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# Biodegradation of Phenol Tainted Industrial Effluent by Exogenous Bacterial Isolates Bio-Mined from Crude Oil Polluted Soil

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## *Abstract*

**Phenol in industrial effluent is a major under-reported pollutant of concern to the aquatic ecosystem. In the current study, the effluent was obtained from Eleme Petrochemical Limited, Rivers State Nigeria. Baseline analyses were conducted to ascertain physico-chemical and microbiological parameters. Exogenous bacterial species were isolated from crude oil polluted in Ogoniland. The inoculum was standardized using the MacFarland approach. About 1% inoculum was seeded into the 100 mL effluent for the *Pseudomonas aeruginosa*, *Micrococcus* sp, *Bacillus tequilensis* and consortia. The setup was monitored using Gas Chromatography-Mass spectrophotometry while the acute toxicity was calculated using the Probit method. The acute toxicity of the set-up seeded with *Pseudomonas aeruginosa* was 718.8 mg/L while the consortia had 941.2 mg/L. The phenol residues were reduced by 100% while the 2-nitrophenol was reduced from 5.13 µg/L to 0.82 µg/L on the 10$^{th}$ day of the study. The remarkable reduction of the phenol residues with the use of microbial cultures goes to show the efficiency of locally sourced feedstock as tools for the degradation of pollutants. There is an urgent need for academia to develop robust microbial bio-mining and culture collection centers for futuristic and commercial use.**

***Keywords***: *Acute Toxicity Effluent, Exogenous Microorganisms, Probit, Phenol, Pollutant, Gas Chromatography-Mass Spectrophotometry*

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## I. INTRODUCTION

Phenol is a top chemical on the priority list of hazardous substances as published by the Agency for Toxic Substances and Disease Registry (USEPA, 1990; ATSDR, 2015). Phenolic compounds are hazardous as a result of their acute toxicity and tendency to remain in the environment for a long duration (Poi, Aburto-Medina, Mok, Ball and Shahsavari, 2017). They are carcinogenic and distort the balance of nature in water bodies (Aljuboury *et al.*, 2017; Poi *et al.*, 2017). The release of phenol into receiving waters has to be closely supervised to ensure that the phenol concentration does not exceed the tolerance limit. Compounds of phenol find their way into water bodies through the discharge of poorly treated wastewater from industries, agricultural and domestic activities. Derivatives of phenol (alkylphenols, halogenated phenols and nitrophenols) are found in wastewaters as a result of industrial processes like petroleum refining, tanning, plastic production etc. Alkylphenols like cresols are employed as additives in industrial processes (Corvini *et al.*, 2006). Dyes, drugs and pesticides industries regularly make use of chlorinated phenol derivatives like monochlorophenols, polychlorophenols, chloronitrophenols, chloroaminophenols and chloromethylphenols (Arora and Bae, 2014). Nitrophenols are variously employed in manufacture of explosives, dyes and pesticides (Duque *et al.*, 2012). Phenolic compounds, whether from natural or anthropogenic sources, are known to pose serious health risks to humans and animals (Bruce *et al.*, 1987). Some of these phenolic compounds often undergo some transformations into other intermediates which could even be more toxic than the parent compound. This



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transformation often results from reactions of these compounds with some physical, chemical and biological factors in the water environment (Kulkarni and Kawere, 2013). This study was aimed at assessing the degradation rate of phenol tainted industrial effluent using exogenous bacterial isolates obtained from crude oil polluted soil.

## II. Materials And Methods

### A. Source of bacteria

The three bacterial isolates were isolated from crude oil polluted creeks of Bodo, in Gokana local government area of Rivers state. After screening for phenol utilization and enrichment in Bushnell Haas media supplemented with different concentrations of phenol. The selected strains were identified using molecular technique.

### B. Preparation of the test organism

Each of the stock pure cultures of the three isolates was re-suspended in fresh nutrient broth and incubated at 30$^{o}$C on a rotary shaker at 120 rpm. After 24 hours, centrifugation was done not less than 3,000 rpm for 20 min was used to harvest the bacterial cells and the resulting pellets were rinsed using normal saline (0.85 % NaCl). The freshly grown isolates were re-suspended using distilled water and compared to 0.1 MacFarland standard prior to inoculation on the media. Turbidity was compared using a Spectrumlab 725 (German) at a wavelength of 600 nm for a bio load confirmation of 1.0 x $10^{8}$. The consortium was formed by mixing equal volumes of the individual isolates in a conical flasks (Iheanacho *et al*., 2019).

