

Phytoremediation Potential of *Ficus Benjamina* for the removal of Naphthalene, Acenaphthene and Phenanthrene in Contaminated Soil

Y. A. Adamu, Y. Musa, S. Nasir

Department of Chemistry,
Federal University Dutse,
Jigawa State, Nigeria.

Email: yamushadis@mail.com

Abstract

This study was undertaken to evaluate the phytoremediation potential of *Ficus Benjamina* plant for the removal of polycyclic aromatic hydrocarbons (PAHs): Acenaphthene (ACN), naphthalene (NAP) and phenanthrene (PHE) from contaminated soil. The plant was transplanted into pot containing 4 kg soil spiked with the PAHs: ACN, NAP and PHE at concentrations of 1600, 2000 and 2400 mg respectively. A separate pot with untreated soil was used as a control. Irrigation was done with 600 mL of water after every three days in the evening hours for eight weeks. Samples of the plant and soil were collected at the end of the phytoremediation process; the plant was washed with tap water and carefully separated into roots and shoots, dried along with the soil, ground and sieved. The sieved soil, roots, shoots of the experimental plant as well as that of the control were analyzed for the levels of PAHs: ACN, NAP and PHE following Soxhlet extraction with 200 mL of dichloromethane-acetone (1:1 v/v) at 60 °C for 6 hours using high performance liquid chromatography (HPLC). The bioconcentration factor (BCF) in mg/kg and the Translocation Factor (TF) in mg/kg of *Ficus Benjamina* for ACN, NAP and PHE were (1.76 and 0.47), (0.54 and 2.7) and (0.93 and 0.68) respectively. The results generated in this study have demonstrated that the different concentrations of the PAHs (NAP, ACN and PHE) in root, only NAP was transferred to the aerial parts of the plant and this suggests the suitability of *Ficus Benjamina* for the phytoremediation of NAP.

Keywords: Phytoextraction, Polycyclic Aromatic Hydrocarbons, Hyperaccumulator, Excluder, Soil

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are sometime referred to as polynuclear aromatic hydrocarbon (Avani *et al.*, 2020). Chemically, these organic pollutants are made up of two or more fused benzene ring molecules which contain only carbon and hydrogen atoms. They are categorized based on their molecular structure as low molecular weight (LMW) and high molecular weight (HMW) PAHs. LMW PAHs consist of < 3 rings, whereas HMW have four or more fused benzene rings (Tahir *et al.*, 2020). Generally, PAHs have low water solubility, low vapour pressure, very soluble in organic solvents and are lipophilic in nature. They also have high melting and boiling points depending on number of fused rings (Choroszy and Tereszkievicz, 2020). The major sources of PAH pollution are natural and anthropogenic emission sources (Mojiri *et al.*, 2019). Natural sources such as volcanic eruptions, natural forest fire, and moorland fire caused by lightning flashes are considered to be negligible relative to anthropogenic sources (Abdel-Shafy and Mansour, 2016). Anthropogenic emission sources

*Author for Correspondence

are the major contributors of PAH pollution to the environment, which includes industrial, automobile, domestic, and agriculture emission sources (Gupte *et al.*, 2016; Mojiri *et al.*, 2019; Patel *et al.*, 2020). The most common and highly toxic PAHs are the 16 PAHs identified by United States Environmental Protection Agency (USEPA) (Tahir *et al.*, 2020; Avani *et al.*, 2020; Masato and Suzuki, 2020).

Accumulation of PAHs in soil is of great concern to human health as they can enter human body through food chain, and the toxic effect on human health is mainly dependent on the time, routes of exposure and concentration of PAHs (Abdel-Shafy and Mansour, 2016). The major toxicities of concern include immunotoxicity, carcinogenicity, mutagenicity, teratogenicity and endocrine disruption (USEPA, 2000; Chunrong *et al.*, 2020). It is also reported by Giri *et al.*, (2016) that the toxicity of PAHs increases with increase in number of fused benzene rings. Hence, lower molecular weight PAHs compounds with 1 to 3 rings are mild toxic whereas higher molecular weight of PAHs with > 4 rings are extremely toxic and can cause genetic damage (Chunrong *et al.*, 2020). Soil remediation is necessary for eliminating risk associated with human health or the environment from toxic PAHs. Majority of conventional remediation technologies are not eco-friendly, costly to implement and cause damage to the environment (Khan *et al.*, 2018; Adamu, 2019). An alternative strategy is the use of plant based system (phytoremediation) to interact with the PAHs for eventual removal or degradation from soil, sediment or water (Adamu, 2019). The advantages of this strategy over conventional remediation processes are: less environmental disturbance, soil erosion prevention, control runoff and windblown dust, increase soil health and fertility, less harmful, broad range of application, environmentally friendly and high public acceptance (Erakhrumen, 2007; Rao and Babu, 2014; Adamu, 2019).

