegetables, as PAH margin of exposure was less than the standard limits, raising concerns about public health risk. However, this is in contradiction with Igbiri et al. (2017) who observed that PAH margin of exposure on mushroom were relatively safe for consumption, as the values are above the 10,000 critical limits. The progressive life cancer risk (PLCR) in this study was utilized to determine the menace of PAH exposure. Progressive life cancer risk that is equal to 1,000,000 were deemed to be inconsequential, while the one of 10,000 were consequential (Moslen et al., 2019). Therefore, supportive action is expected to facilitate the reduction of life cancer threat when the range is within the latter figure. The toxicant-exposed vegetables showed low health risk in terms of progressive life cancer risk through consumption for both adult and children. This is supported by Moslen et al. (2019) who observed that the PAH bioaccumulation of bivalve was of low health risk concern to consumers when considered value of the excess lifetime cancer risk. In consideration of the various method of health risk assessment applied in this study, the guideline may differ depending on region and policy formation of the country (Qu et al., 2015). However, most regions adopt the USEPA standard.

4. Conclusion

The ability of plants to accumulate these toxic pollutants in their tissue does not only depend on concentration level but also on the plant characteristics, as more stress was exerted on *A.hybridus* than in *T.occidentalis*. *Amaranthus hybridus* was adversely affected by these pollutants as its growth and survival rate was repressed when compared to the *Telfairia occidentalis*. This indicates that *A.hybridus* may be utilize in assessing phyto-toxicity of these pollutants while *T.occidentalis* may be beneficial for phyto-monitoring of these pollutants as it was adept in tolerating the stress exerted on it during the period of the experiment. Furthermore, the health risk assessments conducted on the two vegetables exposed to the pollutants in this study was within the low health risk for humans. However, continuous or prolonged exposure to these contaminated vegetables could be detrimental to humans as these pollutants possess the ability to bioaccumulate for an extended period of time.

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