### C. Preparation of the test media

The acute toxicity of phenol to the individual isolates (*Pseudomonas aeruginosa*, *Bacillus tequilensis* and *micrococcus* sp.) and the consortium formed from them was designed using slight modification of the method adopted by Uffort and Odokuma (2018). Different concentrations of phenol (0.10mg/ml, 0.30mg/ml, 0.50mg/ml, 1.0mg/ml and 2.0mg/ml) were prepared and dispersed into 250ml conical flasks using 1.0% Carbon as source of energy. A volume of 99 ml of previously sterilized Bushnell Haas media and one ml of the inoculum were introduced into each of the conical flasks containing the different concentrations. The control had Bushnell Haas media supplemented with the test organisms without phenol. The different set ups were was incubated at 30$^{o}$C for 48 hours with counts taken at zero hour, 24 hours and 48 hours.

### D. The percentage survival of the bacterial consortium in phenol

The percentage log survival of the consortium in the different phenol concentration employed in the study was calculated using the formula adopted from Uffort and Odokuma (2018). This was calculated by obtaining the log of the count in each toxicant concentration and dividing it by the count in the control and then multiplying by 100.

Therefore %log survival $=\frac{\log ci}{\log C}$ x100

Where $Log$ci = log count in each toxicant concentration,

$Log$C = log count in the zero toxicant concentration.

### E. Determination of $LC_{50}$, $LC_{20}$ and $LC_{10}$

The lethal concentration of the toxicant that will kill 50%, 20% and 10% of the test organism ($LC_{50,}$ $LC_{20}$ and $LC_{10}$) were determined by Probit analysis (Uffort and Odokuma, 2018).

## III. Results

The selected strains were identified as *Pseudomonas aeruginosa* (MN294989) with 100% similarity to the isolates deposited to the GenBank, *Bacillus tequilensis* (MN294990) had 99% *and Micrococcus* spp. had 63% similarity to isolates already deposited in the Gen-Bank Library.

### A. Biodegradation of phenol

The GCMS results of the biodegradation of phenol are presented in Figures 2, 3 and 4. While Figure 2 shows the GCMS profile of phenol and some of its derivatives at day zero. The initial concentration of the phenol and some of the derivatives analyzed was 110.79µg/l, with phenol as a compound at a concentration of 23.36µg/l. At day five, there was a reduction in the initial concentration of phenol and all the analyzed derivatives. On day ten, phenol concentration stood at 0.42µg/l while the final residual concentration was 5.73µg/l, a 94.8% reduction in the concentration of phenol and the analyzed phenol derivatives.

Figure 5 shows the removal of phenol and some of the analyzed derivatives over the ten-day test period. On day 10, some of the phenol derivatives, namely: 3-chlorophenol, 4-chlorophenol, 2-methylphenol, 3- methylphenol, 4 - methylphenol, 3-nitrophenol, 4- nitrophenol, cyclohexyl-4, 6-dinitrophenol, 2, 3-Dichlorophenol, 3, 4-Dichlorophenol, 4-Chloro-2-methylphenol, 2-Chloro-5-methylphenol, 2, 4 – Dinitrophenol, 2, 5 – Dimethylphenol, Benzoate, Catechol and Muconic acid had totally been mineralized by the consortium.

The results for the lethal concentrations of phenol against bacterial isolates and consortium are presented in Table 1 while the Probit plot of acute toxicity of phenol presented in Figure 1.

