BIOAVAILABILITY AND GENOTOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS ON TWO EDIBLE VEGETABLES (*Amaranthus hybridus and Telfiaria occidentalis*)

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CERTIFICATION

This is to certify that this project work: *Bioavailability and Genotoxicity of Polycyclic Aromatic Hydrocarbons on Two Edible Vegetables (Amaranthus hybridus and Telfiaria occidentalis)* was carried out by **UKACHUKWU, Chidinma Ogochukwu** with registration number **20184139528** in partial fulfillment of the requirements for the award of Master of Science (M.Sc.) degree in the Department of Biotechnology, School of Biological Sciences, Federal University of Technology, Owerri.

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DEDICATION

This research work is committed to God Almighty for giving me the insight and strength to successfully complete this research and also to my parents Engr. & Mrs. C.C Ukachukwu and my supervisor Prof. (Mrs) A.C Udebuani for their support and guidance during this process.

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ABSTRACT

Bioavailability and genotoxic effect of polycyclic aromatic hydrocarbons (PAHs) were investigated in two edible vegetables (*Amaranthus hybridus* and *Telfairia occidentalis*) using; physicochemical properties, biotolerence of the vegetables to PAH, molecular characterization, and health risk assessment of consumption of exposed vegetables. The study assessed PAH content in different tropical urban environment using soxhlet extraction flame ionization detection (SE-GC-FID) method, physicochemical properties of soil, biotolerance of the two vegetables exposed to different concentrations of spent engine oil, benzo(a)pyrene (B[a]P), benzo(k)fluoranthene (B[k]F) and benzo(ghi)perylene (B[ghi]P) in plant species, and health risk associated with the consumption of contaminated vegetables. The health risk assessment was done using health assessment models such as screening value (SV), incremental lifetime cancer risk (ILCR) and margin of exposure (MOE). Molecular characterization of exposed plant species was carried out to determine the level of toxicity on the DNA, through DNA band breaks. This was carried out with three inter-simple sequence repeat (ISSR) primers (UBC 811, UBC 827, UBC 808). SE-GC-FID method was used for the separation and identification of PAH. The results showed that a total of fourteen PAHs were detected in the different tropical urban environment which includes: acenaphthylene, phenanthrene, 1-2 benzanthene, acenaphthalene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(b)fluoranthene, flourene, naphthalene, dibenzo(a,h)anthracene, anthracene, benzo(ghi)perylene, fluoranthrene and pyrene. The soil physical properties showed that the moisture content and bulk density where higher in the polluted soil, while porosity was higher in the unpolluted soil. However, the soil textural class remains sandy. In soil chemical properties; soil pH was low, effective cation exchange capacity, exchangeable base, exchangeable acidity and organic carbon were higher in the polluted soil samples. The biotolerance of the two vegetables exposed and unexposed to the pollutants showed low growth performance in the polluted plants as concentration increases. However, there was a significant difference ($P<0.05$) between the unexposed and the exposed plants using analysis of variance Dunnet multiple comparison. The result of the genomic effect of these pollutant revealed alteration at genetic level through DNA insertion deletion and changes in band intensity. The three primers showed polymorphism level of 65%, 58% and 18% . The health risk assessment from dietary consumption of PAH contaminated vegetables showed low health risk concern in SV, ILCR and MOE. However, prolong exposure to these pollutants can affect humans as it possesses a high potential to bioaccumulate through the food chain. The study was able to suggest the presence of PAH component in the different tropical urban environs of Owerri. Also the impact of PAH on growth performance of the two plants species showed that it is concentration dependent which was reflected in the DNA of plants species as polymorphism occurred showing variations in DNA.

Keywords: Polycyclic aromatic hydrocarbons, Biotolerance *Bioaccumulation, Molecular characterization, Polymorphism, Telfairia occidentalis, Amaranthus hybridus*

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Polycyclic aromatic hydrocarbons (PAHs), belonging to a group of persistent organic pollutants have become an issue of global concern, due to their persistent nature and as such have the potential to accumulate in the biota. PAHs are produced in all processes of incomplete combustion of organic substance (Kang, Chen, Gao & Zhang 2010). Their production is favored by an oxygen deficient flame, temperature in the range of 650^0-900^0C and fuels which are not highly oxidized. PAHs usually shares the following properties; high melting and boiling points, low vapor pressure, and very low aqueous solubility (Mashir, Singhvi, kumar, Jain & Taneja 2012), and they are usually grouped into two based on their molecular weight; light molecular weight PAHs (two to three rings PAHs) and the high molecular weight PAHs (five or more rings PAHs) (Choi, Baek, & Chang 2010).

PAHs are lipophilic and persistent, including in the soil (Zhang & Fan, 2016). Following their deposition on the surface of the soil, PAHs may accumulate on food crops and other biota, then transferred to humans through the food chain (Kim, Jahan, Kabir & Brown 2013; Bortey-Sam, Ikenaka, Nakayama, Akoto, Yohannes, Baidoo & Ishizuk, 2014) or they can be strongly attached to the soil where they persist for a long period (Banan, Khaled, Mohamad, Halene & Farouk 2018). Several studies have shown the correlation between PAH concentrations and the soil physiochemical properties (such as particle size, total organic carbon etc.) (An, Pei, Zhao, Teng, Li & Li 2016; Zhe, Jing & Shao 2017). According to An *et al*. (2016), factors such as population density, land use type, and urbanization have an effect on soil PAH distribution. This implies that the accumulation and distribution of PAHs in the soil could be affected by soil characteristics and factors associated with urbanization (Wu, Kechavarzi, Li, Sui, Pollard & Coulon 2019).

The uptake of PAHs by plants have harmful effects on the plant which according to researchers can lead to morphological, cytological, metabolic and genetic disorder. PAHs pollution can result in the inhibition and reduction of seed germination (Tomar & Jajoo, 2014), induction of oxidative stress in plants (Salehi-lisar & Daljoo, 2015), and disruption in the function of photosynthesis apparatus (Tomar & Jajoo, 2014). The impact of PAH on humans depend on the length and route of exposure, the amount or concentration of PAHs one is exposed to, the relative toxicity of the

chemical type, the age and pre-existing health status of the individual (Rajan, Singh & Batish 2017). There are various routes of exposure to PAHs such as inhalation, ingestion, and dermal contact in both occupational and non-occupational settings (Kang *et al*., 2010). PAHs have the ability to induce short term exposure effects which have resulted in impairment on pulmonary function in asthmatics and the risk of thrombotic events in patients with heart disease (Kim *et al*., 2013). Mutagenesis and carcinogenesis is a serious concern, associated with PAHs. The International Agency for research on cancer have identified and classified PAH as carcinogens.
Thus, there is insufficient information on the sources, effects and consequences of PAHs presence in our environment. Most literature's have failed to highlight how PAHs are made available for plant uptake and the genotoxic effect of PAHs on food crops that are consumed by humans. Therefore, it is necessary to investigate the bioavailability and genotoxic effects of PAHs on two edible crops plants (*Telfairia occidentalis* and *Amaranthus hybridus*).

1.2 Statement of Problem

Polycyclic aromatic hydrocarbons (PAHs) are one of the major environmental pollutants, which are found almost everywhere, produced from organic materials that are incompletely combusted (Hussein & Mona, 2015). PAHs are persistent, harmful organic pollutants that are detrimental to both the environment and human health (Xing, Qi, Zhang, Wu, Zhang, Yang & Odhiambo 2011). It's exposure to the general population is through the following routes; eating food PAHs contaminated food, inhaling smoke from open fireplaces, from fossil fuels used in cars, cooking, and industries (Cui, Ma, Shi, Lin, Luo & Lui 2015). Occupational exposure to PAHs may occur during roofing using bituminous products, coke production, coal gasification, and oil refining. The most common occupational exposure to PAHs in Nigeria include breathing fumes from exhausts (e.g. mechanics), by street vendors, or motor vehicle drivers and workers involved in mining. Generally, exposure to contaminated food predominates among these routes of exposure. PAHs enter the food chain through deposition from air, soil, or water.

Polycyclic aromatic hydrocarbons are of particular interest due to their potential health concern mainly as a result of their carcinogenic and mutagenic properties. Health problems such as increased risk of lung, skin, gastrointestinal, and bladder cancer, associated with exposure to PAH have been recorded (Diggs, Huderson, Harris, Myers, Banks, Rekhaderi, Niaz & Ramesh 2011). Garcia-Suastegui, Huerta-chagoya, Carrasco-colin, Pratt, John, & Petrosyan (2011) have reported

induced DNA damage as a result of PAH exposure. Also, there are suspicions that long-term exposure to PAHs may increase the risk of cell damage through gene mutation (Kim *et al.*, 2013).

Researches have shown that the highest concentration of atmospheric PAHs can be found in urban environments with high car traffic activity and scarce dispersion of atmospheric pollutants. Human exposure to PAHs may occur through food, water, air or direct contact with materials containing PAHs. Alani, Olayinka & Alo (2013) observed that PAH emission into the air in industrial areas of Lagos and Ogun state was responsible for the adverse health effects of over 23 million people living in the area. PAHs have been identified in sediment cores of some creeks in Delta State (Eguvbe, Iwegbue, Ogala, Nwajei, & Egboh 2014). Toxicological assessment of PAH in sediments from Imo river (Oyo-Ita, Oyo-Ita, Dosunmu, Dominguez, Bayona, & Albaiges 2016) revealed from 409.43 to 41,198ng/g/dw concentration of PAHs as a result of oil spill discharge. PAHs have been identified in sediments from Ubeji, Ifie, and Egbokodo creeks of the Niger Delta (Eguvbe et al., 2014). A research by Ekere, Yakubu, Oparanozie & Ihedioha (2019) also revealed dire concentrations of PAHs in water and fishes from River Niger and Benue confluence in Lokoja

1.3 Aim and Objectives of Study

The aim of the study is to investigate the bioavailability and genotoxic effect of polycyclic aromatic hydrocarbon on selected crop plants.

Objectives

The objectives of the study include:

i. To assess PAH content in different land use in urban environment.
ii. To determine the physical and chemical properties of the soil polluted and unpolluted with PAHs and spent engine oil.
iii. To determine the performance of the two selected vegetables grown on the soil polluted with PAHs.
iv. To determine the bioavailability of different PAHs in soil and some parts of the selected plants species.
v. To determine the effects of PAHs on the genomic DNA of the test vegetables plants species.
vi. To determine or assess the health risk associated with consumption of *A. hybridus* and *T. occidentalis* exposed to PAH polluted soil

1.4 Justification of the Research

Phytoremediation is one of the most commonly applied bioremediation techniques for cleaning environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), and heavy metals. Bioremediation success depends partly on the properties and concentration of the compound (pollutant) and the time required to complete remediation. It is therefore pertinent to investigate the bioavailability of these pollutants, then genomic and phenotypic effect on plants. This can help determine the state in which PAHs are rapidly taken up by plants and how plant respond to stress caused by PAHs.

It is a general knowledge that environmental pollutants and toxicants (such as PAHs) are mostly released from a myriad of anthropogenic activities, and these pollutants eventually settle on and inside the soil (Harris, Banks, Mantey, Huderson & Ramesh 2013). These pollutants are known to affect the physical and chemical properties of the soil, such as the soil moisture content, soil texture, cation exchange capacity, soil pH, soil organic carbon, bulk density, porosity, and soil mineral composition. The effect of polycyclic aromatic hydrocarbons on these soil properties may affect the fertility and biological activities of the soil.

The bioavailability of PAHs can be seen as a fraction of bio-accessible PAHs that are absorbed and enters the circulatory system to elicit effects in a targeted tissues. It is the fraction of PAHs which are systemically available in the body of an organism. Bioavailability is the ultimate indicator of systemic exposure, with bearing on toxicity (PAHs) (Harris *et al*., 2013). Most previous literature's have proposed that the soil is a readily accessible medium in which PAHs could be accumulated. Crops grown in such contaminated soils will carry significant concentration of the contaminants (Ramesh, Archibong, Hood, Guo & Loganathan 2011). The bioavailability of PAHs can be attributed to its physical and chemical properties, the soil properties, and the aging of PAHs in soil and receptor microorganism (Khan, Malik & Muhammed 2011). Yang, Zhang, Xue & Tao (2011), studied the impact of soil organic matter on PAHs distribution in soils, of which they reported that an increase in soil organic matter led to an increase of the average amount of non-bioavailable PAHs.

According to Amodu, Ojumu & Ntwampe (2013), polycyclic aromatic hydrocarbons are the largest known class of carcinogens that do not only cause gene mutation and cancer but are persistent in the environment. There is therefore the need to investigate the genotoxic effect of

PAHs on plants. Higher plants are notable indicators of genotoxicity caused by chemical substances in the environment and can therefore be utilized in determining environmental mutagens (Aksoy, 2017). When these plants are exposed to many stress factors such as chemical pollutants and radiations, they affect their growth, floral, and fruit development. The effect of these stress facts may lead to morphological, physiological, anatomical, biochemical, and molecular damage to plants (Buyuk, Soydam-Aydin & Aras 2012). According to Aksoy (2017), plant bioassays is usually preferred over other available systems in determining the adverse effects caused by pollutants in the environment, because it is simple to carry out, low in cost, and very sensitive. Plant bioassays serve as a means for demonstrating the genotoxic effects of environmental pollutants. This is based on the detection of abnormalities in the chromosome during mitosis, sister chromatid exchanges, and DNA damage analysis.

1.5 Significant of Study

1. The study provides information that is vital to environmentalist, policy makers and stakeholders in making decisions for the regulation of the indiscriminate discharge of such chemical compound in the environment.
2. The study shows the degree of health risk associated with human exposure to PAH.
3. The study showed detailed information of some carcinogenic effect associated with consumption of contaminated vegetables.

1.6 Scope of the Research

Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic pollutants that are ubiquitous in the environment with the potential of accumulating in the biota leading to serious concern. Therefore, this study is focused on the exposure of *Telfeiria occidentalis* (fluted pumpkin) and *Amaranthus hybridus* (green or pigweed) to soil contaminated with different composition of PAHs. Physio-chemical properties of soil exposed and unexposed to PAHs were determined, the performance of the selected crops on polluted and unpolluted soil, bioavailability of different PAH components in soil and crop, genotoxic effect on crop plants exposed and unexposed to PAHs as well as health assessment model used in the investigation of the estimated daily dietary intake, incremental life time cancer risk, and margin of exposure.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Concept of Polycyclic Aromatic Hydrocarbon (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) also known as Polyaromatic hydrocarbons or poly-nuclear aromatic hydrocarbons are groups of organic compounds made up of only carbon and hydrogen that are composed of two or more aromatic rings joined together which do not consist of heteroatom or carry substituent (Ukaogo & Igwe, 2015). PAH can occur in simple forms such as naphthalene which is a two-ring aromatic compound, and anthracene and phenanthrene which are three ring aromatic compounds. PAHs consisting of upto six aromatic rings joined together are called ‘‘small” PAHs, while those with more than six aromatic rings are called ‘‘large” PAHs. They can exist either in particulate and or gaseous phase based on their volatility. Low molecular weight PAHs (LMW PAHs) that have two or three rings are emitted in the gaseous form, while high molecular weight PAHs (HMW PAHs), with five or more rings, have low solubility in water and low volatility thus are present in solid state which are bind in form of particulate; in soils or sediments (Choi *et al*., 2010). The overall properties of PAH include high melting and boiling points, low vapor pressure, and very low aqueous solubility (Mashir *et al.*, 2012). PAHs have been known to be soluble in organic solvents due to the fact that they are highly lipophilic and exhibit a variety of functions that includes heat resistance, light sensitivity, conductivity, corrosion resistance and physiological action (Akyuz & Cabuk, 2010). PAHs can be photo-decomposed under ultraviolet light due to radiation of the sun usually when absorbed as particulate matter or dissolved in water. In the atmosphere it can react with other pollutants such as nitrogen oxide, ozone and sulphur dioxide to yield diones, nitro and dinitro PAHs and sulphonic acid (Ukaogo & Igwe, 2015).

2.2 Sources of PAHs in the Environment

PAHs belong to the group of persistent organic pollutants (POPs), that are resistant to degradation and can remain in environment for a long period and have the potential to cause adverse environmental effects. They occurs naturally in the environment, but their occurrence can be accelerated by anthropogenic activities (Somtrakoon, K., Chaimnangkoon, C., Phalaphol, D., & Chouychai, W. (2015). The different sources of PAH in the environment is shown in figure 2 below.

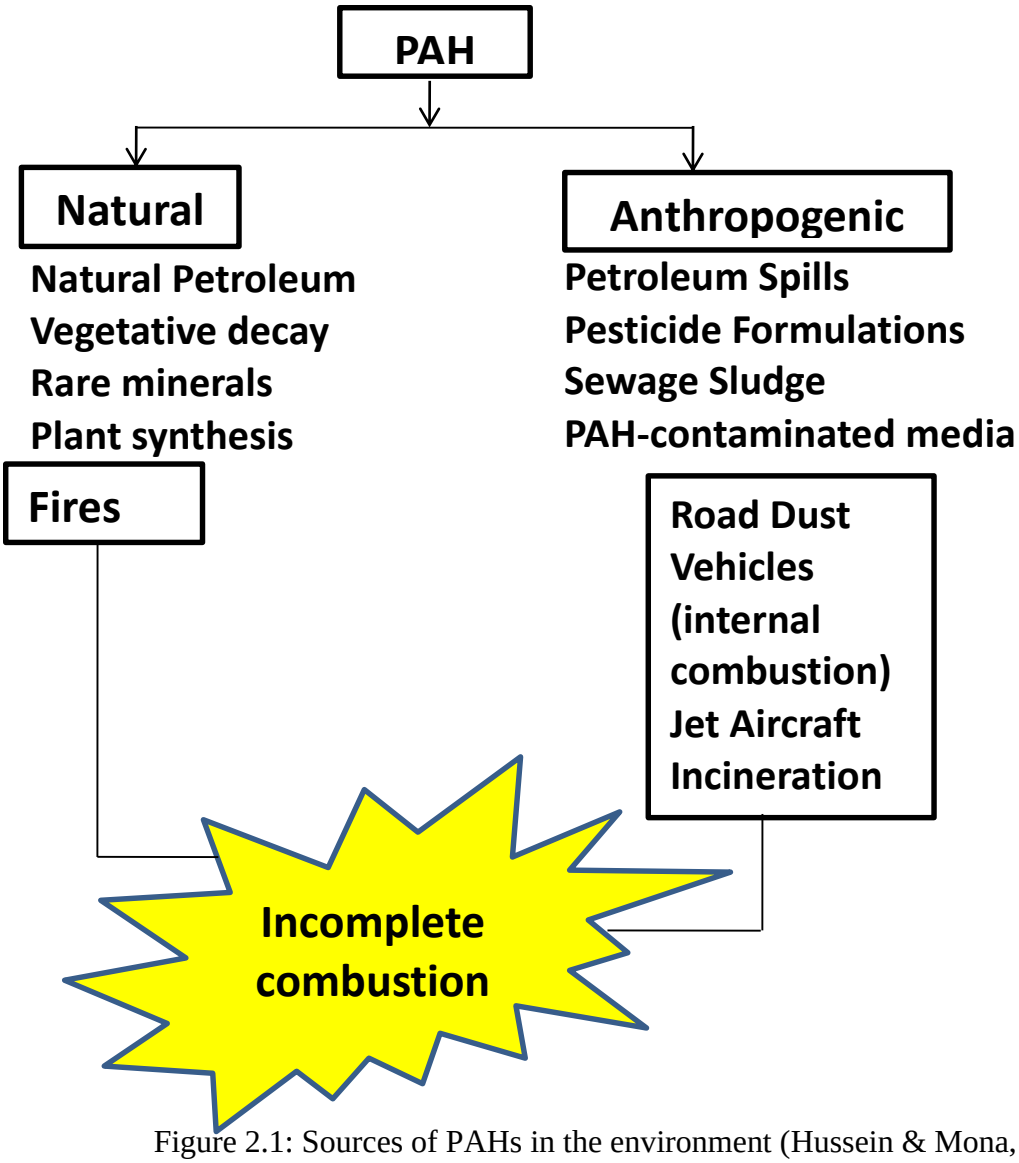


Figure 2.1: Sources of PAHs in the environment (Hussein & Mona, 2015)

2.2.1 Uses of Polycyclic Aromatic Hydrocarbon

PAH are not just chemically produced for industrial uses (Ukaogo & Igwe, 2015). They are used in pharmaceutical, photographic product, thermosetting plastic, agricultural product, lubricating material as intermediaries (ATSDR, 2013). The uses of PAH are presented in table 2.1 below

Table 2.1: Uses of Polycyclic Aromatic Hydrocarbons

PAH	USES
Acenaphthrene	Manufacture of pigments, dyes, plastics, pesticides and pharmaceuticals
Anthracene	Diluent for wood preservatives and manufacture of dyes and pigment. It is used in the manufacture of some dyes and the wood preservation as creosote
Fluoranthrene	Manufacture of agrochemicals, dyes and pharmaceuticals
Fluorene	Manufacture of pharmaceuticals, pigments, dyes, pesticides and thermoset plastics
Phenanthrene	Manufacture of resins and pesticides
Pyrene	Manufacture of pigments

Source: Hussein & Mona (2015)

2.2.2 Types of PAHs

Generally, PAHs can be grouped based on their sources into the environment, and the nature of their chemical structure.

1. Types of PAHs based on their sources into the environment:
   According to Hussein & Mona (2015) the classification of PAHs based on their sources into the environment is represented in table 2.2 below.

Table 2.2: Types of PAHs based on their Formation into the Environment

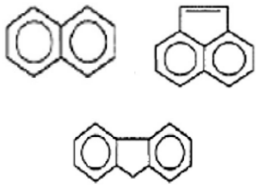
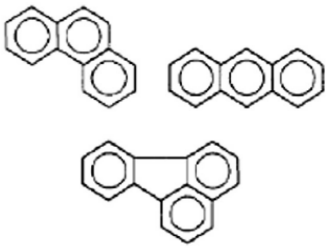
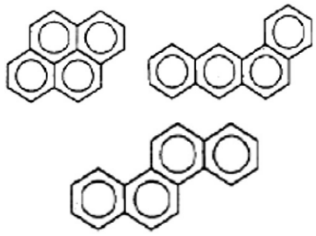
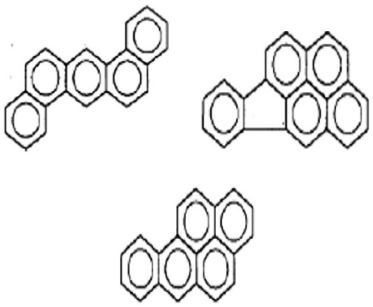
Types of PAHs	Process	Examples
Pyrogenic	Pyrogenic PAHs are formed as a result of exposure of organic matter to high temperature in the presence of little or no oxygen (a process known as pyrolysis). Pyrolysis involves the breakdown of coal to coke and coal tar through distillation or the thermal cracking of petroleum residues to simpler hydrocarbons. Also, incomplete combustion of motor fuels in cars and trucks, incomplete combustion of wood in forest fires and fireplaces, and the incomplete combustion of fuel oils in heating systems. NB: The temperatures at which the pyrogenic processes occurs from about 350 °C to more than 1200 °C.	Phenanthrene, benz(a)anthracene, benzo(b)fluoranthene,benzo (a)pyrene, benzo(a)perylene
Petrogenic	PAHs formed during crude oil maturation and similar process. Petrogenic PAHs are common due to the widespread transportation, storage, and use of crude oil and crude oil products. Some of the major sources of petrogenic PAHs include oceanic and freshwater oil spills, the accumulation of vast numbers of small releases of gasoline, motor oil, and related substances associated with transportation	Naphthalene, acenaphthylene, acenapthene fluorene, anthracene, chrysene
Biological	They can be synthesized by certain plants and bacteria or formed during the degradation of vegetative matter.	Plant bacteria

Source: Hussein & Mona (2015)

2. Types based on the nature of their chemical structure:

   According to Hussein & Mona (2015), the description of the classification of PAHs based on their nature and chemical composition is represented in Table 2.3 below.

Table 2.3: Classification of PAHs based on their nature and chemical structure

Low molecular weight (LMW)		
Double Aromatic ring	Three aromatic ring	Four aromatic ring
		
Naphthaalene, Acenaphthalene, and Fluorene	Phenathrene, Anthracene 1, and Fluoroanthracene 1	Pyrene[1], Benzo(a)anthracene[1,2]
Contains four or less aromatic ring		
High molecular weight (LMW)		
Five aromatic ring		Six aromatic ring
		
Benzo(a)pyrene[1,2] **Dibenz(a,h)anthracene[1,2]** **Indeno(1,2,3cd)pyrene[1,2]**		Benzo(g,h,i)perylene
Contains more than four aromatic rings		

Source: Hussein & Mona (2015)

Natural sources of PAHs formation in the environment can be through erosion of sedimentary rocks containing petroleum hydrocarbon, bacterial and algal synthesis, petroleum seeps, volcanoes, decomposition of vegetative liter fall, and forest and bush fires while the anthropogenic sources can be from the following:

a) Smaller point source: Examples include smoke from wood-burning, cigarette smoke, backyard barbecues as well as automobile emission.
b) Large point sources such as incomplete combustion (e.g. incinerators)

Other anthropogenic sources can be from petroleum product spills, tarry or creosote waste materials and sewage sludge (Hussein & Mona, 2015).

2.3 Transport and Fate of PAHs in the Environment

Primarily, PAHs are released into the atmosphere as a result of incomplete combustion of organic matter. This can be either through natural or anthropogenic sources. PAHs are found in two separate phases when released into the environment, a gaseous phase and a solid phase in which PAHs are sorbet on to particulate matter (Wang, Tian, Zhu, Cheng, Kang & Luo, 2013). The variability of different PAHs in vapor pressures led to the distribution of different concentration of individual PAHs and other sorbet phases (Kuo, Chien, Kuo, Wei & Jui-yeh, 2012). Low vapor pressure PAHs (e.g. benzo[a]pyrene) tend to be sorbet with particle, while high vapor pressure PAHs tends to be associated with gaseous phase.

Atmospheric PAH are deposited continuously either as wet or dry deposition processes. Once PAHs are deposited on the surface of the earth, they become mobile as a result majority of PAHs in the soil will be bound to soil particle (Cachada, Pato, Rocha-Santo, Ferreira & Duante, 2012). The factor that influences the mobility of PAH particulates in the subsurface is the particle size and the pore throat size (smallest opening in between the individual grains of soil) of the soil. PAHs ability to be sorbet with the soil depends on the soil and PAH properties. The movement of individual PAHs in the soil can be due to PAH sorption and soil conductivity (Hussein & Mona, 2015).

