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PHYTOREMEDIATION OF ACENAPHTHENE (ACN), NAPHTHALENE (NAP) AND PHENANTHRENE (PHE) CONTAMINATED SOIL USING *Gardenia Jasminoides* PLANT

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ABSTRACT

In this study, greenhouse pots experiment was conducted to determine the phytoremediation potential of the *Gardenia jasminoides* plant. The plant was transplanted into 4.0 kg soil spiked with three different concentrations of the polycyclic aromatic hydrocarbons (PAHs); 1600 mg Acenaphthene (ACN), 2000 mg naphthalene (NAP) and 2400 mg phenanthrene (PHE) respectively. The Plant was allowed to grow under greenhouse conditions in triplicates with sufficient watering for ten weeks in pots containing soil contaminated with the three PAHs and control. At the end of the experiment, the levels of PAHs in the extracts of soil, roots and shoots were analyzed using high performance liquid chromatography system from Shimadzu equipped with a UV-VIS detector (SPD-20-AV). The results showed that, bioconcentration factor (BCF) values in control Experiment are 0.74 for ACN, 0.57 and 1.64 for NAP which is greater than one. Translocation factors (TF) values in control experiment are 1.23 for ACN, 1.0 for PHE and 1.20 for NAP. BCF values are greater than one at all the three different spiked experiment, 8.66 for ACN, 2.30 for PHE and 4.31 for NAP. The results also showed that the Plant was able to remove NAP with TF=2.32, ACN with TF=2.94 and PHE with TF=3.62 from contaminated soils. High values of one and above for the BCF and TF indicates high accumulation of the PAHs in the shoots of the plant. The plant may therefore be best described as phytoextractor of naphthalene, acenaphthene and phenanthrene in the soil.

Keywords: *Gardenia Jasminoides*, Polycyclic Aromatic Hydrocarbons, Accumulation, Soil

INTRODUCTION

The global problem concerning contamination of the environment as a result of growing rate of industrialization and increase in human population in the twentieth century is gradually leading to deterioration of the environment and pollution is likely to reach disturbing level in the years ahead (Adamu, 2019). Polycyclic aromatic hydrocarbons (PAHs) are groups of persistent organic contaminants formed by incomplete combustion of carbon-containing materials (Mojiri *et al*, 2019). Both natural and anthropogenic sources such as forest and bush fire, volcanic eruption, biosynthesis by plants and bacteria, agricultural waste, combustion of gasoline and diesel by automobile, oil spillage, tobacco smoking, residential wood and coal burning, petroleum catalytic cracking and industrial combustion of fossils fuel contribute to the increase in concentration of PAHs in urban settlements, especially in areas where rapid urbanization is taking place (Siyan *et al.*, 2019; Gupte *et al.*, 2016; Mojiri *et al.*, 2019; Patel *et al.*, 2020). Atmospheric distribution of PAHs between the particulate matter and the gaseous phase influences their fate and migration in the atmosphere to a far distance (Mojiri *et al., 2019*). Thus PAHs are mostly found in air, soil and water. Therefore, they are considered as predominant pollutants in the environment (Menzie *et al.*, 1992; Hussein and Mona, 2016).

PAHs are group of toxic organic compounds composed of two or more benzene rings fused together (Tahir *et al.*, 2020). Their accumulation in soil pose severe threat to human, animal and plant health, and can affect the stability of ecosystem negatively (U.S.EPA, 2000; Chunrong *et al*., 2020). The United State Environmental Protection Agency (U.S.EPA) has identified about 16 PAHs prominent pollutants based on their carcinogenic, cytotoxic, mutagenic and teratogenic nature (U.S.EPA., 2000). The presence of these contaminants in the soil, water and air has encouraged the development of biological method called phytoremediation, which can be used successfully to clean up contaminated environment (Karishma *et al.*, 2018). Phytoremediation is an innovative green technology and best technique which can be used effectively to remove or degrade PAHs contaminants in the environment due to its low cost, wide range application, environmental friendliness and high efficiency (Abdulazeez, 2017; Karishma *et al.*, 2018). Phytoremediation can occur by phytoextraction, phytofilteration, phytostabilization, phytovolatilization, Phytodegradation, rhizodegradation or phytodesalination (Massino *et al*, 2020). Figure 1 summarizes definition and principle characteristics of some of the process.