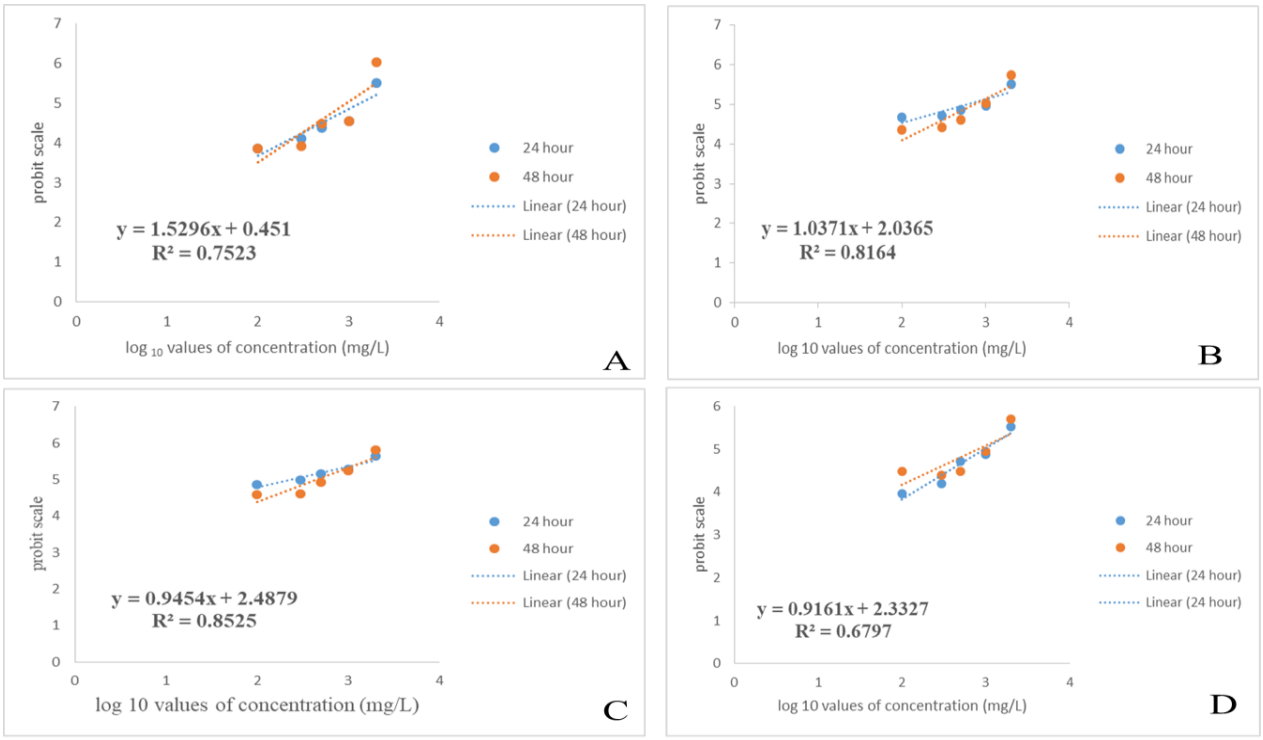



Figure 1. Probit plot of acute toxicity of phenol on: (A) consortium; (B) *Pseudomonas auruginosa*; (C) *Micrococcus* sp. and (D) *Bacillus tequilensis*.

Table 1: Lethal concentrations of phenol against bacterial isolates

| Lethal concentrations in mg/l | | | |
|---|---|---|---|
| *Test organisms* | $LC_{10}$ | $LC_{20}$ | $LC_{50}$ |
| *Pseudomonas aeruginosa* | 42.6±0.28 | 175.9±0.42 | 718.8±0.84 |
| *Micrococcus sp.* | 20.8±1.00 | 58.6±0.21 | 455.3±0.42 |
| *Bacillus tequilensis* | 32.7±0.07 | 98.8±0.24 | 814.9±1.27 |
| Consortium | 137.6±0.63 | 266.0±0.22 | 941.2±1.06 |

