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Effects of *Piper guineense* and *Azadirachta indica* on some Polycyclic Aromatic Hydrocarbons Levels in Leaves of Germinating *Telfairia occidentalis* (ugu)

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Abstract Environmental pollution by petroleum is an increasing global problem with phytoremediation being one of the viable tools for cleansing crude oil polluted soil. The aim of this study was to ascertain the remediating effect of *Piper guineense* and *Azadirachta indica* on the levels of polycyclic aromatic hydrocarbons (PAH) on the leaves of deliberately crude oil polluted germinating *Telfairia occidentalis*. Vessel I contains growing plant and water only, vessel II contains growing plant + crude oil only, vessel III, IV and V had growing plant+crude oil + 100g of *Azadirachta indica*, 77g of *P. guineense* and 100g of *Azadirachta indica* +77g of *P. guineense* leaves respectively. After 8weeks of plant growth, the levels of polyaromatic hydrocarbons (PAH) in the plants were determined using gas chromatography. The result indicates that naphthalene levels were significantly higher ($p<0.05$) in plants grown on vessels with 77 g *P. guineense* (4.2 ± 0.003 µg/Kg) and 77 g *P. guineense* + 100 g *A. indica* (4.2 ± 0.007 µg/Kg) compared to the crude oil only vessel (2.1 ± 0.003 µg/Kg). Phenanthrene levels were significantly lower ($p<0.05$) in plants grown on vessels with 100 g *A. indica* (1.7 ± 0.002 µg/Kg) compared to levels in crude oil only grown plants (2.2 ± 0.003 µg/Kg). Acenaphthylene, acenaphthene, fluorene benzo(k)fluoranthene and benzo(a)pyrene levels were significantly higher ($p<0.05$) in plants grown in vessels III,IV and V compared to the crude oil only vessel. Also, dibenzo(a,b)anthracene and indenol(1,2,3cd)pyrene levels were significantly lower ($p<0.05$) in plants grown on vessels remediated with 100 g *A. indica* and 77 g *P. guineense* + 100 g *A. indica* when compared with plants grown in crude oil only vessel. Therefore, it could be concluded that *P. guineense* and *A. indica* leaves could be good for phytoremediation of phenanthrene, benzo(b)fluoranthene, dibenzo(a,b)anthracene and indeno(1,2,3cd)pyrene.

Keywords: *Azadirachta indica, Piper guineense, Telfairia occidentalis, bioremediation, crude oil*

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1. Introduction

Petroleum is a complex mixture made of thousands of compounds which can be divided into 4 major fractions: the alkanes, the aromatics, the resins and the asphaltenes. The aromatics especially the recalcitrant polycyclic compounds (PAHs) are of concern owing to their toxicity and tendency to bioaccumulation. PAHs are considered as hazardous because of their mutagenic and carcinogenic activities (Kalf *et al*., 1997). Significant amount of PAH compounds are found in crude oil. Oil-polluted soil poses a major environmental and human health problem, and the removal of such compounds has become of particular concern for the protection of the environment.

Microorganisms and plants have complementary roles in phytoremediation of the polluted soil. Phytoremediation refers to the use of plants to clean contaminated soil (Joner *et al*., 2004).

Piper guineense, popularly known as African black pepper or hot leave is widely consumed in some part of West Africa especially Nigeria and Ghana on account of Its' nutritional and medicinal properties (Negbenebor *et al*., 1999). It belongs to the family Piperaceae or Sapotaceae. In traditional herbal medicine, the seeds are put into a variety of uses, for instance, in some parts of Nigeria, the seeds are consumed by women after child birth, to enhance uterine contraction for the expulsion of placenta and other remains from the womb (Udoh *et al*., 1999), as an adjuvant in the treatment of rheumatic pains and as an antiasthma tics (Sofowora, 1982) and also for the control of weight (Mba, 1994). The seed and leaf extracts are capable of exhibiting a depolarizing neuromuscular activity in a concentration related manners (Udoh *et al.*, 1999). The antiparasitic, antimicrobial and antifungal activities of the leaf and seeds of *P. guineense* have also been reported (Ekanem and Obiekezie, 2000; Ngane *et al*., 2003; Ekanem *et al*., 2004) The seed and leaf

extracts are capable of exhibiting a depolarizing neuromuscular activity in a concentration related manners (Udoh *et al.*, 1999). *Piper guineense being* a plant used as spices and condiments, have several other wide applications in the local treatment and management of many diseases. It has significant antioxidant activity owing to its free radical scavenging potential(Etim *et al*.,2013).

Neem (*Azadirachta indica*) is one of the most valuable multi-purpose species least exploited amongst tropical trees for its potential. It is an outstanding example of the species which is highly efficient in restoring soil productivity and simultaneously providing fodder, firewood and other products to meet basic needs in the rural households like medicines, pesticides, mosquito repellants, fertilizer, diabetic food, soaps, lubricants, gums, agricultural implements, toothpaste, toothbrush sticks and even contraceptives. The neem tree contains azadirachtin – a biologically active compound found in its seeds, bark and leaves (Wikipedia, 2007; Makeri *et al.*, 2007) which is responsible for its varied medicinal uses (Schmulterer, 1990), hence its application in this study.

The study was aimed at determining any possible remediating effect of *Piper guineense* and *Azadirachta indica* leaves on the levels of polycyclic aromatic hydrocarbons (PAH) in the leaves of deliberately crude oil polluted germinating *Telfairia occidentalis*.

2. Materials and Methods

2.1. Materials and Equipment

- Analytical weighing balance (Meter PM 2000)
- Drying oven (Genlab)
- Digestion block (Gallenhamp 400)
- Separatory funnel (500ml) with polypropylene with Teflon resin, TFE stopcock (Quick fit)
- Water bath (Grant OLS 200) with temperature control and shaker.
- Chromatographic column, 600mm x 30mm in diameter and coarse frit disk at the bottom.
- Extraction bottles/Glass vials (15-20ml)
- Gas chromatograph (GC-FID) with GC recorder interfaced with an HP Pentium III MMX computer. ATI Unicam 610 series.
- Whatman filter paper (Number 40)
- 50ml beaker Duran glass, weight-28.604g
- Horiba oil content Analyser OCMA 350.
- Pipette and filter, industrial hot plate.
- Kjeldahl flasks.
- Heating mantle.
- pH meter jenway 4010, weighing balance mettle PN163 Max 160g.
- Conductivity Meter (Jenway 4010).