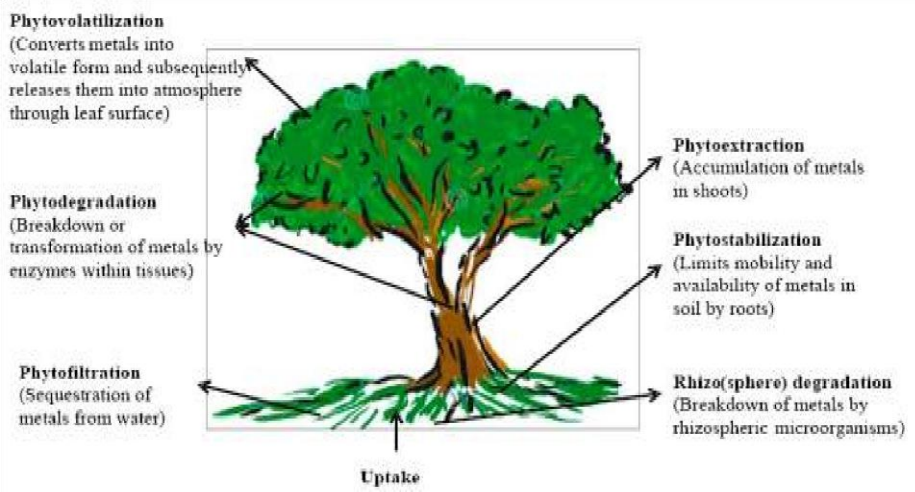

Figure 1: The different techniques of Phytoremediation (Massino *et al.,* 2020).

The favorable plant species to clean up a PAH-contaminated soil are selected based on their depth root system, fast growing rate, high production of biomass, resistance to pathogens, tolerance to contaminant, easy to harvest and age of the plant (Haihua *et al.,* 2017; Asma *et al,* 2019). High levels of pollutant uptake, translocation, and accumulation in harvestable parts of the plant are important plant features for the phytoextraction of organic and inorganic contaminants (Maurizio, 2020; Muhammad *et al,* 2020; Salau *et al*., 2022). Some varieties of plant species used in phytoremediation have shown different capabilities to pick up PAHs in soil. Plants are known to vary widely with respect to root parameters such as morphology, fine root turnover, root exudation, root decomposition, and associated microbial communities (Girihar and Krishan, 2010). If the dominant mechanism of PAHs dissipation in planted soil is associated with the activity of rhizosphere microbial communities, then it would be expected that remediation potential would also vary across plant species and life-history types (Chen *et al.,* 2015; Haihua *et al.,* 2017). Another most important factor which determined the uptake of PAHs from soil into plant is the nature of the contaminant which is dependent on octanol–water partition coefficient (Log Kow), and is defined as the ratio of contaminant concentration in octanol to the concentration of contaminant in water (Zhang *et al,* 2017). Generally, hydrophilic low molecular weight (LMW) PAHs (2-3 rings) with Log Kow < 4 may be accumulated by roots and transported within the plant shoots, whereas hydrophobic non-ionizable high molecular weight (HMW) (above 4 rings) with Log Kow > 4 may strongly bind to plant root with no further translocation to plant shoot, but moderate hydrophobic PAHs with Log Kow between 2-3 may also be accumulated by plant root and transferred to aboveground tissues. However, PAHs that are highly polar and very soluble in water with Log Kow 1.0 are not sufficiently binded onto the root, nor are they actively translocated via plant tissue because of their high polarity (Naidoo and Naidoo, 2018: Wei *et al,* 2020). For instance, naphthalene with 2 rings and log Kow 3.29 will be accumulated by plant roots and translocated to shoot, whereas pyrene with 4 rings and Log Kow 4.58 will bind on to plant roots with no translocation to shoot (Zhang *et al,* 2017; Naidoo and Naidoo, 2018; Wei *et al.,* 2020). Other environmental factors to be considered are nature of the soil, Climate condition, irrigation and soil fertilizing practices (Girihar and Krishan, 2010; Chen *et al,* 2015). The primary objective of this work was to evaluate the accumulation capacity and phytoremediation potential of the *Gardenia jasminoides* plant in removing NAP, ACN and PHE from soil.