PAHs are deposited in sediment by the same process that governs its deposition on soil surface. PAHs in atmospheric particle in rural areas can settle on lake, stream and ocean surface due to dry or wet deposition. It is then integrated into the sediments when carried eventually by current. In urban centers, sediments are influenced by atmospheric depositions of PAH which is as a result of

storm and sanitary sewer effluents as well as roadway runoff. Some of the PAHs deposited will be in particles, which will eventually settle to become part of the sedimentary record (Tehrani, Hshim, Sulaiman, Tavakoly & Salleh, 2012). PAH when incorporated into sediments becomes immobile due to their non-polar structures which prevent them from being dissolved in water. PAHs are not completely insoluble, especially the low molecular weight PAHs. Small molecular weight PAHs do dissolve and become included in water contained in pores of soil where they are bioavailable. The presence of pore water organic colloids can increase the concentrations of PAHs above their aqueous solubility because PAHs will be sorbet onto these organic colloids. These in turn are easily transported through the pore spaces of the sediment. The sorption to colloids can increase the mobility and bioavailability of PAHs in sediments (Veltman, Huijbregt, Rye & Hertwich, 2012).

2.4 Bioavailability of PAHs

PAHs exist in various matrices in the environment due to the fact that they are recalcitrant (as a result of their molecular weight) and hydrophobic nature (Amodu *et al.*, 2013). PAHs are released into the environment through natural and anthropogenic sources as mentioned earlier. The low molecular weight (LMW) PAHs (containing two or three fused benzene ring) are more water soluble than the high molecular weight (HMW) PAHs (four or more fused rings) (Amodu *et al.*, 2012). Thus, the LMW PAHs are generally more bioavailable than HMW PAH.

Bioavailability can simply be defined as a substance ability to interact with the biosystem. According to European Center for Ecotoxicology and Toxicology of chemicals (as cited by Amodu *et al*., 2013), it is a portion of a contaminant in the environment that is available for biological actions. Bioavailability can be seen as a fraction of a compound that is accessible to an organism for absorption or the rate at which a chemical compound is absorbed into a living system (Amodu *et al*., 2013). In terms of soil contamination, Kramer and Ryan (as cite d by Amodu *et al*., 2013), suggest that bioavailability refers to the portion of a compound removed from the soil, through the process of desorption under physiological conditions. Some of the factors that have been reported to affect PAH bioavailability include:

1. Physical and chemical properties of PAHs
2. Soil properties (soil organic matter, moisture content etc).
3. Aging of PAHs in soil

2.4.1 Effect of Physical and Chemical Properties of PAHs on its Bioavailability

The molecular structure and size of PAHs affect its bioavailability (Amodu *et al.*, 2013). LMW PAHs are more bioavailable due to their volatility, higher solubility, and ability to be utilized by microbes as sole carbon source (Yang *et al.*, 2011). Aging refers to the process an organic compound in the soil becomes less susceptible to extractability and biodegradation in a time dependent manner (Chen & Dingi, 2012). Aging leads to low bioavailability of PAHs, in that it makes PAH more recalcitrant to diffusion and mobility (Amodu *et al*., 2013).

2.4.2 Effect of Soil Properties on PAHs Bioavailability

Soil properties such as soil texture, soil depth, soil organic matter content, particle size, pH, porosity, intrinsic permeability, liquid limit and cation exchange capacity, influences PAH bioavailability. Studies have shown that large disparity can occur in soil property from one region to another (Yang *et al*., 2011). Soil structure e.g. aggregation have been observed to decrease PAH availability (Chen & Dingi, 2012). Nom *et al* (as cited by Amodu *et al*., 2013) reported that soil with more than 2% organic matter showed a progressive decline of phenanthrene with respect to time. Also, Yang *et al*. (2011), observed that an increase in organic matter lead to an increase in the non-bioavailability of Anthracene, Acenaphthene, Pyrene, and Fluoranthrene.

Since bioavailability of PAHs in soil can be reflected on its uptake; according to Cachada *et al.* (2012), four main pathways exist through which plant takes up chemical (such as PAHs) from the soil.

1. Root uptake from soil solutions through channels of conduction and translocation into the transpiration stream of xylem.
2. Vapor/aerosols in the surrounding into the plant stomata or absorption from soil air spaces by the root and potentially translocated by the phloem.
3. Contaminants can be deposited on the cuticle by wind or airborne soil
4. Uptake by oil cells or channels of oil-containing plants e.g. carrot, parsaip, and cross

Higher molecular weight PAHs are usually deposited on the cuticle of plant shoot by wind or airborne soil, while lower molecular weight PAHs are less persistent in the environment (Riccardi, Difilippo, Pomata, Di-Basilio & Spicaglia 2013). The roots of vascular plants are the primary site of water and nutrient uptake from the soil and thus the likely point of PAHs entry into the plant as

well. This occurs more actively in the root hairs (root hair zone). Absorbed PAH substance may travel through dead and/or living tissues of the plant through diffusion or mass flow into other parts of the plant. Movement of these substances through the living tissues of plant occurs at a lower rate compared to movement through the tissues of dead plant (Cacheda *et al.*, 2012). The initial uptake of PAH by the root is as a result of PAH concentration in the soil. But generally, PAH uptake and transport from the soil into plant tissues can be illustrated as follows:

Soil solids ⟶ soil water/vapour ⟶ roots ⟶ transpiration stream ⟶ plant stem ⟶ Plant leave

According to Shang, Kim & Haberl (2014), PAH uptake, have two phase process, an initial PAH phase where the compounds diffuses passively into the plant root apparent free space, and a slow accumulation phase where the compound passively permeates the whole volume of exposed tissue. PAHs can be bound within soils, mineralized, or metabolized within or outside the plant. As a result, PAHs can be found in a chemically free state (extractable) or as non-extractable residue in plants, or extractable conjugates that are bound to plant material like carbohydrate (Riccardi *et al.*, 2013).

2.5 Effects of PAH Pollution in the Environment

Polycyclic aromatic hydrocarbon (PAH) are compound which have been revealed to be toxic to life forms and environments. However, the toxicity level of PAH cannot be over-emphasized as its damaging effect have been witnessed in every aspect of our environment which includes but not limited to aquatic, terrestrial and arial components.

2.5.1 Effects of PAHs in Aquatic Environment

There is a high likelihood of receiving and accumulating contaminants in aquatic ecosystem. The deterioration of the aquatic ecosystems has been identified to be caused by PAH (Baali &Yahyaoui, 2019). United States Environmental protection agency (EPA) recognizes PAH as one of the most toxic organic pollutants due to its persistence in the environment and are said to be toxic to fishes (Beyer, Jonsson, Porte, Krahn & Ariese 2010). The degradation products of PAHs that are produced intermittently are potential in generating toxic or mutagenic effects in fish (Brinkmann, Hudjetz, Cofalla, Roger, Kammann & Zhang 2010). PAHs toxicity to aquatic organisms is affected by metabolism and photo-oxidation, which are harmful in the presence of ultraviolent light. The toxicity of PAH to aquatic organisms and birds can be moderate to highly

acute (Baali & yahyaoui, 2019). PAHs in various biological tissues (bivalve, crustaceans and fish) have a half-life time of about a few days to 10days which are five times greater for heavy PAHs relative to lower PAHs. The uncertainty of PAHs to fish and other aquatic organisms in the natural system are due to complex, incomplete characterization of mixtures of chemical, the physical and biological controls on fish exposure to UV light, bioaccumulation of PAHs and large spatial heterogeneity in exposure concentrations (Baali & Yahyaoui, 2019). The concentrations of PAHs found in fish are usually expected to be much higher, than in the environment from which they are absorbed due to bioaccumulation.

Studies have shown a decrease in growth, weight loss, gonado-somatic index (GSI) increase in *Oryziaslatipes*, and breakage in DNA in oyster (*Crassostie agigas*) and zebra fish as a result of Benzo[a]pyrene (Baali & Yahyaoui, 2019). PAHs are known to cause teratogenic effect on the heart of sardine and zebra fish (Hicken, Linbo, Baldwin, Willis, Myers, Holland 2011), as well as in scorpion fish (*Sebastes schlegelii).* Benzo[a]pyrene can disrupt aromatase (enzyme necessary for the conservation of androgen such as estrogen isosterone) expression in female mummichog (*Fundulus heteroditus*) and stops the production of testosterone and estradiol in flounder (*Platichthys flesus L*) (Baali & Yahyaoui, 2019).

2.5.2 Effect of PAH Pollution on Soil

Soil, being a major sink for contaminants, is prone to the effect of these contaminants on the soil physical, chemical and biological properties (Sakshi, Haritash & Singh 2019). The effect on soil properties differs based on the type of contaminant. Long contamination of the soil by PAH may affect the soil geotechnical properties (such as permeability, consolidation, hydraulic conductivity, compaction, and shear strength) and biological properties (biomass and enzyme activity). Due to PAH toxicity on the soil microbes, contaminated soil has lower self-purification capacity (Hreniuc, Coman & Cioruta 2015). The replacement of soil pores liquid with PAH in contaminated soil reduces soil aeration and water infiltration, which can lead to adverse effects on the soil properties (Sakshi *et al.*, 2019). The effect of PAH on soil is regulated by certain parameters of the soil which include soil texture, soil organic carbon, soil pH (Sakshi *et al*., 2019).

2.5.3 Effect of PAHs on Soil Microbial Processes

Based on previous studies, the toxicity of fluorene, pyrene, fluoranthene, and phenanthrene have been documented (Sakshi *et al*., 2019). PAHs have been shown to affect soil nitrification process, and at high concentrations it can affect soil protozoan population and also the bacterial diversity (Cachada *et al.*, 2012). Researchers have shown that relationship exist between PAH biodegradation and microbial biochemistry, physiology, soil properties, and bioavailability to microorganisms (Riccardi *et al*., 2013). In general, the presence of PAHs in the soil can reduce microbial activity. Although, certain microbial and fungal groups can utilize some PAHs (such as; phenanthrene, naphthalene, and pyrene) as energy source (Shang *et al*., 2014). At high concentration of PAH, microbial activity is suppressed as PAH toxicity overcomes the value of PAH as an energy source to the bacteria (Shang *et al.*, 2014). Due to the effect of PAH contamination on the soil pore space, it may cause an anaerobic concentration which affects microbial community of the soil (Suttun, Maphosa, Morillo, Abu Al-Soud, Langenhoff, Grotenhuis, *et al.*, 2013). Researchers have shown that PAH contamination of the soil have significant effect on the soil bacterial community (Khomurbughi, Shavandi, Amvozegar & Dastgheib 2014). In some cases, PAH contamination can lead to the total loss of a particular species of microorganism in the soil (Sakshi *et al*., 2019).

2.5.4 Effect of PAH Pollution on Plant

Polycyclic aromatic hydrocarbons (PAHs) can be taken up by plants from contaminated soil and water. The uptake of PAHs by plants in the soil is dependent on factors such as; the initial concentrations and chemical properties of PAHs, Physical and chemical properties of the soil, the presence of other pollutant in the soil, the time of exposure, the plant species, and the morphological property of the plant (Salehi-lisar & Deljoo, 2015). Plants can take up PAHs by roots from soil or shoot from atmosphere. PAHs have harmful effects on plant that could lead to morphological, cytological, genetical and metabolic disorder in plant. PAH can also result in the inhibition and reduction of seed germination (Tomar & Jajoo, 2014), induction of oxidative stress in plants (Salehi-lisar & Daljoo, 2015), and disruption in photosynthesis apparatus function (Tomar & Jajoo, 2014).

Studies on the exposure of lettuce (*Lectuca sativa*) and garden radish (*Raphanus sativus*) to synergistic effect of fluorene, fluoranthrene and anthracene contaminated soil showed decrease in

the rate of photosynthesis (Somtrakoon, Chaimnangkoon, Phalaphol & Chouychai 2015). Also studies on wheat (*Triticum aestivum*), sunflower (*Medicago sativum*) and alfalfa (*Helianthus annus*) led to growth reduction of the root and shoot, deformed trichomes, reduced chlorophyll level and reduced root hair in the three plants species especially on wheat plant due to exposure to high concentration of fluorine (Salehi-lisar & Daljoo, 2015). Jin, Chang, Li, Gao & Shijie (2017) demonstrated the effect of Phenanthrene on photosynthetic apparatus of cucumber leaves, revealed inhibition of carboxylation activities, and decrease photosynthesis rate due to the inhibition of photosystem II activities, thereby causing a blockage in the photosynthetic electron transport. The inhibition of the light and dark reactions decreased the photosynthetic electron transport rate.

2.5.5 Effect of PAH Pollution on Human Health

Polycyclic aromatic hydrocarbons are known to be of serious concern due to the adverse health effect and its exposure on humans. The impact of PAH on humans depend on the length and route of exposure, the amount or concentration of PAHs one is exposed to, the relative toxicity of the chemical, the age and pre-existing health status of the individual, (Rajan *et al*., 2017). There is various route of exposure to PAH which can be through inhalation, ingestion, and dermal contact in both occupational and non-occupational settings. PAH exposure may also involve more than one route simultaneously, affecting the total absorbed dose (such as dermal and inhalation exposure from contaminated air) (Wang, Tian, Zhu, Cheng, Kang & Luo 2012). The ability of PAH to induce short term exposure effect have been recorded to result in impairment on pulmonary function in asthmatics and the risk of thrombotic events in patient with heart disease (Kim *et al*., 2013). Mutagenesis and carcinogenesis is a serious concern, associated with PAHs. The International Agency for research on cancer have identified and classified PAH as carcinogens e.g. benzo[a]pyrene has been included in group A (Carcinogenic to humans) while benz[a]anthracene, benzo[k]fluoranthrene, benzo[b]fluoranthrene, naphthalene and chrysene in group 2B (possibly carcinogenic to human) (Rajan *et al.,* 2017). PAHs being lipophilic in nature, has the capacity to accumulate in the human body, and in adipose tissues. Studies have shown high risk of lung, skin, bladder and gastrointestinal cancer as a result of exposure to PAHs (Diggs *et al*., 2011). The mutagenic ability of PAHs is due to the fact that they interact covalently with DNA and form adducts which leads to base pair substitutions and frame shift mutagen as a result of their electrophilic metabolites (Jung, Kim, Noh, Eun, Bae, Kim *et al.*, 2013).

Table 2.4: Adverse effects of PAH on human health due to exposure

Adverse effects due to short term exposure	Adverse effects due to long-term exposure
Nausea	Cataracts
Vomiting	Reduce immunity
Loose motions	Kidney damage
Confusion	Liver damage
Eye irritation	Haemolysis due to naphthalene
Skin irritation	Breathing problems and asthma-like symptoms
	Pulmonary abnormality
	Cancer-lung, skin, bladder, gastrointestinal tract

Source: (Rajan *et al.*, 2017)

2.6 Bioindicators and Biomarkers of PAHs

For an organism to be a good bio-indicator, it must possess the following features; good representative, sensitivity, and functional importance in the ecosystem. Also, the organism should be easily collected, identified, and analyzed (Fontanetti, Nogarol, Bustade, Perez & Maziviero 2011).

2.6.1 Terrestrial Invertebrates as Bio-indicators

Saprophytic fauna of terrestrial arthropods such as; Isopoda, diplopoda and collembolan are among the group of organisms that are most appropriate in evaluating the effects of contaminants accumulated in the soil, due to the proximity of these organism with the contaminants in the soil (Fontanetti *et al*., 2011). Annelids (the oligochaete), are commonly used in the toxicity test due to their contact with pollutants present in the soil through their movement and ingestion of contaminated soil or leaf litter (Somtrakoon et al., 2015). Several factor such as the knowledge of their habitats and trophic position, made earthworms an excellent bioindicators of the toxicity of PAHs in the soil (Somtrakoon et al., 2015). As a result of their importance in the soil, their distribution, and the previously stated factors, earthworms (mainly *Eisenia fetida* and *Eisenia andrei*) are used for several contaminants toxicity test.

Collembola are involved in the decomposition process that occurs in the soil and as such are members of the soil meso-fauna. They are vulnerable to the toxicity of soil organic contaminants making them suitable bioindicators (Fontanetti *et al*., 2011). Collembola have a short lifecycle, so they respond rapidly to environmental changes and are more sensitive to certain types of stresses due to their direct contact with the soil (Stefunova, Bezo, Ziarovska & Razna 2015). Diplopods play an important role in soil aeration, nutrient recycling, and fertilization of soil has been observed in various studies. Due to these habits (soil colonization), diplopods can greatly influence the deposition of organic contaminants and complex substances in the soil. Researchers have shown that diplopods (*Glomeris conspersus*) can be used as bioindicators of soil contaminants (Souza & Fontanetti, 2011). Examples of diplopods studies as a good bioindicators of soil contaminants include; *Glomeris conspersa* and *Rhinocricus padbergi* (Nogarol & Fontanetti, 2011)

2.6.2 Higher Plant as Bioindicators

According to Rodriques *et al*. (as cited by Fontanetti *et al*., 2011), plants can provide important information on the cytotoxic, genotoxic and mutagenic potential of substances. In some researches, plants have been observed to be sensitive enough to detect the effects of complex mixtures of contaminants (Perez & Fontanetti, 2011). Plants can be in direct contact with the contaminants, and as such, are suitable for assessing the genotoxicity potential of the contaminants, in in-situ monitoring (Perez & Fontanetti, 2011). One of the limitations of using plants as bioindicators is its insensitivity to some classes of PAHs. Although, *Allium cepa,* have been studied to be sensitive to some of these classes of PAHs (nitro-aminobenzene, benzene, toluene, ethylbenzene and xylene) and are the most plant used in determining the toxic effects (cytoxicity, genotoxicity) of many soil contaminants (Fontanetti *et al*., 2011). Also, using different species of the genus *Tradescentia* the genotoxicity of contaminants in the air, soil and water can be assessed in the cell on the pollen grain (Godoy & Fontanetti, 2010).

Vicia faba is a popular plant that has been extensively used in cytological and physiological investigation of the effects of contaminants. The plant was initially used for radiobiological test to investigate the chromosomal aberration effect of contaminants. *V. faba* has been extensively used for bioindication of contaminant genotoxicity (Fontanetti *et al.*, 2011).

2.7 Biomarkers of PAHs Pollution

Lam and gray (as cited by Fontanetti *et al.*, 2011), defined "biomarkers as biochemical, or molecular alterations or physiological changes in the cells body fluids, tissues or organs of an organism that are indicative of exposure or effect of a xenobiotic". Biomarkers can be seen as biological responses that are adaptive to stressors and are evidenced as cellular, biochemical, histological, behavioral and physiological alterations (Fontanetti *et al*., 2011).

2.7.1 Morphological biomarkers: Morphological biomarkers have made it possible to detect many damages caused by contaminants in tissues and cells (Nogarol & Fontanetti, 2010). These morphological alterations can provide evidence of a functional adaptation to the external environment (Nogarol & Fontanetti, 2010). Studies have shown that soil contaminants may be selectively concentrated in one or few organs of soil invertebrates. These organs are successfully used as biomarkers in the assessment of soil contaminants (Godoy & Fontanetti, 2010). Examples of these invertebrate organs include: the epithelium of the midgut of millipeds, isopods and springtail, the digestive tube and fat body of diplopods (Perez & Fontanetti, 2011).

2.7.2 Genotoxicity biomarker: The genetic material of bioindicator bacteria can be used to assess the genotoxicity of soil contaminants. Also, fungi, plants and insects can also be used (Wolz, 2011). Studies have shown that the meristematic cells of *V. feda* and *A. cepa* constitute an efficient cytogenetic material that can be used to analyze chromosome aberration caused by soil pollution. Chromosome aberrations test helps discover the mechanism of action of a particular pollutant and can be used as a tool for quantifying and monitoring genetic alterations in soils that are radioactively contaminated (Misik, Ma, Nersesyan, Monarca, Kim & Kassmueller 2011).

2.8 Fluted pumpkin (*Telfairia occidentalis)*

Telfairia occidentalis Hook F. (Fluted pumpkin) is a member of the Cucurbitaceae family. It is native to West Africa and predominantly grown in Sierra Leone, Ghana, and Nigeria (Odiaka, Akoroda & Odiaka 2008). *T. occidentalis* is one of the main vegetable crops cultivated in the Southern part of Nigeria and used primarily in soups and herbal medicine (Nwanna and Oboh, 2007). it is popularly known in different languages and countries such as fluted pumpkin, oyster nut, oil nut, fluted gourd and Telfairia nut (English); Costillada (Spanish); Krobonko (Ghana); Oroko, pondokoko and Gonugbe (Sierra Leone); Ug u (Igbo-Nigeria), Aworoko, Eweroko

(Yoruba-Nigeria) and Ikong (Efik/Ibibio-Nigeria) (Eseyin, Sattar & Rathore, 2014). It has about 90 genera and more than 700 species which originated from tropical West Africa (Odiaka *et al.*, 2008).

2.8.1 Description of *T. occidentalis*

T. occidentalis is drought-tolerant, dioecious, perennial, tropical vine grown for its leaves and edible seeds (Philip, 2008). It is a creeping herbaceous vegetable with lobed leaves and twisted tendrils that extends over the soil. T. occidentalis leaves possess high curative, industrial, and nutritional values. According to Akanbi, Adeboye, Togun, Ogunrinde & Adeyeye (2007) the leaves are abundant in fat (18%), protein (29%), and minerals and vitamins (20%). The leaves are also a rich source of phosphorus, calcium, zinc, iron, and copper (Eseyin, Ebong, Igboasoiyi & Oforah 2007). Phytochemical screening of fluted pumpkin leaf extracts has been confirmed to contain saponins, alkaloids, tannins, and phenolics (Oyewole and Abalaka, 2012). The seeds contain 45% fat, 23% carbohydrates, 20.5% protein, 2.2% fibers, and 4.8% total ash (Akanbi *et al.*, 2007). In addition, the seeds contain phospholipid, glycolipid, and neutral lipid contents of 58%, 26%, and 15%, respectively. Fluted pumpkin seed oil contains 61% unsaturated fatty acids (Eseyin *et al.*, 2007).

Table 2.5: Uses of *T.occidentalis*

S/N	Uses	Citation
1	It contains antimicrobial and antiplasmodial properties which helps in the treatment of malaria.	Adekunle, 2016
2	It has antioxidant properties which helps to reduce oxidative damage	Kayode, Kayode & Odetola 2010
3	It is use to boost blood level which reduces the risk of anaemia and also beat diabetes by reducing the production of free radicals.	Kayode and Kayode, 2011

2.9 Green or pigweed (*Amaranthus hybridus*)

A. hybridus (spinach) belongs to the family of Amaranthaceae of the order caryophyllales, a native of North America and has been naturalized in many places of warmer climates. It is popularly known by many common names such as smooth amaranth, smooth pigweed, green amaranth, green pigweed or hybrid amaranth (Assad, Reshi, Jan & Rashid 2017).

2.9.1 Description of *A. hybridus*

A. hybridus popularly called amaranth or pigweed is an annual herbaceous plant of 1-6 feet high. The leaves are alternate petioled, 3-6 inches long, dull, green and rough, hairy, ovate or rhombic with wavy margins. The flowers are small, with greenish or red terminal panicles (Assad *et al.*, 2017). Taproot is long, fleshy red or pink. The seeds are small lenticellular in shape: with each seed averaging 1-1.5 mm in diameter and 1000 seeds weighing 0.6-1.2g. The plant contains large amount of squalene, a compound that has both health and industrial benefits (Akubugwo, Obasi, Chinyere & Ugbogu 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study 1: Assessment of PAH in Different Land Uses in Urban Environment

3.1.1 Sample Collection

Polycyclic aromatic hydrocarbon polluted soil was collected from three different areas;

I. Spent engine oil polluted soil from Chukwuma Nwoha mechanic workshop.
II. Soil from meat roasting section of relief market.
III. Water and sediment sample were collected from urban city surface water, Nworie River in a plastic container.
IV. The unpolluted soil sample was collected from an Agricultural farm in Federal University of Technology Owerri (FUTO). The soil sample was collected at a range of 0-15cm deep using a spade into a sterile polyethylene bag, tied and properly labeled. The freshly collected sample was transported to the laboratory for both physical and chemical analysis.

3.1.2 Sample Preparation

The soil samples used were air dried under the sun for a period of 7-days. Thereafter, the soil sample was passed through a 2mm sieve and all the large particles were discarded. The fractions that pass through a 2mm sieve were fine soil, which was used for subsequent analysis. The soil analysis was carried out in the department of Agricultural Science laboratory, Federal University of Technology Owerri (FUTO).

3.1.3 Procurement of three benzo(a)pyrene, benzo(k)fluoranthene and benzo(ghi)perylene and seeds

The three PAH components (benzo(a)pyrene, benzo(k)fluoranthene and benzo(ghi)perylene) were sourced from Peninsula University, South Africa through Chemiscience laboratory, Owerri, Imo State. The seeds of *Telfairia occidentalis* and *Amaranthus hybridus* used in the study were procured from Ekeonunwa market in Owerri-municipal, Imo State, and were taken to the screen house at International Institutes of Tropical Agriculture (IITA), Ibadan.

3.2 Study 2: Analysis of the Physical Characteristics of Spent engine oil, combine effect of benzo(a)pyrene, benzo(k)fluoranthene and benzo(ghi)perylene Polluted and Unpolluted Soil

3.2.1 Determination of Soil Moisture Content

This was obtained based on the analysis related to oven dry using the method described by Messers, *et al.,* (2002). About 10g of the finely sieved soil was weighed and 5ml of 5% $NaPO_3$ in 100ml of purified water was added. The suspension was mixed vigorously for 15minutes and transferred into a 1 liter measuring cylinder. The temperature of the suspension was recorded in order to determine the settling of time for the fraction. The cylinder was covered with left palm and shaken vigorously by inversion 10times and allowed to settle for 4mins 15seconds at 25^{o}C. Pipette was lowered into the solution to the depth of 10cm and 25ml sample was withdrawn. The pipetted sample was transferred into a weighed moisture can and oven dried at 105^{o}C and weighed periodically until a constant weight was obtained.

$$\%\ Moisture\ in\ Soil = \frac{Wet\ soil\ (g) - Dry\ soil\ (g)}{Drysoil}\ x\ 100 \qquad (1)$$

3.2.2 Determination of Particle Size Distribution

The 40g of the air-dried soil of 2mm in size was measured into a 600ml beaker and 60ml of dispersing solution was added to it. This was enclosed inside a watch-glass and left overnight. The next day, the beaker content was measured and transferred into a soil-mixing cup, and filled with water, three quarter of the cup. The soil suspension was mixed at high frequency for 3minutes using the special stirrer. The mixing paddle was rinsed into a cup and allowed to settle for a minute. Thereafter, the suspension was quantitatively transferred into a hydrometer jar and brought to volume with distilled water (Messers *et al.,* 2002).

(a) Determination of Blank

Dilution of 60ml dispersing solution into a hydrometer jar with water was used to determine blank. The suspension in the hydrometer jar was carefully mixed, a hydrometer was inserted thereafter and reading, (R_b) was taken.