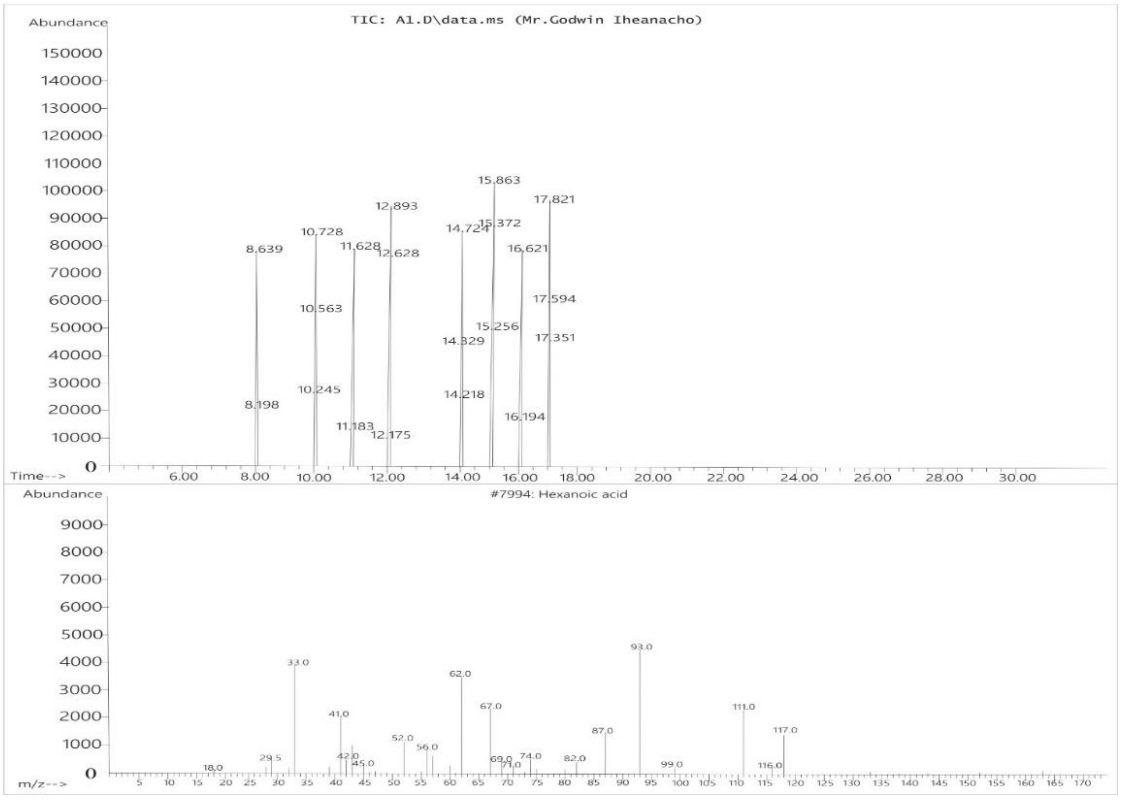
Data presented as Mean ± Standard deviation of triplicate samples

## IV. Discussion

The presence of phenol and its derivatives as pollutants is a long-term environmental issue faced by worldwide population. Phenol is known to be toxic even at low concentrations and has been listed as a chemical of priority concern by many regulatory agencies. A number of bacteria possess the ability to metabolize phenolic compounds under aerobic and anaerobic conditions (Nasvera *et al.*, 2015). Phenol is an organic molecule and acts as energy source for bacteria. Bacteria is able to oxidize these molecules and in the process rid the contaminated media of phenol pollutant. The result of the acute toxicity test of the phenol to the consortium and the individual isolates that made up the consortium indicated that the bacteria employed in this study were well adapted to utilize phenol as the sole source of carbon. The phenol degradation over a ten – day period monitored with the aid of GC-MS further underscores the ability of the selected bacteria strains to biodegrade phenol. At day 10, a number of phenol derivatives had totally disappeared from the sample.