MATERIALS AND METHODS

Sampling Area

The plant sample (*Gardenia jasminoides)* along with the soil that support the growth of the plant were collected from botanical garden, Federal University, Dutse with a geographical coordinates 11º 45’ 22.25” N and 9º 20’ 20.26” E situated at Ibrahim Aliyu Bypass, Dutse Local Government Area, Jigawa state, Nigeria.

Green House Pot Experiment

Green house pot experiment containing 4 kg soil was conducted according to the method described by Seniyat *et al.* (2001); 1600 mg acenaphthene > 97 % purity, 2000 mg naphthalene > 97 % purity and 2400mg phenanthrene > 96 % purity were dissolved in 200 mL of acetone and then mixed with each soil sample. The same amount of acetone was used for all treatments, including the control (without PAHs). After evaporation of acetone from the soils in fume hood, soils in each pot were thoroughly mixed and then irrigated for one day before transplanting the plants into the pots. A separate pot containing untreated soil was used to serve as a control. Experiments were exposed to natural conditions for ten weeks. Watering of the pots was done with 750 mL of water per pot every two days in the morning. Three replicate of each pot of the plant were planted for data handlings.

Sample Preparation

The plant and soil were collected at the end of the experiment, washed thoroughly in the laboratory with distilled water and carefully separated into roots and shoots. These were air-dried at room temperature to a constant weight, ground, homogenized and sieved through a 2 mm sieve according to Mang *et al,* (2014). The sieved root, shoot and soil samples were stored in black polyethylene bags in dark at 4ºC to prevent microbial degradation, evaporation and photo-oxidation of PAHs before further analysis as reported by Khan *et al., (*2018).

Soxhlet Extraction of PAHs from Plant and Soil Sample

Ten grams (10 g) of each air-dried soil sample containing PAH with ten grams (10 g) anhydrous sodium sulphate powder was extracted in a soxhlet extractor using 180 mL of dichloromethane-acetone (1:1 v/v) set to 60ºC and run for 4 hours. PAHs in shoot and root of the plant sample were

extracted in the same way. Both of the extract from soil and plant were evaporated (concentrated) to near dryness (up to 20 mL) in rotary evaporator set at 69ºC as described by Khanitta *et al.,* (2014).

Clean-up Procedure

The plant and soil extracts containing the naphthalene, acenaphthene and phenanthrene were purified using column chromatography packed with silica gel and anhydrous sodium sulphate saturated with 2.0 mL of dichloromethane and acetone 1:1 (v/v). Each extract was loaded into the column and eluted with dichloromethane. The first 1.0 mL of eluate was discarded before 5.0 mL of eluate was collected into an amber coloured vial as described by Khanitta *et al.,* (2014) and Itodo *et al., (*2020).

Quantification of PAHs

The levels of PAHs in the extracts of soil, roots and shoots were analyzed using high performance liquid chromatography (HPLC) system from Shimadzu (prominence) equipped with a UV-VIS detector (SPD-20-AV), Aproma (Promasil) C18 150 mm x 4.6 mmm, µm pores column and CTO-20AC column oven, 20 µL of each sample were injected into the HPLC column by the aid of a syringe.