(b) Determination of Silt and Clay

A hydrometer jar was used to mix the soil suspension, using a special paddle. Then the paddle was carefully removed, and the immediate insertion of the hydrometer. Froth, on the surface of the jar was removed with the addition of one drop of amyl alcohol. The hydrometer reading was taken 40 seconds after removal of paddle. The hydrometer reading gave (R_{sc}).

Percentage silt plus clay was then calculated using the formula

$$\% \ [Silt + Clay] \ (w/w) = (R_{sc} - R_b) \ x \ \frac{100}{Ovendrysoil \ (g)} \qquad (2)$$

Where: R_c = Hydrometer reading before withdrawing paddle

R_{sc} = Hydrometer reading 40sec after withdrawing the paddle.

(c) Determination of Clay

The soil sample suspension in the hydrometer jar was mixed with paddle and removed from the hydrometer jar with care and the suspension was left undisturbed. After 4hours, the hydrometer was inserted and reading R_c taken. Percentage clay and silt in the soil was calculated using the formula below.

$$\% \ Clay \ (w/w) = (R_c - R_b) \ x \frac{100}{Ovendry \ soil \ (g)} \qquad (3)$$

Where: R_b = Hydrometer reading before withdrawing paddle

R_c = Hydrometer reading 4 hours after withdrawing the paddle.

% Silt *(w/w) = [%Silt + Clay] (w/w)] – [% Clay (w/w)]*

(d) Determination of Sand

After taking the reading for clay and silt, the suspension was measured and poured using a 50µm sieve. The sieve was then properly washed of any remnant. Then, sand was measured and transferred from sieve into 50ml beaker of a known weight. The sand was allowed to settle in the beaker and excess water was discarded.

The beaker containing the sand was dried overnight at 105^{o}C. This was then cooled in desiccators. Thereafter, it was re-weighed and percentage sand in the soil was calculated with the formula below.

$$\% \, Sand \, (w/w) = Sand \, weight \times \frac{100}{Oven-dry \, soil \, (g)} \quad (4)$$

Where: weight of sand was measured by subtracting the weight of the beaker from the weight of the soil and beaker.

Thus, the following: Sand weight (g) = [beaker + Sand (g)] – [Beaker(g)]

3.2.3 Determination of Bulk Density

Soil bulk density was conducted according to Fernandez & Lopez (2019). The sample was pressed not too far as compressing the soil in the confined space of the sampler. The content of the sampler was carefully removed in other to reserve soil structure and compartment. The two cylinders were then separated, retaining the undisturbed soil in the inner cylinder. The soil extended beyond each end with a straight edge knife. At this time, the soil sample volume was established to be the same as the volume of the sample holder. The soil was then transferred to a container, weighed and placed in an oven at 105^0C. The soil sample was weighed and reweighed again until a constant weight will be reached. The bulk density was calculated as the ratio of the oven dry mass of the soil sample to the sample volume.

Bulk density was calculated as

$l\beta = Ms/Vt$ (4)

Where:

Ms = Mass of oven dry soil (g)

Vt = total soil volume (cm^3) assumed to be equal to volume of cylinder and this was calculated from formula

$Vt = \pi r^2 h$

Where $\pi = 22/7$, r = radius and h = height.

3.2.4 Determination of Porosity

Porosity of the soil was calculated using bulk density using the method of Fernandez & Lopez (2019). The figure obtained from bulk density and particle density was used for calculating porosity. Porosity is then calculated using the formula.

Porosity (f) was calculate from bulk density value, thus

$$f = (1 - e_O/e_S) \times 100 \quad (5)$$

Where: f = Porosity

e_O= dry bulk density

e_S = particle density

3.2.5 Chemical Characteristics of the Polluted and Unpolluted Soil

3.2.5.1 Determination of Soil pH

This was determined according to the method of Al-Busaidi, Cookon & Yamamolo (2005).

A 20g of air dried soil was weighed into 50ml of glass beaker and addition of 20ml of distilled water using a calibrated cylinder. The suspension was thoroughly stirred with a glass rod and allowed to settle for 30minutes to maintain equilibrium. The suspension was stirred occasionally with glass rod. The pH meter electrode was inserted into the partially settled suspension (to a depth of 3cm) to measure the pH, and reading taken.

3.2.5.2 Determination of Soil Nitrogen

Nitrogen was determined via Macro Kjeldahl method as reported by Kalambe (2021). The 10g of air dried soil was weighed into 500ml macro kjeldahl flask and 20ml distilled water was added and swirled for 3 minutes and then allowed to stand for 30 minutes. A tablet of 1g of $K_2SO_4 – H_2O$ mixture catalyst and 10g of K_2SO_4 was added to the suspension. 30ml of concentrated H_2SO_4 was also added through an automatic pipette. The flask was heated cautiously at low heat on the digestion stand, when the water has been removed and frothing has ceased. The temperature was increased until the digest has cleared. Then the mixture was boiled for 5 hours. During boiling, the heating was regulated to prevent condensation of about half up to the neck of the flask. The flask was allowed to cool, then 100ml of water was added to the flask. The content in the flask was carefully transferred into another Macro-kjeldahl flask (750ml). All sand particles were retained in the original digestion flask. Sand residue was washed 4x with 50ml of distilled water and the aliquot was transferred into the same flask. 50ml of H_3BO_3 indicator solution was put into a 500ml Erlenmeyer flask which was placed on the condenser of the distillation apparatus. The 750ml of the Kjeldahl flask was attached to the distillation apparatus. Then 150ml of 10N NaOH was poured through the distillation flask opening the funnel stopcock and distillation process was commenced.

The condenser was kept cool by allowing sufficient cold water to flow through and regulate heat to minimize frothing and preventing suck back. 150ml distillate was collected and distillation was stopped.

NH_4-N in the distillate was determined by titrating with 0.01N standard HCl using a 25ml burrette graduated at 0.1ml intervals. The color change at the end point was green to pink.

3.2.5.3 Determination of Available Phosphorus

Available phosphorus was done according to Koralage, Weerasinghe, Silva & De Silva (2015). The 1g of air dried soil sample was weighed into 15ml centrifuge tube and 7ml of the extracting solution was added into it. This was shaken for 1 minute on a reciprocating shaker after which the suspension was centrifuged at 2000rpm for 15 minutes. 2ml of the clear supernatant was pipette out into a 20ml test tube. Then 5ml of distilled water and 2ml of ammonium molybdate solution was added. The content was properly mixed and 1ml of $SnCl_2.2H_2O$ dilute solution was added and mixed again.

After 5 minutes, the percentage (%) transmittance was measured on the electrophotometer (fisher scientific electrophotometer ii model 81) at a wavelength of 660nm. The standard curve was prepared with 1µgP/ml (or ppm P). The optical density (OD) of the standard deviation was plotted against µgP/ml (or ppm P) and the content of the extractable P in soil was calculated.

3.2.5.4 Determination of Organic Carbon and Organic Matter

This is the method of wet digestion by Poudel (2020). A representative sample was taken, grinded and passed through a 0.5mm sieve. Soil sample was weighed out in duplicates and transferred to 250ml Erlenmeyer flask. 10ml of 1N $K_2Cr_2O_7$ solution was pipette accurately into each flask and swirled gently to disperse the soil. A 20ml of concentrated H_2SO_4 was added rapidly using an automatic pipette, directing the stream into the suspension. Immediately the flask was gently swirled until soil and reagent are mixed and then swirled vigorously for 1 minute. The flask was allowed to stand on a sheet of asbestos for about 30 minutes. 3 drops of indicators were added and titrated with 0.5 $FeSO_4$ solutions. As the end point approached, the solution turned to greenish cast and then changes to dark green. At this point, $FeSO_4$ was added in drops until color change occurs sharply from green to red. Blank titration was also made in the same manner, but without soil to standardize the dichromate. The result was calculated using the formula;

$$\% \, organic \, C = \frac{(MeK_2Cr_2O_7 - FeSO_4) \times 0.003 \times 100 \times f}{Weight \, of \, air \, dry \, soil} \quad (6)$$

Where Correction factor, f, =1.33

Me = Normality of solution x ml of solution used.

% Organic matter was calculated by multiplying with Van Denmelen's correction factor.

% organic matter = % organic carbon x 1.724

Where: 1.724 = Van Denmelen's correction factor.

3.2.5.5 Determination of Total Available Bases, and Effective Cation Exchange Capacity (ECEC)

This was carried out using the method of Pilarski, Kaliszan, Wyrzykowski & Mlodzianowski (2015). The 30ml of 1N NH_4OAC was added into 5g of air dry soil and this was shaken on a mechanical shaker for 2 hours. The suspension was centrifuged at 2000rpm for 10mins, the clear supernatant was carefully decanted into a 100ml volumetric flask.

Another 30ml of NH_4OAC solution was added and shaken for 30 minutes, centrifuge and the supernatant were transferred into the same volumetric flask. The solution was made up to mark by adding more NH_4OAC solution. K and Na were determined on a flame photometer (bulk scientific PFP-7) while Mg and Ca was determined on an atomic absorption spectrometer (bulk scientific model 210).

Effective cation exchange capacity was thus calculated by summing up the exchangeable bases (Ca, Mg, K, Na) and exchangeable Al and H.

Blends of soil and polycyclic aromatic hydrocarbon was developed at 3 levels each with the following rates of PAH; 10%, 20%, and 30% as shown in Table 3.1. Each treatment was replicated thrice. This brought the total experimental pots to 27. These will be arranged in a completely randomized design (CRD) (Gomez & Gomez, 1984).

3.2.5.6 Determination of Available Acidity

Available acidity was carried out using the method of Koralage *et al.,* (2015). The 5g of air dried soil was weighed and transferred into a 50ml centrifuge tube. A 30ml of 1N potassium Chloride was added to each centrifuge tube and covered tightly with a rubber stopper. The centrifuge tube

and its contents will be shaken intermittently for one hour using a silent shake. Thereafter, the centrifuge was centrifuged at 2000rpm for 15 minutes. The clear supernatant was carefully transferred into a volumetric flask and titrated with 1N HCl using 3 drops of Bromocresolgren indicator. The color change was from initial deep yellow – green due to the combine color paranitrophenol, became colorless and more addition of indicator and a few drops of acid turned it yellow. After that, exchangeable acidity was calculated using the formula;

$$\text{Exchangeable acidity (Meq /100g)} = (T - B) \times \frac{E}{A} \times \frac{100}{W} \times N \qquad (7)$$

Where:

T = ml of NaOH sample
E = Extract volume
A = Aliquot volume taken
W = weight of soil used
N = Normality of base

3.3 Study 3: Performance of *Amaranthus hybridus* and *Telfairia occidentalis* on Soil Polluted with Polycyclic Aromatic hydrocarbons

3.3.1 Raising of Seedlings

Seedlings were raised from the selected crop plant (*Telfairia occidentalis and Amaranthus hybridus*) seeds on a sandy loamy nursery bed ($1 \times 3m^2$) at the nursery section of IITA, Ibadan. The nursery bed was kept moist by adding water when necessary. Seedlings were allowed to grow on the nursery for 2 weeks before they were transplanted and subjected to the treatment.

3.3.2 Blending of Soil and PAH

Table 3.1 Experimental Design

Treatments	Measurement
Treatment 1(spent engine oil)	100ml SEO 10kg Soil
	200ml SEO 10kg Soil
	300ml SEO 10kg Soil
Treatment 2 (PAH)	20mg/l B(a)P 2kg Soil
	20mg/l B(k)F 2kg Soil
	20mg/l B(ghi)P 2kg Soil

The soil samples and PAH were mixed thoroughly (Amadi & Bari, 2010). Soil of 10kg was placed in perforated plastic buckets and stored in screen house in the IITA, Ibadan.

3.3.3 Growth Experiment

All growth experiments were conducted in a controlled environment in the screen house located at IITA, Ibadan. Plantlets of equal height (10cm) were selected and used for the study. Three seedlings of each species were planted in each of the perforated plastic bucket representing each treatment (Table 3.1) and this was replicated three times. Similarly, the two-plant species were also be planted in the spent engine oil (SEO) and PAH polluted soil and unpolluted soil. The unpolluted soil sample served as control and was replicated thrice. The plastic buckets containing the experimental plant were thereafter set up on a bench top in the screen house, in a completely randomized design. The temperature and relative humidity of the screen house was maintained at $20 \pm 0.25^{o}C$ and $79.79 \pm 4.07\%$ respectively.

3.3.3.1 Growth Performance Parameters

Growth parameters such as number of leaf, plant height, leaf area, fresh matter weight, and dry matter weight etc. were determined at 7days interval starting from the day of transplant (Pandey, Paul, Das, meena & Meena 2017). 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 and recorded.

3.3.3.2 Number of leaves

The number of leaves on the *Telfairia occidentalis* and *Amaranthus hybridus* were counted on weekly basis for each plant stand and the average number of leaves was calculated and recorded.

3.3.3.3 Plant Height (cm)

Plant height was measured using a meter rule. It was measured from the base of the stem to the tip of the plant (AOAC, 2005).

3.3.3.4 Leaf Area (cm^3)

Leaf area was measured using meter rule. This was carried out by tracing the outline of a leaf on a graph sheet of paper, and then counting the number of square centimeters covered (Pandey *et al.,* 2017). The leaf area was measured by multiplying the length and the width, while the total leaf area was calculated using the formula

Leaf area = length × width

3.3.3.5 Fresh and Dry Matter Weight (g)

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 Mettle PM 34) to determine the fresh matter weight. After weighing, the plants was labelled accordingly and then air dried at room temperature for 14 days and then weighed again.

3.4 Study 4: Bioavailability of Three Different Polycyclic Aromatic Hydrocarbon (PAH) Components and various Concentrations of Spent Engine Oil to Soil and Crop Plants

3.4.1 Determination of PAH from Soil Samples

This was done according to the method of Snezana, Marjanovic, Mira & Suturovic (2010). A 10g each of homogenized soil was extracted in a soxhlet apparatus for 24 hours using 300ml of n-hexane. The extract was mix with 60g of anhydrous sodium sulphate in agate mortar to absorb moisture. The homogenate was place into a 500ml beaker. Crude extract obtained was evaporated using the rotary vacuum evaporator at 40^{o}C and 48 mbar to dryness. Residue was transferred with n-hexane onto a 5ml flosiril column for clean-up. The clean-up was done with florisil heated in an

oven at 130^0C overnight and transferred to a 250ml size beaker and placed in a desicator. A 0.5g anhydrous $NaSO_4$ was added to 1.0g of activated florisil (magnesium silicate) (60-100nm mesh) on an 8ml column plugged with glass wool. Packed column was filled with 5ml n-hexane for conditioning. Stopcock was opened to allow n-hexane run out until it reaches top of sodium sulphate into a receiving vessel whilst tapping the top of the column gently till the flosiril settle well in the column. Extract was then transferred onto the column with disposable Pasteur pipette from an evaporating flask. The evaporating flask was rinsed twice with 1ml portions of n-hexane and added to the column. Eluate was collected into an evaporating flask and rotary evaporated to dryness. Dry eluate was then dissolved in 1ml n-hexane for PAH chromatographic analysis. 20µL aliquot was injected into the Buck M910 gas chromatography (GC) equipped with an on-column, automated injector, flame ionization detector, HP 88 capillary column (100m x 0.25µm film thickness), CA, and USA. Detected temperature 250˚C and injection temperature 22˚C.

3.4.2 Determination of Polycyclic Aromatic Hydrocarbons (PAH) in Plant Sample

PAH extraction from plant tissues was carried out using the method of Adekule, Oyekunle, Ola, Obisessan & Maxakato (2018). The pulverized plant tissues were freeze-dried and then extracted for 1 hr using an acetone and Hexane mixture (vol/vol = 1:1), followed by 1 hr of ultrasonic extraction. This acetone/hexane extraction step was repeated thrice. The solvent will be evaporated using a rotary evaporator and exchanged to 2 mL hexane followed by a clean-up procedure through a 2g silica gel column using 11µL, 1:1 (v/v) elution of hexane and dichloromethane. The samples were then evaporated and exchanged to ethanol with a final volume of 2mL. PAH was analyzed using GC equipped with flame ionization detector. The UV detector wavelength was set at 254 nm. The mobile phase was spectro pure methanol with a flow rate at 1.0mL/min and the column temperature will be 30ºC.

The bioavailability of PAH in the soil can be assessed using the following formula

Bioaccumulation factor (BAF)

$$= \frac{PAH\ concentration\ in\ plant\ tissue}{Initial\ concentration\ of\ PAH\ in\ substrate\ (soil)} \quad (8)$$

BAF can be used to determine the quantity of PAH absorbed by plant from the soil (i.e. the concentration of PAH that is bioavailable to the plant). This is an index of the ability of the plant to accumulate PAH with respect to its concentration in the soil.

3.5 Study 5: Genomic Effects of Spent engine oil, Benzo(a)pyrene, Benzo(k)fluoranthene, Benzo(ghi)perylene on *A. hybridus* and *T.* occidentalis

3.5.1 DNA Extraction of exposed and unexposed plants samples

Young leaves of the plant samples were collected from the screen house and transferred to the laboratory in an ice box and stored at -20^{o}C. Samples were prepared by putting two steel balls and approximately 100mg of lyophilized tissues into 2ml Eppendorf tube. Lyophilized plant tissues were grinded into fine powder by using genogrinder-2000 for 30-40seconds at 1500rpm. 700µl of SDS extraction buffer was added into the samples and mixed gently to homogenize. Incubation in water bath was done at 65^{0}C for 20 minutes and mixed gently by rocking intermittently. Tubes were removed from water bath and allowed to cool for 3-5 minutes and 200µl of 5M ice cold potassium acetate was added and mixed gently. Incubation on ice was done for 20 minutes and 700µl of chloroform isoamyl alcohol (24:1) was added and mixed gently by continuous inversion. Centrifugation was done at 10,000rpm for 10 minutes. Supernatant was aliquot into a new Eppendorf tube. Ice cold isopropanol of 700µl was added into each sample and mixed gently. Samples were then incubated at -80^{0}C for 15 minutes and then centrifuged at 10,000rpm for 10 minutes. Supernatants were decanted and 500µl of ethanol was added and centrifuge at 10,000rpm for 10 minutes. Ethanol was decanted in each sample and air dried. DNA was then resuspended with 5% RNase and 95% autoclaved water (Dharajiya, Khadia, Pagi, Khatrani, Jasani Khunt *et al*., 2017).

3.5.2 DNA Quantification of Exposed and Unexposed Plants Samples

DNA quantification was done according to the method of Garcia-Alegria, Corona, Perez-martinez, Corella-Madueno, Duran & Garcia (2020). DNA quantification was carried out using Nanodrop 2000 spectrophotometer. The nanodrop spectrophotometer was first blanked with autoclave water and wiped properly with soft tissues in other to eliminate false result. The concentration and purity of the DNA was determined by putting 2µl of the DNA form each sample at a time on the nanodrop machine. This then measures the absorbance of the diluted DNA solution at 260/280nm.

3.5.3 Gel Electrophoresis of DNA Exposed and Unexposed Plants Samples

This was done using the method of Lee, Costumbrado, Hsu & Kim (2012). A 1% agarose was dissolved in 100ml 0f SBE buffer and microwaved at 650 wavelengths for 3 minutes until a clear crystal solution was seen. The gel was then cooled down under running tap water. Ethidium bromide of 14µl was added into the gel and mixed gently to allow visibility. The gel was cast on the already prepared tray containing the comb and base. The gel was left to solidify for 30 minutes. The comb was gently removed inside the electrophoretic chamber containing SBE buffer solution to ensure that the wells were not damaged. DNA of 6µl from each sample were gently loaded into the wells. After loading, the electrophoretic chamber was then closed and connected to power supply. The gel was run at 100V for 45 minutes and the DNA bands was photographed under aplegen UV light transilluminator.

3.5.4 PCR Amplification Using ISSR Marker on Exposed and Unexposed Plants Samples

PCR amplification was done using ISSR marker according to (Gelotar, Dharajiya &Tiwari 2019; Stefunova *et al*. 2015). PCR reaction was carried out in a volume of 25µl containing 3µl template DNA, 2.5µl PCR buffer, 2.0µl of dNTPs, 1.0µl $MgCl_2$, 1.0µl $DMSO_4$, 2µl of ISSR primer, 0.06µl of Taq DNA polymerase and 13.44µl of nuclease free water. The primers used for the PCR amplification include UBC 811, UBC 827 and UBC 808 respectively. The PCR amplification was performed as follows: initial denaturation (at 94^{o}C for 1mins), annealing (at melting temperature of 50^{0}C for 1min), extension (at final extension at 72^{o}C for 3mins and then cool down to 4^{o}C. Amplification will be performed in a mycycler (Biorad). Each reaction was repeated three times. The PCR product was separated by electrophoresis on 1.5% agarose gel containing 14µg ethidium bromide in a 1xSBE buffer. The molecular ladder used was 50bp. The gel was run at 100v for 1hr 30min and then photographed under aplegen UV light using transilluminator.

The bands from the PCR products were scored using 1 and 0 to indicate its presence and absence which was used in the formulation of binary matrix. Polymorphism in the band was calculate using the formula (Darinka, Tatjana, Tatjana, Katerina and TrajIe, 2012) below;

$$P = [\frac{a+b}{c}] \times 100 \quad (9)$$

Where; P = Polymorphism

a = number of newly appeared bands

b = number of disappeared bands

c = total number of bands

3.6 Study 6: Human Health Risk Assessment of Exposure to Spent Engine Oil, Benzo(a)pyrene, Benzo(k)fluoranthene and Benzo(ghi)perylene

3.6.1 Health Risk Assessment Model

To assess the health risks associated with polycyclic aromatic hydrocarbons (PAHs), through the consumption of the PAH contaminated crop plants fluted pumpkin and green. A deterministic model proposed by USEPA was employed to assess the potential human health risks posed by PAH. By this method benzo(a)pyrene is used as a marker for the occurrence and effect of carcinogenic PAHs in foods and, therefore, the overall carcinogenic health risk from the measured PAH was estimated based on the Toxic equivalent factors (TEFs) derived from the cancer potencies of individual PAH compounds relative to the cancer potency of benzo(a)pyrene equivalent concentration (B[a]Peq) for each PAH (Okereke, Essien & Wegwu 2016). The incremental lifetime cancer risk (ILCR) was used to evaluate non-carcinogenic health risks.

Benzo(a)pyrene equivalent was calculated using the formula stated in Eq. 10

$$\text{B(a)PeqC} = \sum(TEF\ x\ Conc) \quad (10)$$

Where B(a)PeqC = Benzo(a)pyrene equivalent concentration

TEF = Toxic equivalent factor

$$\text{SV} = \frac{\left(\frac{RL}{SF}\right) x\ BW}{CR} \quad (11)$$

Where: SV = Screening vaue

RL = Maximum acceptable risk level (0.000001)

SF = USEPA oral slope factor (0.0073µg/kg/d-adult/0.0061-children)

BW = Body weight (adult = 60kg/ children = 35kg)

CR = Consumption rate (g/day)

3.6.2 Dietary Daily Intake (EDI): The dietary daily intake of PAH contaminated vegetables through consumption was calculated by multiplying the respective PAHs concentration in each

plant by the weight of the plant consumed by an average individual. Total dietary intake of B(a)Peq was calculated by multiplying concentrations for each sample with the ingestion rate (IR) of 0.5kg (Fang *et al.*, 2014). The daily dietary exposure dose level (ED) was calculated in equation 12 below. The dietary daily intake was calculated based on Halek, Nabi & Kavousi (2007).

$$ED = DDIB(a)Peq = B(a)Peq \times IR \quad (12)$$

Where: ED = Exposure dose

DDI B(a)Peq = Dietary daily intake of Benzo(a)pyrene equivalent

B(a)Peq = Benzo(a)pyrene equivalent

IR = Ingestion rate

The incremental life time cancer risk (ILCR) was calculated as described by Xia, Duan & Qiu (2010). The incremental life time risk caused by PAH dietary exposure was calculated using the following equation.

$$ILCR = \frac{ED \; x \; EF \; x \; EDB[a]Peq \; x \; SF \; x \; CF}{BW \; x \; AT} \quad (13)$$

Where; ILCR = is measured in µg/kg body wt/d

ED = exposure duration = life expectancy (70yrs)

EF = exposure frequence (365days/yr)

EDB[a]Peq = exposure dose for B[a]P

SF = oral slope factor of benzo[a]pyrene (0.0073µg/kg/d-adult/0.0061-children)

CF = conversion factor

BW = body weight (adult = 60kg/ children = 35kg) (Tongo, Ogbeide & Ezemonye 2017)

AT = average life span (70yrs)

Margin of exposure was evaluated to determine the genotoxic and carcinogenic levels of the PAH. This is an acceptable method of risk assessment approved by the Joint FAO/WHO expert committee on food additives (JECFA) and European food safety authority (EFSA) (Wu, Xia, Zhang, Yin, Zhou & Yang 2016).

$$MOE = \frac{BMDL \; x \; BW}{DDI} \quad (14)$$

Where: MOE = Margin of exposure

BMDL = Bench mark dose level

BW = Body weight (60kg for adult/35kg for children)

EED = Estimated exposure dose

3.7 **Statistical Analysis**

Data collection for all parameters was subjected to analysis of variance (ANOVA). Where least significant difference (LSD) was established among means using Dunnet multiple comparison. They were separated using Minitab statistical software (2017). Genomic analysis was done using fingerprint analysis of missing data (FAMD) software version 1.31β.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Different PAH Component in a Typical Urban Environment

The results of the different PAH components obtained from different land use and surface water in a typical urban city center are presented in Table 4.1. Results obtained showed that different concentrations of PAHs were present in the samples analyzed. Elev en (11) chemical components of PAH were detected in water sample from an urban surface water, compared to those obtained from other land uses. These PAH include acenaphthylene, phenanthrene, 1-2 benzanthrene, acenaphthene, benzo (k) fluoranthene, benzo (a) pyrene, fluorene, naphathalene, dibenzyl (a-h) anthracene, anthracene and benzo (g-h-i) perylene. Of all the PAH obtained in water sample, acenaphthylene and phenanthrene had the highest value (0.8737mg/ml and 0.6850mg/ml), compared to other substances, however, anthracene had the least value (0.0030mg/ml). Sediment sample obtained from urban surface water showed seven (7) PAHs components. These include; acenaphthylene, phenanthrene, 1-2 benzanthrene, acenapthlene, benzo (k) fluoranthene, benzo (a) pyrene and benzo (b) fluoranthene. These PAH components were also found in the water body. Apart from acenapthalene and phenanthrene that were higher in the water body, every other PAH found in the sediment were higher than that obtained in water body.