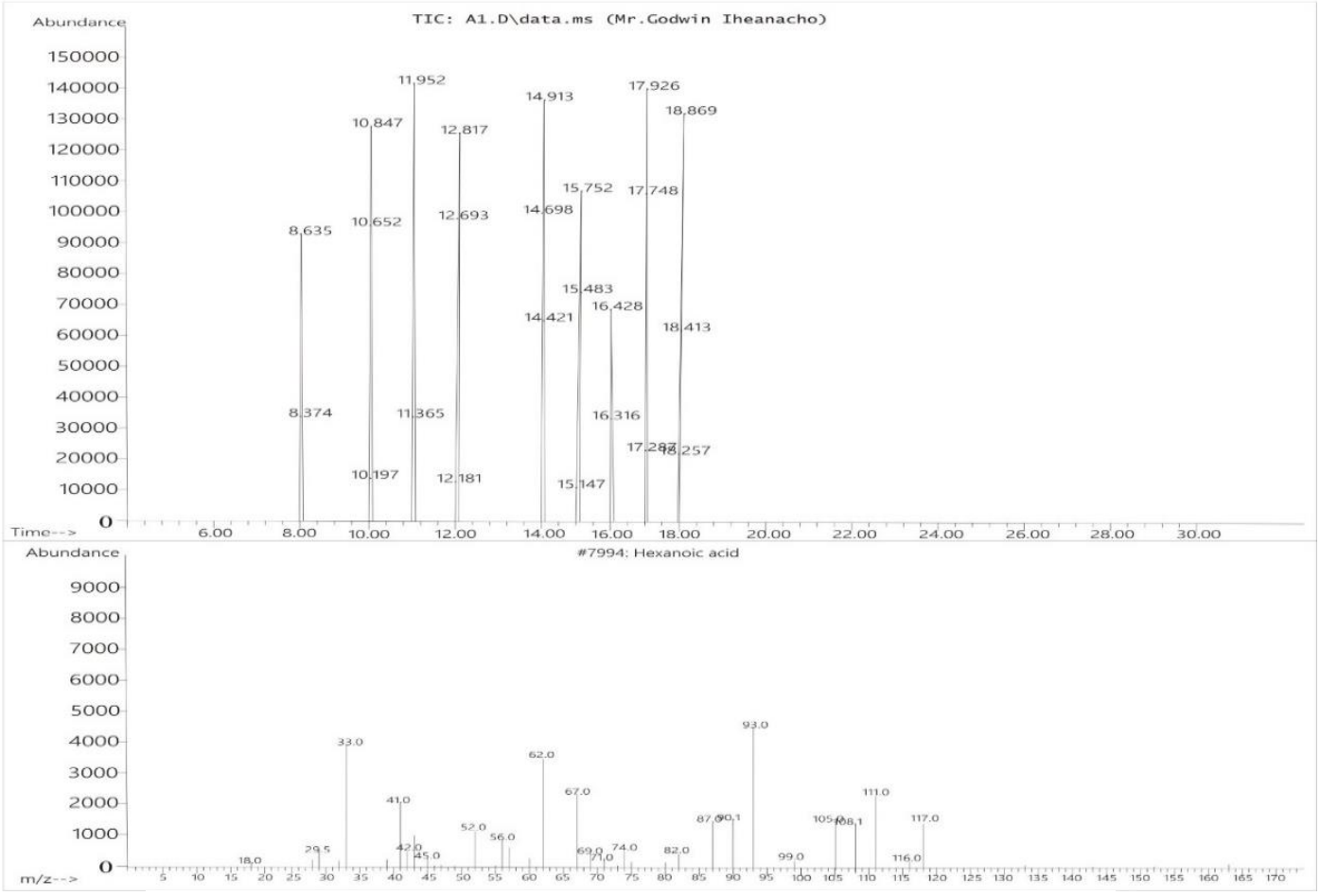
The findings of this work were consistent with some literatures on bacterial degradation of phenol and its derivatives. *Pseudomonas* had severally been implicated in the biodegradation of chlorophenol (Arora and Bae, 2014; Olaniran and Igbinosa, 2011). Olaniran and Igbinosa (2011) reported that chlorophenols are more soluble in water than the parent compound but are equally very recalcitrant.

Similarly, Arora and Bae (2014) had reported bacterial degradation of nitrophenol by a *Pseudomonas* sp. while Kolvenbach and Corveni (2012) reported the degradation of alkylphenols by *Shingomonas* sp. strain TTNP3.