Handling Statistical Analysis

Statistical analysis was done by comparing the chromatogram of sample with the chromatogram of standard in term of peak height. Microsoft Excel 2010 was used to calculate sample mean concentration and standard deviation (n =3). One-way ANOVA analysis was used to compare the significant means between the soil and the two parts of plant treatments. Subsequent multiple comparisons of means were performed using the Turkey test comparison method. A statistical significance level of ($P \leq 0.05$) was considered throughout the analysis.

RESULTS AND DISCUSSION

Physicochemical Properties of the Soil

The results of the physicochemical properties of the experimental soil are presented in Table 1 below. The result shows that, the soil was predominantly loamy sand in texture. Soil texture indicates the particle size distribution of the soil and the content of fine particles like clay and oxides. As a result, PAHs uptake by plants is more favorable in soil with low level of clay and oxides than in soil with higher level of clay and oxide (Balasubramaniyam, 2020). The soil pH is found to be within 6.5 to 6.8, which is slightly acidic in nature and is within the recommended range for proper growth and efficient uptake of nutrient and compound from the soil by Gardenia *jasminoides* (Sydney and Joan, 2020). Soil pH play an important role in the absorption of PAHs, it controls the solubility and mobility of organic compounds. Thus, at high pH, organic bioavailability increases as more PAHs are released into the soil solution due to increase in the solubility of soil organic matter (Merkl *et al,* 2005). At low pH, PAHs are less available, they are strongly bound by soil organic matter, retarding uptake by the plant root (Balasubramaniyam, 2020). The experimental soil is non-saline with a very low electrical conductivity (EC) of 0.17 mS/cm. Low organic matter content (OM %) of 0.52 % was also observed in the experimental soil as well as very low cation exchange capacity (9.51 Cmol/100 kg of soil). Soil-plant transfer of persistent organic compounds like PAHs also depends on soil organic matter (Merkl *et al,* 2005). Normally, PAH uptake by plants is favoured under moist conditions in soils with low organic matter content. Increasing the amount of organic matter content in the soil reduces the uptake of PAHs by plant root (Balasubramaniyam, 2020). The low level of clay and CEC indicate the bioavailability of PAHs in the soil and the possibility of plants to pick up PAHs from the soil (Atarfar *et al.*, 2010).

Table 1: Physicochemical Properties of the Soil

Parameters	Results
pH	6.5 ±0.10
EC(mS/cm)	0.17±0.01
CEC ((cmol/100kg Soil)	9.51±0.10
Organic Carbon (%)	0.30±0.01
Organic Matter (%)	0.52±0.01
Silt (%)	6.0±0.01
Clay (%)	17.7±0.57
Sand (%)	76.30±0.01
Textural Class	Sandy Loamy

Data are presented as Mean ± SD. Where SD = Standard Deviation with n = 3

Polycyclic Aromatic Hydrocarbons Accumulation in the Root and Shoot of G*ardenia jasminoides*

Acenaphthene (ACN)

In this research, absorption and accumulation of acenaphthene by *Gardenia jasminoides* plant at spiked concentration of 16000mg were found to be high in the shoot than in the root. Although low concentration of ACN was observed in the root of the plant in the control experiment (Table 2). This result is in agreement with the result of Tian *et al* (2019), who reported high level of low molecular weight (LMW) PAHs in the shoot of *Sabina chinesis* plant. This observation could be due to the high level of ACN in the soil. Following absorption by the root, ACN is rapidly transferred through the xylem to the shoot of *Gardenia jasminoides* plant. It has been reported in literature that, decreased root cell sequestration may facilitate translocation of PAHs from root-to-shoot in the hyperaccumulator plants, whereas in non-accumulator plants much of PAHs absorbed are sequestered in the root, possibly via storage in the vacuoles and rendered PAHs translocation from root to the shoot (Napoli *et al,* 2019).

