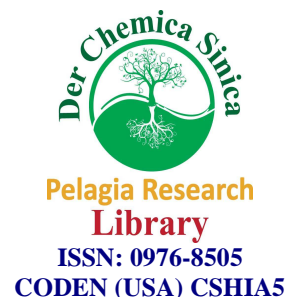




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### The Role of pH in Nitrate-reducing Conditions in Degradation of Polycyclic Aromatic Hydrocarbons in Contaminated Soil Extract

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#### ABSTRACT

Degradation of polycyclic aromatic hydrocarbons (PAHs) was investigated by applying different  $\text{NaNO}_3$  mass at varying pH levels (2.0, 4.0, 6.0 and 8.0) to contaminated soil extract. Results obtained indicated that using 2 g  $\text{NaNO}_3$ , 31.1% of 2-methylnaphthalene was regarded as the overall highest PAH degraded (pH 6.0), while 1.4% anthracene at pH 2.0 was the overall lowest degraded PAH. However, using 4 g  $\text{NaNO}_3$ , 20% of 2-methylnaphthalene was degraded as the highest PAH at pH 6.0, while 1.0% of acenaphthalene (pH 2.0) was observed as the least degraded PAH. A general increase in trend in PAH degradation was noted using 2 g  $\text{NaNO}_3$  as the pH was increased from 2.0 to 6.0, though, at pH 8.0 there was a remarkable decrease in PAHs degradation. The trend in PAHs degradation observed using 2 g  $\text{NaNO}_3$  was replicated using 4 g  $\text{NaNO}_3$  except for phenanthrene, fluoranthrene and pyrene. It was also found that increasing the mass of  $\text{NaNO}_3$  from 2 to 4 g didn't have any positive impact on the rate of degradation of PAHs.

**Keywords:** acenaphthene, anaerobic, bioavailability, contaminant, compounds, environment, sorption.

#### INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are significantly products of incomplete combustion of fossil fuels [1]. While PAHs are produced from various combustion sources, it has been reported that elevated levels are mostly found in cities and urban areas inferring that PAHs levels in the environment is a function of population density [2]. These compounds enter the environment either adsorbed onto soil particles by emissions from combustion processes or from spilling of mineral or tar oils. PAHs frequently adhere to carbon particles in the soil and dust or the or the lipophilic surfaces of vegetation. The importance of black carbon to sorption of native PAHs in harbor sediments has been studied by Lohmann et al., [3]. It was observed that sorption of PAHs to the sedimentary black carbon phase dominated the solid-water partitioning of the PAHs. Adsorption of hydrophobic organic compounds such as PAHs by carbon nanomaterials may enhance their toxicity and affect the fate, transformation, and transport of PAHs in the environment [4]. For different PAHs tested, adsorption appears to relate with their molecular size, i.e., the larger the molecular size, the lower the adsorbed volume capacity. However, it was noted that high adsorption capacity of PAHs by carbon nanotubes may add to their high environmental risks and once released to the environment, may result in potential alteration of PAHs fate and bioavailability in the environment. Another study to assess the sorption of PAHs to carbonaceous materials indicated that accessibility of indigenous background PAHs was reduced when coal and coal-derived particles were associated with the contaminated soil substrate [5]. Polyoxymethylene (POM) is a polymer material that has been used successfully in passive sampling of hydrophobic organic contaminants such as PAHs in soil and water sediments. A study examining the sorption behavior of 12

PAHs compounds on to POM revealed that the uptake rates for PAHs were controlled by the POM phase and the hydrophobicity of PAHs compounds. In this regard, POM partitioning technique was demonstrated to agree well with procedures for measuring polycyclic aromatic hydrocarbon (PAH) soil-water distribution coefficients in oil-soot (lampblack) containing soil samples [6]. There is strong field evidence suggesting colloid-facilitated transport of hydrophobic organic compounds. Mackay & Gschwend [7] had noted that excess pyrene concentrations are associated with colloids that passed an ultrafilter at ambient pH (< 1).