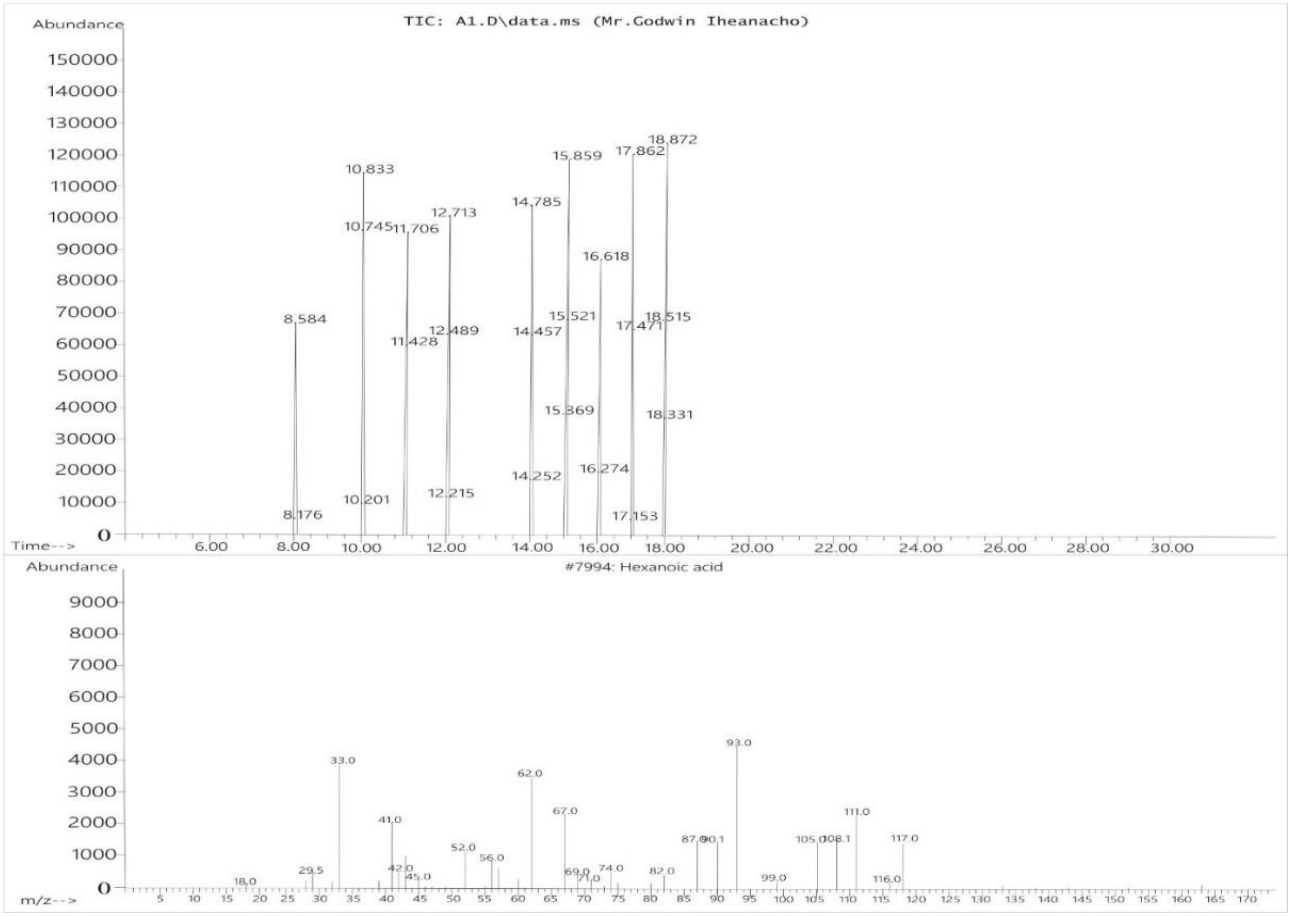

| Compound | R.T (min) | #CAS# NO | Conc. (µg/l) |
|---|---|---|---|
| Phenol | 8.198 | 108-95-2 | 23.36 |
| 2-Chlorophenol | 8.639 | 92-48-7 | 3.02 |
| 3-Chlorophenol | 10.245 | 108-43-0 | 9.74 |
| 4-Chlorophenol | 10.563 | 106-48-9 | 6.25 |
| 2-Methylphenol | 10.728 | 95-57-8 | 1.41 |
| 3-Methylphenol | 11.183 | 108-39-4 | 2.76 |
| 4-Methylphenol | 11.628 | 106-44-5 | 0.52 |
| 2-Nitrophenol | 12.175 | 88-75-5 | 9.69 |
| 3-Nitrophenol | 12.628 | 554-84-7 | 1.16 |
| 4-Nitrophenol | 12.893 | 100.02-7 | 3.42 |
| Cyclohexyl-4,6-dinitrophenol | 14.218 | 131-89-5 | 4.94 |
| 2,3-Dichlorophenol | 14.329 | 576-24-9 | 1.63 |
| 2,4-Dichlorophenol | 14.724 | 120-83-2 | 5.15 |
| 2,5-Dichlorophenol | 15.256 | 583-78-8 | 11.46 |
| 3,4-Dichlorophenol | 15.372 | 95-77-2 | 2.13 |
| 3,5-Dichlorophenol | 15.863 | 591-35-5 | 8.42 |
| 4-Chloro-2-methylphenol | 16.194 | 1570-64-5 | 4.86 |
| 2-Chloro-5-methylphenol | 16.621 | 615-74-7 | 1.23 |
| 2,3-Dimethylphenol | 17.351 | 526-75-0 | 5.17 |
| 2,4-Dinitrophenol | 17.594 | 95-87-4 | 2.85 |
| 2,5-Dimethylphenol | 17.821 | 95-87-4 | 1.62 |
| TOTAL | | | 110.79 |

Figure 2: GC-MS result for phenol biodegradation on day zero




| Compound | R.T (min) | #CAS# NO | Conc. (µg/l) |
|---|---|---|---|
| Phenol | 8.374 | 108-95-2 | 1.84 |
| 2-Chlorophenol | 8.635 | 92-48-7 | 0.53 |
