

Accumulation of Polycyclic Aromatic Hydrocarbon Content in *Talinium Triangulare* Grown in Spent Engine Oil Polluted Soil

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ABSTRACT

Polycyclic Aromatic hydrocarbons constitute a large class of organic compound that are composed of two or more fused aromatic rings. They are mostly produced by the pyrolysis or incomplete combustion of organic materials. The level of polycyclic aromatic hydrocarbons (PAHs) were determined in spent motor-cycle engine oil, spent sport car engine oil, *Talinium triangulare* (water leaf) grown on soil contaminated with spent sport car engine oil, and spent motor-cycle oil. The PAHs were analyzed using gas chromatograph-Flame Ionization Detector (GC-FID). Additionally, the growth factors were established using *Talinium Triangulare* grown in the unpolluted soil and exposed to both spent sport car engine oil and motor-cycle oil. In terms of their bio-tolerance, the uncontaminated *talinium triangulare* displayed a superior growth performance in comparison to the exposed plant. Nonetheless, there was a notable difference between uncontaminated and contaminated plants ($P < 0.05$). The PAHs results indicated that spent sport car engine oil had higher total PAHs concentration of 5.5518 mg/L than spent motor-cycle engine oil, which had a total PAHs concentration of 2.7490 mg/L. *Talinium Triangulare* grown on soil contaminated with spent sport car engine oil accumulated 0.9108 mg/kg of total PAHs, while spent motor-cycle oil accumulated a total PAHs concentration of 0.5276 mg/kg. In spent sport car engine oil benzo (b) fluoranthene had the highest concentration, while in spent motor-cycle engine oil, the highest concentration was benzo (a) pyrene. Out of the sixteen PAHs USEPA targets, kylene was the only one that wasn't found in any of the samples. These findings demonstrated the presence of hazardous PAHs in the spent engines oil of both motor-cycles and sport car, which are carelessly discarded into the environment and can bioaccumulate in edible plants. It implies that eating vegetables grown on soil tainted with used engine oil poses an inherent risk and danger to consumers.

Keyword: polycyclic aromatic hydrocarbon; spent engine oil; *Talinium triangulare*;

INTRODUCTIONS

The need for energy will keep growing as the world's population expands and developing nations become increasingly industrialized, mechanized in agriculture, and more cars. Petroleum will remain the primary energy source for the next few decades, despite advancements in alternative fuels. Base oils derived from petroleum fractions are the source of motor oil. Only a tiny fraction (2-4%) of petroleum, though, may be used for this purpose (Pawlak *et al.*, 2010)

Petroleum product contamination of the environment is acknowledged as one of the most pressing issues today, resulting in a significant shift in the zoosphere, phytosphere, and microbiome, which causes several species to proliferate, reduces soil fertility and support for plant and animal life, and ultimately harms humans who depend on those plants and animals.

Petroleum products have been demonstrated in several studies to have a negative impact on plants, including decreased seed germination, plant survival, and plant production (Akinola *et al.*, 2004; Andrade *et al.*, 2004). Majority of research on the impact of petroleum products on plants has concentrated on crude oil, diesel, and gasoline (Siddiqui and Adams, 2002; Inoni *et al.*, 2006), but a few has been done on spent engine oil.

Every day, millions of gallons of used engine oil are dumped in garbage on land and in bodies of water (sewers). The U.S. EPA defines "spent oil" as any petroleum or synthetic oil that has been used and is consequently polluted by its physical or chemical characteristics (USEPA, 2015).

Spent oil is a prevalent and harmful environmental pollutant that is not naturally occurring in the environment (Dominguez-Rosado and Pichtel, 2004). In actuality, used engine oil contamination is a widespread issue in the majority of African nations, because developing nations lack the infrastructure for recycling used engine oil, it is released into the environment without sufficient treatment. Used engine oil enters the environment via leaks in the exhaust system during engine use and discharge by motor and generator mechanics (Odjegba and Sadiq, 2002) (Anoliefo and Edegai, 2000; Osubor and Anoliefo, 2003).

