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Bacterial Communities Utilizing Naphthalene in Soil Contaminated by Auto-Mechanic Waste: A Study in Agbor, Delta State

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ABSTRACT

Naphthalene, a primary polycyclic aromatic hydrocarbon, represents a significant pollutant in petroleum contaminated soils. This study investigated the abundance of naphthalene-utilizing bacteria in soils contaminated with automobile waste in Agbor, Delta State. Soil samples were collected from automobile workshops located in Agbor, specifically Orubor, Memeh, and Odim, utilizing a soil auger. Enumeration and characterization of naphthalene-utilizing bacterial populations were conducted employing standard microbiological methods. During both dry and wet seasons, the total heterotrophic bacterial counts was between 0.5-2.25 × 10^6 CFU/g and 0 - 9.20 × 10^6 CFU/g, respectively. Meanwhile, the total counts of naphthalene-utilizing bacteria varied between 0- to 0.73 × 10^5 CFU/g and 0.019 - 1.47 × 10^6 CFU/g respectively. Although the total heterotrophic bacterial counts generally exceeded those of naphthalene-utilizing bacteria, the difference was not statistically significant across most locations. The genera of naphthalene-utilizing bacteria identified in the automobile-contaminated soil encompassed Pseudomonas, Serratia, and Enterococcus. Notably, Pseudomonas species predominated in all sampling locations. This study underscores the presence of diverse naphthalene-utilizing bacterial communities in automobile-contaminated soil environments, with Pseudomonas species notably abundant. These findings suggested the potential of Pseudomonas species as a promising candidate for bioremediation strategies aimed at mitigating naphthalene contamination.

1.0 INTRODUCTION

The major Polycyclic Aromatic Hydrocarbons (PAHs) compounds of environmental significance include acenaphthene, acenaphthylene, anthracene, benz (a) anthracene, benzo (a) pyrene, benzo (b) flouranthene, benzo (g, h, i) perylene, benzo (k) flouranthene, chrysene, dibenz (a,h) anthracene, flouranthene, flourene, indeno(1,2,3-c,d)pyrene, naphthalene, phenanthrene and pyrene. (USEPA, 2001). Among these compounds, naphthalene is the simplest and most studied PAH and is generally considered as a vital chemical model for PAH's degradation (Karimi *et al.,* 2015). Naphthalene is one of the most important environmental PAH pollutants with genotoxic, carcinogenic and mutagenic characteristics and thus of public health significance (Aranda *et al*., 2009; Pawar*et al,* 2013; Karimi *et al.,* 2015). Studies reported that exposure to relatively large doses of naphthalene via food and water could lead to diverse health hazards such as hemolytic anemia, gastroenteritis, nephrotoxicity, cataract, dermatitis, malignant cell growths among others. Naphthalene binds covalently to molecules in the liver, kidney and lung tissues thereby enhancing its toxicity. It is also an inhibitor of mitochondrial respiration (Falahatpisheh *et al.,* 2001).

Literature review have shown that bacteria capable of degrading naphthalene and other PAHs can be isolated from hydrocarbon contaminated soils and water (Kim *et al.,* 2005; Kafilzadeh *et al.,* 2011; Pawar *et al*., 2013; Karimi *et al,* 2015; Fulekar, 2017). One of such hydrocarbon polluted soil environment is auto-mechanic workshops because spent oil from automobiles and serviced engine oil are constantly discharged thereby. Hence, this study was carried to investigate the naphthalene-utilizing bacterial population of auto-mobile waste contaminated soils in Agbor, Delta State.

2.0 Materials and methods

Study Area

The study was carried out in Agbor Delta State. Agbor is a semi-urban community located in the South- South region of Nigeria. It lies within Latitude $6^0 07^l$ and $6^0 20^l$N and longitudes 6^0 05 and $6^0 20^l$E and covers about 650 km^2. The population at the 2006 Census was 162,594. (Olobaniyi *et al* 2007: Edjere and Iyekowa 2017).