2.2. Chemicals/ Reagents

- All reagent used in this analysis were of analytical grade quality and were procured from Sigma Aldrich Chemical Company, USA.
- Hexane (Sigma Aldrich Chemical Company USA)
- Dichloromethane (Sigma Aldrich Chemical Company USA)
- Borosilicate
- Anhydrous sodium sulphate, Na2SO4
- Deionized water
- Alumia
- Nitric acid and hydrochloric acid

2.3. Experimental Design

Vessel 1	plant + water
Vessel 2	plant + water + crude oil
Vessel 3	plant + water + crude oil + 77g of *P. guineense*
Vessel 4	plant + water + crude oil + 100g of *A. indica*
Vessel 5	plant + water + crude oil + 77g of *P. guineense* + 100g of *A. indica*

The seeds of *Teffairia Occidentalis*, was procured from one of the farmlands in Madonna University, Elele,Rivers State. They were dried and made ready for planting.

Bonny light crude oil was obtained from the Nigerian National Petroleum Corporation (NNPC). The leaves of *Piper guineense* and *Azadirachta indica* were collected from an area in Elele, Rivers State. They were room dried and grinded. The seeds were planted and monitored for eight weeks using sterilized cotton wool as soil. After harvest, the leaves were dried and prepared for assay.

2.4. Methodology

2.4.1. Extraction

The extraction method for the analysis of PAH profiles in samples was followed by employing the modified methods of ASTM D3328 and ASTM 3415.

0.2g of the pulverized sample was weighed into a 250ml capacity beaker of borosilicate material and 20ml of the ratio 3:1 redistilled hexane: dichloromethane was added. The beaker and its content is placed in the sonicator to extract the hydrocarbons for about 2 hours.The organic layer was filtered into the 250ml capacity borosilicate beaker. The extract was dried by passing the filtrate through the funnel containing anhydrous sodium sulphate. The dried extract was concentrated with a stream of nitrogen gas.

2.4.2. PAH Separation

The concentrated oil was separated into the aliphatic profiles and PAH profiles by packing the glass column with activated alumina, neutral and activity/grade 1. 10ml of the treated alumina was packed into the column and cleaned properly with redistilled hexane. The extrct was poured onto the alumina and was allowed to rundown with the aid of the redistilled hexane to remove the aliphatic profile into a pre-cleaned 20ml capacity of glass container. The aromatic fraction was recovered by allowing the mixture of hexane and dichloromethane in ratio 3:1 and finally removed the most polar PAH by removing with the dichloromethane into the pre-cleaned borosilicate beaker. The mixture was concentrated to 1.0ml by stream of the nitrogen gas before the gas chromatography.