When a new mineral-based crankcase is exposed to high temperature and high mechanical strain, engine oil is produced (ATSDR, 1997). It's a combination of several compounds (Wang *et al.*, 2000). Chlorinated biphenyls, chlorodibenzofurans, and lubricative additives are typically referred to as engine oil, which is made from petroleum and is made up of complex mixtures of hydrocarbon molecules, including isoalkanes with slightly longer branches and monocycloalkanes and monoaromatics with a few short branches on the ring (Cotton, 1982).

Engine oil is called lubricating oil with others like (gear, hydraulic oil and turbine oil) (Olugboji and Ogunwole, 2008). They help to reduce friction between moving surfaces and prevent corrosion. They also serve to remove heat from moving parts in machinery, get rid of wear debris produced by moving surfaces, and offer a protective layer on the metal surfaces to prevent corrosion.

Due to the breakdown of additives, contamination with the product of combustion, and the addition of metals from the wear and tear of the engine, engine oil is changed when used by cars, motorcycles, generators, and other equipment. The spent oil is mixed with contaminants such salt, deteriorated additive components, varnish, gum, and other materials (Durrani *et al.*, 2011; Ogbeide, 2010). Additionally, many contaminants are produced in lubricating oil during its usage in internal combustion engines due to oxidation or thermal deterioration. The following pollutants are present in these impurities: alcohols, acids, phenolic compounds, aldehydes, unsaturated hydrocarbons, and non-stable hydrocarbon by-products. Furthermore, used oil absorbs nitrogen oxides and the acidic flue gas produced by the combustion of fuel.

Spent oil contaminates soils, resulting in a notable decrease in soil moisture (Akoachere *et al.*, 2008). According to Achuba and Peretiemo-Clarke (2008), spent oil severely slowed the activity of soil dehydrogenase and catalase. Spent oil retards seed germination and inhibits plant development (Adenipekun *et al.*, 2008).

The incomplete combustion of petroleum products produces PAHs, which are primarily produced by waste incinerators, car emissions, and fossil fuel burning during heating procedures. They are pervasive environmental pollutants that have harmful biological impacts, toxicity, mutagenicity, and carcinogenicity (Vazquez, 1989). Spent oil's PAHs have been shown to have indirect secondary effects, such as impacts on microorganisms like mycorrhizal fungi (Nicolotti and Egli, 1998) and disruption of plant water-air interactions (Renault *et al.*, 2000).

The unusual vegetable crop of the Portulacaceae family, *triangulare talinium* (water leaf), is native to tropical Africa and is widely cultivated in West Africa, Asia, and South America (Schippers, 2000). In Nigeria, it is used as a vegetable and sauce ingredient. *Talinium triangulare* has been shown to have the necessary nutrients, such as B-carotene, minerals (such calcium, potassium, and magnesium), pectin, protein, and vitamins, according to Ezekwe *et al.*, 2001. Additionally, *talinium triangulare* has been linked to the medical treatment of cardiovascular conditions like stroke, obesity, and others (Adewunmi and Sofowora, 1980). *Triangulare talinium* a key leaf vegetable in Nigeria, and it is traditionally used as a softener for other vegetable species. This research is necessary because there is currently no data on the absorption of PAHs by locally eaten leafy Nigerian vegetables (*talinium triangulare*) from soil that has been contaminated by used engine oil.

MATERIALS AND METHODS

Sample Collection and Preparation

The spent motor-cycle engine oil and spent sport car engine oil were obtained from automobile mechanic shop at Awka. They were all collected in a bottle and sterilized container.