Spent engine oil polluted soil (SEOPS), contained nine (9) different PAH components. These individual components include; acephthylene, phenathrene, 1-2 benzanthrene, benzo (k) fluoranthene, benzo (a) pyrene, benzo (b) fluoranthene, naphthalene, benzo (g-h-i) perylene and fluoranthene, with fluoranthene having the highest value of 2.25mg/ml and phenanthrene having the least value (0.16mg/ml). Spent engine oil polluted soil had the highest cumulative total concentration of 6.04mg/ml compared to the total concentration obtained from other samples. Furthermore, the low molecular weight (LMW) PAHs (acenaphthalene, phenanthrene, acenaphthalene, fluorene, naphathalene and anthracene) where observed to occur more in water samples, while the high molecular weight (HMW) PAHs (benzo (k) fluoranthene, benzo (a) pyrene, benzo (b) fluoranthene, benzo (g-h-i) perylene and fluoranthene) occurred more in spent engine oil polluted soil, compared to the different PAHs compounds obtained from other environmental components.

Kanda processing soil (KPS) had the least cumulative total (PAH) concentration (1.0701mg/ml). Only five (5) individual PAH compounds were detected in KPS, these include; acenaphthylene, 1-2 benzanthene, benzo (k) fluoranthene, benzo (g-h-i) perylene and pyrene. Pyrene which is a high molecular weight PAH was only found present in KPS. Acenaphthalene, 1-2 benzathene, and benzo (k) fluoranthene were the only PAH components detected in all the environmental components.

It was observed that four (4) out of the eight carcinogenic PAH recognized by USEPA were detected in spent engine oil polluted soil when compared to other environmental components. This include benzo(k)fluoranthene, benzo(a)pyrene, benzo(b)fluoranthene and fluoranthene. Out of all the carcinogenic PAH obtained from spent engine oil polluted soil, fluoranthene and benzo(a)pyrene had the highest value (2.25mg/ml and 0.281mg/ml) respectively. In sediment sample, the carcinogenic PAH detected include benzo(k)fluoranthene, benzo(a)pyrene and benzo(b)fluoranthene, with benzo(b)fluoranthene having the highest value of 0.44mg/ml. Benzo(k)fluoranthene, benzo(a)pyrene, benzo(a)pyrene and dibenzyl (a-h) anthracene were detected in water samples with dibenzy (a-h) anthracene having the highest value (0.31mg/ml).

Table 4.1: PAHs concentration from different land use in a typical urban environmental component.

PAH Components	SUSW(mg/ml)	USW(mg/ml)	SEOPS(mg/ml)	KPS(mg/ml)
Acenaphthylene	0.5302 ±0.010	0.8737 ±0.108	0.1720 ±0.051	0.4983 ±0.014
Phenanthrene	0.5489 ±0.111	0.6850 ±0.101	0.1619 ±0.052	ND
1-2 Benzanthrene	0.2141 ±0.016	0.0222 ±0.141	0.3219 ±0.077	0.0007 ±0.000
Acenaphthene	0.3788 ±0.119	0.0047 ±0.001	ND	ND
Benzo (k) fluoranthene	0.1810 ±0.031	0.1801 ±0.007	0.1643 ±0.057	0.1294 ±0.026
Benzo (a) pyrene	0.1950 ±0.097	0.1246 ±0.019	0.2806±0.028	ND
Benzo (b) fluoranthene	0.4371 ±0.047	ND	0.2391 ±0.040	ND
Fluorene	ND	0.0048 ±0.001	ND	ND
Naphthalene	ND	0.0047 ±0.000	0.2607 ±0.122	ND
Dibenzyl (a-h) anthracene	ND	0.3067 ±0.153	ND	ND
Anthracene	ND	0.0030 ±0.001	ND	ND
Benzo (g-h-i) perylene	ND	0.2016 ±0.064	0.1871 ±0.023	0.2627 ±0.078
Fluoranthene	ND	ND	2.2493 ±0.110	ND
Pyrene	ND	ND	ND	0.1789 ±0.007
Cumulative Total Mean Concentrations	2.4851 ±0.431	2.6032 ±0.596	6.0369 ±0.560	1.0701 ±0.125

Legend: ± Standard deviation; n = 14. ND = not detected; SUSW: Sediment from urban surface water; USW: Urban surface water; SEOPS: Spent engine oil polluted soil; KPS: Kanda processing site.

The different PAH concentrations obtained from the different land use and surface water of urban environment was compared with the United State environmental protection agency (USEPA) threshold limit of PAH in water and sediments, and the classification of PAH as strong, weak and non-carcinogenic as shown in Table 4.2. Carcinogenic PAH identified in surrounding urban environment include; benzo(k)fluoranthene, benzo(b)fluoranthene and dibenyl(a, h)anthracene. Of all the three PAH benzo(k)fluoranthrene was found in all the different land use and water body in higher quality. The kanda processed soil did not record any benzo(a)pyrene and benzo(b)fluoranthrene. Benzo(a)pyrene which was classified as strongly carcinogenic was also obtained in water, sediment and spent engine oil polluted soil in quantities greater than the standard limit of USEPA (2006). Fluoranthene which is a weak carcinogenic substance was also obtained in spent engine oil polluted soil.

The United State environmental protection agency (USEPA) threshold limits of PAH in water and sediment for the protection of aquatic life are present in Table 4.2.

Table 4.2: Quality threshold limits of PAH in water and sediment for the protection of aquatic life

PAH Component	Carcinogenicity	Water mg/ml	Sediment (μg/g)		
		WOG ($x10^{-5}$)	ISOG	PEL	FSSB
Acenaphthylene	NC	-	0.00587	0.12800	0.0059
Phenanthrene	NC	0.4	0.04190	0.51500	0.2040
1-2 Benzanthrene	NC	-	-	-	-
Acenaphthene	NC	5.8	0.00671	0.08890	0.0067
Benzo (k) fluoranthene	C	-	-	-	0.2400
Benzo (a) pyrene	SC	0.015	0.03190	0.78200	0.1500
Benzo (b) fluoranthene	C	-	-	-	0.0272
Fluorene	NC	3.0	0.02120	0.14400	0.0774
Naphthalene	NC	1.1	0.03460	0.39100	0.1760
Dibenzyl (a-h) anthracene	C	-	0.00622	0.13500	0.0330
Anthracene	NC	0.12	0.04690	0.24500	0.0572
Benzo (g-h-i) perylene	NC	-	-	-	0.0170
Fluoranthene	WC	0.04	0.11100	2.35500	0.4230
Pyrene	NC	0.025	0.05300	0.87500	0.1950

NC: Non-Carcinogenic, C: Carcinogenic, WC: Weakly Carcinogenic, SC: Strong Carcinogenic. ISQG: Interim Sediment Quality Guideline. PEL: Probable Effect level. FSSB: Freshwater Sediment Screening Benchmark. WOG: Water Quality Guideline. Adapted from USEPA 2006.

4.1.2a Physical Properties of Spent Engine Oil Polluted and Unpolluted Soils as Source of PAH

The soil physical properties of spent engine oil polluted and unpolluted soils are presented in Figure 4.1. Results obtained showed that the percentage moisture content and bulk density were higher in the polluted soil compared to the unpolluted soil. However, the result of porosity showed little difference, as the values obtained in the unpolluted soil were higher than those obtained from the polluted soil samples. The percentage soil particle size distribution shows that there were variations in the percentage sand, silt and clay distribution for the polluted and unpolluted soil sample. Unpolluted soil samples recorded higher values in clay and silt in the range of 2.46 and 5.1 respectively compared to the polluted (1.71 and 3.50). Further, the soil particle size distributions showed higher values of sand in the polluted and unpolluted samples as 87.44 and 90.62 respectively. This shows that the soil textural class for both polluted and unpolluted soil samples were sandy.

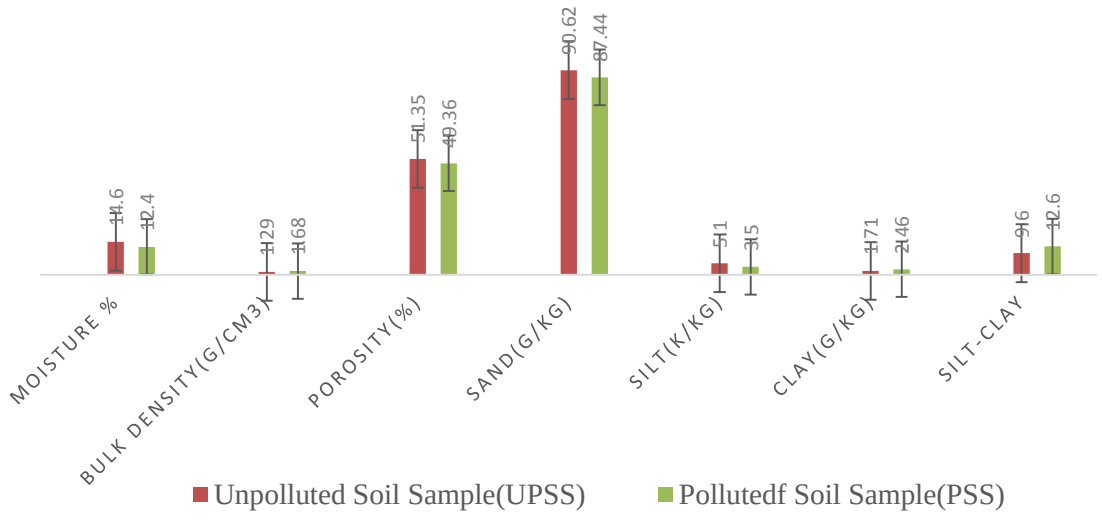


Figure 4.1: Physical properties of polluted and unpolluted soil sample.

LEGEND: PSS = Polluted soil sample
UPSS = Unpolluted soil sample.

4.1.2b Chemical Properties of Spent Engine Oil Polluted and Unpolluted Soils as Source of PAH

Soil chemical properties of spent engine oil polluted and unpolluted soils are presented in Figure 4.2. Results of soil pH values showed the polluted to be lower (5.1) compared to the unpolluted, which was higher (7.4). The ECEC was higher in the polluted (9.27g/kg) compared to the values obtained in the unpolluted (7.36) soil samples. Organic carbon and organic matter content where higher in spent engine oil polluted soil compared to that obtained from unpolluted soil. The percentage nitrogen content was higher (0.429%) in the spent engine oil polluted soil compared to the unpolluted soil (0.294%). Available phosphorus was higher in the unpolluted soil (28mg/kg) but reduced in the polluted (19mg/kg). Generally, the result showed, exchangeable acidity, exchangeable base, organic matter and organic carbon values to be higher in spent engine oil polluted soil samples compared to the unpolluted which had higher values in pH, effective cation exchange capacity and available phosphorus.

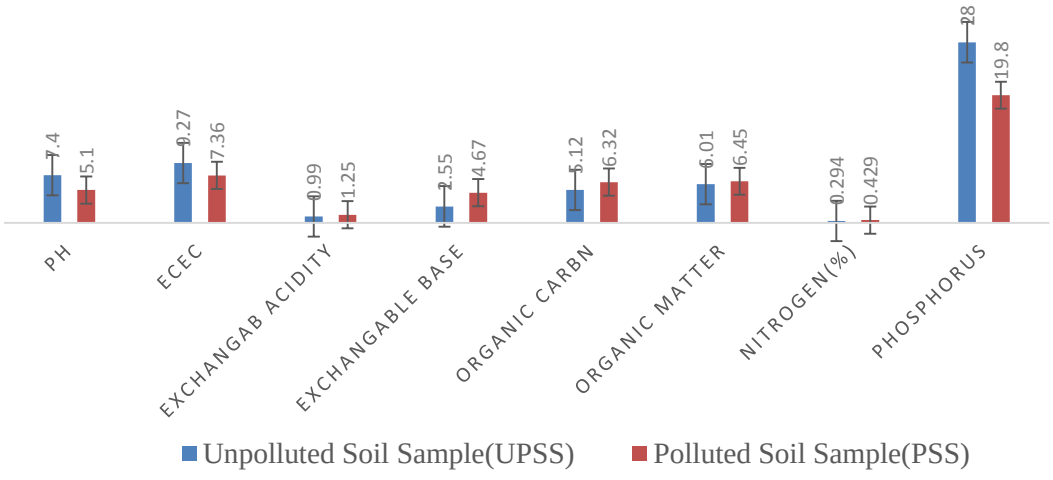


Figure 4.2: Chemical properties of polluted and unpolluted soil samples.

LEGEND: PSS = Polluted soil sample
UPSS = Unpolluted soil sample
ECEC = Effective cation exchange capacity.

4.1.3a Growth Performance of *Amaranthus hybridus* on Polluted and Unpolluted after Seven (7) Days

The growth performance of *A. hybridus* after 7 days of exposure to benzo(a)pyrene, benzo(k)fluoranthrene, benzo(ghi)perylene and the different concentrations of spent engine oil polluted soil is presented in Table 4.3. Results obtained showed that plants grown in benzo(ghi)perylene and benzo(k)fluoranthrene polluted soils had the highest plant height. The least values of plant height were obtained in soils polluted with the highest concentrations of spent engine oil (SEOPS C). The study showed high values in the number of leaves, leaf area, fresh and dry matter weight for benzo(ghi)perylene, and soil polluted with low concentration of spent engine oil (SEOPS A) compared to that polluted with high concentrations of spent engine oil and those of benzo(a)pyrene and benzo(k)fluoranthrene. The least values in number of leaves, leaf area, dry and fresh weight were obtained from SEOPS C (soils polluted with high concentrations of spent engine oil) and benzo(a)pyrene.

Furthermore, the result revealed that data obtained from unpolluted soil samples were significantly different ($P<0.05$) from that obtained from different PAH component used and the various concentrations of spent engine oil applied. However, no significant difference was obtained from the values of fresh wet matter of SEOPS A compared with fresh and dry weight matter of benzo(ghi)perylene.

Table 4.3: Growth performance of *A. hybridus* in benzo(a)pyrene, benzo(k)fluoranthrene benzo(ghi)perylene and different concentrations of Spent engine oil after 7days of exposure.

Parameters	**UPS**	**B(a)P**	**B(k)F**	**B(ghi)P**	**SEOPS A**	**SEOPS B**	**SEOPS C**
Plant Height(cm)	12.7 ±1.39[a]	8.7 ±0.23	10.1 ±0.31	10.2 ±0.66	9.3 ±0.58	8.6 ±0.51	8.2 ±0.20
Leaves Number	7 ±0.58[a]	4 ±0.58	4 ±1.16	5 ±1.16[a]	5 ±0.58[a]	4 ±0.58	4 ±0.58
Leave Area(cm^3)	4.3 ±0.27[a]	1 ±0.44	1 ±0.44	2.8 ±0.15	2.4 ±0.40	1.3 ±0.20	0.7 ±0.20
Fresh Weight(g)	0.25 ±0.09[a]	0.09 ±0.02	0.09 ±0.02	0.2 ±0.02[a]	0.17 ±0.04[a]	0.09 ±0.04	0.06 ±0.03
Dry Weight(g)	0.07 ±0.05[a]	0.01 ±0.00	0.01 ±0.00	0.03 ±0.014[a]	0.02 ±0.006	0.002 ±0.006	0.005 ±0.003

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

LEGEND:

±Standard deviation; UPS: Unexposed plant species

B(a)P: Benzo(a)pyrene polluted plant

B(k)F: Benzo(k)fluoranthrene polluted plant

B(ghi)P: Benzo(ghi)perylene polluted plants

SEOPS A: 100ml spent engine oil polluted samples

SEOPS B: 200ml spent engine oil polluted sample

SEOPS C: 300ml spent engine oil polluted sample

4.1.3b Growth Performance of *Amaranthus hybridus* on Polluted and Unpolluted Soil after Fourteen (14) Days

The growth performance of *A. hybridus* after 14days of exposure to benzo(a)pyrene, benzo(k)fluoranthrene, benzo(ghi)perylene and various concentrations of spent engine oil polluted and unpolluted soil of is shown on Table 4.4. The result obtained showed higher plant height of benzo(ghi)perylene and benzo(k)fluoranthrene polluted and unpolluted soil. The least value of plant height was observed in polluted soil with the highest concentration of spent engine oil (SEOPS C). Furthermore, there was an increase in the number of leaves, leaf area, fresh and dry matter weight of benzo(ghi)perylene, benzo(k)fluoranthrene and SEOPS A polluted soil samples. However, the least number of leaves, leaf area, and fresh and dry matter weight was observed in SEOPS C.

The result from the unpolluted soil samples were significant different from that obtained from the different PAH components used and the various concentrations of spent engine oil applied. However, there was no significant difference obtained from fresh matter of benzo(ghi)perylene, benzo(k)fluoranthrene, benzo(k)fluoranthrene and SEOPS A polluted samples. Also, there was no significant difference in the dry matter weight of benzo(ghi)perylene and SEOPS A with that of the unpolluted sample.

Table 4.4: Growth performance of *A. hybridus* in different concentrations of Spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene after 14days of exposure.

Parameters	**UPS**	**B(a)P**	**B(k)F**	**B(ghi)P**	**SEOPS A**	**SEOPS B**	**SEOPS C**
Plant Height(cm)	14.3 ±0.03[a]	10.3 ±1.31	11.7 ±0.27	12.4 ±0.56	10.3 ±0.25	8.9 ±0.59	8.5 ±0.20
Leaves Number	9 ±1.00[a]	5 ±0.00	7.0 ±0.58	7 ±1.16	7.0 ±0.00	6 ±0.58	3 ±0.58
Leave Area(cm^3)	6.5 ±0.25[a]	1.60 ±0.27	3.1 ±0.40	4.2 ±0.62	4 ±0.31	2.4 ±0.35	1.4 ±0.10
Fresh Weight(g)	0.48 ±0.22[a]	0.12 ±0.03	0.27 ±0.03[a]	0.34 ±0.05[a]	0.28 ±0.07[a]	0.13 ±0.07	0.07 ±0.08
Dry Weight(g)	0.07 ±0.02[a]	0.02 ±0.003	0.03 ±0.01	0.05 ±0.03[a]	0.04 ±0.015[a]	0.02 ±0.006	0.006 ±0.005

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

LEGEND:

±Standard deviation; UPS: Unexposed plant species

B(a)P: Benzo(a)pyrene polluted plant

B(k)F: Benzo(k)fluoranthrene polluted plant

B(ghi)P: Benzo(ghi)perylene polluted plants

'a': Shows significant difference

SEOPS A: 100ml spent engine oil polluted samples

SEOPS B: 200ml spent engine oil polluted sample

SEOPS C: 300ml spent engine oil polluted sample

4.1.3c Growth Performance of *Amaranthus hybridus* on Polluted and Unpolluted Soil after Twenty-One (21) Days

The growth performance of *A.hybridus* after 21 days of exposure to benzo(a)pyrene, benzo(k)fluoranthrene, benzo(ghi)perylene and the different concentrations of spent engine oil polluted and unpolluted soil is depicted on Table 4.5. The result obtained showed that there was a decrease in plant height grown in soil polluted with different concentrations of spent engine oil (SEOPS B & SEOPS C), benzo(a)pyrene and benzo(k)fluoranthrene after 21 days of exposure when compared to that of 7days and 14days of exposure. Except in benzo(ghi)perylene and SEOPS A polluted soil. Furthermore, there was a decrease in the number of leaves, leaf area, fresh and dry matter weight of SEOPS A, SEOPS B, SEOPS C, B(a)P, B(k)F and B(ghi)P. With exception to the number of leaf of B(ghi)P that remained the same with that of 14days, an increase in its fresh matter weight was also observed. However, there was a significant difference from the result of the unpolluted samples and that of the polluted samples.

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Table 4.5: Growth performance of *A. hybridus* in different concentrations of Spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene polluted soil 21days after transplanting.

Parameters	**UPS**	**B(a)P**	**B(k)F**	**B(ghi)P**	**SEOPS A**	**SEOPS B**	**SEOPS C**
Plant Height(cm)	16.7 ±0.42[a]	10.8 ±0.65	10.9 ±0.16	12.4 ±0.20	10.7 ±0.29	8.8 ±0.27	7.3 ±0.06
Leaves Number	11 ±0.58[a]	4 ±0.58	5 ±1.0	7 ±0.58	6 ±0.00	4 ±0.00	2 ±0.00
Leave Area(cm^3)	8.5 ±0.55[a]	1.4 ±0.20	2.7 ±0.45	3.8 ±0.55	3.7 ±0.10	2.2 ±0.15	1.1 ±0.10
Fresh Weight(g)	0.83 ±0.08[a]	0.22 ±0.02	0.31 ±0.03	0.45 ±0.05	0.23 ±0.07	0.02 ±0.02	0.08 ±0.21
Dry Weight(g)	0.10 ±0.05[a]	0.009 ±0.001	0.006 ±0.003	0.04 ±0.03	0.02 ±0.007	0.006 ±0.002	0.004 ±0.001

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

LEGEND:

±Standard deviation; UPS: Unexposed Plant Species

B(a)P: Benzo(a)pyrene polluted plant

B(k)F: Benzo(k)fluoranthrene polluted plant

B(ghi)P: Benzo(ghi)perylene polluted plants

'a': Shows significant difference

SEOPS A: 100ml spent engine oil polluted samples

SEOPS B: 200ml spent engine oil polluted sample

SEOPS C: 300ml spent engine oil polluted sample

4.1.3d Growth Performance of *Amaranthus hybridus* on Polluted and Unpolluted Soil after Twenty-Eight (28) Days

The growth performance of *A. hybridus* after 28 days of exposure to benzo(a)pyrene, benzo(k)fluoranthrene, benzo(ghi)perylene and the different concentrations of spent engine oil polluted and unpolluted soil is depicted in Table 4.6. The result obtained showed that plant grown on B(ghi)P and B(k)F polluted soil had the highest plant height when compared to other polluted soil. The least plant height was observed in SEOPS C (soil with the highest concentrations of spent engine oil). The result from the study of *A. hybridus* after 28days of exposure to B(a)P, B(k)F, B(ghi)P and different concentrations of spent engine oil polluted soils showed a general decrease in the plant height, number of leaves, leaf area, fresh and dry matter weight. The results from the data obtained from the unpolluted soil samples showed a significant difference ($P<0.05$) from that obtained from the different PAH components and the various concentrations of spent engine oil.

Generally, the values of all the growth parameters obtained from *A. hybridus* grown in unpolluted samples were higher when compared to that grown in the various polluted soil samples. There was also a decrease value in growth parameters of *A. hybridus* as the concentration of spent engine oil in the soil increases. It was further observed that plant grown on benzo(ghi)perylene (B[ghi]P) polluted soil had the highest growth performance when compared to those grown in B(a)P, B(k)F and the various concentrations of spent engine oil (SEOPS A, B & C). The least plant growth was recorded in SEOPS C throughout the experimental growth period.

Table 4.6: Growth performance of *A. hybridus* in different concentrations of Spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene polluted soil after 28days of exposure.

Parameters	**UPS**	**B(a)P**	**B(k)F**	**B(ghi)P**	**SEOPS A**	**SEOPS B**	**SEOPS C**
Plant Height(cm)	19.6 ±0.51[a]	9.8 ±0.91	10.3 ±0.35	11.4 ±0.21	9.5 ±0.25	8.2 ±0.38	6.9 ±0.44
Leaves Number	13 ±0.58[a]	4 ±0.58	5 ±0.00	6 ±0.58	5 ±0.58	5 ±0.58	3 ±0.00
Leave Area(cm^3)	10.5 ±0.46[a]	1.3 ±0.1	2.5 ±0.27	3.6 ±0.30	3.2 ±0.35	1.7 ±0.31	0.9 ±0.10
Fresh Weight(g)	1.32 ±0.30[a]	0.07 ±0.02	0.19 ±0.01	0.21 ±0.04	0.15 ±0.05	0.06 ±0.04	0.02 ±0.01
Dry Weight(g)	0.25 ±0.06[a]	0.004 ±0.02	0.004 ±0.003	0.007 ±0.004	0.02 ±0.01	0.005 ±0.002	0.005 ±0.001

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

LEGEND:

±Standard deviation; UPS: Unexposed plant species

B(a)P: Benzo(a)pyrene polluted plant

B(k)F: Benzo(k)fluoranthrene polluted plant

B(ghi)P: Benzo(ghi)perylene polluted plants

'a': Shows significant difference

SEOPS A: 100ml spent engine oil polluted samples

SEOPS B: 200ml spent engine oil polluted sample

SEOPS C: 300ml spent engine oil polluted sample

4.1.3e Growth Performance of *Telfairia occidentalis* on Polluted and Unpolluted Soil after Seven (7) Days

The growth performance of *T. occidentalis* after 7 days of exposure to benzo(a)pyrene, benzo(k)fluoranthrene, and different concentrations of spent engine oil polluted and unpolluted soil is presented in Table 4.7. The result shows that plant grown on benzo(ghi)perylene polluted soil had the highest plant height values (28.1cm) compared to other polluted soil. The least plant height was observed in SEOPS C (soil with highest concentration of spent engine oil) (6.9cm). The study showed higher mean values in the numbers of leaves, leave area, fresh and dry matter weight of plant grown on benzo(ghi)perylene polluted soil compared to other polluted soils. It was observed that the plant height of benzo(k)fluoranthrene and SEOPS A (least concentration of spent engine oil polluted soil) were almost of the same value. The least number of leaf, leaf area, fresh and dry matter weight was observed in SEOPS C and benzo(a)pyrene.

Furthermore, the data obtained from the unpolluted soil samples were significantly different from that obtained from different PAH component and the various concentration of spent oil applied. However, no significant difference was obtained from the leaf area and fresh weight of benzo(ghi)perylene.

Table 4.7: Growth performance of *T. occidentalis* in different concentrations of Spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene polluted soil after 7 days of exposure.