The processes which are responsible for PAHs entrance in to plants are dependent on soil organic matter (SOM), octanol - water partition coefficient (Kow) and plant species (Balasubramaniam, 2020). Normally, PAH uptake by plants is favoured under moist conditions in soils with low organic matter content. An increase in log Kow of the organic contaminant decreases the uptake of PAHs by plants in soil (Merkl *et al.*, 2005). The favorable plant properties for phytoremediation of organic pollutants are fast growing rate, high production of biomass, widely distributed deep root system, tolerance of high level of contaminant, easily to harvest and process, resistance to disease and pest, and repulsive to herbivores to avoid food chain contamination (Giridhar and Krishna, 2010; Chen *et al.*, 2015; Haihua *et al.*, 2017). The primary objective of this work was to evaluate the accumulation capacity of the *Ficus benjamina* plant in removing NAP, CAN and PHE from soil. However, no previous studies have targeted NAP, CAN and PHE contaminated soil.

MATERIALS AND METHODS

Sampling Area

The plant sample (*Ficus Benjamina*) and the soil used in this study were collected from botanical garden, Federal University, Dutse with a geographical coordinate 11 ° 45' 22.25" N and 9 ° 20' 20.26" E. The University is located at Ibrahim Aliyu Bypass, Dutse Local Government Area, Jigawa state, Nigeria. After collection of soil samples, each soil sample was divided into two and transferred into a black polyethene bags. One part of the soil was used differently for phytoremediation, while the other half was dried, sieved with 2 mm diameter mesh and was used for physicochemical analysis.

Green House Pot Experiment

The pot experiment was conducted according to the method described by Seniyat *et al.* (2001); 1600 mg ACN, 2000 mg NAP and 2400 mg PHE were dissolved in 250 mL of acetone and then mixed each with about 4 kg soil sample. The same amount of acetone was used for the control (0 mg PAHs). After evaporation of acetone from the soils in fume hood, soils in each pot were mixed thoroughly and then irrigated for 12 hours before the plants were transplanted into the pots containing spiked soils. A different pot without PAHs was used to serve as a control. The experiments were conducted under natural conditions for eight weeks. Watering of the pots was done with 600 mL of water per pot every three days in the evening. For statistical handling, three replicate of each pot of the plant were planted.

Sample Preparation

At the end of the phytoremediation process; the plants were harvested from the pots and the soils were homogenized, dried and sieved through a 2 mm sieve. The plants were carefully separated into roots and shoots, and washed thoroughly in the laboratory with distilled water. These were dried in the oven at 20 °C to a constant weight, ground, homogenized and sieved through a 2 mm sieve according to Mang *et al.*, (2014). The sieved soil, shoot and root samples were stored in black polyethylene bags in dark at 4°C to prevent photo-oxidation, evaporation and microbial degradation of PAHs prior to analysis as reported by Khan *et al.* (2018).

Soxhlet Extraction of PAHs from Plant and Soil Sample

Soxhlet extraction (EPA method 3540c)

Ten (10) g of each air-dried soil sample containing PAHs and 10 g of anhydrous sodium sulphate powder were mixed. 50 µL of 200 mg/L PAH was dissolved in dichloromethane and then added as an internal standard. The above mixture was placed into the extraction thimble and extracted in a soxhlet extractor using 200 mL of dichloromethane-acetone (1:1 v/v) set at 60 °C for 6 hours. NAP, ACN and PHE in the shoot and root of each plant sample were extracted using the same procedure as described above. Both extracts from plant and soil were concentrated to 20 mL in rotary evaporator set at 69 °C as described by Bhupander *et al.*, (2014).

Clean-up procedure

Column chromatography packed with mixture of silica gel and anhydrous sodium sulphate saturated with 2.0 mL of acetone and dichloromethane 1:1 (v/v) were used to purify the plant and soil extracts containing the NAP, ACN and PHE. Each extract was loaded onto the column and eluted with dichloromethane (Khanitta *et al.*, 2014). The first 1.5 mL of eluate was discarded before 10 mL of eluate was collected into an amber coloured vial as described by Ito *et al.* (2020).