Experimental procedure

The soil sample came from the pristine garden of Nnamdi Azikiwe University, Awka, and the *talinium triangulare* seedlings came from a nearby farm in Ifite, Awka, Anambra State. Using a hand trowel, the soil sample was collected at a depth of 0–15 cm. Seedlings of chosen (*talinium triangulare*) were planted in various concentrations of spent engine oil (motor-cycle and sport car) consisting of 10ml, 20ml, 50ml, and non-contaminated soil samples placed in a perforated plastic bag. The study used seedlings of the same height. All experimental analysis was performed in triplicate under regulated conditions at Science village, Nnamdi Azikiwe University, Awka, Anambra State. The experimental plant was thereafter set up on a bench top in the screen house.

Seed and soil sample

Weekly measurements of growth parameters from the time of transplant were used to assess the performance of the plant samples. The values for each plant in a bag were calculated and summed in this instance. The average for the bag was then computed. The leaves number on the *talinium triangulare* were counted once a week for each plant bag, and the average number of leaves was calculated. A meter stick was used to measure plant height. The distance between the base and tip of the plant was measured. A meter rule was used with a paper graph sheet to determine leaf area. The leaf area was then calculated using the formula: leaf area = length × width.

Methods for Samples Extraction (Spent Engine Oil and Talinium Triangulare) For PAHS

The mixture, which was made up of around 20g of the homogenized sample and 60g of anhydrous sodium sulphate (Na₂SO₄) to absorb moisture, was put in a 500ml beaker after being well combined in an agate mortar. This was left to extract for 24 hours in a round bottom flask using 300 ml of n-hexane.

The raw extract was dried using a rotary vacuum evaporator at 40°C. The residue was then transferred with n-hexane onto a 5 mL florisil column for purification.

Methods for Florisil Clean Up Process

Florisil was heated in an oven at 130°C overnight (ca. 15h) and transferred to a 250ml size beakers and placed in a designator. 0.5g anhydrous sodium Sulphate (Na₂So₄) 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. Stopwatch was opened to allow N-hexane run out until it just reaches top of sodium Sulphate into a receiving vessel whilst tapping gently the top of the column till the florisil settled well in the column. The extract was transferred to the column with disposable pasteur pipette from an evaporating flask. Each evaporating flask was rinsed twice with 1ml portions of n-hexane and added to column. Eluate was collected into an evaporating flask and rotary evaporated to dryness. Dry eluate was dissolved in 1ml of n-hexane for PAH chromatographic analysis

PAH Detection Using Buck 530 Gas Chromatography

Generally, there is an adjustment of gas flows to the columns, the inlets, the detectors, and the split ratio. In addition, the injector and detector temperatures must be set. The detectors are generally held at the high end of the oven temperature range to minimize the risk of analyze precipitation. Agilent 6890 gas chromatograph CA, U.S.A was used. It is equipped with an on-column automatic injector, flame ionization detector, HP 88 capillary

column (100m x 0.25µm film thickness,). Detector Temperature is set at 250°C, injector temperature at 220°C and integrator chart speed: 2cm/min. The Oven temperature of the GC is set at 180°C and then allowed to warm up. While it's warming, the settings were adjusted as follows:

FINAL TEMPERATURE at 280°C
 INITIAL TEMPERATURE at 180°C
 FINAL TIME at 45minutes

The "NOT READY" light will turn off when the instrument is ready, inject a 1µL of the sample into the Buck 530-GC column and begin your run. The detection limit of the equipment is 0.001µg/ml.

Statistical Analysis

The data were expressed as mean ± SD and a test of statistical significance was carried out using a one-way analysis of variance (ANOVA). The data obtained were analyzed using Statistical Product and Service Solutions (SPSS), version 18. P < 0.05 was considered significant.