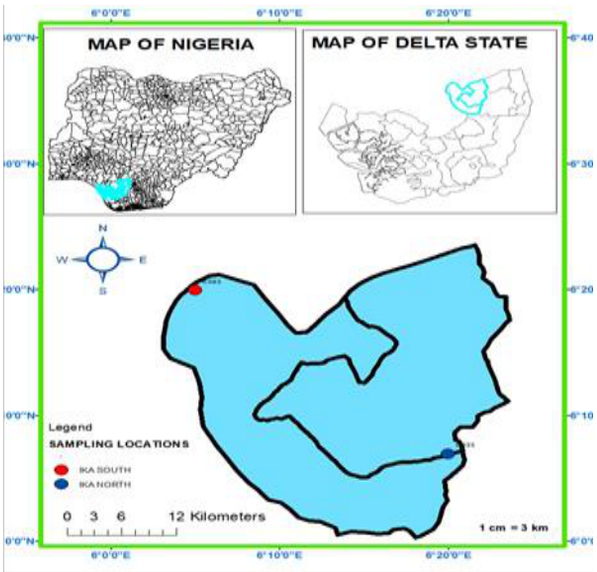

Figure 1: Map of sampling location
Source: Igborgbor *et al.,* 2022

Sample Collection

Top soils were collected randomly from three mechanic workshops in Agbor metropolis, Delta State. The mechanic workshops are aged above ten years and located in Orubor Street, Odim Street and Memeh Street. Samples were collected using improvised soil auger at a depth of 0 cm-5 cm, and 6 cm-10 cm as described by Raissa *et al* (2012). Samples were collected in both dry season and raining season. A total of thirty-six (36) samples were collected. Eighteen (18) samples in dry season and eighteen (18) samples in raining season. All the samples were transported in ice-packed containers to the laboratory for further analysis.

Enumeration and isolation of bacterial from the soil samples

One gram (1g) soil samples from automobile workshops were weighed into 10 ml of sterile distilled water as stock solution. Six flasks containing 9 ml each of sterile distilled water was used for the dilution. One milliliter of the initial dilution was introduced into the first 9 ml text tube to give 10^{-1} suspension up to 10^{-6} suspension. Aliquot of 1ml of the appropriate dilution from each contaminated soil was plated in nutrient agar for the isolation of bacteria. The nutrient agar plates were incubated at 37°C for 24 to 48 hours for the cultivation of heterotrophic bacteria while the Bushnell Haas agar was incubated at 37°C for 48 to 72 hours for the cultivation of naphthalene utilizing bacteria. After incubation, the number of discrete colonies was counted in colony forming units per gram (CFU/g). A single isolated colony of the visibly grown bacteria was picked with the help of sterilized wire loop and streaked on fresh nutrient agar medium. The nutrient agar plates were incubated at 37°C for 24 hours. The isolated and purified bacterial cultures were stored in already prepared slants in refrigerator at 4°C. Cultural, microscopic and biochemical characterization of bacterial isolates were carried out following standard microbiological methods described (Cheesbrough, 2000).

3.0 Data analysis

All analyses were carried out in triplicates and values were expressed as means with their standard deviations. Results were presented in tabular and graphical formats. Where necessary, data obtained were statistically analyzed using different analysis of variance (ANOVA) adopting probability levels below 5%. Differences in means were analyzed using the Duncan's Multiple Range Test (Paulson, 2008).

4.0 Results

The total heterotrophic bacteria counts of the soil samples from the Orubor automobile workshop in Agbor during the dry season was lowest (1.92×10^5 CFU/g) at point C_{6}-10 and highest (9.67×10^5 CFU/g) in point $A_{6\text{-}10}$. The total naphthalene utilizing bacterial count was zero at point $C_{6\text{-}10}$, while the highest (5.4×10^4CFU/g) was recorded at point $A_{6\text{-}10}$ (Fig. 2), while the total heterotrophic bacterial counts of the soil samples from Orubor automobile workshop in Agbor during the wet season was zero in point $C_{6\text{-}10}$ and highest (3.83×10^5 CFU/g) at point $C_{0\text{-}5}$. The total naphthalene utilizing bacterial counts was lowest (0.19×10^4 CFU/g) at point $B_{0\text{-}5}$ and highest (3.13×10^4 CFU/g) at point $C_{0\text{-}5}$ (Fig. 5).

The total heterotrophic bacterial load of the soil samples from Memeh automobile workshop in Agbor during the dry season was lowest (1.70×10^5 CFU/g) at point $D_{6\text{-}10}$ and highest (6.25×10^5 CFU/g) at point $D_{0\text{-}5}$. The total naphthalene utilizing bacterial counts was lowest (0.16×10^4 CFU/g) at point $D_{6\text{-}10}$ and highest (5.50×10^4 CFU/g) at point $D_{0\text{-}5}$ (Fig. 3), While the total heterotrophic bacterial counts of the soil samples from Memeh automobile workshop in Agbor during the wet season was lowest (2.27×10^5 CFU/g at point $F_{6\text{-}10}$ and highest (3.80×10^5 CFU/g) at point $E_{0\text{-}5}$. The total naphthalene utilizing bacterial counts was lowest (2.00×10^4 CFU/g) at point $F_{6\text{-}10}$ and highest (3.43×10^5 CFU/g) at point $F_{0\text{-}5}$. (Fig. 6).