3. Results

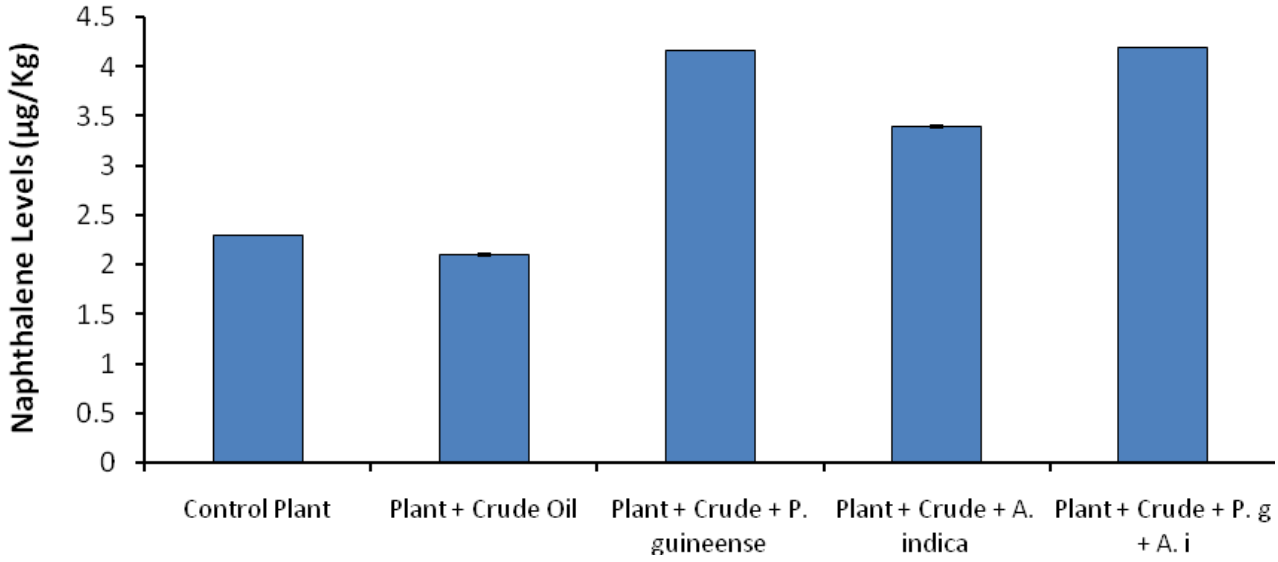

Figure 1. Naphthalene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

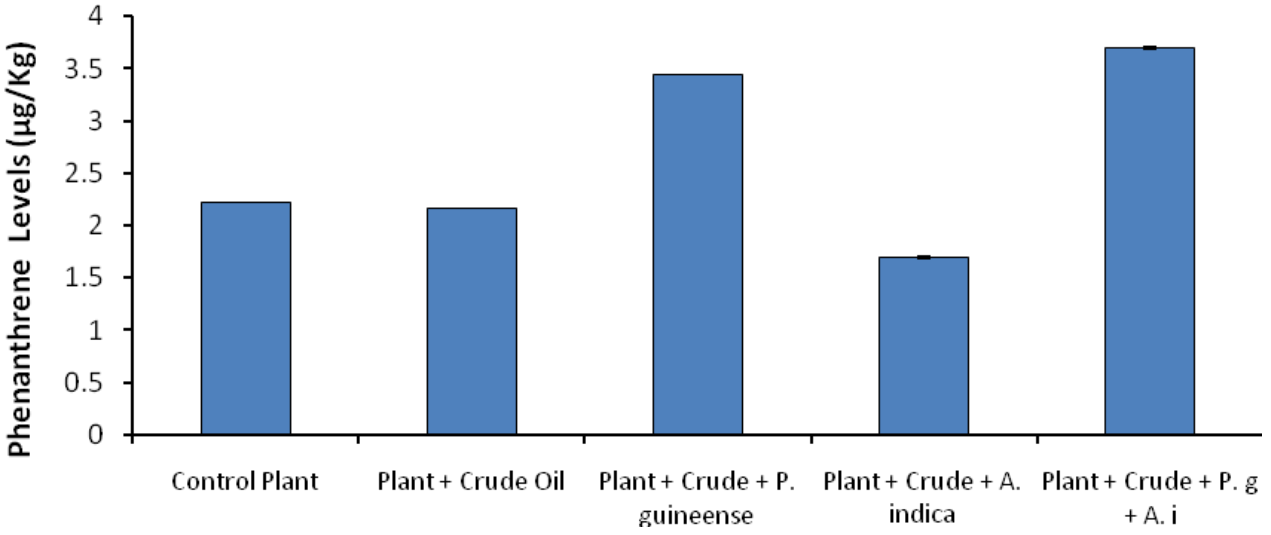

Figure 2. Phenanthrene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

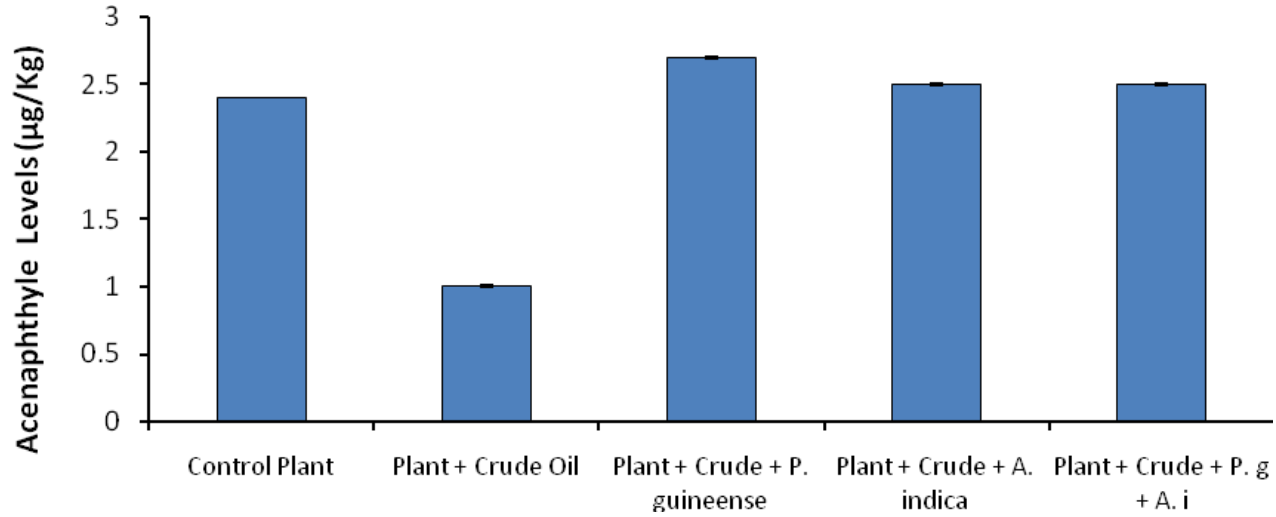

Figure 3. Acenaphthylene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