Nitrate-rich surface waters are good systems to examine direct and indirect photolysis of PAHs. Direct photolysis of PAHs has been observed under simulated solar radiation with pyrene degrading at a faster rate than phenanthrene and naphthalene [8]. However, while Kulik *et al.*, [9] had used combined pre-oxidation and bioremediation to illustrate degradation of PAHs in contaminated soils, other authors [10],[11] have attempted various PAHs degradation protocols in soils. Bertilsson & Widenfalk[10], researched photochemical degradation where solar ultraviolet radiation was used to degrade and alter the quality of PAHs, while Manariotis *et al.*, [11], recently investigated high frequency ultrasound as a possible index to be employed successfully to degrade phenanthrene, naphthalene and pyrene. Electrochemical reactors deployed by Alshawabkeh & Sarahney [12] were used to evaluate the effect of current densities on the transformation of naphthalene. It was shown that most of naphthalene degradation occurred in the first 4 hrs under applied currents of between 9 and 18.2 mA/L. However, recent published data by Zhang *et al.*, [13] has revealed photodegradation of pyrene in soil using ultraviolet light irradiation under the influence of temperature, soil particle size, soil depth, and humic acid concentration. Nevertheless, other operating conditions such as liquid phase temperature, applied power, ultrasound frequency, and presence of matrix components have been found to impart on the sonochemical degradation of naphthalene, acenaphthylene, and phenanthrene in aqueous systems [14]. The study noted that addition of 1-butanol substantially suppressed degradation of the PAH compounds while addition of Fe<sup>2+</sup> ions at low concentrations enhanced degradation through a Fenton-like reaction.

As early as the eighties, evidence have been accumulating suggesting that PAHs especially naphthalene and acenaphthene could be degraded under nitrate-excess conditions [15]. Several authors including Rochne *et al.*, [16] also lent credence to these findings. However, these investigators are of the opinion that several factors do influence the probability and rate of PAHs degradation. Chiefly, among these factors is the pH of the contaminated substrate. In the light of this development, the present study aimed at evaluating the effect of pH on nitrate-reducing conditions in PAHs degradation in contaminated soil extract.

## MATERIALS AND METHODS

The soil sample used in this experiment was obtained from the commercial bus park of the Federal University of Technology, Owerri, Nigeria. Soil pre-treatment include sieving to select particle size of 10 mm and air dried for 48 hrs [17]. Obtaining this particle size is important to facilitate contact between soil and contaminant as well as organic solvent used for extraction. The soil sample was dried in an autoclave (Ac 064) for 30 mins at 105 °C. About 5 kg of the dried soil sample was placed into a 25 L plastic bucket previously washed and rinsed with deionised water. About 500 g of the dried soil sample was also separately weighed and placed in a 1 L beaker. This sample in the 1 L beaker was used for the control experiment.

About 5 L of waste automobile engine oil was poured into the soil sample in the 25 L plastic bucket. It has been previously reported that waste automobile engine oil contain 2-6 ring PAH compounds [18],[19]. The mixture was triturated for 10 mins and 5 L of n-hexane solution was added to the triturated mixture and further trituration was performed for another 10 mins. About 10 L of distilled water was then added to the mixture and the content was stirred for a further 10 mins and allowed to stand for 2 hrs. The supernatant was decanted and filtered through Whatman No. 42 filter papers. About 9 L of the filtrate was recovered to serve as the stock solution.

To 100 ml of the stock solution in a 250 ml beaker was added 2 g of NaNO<sub>3</sub> powder. The mixture was stirred for 2 mins and the pH of the mixture was adjusted to 2.0 using 1 M NaOH/HCl as appropriate and filtered. The filtrate was then transferred into a 500 ml separatory funnel and 500 ml of n-hexane solution was further added. The mixture was shaken for 2 mins with periodic venting to release excess pressure. The organic layer was allowed to separate from the water phase and collected through a funnel containing solvent-moistened filter paper containing anhydrous sodium sulphate into a 1 L Erlenmeyer flask. The solvent was evaporated on a water bath at 110 °C to 10 ml and concentrated with a stream of nitrogen gas to 2 ml.

PAHs concentration in the extract was determined using Gas Chromatography/Mass Spectroscopy (GC/MS), Shimadzu QP2010 GCMS, Japan, at flow rate 1.18 ml/min with a helium carrier gas, column oven temperature was ramped at 80 to 280 °C at 5 °C/min with 5 min holding time, then to 300 °C at 10 °C/min with 10 min holding time. HP5MS column (30m x 0.25µm x 0.25 mm ID) was used. Three replicate sample treatments were processed and mean PAHs concentration was obtained.

The above procedure was repeated at pH 4.0, 6.0, and 8.0. However, the procedure was also repeated at the above pH range using 4 g NaNO<sub>3</sub>.

The control sample was also processed as in above at pH 2.0 without addition of NaNO<sub>3</sub>.

Physicochemical characteristics of the soil substrate included the following tests; pH, organic matter, trace elements, temperature, bulk density, and soil texture. Analyses of physicochemical properties were performed according to methods described in USAID [20]. The values are shown in **Table 3**.

### 3.0 Statistical Analysis

Data are given as arithmetic mean and standard deviation. The *F*-test was used to estimate significant difference in mean PAHs concentration between pH level (2.0) and control experiment in both 2 and 4 g NaNO<sub>3</sub>.