Parameters	**UPS**	**B(a)P**	**B(k)F**	**B(ghi)P**	**SEOPS A**	**SEOPS B**	**SEOPS C**
Plant Height(cm)	35.7 ±0.91[a]	18. ±0.70	24.7 ±0.61	28.1 ±0.29	24.1 ±1.21	20.1 ±0.42	16.5 ±0.87
Leaves Number	13 ±0.58[a]	8 ±1.00	10 ±0.58	11 ±1.00	9 ±0.58	8 ±0.58	7 ±0.00
Leave Area(cm^3)	12.8 ±2.16[a]	8.2 ±0.40	8.8 ±0.35	10.9 ±0.06[a]	10.2 ±0.38	8.3 ±0.35	7.3 ±1.35
Fresh Weight(g)	8.5 ±0.82[a]	4.89 ±0.90	5.56 ±0.92	6.13 ±1.14[a]	5.34 ±1.94	4.79 ±1.05	3.43 ±1.15
Dry Weight(g)	2.91 ±0.20	1.02 ±0.05	1.49 ±0.05	1.86 ±0.10	1.44 ±0.34	1.16 ±0.15	0.72 ±0.10

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

LEGEND:

±Standard deviation; UPS: Unexposed plant species

B(a)P: Benzo(a)pyrene polluted plant

B(k)F: Benzo(k)fluoranthrene polluted plant

B(ghi)P: Benzo(ghi)perylene polluted plants

'a': Shows significant difference

SEOPS A: 100ml spent engine oil polluted samples

SEOPS B: 200ml spent engine oil polluted sample

SEOPS C: 300ml spent engine oil polluted sample

4.1.3f Growth Performance of *Telfairia occidentalis* on Polluted and Unpolluted Soil after Fourteen (14) Days

The growth performance of *T.occidentalis* after 14days of exposure to benzo(a)pyrene, benzo(k)fluoranthrene, and different concentrations of spent engine oil polluted and unpolluted soil is presented in Table 4.8. The result showed that benzo(ghi)perylene and benzo(k)fluoranthrene had the highest plant heights (42.7cm & 35.2cm) when compared to plant in other polluted soil samples. However, plant with the highest pollutant concentration in the soil (SEOPS C) had the least plant height (23.3cm). This was followed by plant grown on benzo(a)pyrene. The number of leaves and fresh matter weight were higher in benzo(ghi)perylene and SEOPS A. Furthermore, the values of the leaf area were higher in benzo(ghi)perylene, benzo(k)fluoranthrene and SEOPS A. In addition, the values of dry matter weight were higher in benzo(ghi)perylene, benzo(k)fluoranthrene and benzo(a)pyrene treated samples. However, the data obtained from the unpolluted soil samples were significantly different from that obtained from the different PAH component and the various concentrations of spent engine oil applied.

Table 4.8: Growth performance of *T. occidentalis* in different concentrations of Spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene polluted soil after 14 days of exposure.

Parameters	**UPSS**	**B(a)P**	**B(k)F**	**B(ghi)P**	**SEOPS A**	**SEOPS B**	**SEOPS C**
Plant Height(cm)	69.3 ±2.00[a]	27.6 ±1.85	35.2 ±1.41	42.7 ±1.97	30.3 ±1.02	27.4 ±2.44	23.3 ±2.44
Leaves Number	19 ±0.58[a]	9 ±1.00	11 ±1.16	13 ±0.00	12 ±0.58	10±0.58	8±0.58
Leave Area(cm^3)	15.6 ±1.56[a]	10.7 ±0.76	11.1 ±0.58	12 ±0.66	11.1 ±0.64	10.6 ±151	8.5 ±1.36
Fresh Weight(g)	13.5 ±1.68[a]	5.69 ±0.48	7.70 ±0.71	9.3 ±0.88	8.10 ±0.56	6.63 ±0.47	5.08 ±0.06
Dry Weight(g)	4.56 ±0.83[a]	1.74 ±0.23	1.79 ±0.08	2.49 ±0.15	1.58 ±0.44	1.21 ±0.58	0.89 ±0.25

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

LEGEND:

±Standard deviation; UPS: Unexposed plant sample

B(a)P: Benzo(a)pyrene polluted plant

B(k)F: Benzo(k)fluoranthrene polluted plant

B(ghi)P: Benzo(ghi)perylene polluted plants

'a': Shows significant difference

SEOPS A: 100ml spent engine oil polluted samples

SEOPS B: 200ml spent engine oil polluted sample

SEOPS C: 300ml spent engine oil polluted sample

4.1.3g Growth Performance of *Telfairia occidentalis* on Polluted and Unpolluted Soil after Twenty-One (21) Days

The growth performance of *T.occidentalis* after 21days of exposure to benzo(a)pyrene, benzo(k)fluoranthrene, and different concentrations of spent engine oil polluted and unpolluted soil is presented in Table 4.9. The result obtained showed that there was a decrease in plant height at the end of 21 days with exception to plant grown on SEOPS A polluted soil. Furthermore, there was a slight increase in the leaf area and fresh matter weight of SEOPS A, when compared with 7days and 14days. Also, the number of leaves, leave area, fresh and dry matter weight of plant grown on soil polluted with different PAH components and the various concentrations of spent engine oil after 21days of exposure when compared to 7 days and 21 days. Furthermore, the data obtained from the unpolluted soil samples were significantly different from that obtained from the different PAH component and the various concentrations of spent engine oil applied. However, there was no significant difference obtained from the values of the dry weight of benzo(k)fluoranthrene and SEOPS A (least concentration of spent engine oil polluted soil) with that of the unpolluted samples.

Table 4.9: Growth performance of *T. occidentalis* in different concentrations of Spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene polluted soil after 21 days of exposure.

Parameters	**UPS**	**B(a)P**	**B(k)F**	**B(ghi)P**	**SEOPS A**	**SEOPS B**	**SEOPS C**
Plant Height(cm)	99.6 ±0.87[a]	31.8 ±1.63	33.8 ±1.07	38.9 ±1.28	32.2 ±1.50	26.6 ±1.12	21.1 ±1.22
Leaves Number	28 ±1.53[a]	10 ±1.15	12 ±0.00	12 ±0.58	10 ±0.58	8 ±0.58	7 ±1.00
Leave Area(cm^3)	18.1 ±1.39[a]	11.9 ±0.64	13.5 ±0.47	14.8 ±0.61	11.4 ±0.40	9.6 ±0.55	7.3 ±0.53
Fresh Weight(g)	17.9 ±1.76[a]	8.90 ±0.99	6.48 ±0.58	8.78 ±0.81	8.64±0.22	6.35 ±0.88	3.88 ±0.68
Dry Weight(g)	4.78 ±0.76[a]	2.00 ±0.51	1.69 ±0.12[a]	1.86 ±0.282	1.06 ±0.08[a]	0.83 ±0.15	0.33 ±0.25

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

LEGEND:

±Standard deviation; UPS: Unexposed plant species

B(a)P: Benzo(a)pyrene polluted plant

B(k)F: Benzo(k)fluoranthrene polluted plant

B(ghi)P: Benzo(ghi)perylene polluted plants

'a': Shows significant difference

SEOPS A: 100ml spent engine oil polluted samples

SEOPS B: 200ml spent engine oil polluted sample

SEOPS C: 300ml spent engine oil polluted sample

4.1.3h Growth Performance of *Telfairia occidentalis* on Polluted and Unpolluted Soil after Twenty-Eight (28) Days

The growth performance of *T.occidentalis* after 28days of exposure to benzo(a)pyrene, benzo(k)fluoranthrene, and different concentrations of spent engine oil polluted and unpolluted soil is presented in Table 4.10. The result obtained showed that plant grown on B(ghi)P and B(k)F polluted soil had the highest plant height when compared to other polluted soil. The least plant height was observed in SEOPS C (soil it the highest concentrations of spent engine oil). The result from the study of *T.occidentalis* after 28days of exposure to B(a)P, B(k)F, B(ghi)P and different concentrations of spent engine oil polluted soils showed a general decrease in all the plant growth parameters. The results from the data obtained from the unpolluted soil samples showed a significant difference ($P<0.05$) from that obtained from the different PAH components and the various concentrations of spent engine oil.

In general, it was observed that plant grown on benzo(ghi)perylene polluted soil had the highest growth performance throughout the 28days experimental period when compared to those grown on other polluted soil. There was also a decrease in plant growth with increase concentration of spent engine oil in the soil. However, in the different PAH component used, benzo(a)pyrene had the least growth performance. Furthermore, the unpolluted soil samples had the best growth performance in all the growth parameters when compared to the unpolluted soil samples.

Table 4.10: Growth performance of *T. occidentalis* in different concentrations of Spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene polluted soil after 28 days of exposure.

Parameters	**UPSS**	**B(a)P**	**B(k)F**	**B(ghi)P**	**SEOPS A**	**SEOPS B**	**SEOPS C**
Plant Height(cm)	114.5 ±0.70[a]	28.8±1.27	30.8 ±2.03	37.7 ±0.50	28.8 ±1.40	24.3 ±1.06	17.4 ±0.85
Leaves Number	34 ±0.58[a]	10 ±1.00	10 ±0.58	11 ±0.58	9 ±0.58	7 ±0.58	7 ±0.85
Leave Area(cm^3)	22.9 ±0.35[a]	11.0 ±0.40	12.4 ±0.72	13.1 ±0.36	9.8 ±0.20	8.2 ±0.93	6.8 ±0.70
Fresh Weight(g)	20.0 ±1.75[a]	7.08 ±1.02	5.67 ±0.97	8.39 ±0.59	7.65 ±0.25	5.48 ±0.64	3.01 ±0.41
Dry Weight(g)	6.0 ±0.28 [a]	1.12 ±0.04	1.08 ±0.08	1.53 ±0.58	0.94 ±0.06	0.53 ±0.58	0.06 ±0.40

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

LEGEND:

±Standard deviation; UPS: Unexposed plant species

B(a)P: Benzo(a)pyrene polluted plant

B(k)F: Benzo(k)fluoranthrene polluted plant

B(ghi)P: Benzo(ghi)perylene polluted plants

'a': Shows significant difference

SEOPS A: 100ml spent engine oil polluted samples

SEOPS B: 200ml spent engine oil polluted sample

SEOPS C: 300ml spent engine oil polluted sample

4.1.4a The Concentrations of PAH in *A. hybridus* and *T. occidentalis* after 14days of exposure to Spent Engine Oil Polluted Soil

The concentration of PAH components in *A.hybridus* and *T.occidentalis* leaf samples after 14days of exposure to different concentrations of spent engine oil polluted soil are shown in Table 4.11. The results obtained showed that a total of 14 PAH component were detected in the plants samples. This includes; acenaphthylene, phenanthrene, benzo(k)fluoranthrene, benzo(ghi)perylene, 1-2 benzanthrene, fluoranthrene, dibenzyl(a,h)anthracene, benzo(a)pyrene, florene, acenaphthalene, anthracene, pyrene, naphthalene and benzo(b)fluoranthrene. However, in *A. hybridus*, phenanthrene and benzo(k)fluoranthrene were not detected in both unpolluted and polluted plants samples. Also, the unpolluted plant samples of *A.hybridus* did not contain benzo(ghi)perylene, 1-2 benzanthrene, florene, acenaphthalene, anthracene, pyrene, naphthalene and benzo(k)fluoranthrene. Of all the PAH components from the different concentrations of spent engine oil, it was observed that fluoranthrene and benzo(a)pyrene was detected in the unpolluted and polluted plants samples of *A.hybridus* and *T. occidentalis*. It was observed that in *A. hybridus*, benzo(a)pyrene from SEOP A had the highest concentration (0.3160mg/ml) when compared to the other PAH component. However, acenaphthylene had the least concentration (0.0003mg/ml) from SEOPS A. Furthermore, in *T. occidentalis* benzo(ghi)perylene and benzo(b)fluoranthrene were not detected in both the unpolluted and polluted samples. Anthracene had the highest concentration (0.2978mg/ml) in *T. occidentalis* when compared to other PAH components. However, naphthalene had the least concentrations in all samples.

Table 4.11: Concentrations of PAHs in *A. hybridus* and *T. occidentalis* after 14days in spent engine oil polluted soil (mg/ml).

PAH components	*A.hybridus* UPS	SEOPS A	SEOPS B	SEOPS C	*T.occidentalis* UPSS	SEOPS A	SEOPS B	SEOPS C
Acy	0.0003 ±0.000	0.0134 ±0.008	0.0098 ±0.003	0.0133±0.023	0.0001±0.00	0.0298 ±0.022	0.0321 ±0.013	0.0096 ±0.006
Phen	ND	ND	ND	ND	ND	0.1048 ±0.005	0.0451 ±0.035	0.0805 ±0.052
B(k)F	ND	ND	ND	ND	ND	0.0969 ±0.008	0.1356 ±0.015	0.1107 ±0.023
B(ghi)P	ND	0.0276 ±0.016	0.0336 ±0.017	0.1141 ±0.012	ND	ND	ND	ND
1-2Benz	ND	0.0201 ±0.014	0.0101 ±0.015	0.0283 ±0.015	ND	0.0316 ±0.009	0.0845 ±0.027	0.0812 ±0.017
Flu	0.0080 ±0.014	0.0881 ±0.055	0.1805 ±0.025	0.1830 ±0.014	0.0099 ±0.005	0.2161 ±0.005	0.1541 ±0.130	0.3220 ±0.054
D(ah)A	0.0108 ±0.032	0.1876 ±0.082	0.0182 ±0.021	0.0190 ±0.014	0.0961 ±0.002	ND	0.0098 ±0.007	0.0288 ±0.020
B(a)P	0.0689 ±0.032	0.3160 ±0.092	0.1415 ±0.032	0.1427 ±0.015	0.0561 ±0.001	0.0121 ±0.013	0.0115 ±0.009	0.0125 ±0.009
Flo	ND	0.1307 ±0.055	0.1344±0.022	0.1461 ±0.019	ND	0.1148±0.007	0.1569 ±0.019	0.1851 ±0.110
Ace	ND	0.1771 ±0.156	0.1772 ±0.021	0.1785 ±0.113	ND	0.2138 ±0.017	0.1361 ±0.103	0.1394 ±0.215
Ant	ND	0.0914 ±0.008	0.0921 ±0.024	0.9537 ±0.019	ND	0.2978 ±0.037	ND	0.3801 ±0.120
Pyr	ND	0.0296 ±0.018	0.0351 ±0.022	0.0369±0.020	ND	0.0139 ±0.002	0.0421 ±0.029	0.0619 ±0.048
Nap	ND	0.1149 ±0.101	0.1217 ±0.007	0.1392 ±0.010	ND	0.0001 ±0.000	0.0056 ±0.001	0.0077 ±0.002
B(b)F	ND	0.0990 ±0.010	0.1793 ±0.089	0.1845 ±0.019	ND	ND	ND	ND

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)a nthracene; B(a)P-benzo(a)pyrene; Flo-Florene; Ace-acenaphthene; Ant-anthracene; Pyr-pyrene; Nap-Naphthalene; B(b)F-benzo(b)Fluoranthene; UPS: Unexposed plant species; SEOPSS A: 100ml Spent engine oil polluted soil sample; SEOPSS B: 200ml Spent engine oil polluted soil sample: SEOPSS C: 300ml Spent engine oil polluted soil sample. ND- not detected. All units are in mg/ml.

4.1.4b The Concentrations of Benzo(a)pyrene, Benzo(k)fluoranthrene and Benzo(ghi)perylene in *A.hybridus* and *T.occidentalis* after 14days

The concentrations of benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene in *A. hybridus* and *T. occidentalis* leaf samples after 14 days of exposure is presented on Table 4.12. The result showed that benzo(k)fluoranthrene had higher concentration value of 0.8476mg/ml in *T.occidentalis* when compared to *A.hybridus*. However, in *A. hybridus*, benzo(a)pyrene and benzo(ghi)perylene had higher concentrations values of 1.6581mg/ml and 0.6475mg/ml when compared to those of *T. occidentalis*.

Table 4.12: Concentrations of Benzo(a)pyrene, Benzo(k)fluoranthene and Benzo(ghi)perylene in *A.hybridus* and *T.occidentalis* after 14days of exposure

PAH components	***A. hybridus*** **UPS**	**B(a)P**	**B(k)F**	**B(ghi)P**	***T. occidentalis*** **UPS**	**B(a)P**	**B(k)F**	**B(ghi)P**
B(k)F	ND	ND	0.5816 ±0.89	ND	ND	ND	0.8476 ±0.48	ND
B(ghi)P	ND	ND	ND	0.6475 ±0.026	ND	ND	ND	0.4758 ±0.116
B(a)P	ND	1.6581 ±0.825	ND	ND	ND	0.1866 ±0.017	ND	ND

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; UPS- Unexposed plant species; PAH A- Benzo(a)pyrene; PAH B- Benzo(k)fluoranthene; PAH C- Benzo(ghi)perylene. ND- not detected. All units are in mg/ml

4.1.4c The Concentrations of PAH in *A. hybridus* and *T. occidentalis* after 28days of exposure to Spent Engine Oil Polluted Soil

The Concentrations of PAH components in *A. hybridus* and *T. occidentalis* leaf after 28 days of exposure to spent engine oil polluted and unpolluted soil is presented on table 4.13. 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 both *A. hybridus* and *T. occidentalis*. However, higher concentration values were detected in unexposed plant species of *A. hybridus* compared to *T. occidentalis*. It was observed that phenanthrene and benzo(k)fluoranthene were not detected in the unexposed and exposed of *A. hybridus* after 28days. Also, benzo(ghi)perylene and benzo(b)fluoranthene were not detected in *T. occidentalis* after 28days. The two to four ring PAH components such as acenaphthylene, naphthalene, acenaphthalene, anthracene, flourene, and fluoranthrene were more detected compared to the five to six ring PAH (benzo(a)pyrene, dibenzy(a,h)anthracene, benzo(ghi)perylene, benzo(k)fluoranthrene and benzo(b)fluoranthrene) in *A. hybridus* and *T. occidentalis*. Furthermore, it was observed that acenaphthylene and acenaphthalene from SEOPS A had higher concentration values of 0.4331mg/ml and 0.5249mg/ml, while fluoranthracene, anthracene, and flourene from SEOPS C had higher concentration values of 0.4360mg/ml, 0.5001mg/ml and 0.2017mg/ml all in *T.occidentalis* when compared to other concentrations of spent engine oil and *A. hybridus*. However, naphthalene, benzo(a)pyrene, and dibenzyl(a,h)anthracene from SEOPS A and benzo(b)fluoranthrene from SEOPS B had higher concentration values of 0.1441mg/ml, 0.5460mg/ml and 0.2491mg/ml, and 0.1893mg/ml in *A.hybridus* when compared to *T. occidentalis*. In general *T. occidentalis* had higher concentrations of PAH component in leaf samples when compared to *A.hybridus*. Also, the concentration PAH content increased in both *A. hybridus* and *T. occidentalis* at the end of 28days.

Table 4.13: Concentrations of PAHs in *A. hybridus* and *T. occidentalis* after 28days of exposure to spent engine oil polluted soil.

PAH components	*A.hybridus* UPS	SEOPS A	SEOPS B	SEOPS C	*T.occidentalis* 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	ND	ND	ND	ND	ND	0.1243 ±0.005	0.0930 ±0.035	0.1005 ±0.052
B(k)F	ND	ND	ND	ND	ND	0.2038 ±0.008	0.1948 ±0.015	0.1457 ±0.023
B(ghi)P	ND	0.0946 ±0.016	0.0724 ±0.017	0.1545 ±0.012	ND	ND	ND	ND
1-2Benz	ND	0.0841 ±0.014	0.0421 ±0.015	0.0833 ±0.015	ND	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	ND	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	ND	0.1667 ±0.055	0.1210 ±0.022	0.1651 ±0.019	ND	0.1343±0.007	0.1843 ±0.019	0.2017 ±0.110
Ace	ND	0.2961 ±0.156	0.2629 ±0.021	0.2970 ±0.113	ND	0.5249 ±0.017	0.4726 ±0.103	0.4769 ±0.215
Ant	ND	0.1016 ±0.008	0.1904 ±0.024	0.2038 ±0.019	ND	0.4994 ±0.037	ND	0.5001 ±0.120
Pyr	ND	0.0577 ±0.018	0.0558 ±0.022	0.0549 ±0.020	ND	0.0479 ±0.002	0.0621 ±0.029	0.0957 ±0.048
Nap	ND	0.1441 ±0.101	0.1217 ±0.007	0.1172 ±0.010	ND	0.0005 ±0.000	0.0032 ±0.001	0.0044 ±0.002
B(b)F	ND	0.1890 ±0.010	0.1893 ±0.089	0.1872 ±0.019	ND	ND	ND	ND

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; UPS: Unexposed plant specie; SEOPSS A: 100ml Spent engine oil polluted soil sample; SEOPSS B: 200ml Spent engine oil polluted soil: SEOPS C: 300ml Spent engine oil polluted soil sample. ND- not detected. All units are in mg/ml.

4.1.4d The Concentration of Benzo(a)pyrene, Benzo(k)fluoranthrene and Benzo(ghi)perylene in *A.hybridus* and *T.occidentalis* after 28days

The concentrations of the benzo(a)pyrene (B[a]P), benzo(k)fluoranthene (B(k)F) and benzo(ghi)perylene (B(ghi)P) in *A. hybridus* and *T. occidentalis* leaf samples after 28 days of exposure are presented in Table 4.14. The result showed higher concentrations of benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene in both *A.hybridus* (2.7164; 1.7381 and 1.0635) and *T.occidentalis* (2.0815; 1.7376 and 0.9604) after 28days. However, *A.hybridus* had higher concentrations of these pollutant when compare to *T.occidentalis*.

Table 4.14: Concentrations of PAHs in *A.hybridus* and *T.occidentalis* at 28days of pollution with B(a)P, B(k)F and B(ghi)P polluted samples.

PAH components	***A. hybridus* UPSS**	**B(a)P**	**B(k)F**	**B(ghi)P**	***T. occidentalis* UPSS**	**B(a)P**	**B(k)F**	**B(ghi)P**
B(k)F	ND	ND	1.7381 ±0.89	ND	ND	ND	1.7376 ±0.48	ND
B(ghi)P	ND	ND	ND	1.0635 ±0.026	ND	ND	ND	0.9604 ±0.116
B(a)P	ND	2.7164 ±0.825	ND	ND	ND	2.0815 ±0.017	ND	ND

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. ND- not detected. All units are in mg/ml.

4.1.4e Bioaccumulation Factor of PAH Components from Spent engine (SEO) oil polluted soil in *A. hybridus* and *T. occidentalis* at 14days of exposure.

The bioaccumulation factor of PAH in SEO in *A.hybridus* and *T.occidentalis* after 14days of exposure are presented in Table 4.15. The results showed that the bioaccumulation factor (BAF) of the individual PAH components in the unpolluted soil had higher values in *T. occidentalis* compared to *A. hbridus*. However, acenaphthylene and benzo(a)pyrene had higher BAF values in *A. hybridus*. In *A. hybridus*, benzo(a)pyrene had the highest BAF values (2.11) in SEOPS C (soil with the highest concentration of spent engine oil). This was followed by pyrene in SEOPS A (soil with the least concentrations of spent engine oil). The least BAF value was recorded in acenaphthylene for *A. hybridus* in all the different concentrations of spent engine oil used. Furthermore, in *T. occidentalis*, fluoranthene had the highest BAF value (2.47) in SEOPS B. this was followed by phenanthrene in SEOPS A. The least BAF value was obtained in benzo(a)pyrene in all the concentrations of spent engine oil applied.

Generally, from the result the high molecular weight (HMW) of PAHs (B[ghi]P, D[a,h]A, B[a]P, B[b]F and pyrene) had higher BAF values in *A. hybridus*. However, in *T. occcidentalis*, the low molecular weight (LMW) PAH (Phen, 1,2 Benz, Flo, and Ant) had higher BAF values. In addition, the total bioaccumulation factor (BAF_{Total}) were all greater than one (<1). However, *A. hybridus* grown on SEOPS A had higher BAF_{Total} (7.96) compared to those grown on SEOPS B and SEOPS C, while *T. occidentalis* grown on SEOPS C had higher BAF_{Total} (8.89) than those grown in SEOPS A and SEOPS B. The least BAF_{Total} for both *A. hybridus* and *T. occidentalis* was observed in their unpolluted samples.

Table 4.15: Bioaccumulation Factor of PAH components from spent engine oil polluted soil in *A.hybridus* and *T.occidentalis* after 14days.

PAH components	***A.hybridus***				***T.occidentalis***			
	UPS	**SEOPS A**	**SEOPS B**	**SEOPS C**	**UPS**	**SEOPS A**	**SEOPS B**	**SEOPS C**
Acy	0.05	0.05	0.04	0.04	0.02	0.11	0.10	0.03
Phen	ND	ND	ND	ND	ND	1.63	0.62	1.21
B(k)F	ND	ND	ND	ND	ND	0.60	0.67	0.74
B(ghi)P	ND	0.64	0.48	2.11	ND	ND	ND	ND
1-2Benz	ND	0.29	0.16	0.45	ND	0.46	1.36	1.30
Flu	0.004	0.48	0.65	0.04	0.49	1.17	2.47	1.17
D(ah)A	0.07	1.05	0.10	0.08	0.59	ND	0.05	0.17
B(a)P	0.95	0.84	0.40	0.33	0.77	0.03	0.03	0.03
Flo	ND	0.89	0.52	0.90	ND	0.78	0.89	1.14
Ace	ND	0.67	0.51	0.83	ND	0.81	0.55	0.55
Ant	ND	0.36	0.37	0.62	ND	1.18	ND	1.53
Pyr	ND	1.38	1.06	0.50	ND	0.65	1.31	0.97
Nap	ND	0.70	0.75	0.65	ND	0.00	0.05	0.05
B(b)F	ND	0.61	0.90	0.86	ND	ND	ND	ND
BAF_{Total}	1.07	7.96	5.94	7.41	1.87	7.42	8.1	8.89

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; UPS- Unexposed plant species; PAH A- Benzo(a)pyrene; PAH B- Benzo(k)fluoranthene; PAH C- Benzo(ghi)perylene. ND- not detected. All units are in mg/ml.

4.1.4f Bioaccumulation Factor of benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene in *A.hybridus* and *T.occidentalis* at 14days of exposure.

The bioaccumulation factor of PAH from SEOPS in *A.hybridus* and *T.occidentalis* after 14 days exposure, are presented in Table 4.16. The result obtained compares the BAF values of B(a)P, B(k)F and B(ghi)P and that from spent engine oil polluted soil. It was observed that the BAF values of the three individual PAHs component were less than one with exception to B(a)P which was slightly above one and that of B(ghi)P, both in *A. hybridus*. The BAF_{Total} of the three PAH components was slightly greater than one in *A.hybridus* while PAH from Spent engine oil polluted soil only BAF_{Total} in A.hybridus was above one. However, *A.hybridus* had a higher BAF_{Total} value (1.88 mg/ml) when compared to *T. occidentalis* (1.03 mg/ml). While the BAF_{Total} of the three PAH from spent engine oil polluted soil was greater than one in *A. hybridus* (1.60mg/ml) and that of *T. occidentalis* was less than one (0.7mg/ml).

Table 4.16: Bioaccumulation Factor of B(a)P, B(k)F and B(ghi)P pollution in *A. hybridus* and *T. occidentalis* and that from spent engine oil polluted soil were compared after 14days.

Pollutants	Parameters	*A.hybridus* (mg/ml)	*T.occidentalis* (mg/ml)
PAH	B(a)P	1.04	0.12
	B(k)F	0.41	0.60
	B(ghi)P	0.43	0.31
	BAF_{Total}	1.88	1.03
SEO	B(a)P	0.52	0.03
	B(k)F	ND	0.67
	B(ghi)P	1.08	ND
	BAF_{Total}	1.60	0.70

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

4.1.4g Bioaccumulation Factor of PAH Components from Spent engine oil polluted soil (SEOPS) in *A. hybridus* and *T. occidentalis* at 28days of exposure.