The total heterotrophic bacterial counts of the soil samples from Odim automobile workshop in Agbor during the dry season was lowest (0.58×10^5 CFU/g) at point $H_{6\text{-}10}$ and highest (2.25×10^6 CFU/g) at point $C_{6\text{-}10}$. The total naphthalene utilizing bacterial counts was lowest (0.96×10^4 CFU/g) at point $1_{0\text{-}5}$ and highest (7.25×10^4 CFU/g) at point $H_{0\text{-}5}$. (Fig 4), while the total heterotrophic bacterial counts of the soil samples from Odim automobile workshop in Agbor, during the wet season revealed zero count for point $I_{6\text{-}10}$ and the highest count (9.20×10^6 CFU/m) at point $G_{6\text{-}10}$. And the total naphthalene utilizing bacterial counts was lowest (4.00×10^4 CFU/g) at point $I_{0\text{-}5}$ and highest (1.47×10^6 CFU/g) at point $H_{0\text{-}5}$ (Fig.7).

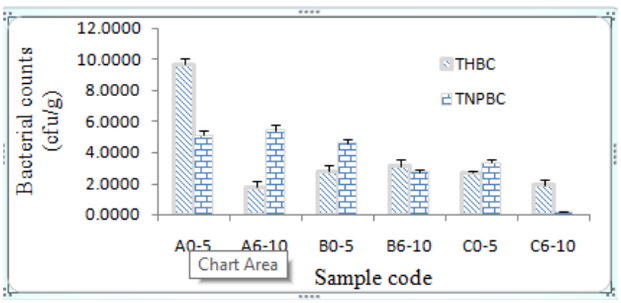

Fig.2: Total heterotrophic and total naphthalene utilizing bacterial counts of soils sampled during dry season from Orubor mechanic workshop, Agbor. Dilution factors THBC: × 10^5CFU/g, TNPBC ×10^4 CFU/g, n=3, values in mean ± standard error of mean

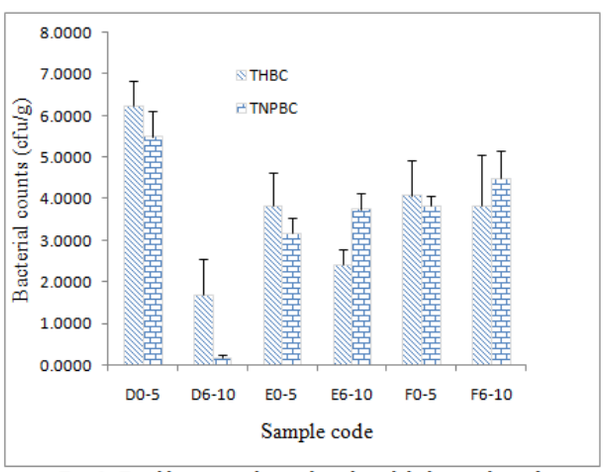

Fig. 3: Total heterotrophic and total naphthalene utilizing bacterial counts of soils sampled during dry season from Memeh mechanic workshop, Agbor. Dilution factors THBC: ×10^5CFU/g, TNPBC ×10^4 CFU/g, n=3, values in mean ± standard error of mean

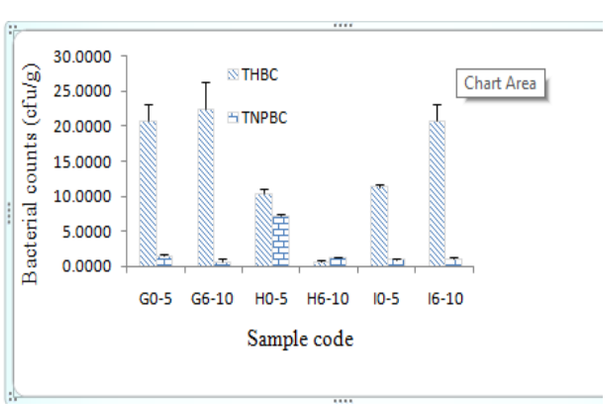

Fig. 4: Total heterotrophic and total naphthalene utilizing bacterial counts of soils sampled during **dry season** from **Odim** mechanic workshop, Agbor. Dilution factors THBC: ×10^5CFU/g, TNPBC ×10^4 CFU/g, n=3, values in mean ± standard error of mean

