

Degradation of Polycyclic Aromatic Hydrocarbons from Soil Leachate by Water Hyacinth (*Eichhornia crassipes*)

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Abstract

The ability of water hyacinth (*Eichhornia crassipes*) to adsorped polycyclic aromatic hydrocarbons from contaminated soil leachate was examined. Results obtained from analysis using GC/FID indicated that naphthalene (8.350 µg/g) was the overall highest polycyclic aromatic hydrocarbon adsorped at pH 4.0, while pyrene (0.057 µg/g) was the least adsorped polycyclic aromatic hydrocarbon overall at pH 6.0. In ascending the pH from 2.0 to 6.0, there wasn't any trend observed in PAH degradation, though, it was noted that polycyclic aromatic hydrocarbons degradation increased from pH 2.0 to 4.0, and then, sharply decreased at pH 6.0. This results thus lend credence to the fact that *E. crassipes* is a potential phytoremediator in removing polycyclic aromatic hydrocarbons from contaminated waste streams.

Keywords: accumulation, anthracene, contamination, petroleum, plants, pollution, uptake, rhizoremediation

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are fused aromatic ring compounds that have been reported to pose great risk to the environment and human health due to their mutagenic, teratogenic and carcinogenic effects (Ukiwe, Egereonu, & Nwoko, 2012a) and (Ukiwe & Egereonu, 2012b). PAHs could be produced mainly from incomplete combustion of organic materials, during discharge of petroleum products or from biological transformation precursors. The efficiency of plants to adsorp PAHs from contaminated systems have been documented (Haritash & Kaushik, 2009). Phytoremediation has become one of the most effective practical approaches used to remove PAHs from polluted substrates. Applying this approach involve two fundamental processes responsible for PAHs transfer and distribution in plants tissues. These processes include;

- 1) That transfer between plant tissues and cells is driven by PAHs concentration gradient across plant cell components (Providenti, Lee, & Trevors, 1993).
- 2) That accumulation of PAHs in plant tissues is a function of plant lipid content (Providenti et al., 1993).

Plant uptake of PAHs from polluted media is primarily through the roots and secondarily through leaves. Adsorption from water and soil via plant roots is a major pathway of polycyclic aromatic hydrocarbon (PAH) entry into plants. Rhizoremediation is an aspect of phytoremediation involving the breakdown of contaminants in soil due to activities by microbes in the presence of the plant root zone (rhizosphere) (Gaskin, Soole, & Bentham, 2008). Plant root exudates also help to enhance rhizoremediation by stimulating the growth of microorganism in the soil/water media which further degrade pollutants in the plant rhizosphere. Evidence abound showing that selection of plants for rhizoremediation depend on several factors namely; the general plant type, pH, temperature, microbial population (Frick, Farrel, & Germida, 1999; Hutchinson, Schwab, & Banks, 2003), and variations in plant morphology and physiology (Pichtel & Liskanen, 2001). Grasses and leguminous plants are candidate species for effective rhizoremediation (Adam & Duncan, 2003). For a plant to qualify as candidate rhizoremediator, it must posses highly branched, fibrous root system, it must also posses the ability to grow successfully in low nutrient systems, be native to remediation site as well as be tolerant to high hydrocarbon levels (Anderson, Guthrie, & Walton, 1993). Researches have indicated that tropical grasses show special applicability to rhizoremediation most especially since they are tolerant to drought and low nutrient availability (Aprill & Sims, 1990).