The bioaccumulation factor of PAH from SEOPS in *A.hybridus* and *T.occidentalis* after 28days of exposure are presented in Table 4.17. The result shows that the BAF value of the five PAH in the unpolluted soil sample of *A. hybridus*, were higher than those of *T. occidentalis*. In *A.hybridus*, the BAF value was observed to be higher in benzo(ghi)perylene from SEOPS C compared to other individual PAH component obtained in SEOPS A and SEOPS B, this was followed by 1,2-benzanthene. However, the least BAF among the polluted samples was recorded in acenaphthylene. Furthermore, in *T. occidentalis*, fluoranthene had the highest BAF value (1.25) from SEOPS B, compared to other components in the different spent engine oil concentrations (SEOPS A & B). Naphthalene had the least BAF value in SEOPS A for *T. occidentalis*. Generally higher BAF value were more detected in *T. occidentalis* when compared to *A. hybridus* with exceptions to dibenzy(a,h)anthracene (D[a,h]A), Benzo (a)pyrene (B[a]P), and Naphthalene (Nap). The total bioaccumulation factor (BAF_{Total}) was observed to be higher in SEOPS A for both *A. hybridus* and *T. occidentalis* (7.2mg/ml and 7.6mg/ml) respectively.

Table 4.17: Bioaccumulation Factor of PAH components from spent engine oil pollution in *A.hybridus* and *T.occidentalis* at 28days of exposure

PAH components	*A.hybridus* UPSS	SEOPS A	SEOPS B	SEOPS C	*T.occidentalis* UPSS	SEOPS A	SEOPS B	SEOPS C
Acy	0.05	0.06	0.02	0.12	0.04	0.78	0.11	0.01
Phen	ND	ND	ND	ND	ND	0.85	0.58	0.58
B(k)F	ND	ND	ND	ND	ND	0.67	0.47	0.35
B(ghi)P	ND	0.83	0.58	0.91	ND	ND	ND	ND
1-2Benz	ND	0.86	0.34	0.53	ND	0.84	0.84	0.74
Flu	0.32	0.59	0.40	0.43	0.22	1.25	0.68	0.73
D(ah)A	0.31	0.76	0.09	0.10	0.30	ND	0.04	0.09
B(a)P	0.78	0.76	0.86	0.88	0.04	0.07	0.08	0.08
Flo	ND	0.55	0.39	0.51	ND	0.44	0.57	0.62
Ace	ND	0.52	0.45	0.52	ND	0.91	0.82	0.43
Ant	ND	0.22	0.40	0.41	ND	1.09	-	1.01
Pyr	ND	0.88	0.64	0.43	ND	0.73	0.71	0.75
Nap	ND	0.54	0.41	0.38	ND	0.001	0.01	0.01
B(b)F	ND	0.61	0.55	0.50	ND	ND	ND	ND
BAF_{Total}	1.46	7.2	4.8	5.1	1.2	7.6	4.9	5.4

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; UPS- Unexposed plant species; PAH A- Benzo(a)pyrene; PAH B- Benzo(k)fluoranthene; PAH C-Benzo(ghi)perylene. ND- not detected. All units are in mg/ml.

4.1.4h Bioaccumulation Factor benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene in *A. hybridus* and *T. occidentalis* after 28days of exposure.

The bioaccumulation of B(a)P, B(k)F and B(ghi)P and that from spent engine oil (SEO) polluted soil in *A. hybridus* and *T. occidentalis* are presented on Table 4.18. The result obtained compares the BAF values of B(a)P, B(k)F and B(ghi)P and that from spent engine oil polluted soil. It was observed that the BAF values of individual PAHs and that from SEO polluted soil were less than one. Although, the individual PAH had a higher BAF values when compared with that from SEOPS in both plants. The BAF_{Total} of the three PAH components was greater than one in both *A. hybridus* and *T. occidentalis*. However, *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 that of *T. occidentalis* was less than one (0.5).

Table 4.18: Bioaccumulation Factor of B(a)P, B(k)F and B(ghi)P pollution in *A. hybridus* and *T. occidentalis* after 28days.

Pollutants	**Parameters**	***A.hybridus* (mg/ml)**	***T.occidentalis* (mg/ml)**
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}	1.8	1.5
SEO	B(a)P	0.51	0.04
	B(k)F	ND	0.49
	B(ghi)P	0.78	ND
	BAF_{Total}	1.3	0.5

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

4.1.5a DNA Quality Assessment Test of Extracted Plants Sample

The gel electrophoresis picture of DNA bands of *A. hybridus* and *T. occidentalis* exposed to B(a)P, B(k)F, B(ghi)P and different concentrations of spent engine oil polluted soil are presented on Plate 4.1. The gel picture shows the quality of the DNA of *Amaranthus hybridus* and *Telfairia occidentalis* exposed and unexposed to these different pollutants. It was observed that 1(control), 2, 6, & 10, 12 showed high light intensity compared to others. The light intensity shows the state and integrity of the DNA.

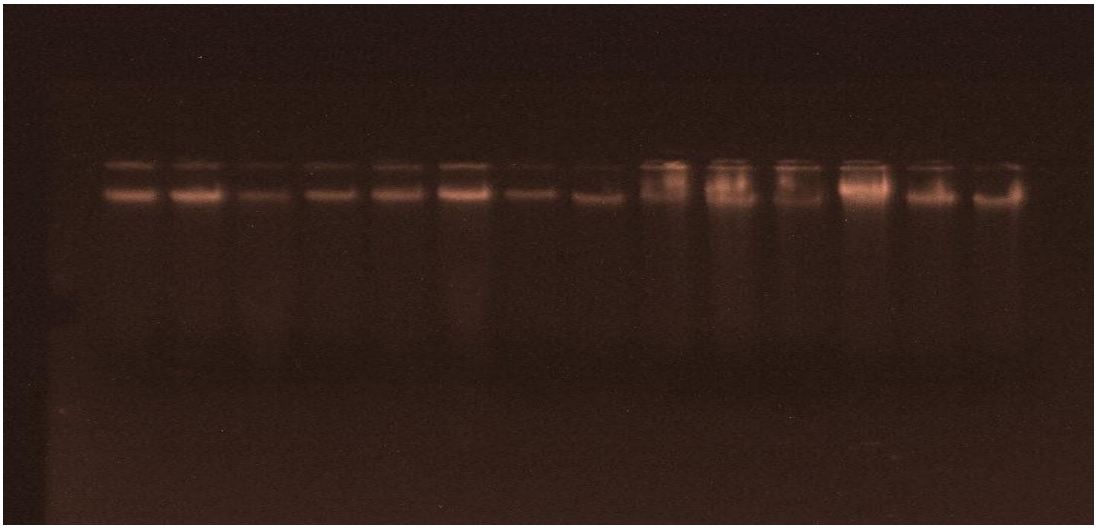

Plate 4.1: Gel pictures, depicting light intensity of DNA bands and quantification of DNA from crop plants exposed to B(a)P, B(k)F, B(ghi)P and spent engine oil polluted soil. lane 1 and 8: Control (*A. hybridus* and *T. occidentalis*), Lanes 2, 3, 4 (*A. hybridus*)/9, 10, 11 (*T. occidentalis*): were treated with B(a)P, B(k)F and B(ghi)P. Lanes 5, 6, 7 (*A. hybridus*)/12, 13, 14 (*T. occidentalis*) were treated with different concentrations of SEOPS.

4.1.5b Assessment of Genotoxic effect of Different toxicants on the Genomic DNA of *A. hybridus* and *T. occidentalis* using Inter Simple Sequence Repeat (ISSR) Marker

The gel picture showing the DNA band breaks or DNA fragments of *Amaranthus hybridus* and *Telfairia occidentalis* plant exposed to the different PAH components (benzo(a)pyrene, benzo(k)fluoranthrene, benzo(ghi)perylene) and various concentrations of spent engine oil is shown on Plate 4.2(a, b & c). The bands generated were primer dependent. From the gel pictures lane 1-7 represents *A. hybridus*, while lane 8-14 represent *T. occidentalis*. The gel picture generated in Figure 4.4a using UBC 811 primer; lane 3 & 4 had the same number of DNA band fragments (three each) as the lane 1(unpolluted). Lane 2 & 5 had band insertion and deletion. However, lane 2 had two DNA band fragments and lane 5 had three DNA band fragment with appearing band above and disappearing band below. In lane 7, two DNA band fragment were observed, however, band disappeared below. Furthermore, lane 8(control), 10, 12 & 13 each had one DNA band fragment. However, lane 11 and 14 had four DNA band fragments indicating that new band appeared when compared to the unpolluted for *T. occidentalis*. It was further observed in both *A. hybridus* (lane 6) and *T. occidentalis* (lane 9), there was a complete disappearance of DNA band. In all, the total number of bands scored using UBC 811 primer is 26 for both *A. hybridus* and *T. occidentalis*. However, the total number of newly appeared band was 8 and the disappeared band was 9. The percentage polymorphism that occurred in UBC 811 primer is 65%. The total number of DNA frangments that occurred in each lane is recorded in Table 4.19

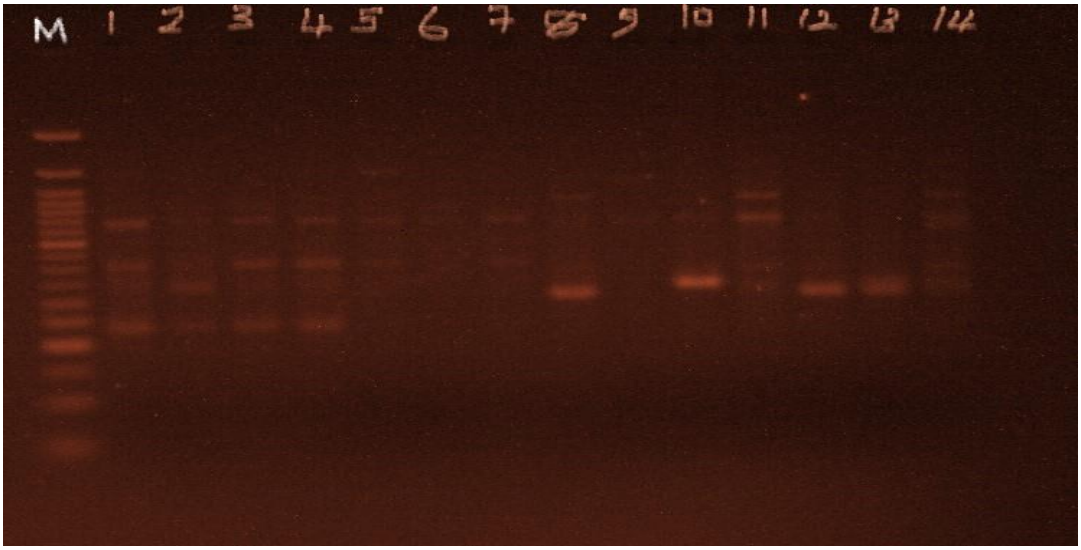

Plate 4.2a: ISSR PCR amplification products for *A. hybridus* and *T. occidentalis* treated with three components of PAH and various concentrations of SEOPS using UBC-811 primer. Lane M: 50bp DNA ladder, lane 1 and 8: Control (*A. hybridus* and *T. occidentalis*), Lanes 2, 3, 4 (*A. hybridus*)/9, 10, 11 (*T. occidentalis*): were treated with B(a)P, B(k)F and B(ghi)P. Lanes 5, 6, 7 (*A. hybridus*)/12, 13, 14 (*T. occidentalis*) were treated with different concentrations of SEOPS.

Table 4.19: Numbers of DNA band breaks in *A. hybridus* and *T. occidentalis* exposed and unexposed to B(a)P, B(k)F, B(ghi)P and different concentrations of spent engine oil polluted soil using UBC 811.

Lane	**Sample ID**	**No. of DNA Fragments**
1	UPS (A.hybridus)	3
2	B(a)P (A.hybridus)	2
3	B(k)P (A.hybridus)	3
4	B(ghi)P (A.hybridus)	3
5	SEOPS A (A.hybridus)	3
6	SEOPS B (A.hybridus)	0
7	SEOPS C (A.hybridus)	2
8	UPS (T.occidentalis)	1
9	B(a)P (T.occidentalis)	0
10	B(k)P (T.occidentalis)	1
11	B(ghi)P (T.occidentalis)	4
12	SEOPS A (T.occidentalis)	1
13	SEOPS B (T.occidentalis)	1
14	SEOPS C (T.occidentalis)	4

The gel picture generated using UBC 827 primer is shown in Plate 4.2b. From the result it was observed that in *A. hybridus*, lane 1(control) had three DNA band fragments. Lane 2 showed two DNA band fragments with one having high light intensity. Lane 3 4, 5, 6 & 7 all had appearance of new DNA band when compared to control (lane 1) for *A. hybridus*. In lane 3 and 6 only one band was observed; lane 4 and 7 had three bands with bands having different positions when compared to the control (lane 1). However the highest DNA band fragments was observed in lane 5 (four bands). Moreover, in *T.occidentalis*, it was observed that only one band occurred in lane 8-14 except in lane 12 which had three bands. Also it was observed that lane 8-11 had high light intensity when compared with lane 12-14. In general, there was appearance of new DNA band in *A.hyridus* compared to *T. occidentalis.* The total number of bands present in UBC 827 is 25 for both *A.hybridus* and *T. occidentalis*. However, the number of new bands that appeared is 9, while the ones that disappeared was 6. The percentage polymorphism detected in UBC 827 primer is 58%. The total number of DNA frangments that occurred in each lane is recorded in Table 4.20

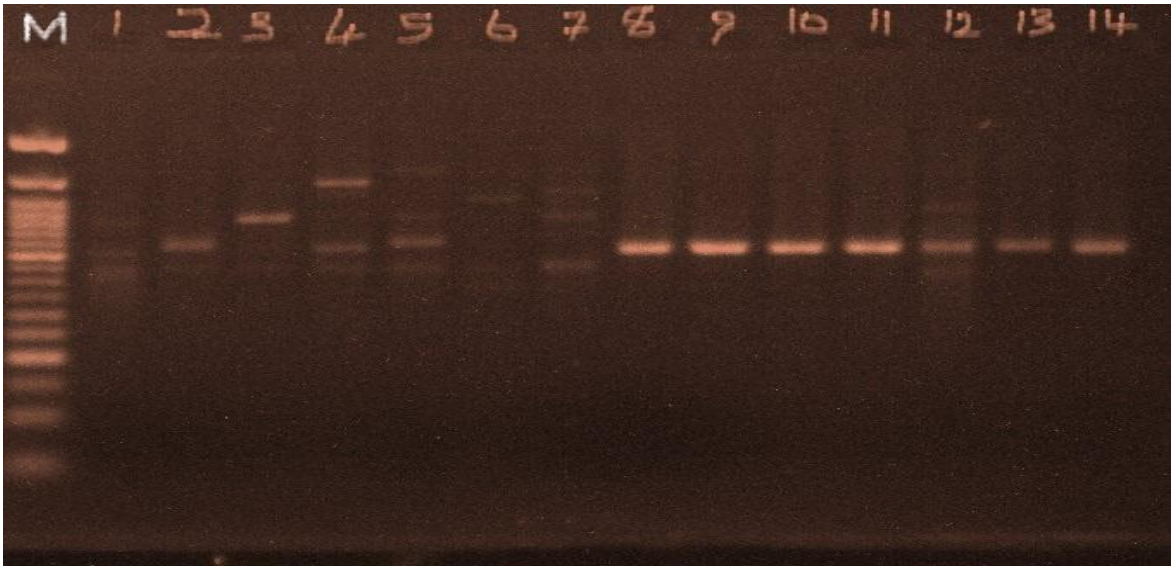

Plate 4.2b: ISSR PCR amplification products for *A. hybridus* and *T. occidentalis* treated with three components of PAH and various concentrations of SEOPS using UBC-827 primer. Lane M: 50bp DNA ladder; lane 1 and 8: control (*A. hybridus* and *T. occidentalis*): Lanes 2, 3, 4 (*A. hybridus*)/9, 10, 11 (*T. occidentalis*): were treated with B(a)P, B(k)F and B(ghi)P. Lanes 5, 6, 7 (*A. hybridus*)/12, 13, 14 (*T. occidentalis*) were treated with various concentrations of SEOPS.

Table 4.20: Numbers of DNA band breaks in *A.hybridus* and *T.occidentalis* exposed and unexposed to B(a)P, B9k)F, B(ghi)P and different concentrations of spent engine oil polluted soil using UBC 827 primer.

Lane	Sample ID	No. of DNA Fragments
1	UPS (A.hybridus)	3
2	B(a)P (A.hybridus)	2
3	B(k)P (A.hybridus)	1
4	B(ghi)P (A.hybridus)	3
5	SEOPS A (A.hybridus)	4
6	SEOPS B (A.hybridus)	1
7	SEOPS C (A.hybridus)	3
8	UPS (T.occidentalis)	1
9	B(a)P (T.occidentalis)	1
10	B(k)P (T.occidentalis)	1
11	B(ghi)P (T.occidentalis)	1
12	SEOPS A (T.occidentalis)	3
13	SEOPS B (T.occidentalis)	1
14	SEOPS C (T.occidentalis)	1

The gel picture generated using UBC 808 primer is shown in Plate 4.2c. From the result it was observed that in *A.hybridus*, lane 1(contol) to 7 showed five DNA band fragments each. Although the first band in lane 5 to 7 were very faint. However, in *T. occidentalis*, the highest number of DNA fragments was observed to occur in lane 8(control) with six bands; In lane 9-14 a total of four band each was observed except in lane 11 which had only two bands, hence indicating that DNA band disappeared at different positions when compared to the control (lane 8). The number of new bands that appeared is 0, while the deleted one's was 11. The percentage polymorphism detected in UBC 808 primer is 19%.

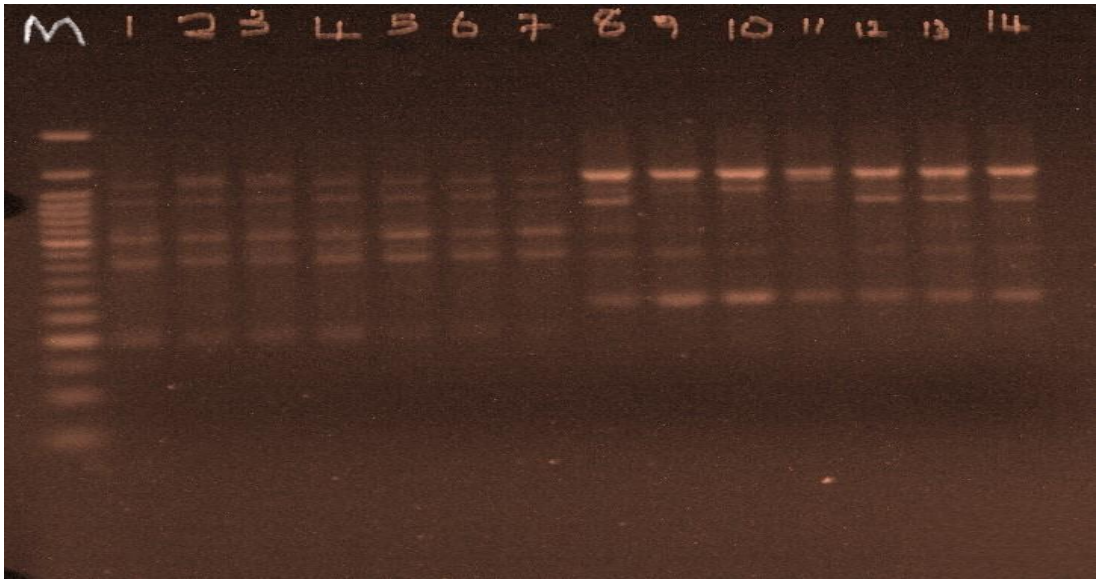

Plate 4.2c: ISSR PCR amplification products for *A. hybridus* and *T. occidentalis* treated with three components of PAH and various concentrations of SEOPS using UBC-808 primer. Lane M: 50bp DNA ladder; lane 1 and 8: control (*A. hybridus* and *T. occidentalis*): Lanes 2, 3, 4 (*A. hybridus*)/9, 10, 11 (*T. occidentalis*): were treated with B(a)P, B(k)F and B(ghi)P. Lanes 5, 6, 7 (*A. hybridus*)/12, 13, 14 (*T. occidentalis*) were treated with various concentrations of SEOPS.

Table 4.21: Numbers of DNA band breaks in *A. hybridus* and *T. occidentalis* exposed and unexposed to B(a)P, B(k)F, B(ghi)P and different concentrations of spent engine oil polluted soil using UBC 827.

Lane	Sample ID	No. of DNA Fragments
1	UPS (A.hybridus)	5
2	B(a)P (A.hybridus)	5
3	B(k)P (A.hybridus)	5
4	B(ghi)P (A.hybridus)	5
5	SEOPS A (A.hybridus)	5
6	SEOPS B (A.hybridus)	5
7	SEOPS C (A.hybridus)	5
8	UPS (T.occidentalis)	6
9	B(a)P (T.occidentalis)	4
10	B(k)P (T.occidentalis)	4
11	B(ghi)P (T.occidentalis)	2
12	SEOPS A (T.occidentalis)	4
13	SEOPS B (T.occidentalis)	4
14	SEOPS C (T.occidentalis)	4

4.1.5c Dendrogram of the Plant Species

The distance matrix dendrogram showing hierarchical clustering of the plants sample was constructed. Unexposed *Amaranthus hybridus* (1A) was closely related with benzo(ghi)perylene pollutant (4A) than other pollutants. Benzo(a)pyrene (2A), spent engine oil polluted soil A (5A), and spent engine oil polluted soil B (6A) has the most distant relationship when compared to control(1A). However, *T. occidentalis* most distantly clustered is spent engine oil polluted soil C (14T) and spent engine oil polluted soil B (13T) shares the most similarities when compared to control (8T) while spent engine oil polluted soil A (12T) are inter-related with spent engine oil A(12T). Hence, this dendrogram illustrate the relationship between the numerous clusters.

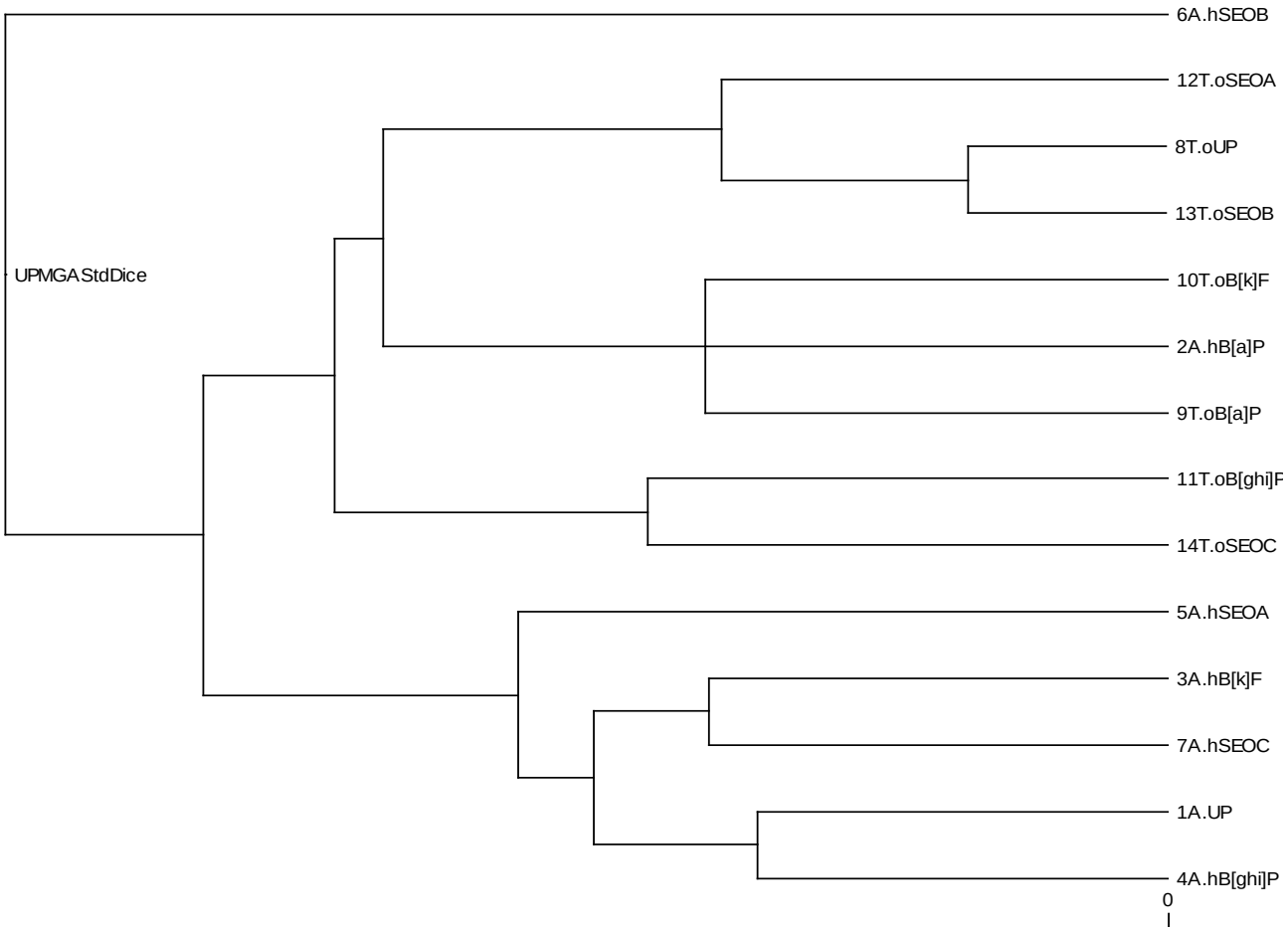

Figure 4.3: The UPGMA Dendrogram of DNA bands relationship between *A. hybridus* (1A-7A) and *T. occidentalis* plants (8T-14T) exposed to different PAH components.