Table 2: Concentration (mgkg^{-1}) of ACN in the Soil, Shoot and Root of *Gardenia jasminoides* plant

Amount Spiked *(mg/4kg)*	Soil	Root	Shoot	BCF	TF
1600mg	90.8±0.89	267.21 ± 2.80	786 ± 2.93	8.86	2.94
Control	19.62 ± 0.01	14.58.5± 0.02	11.88 ± 0.01	0.74	1.23

Data are presented as Mean ± SD. No significant difference was observed at P < 0.05 using one-way Anova analysis and multiple comparison according to Tukey Test, SD = Standard Deviation, BCF = Bioconcentration Factor and TF = Translocation Factor.

Naphthalene (NAP)

Accumulation of the naphthalene in soil spiked with 2000mg by *Gardenia jasminoides* was found to be higher in the shoot compared to the root (Table 3). This could be due to the hydrophilic nature and low octanol-water partition coefficient (Log Kow < 4) of naphthalene which makes it more soluble and easier to absorb by the plant in the soil (Wei *et al.,* 2020). This suggested that, at high level of NAP in the soil, *Gardenia jasminoides* may translocate most of the absorbed NAP from the root to shoot. This observation agrees well with the report of Tian *et al,* (2019) who observed that naphthalene was highly accumulated in the shoot than in root of *Oryza sativa* (957µg/kg and 153 µg/kg). Again, higher level of naphthalene was also observed in the shoots of the plant in the control experiment (Table 3). Gao and Ling (2006) found phenanthrene and pyrene not only in the plants grown on spiked soil, but also in the above ground parts of control plants cultivated in pots with unpolluted soil. Binet *et al,* (2000); Gao and Ling (2006) showed that the presence of PAHs in control plants proved the contribution of the direct uptake of these contaminants from air (Atmospheric deposition).

Table 3: Concentration (mgkg^{-1}) of NAP in the Soil, Shoot and Root of *Gardenia jasminoides* plant.

Amount Spiked *(mg/4kg)*	Soil	Root	Shoot	BCF	TF
2000mg	126.18±03.06	234.63 ± 10.56	544.42 ± 9.80	4.31	2.32
Control	20.16 ± 0.01	27.54± 0.02	33.12± 0.01	1.64	1.20

Data are presented as Mean ± SD. No significant difference was observed at P < 0.05 using one-way Anova analysis and multiple comparison according to Tukey Test, SD=Standard Deviation, BCF = Bioconcentration Factor and TF = Translocation Factor

Phenanthrene (PHE)

In this study, absorption and accumulation of Phenanthrene at concentration 2400mg by *Gardenia jasminoides* showed high level of Phenanthrene in the shoot compared to the root (Table 4). This observation agrees with the report of Tian *et al*. (2019). These authors reported that, high level of *phenanthrene* was found in the shoot of *Sabina chinesis* plant especially the stem (52 %). Translocation of PAHs from root to shoot has been studied in several plant species including *Sorghun bicola*, *Brassics napus* (Petrova *et al*., 2017), *Magnolia grandiflora* (Tian *et al.,* 2019), *Racomitrium species* (Oshi., 2018).

Table 4: Concentration (mgkg^{-1}) of PHE in the Soil, Shoot and Root of *Gardenia jasminoides* plant

Amount Spiked *(mg/4kg)*	Soil	Root	Shoot	BCF	TF
2400mg	221.58±0.76	140.94±1.02	510.48±7.12	2.30	3.62
Control	12.42±0.01	7.00± 0.02	7.20± 0.01	0.57	1.00

Data are presented as Mean ± SD. No significant difference was observed at P < 0.05 using one-way Anova analysis and multiple comparison according to Tukey Test, SD=Standard Deviation, BCF = Bioconcentration Factor and TF = Translocation Factor