RESULTS AND DISCUSSION

Table 1 Growth performance of *talinium triangulare* at weeks four after transplanting

Plant Parameters	Non-contaminated soil	10mlT MC(SEO)	20ml MC(SEO)	50ml MC(SEO)	100ml SC(SEO)	200ml SC(SEO)	500ml SC(SEO)
Plant height(cm)	43.7±0.01	29.8±0.04	24.4±0.61	15.3±0.01	23.6±0.44	26.2±0.67	13.7±0.02
Leaves numbers(cm)	40±0.22	26±0.24	24±0.53	11±0.46	31±0.66	29±0.04	08±0.56
Leaf Area (cm ³)	12.3±0.08	10.6±0.48	4.56±0.01	4.23±0.64	8.2±0.22	4.8±0.01	3.9±0.84
Fresh weight (g)	1.9±0.64	0.8±0.01	0.7±0.00	0.4±0.01	1.2±0.02	0.8±0.01	0.3±0.01

MC (SEO) = motor-cycle spent engine oil, SC(SEO) = sport car spent engine oil

Table 2 The Polycyclic aromatic hydrocarbons content of spent engine oil of motor-cycle and Toyota car

PAHs component	Spent motor-cycle engine oil	Spent sport car engine oil
Acenaphthylene	0.002	0.4162
Fluorene	0.049	0.6849
Fluoranthene	0.3742	0.5288

Phenanthrene	0.317	ND
Dibenzyl (a-h) anthracene	0.2331	0.2216
1-2 benzoanthracene	0.2659	0.3476
Acenaphene	0.3285	0.6452
Benzo (k) fluoranthene	0.0798	0.6785
Benzo (a) pyrene	0.6237	ND
Pyrene	0.1235	0.4639
Benzo (b) fluoranthene	0.3523	0.1884
Naphthalene	ND	1.0452
Anthracene	ND	0.3315
Kylene	ND	ND
Total mg/L	2.7490	5.5518
Mean value	0.2499	0.5047

Table2: the Polycyclic aromatic hydrocarbon content of water leaf grown on contaminated and uncontaminated soil sample

PAHs component	Non- contaminated soil	50ml MC(SEO)	50ml SC(SEO)
Acenaphthylene	0.0001	0.1442	0.117
Fluorene	ND	ND	0.1126
Fluoranthene	0.0001	0.0208	0.1392
Phenanthrene	ND	ND	ND
Dibenzyl (a-h) anthracene	ND	0.0188	0.1417
1-2 benzoanthracene	ND	0.1481	0.1114
Acenaphene	ND	ND	0.0356
Benzo (k) fluoranthene	ND	ND	0.1310
Benzo (a) pyrene	ND	ND	ND
Pyrene	ND	ND	0.0416

Benzo (b) fluoranthene	ND	0.1957	0.0807
Naphthalene	0.0001	ND	ND
Anthracene	ND	ND	ND
Kylene	ND	ND	ND
Total mg/kg	0.0003	0.5276	0.9108
Mean valu	0.0001	0.10552	0.8391

MC (SEO) = motor-cycle spent engine oil, SC(SEO) = sport car spent engine oil

Soil contamination is one of the most pressing environmental issues facing the world today, due to its poor self-purification ability, lengthy degradation period, and high expense of remediation, (Zhang and Zhang, 2022). The indiscriminate dumping of spent engine oil has been shown to be detrimental to plant life and living creatures. The negative effects on plant development can manifest in a variety of ways, including stomatal defects, biomass loss, and morphological abnormalities (Sharma *et al.*, 1980).

The plant capacity to tolerate the stress caused by spent engine oil pollutants was shown in its performance. Compared to control, the concentrations of the pollutants had a clear impact on the growth parameters of plants cultivated on contaminated soil, demonstrating its negative impact on plant development. Additionally, the concentration of SC(SEO) seems to be more harmful to the plant since it is considerably greater than MC(SEO). The percentage reduction in seedling height, leaf area, and shoot height were used to demonstrate that increasing the concentration of used engine oil in the soil caused a decrease in the mean plant shoot height and mean leaf area of the crops.