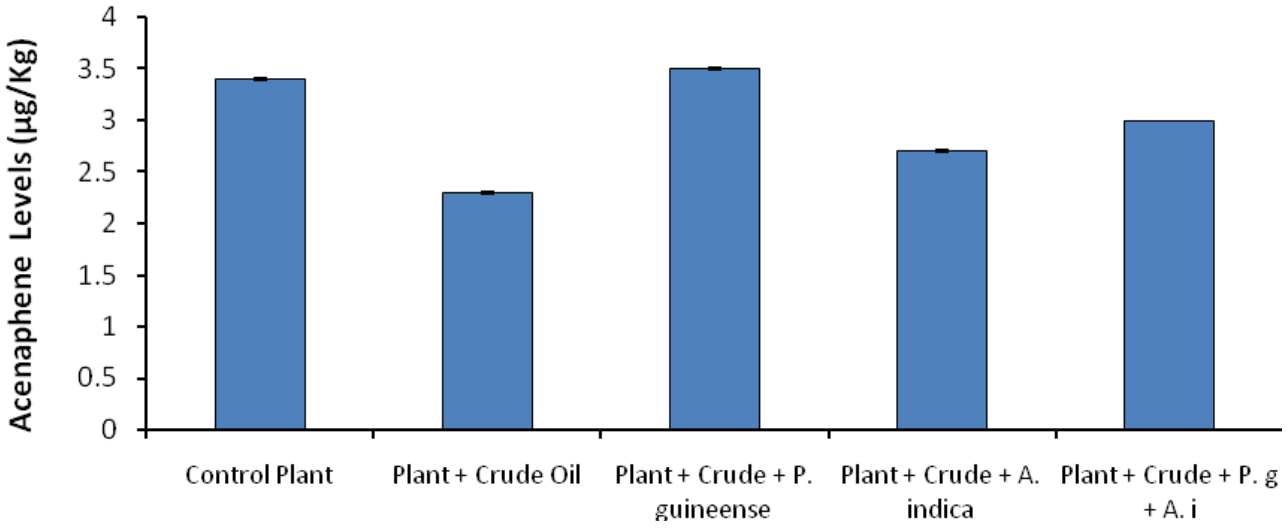

Figure 4. Acenaphthene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

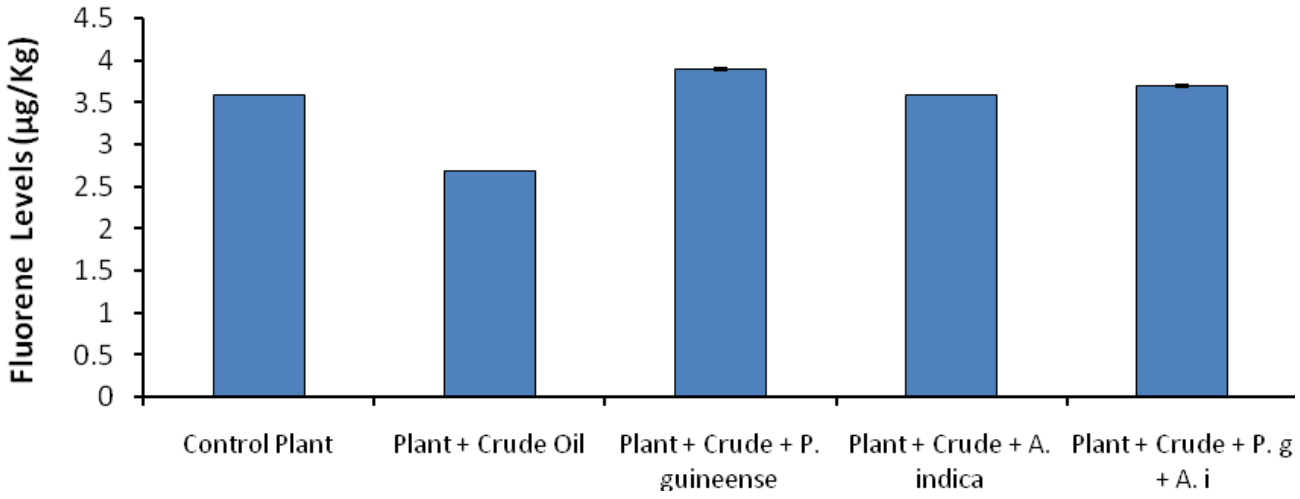

Figure 5. Fluorene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

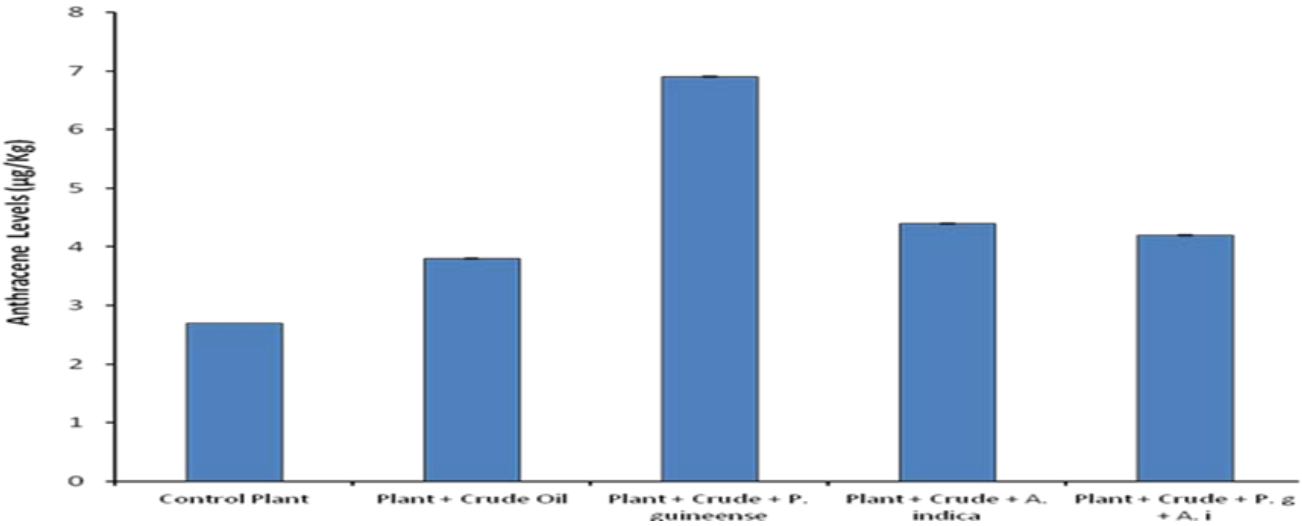

Figure 6. Anthracene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