## RESULTS AND DISCUSSION

PAHs vary widely in molecular structure ranging from two-rings low molecular weight (LMW) naphthalene to six-rings high molecular weight (HMW) coronene compounds. They are surrounded by dense clouds of  $\pi$  (Pi) electrons and this resistant to nucleophilic attack. Certain physical properties act against PAHs microbial utilization or degradation, including their low aqueous solubility and high solid-water distribution ratios [21]. The bioavailability of PAHs is believed to decrease almost logarithmically with increasing molecular mass [22]. LMW PAHs tend to be absorbed directly through plant cuticles whereas HMW PAHs adhere to surface soil [22], while decaying organic matter provides microcrevices for concentrated exposure for soil microorganisms. However, some high order PAHs are not accessible to microbes as food due to their absorption inside organic particles or location in small pores that are inaccessible for bacteria [21]. Biofilm formation on PAH-containing sorbents has been noted as an efficient way of increasing the PAH flux to biofilm cells [21]. Biofilm cells on surface soil craters make the PAH compound more bioavailable. The degree to which these cells accelerate degradation is unclear [21]. However, the addition of nitrogen containing soil amendments has been described as the best way to facilitate the growth of biofilm cells given supportive soil pH [21].

**Table 1** give values of PAHs in µg/g of soil extract using 2 g NaNO<sub>3</sub> at pH 2.0, 4.0, 6.0, and 8.0 respectively. 2-methylnaphthalene (31.1%) was observed as the highest PAH degraded at pH 6.0, while anthracene (1.4%) was the least degraded PAH at pH 2.0. It was generally noted that PAHs degradation followed an increasing trend as the pH was increased from 2.0 to 6.0, but PAHs degradation dropped sharply at pH 8.0. Degradation of PAHs was statistically significant when compared with the control experiment since  $P > 0.05$  at 8 and 8 degree of freedom (*F*-test = 21.4). **Table 2** also shows values of PAHs degradation in µg/g of soil extract using 4 g NaNO<sub>3</sub> at the previously mentioned pH range. About 20% of 2-methylnaphthalene was again noted as the highest degraded PAH at pH 6.0, while 1.0% of acenaphthylene at pH 2.0 was observed as the lowest degraded PAH. Just like the experiment conducted using 2 g NaNO<sub>3</sub>, PAH degradation did follow an increasing trend as the pH was increased from 2.0 to 6.0 except for phenanthrene, fluoranthrene, and pyrene respectively. It is not clear if the lack of degradation trend in PAH in these three compounds is associated with the increase in NaNO<sub>3</sub> mass from 2 to 4 g, it was however noted that increasing the mass of NaNO<sub>3</sub> from 2 to 4 g didn't actually have any positive impact on PAHs degradation. As in 2 g above, at pH 8.0, using 4 g NaNO<sub>3</sub> PAHs degradation had a sharp decrease in trend. Significant statistical amount of PAHs degradation was also observed across pH levels using 4 g NaNO<sub>3</sub> as compared to control experiment since at pH 2.0, the *F*-test value (38.0) when tested at 8 and 8 degree of freedom ( $P > 0.05$ ).

It has been documented that denitrifying conditions are responsible for remarkable increase in degradation rates of acenaphthylene, acenaphthene, fluorene, and anthracene [23]. Nitrates have a high aqueous solubility than oxygen, and can be added in the form of nitrate salts to enhance bioremediation of contaminated soils [24]. Though contributions from researchers have indicated that nitrites could be toxic to nitrate reducing bacteria, thus limiting

the effectiveness of nitrate-reduction in sub-surface soil remediation [24]. For anaerobic processes, PAHs degradation coupled to nitrate reduction has been documented, although in this regard, nitrate concentration has been reported to be typically low [25]. To enhance biodegradation rate of PAHs under anaerobic conditions, sediment samples could be amended with biostimulating agents alone or in combination with nitrogen in the form of slow-release fertilizer [26]. Genthner *et al.*, [27], in a related study, noted that nitrate and sulphate concentrations in the 15-20 mM range are required to biodegrade 2 or 3-ring PAHs in anaerobic sediments. To evaluate chemical degradation of hydrophobic organic compounds in the presence of manganese oxide, pH of the soil, solute concentration, the concentration of the oxide suspension as well as the suspension age have been demonstrated to affect the rate of the reaction [28]. Denitrification and low temperature have been employed successfully in PAHs degradation [29]. It was revealed that for 2-methylnaphthalene and fluorene, degradation was coupled to denitrification on the basis of stoichiometry removal ratio of the PAH compounds and nitrate amount. Previous studies demonstrating the biodegradation of hydrophobic organic compounds under nitrate-reducing conditions have been reported by Dou & Liu [30], and Dou *et al.*, [31]. However, effective biodegradation of benzene series compounds under nitrate and sulphate-reducing conditions by mixed bacterial consortium enriched from a gasoline contaminated soil has been investigated [30]. It was however evident that under these reducing conditions the degradation rates of most benzene series compounds decreased. Nitrate was documented as a more favorable electron acceptor compared to sulphate in degradation action.