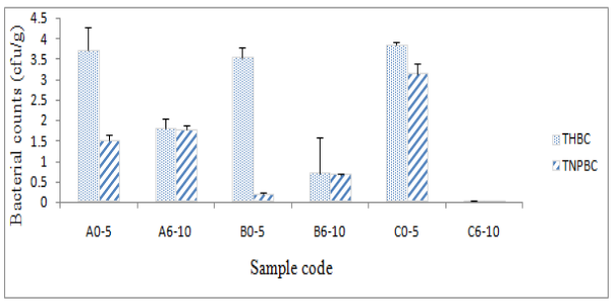

Fig. 5: Total heterotrophic and total naphthalene utilizing bacterial counts of soils sampled during wet season fromOrubormechanic workshop, Agbor. Dilution factors THBC: × 10^5 CFU/g, TNPBC ×10^4 CFU/g, n=3, values in mean ± standard error of mean

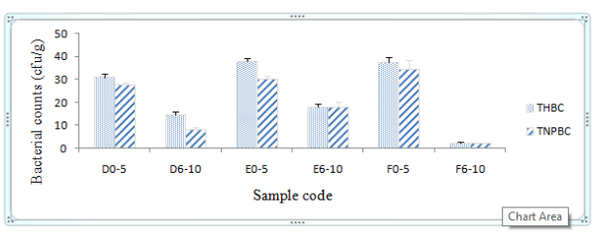

Fig.6: Total heterotrophic and total naphthalene utilizing bacterial counts of soils sampled during wetseasonfrom Memeh mechanic workshop, Agbor. Dilution factors THBC: × 10^5 ×10^5CFU/g, TNPBC ×10^4CFU/g, n=3, values in mean ± standard error of mean

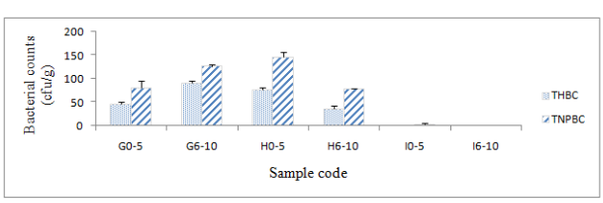

Fig. 7: Total heterotrophic and total naphthalene utilizing bacterial counts of soils sampled during wetseason fromOdimmechanic workshop, Agbor. Dilution factors THBC: ×10^5 CFU/g, TNPBC ×10^4 CFU/g, n=3, values in mean ± standard error of mean

Characterization of the bacterial isolates reveled that majority of them were recovered during wet season and belonged to the genera of *Pseudomonas, Serratia* and *Enterococcus* (Table 1a and 1b). Among the isolates, *Pseudomonas* species were the predominant naphthalene-utilizing bacteria recovered from the auto-mobile contaminated soil.

Table 1a. Phenotypic characterization of bacterial isolates

	A	B	C	D	E
Colour	Cream	Green	Green	Green	Cream
Elevation	Flat	Flat	Flat	Flat	Flat
Wet/dry	Wet	Wet	Wet	Wet	Wet
Gram stain	(-)	(-)	(-)	(-)	(-)
Shape	rod	Rod	Short rod	Short rod	Rod
Motility	(+)	(+)	(+)	(+)	(+)
Catalase	(+)	(+)	(+)	(+)	(+)
Oxidase	(+)	(+)	(+)	(+)	(+)
Indole	(-)	(-)	(-)	(-)	(-)
Urease	(-)	(-)	(-)	(-)	(-)
Citrate	(+)	(-)	(+)	(-)	+
VP	(-)	(-)	(-)	(-)	(-)
Centrimide	(+)	(+)	(+)	(+)	(-)
Lactose	(-)	(-)	(+)	(+)	(-)
Sucrose	(+)	(-)	(+)	(-)	(-)
Maltose	(+)	(+)	(+)	(+)	(+)
Galactose	(+)	(+)	(-)	(+)	(-)
Glucose	(+)	(+)	(+)	(+)	(+)
Suspected isolates	*Pseudomonas* sp	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Serratia* sp.