Various plants species have been identified for their ability to enable rhizoremediation of sites contaminated with petroleum hydrocarbon (Reilley, Banks, & Schwab, 1996). Recent studies include that of Qiu, Leland, Shah, and Sorensen (1997) and Gunther, Dornberger, and Fritsche (1996). Chen, Banks, and Schwab (2003) also suggested that tall fescue (*Festuca arundinacea*) and switch grass (*Panicum virgatum*) both could degrade about 1.6 and 8.7% pyrene in 190 days of incubation. In a related study, Chouychai et al. (2009) indicated that corn plant (*Zea mays*) has the ability to enhance the degradation of phenanthrene and pyrene in acidic soil inoculated with *Pseudomonas putide*. However, in conducting investigations into the capability of four plants species namely; *F. arundinacea*, ryegrass (*Lolium perenne*), alfalfa (*Medicago sativa*) and oil-rape seed (*Brassica napus*) grown alone and in combination to degrade phenanthrene and pyrene in spiked soil, Cheema et al. (2009) had noted higher degradation rates of PAHs in the combined plant cultivation (99% phenanthrene and 88% pyrene), compared to the single plant cultivation (97 and 79% of phenanthrene and pyrene), suggesting that enhanced rhizoremediation could be achieved using combined plant cultivation. As a viable clean up alternative for soils contaminated with petroleum products, Cofield, Schwab, and Banks (2007) evaluated the potential of *F. arundinacea*, *P. virgatum*, and zucchini (*Curcubita pepo*) in remediating soil from a gas plant. It was revealed that concentrations of PAHs decreased significantly in all treatments after 90 days. It has also been documented that PAHs degradation could be promoted in the rhizosphere of *F. arundinacea* with *Pseudomonas fluorescens* (Ho, Applegate, & Banks, 2007). Nevertheless, using native species for rhizoremediation, plants and their associated rhizosphere microbes can enhance PAH dissipation, hence, offering an economic and ecologically attractive remediation technique (Olson et al., 2007). However, according to Nedunuri, Lowell, and Meade (2010), for rhizoremediation to be more ecologically beneficial and cost-effective, it is advisable to allow contaminated sites to re-vegetate with the native grasses.

Water hyacinth (*Eichhornia crassipes*) can significantly accumulate petroleum hydrocarbons from contaminated waste substrates (Ndimele, 2010). The plant posses dense highly branched, fibrous root system, it floats on water, and has been shown to tolerate low nutrient concentration (Ukiwe & Ogukwe, 2007). *E. crassipes* has been tested by several researchers for its ability to adsorb aromatic hydrocarbons in effluents (Wolverton & Mckown, 1976; O'Keeffe, Wiese, Brummet, & Miller, 1987; Vaidyanathan, Kavadia, Rao, Basu, & Mahajan, 1983). These studies all demonstrated that the purification performance of the effluent improved by over 89% and that the root of the plant played a critical role in the rhizoremediation process. Xia (2008) also examined the effectiveness of *E. crassipes* in removal of dicofol in contaminated waters. The study concluded that the plant was very effective in the phytoremediation process, accumulating about 50% of dicofol in 7 days demonstrating the fact that the plant may thus be a viable candidate for development as a phytoremediator for systems contaminated by organics.

The present study investigates the effectiveness of *E. crassipes* in adsorbing PAHs from contaminated soil leachate.

2. Materials and Method

2.1 Preparation of Stock Solution

The soil sample used in this experiment was obtained from the commercial bus park of the Federal University of Technology, Owerri, Nigeria in April, 2011, while the *E. crassipes* plant also used in the present experiment was harvested from Enyioha farms in Imerienwe, Ngor Okpala LGA, Nigeria, in March, 2012 and identified Dr C.E. Ihejirika, of the Department of Environmental Technology, Federal University of Technology, Owerri, Nigeria. The plant specimen number was given as; EVS 00100029. Stratification sampling method was used to select the sample site, while random sampling was then applied within each stratification sub-groups to improve representativeness of the sample. Soil pre-treatment included sieving to select particle size of 10 mm and air dried for 48 hrs (Valentin et al., 2007). Obtaining this particle size was important to facilitate contact between soil and contaminant as well as organic solvent to be used for extraction.

The soil sample was autoclaved for 1hr at 110°C. About 5 kg of the dried soil sample was placed into a 25 L plastic bucket previously washed and rinsed with deionized water. About 5 L of waste engine oil was measured and poured into the 25 L plastic bucket and the entire mixture was mixed thoroughly. It has been reported that waste engine oil contain appreciable amount of PAHs (Carmichael, Jacob, Grimmer, & Phillips, 1990; Wong & Wang, 2001). About 5 L of n-hexane was further added to the triturated mixture and further trituration performed for another 10 mins. About 10 L of distilled water was then added to the mixture and the entire content stirred for another 10 mins and allowed to stand for 2 hrs. The supernatant was decanted and filtered through white cheese cloth and then through Whatman No. 42 filter papers. About 10 L of this filtrate was reserved to serve as the stock solution.