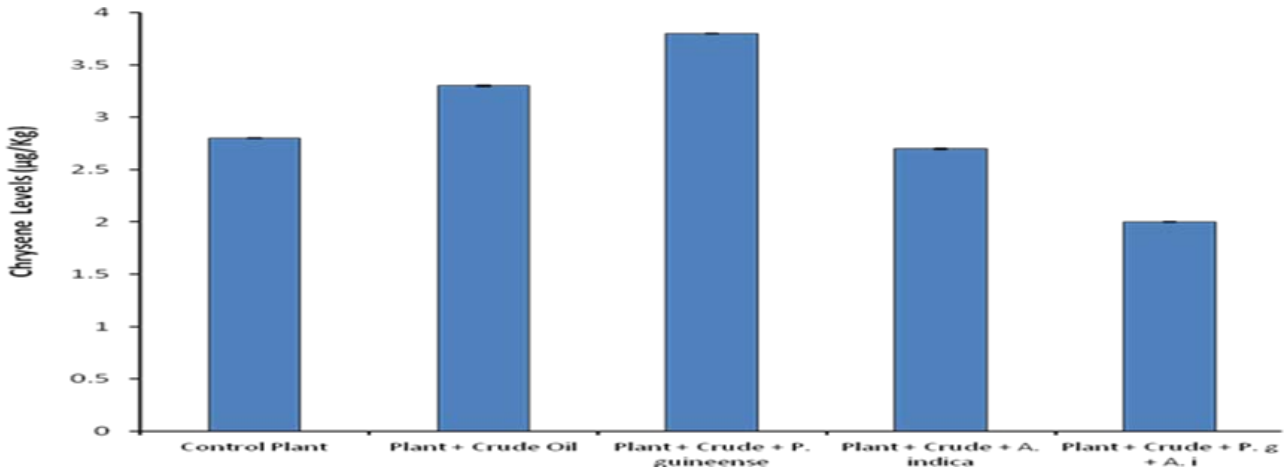

Figure 7. Chrysene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

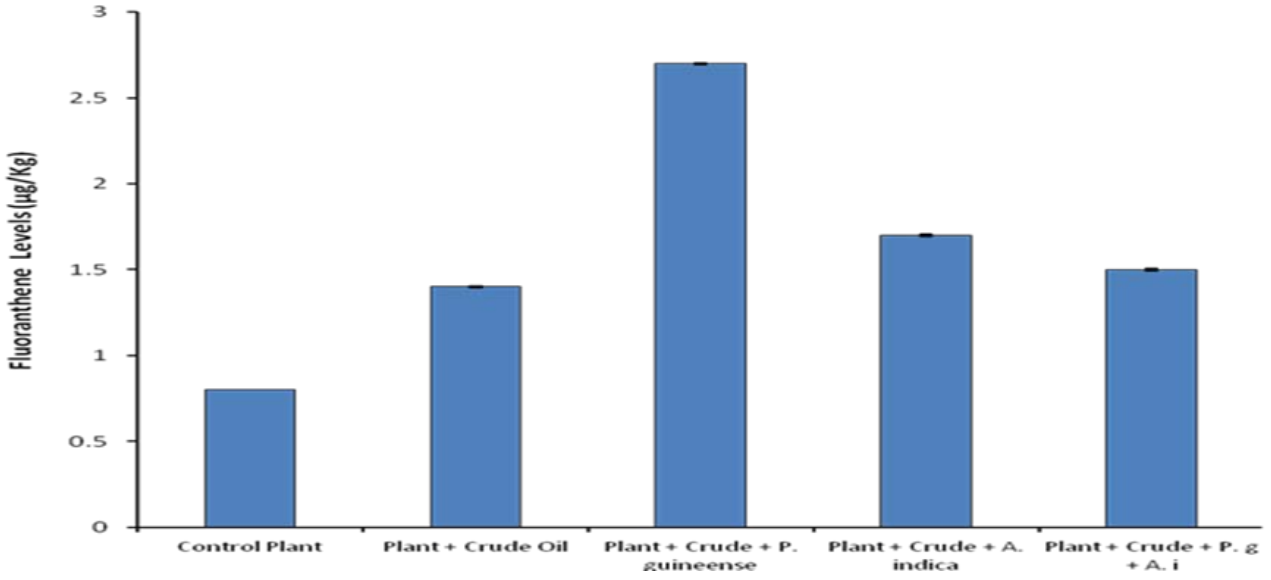

Figure 8. Fluoranthene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

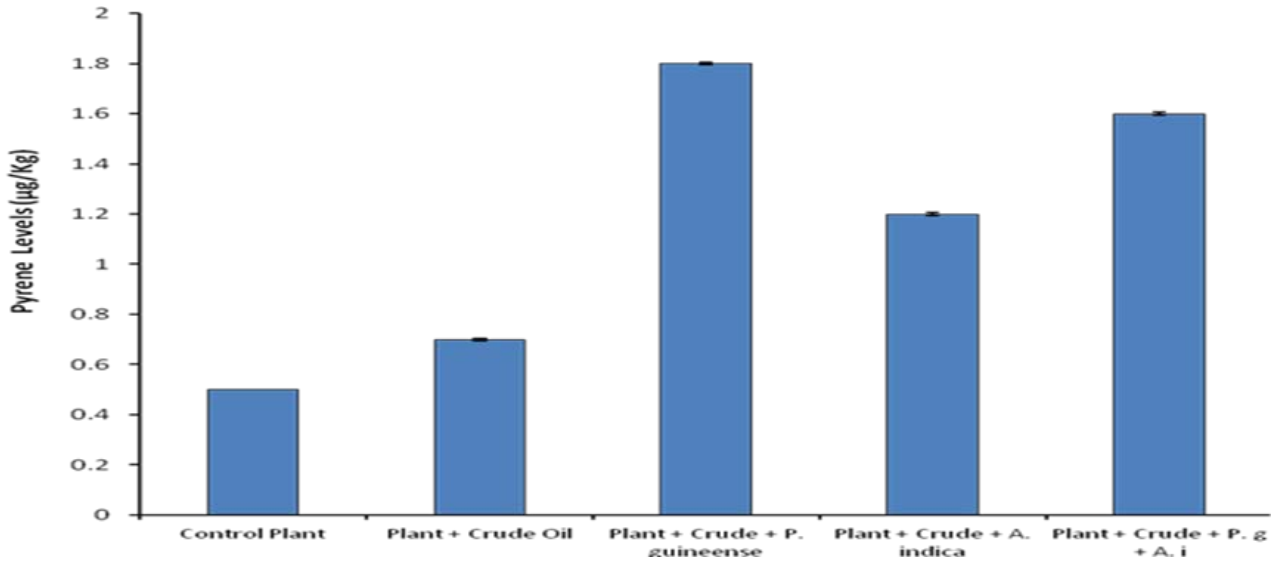

Figure 9. Pyrene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