4.1.6 Human health risk associated with dietary intake of PAH from various sources for *A.hybridus*

The human health risk associated with dietary consumption of *A. hybridus* exposed to benzo(a)pyrene, benzo(k)fluoranthene, benzo(ghi)perylene and that from different spent engine oil polluted soil are presented on Table 4.22a. The result showed that benzo(a)pyrene had the highest dietary daily intake (DDI) value of 1.358 $x10^{-3}$mg/kg when compared to that from spent engine oil polluted soil benzo(a)pyrene, benzo(k)fluoranthene and benzo(ghi)perylene. This was followed by benzo(a)pyrene from spent engine oil polluted soil. In benzo(a)pyrene toxic equivalent (B(a)Pteq), benzo(a)pyrene had the highest value when compared with that of benzo(k)fluoranthene and benzo(ghi)perylene. Furthermore, the value of benzo(a)pyrene value exceeded that of the screening values of adult and children. Furthermore, the incremental lifetime cancer risk (ILCR) values from the three PAH components and that from spent engine oil polluted soil for children were higher when compared 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 4.22a: Health risk associated with consumption of *Amaranthus hybridus* exposed to PAH component from different sources

Pollutants	PAH components	Mean ±SD (mg/ml)	TEF	ED=DDI (Ci x IR) mg/kg	B(a)Pteq (Ci x TEFi) (mg/kg)	ILCR Adult	ILCR Children	MOE Adult	MOE Children
SEOPS	B(a)P	0.372 ±0.05	0.1	1.86×10^{-4}	3.7×10^{-5}	8.30×10^{-9}	1.18×10^{-8}	93.5	54.6
	B(k)F	ND	0.03	ND	ND	ND	ND	ND	ND
	B(ghi)P	0.107 ±0.02	0.009	0.054	1×10^{-6}	2.40×10^{-9}	3.44×10^{-9}	244.4	142.6
PAH	B(a)P	2.716 ±0.83	0.1	1.358×10^{-3}	2.72×10^{-4}	6.03×10^{-8}	8.64×10^{-8}	12.8	3.8×10^{-3}
	B(k)F	1.738 ±0.89	0.03	0.869×10^{-3}	5×10^{-5}	3.86×10^{-8}	5.53×10^{-8}	9.7	5.6
	B(ghi)P	1.064 ±0.03	0.009	0.532×10^{-3}	1.0×10^{-5}	2.36×10^{-8}	3.38×10^{-8}	24.8	14.5
SV-Adult	8.4×10^{-5} mg/kg								
SV-Children	5.6×10^{-5}mg/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
SV = Screening value
ILCR = Incremental lifetime cancer risk
MOE = Margin of exposure
DDI = Dietary daily intake

4.1.7 Human health risk associated with dietary intake of PAH from various sources for *T.occidentalis*

Human health risk associated with dietary consumption of *T. occidentalis* exposed to benzo(a)pyrene, benzo(k)fluoranthene and benzo(ghi)perylene and that from spent engine oil polluted soil are presented on Table 4.22b. The results showed that benzo(a)pyrene and benzo(k)fluoranthene had the highest dietary daily intake (DDI) value ($1.4x\ 10^{-5}$mg/kg and 0.869ng/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 2.08×10^{-4}mg/kg which exceeded the screening value for both adult (8.4×10^{-5} mg/kg) and children (5.6×10^{-5}mg/kg). The incremental lifetime cancer risk from PAH from spent engine oil polluted soil, benzo(a)pyrene, benzo(k)fluoranthene and benzo(ghi)perylene for children were higher when compared with that of adult. Furthermore, the margin of exposure value was higher in benzo(a)pyrene from the spent engine oil polluted soil compared to that of the three different PAH components. However, the margine of exposure values of adult were higher than that of children, except for benzo(ghi)perylene which was not detected.

Table 4.22b: Health risk associated with consumption of *Telfaria occidentalis* exposed to PAH component from different sources

Pollutants	PAH components	Mean ±SD (mg/ml)	TEF	ED=DDI (Ci x IR) mg/kg	B(a)Pteq (Ci x TEFi) mg/kg	ILCR Adult	ILCR Children	MOE Adult	MOE Children
SEOPS	B(a)P	0.028 ±0.02	0.1	$1.4x\ 10^{-5}$	2.8×10^{-6}	6.00×10^{-10}	8.90×10^{-10}	1242.9	725
	B(k)F	0.181 ±0.01	0.03	$9.1x\ 10^{-5}$	5.4×10^{-6}	4.00×10^{-9}	5.79×10^{-9}	92.3	53.8
	B(ghi)P	ND	0.009	ND	ND	ND	ND	ND	ND
PAH	B(a)P	2.082 ±0.01	0.1	1.04×10^{-3}	2.08×10^{-4}	4.62×10^{-8}	6.62×10^{-8}	16.7	9.8
	B(k)F	1.738 ±0.48	0.03	8.69×10^{-4}	5.2×10^{-5}	3.86×10^{-8}	5.52×10^{-8}	9.67	5.6
	B(ghi)P	0.960 ±0.12	0.009	4.82×10^{-4}	9×10^{-6}	2.14×10^{-8}	3.07×10^{-8}	27.3	15.9
SV-Adult	$8.4x\ 10^{-5}$ mg/kg								
SV-Children	5.6×10^{-5}mg/kg								

Legend:
SEO = Spent engine oil
PAH = Polycyclic aromatic hydrocarbons
B(a)Peq = Benzo(a)pyrene equivalent
B(a)P = Benzo(a)pyrene
B(k)F = Benzo(k)fluoranthrene
B(ghi)P = Benzo(ghi)perylene
TEF = Toxic equivalent factor
SV = Screening value
ILCR = Incremental lifetime cancer risk
MOE = Margin of exposure
DDI = dietary daily intake

4.2 DISCUSSION

4.2.1 Assessment of PAH Content in different Environment

The total PAH samples in the environment shows that they were higher than the permissible limit as indicated by United State Environmental Protection Agency (USEPA). The PAH levels in water samples of Nworie river were observed to be higher than those measured in corresponding sediment samples. This is in contradiction to the high levels of PAH that have been reported in sediments (Ma, *et al.*, 2013; Hong, Jia, Li, Sun, Liu & Wang, 2016). The difference can be attributed to presence of the low molecular weight (LMW) PAH (2-3 rings PAH) which are soluble and volatile in water and have been more detected in water samples of Nworie river. According to Nasr, Arief, Abdel & Malhat (2013); Mohammed, Al-Taee & Hassan (2009), the composition pattern of PAH in water samples are dominated by three rings PAHs while sediment is mostly dominated by four ring PAHs. The predominance of the low molecular weight PAHs relative to high molecular weight PAHs in water and sediment samples can be due to petrogenic PAHs from oil spills and road construction materials (such as production of coke, coal tar and asphalt) (CCME, 2010; Brazkova & Krastarov 2013; Oluseyi, Okyinka, Alo & Smith 2011). Both water and sediment samples from Nworie River were considered to be highly contaminated with PAHs, as some of the detected PAH components exceeded the water quality guideline threshold recommended by USEPA for the protection of aquatic life (USEPA, 2006). In sediment samples, the high level of phenanthrene (3-ringed PAH) detected shows a major constituent of crude oil and coal tar. According to Santos, Souza, Vilela, Soares, Frena & Alexandre (2018), an increase level of phenanthrene in aqueous sediment was linked to atmospheric deposition and petroleum contamination. Phenanthrene is known to cause endocrine and reproductive disruption, neurotoxicity, genotoxicity, oxidative damage, cytotoxicity and growth impairment in fish has been reported (Machado, De, Hoff, Klein, Cordeiro, Lencina *et al.*, 2014). In spent engine oil polluted soil (SEOPS), the HMW PAH (4-5) had the highest proportion. The predominance of the HMW PAHs relative to LMW PAH in SEOPS had been attributed to pyrogenic PAH from activities of the mechanic workshop such as photochemical smog from automobile exhaust (Nekhavhambe, van Ree & Fatoki 2014).

4.2.2 The Impact of PAH from Spent Engine Oil and the three PAH components on Soil Samples

Physicochemical characteristics of soil polluted with spent engine oil indicates the trends of soil textural class, moisture content, bulk density, pH, organic carbon, nitrogen, etc. This reveals that the polluted soil sample has lower moisture content when compared to the unpolluted soil sample. This might be as a result of the pollutant present in the soil as its hydrophobic nature facilitates the loss of water through evaporation. Ahamefule, Obi, Amana, Peter, Eifediyi & Nwokocha (2014) reported that the hydrophobic property of spent engine oil impedes the adherence of water molecule to soil particles which leads to water loss through evaporation. Bulk density was shown to be high when compared to the unpolluted soil samples. The consequence of increased bulk density leads to soil compaction thereby affecting soil porosity (Grossman and Reinsch, 2002). However, the textural class which is sandy was not affected by the spent engine oil pollution. This agrees with Onweremadu (2012) who observed that the textural class of contaminated soil in the region is not affected by pollutant. The pH of the polluted soil is acidic which indicates its necessity as metal cations are made soluble and available for plant uptake. However, the input of microbial degradation in the formation of organic acid can also make the soil pH acidic (Osuji and Nwoye, 2007). Uchendu and Ogwo (2014) reported that plants grown in acidic soil can experience a variety of stress as a result of heavy metal availability for root absorption. The increase in organic carbon and organic matter in the polluted soil was probably due to hydrocarbon content in spent engine oil polluted soil while increase in nitrogen may be due to the high organic matter content of the contaminated soil. This agrees with Tanimu, Michael & James (2019) who reported increase in organic carbon and nitrogen content of oil polluted soil. The effective cation exchange capacity (ECEC) which shows the capability of Ca, Mg, Na and K to displace other cations was low, probably because of the sandy textural class of the polluted soil and its low pH. This indicates low fertility of the contaminated soil (Uchendu and Ogwo, 2014). The available phosphorus of polluted soil was low indicating that the soil physicochemical property was altered by the spent engine oil as it inhibits microbial transformation of organic matter (Nwite and Alu, 2015).

4.2.3 Effect of PAH from spent engine oil polluted soil, Benzo(a)pyrene, Benzo(k)fluoranthrene, and Benzo(ghi)perylene

The plants ability to withstand the stress caused by the pollutants was shown in its performance. In *Amaranthus hybridus*, there was significant difference in concentrations of the pollutants on growth parameters when compared to control and this indicates its detrimental effect to the plant growth. Moreover, concentrations SEOPS C and PAH A appear to be more detrimental to the plant as they

are significantly higher than other concentrations. Although, 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 two weeks interval. Furthermore, the effects of the pollutants were visible in *Amaranthus hybridus* as it was not able to withstand stress within the period of the experiment as yellowing of leaves and stunted growth was observed.

The Effect of spent engine oil pollutant shows a trend of concentration dependent as SEOPS 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 aeration 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 as it reduces the germination and seedling growth of *Z. mays*, *A. hypogea* and *V. unguiculata*. Wyszkowski, Wyszkowski & Zirilkowska. (2004) reported hydrocarbons smearing root plants with oily substances reduce the permeability of cell membrane and upsetting metabolic processes which leads to changes in the chemical composition and finally exact toxic effects on the plants. The fresh and dry weight level of the plants samples were because of the pollutant. Therefore, absorption of water and ions take place in the root which has a direct contact with spent engine oil and PAH polluted soil. This is in line with work of Agbogidi and Ejemeta (2005) who reported on the reduced dry mass accumulation following spent oil application on garden soil.

4.2.4 Bioaccumulation of Spent Engine Oil and Benzo(a)pyrene, Benzo(k)fluoranthrene, and Benzo(ghi)perylene pollutants on Plant Samples

Some plants have shown potentials to bioaccumulate some of these pollutants and as such can be toxic to the food chain. This is because of PAH carcinogenicity, mutagenicity, teratogenicity and acute toxicity which can damage the endocrine system. The three different PAH components analyzed because of their high toxicity level for the two plants samples revealed that *Amaranthus hybridus* accumulated more of the PAH components B(ghi)P and B(a)P than *Telfairia occidentalis* which accumulated more of B(k)P then less of B(a)P both from spent engine oil. However, in soil polluted with purchased PAH, *Amaranthus hybridus* accumulated more of it as well. In general, *amaranthus hybridus* bioaccumulate more PAH than *Telfaiiar occidentalis* even though it couldn't withstand the toxicity exerted by PAH within 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 pollutants concentration cannot be over-emphasized as the level of accumulation in the plants are concentration dependent. Pretorius, Charest, Kimpe & Blais (2018) worked on accumulation of metal, PAH and alkyl PAH, and reported that increase in PAH accumulation was observed in the roots of *E. purprea*. However, there is possibility of Phyto-immobilization occurrence where restriction of absorbed pollutant mobility occurs within the plant root, thereby confining it to a specialized vacuole. This could explain the decrease PAH accumulation observed in *Telfairia occidentalis*. This is in contrast with the report of Aniefiok and Udo, (2019), on the role of plants and microbes in bioremediation of petroleum hydrocarbons contaminated soils.

4.2.5 Genotoxic Effect of Pollutants on Plants

Quantification of DNA extracted from unpolluted and polluted plants sample was to determine its purity and integrity when compared to the control. However, the band shows that polymorphism occurred as insertion, deletion and changes in band intensity was observed. The dendrogram obtained from dice similarity matrix separating the DNA of plants sample showed the relationship between treatments. The genetic differentiation value from *Amaranthus hybridus* was more than *Telfairia occidentalis* as it seems to possess more activities of spent engine oil pollutant than *Telfairia occidentalis* while PAH pollutants occurred more in *Telfairia occidentalis*. This reveals pollutant absorption at the genetic level. These changes could be attributed to the stress induced by pollutants presence in the soil as it possesses the capability to modify structural DNA or alter the expression of regulatory genes. The components of the pollutants involved can cause mutational changes in the DNA which could be inhibitive or change inherent expression thereby leading to differences in band and band intensities. Hardonniere, Saunier & Lemarie (2016) reported that DNA damage induced by PAH are through formation of adducts and reactive oxidative species. During PAH degradation, diol epoxide enantiomers are formed which can bind to DNA thereby causing mutagenicity and carcinogenicity. Benzo(a)pyrene possess the ability to generate reactive oxygen species in redox cycle leading to oxidative stress. The auto-oxidation of 6-OH-BaP which are precursors of benzo(a)Pyrene cations can generate BaP quinones (An, Yin & Shang 2011). PAH can also absorb ultra violet rays which can induce reactive oxygen species also causing DNA damage. However, DNA internal repair mechanism can revert or repair the genes saddled with cell control cycle but constant exposure to these pollutants can overwhelm the repair system which

definitely will lead to DNA damage. Mustapha,Njoku, Adesuyi & Jolaoso (2019) reported the genotoxicity ability of PAH and heavy metals obtained from mechanic workshop and dumpsite to induce mutational changes. Zeid and Abou El Ghate (2007) also reported the toxic potentials of heavy metals in transcription of stress-induced genes and accumulation of their polypeptides. Toxicity of the pollutants appear to be more severe in *Amaranthus hybridus* probably because of its low tolerance threshold in both spent engine oil and PAH polluted soil. The mutational effect of pollutants was more from the spent engine oil polluted soil than from the PAH polluted soil and this was seen in the DNA bands distortion. Therefore, spent engine oil polluted soil was more toxic to the plants and this may be because of other toxic substances imbedded in the pollutant e.g. heavy metals etc.

4.2.6 Health Risk Assessment Model

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 screening value determine the level of toxic chemical in edible substances which is of public health concern (Wu, Zhao, Sun, Tan Tang, Nie *et al* 2012). The toxicity level of the plants was evaluated using screening value (SV) threshold which was used to assess the health implication of PAH on humans 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 screening 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 component of interest in this study possess low potential health risk. However, benzo(a)pyrene was more when compared to the screening value and this portend imperilment as its indicates high potential health risk for consumers within the polluted area. According to Okereke *et al*. (2016) who studied the risk assessment of PAH especially benzo(a)pyrene in River state, Nigeria, he observed that consumable vegetables in farmlands within an industrial cite area had toxic equivalent quotients higher than the screening value indicating significant health risk concern.

Furthermore, the genotoxic and carcinogenic level of the vegetables exposed to PAH from both toxicant was assessed through margin of exposure (MOE). The margin of exposure is the benchmark dose lower limit of estimated exposure dose or concentration. This was developed by joint committee on food additives (JECFA) and European food safety authority (EFSA) in 2005, as a

guidance for risk assessment of genotoxic and carcinogenic compounds (EFSA, 2005). PAH from vegetables exposed to toxicants 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 low health risk concern for consumers of this vegetables contaminated with PAH toxicant. This supports the study conducted by Minmin, Zhanghuan & Qianaian (2016) from Nanjing China on edible vegetables as PAH margin of exposure was less than the standard limits making it of low risk concern for public health risk. However, this is in contradiction with the study of Sorbari, Udowelle, Ekhator, Asomugha, Igweze & Orisakwe (2019) who reported that PAH margin of exposure on mushroom were relatively unsafe for consumption as the values are above the 10,000 critical limits.

The incremental life cancer risk (ILCR) in this study was used to quantitatively determine the exposure risk of PAH. Incremental life cancer risk of $\geq 10^{-6}$ were considered to be insignificant while $\geq 10^{-4}$ were significant (Mana, Kang, Wang, Laub, Li, Sunb *et al* 2013). Therefore, action is expected to be taken in other to reduce life cancer risk when the range is within the latter figure. The vegetables exposed to toxicant showed low health risk in terms of incremental life cancer risk through consumption for both adult and children. This is supported by Moslen, Mieba & Boisa (2019), who observed that the PAH bioaccumulation of bivalve were of low health risk concern to consumers when considered value of the excess lifetime cancer risk. According to Qu, Qi, Yang, Huang, Zhang, Chen, Yoannes *et al.,* (2015) considering the various method of health risk assessment applied in this study, the guideline may differ depending on region and policy formation of the country.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

It was observed that in the assessment of PAH content in different urban city center the lower molecular weight PAHs were more detected in water samples even though PAH are generally not soluble in water while the high molecular weight PAH were more in polluted soil. This indicates that even though PAH are lipophilic in nature, the LMW PAH (2-3rings) are known to have high solubility and volatility rate and as such accumulate in the water bodies. The study also established that PAH components detected includes; weak carcinogenic, strong carcinogenic and non carcinogenic PAH in the land use in owerri environs.

The use and management of soil is largely dependent on its characteristics and qualities. The study showed that spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)peryene had significant influence on the soil properties and plants growth performance when compared to the unpolluted soil.

The impact of PAH on growth performance of the two plants samples showed that it is dependent on the plant characteristics and concentration involved. However, *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 used in assessing phyto-toxicity of these pollutants while *T. occidentalis* may be useful for phyto-monitoring of these pollutants as it was able to withstand the stress exerted on it during the period of the experiment.

The study also observed appearance, disappearance and changes in DNA band intensity of the vegetables exposed to the B(a)P, B(k)F, B(ghi)P and different concentrations of SEO, which lead to genetic alterations. This is due to the accumulation potential of these toxic pollutant in the plant tissue which are concentration dependent.

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 prolong exposure to these contaminated vegetables could be detrimental to humans as these pollutants possess the ability to bioaccumulate over a long period of time.

5.2 Recommendations

This study identified that exposure of crop plants to spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene could have adverse effect on plants as well as human life. As the pollutants have the ability to bioaccumulate and can also induce mutation and genotoxicity effects on plants. Therefore, it is recommended that:

a) Awareness of the effects of disposal of this pollutants in the environment and the potential risk on our ecosystem should be created.
b) By educating the users of this pollutants and general public at large on the need for proper disposal methods and the consequence of failure to remedy the environment of this pollutants.
c) Laws which aim at proper disposal should be enacted to stop the indiscriminate release of this toxicants and penalty be impose on offenders.
d) Further research should be conducted to ascertain the magnitude of mutation these pollutants can exert on other crop plants and possibly animals.

5.3 Contribution to Knowledge

a) The study has shown that indiscriminate discharge of PAH and spent engine oil have left the different law use in Owerri with PAH components.

b) The study also showed that PAH accumulation in plant can lead to band appearing, disappearing and change in DNA intensity of the plant exposed.

c) The study established that there is no much risk associated with short term exposure to B(a)P, B(k)F, B(ghi)P and differeent concentrations of spent engine oil.

d) The study shows the relationship in the toxicity level of polluted plants.

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APPENDIX

Appendix 1a: Soil physical properties of polluted and unpolluted soil sample

Parameters	UPSS	PSS	SEM
% Moisture content	14.6	12.4	0.664
Bulk density(g/cm^3)	1.29	1.68	0.115
Porosity(%)	51.35	49.36	0.980
Sand(g/kg)	90.62	87.44	-
Silt(g/kg)	5.10	3.50	0.462
Clay(g/kg)	1.71	2.46	0.191
Silt-clay	9.6	12.6	0.580

Legend: ±Standard deviation; PSS: Polluted soil sample; UPSS: Unpolluted soil sample

Appendix 1b: Chemical properties of polluted and unpolluted soil samples

Parameters	UPSS	PSS	SEM
pH	7.4	5.1	0.202
ECEC	9.27	7.36	0.558
Exchangeable acidy	0.99	1.25	0.219
Exchangeable base	2.55	4.67	0.384
Organic carbon	5.12	6.32	0.208
Organic matter	6.01	6.45	0.408
% Nitrogen	0.294	0.429	0.047
Available phosphorus	28.0	19.8	1.008

Legend: PSS: Polluted soil sample; UPSS: Unpolluted soil sample; ECEC: Effective cation exchange capacity.

Appendix 2

Plate 1: *A.hybridus* at 7days in nursery

Plate 2: *A.hybridus* at 14days of Transplanting

Plate 3: *T.occidentalis* at 7days in nursery

Plate 4: *T.occidentalis* at 14days of transplanting

Appendix 3a: Concentrations of PAH components from Soil Polluted with Different Concentrations of spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene at 14days

PAH components	UPSS	SEOPS A	SEOPS B	SEOPS C	B(a)P	B(k)F	B(ghi)P
Acy	0.0060	0.2689	0.2515	0.3118	-	-	-
Phen	0.0034	0.0641	0.0725	0.0664	-	-	-
B(k)F	0.0326	0.1611	0.2015	0.1498	-	1.4212	-
B(ghi)P	0.05155	0.0428	0.0698	0.0541	-	-	1.5060
1-2Benz	ND	0.0685	0.0623	0.0625	-	-	-
Flu	0.0200	0.1839	0.2756	0.2745	-	-	-
D(ah)A	0.1629	0.1785	0.1851	0.1743	-	-	-
B(a)P	0.0728	0.3781	0.3556	0.3719	1.5956	-	-
Flo	-	0.1475	0.1758	0.1622	-	-	-
Ace	-	0.2647	0.2467	0.2145	-	-	-
Ant	-	0.2518	0.2168	0.2486	-	-	-
Pyr	-	0.0214	0.0321	0.0640	-	-	-
Nap	-	0.1636	0.1219	0.1381	-	-	-
B(b)F	-	0.1615	0.1984	0.1675	-	-	-

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. ND- not detected. All units are in mg/ml.

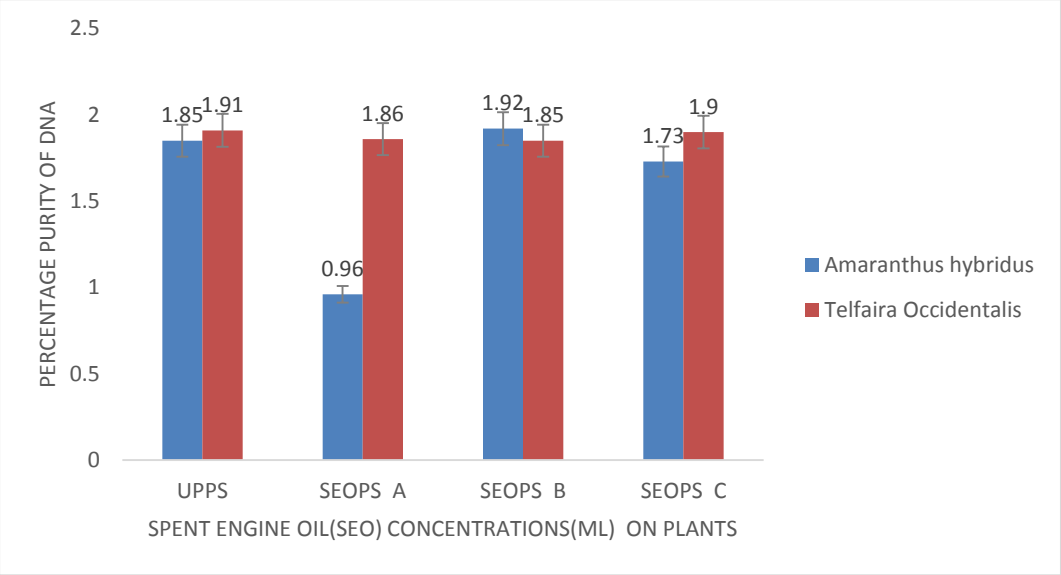
Appendix 3b: Concentrations of PAH components from Soil Polluted with Different Concentrations of spent engine oil, benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene at 28days

PAH components	UPSS	SEOPS A	SEOPS B	SEOPS C	B(a)P	B(k)F	B(ghi)P
Acy	0.0132	0.5541	0.5781	0.5970	-	-	-
Phen	0.0078	0.1462	0.1593	0.1735	-	-	-
B(k)F	0.0632	0.3021	0.4113	0.4152	-	3.1845	-
B(ghi)P	0.1011	0.1134	0.1258	0.1690	-	-	3.0120
1-2Benz	ND	0.0978	0.1245	0.1562	-	-	-
Flu	0.0578	0.3351	0.5709	0.6002	-	-	-
D(ah)A	0.3584	0.3276	0.3545	0.3652	-	-	-
B(a)P	0.1214	0.7189	0.7311	0.7510	3.1912	-	-
Flo	-	0.3019	0.3105	0.3243	-	-	-
Ace	-	0.5668	0.5782	0.5734	-	-	-
Ant	-	0.4601	0.4724	0.4975	-	-	-
Pyr	-	0.0656	0.0878	0.1280	-	-	-
Nap	-	0.2651	0.2983	0.3108	-	-	-
B(b)F	-	0.3123	0.3456	0.3718	-	-	-

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. ND- not detected. All units are in mg/ml.

Appendix 4a: Percentage Purity of Plants Sample Exposed and Unexposed to different concentrations of Spent Engine Oil Polluted Soil.

The percentage purity of *Amaranthus hybridus* and *Telfairia occidentalis* exposed and unexposed to different concentrations of spent engine oil is presented in Figure 4.5. The result shows the DNA quantification in order to determine its percentage purity. The result of the purity of DNA from crop plants (*A.hybridus* and *T.occidentalis*) showed the percentage integrity of the DNA of both plants from unpolluted soil samples (UPPS) were slightly higher than the percentage purity of the polluted soils. The result also showed that the DNA of *A.hybridus* exposed to spent engine oil polluted sample A (SEOPS A) had a higher percentage integrity that is slightly higher than the percentage purity of DNA obtained from same polluted soil. A similarly result, though slightly decreased percentage integrity was obtained in SEOPS B of all the percentage purity of DNA obtained from various exposure SEOPS C. *A.hybridus* showed the lowest percentage purity (0.96%) compared to the counterpart exposed in the same polluted soil and other plants from polluted soil. The study has shown reduced percentage purity as the concentration of spent engine oil increases.