However, the effects of the pollutants were visible in the 50ml of spent engine oil of motorcycle and spot car as it was not able to withstand the stress within the period of experiment as yellowing of leaves and stunted growth was observed. Furthermore, the poor growth parameters (plant height and leave area) recorded in *talinium triangulare* exposed to the pollutant could be attributed to decline in soil nutrients, organic matters and interference on the moisture content of the soil which can also lead to nutrient immobilization and poor mineral uptake Tanimu *et al.* (2019) reported a decrease in most soil nutrient in spent engine oil contaminated soil

Consequently, reduced leave number obtained may be because of PAHs toxicity and insufficient ventilation of the soil which can limit the transpiration and respiration by plant. Damage to plants exposed to 50ml of spent engine oil contamination may also have resulted from increase in temperature due to the dark nature of contaminated soils. It was observed by Ezenwa *et al.*, 2017 in their study that the contaminated soils were darker than the control, and dark soils absorb more heat than light ones. Donahue *et al.* (1990) reported that some black coal mining wastes and dark colored oil-shale residues reached temperatures of 65-70o C, which are lethal to many plants that would otherwise grow in those soils

Osuagwu *et al.* (2017) in his work observed the negative impact of spent engine oil on its proficiency to moderate the sprouting and seedling growth of *Z. mays*, *A. hypogea* and *V. unguiculata*. Adenipekun and Kassim, 2006 reported that used engine oil affect plant height, stem girth, moisture content, leaf area and number of leaves in *Celosia argentea*. Also, Okonokhua *et al.*, 2007 reported reduced grain yield and negative effect (reduction in vegetative growth) of spent engine oil on the maize plant.

Wyszkowski *et al.* (2004) claim that hydrocarbons' ability to coat the plant root with a greasy material reduces cell membrane absorbency, which disrupts metabolic activity and makes the cell harmful. The differences in the fresh weight of the plant specimens were caused by the contaminant in the soil. The root,

which is in direct contact with PAH-polluted soil and used engine oil, absorbs water and ions. These results are consistent with the research on Glycine max and Vigna conducted by Ekpo *et al.*, 2012, as well as the study on *uniguiculata* and *Z. mays L.* carried out by Kayode *et al.* 2009.

PAHs are common in the environment and represent a significant risk to the soil environment (Ji *et al.*, 2022). PAHs are chemical compounds that are known as carcinogens and mutagens, and they are also bad for people. Diet has been recognized as a significant source of human exposure to polycyclic aromatic hydrocarbons (PAHs), particularly among non-smokers (Wang *et al.*, 20011; Tao *et al.*, 2004). Vegetable consumption may result in indirect exposure to people through the bioaccumulation of PAHs. PAHs are absorbed by plants from soil through their roots, bioconcentrated, and moved to their many organs, which are often consumed by people and other living things (Essumang *et al.*, 2011).

From our findings in Table 2, we discovered that 11 of the 14 PAHs studied were present in spent motor-cycle oil: acenaphthene, benzo (k) fluoranthene, benzo (a) pyrene, pyrene, benzo (b) fluoranthene, acenaphthylene, fluorene, fluoranthene, phenanthrene, dibenzyl (ah) anthracene, and 1-2 benzanthracene. With a total PAHs concentration of 2.7490 mg/l and benzo (a) pyrene, having highest concentration. Eleven PAHs components were found in used sport car oil, with the majority of four and six ringed PAHs present: acenaphthylene, fluorine, fluoranthene, dibenzyl (ah) anthracene, 1-2 benzanthracene, acenaphthene, benzo (k) fluoranthene, pyrene, benzo (b) fluoranthene, naphthalene, anthracene, and benzo (ghi) perylene. With a total PAHs of 5.5518 mg/l, benzo (b) fluoranthene had the highest concentration. Sport car spent engine oil had the highest concentration of PAHs than spent motor-cycle oil and this could be attributed to size of the engine and the duration of use. The PAH concentration in unused motor oil is rather low, but it rises as the engine runs (Pasquini and Monarca 1983). Used engine oil can have 670 times more PAH than new motor oils (Hoffman *et al.*, 1982). The PAH content of used engine oil from gasoline engines can be 180 times higher than that of fresh oil (Grimmer *et al.*, 1981).