Quantitative Determination of Naphthalene, Acenaphthene and Phenanthrene

The levels of three PAHs from soil and plant extracts were analyzed using high performance liquid chromatography (HPLC) system from Shimadzu equipped with a UV-VIS detector (SPD-20-AV), Aproma C18 150 mm x 4.6 mm, µm pores column and CTO-20AC column oven. 20 µL of each liquid sample were injected into the HPLC column by the aid of a syringe. The mobile phase consists of solvent A (Water) and solvent B (Acetonitrile). The flow rate was 1.5 mL/min. Three PAHs concentrations were determined by relating the peak height of PAHs standards with the peak height of samples (Bhupander *et al.*, 2019).

Statistical Analysis

Data analysis was done by relating the chromatogram of standard with the chromatogram of sample in term of peak height. Microsoft Excel 2010 was used to determine sample mean

concentration and standard deviation (n =3) while one-way ANOVA was used to compare the significant mean difference between the soil and the two parts of plant treatments. Multiple comparisons of means were conducted using Turkey test comparison method. A statistical significance level of $P \leq 0.05$ was considered throughout the statistical analysis.

RESULTS AND DISCUSSION

Soil Physicochemical Parameters

The results of soil physicochemical analysis are presented in Table 1. The taxonomy classification of the soil was found to be loamy sand with pH of 7.6. The less acidic nature of the soil is within the recommended range for proper uptake of compounds from soil by plants; soil pH plays a vital role in the solubility and the absorption of PAHs from soil (Balasubramaniyam, 2020). This result is in agreement with the report of Dora (2019). A very low electric conductivity of 170 $\mu\text{S}/\text{cm}$ was observed in soil. The results also showed low amount (4.70 mg/kg Soil) of available phosphorus and moderate level of total nitrogen content of 0.25%. Phosphorus is an essential constituent of numerous substances involved in photosynthesis and respiration in plants while nitrogen plays a fundamental role in energy metabolism and protein synthesis for plant development (Nwakife *et al.*, 2022). Low organic matter content (OM %) of 0.52 % was also observed in the experimental soil as well as very low cation exchange capacity (CEC) (9.51 Cmol/100 kg of soil). Organic matter is largely responsible for much of the physical and chemical fertility of a soil. The low level of organic matter showed very poor structural condition and very low structural stability of the experimental soil (Atafar *et al.*, 2010). CEC is a major controlling agent of stability of soil structure, nutrient availability for plant growth, soil pH, and the soil's reaction to fertilizers and other nutrients (Atafar *et al.*, 2010). The low level of clay and CEC indicate the mobility of PAHs in the soil and the ability of plants to pick up PAHs from the soil. This observation is also in agreement with the assertion by Atafar *et al.*, (2010).

Table 4.1 Physicochemical Properties of the Experimental Soil

Parameters	Results	Interpretation
pH	6.50 \pm 0.10	Slightly Acidic
EC ($\mu\text{S}/\text{cm}$)	170 \pm 0.01	Non Saline
Organic Carbon (%)	0.30 \pm 0.01	Low
Organic Matter (%)	0.52 \pm 0.01	Low
Nitrogen (%)	0.25 \pm 0.03	Moderate
Available P (mg/kg Soil)	4.70 \pm 0.02	Very Low
Al ³⁺ + H ⁺ (Cmol (+)/100 kg Soil)	2.0 \pm 0.01	Low
Na ⁺ (Cmol (+)/100 kg Soil)	0.13 \pm 0.02	Low
K ⁺ (Cmol (+)/100 kg Soil)	0.07 \pm 0.01	Very Low
Ca ²⁺ (Cmol (+)/100 kg Soil)	5.24 \pm 0.03	Moderate
Mg ²⁺ (Cmol(+)/100kgSoil)	CEC 2.10 \pm 0.01	Moderate
(Cmol (+)/100 kg Soil)	9.54 \pm 0.03	Low
Clay (%)	17.7 \pm 0.57	Low
Silt (%)	6.0 \pm 0.01	Low
Sand (%)	76.30 \pm 0.01	Very High
Textural Class	Loamy Sand	Loamy Sand

Data are presented as Mean \pm SD (n=3), SD=Standard Deviation, P = Phosphorus, EC=Electric Conductivity, CEC=Cation Exchange Capacity

Polycyclic Aromatic Hydrocarbons Accumulation in the Root and Shoot of *Ficus benjamina* Plant

This research work showed the uptake and accumulation of different PAHs by *Ficus Benjaminina* at concentrations of 2000 mg, 1600 mg and 2400 mg for NAP, ACN and PHE, spiked in to the experimental pots. The results showed that most of the PAHs absorbed were retained in the root including the control. Tables 2, 3 and 4 show that, the experimental pot spiked with 2000 mg NAP had the highest TF value of (2.70) whereas the experimental pot spiked with 1600 mg ACN and 2400 mg PHE had the lower translocation factor (TF) values of 0.47 and 0.68 respectively. This report agrees with the observation reported by Wei *et al.* (2020) that high molecular weight PAHs above 4 rings with Log Kow > 4 may be strongly retained in the plant root with no further translocation to plant shoot whereas lower molecular weight PAHs (2-3 rings) with Log Kow < 4 may be accumulate by roots and transported within the plant shoots.