Table 1b. Phenotypic characterization of bacterial isolates

Parameter	F	G	O	P	Q
Colour	Green	Cream	Cream	Green	Brown
Elevation	Raised	Raised	Raised	Flat	Raised
Wet/dry	Wet	Wet	Dry	Wet	Wet
Gram stain	(-)	(-)	(-)	(-)	(+)
Shape	Rod	Short rod	Rod	Rod	Cocci
Motility	(+)	(+)	(+)	(+)	(+)
Catalase	(+)	(+)	(+)	(+)	(+)
Oxidase	(+)	(+)	(+)	(+)	(+)
Indole	(-)	(-)	(-)	(-)	(-)
Urease	(-)	(-)	(-)	(-)	(-)
Citrate	(+)	(-)	(-)	(-)	(+)
VP	(-)	(-)	(-)	(-)	(-)
Centrimide	(+)	(-)	(+)	(+)	(-)
Lactose	(-)	(+)	(+)	(+)	(-)
Sucrose	(+)	(+)	(+)	(-)	(+)
Maltose	(+)	(+)	(-)	(+)	(+)
Galactose	(-)	(+)	(+)	(+)	(-)
Glucose	(+)	(+)	(+)	(+)	(+)
Suspected isolates	*Providencia* sp.	*Serratia* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Enterococcus* sp.

Discussion

Microorganisms are found in all environments where hydrocarbons are present. They use the hydrocarbon as source of carbon and energy (Fulekar 2017). In this study, it was observed that hydrocarbon utilizing bacteria were abundant in soil contaminated by auto-mechanic wastes.The variations in the total heterotrophic bacterial counts and naphthalene-utilizing bacterial counts across different workshops and seasons suggest differences in microbial activity and pollution levels. (Aigberua *et al.,* 2016). Higher counts during the wet season, particularly in the Odim workshop, may indicate favourable conditions for microbial growth due to increased moisture. The presence of naphthalene-utilizing bacteria indicates the biodegradation potential of the microbial communities in response to hydrocarbon contamination, particularly from petroleum-based products often present in automobile workshops. These findings highlight the need for regular monitoring and potential bioremediation strategies to manage environmental pollution in such areas. The hydrocarbon, naphthalene was found to be utilized by most of the bacterial community under study. The presence of higher levels of naphthalene utilizing bacterial population in some of the locations suggested higher concentration of naphthalene degraders in the soils compared to other heterotrophs. This may be attributed to the adaptation of some of the indigenous bacteria to auto-mobile wastes which consist of crankcase oil and other petroleum hydrocarbons. The high population of naphthalene utilizers is also an indication that the soil contained high level of residual naphthalene and possibly other polycyclic aromatic hydrocarbons which accounted for their ability to proliferate in these workshop soils. These results agree with the reports of Ebakota *et al.* (2017) who reported higher oil degrading bacterial counts than heterotrophic bacterial counts and also reduction in both heterotrophic and oil degrading bacterial counts with increasing depths. This was further buttressed by Tirkey *et al*., (2021) who reported higher oil degrading bacteria populations and low heterotrophic bacterial counts which was attributed to stimulatory effect of additional carbon and energy source in form of automobile wastes (crankcase oil and other petroleum hydrocarbon components).

The genera of naphthalene-utilizing bacteria recovered from the auto-mobile contaminated soil included *Pseudomonas, Serratia* and *Enterococcus.* Among the isolates, *Pseudomonas* species were predominant in the samples. This finding is in consonance with previous studies who reported *Pseudomonas* sp. as versatile bacteria in hydrocarbon polluted systems (Raissa*et al*., 2012; Kumar *et al*., 2018: Ebakota *et al*., 2017; Tirkey *et al*., 2021). Hence, they could be responsible for the biodegradation of various hydrocarbons of engine oil and other PAH in polluted environments.

Conclusion

This study has provided compelling evidence demonstrating the diverse array of naphthalene-utilizing bacterial communities present within automobile-contaminated soil environments in Agbor, Delta State. Among these communities, *Pseudomonas* species emerged as particularly noteworthy due to their prevalence and adaptability in the naphthalene contaminated soils. The abundance of *Pseudomonas* species suggested their potential efficacy as a remediation tool for addressing environments polluted by naphthalene and related compounds. Also, this study has further shown that auto-mobile soil environment contained various naphthalene-utilizing bacterial communities, which could be considered as remediation tool for bioremediation of environments polluted by naphthalene and related compounds.

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