2.2 Preparation of *E. Crassipes* Samples

The choice of *E. crassipes* for this experiment is based on the following desirable criteria;

- 1) Dense aggressive and fibrous root system for maximum surface area to trap hydrophobic contaminants (Ukiwe & Oguke, 2007).
- 2) Dense coverage of contaminated site (Ukiwe & Aneke, 2010).
- 3) High hydrocarbon tolerance (Ukiwe, Oguzie, & Ajaero, 2008a).
- 4) Ability to tolerate low nutrient availability (Ukiwe, Nwoko, & Enenebeaku, 2008b).

About four *E. crassipes* plants were potted in four 2 L plastic sample holder vessels previously washed and rinsed with distilled water. The potted plants were prepared and placed in the Project Laboratory of the Department of Chemistry, Federal University of Technology, Owerri, Nigeria. To each of three of the potted plants was added 500 ml of distilled water and 500 ml each of the stock solution and the mixture triturated for 5 mins and the pH of the mixture in the three potted plants maintained each at 2.0, 4.0, 6.0, respectively using 1M NaOH/HCl as appropriate. These pH levels were maintained for two weeks. After this period, the plants were separately harvested, cut into tiny pieces, dried and mashed separately in a porcelain mortar. The resultant substance in each of the three potted plants was separately placed in six 500 ml beakers and 50 ml each of n-hexane solution was poured separately into the three beakers. The mixtures were separately triturated for 10 mins and filtered separately through clean cheese cloth and Whatman No. 42 filter papers into another three 500 ml beakers. The filtrates were then concentrated to 2 ml in a water bath at 40°C. A Hewlett-Packard GC 6890 series 11 was used with an FID and a Hewlett-Packard HP-5 column (length, 10 m; inner diameter (ID), 0.25 mm; film thickness, 0.25 µm). Methylene chloride was used for column preparation. The carrier gas was helium at a pressure of 7.5 lb/inch and a flow rate of 2.5 ml/min. The temperature program was as follows: 40°C for 3 min, 40°C /min to 300°C, hold for 10 min. The injector and detector temperature were 290 and 300°C respectively. Samples of 2 µl were injected in splitless mode for 1 min. Three replicate treatments each in each of the six beakers were processed and the mean PAHs concentration determined separately. The fourth potted plant was processed as in above without adding the stock solution. This last experiment was the control which enabled us to know if originally there were any PAHs present in the *E. crassipes* plant.

2.3 Statistical Analysis

Data is presented as arithmetic mean and standard deviation. The one-way Analysis of Variance (ANOVA) was employed to determine the difference in PAHs concentration between pH levels. The *F-test* was used to estimate significant difference in mean PAHs concentration between pH levels.

3. Results

Table 1. Values of PAHs in µg/g of water hyacinth extract at various pH range

PAHs	pH 2.0 ⁽ⁱ⁾	pH 4.0 ⁽ⁱⁱ⁾	pH 6.0 ⁽ⁱⁱⁱ⁾	Control
	Mean (µg/g) ± SD	Mean (µg/g) ± SD	Mean (µg/g) ± SD	Mean (µg/g) ± SD
Naphthalene	7.686 ± 0.2	8.350 ± 0.1	4.343 ± 0.2	ND
2-methyl naphthalene	6.384 ± 0.1	6.614 ± 0.2	4.202 ± 0.1	ND
Acenaphthylene	1.336 ± 0.3	1.416 ± 0.1	1.404 ± 0.1	ND
Acenaphthrene	1.214 ± 0.0	1.362 ± 0.1	1.326 ± 0.1	ND
Fluorene	1.126 ± 0.1	1.282 ± 0.1	1.312 ± 0.1	ND
Phenanthrene	1.215 ± 0.1	1.462 ± 0.2	1.252 ± 0.1	ND
Anthracene	1.184 ± 0.2	1.282 ± 0.1	1.388 ± 0.2	ND
Fluoranthrene	1.131 ± 0.2	1.286 ± 0.2	0.221 ± 0.0	ND
Pyrene	1.001 ± 0.1	1.105 ± 0.0	0.057 ± 0.0	ND

F-test i and ii = 1.18; Not significantly different [standard deviation of nth (8) samples; n = 8 and 8] at p < 0.05
ND = Not Detected

4. Discussion

In the present study, two rings PAHs were mostly degraded than three ring PAHs compounds. Naphthalene (8.350 µg/g) was the overall highest degraded PAH at pH 4.0, while pyrene (0.057 µg/g) was the overall least degraded PAH at pH 6.0 (Table 1). No significant trend was observed in total PAHs degradation as pH was

exceeded from pH 2.0 to 6.0. However, an increase in PAHs degradation was observed as the pH was increased from 2.0 to 4.0, and reduced as the pH increased from 4.0 to 6.0. (Table 1). According to Nesterenko, Kirzher, Zimmels, and Armon (2012), *E. crassipes* devoid of rhizospheric bacteria could reduce about 45% naphthalene in waste water in 7 days. Nevertheless, the present study agrees with that claim though noting that *E. crassipes* could only reduce about 5% naphthalene within the same time frame.