Uptake and Translocation of Naphthalene in the soil, root and shoot of *Ficus benjamina*.

Table 2 shows the uptake and translocation of NAP in the soil, root and shoot of *Ficus benjamina*. High level of NAP was recorded in the shoots of *Ficus benjamina* for both spiked and control experimental plants. The spiked experiment accumulated high levels of NAP in the shoot (165.06 ± 12.22 mgkg⁻¹). This level was higher than what was observed in the roots of both spiked (60.39 ± 4.00 mgkg⁻¹) and control (11.54 ± 0.02) plants respectively. This result agrees with the observation reported by Tian *et al.* (2019) where they reported that high level of LMW PAHs with 2 – 3 rings were translocated to the shoot of *Brassica napus*. Despite the high concentration of the NAP in the experimental pots, no physical sign of toxicity was observed on the *Ficus benjamina* plant as compared with the control.

Table 2: Concentration (mgkg⁻¹) of Naphthalene in the Soil, Root and Shoot of *Ficus benjamina*

Amount (mg/4kg)	Spiked	Soil	Root	Shoot	BCF	TF
2000		103.75 ± 2.62	60.39 ± 4.00	165.06 ± 12.22	1.59	2.70
Control		11.16 ± 0.01	11.54 ± 0.02	17.46 ± 0.01	1.03	1.51

Data are presented as Mean ± SD (n=3). No significant difference was observed at P < 0.05 using ANOVA and Multiple Comparison according to Tukey Test, SD = Standard Deviation

Uptake and Translocation of Acenaphthene in the soil, root and shoot of *Ficus benjamina*.

Table 3 shows the distribution of ACN in the parts of *Ficus benjamina* spiked with concentration of 1600 mg in the experimental pots. The plant accumulated high level of ACN in the root. There was less translocation to the shoot at the experimental pot with 578.07 ± 6.75 mgkg⁻¹ in the root and 274.05 ± 4.96 mgkg⁻¹ in the shoot. This agrees with the report of Alagic *et al.* (2017), who observed that ACN was highly accumulated by the root than the shoot of *Rubus fruticosus L* with concentrations of 454 µg/kg and 156 µg/kg respectively. Patowary *et al.* (2017) also reported that *Oriza sativa L*, accumulated high level of ACN in the root than in the shoot, but this result is in disagreement with Oishi (2016), who reported high level of ACN in the shoot than in the root of *Racomitrium* specie. The high amount of acenaphthene in the roots and the poor translocation to the shoot in *Ficus benjamina* may be explained by a sequestration of acenaphthene by the xylem parenchyma vessel walls in roots and immobilization in the vacuoles of the root cells (Patowary *et al.*, 2017). This could also be the reason why high level of ACN was recorded in the root of *Ficus benjamina* in this study.

Table 3: Concentration (mgkg⁻¹) of Acenaphthene in the Soil, Root and Shoot of *Ficus benjamina*

Amount (mg/4kg)	Spiked	Soil	Root	Shoot	BCF	TF
2000		156.06 ± 5.35	578.07± 6.75	274.05 ± 4.96	1.76	0.47
Control		4.32 ± 0.01	33.12 ± 0.02	21.42 ± 0.01	4.96	0.65

Data are presented as Mean ± SD (n=3). No significant difference was observed at P < 0.05 using ANOVA and Multiple Comparison according to Tukey Test, SD = Standard Deviation

Uptake and Translocation of Phenanthrene in the soil, root and shoot of *Ficus benjamina*