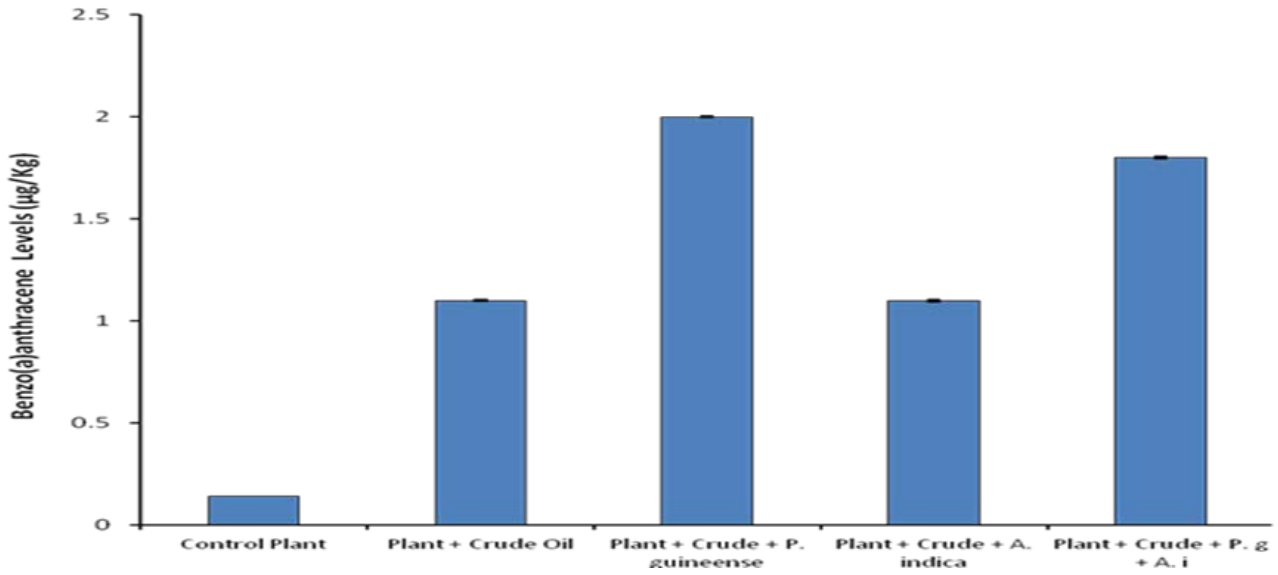

Figure 10. Benzo(a)anthracene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

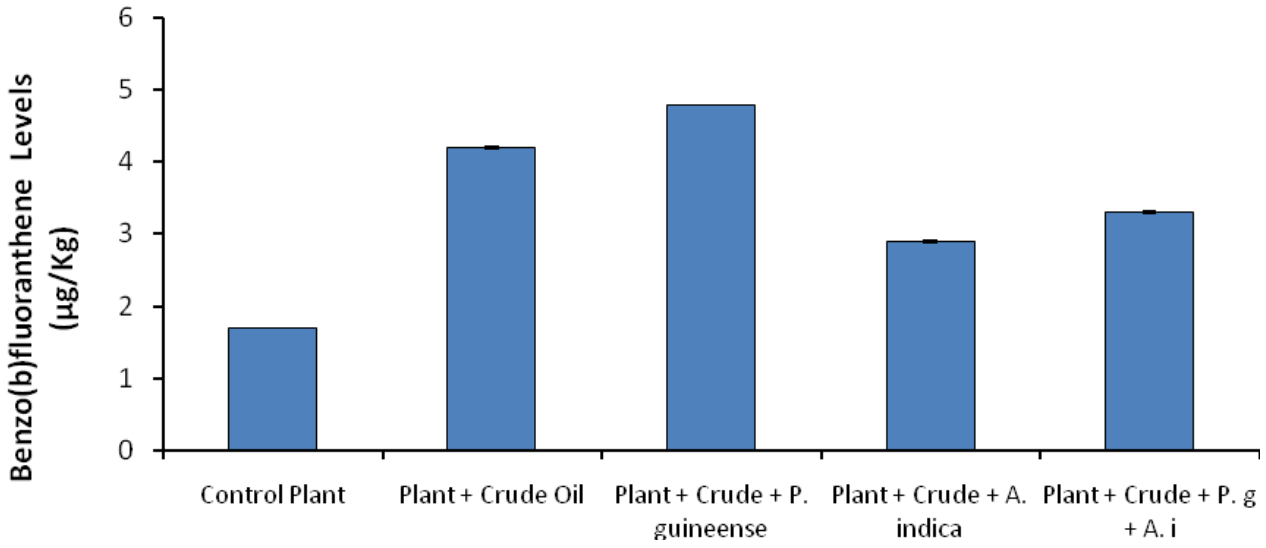

Figure 11. Benzo(b)fluoranthene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

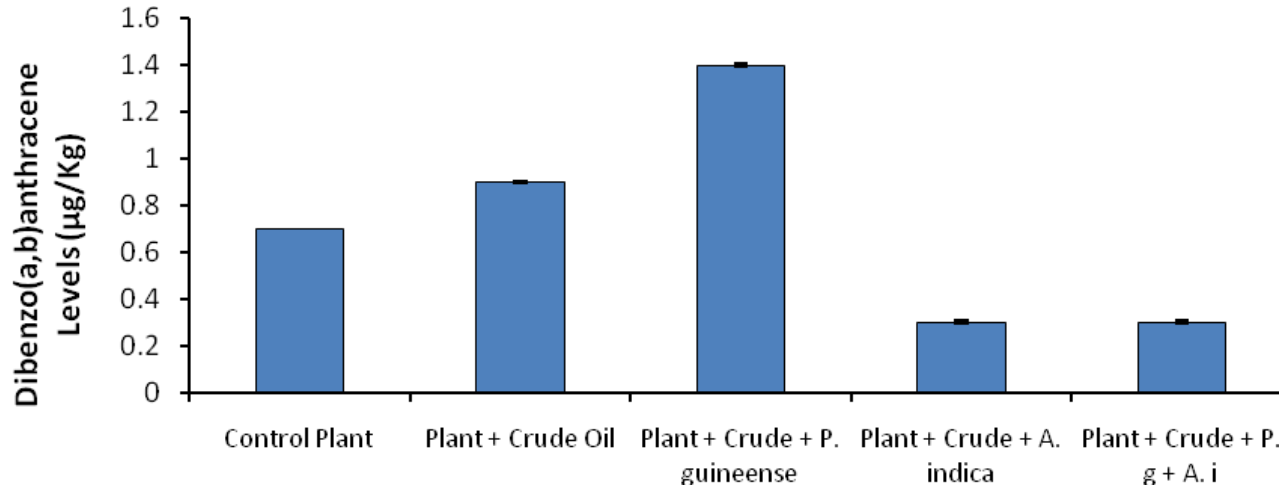

Figure 12. Dibenzo(a,b)anthracene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