Direct degradation of petroleum hydrocarbon by plants have been studied by many authors including Frick et al. (1999) and Durmishidze (1977) who examined the ability of poplar trees to assimilate n-alkanes and liberate $^{14}\text{CO}_2$. However, evidence has been accumulating on indirect degradation of hydrocarbons by plants. Gunther et al. (1996) indicated that exudates from root of plants enable rhizosphere co-metabolic degradation of petroleum hydrocarbons. In further studies, Wolverton and Mckown (1996) showed that one hectare of the plant is potentially capable of removing about 160 kg of petroleum hydrocarbon from polluted water in about 4 days. Depending on the value of chemical oxygen demand (COD) of waste waters, it has been shown that *E. crassipes* could improve the quality of oil-refinery waste water by decreasing petroleum hydrocarbons by about 18% (Tang & Lu, 1993). The study pointed out that when the COD of the waste water was below 131 mg/l. *E. crassipes* degraded several hydrocarbons through adsorption and uptake of the hydrocarbon compounds leading to improved and transparent water. In an attempt to investigate the removal of phenol using *E. crassipes* in the presence of trace metals, Nor (1994) demonstrated that the plant has tremendous capability to adsorb phenolic compounds as well as heavy metals simultaneously. The study noted that bioassays involving filtered *E. crassipes* root extract revealed that phenol was removed by the aqueous root extract indicating involvement of cellular constituents of roots of the plant in the adsorption process. Della Greca, Previtera, and Zarrelli (2009) extensively documented evidence showing that certain structures of the PAHs compounds are involved in the ability of *E. crassipes* to adsorb PAHs from contaminated substrates. Two compounds identified as methyl derivatives of phenyl naphthalenedicarboxylic acid and phenyl naphthalenecarboxylic acid were linked to a phenylphenylene unit isolated from extracts of the plant. The study further suggested that given the chemistry of these compounds, they may have enabled the plant to adsorb hydrocarbons from waste streams. To the extent that certain factors (temperature, pH, redox potential) are applied within acceptable limits, will the plant be able to adsorb petroleum hydrocarbons from waste systems. However, recent advances have suggested that the adsorption of hydrocarbons by *E. crassipes* is dominated by Van Der Waals forces (Lin & Zheng, 2003), hence, the plant has capability to adsorb other compounds including inorganic nutrients and pesticides (Reddy, Agami, & Turker, 1989; Xia & Ma, 2006; Rosas, Carbajal, Gómez, Belmont, & Villalobos, 1984; Ebel, Evangelou, & Schaeffer, 2007; Ghabbour, Davies, Lam, & Vozzella, 2004). Reddy et al. (1989), Rosas et al. (1984) and Ebel et al. (2007) all investigated the adsorption of phosphorus, potassium and cyanide by *E. crassipes*, while Ghabbour et al. (2004) noted that the presence of humic acids in the leaves, stem and root of the plant is related to the binding properties of these acids, while Xia and Ma (2006) also examined the ability of the plant to adsorb organo-pesticides and concluded that *E. crassipes* has the ability to adsorb about 81% of ethion in one week using its roots.

Despite these recognized studies, the present study however notes that studies relating to PAHs adsorption by *E. crassipes* are rare.

5. Conclusion

Phytoremediation using *E. crassipes* was performed in degrading PAHs from contaminated soil leachate. Naphthalene and 2-methylnaphthalene were highly degraded by the plant, while pyrene and fluoranthrene were least degraded, showing that *E. crassipes* is effective as candidate plant in removing PAHs from contaminated streams. The present study thus agrees with a growing body of literature beginning to accumulate over the years that two rings PAHs are easily degraded by plants species than three and above ring PAHs.

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