Phytoremediation Potential of *Gardenia jasminoides* Plant

The most important criteria for identifying the polycyclic aromatic hydrocarbons accumulator plants, is the concentrations of PAH in the aboveground parts of the plant and in the soil (Rajput *et al,* 2020). According to Muhammad *et al.,* (2020), a successful phytoextraction process depends on contaminant removal by the shoots (Salau *et al*., 2022). The ability of the plant to accumulate a particular PAH with respect to its concentration in the soil can be calculated using the bioconcentration factor (BCF), which is the ratio of the PAH concentration in the shoot to the concentration in the soil. The higher the BCF value the more suitable is the plant for phytoextraction. BCF Value > 1 is regarded as high values as described by (Sesan *et al*., 2013).

$$BCF = \frac{\text{PAH concentration in the shoot}}{\text{PAH concentration in the soil}}$$

The transfer of PAHs from roots to shoots is estimated using the translocation factor (TF). This ratio is an indication of the ability of the plant to translocate PAHs from the roots to the shoots of the plant (Sesan *et al*, 2013). TF is calculated by the relation: - ratio of concentration of PAHs in the shoot to the concentration of PAHs in the roots. PAHs that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1, whereas values > 1 indicate that the PAHs are stored in the stems and leaves (shoot) (Sesan *et al.,* 2013).

$$TF = \frac{\text{PAH concentration in the shoot}}{\text{PAH concentration in the root}}$$

Some plants accumulate polycyclic aromatic hydrocarbons; others exclude them while other plant species are indicators that show poor control over organic contaminants uptake and transport processes (Muhammad *et al.,* 2020; Sesan *et al.,* 2013). In this research work, the BCF and TF values for the acenaphthene, naphthalene and phenanthrene are presented in Table 2, 3 and 4 for the experimental and control plant.

For ACN, the BCF and TF values at 1600 mg are; BCF = 8.86 and 0.74 for sample and control respectively. TF 2.94 and 1.23 respectively (Table 2). BCF is used in the estimation of the degree of uptake and storage of contaminants in plants (Rajput *et al,* 2020). For having the BCF and TF values greater than one (1), with exception of the control, the plant, *Gardenia jasminoides* may be suggested as hyperaccumulator of ACN. This suggests that, at high concentration of ACN, *Gardenia jasminoides* can translocate most of the absorbed ACN to shoot.

The BCF and TF values for NAP at 2000 mg are; BCF = 4.31 and 1.64 for the sample and control respectively. TF 2.23 and 1.20 respectively as shown in Table 3. This result indicated greater uptake and accumulation of NAP in the above ground parts of the experimental plant with less or poor stabilization in the root tissues. Such plant that has BCF and TF value above one could be described as potential NAP accumulator. This process of accumulating contaminants in the shoots just as in the ACN above is called phytoextraction (Muhammad *et al.*, 2020). Thus, *Gardenia jasminoides* may best be described as NAP phytoextractor.

Phenanthrene BCF and TF values at the concentration of 2400 mg are; BCF = 2.30 and 0.57 for the sample and control respectively, whereas the TF values are 3.62 for sample and 1.0 for the control (Table 4). Plants with both bioaccumulation and translocation factors greater than one (BCF and TF >1) have the potential to be used as phytoextractors (Adamu, 2019). PHE has high BCF and TF greater than one. This means, the plant can serve as hyper-accumulator of PHE.

CONCLUSION

In this study an assessment of remediation of contaminated soil was done using house plant, called *Gardenia jasminoides* without the need for soil excavation. *Gardenia jasminoides* demonstrated its ability to absorb and accumulate PAHs (naphthalene, acenaphthene and phenanthrene) from soil. From the results obtained *Gardenia jasminoides* plant may successfully be used as a good accumulator or hyperaccumulator of naphthalene, acenaphthene and phenanthrene using the proper agronomic practices. The results of this work demonstrated that, the elevated concentration of the PAHs in roots and translocation to the above ground aerial parts of the plant suggest the suitability of *Gardenia ja*sminoides for phytoremediation of naphthalene, acenaphthene and phenanthrene.

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