Table 4 shows the concentrations of the PHE in the soil, root and shoot of *Ficus benjamna*. The results showed lower concentrations in the shoot of plant for both spiked and control experiment. At 2400 mg level of the spiked PHE in the soil, highest proportion was observed in the root (389.70* ± 0.51) with very little translocation to the shoot part (263.88* ± 0.76) as presented in Table 4. Once PHE has penetrated into the root system, it may accumulate there due to its low solubility and high octanol-water coefficient (Log Kow > 4), thereby leaving only small concentration to be translocated to above ground plant parts. For most plant species studied, the majority of absorbed PHE (approximately 75% or more) was accumulated in the roots, and only a small fraction was translocated to shoot of the plant (Alves *et al.*, 2017). This observation agrees with the report of *Sushkova et al.* (2020). These authors reported that *Phragmites australis*, accumulated high level of PHE in the root (308.0 µg/kg) than in shoot (239.8 µg/kg). Studies by Li and Ma (2016) on *Triticum aestivum* plant found out that 700 mg/kg was accumulated in the root. However, a few plant species have been reported to accumulate high concentrations of PHE in the above-ground parts and are called hyperaccumulators (Kvesitadze *et al.*, 2006; Guarino *et al.*, 2019; Rajput *et al.*, 2020).

Table 4: Concentration (mgkg⁻¹) of phenanthrene in the Soil, Root and Shoot of *Ficus benjamina*

Amount (mg/4kg)	Spiked	Soil	Root	Shoot	BCF	TF
2400		284.13* ± 6.49	389.70*± 0.51	263.88* ± 0.76	0.93	0.68
Control		28.62* ± 0.01	10.08* ± 0.02	7.02* ± 0.01	0.25	0.69

Data are presented as Mean ± SD (n=3). Means with asterisks within a row are significantly difference at P < 0.05 using ANOVA and Multiple Comparison according to Tukey Test, SD = Standard Deviation

Determination of Bioconcentration Factor (BCF)

BCF of PAHs was used to determine the amount of PAHs that is absorbed by the plant from the soil (Sesan *et al.*, 2013). This is an ability of the plant to accumulate a particular PAH with respect to its concentration in the soil (Sesan *et al.*, 2013). BCF is calculated from the ratio of the PAH concentration in the shoot to the concentration in the soil (Sesan *et al.*, 2013). The higher the BCF value the more suitable is the plant for phytoextraction. BCF Values greater than one is regarded as high values as described by (Sesan *et al.*, 2013).

$$BCF = \frac{\text{Average PAH concentration in the shoot or root (mg/kg)}}{\text{PAH concentration the soil (mg/kg)}}$$

Determination of Translocation Factor (TF)

The transfer of PAHs from root to shoot was calculated using the translocation factor (TF). This ratio is an indication of the effectiveness of a plant to translocate PAHs accumulated in the roots to the aerial parts of the plant (Sesan *et al.*, 2013). TF is determined from the ratio of concentration of PAHs in the shoot to the concentration of PAHs in the roots. PAHs that are

accumulated by plants and largely transferred to shoots of plants are indicated by TF values greater than one whereas TF values below one indicating that the PAHs are stored in the root (Sesan *et al.*, 2013).

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

In this study, the BCF and TF values for the PAHs; NAP, ACN and PHE are presented in the Tables 2, 3 and 4 at different concentrations of the PAHs in the experimental pots.

For NAP, the TF value was found to be 2.70 at 2000 mg whereas in control the TF value was 1.51 as shown in Table 2. This shows that *Ficus benjamina* can absorb and translocate NAP to the shoot. The BCF values are all greater than 1 which also indicates the ability of the plant to absorb NAP as shown in Table 2. The study shows that experimental plant may serve as hyperaccumulator of NAP contaminated soil. At 2000 mg the TF value was found to be 0.47 which is less than unity as presented in Table 3. The BCF value is also less than unity which indicates the degree of ACN absorption from the soil and retention in the root of the plant. *Ficus benjamina* may serve as ACN phytostabilizer or excluder. The results from this study show that at 2400 mg of PHE in the soil, *Ficus benjamina* plant had low TF (Table 4) indicating that the plant had difficulty in transferring PHE from the root to upper parts of the plant. The BF value was 0.69 indicating high retention of PHE in the root without translocating it to the shoot. Owing to this result, *Ficus benjamina* plant may serve as PHE phytostabilizer or excluder.

CONCLUSION

Phytoremediation is a green technology that uses plants to pick up and/or degrade inorganic and organic contaminants in the environment. In this study an assessment of remediation of contaminated soil was conducted using house plant (*Ficus benjamina*) without the need for conventional techniques. *Ficus benjamina* plant demonstrated its ability to absorb and accumulate PAHs (NAP, ACN and PHE) in either their roots or shoots the plant with no noticeable symptoms of toxicity in this study. From results obtained coupled with TF and BCF recorded, suggest the suitability of *Ficus benjamina* as hyperaccumulator of NAP in the soil for having higher values of TF and BCF. The plant may also serve as phytostabilizer or excluder for ACN and PHE in soil for having TF and BCF values less than unity.

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