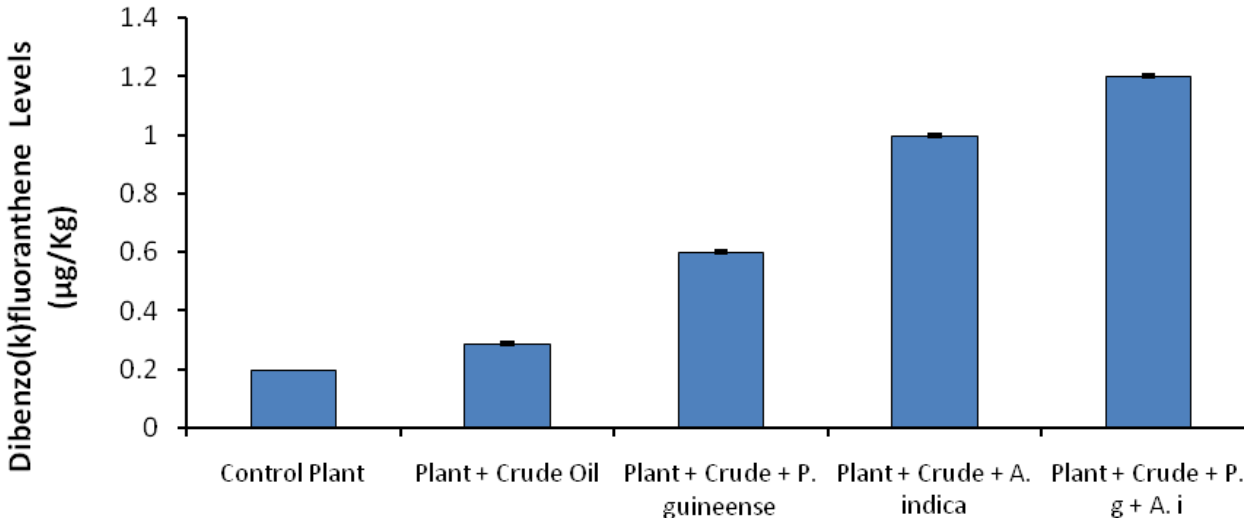

Figure 13. Benzo(k)fluoranthene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

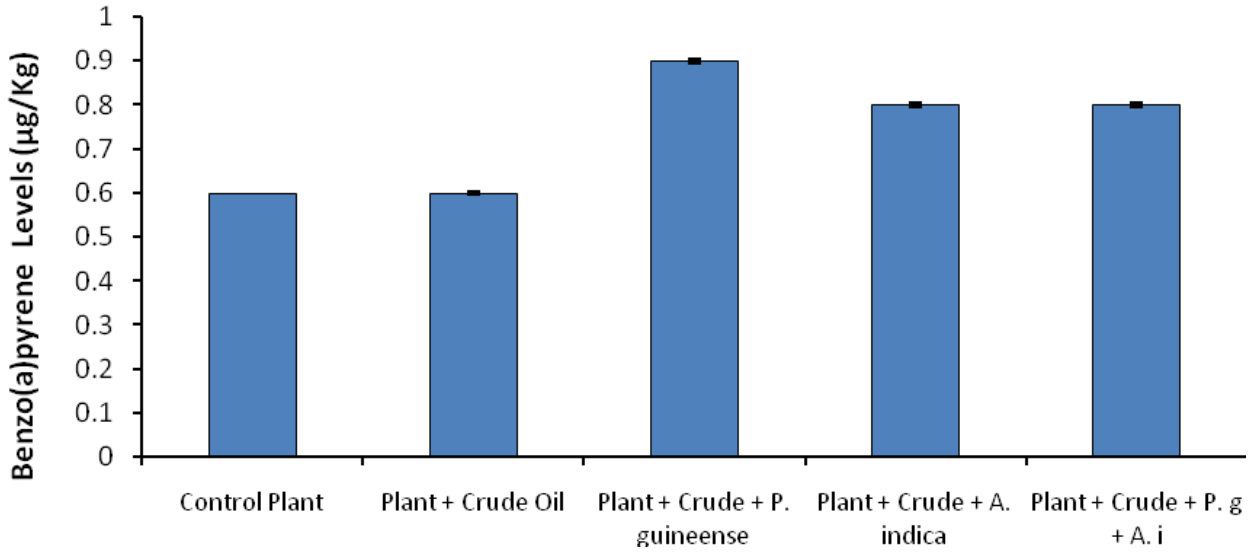

Figure 14. Benzo(a)pyrene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

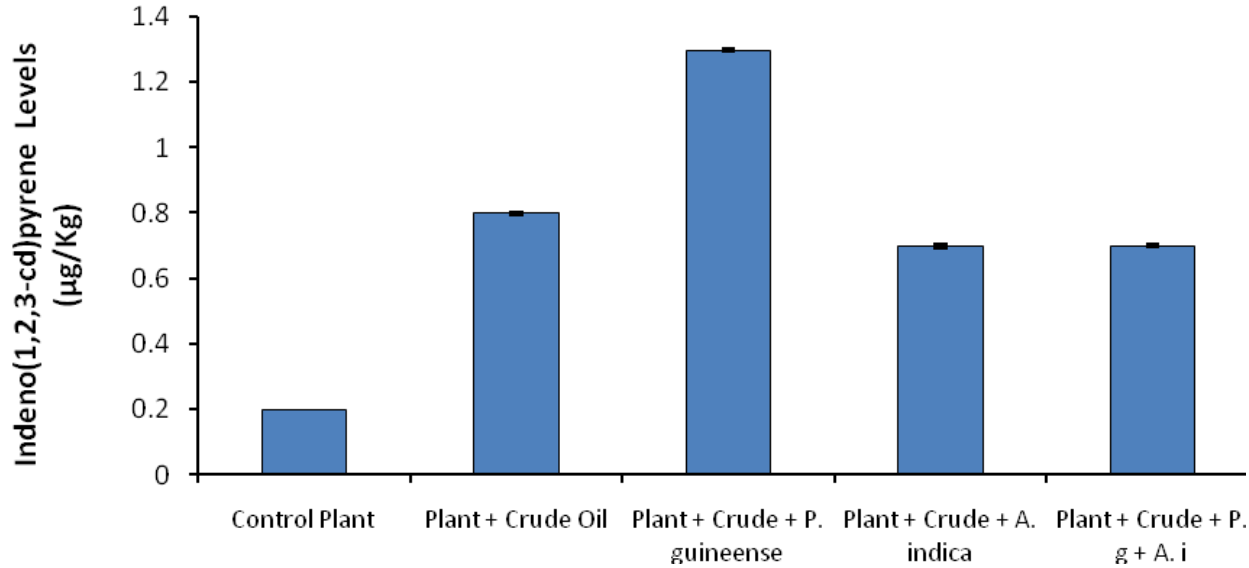

Figure 15. Indeno(1,2,3-cd)pyrene Levels in Plants Grown on Crude Oil Polluted Soil Bioremediated with *Piper guineense* and *Azadirachta indica* Leaves