Some researcher has also reported, different concentration of PAHs from spent engine oil contaminated soil in other towns. Oko *et al.*, (2024) reported a total PAHs concentration of 333.6820mg/kg and 104.8540mg/kg in spent motor oil soil treated with NaOH and heat respectively. Adesina *et al.*, 2023 reported a total PAHs ranged of 5.58 - 6.40 ug/g in automobile repair workshops in Ado-Ekiti. Also Obini *et al.*, 2013 stated a total PAHs ranged of 0.0184 - 0.1385mg/kg in soil contaminated with spent motor engine oil.

There was accumulation of PAHs in *talinium triangulare* grown in soil contaminated with both spent engine oil. *Talinium triangulare* grown in non-contaminated soil showed very low total PAHs concentration when compared to those grown on spent engine oil contaminated soil as presented in table 3. Our result showed five PAHs components out of the fourteen PAHs analysed in *talinium triangular* grown in motor-cycle contaminated soil: Acenaphthylene, fluoranthene, dibenzyl (ah) anthracene, 1-2 benzanthracene, benzo (b) fluoranthene, with a total PAHs concentration of 0.5276mg/kg and benzo (b) fluoranthene having the highest concentration. While nine PAHs component was present in *talinium triangular* grown in sport car spent engine oil contaminated soil: Acenaphthylene, fluorene, fluoranthene, dibenzyl (ah) anthracene, 1-2 benzanthracene, acenaphthene, benzo (k) fluoranthene, pyrene, and benzo (b) fluoranthene. Dibenzyl (ah) anthracene had the highest concentration with a total PAHs concentration of 0.9103mg/kg.

Similar to our findings is the study of Ukachukwu *et al.*, 2023, who reported *Telfairia occidentalis* and *Amaranthus hybridus* absorbed PAHs from soil that had been tainted with spent motor oil. The following seven PAHs compounds—Benzo (a) anthracene, Benzo (a) pyrene, Benzo (b) fluoranthene, Benzo (k) fluoranthene, chrysen, and dibenzyl (ah) anthracene—have been identified by the environmental protection agency as potential carcinogens for humans (USEPA, 2008). For the following individuals PAHs, the USEPA has established reference doses: anthracene (0.3 mg/kg/day), acenaphthene (0.06 mg/kg/day), fluoranthene (0.04 mg/kg/day), fluorene (0.04 mg/kg/day), and pyrene (0.03 mg/kg/day). It is unlikely that consuming these amounts of individual PAHs daily will have negative health consequences.

From our results, three out of the seven PAHs classified by EPA as probable human carcinogen are present in *T. triangular* grown in the spent oil contaminated soils. They are benzo (k) fluoranthene, dibenzyl (ah) anthracene and benzo (b) fluoranthene. As such consumers of these vegetable are exposed to PAHs through consumption. In Nigeria exposure risks are usually estimated without contribution from eating home grown vegetables. Polycyclic aromatic hydrocarbons are very dangerous to health because some are known to be mutagenic and carcinogenic (Vazquez, 1989).

CONCLUSION

From the findings of this study, it is crystal clear that *talinium triangulare* grown on soil contaminated with spent engine oil is capable of becoming detrimental to human health because of the large amount of polycyclic aromatic hydrocarbons in spent oil. Conclusively, *talinium triangulare* grown in soil contaminated with spent engine oil is harmful to human health, hence there is need to enlighten the public on the hazard of indiscriminate disposal of spent engine oil pollutant into our agricultural land. This will go a long way in ensuring human health and food safety.

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CONFLICT OF INTERESTS

The authors hereby declare no conflict of interests

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