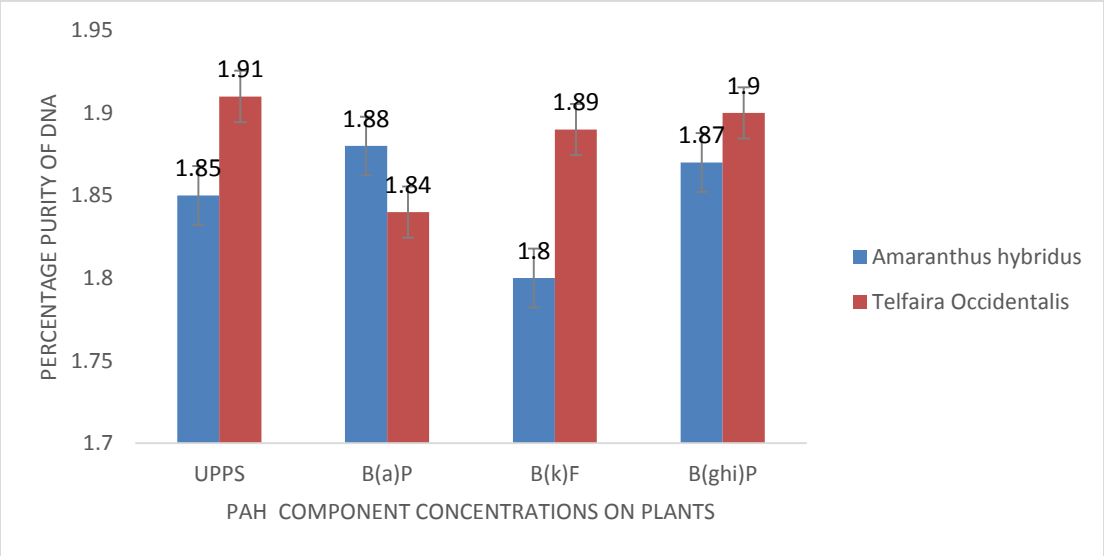


Percentage purity of spent engine oil polluted and unpolluted plants.

LEGEND: Unpolluted plant sample (UPPS)
100ml spent engine oil polluted sample (SEOPS A)
200ml spent engine oil polluted sample (SEOPS B)
300ml spent engine oil polluted sample (SEOPS C)

Appendix 4b: Percentage Purity of Plant Samples Exposed and Unexposed to benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene.

The percentage purity of *Amaranthus hybridus* and *Telfairia occidentalis* exposed and unexposed to benzo(a)pyrene, benzo(k)fluoranthrene and benzo(ghi)perylene is presented in Figure 4.6. The result obtained from the percentage purity of DNA from *A.hybridus* ranged from 1.8-1.88 and *T.occidentalis* from 1.84-1.91. The DNA of crop plant from unpolluted soil sample had increase percentage purity in *T.occidentalis* compared to percentage purity from other exposed DNA. However, the percentage purity for DNA of *A.hybridus* exposed to benzo(a)pyrene ha the highest percentage purity values. The study showed that the percentage purity of DNA of both plants unexposed to the different PAH component were almost of the same values with that exposed to benzo(ghi)perylene. The smallest percentage purity values were obtained from DNA of *A.hybridus* exposed to benzo(k)fluoranthene while the smallest percentage purity of DNA from *T.occidentalis* was observed in that exposed to benzo(a)pyrene. Generally, the percentage purity of *T.occidentalis* where higher and in the range of 1.84-1.9% compared to that of *A.hybridus*.



Percentages purity of DNA from A.hybridus and T.occidentalis exposed to concentrations of PAH.

LEGEND:
B(a)P (Benzo(a)pyrene),
B(k)F (Benzo(k) Fluoranthene),
B(ghi)P (Benzo(ghi)perylene.

Appendix 4c: The percentage purity, nucleic acid and absorbance of DNA of *Amaranthus hybridus* and *Telfairia occidentalis* exposed to spent engine oil polluted soil (ml).

Samples	**Concentration exposure**	**Nucleic acid (ng/µl)**	**DNA Absorbance (A260)**	**DNA Absorbance (A280)**	**DNA Purity (260/280)**
Amaranthus hybridus	UPS	984.20±27.30	19.69 ±1.59	10.62 ±0.62	1.85 ±0.46
	SEOPS A	1089.30 ±71.95	21.79 ±1.80	22.71 ±1.60	0.96 ±0.24
	SEOPS B	1618.30 ±181.80	32.37 ±0.10	16.89 ±2.53	1.92 ±0.09
	SEOPS C	552.80 ±96.05	11.06 ±1.95	6.38 ±1.10	1.73 ±0.03
Telfairia occidentalis	UPSS	1898.40 ±90.90	37.97 ±4.57	19.89 ±1.20	1.91 ±0.88
	SEOPSSA	1468.70 ±230.80	29.37 ±1.38	15.78 ±0.32	1.86 ±0.22
	SEOPSSB	1320.60 ±233.00	26.41 ±0.54	14.29 ±3.52	1.85 ±0.16
	SEOPSSC	1326.40 ±48.30	26.53 ±2.80	13.93 ±2.22	1.90 ±0.28

Legend: ±: Standard deviation
UPS: Unpolluted sample
SEOPS A: 100ml spent engine oil polluted sample;
SEOPS B: 200ml spent engine oil polluted sample;
SEOPS C: 300ml spent engine oil polluted sample

Appendix 4d: the percentage purity, nucleic acid and absorbance of DNA of *Amaranthus hybridus* and *Telfairia occidentalis* exposed to different components of polycyclic aromatic hydrocarbon polluted soil.

Samples	**Concentration exposure**	**Nucleic acid (ng/µl)**	**DNA Absorbance (A260)**	**DNA Absorbance (A280)**	**DNA purity (260/280)**
Amaranthus hybridus	UPSS	984.20 ±27.30	19.69 ±1.59	10.62 ±0.62	1.85 ±0.46
	B(a)P	1119.70 ±130.60	22.40 ±1.71	11.94 ±2.93	1.88 ±0.13
	B(k)F	2479.90 ±816.50	49.60 ±9.61	27.53 ±1.64	1.80 ±0.20
	B(ghi)P	1006.70 ±258.50	20.13 ±1.96	10.78 ±1.31	1.87 ±0.63
Telfairia occidentalis	UPSS	1898.40 ±90.90	37.97 ±4.57	19.89 ±1.20	1.91 ±0.88
	B(a)P	1539.90 ±188.60	30.80 ±1.83	16.70 ±2.61	1.84 ±0.15
	B(k)F	1948.80 ±215.10	38.98 ±1.68	20.63 ±1.36	1.89 ±0.30
	B(ghi)P	2364.00±404.90	47.28 ±1.30	24.85 ±1.18	1.90 ±0.20

Legend: ±: Standard deviation
UPS: Unpolluted sample
SEOPS A: 100ml spent engine oil polluted sample;
SEOPS B: 200ml spent engine oil polluted sample;
SEOPS C: 300ml spent engine oil polluted sample

Appendix 5a: The band scoring for *A.hybridus* and *T.occidentalis* exposed and unexposed to different pollutants using UBC 811 is presented in table 4.6a. The table shows the absence and presence of bands using 0 and 1 which is a representative of the gel picture in figure 4.5a. It was observed that in *A.hybridus*, lane 1(control), 3, 4 &5 had three bands at the same position except for lane 5, lane 2 & 7 had two bands at different positions. However, in lane 6 no band was detected. In *T.occidentalis*, lane 8(control), 10, 12 & 13 had one band at same position except in lane 10. Lane 14 had four bands, while in lane 9, no band was detected. The table also gives an accurate number of band that is either deleted or form as a result of the individual pollutant.

Appendix 5a: DNA Band Scoring for *Amaranthus hybridus* and *Telfairia occidentalis* exposed and unexposed to different pollutants using primer UBC 811

S/N	Sample ID	UBC 811	UBC 811	UBC 811	UBC 811	UBC 811
1	UPS (A.hybridus)	1	0	1	1	0
2	B(a)P (A.hybridus)	1	1	0	0	0
3	B(k)F (A.hybridus)	1	0	1	1	0
4	B(ghi)P (A.hybridus)	1	0	1	1	0
5	SEOPS A (A.hybridus)	0	0	1	1	1
6	SEOPS B(A.hybridus)	0	0	0	0	0
7	SEOPS C (A.hybridus)	0	0	1	1	0
8	UPS (T.occidentalis)	1	0	0	0	0
9	B(a)P (T.occidentalis)	0	0	0	0	0
10	B(k)F (T.occidentalis)	0	1	0	0	0
11	B(ghi)P (T.occidentalis)	0	0	1	1	0
12	SEOPS A (T.occidentalis)	1	0	0	0	0
13	SEOPS B (T.occidentalis)	1	0	0	0	0
14	SEOPS C (T.occidentalis)	1	1	1	1	0

Legend:

1= presence of band

0= absence of band

Appendix 5b: The DNA band scoring for *A.hybridus* and *T.occidentalis* exposed and unexposed to different pollutants using UBC 827 is presented in table 4.6b. From the Table it was observed that in *A.*hybridus, lane 1(control) had two bands, lane 2, 3 & 6 had one band each in different positions. Lane 4&7 had three bands at different position while lane had four bands. In addition, lane 8(control), 9, 10,11, 13 & 14 had one bands each at the same position except for lane 12 which had three bands in *T.occidentalis*. It is also observed that band insertion and deletion occurred at several positions in *A.hybridus* than in *T.occidentalis*.

Appendix 5b: DNA Band Scoring for *Amaranthus hybridus* and *Telfairia occidentalis* exposed and unexposed to different pollutants using primer UBC 827

S/N	Sample ID	UBC 827	UBC 827	UBC 827	UBC 827	UBC 827	UBC 827
1	UPS (A.hybridus)	1	1	0	0	0	0
2	B(a)P (A.hybridus)	0	1	0	0	0	0
3	B(k)F (A.hybridus)	0	0	1	0	0	0
4	B(ghi)P (A.hybridus)	1	1	0	0	1	0
5	SEOPS A (A.hybridus)	1	1	1	0	0	1
6	SEOPS B(A.hybridus)	0	0	0	1	0	0
7	SEOPS C (A.hybridus)	1	0	1	1	0	0
8	UPS (T.occidentalis)	0	1	0	0	0	0
9	B(a)P (T.occidentalis)	0	1	0	0	0	0
10	B(k)F (T.occidentalis)	0	1	0	0	0	0
11	B(ghi)P (T.occidentalis)	0	1	0	0	0	0
12	SEOPS A (T.occidentalis)	1	1	1	0	0	0
13	SEOPS B (T.occidentalis)	0	1	0	0	0	0
14	SEOPS C (T.occidentalis)	0	1	0	0	0	0

Legend:
1= presence of band
0= absence of band

Appendix 5c: The band scoring for *A.hybridus* and *T.occidentalis* exposed and unexposed to different pollutants using UBC 808 is presented in table 4.6c. It was observed that lane 1 to 7 had five bands each for *A.hybridus*. However, in *T.occidentalis* lane 8(control) had five bands, lane 10, 12, 13 & 14 had three bands at the same position except for lane 10. While lane 9 had only two bands. This table shows the position in which band deletion and insertion occurred in each polluted sample.

Appendix 5c: DNA Band Scoring for *Amaranthus hybridus* and *Telfairia occidentalis* exposed and unexposed to different pollutants using primer UBC 808

S/N	Sample ID	UBC 808	UBC 808	UBC 808	UBC 808	UBC 808
1	UPS (A.hybridus)	1	1	1	1	1
2	B(a)P (A.hybridus)	1	1	1	1	1
3	B(k)F (A.hybridus)	1	1	1	1	1
4	B(ghi)P (A.hybridus)	1	1	1	1	1
5	SEOPS A (A.hybridus)	1	1	1	1	1
6	SEOPS B(A.hybridus)	1	1	1	1	1
7	SEOPS C (A.hybridus)	1	1	1	1	1
8	UPS (T.occidentalis)	1	1	1	1	1
9	B(a)P (T.occidentalis)	1	1	0	0	0
10	B(k)F (T.occidentalis)	1	1	0	1	0
11	B(ghi)P (T.occidentalis)	1	1	0	0	0
12	SEOPS A (T.occidentalis)	1	1	1	0	1
13	SEOPS B (T.occidentalis)	1	1	1	0	1
14	SEOPS C (T.occidentalis)	1	1	1	0	1

Legend:
1= presence of band
0= absence of band

Appendix 6: Dice Coefficient Similarity Index

The genetic similarity of *A. hybridus* (1A-7A) and *T. occidentalis* (8T-14T) was measured using Dice coefficient matrix. The similarity value in *A. hybridus* ranged from 0.84-0.78 *for* sample 4A-7A while in *T. occidentalis*, the similarity index was within 0.78-0.86 in sample 14T-8T. Additionally, the highest genetic similarity (0.86) was observed in sample 2A and 12T while the lowest was 0.43 in sample 1A-7A respectively.

Appendix 6: Dice similarity index of plants sample.

	1A	2A	3A	4A	5A	6A	7A	8T	9T	10T	11T	12T	13T	14T
1A	1.00													
2A	0.63	1.00												
3A	0.82	0.68	1.00											
4A	0.84	0.71	0.78	1.00										
5A	0.73	0.59	0.78	0.80	1.00									
6A	0.57	0.68	0.62	0.53	0.53	1.00								
7A	0.78	0.50	0.82	0.74	0.84	0.71	1.00							
8T	0.75	0.86	0.76	0.71	0.59	0.67	0.50	1.00						
9T	0.43	0.83	0.50	0.53	0.53	0.60	0.43	0.67	1.00					
10T	0.57	0.83	0.62	0.67	0.67	0.80	0.57	0.83	0.80	1.00				
11T	0.67	0.62	0.71	0.75	0.75	0.55	0.67	0.62	0.73	0.73	1.00			
12T	0.71	0.67	0.63	0.67	0.67	0.46	0.59	0.80	0.62	0.62	0.57	1.00		
13T	0.68	0.77	0.57	0.63	0.50	0.55	0.40	0.92	0.73	0.73	0.67	0.86	1.00	
14T	0.78	0.75	0.71	0.74	0.63	0.43	0.56	0.75	0.71	0.57	0.80	0.71	0.80	1.00

Appendix 7

The screening value was calculated using the equation below:

$$SV = \frac{\left(\frac{RL}{SF}\right) X\ BW}{CR} \quad (1)$$

RL = 0.00001

SF = (0.00730µg/kgday-adult/0.0061kg-children)

BW = (60kg-adult/35kg-children)

CR = 1g

$$SV = \frac{\left(\frac{0.00001}{0.0073}\right) X\ 60}{1} \text{ (adult)}$$

$$SV = \frac{0.0014\ X\ 60}{1} = 0.084$$

$$SV = \frac{\left(\frac{0.00001}{0.0061}\right) X\ 35}{1} \text{ (Children)}$$

$$SV = \frac{0.0016\ x\ 35}{1} = 0.0056$$

The benzo(a)pyrene equivalent concentrations (B[a]PeqC) of the vegetables samples using benzo(a)pyrene B(a)P, benzo(k)fluoranthrene B(k)F, and benzo(ghi)pyrene B(ghi)P from both Spent engine oil (SEO) and purchase PAH was calculated using the equation below

$$B(a)PEQC = \sum(TEF\ x\ Conc) \quad (2)$$

Parameters	**TEF**
B(a)P	0.1
B(k)F	0.03
B(ghi)P	0.009

The sum of the concentrations of B(a)P, B(k)F, and B(ghi)P For *A.hybridus* from SEO;

∑B(a)P = 0.5460 + 0.3912 + 0.1802 = 0.372

∑B(k)F = ND

∑B(ghi) = 0.0946 + 0.0724 + 0.1545 = 0.107

For *T.occidentalis* from SEO;

∑B(a)P = 0.0308 + 0.0224 + 0.0309 = 0.028

∑B(k)F = 0.1243 + 0.0930 +0.1005 = 0.181

∑B(ghi)P = ND

The sum of the concentrations of B(a)P, B(k)F, and B(ghi)P for *A.hybridus* from purcased PAH*;*

∑B(a)P = 2.716

∑B(k)F = 1.738

∑B(ghi)P = 1.064

For *T.occidentalis* from purchased*;*

∑B(a)P = 2.082

∑B(k)F = 1.738

∑B(ghi)P = 0.960

B(a)Pteq concentrations of B(a)P, B(k)F, and B(ghi)P for *A.hybridus* from SEO;

B(a)Pteq for B(a)P = 0.1 x 0.372 = 0.0372

B(a)Pteq for B(k)F = ND

B(a)Pteq for B(ghi)P = 0.009 x 0.1072 = 0.001

For *T.occidentalis* from SEO;

B(a)Pteq for B(a)P = 0.1 x 0.028 = 0.028

B(a)Pteq for B(k)F = 0.03 x 0.1.738 = 0.0054

B(a)Pteq for B(ghi)P = ND

B(a)Pteq concentrations of B(a)P, B(k)F, and B(ghi)P for *A.hybridus* from purchased PAH;

B(a)Pteq for B(a)P = 0.1 x 2.72 = 0.272

B(a)Pteq for B(k)F = 0.03 x 1.738 = 0.05

B(a)Pteq for B(ghi)P = 0.009 x 1.064 = 0.01

For *T.occidentalis* from purchased;

B(a)Pteq for B(a)P = 0.1 x 2.082 = 0.208

B(a)Pteq for B(k)F = 0.03 x 1.7376 = 0.05

B(a)Pteq for B(ghi)P = 0.009 x 0.960 = 0.009

$$DDIB(a)PEQ = B(a)P \times IR \quad (3)$$

Dietary daily intake of B(a)Peq of B(a)P, B(k)F and B(ghi)P for A.hybridus from SEO;

DDI B(a)Peq for B(a)P = 0.372 x 0.5 = 0.186

DDI B(a)Peq for B(k)F = ND

DDI B(a)Peq for B(ghi)P = 0.107 x 0.5 = 0.054

For T.occidentalis from SEO

DDI B(a)Peq for B(a)P = 0.028 x 0.5 = 0.014

DDI B(a)Peq for B(k)F = 0.181 x 0.5 = 0.091

EDB(a)Peq for B(ghi)P = ND

Dietary daily intake of B(a)Peq of B(a)P, B(k)F and B(ghi)P for *A.hybridus* from purchased PAH;

DDI B(a)Peq for B(a)P = 2.716 x 0.5 = 1.358

DDI B(a)Peq for B(k)F = 1.738 x 0.5 = 0.869

DDI B(a)Peq for B(k)F = 1.064 x 0.5 = 0.532

For *T.occidentalis* from purchase

E = DDI B(a)Peq for B(a)P = 2.08 x 0.5 = 1.04

E = DDI B(a)Peq for B(k)F = 1.738 x 0.869 = 0.025

E = DDI B(a)Peq for B(ghi)P = 0.960 x 0.5 = 0.005

$$\text{ILCR (Adult)} = \frac{\text{ED x EF x EDB(a)Peq x SF x CF}}{BW\ x\ AT} \quad (4)$$

Incremental life time cancer risk of B(a)P, B(k)F and B(ghi)P for *A.hybridus* from SEO;

$$\text{ILCR for B(a)P} = \frac{70\ x\ 365\ x\ 0.186 \text{ x } 0.0073 \text{ x } 0.000001}{60\ x\ 70} = 8.30 \text{ x } 10^{-9}$$

ILCR for B(k)F = ND

$$\text{ILCR for B(ghi)P} = \frac{70\ x\ 365\ x\ 0.054 \text{ x} 0.0073 \text{ x } 0.000001}{60\ x\ 70} = 2.40\text{x } 10^{-9}$$

Incremental life time cancer risk of B(a)P, B(k)F and B(ghi)P for *A.hybridus* from purchased;

$$\text{ILCR for B(a)P} = \frac{70\ x\ 365\ x\ 1.358 \text{ x } 0.0073 \text{ x } 0.000001}{60\ x\ 70} = 6.03 \text{ x } 10^{-8}$$

ILCR for B(k)F = $\frac{70\ x\ 365\ x 0.869 \text{ x } 0.0073 \text{ x } 0.000001}{60\ x\ 70}$ = 3.86 x 10^{-8}

ILCR for B(ghi)P = $\frac{70\ x\ 365\ x\ 0.532 \text{ x } 0.0073 \text{ x } 0.000001}{60\ x\ 70}$ = 2.36 x 10^{-8}

Incremental lifetime cancer risk of B(a)P, B(k)F and B(ghi)P for *T.occidentalis* from SEO

ILCR for B(a)P = $\frac{70\ x\ 365\ x\ 0.014 \text{ x } 0.0073 \text{ x } 0.000001}{60\ x\ 70}$ = 6.00 x 10^{-10}

ILCR for B(k)F = $\frac{70\ x\ 365\ x\ 0.091 \text{ x } 0.0073 \text{ x } 0.000001}{60\ x\ 70}$ = 4.00 x 10^{-9}

ILCR for B(ghi)P = ND

Incremental lifetime cancer risk of B(a)P, B(k)F and B(ghi)P for *T.occidentalis* from purchased;

ILCR for B(a)P = $\frac{70\ x\ 365\ x\ 1.04 \text{ x } 0.0073 \text{ x } 0.000001}{60\ x\ 70}$ = 4.62 x 10^{-8}

ILCR for B(k)F = $\frac{70\ x\ 365\ x\ 0.869 \text{ x } 0.0073 \text{ x } 0.000001}{60\ x\ 70}$ = 3.86 x 10^{-8}

ILCR for B(ghi)P = $\frac{70\ x\ 365\ x\ 0.482 \text{ x } 0.0073 \text{ x } 0.000001}{60\ x\ 70}$ = 2.14 x 10^{-8}

ILCR (Children) = $\frac{\text{ED x EF x EDB(a)Peq x SF x CF}}{BW\ x\ AT}$

Incremental life time cancer risk of B(a)P, B(k)F and B(ghi)P for *A.hybridus* from SEO;

ILCR for B(a)P = $\frac{70\ x\ 365\ x\ 0.186 \text{ x } 0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 1.18 x 10^{-8}

ILCR for B(k)F = ND

ILCR for B(ghi)P = $\frac{70\ x\ 365\ x\ 0.054 \text{ x}0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 3.44x 10^{-8}

Incremental life time cancer risk of B(a)P, B(k)F and B(ghi)P for *A.hybridus* from purchased PAH;

ILCR for B(a)P = $\frac{70\ x\ 365\ x\ 1.358 \text{ x } 0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 8.64x 10^{-8}

ILCR for B(k)F = $\frac{70\ x\ 365\ x 0.869 \text{ x } 0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 5.53 x 10^{-8}

ILCR for B(ghi)P = $\frac{70\ x\ 365\ x\ 0.532 \text{ x } 0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 3.38 x 10^{-8}

Incremental lifetime cancer risk of B(a)P, B(k)F and B(ghi)P for *T.occidentalis* from SEO

ILCR for B(a)P = $\frac{70\ x\ 365\ x\ 0.014 \text{ x } 0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 8.90 x 10^{-10}

ILCR for B(k)F = $\frac{70\ x\ 365\ x\ 0.091 \text{ x } 0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 5.79 x 10^{-9}

ILCR for B(ghi)P = ND

Incremental lifetime cancer risk of B(a)P, B(k)F and B(ghi)P for *T.occidentalis* from purchased;

ILCR for B(a)P = $\frac{70\ x\ 365\ x\ 1.04 \text{ x } 0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 6.62 x 10^{-8}

ILCR for B(k)F = $\frac{70\ x\ 365\ x\ 0.869 \text{ x } 0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 35.52 x 10^{-8}

ILCR for B(ghi)P = $\frac{70\ x\ 365\ x\ 0.482 \text{ x } 0.0061 \text{ x } 0.000001}{35\ x\ 70}$ = 3.07 x 10^{-8}

MOE (Adult) = $\frac{BMDL\ x\ BW}{Exposure\ dose}$ (5)

Margin of exposure (MOE) of B(a)P, B(k)F and B(ghi)P for *A.hybridus* from SEO

BMDL for B(a)P = 0.29

BMDL for B(k)F = 0.14

BMDL for B(ghi)P = 0.22

MOE of B(a)P = $\frac{0.29\ x\ 60}{0.186}$ = 93.5

MOE of B(k)F = ND

MOE of B(ghi)P = $\frac{0.22\ x\ 60}{0.054}$ = 244.4

Margin of exposure (MOE) of B(a)P, B(k)F and B(ghi)P for *A.hybridus* from purchased PAH

MOE of B(a)P = $\frac{0.29\ x\ 60}{1.358}$ = 12.8

MOE of B(k)F = $\frac{0.14\ x\ 60}{0.869}$ = 9.7

MOE of B(ghi)P = $\frac{0.22\ x\ 60}{0.532}$ = 24.8

Margin of exposure (MOE) of B(a)P, B(k)F and B(ghi)P for *T.occidentalis* from SEO;

MOE of B(a)P = $\frac{0.29\ x\ 60}{0.014}$ = 1242.9

MOE of B(k)F = $\frac{0.14\ x\ 60}{0.091}$ = 92.3

MOE of B(ghi)P = ND

Margin of exposure (MOE) of B(a)P, B(k)F and B(ghi)P for *T.occidentalis* from Purchased;

MOE of B(a)P = $\frac{0.29\ x\ 60}{1.04}$ = 16.7

MOE of B(k)F = $\frac{0.14\ x\ 60}{0.869}$ = 9.67

MOE of B(ghi)P = $\frac{0.22\ x\ 60}{0.489}$ = 27.3

MOE (Children) = $\frac{BMDL\ x\ BW}{Exposure\ dose}$

Margin of exposure (MOE) of B(a)P, B(k)F and B(ghi)P for *A.hybridus* from SEO

MOE of B(a)P = $\frac{0.29\ x\ 35}{0.186}$ = 54.6

MOE of B(k)F = ND

MOE of B(ghi)P = $\frac{0.22\ x\ 35}{0.054}$ = 142.6

Margin of exposure (MOE) of B(a)P, B(k)F and B(ghi)P for *A.hybridus* from purchased PAH

MOE of B(a)P = $\frac{0.29\ x\ 35}{1.358}$ = 7.5

MOE of B(k)F = $\frac{0.14\ x\ 35}{0.869}$ = 5.6

MOE of B(ghi)P = $\frac{0.22\ x\ 35}{0.532}$ = 14.5

Margin of exposure (MOE) of B(a)P, B(k)F and B(ghi)P for *T.occidentalis* from SEO;

MOE of B(a)P = $\frac{0.29\ x\ 35}{0.014}$ = 725

MOE of B(k)F = $\frac{0.14\ x\ 35}{0.091}$ = 53.8

MOE of B(ghi)P = ND

Margin of exposure (MOE) of B(a)P, B(k)F and B(ghi)P for *T.occidentalis* from Purchased;

MOE of B(a)P = $\frac{0.29\ x\ 35}{1.04}$ = 9.8

MOE of B(k)F = $\frac{0.14\ x\ 35}{0.869}$ = 5.6

MOE of B(ghi)P = $\frac{0.22\ x\ 35}{0.489}$ = 15.9