Data represented as Mean ± SEM

4. Discussion

Plants can take up, translocate, and tolerate increased levels of certain polycyclic aromatic hydrocarbons and heavy metals that could be toxic to any other known organisms (Flathmann, 1994). Plants contribute to biodegradation by their ability to fix hydrocarbons and metals due to the presence of proteins. This ability can be harnessed to extract hydrocarbons from polluted areas (Chavan, 2008). Phytoremediation can be applied at moderate contamination levels or after the application of other remediation measures as a polishing step (Frick *et al.*, 1999).

As shown in Figure 1, naphthalene levels were significantly higher ($p<0.05$) in plants grown on vessels with 77 g *P. guineense* (4.2 ± 0.003 µg/Kg) and 77 g *P. guineense* + 100 g *A. indica* (4.2 ± 0.007 µg/Kg) compared to the crude oil only vessel (2.1 ± 0.003 µg/Kg).

Figure 2 shows that phenanthrene levels were significantly lower ($p<0.05$) in plants grown on vessels with 100 g *A. indica* (1.7 ± 0.002 µg/Kg) compared to levels in crude oil only grown plants (2.2 ± 0.003 µg/Kg). These results demonstrate successful phytoremediation process as compared to the increase in levels observed in some PAHs. Addition of *P. guineense* however significantly ($p<0.05$) increased the phenanthrene levels in the plants relative to the control and the crude oil only grown plants.

Figure 3 indicates that, acenaphthylene levels were significantly higher ($p<0.05$) in plants grown on vessels with 77 g *P. guineense* (2.7 ± 0.003 µg/Kg), 100 g *A. indica* (2.5 ± 0.0003 µg/Kg) and 77 g *P. guineense* + 100 g *A. indica* (2.5 ± 0.0007 µg/Kg) compared to the crude oil only vessel (1.1 ± 0.0003 µg/Kg).

Acenaphthene levels shown in Figure 4 were significantly higher ($p<0.05$) in plants grown on vessels with 77 g *P. guineense* (3.5 ± 0.0007 µg/Kg), 100 g *A. indica* (2.7 ± 0.0006 µg/Kg) and 77 g *P. guineense* + 100 g *A. indica* (3.0 ± 0.000 µg/Kg) compared to the crude oil only vessel (2.3 ± 0.0001 µg/Kg).

As shown in Figure 5, fluorene levels were significantly higher ($p<0.05$) in plants grown on vessels with 77 g *P. guineense* (3.9 ± 0.0007 µg/Kg), 100 g *A. indica* (3.6 ± 0.0006 µg/Kg) and 77 g *P. guineense* + 100 g *A. indica* (3.7 ± 0.000 µg/Kg) compared to the crude oil only vessel (2.7 ± 0.0001 µg/Kg).

Results showed that in Figure 7, chrysene levels were significantly lower ($p<0.05$) in plants grown on vessels bioremediated with 100 g *A. indica* (2.7 ± 0.002 µg/Kg) and 77 g *P. guineense* + 100 g *A. indica* (2.0 ± 0.0009 µg/Kg) compared crude oil only grown plants (3.4 ± 0.003 µg/Kg). Anthracene, fluoranthene, pyrene and benzo(a)anthracene levels were however, significantly higher ($p<0.05$) in plants grown on vessels remediated with 77 g *P. guineense*, 100 g *A. indica*, and 77 g *P. guineense* + 100 g *A. indica* compared to the crude oil only vessel and the control.

Certain factors like nutrients and temperature changes may however be responsible for the significant levels in some of the PAHS as shown in the results. Temperature plays a major role in controlling the nature and extent of hydrocarbon metabolism. Temperature increase leads to increase in diffusion rate of the organic compounds by decreasing their viscosity which leads to increase in bioavailability by increasing solubility, diffusion and reaction rate(Mohan et al., 2006; Northcott and Lones,2001).Increament in the levels of some of the PAHS may equally result from input from remediating plants used being grown in the oil producing regions.

Benzo(b)fluoranthene levels were significantly lower ($p<0.05$) in plants grown on vessels bioremediated with 100 g *A. indica* (2.9 ± 0.002 µg/Kg) and 77 g *P. guineense* + 100 g *A. indica* (3.3 ± 0.009 µg/Kg) compared crude oil only grown plants (4.2 ± 0.003 µg/Kg). Also dibenzo(a,b)anthracene and indeno(1,2,3-cd)pyrene levels were significantly lower ($p<0.05$) in plants grown on vessels bioremediated with 100 g *A. indica* and 77 g *P. guineense* + 100 g *A. indica* compared to plants grown in crude oil only vessel.

5. Conclusion

The biodegradation of polycyclic aromatic hydrocarbons in the environment is a complex process. Microorganisms such as bacteria and fungi are the key agents of bioremediation especially on administration with plants which can enhance translocation of these toxic organic compounds, with bacteria assuming the dominant role in marine ecosystems and fungi becoming more important in freshwater and terrestrial environments (Leahy and Colwell, 1990). The result findings suggest that, though *Azadirachta indica* and *Piper guineense* leaves could be used to bioremediate or reduce phenanthrene, chrysene, benzo(b)fluoranthene, dibenzo(a,b)anthracene and indeno(1,2,3-cd)pyrene levels, they seem not to be a good remediating agent for naphthalene, fluorene, Acenaphthylene, acenaphthene, anthracene, fluoranthene, and benzo(a)anthracene.

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