**Table 1: Values of PAHs in µg/g of soil extract using 2 g NaNO<sub>3</sub> at various pH range**

PAHs	pH 2.0 <sup>(i)</sup>	pH 4.0 <sup>(ii)</sup>	pH 6.0 <sup>(iii)</sup>	pH 8.0 <sup>(iv)</sup>	Control <sup>(v)</sup>
	Mean (µg/g) ± SD	Mean (µg/g) ± SD	Mean (µg/g) ± SD	Mean (µg/g) ± SD	Mean (µg/g) ± SD
Naphthalene	20.687 ± 0.3	35.975 ± 0.4	38.775 ± 0.5	10.814 ± 0.5	143.9 ± 0.1
2-methyl naphthalene	16.974 ± 0.1	29.901 ± 0.2	29.945 ± 0.1	9.078 ± 0.3	96.41 ± 1.2
Acenaphthylene	0.236 ± 0.1	0.389 ± 0.2	0.395 ± 0.1	0.332 ± 0.3	12.98 ± 1.2
Acenaphthrene	0.226 ± 0.2	0.283 ± 0.1	0.379 ± 0.1	0.216 ± 0.1	10.66 ± 0.1
Fluorene	0.333 ± 0.1	0.573 ± 0.2	0.580 ± 0.2	0.432 ± 0.1	6.937 ± 0.3
Phenanthrene	0.756 ± 0.1	1.321 ± 0.2	1.363 ± 0.4	0.564 ± 0.1	5.993 ± 1.1
Anthracene	0.097 ± 0.0	0.147 ± 0.1	0.146 ± 0.2	0.482 ± 0.1	6.728 ± 1.4
Fluoranthrene	0.141 ± 0.2	0.203 ± 0.2	0.215 ± 0.1	0.204 ± 0.1	3.625 ± 1.4
Pyrene	0.199 ± 0.5	0.269 ± 0.1	0.288 ± 0.1	0.212 ± 0.2	4.921 ± 0.6

F-test; i and v = 21.4

**Table 2: Values of PAHs in µg/g of soil leachate using 4 g NaNO<sub>3</sub> at various pH range**

PAHs	pH 2.0 <sup>(a)</sup>	pH 4.0 <sup>(b)</sup>	pH 6.0 <sup>(c)</sup>	pH 8.0 <sup>(d)</sup>	Control <sup>(e)</sup>
	Mean (µg/g) ± SD	Mean (µg/g) ± SD	Mean (µg/g) ± SD	Mean (µg/g) ± SD	Mean (µg/g) ± SD
Naphthalene	9.589 ± 0.2	22.894 ± 0.4	23.088 ± 0.7	10.254 ± 0.1	143.9 ± 0.1
2-methyl naphthalene	8.808 ± 0.3	17.061 ± 0.5	19.265 ± 0.2	7.792 ± 0.3	96.41 ± 1.2
Acenaphthylene	0.132 ± 0.2	0.226 ± 0.2	0.252 ± 0.1	0.133 ± 0.1	12.98 ± 1.2
Acenaphthrene	0.136 ± 0.1	0.165 ± 0.1	0.236 ± 0.1	0.117 ± 0.1	10.66 ± 0.1
Fluorene	0.181 ± 0.1	0.310 ± 0.1	0.351 ± 0.1	0.268 ± 0.1	6.937 ± 0.3
Phenanthrene	0.860 ± 0.1	0.731 ± 0.2	0.822 ± 0.2	0.472 ± 0.2	5.993 ± 1.1
Anthracene	0.085 ± 0.0	0.097 ± 0.0	0.104 ± 0.1	0.103 ± 0.0	6.728 ± 1.4
Fluoranthrene	0.153 ± 0.1	0.138 ± 0.1	0.142 ± 0.1	0.136 ± 0.1	3.625 ± 1.4
Pyrene	0.135 ± 0.1	0.175 ± 0.2	0.125 ± 0.0	0.110 ± 0.0	4.921 ± 0.6

F-test; a and e = 38.0

**Table 3: Physicochemical characteristics of soil sample.**

S/N	Parameters	Values
1	Ph	5.91
2	Organic matter (%)	0.55
3	Mg <sup>2+</sup> (mg/100g)	19.17
4	K <sup>+</sup> (mg/100g)	0.07
5	Ca <sup>2+</sup> mg/100g)	17.06
6	Temperature (°C)	29.0
7	Bulk density (g/cm <sup>3</sup> )	1.50
8	Soil texture (mm)	10

**CONCLUSION**

It is hoped that the results obtained in the present study would contribute to the development of new approaches and research in factors affecting chemical degradation of PAHs in contaminated soil substrates.

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