| 3-Chlorophenol | 10.197 | 108-43-0 | 2.18 |
| 4-Chlorophenol | 10.652 | 106-48-9 | 1.35 |
| 2-Methylphenol | 10.847 | 95-57-8 | 0.17 |
| 3-Methylphenol | 11.365 | 108-39-4 | 0.28 |
| 4-Methylphenol | 11.952 | 106-44-5 | 0.00 |
| 2-Nitrophenol | 12.181 | 88-75-5 | 3.13 |
| 3-Nitrophenol | 12.693 | 554-84-7 | 0.00 |
| 4-Nitrophenol | 12.817 | 100.02-7 | 1.08 |
| Cyclohexyl-4,6-dinitrophenol | 14.421 | 131-89-5 | 0.74 |
| 2,3-Dichlorophenol | 14.698 | 576-24-9 | 0.00 |
| 2,4-Dichlorophenol | 14.913 | 120-83-2 | 1.09 |
| 2,5-Dichlorophenol | 15.147 | 583-78-8 | 4.53 |
| 3,4-Dichlorophenol | 15.483 | 95-77-2 | 0.27 |
| 3,5-Dichlorophenol | 15.752 | 591-35-5 | 3.19 |
| 4-Chloro-2-methylphenol | 16.316 | 1570-64-5 | 0.47 |
| 2-Chloro-5-methylphenol | 16.428 | 615-74-7 | 0.16 |
| 2,3-Dimethylphenol | 17.287 | 526-75-0 | 1.03 |
| 2,4-Dinitrophenol | 17.748 | 95-87-4 | 0.00 |
| 2,5-Dimethylphenol | 17.926 | 95-87-4 | 0.00 |
| Benzoate | 18.257 | 532-32-1 | 0.83 |
| Catechol | 18.413 | 120-80-9 | 1.05 |
| Muconic acid | 18.869 | 3588-17-8 | 0.58 |
| TOTAL | | | 24.05 |

Figure 3: GC-MS results for phenol biodegradation on day 5




| Compound | R.T (min) | #CAS# NO | Conc. (µg/l) |
|---|---|---|---|
| Phenol | 8.176 | 108-95-2 | 0.42 |
| 2-Chlorophenol | 8.584 | 92-48-7 | 0.00 |
| 3-Chlorophenol | 10.201 | 108-43-0 | 0.58 |
| 4-Chlorophenol | 10.745 | 106-48-9 | 0.00 |
| 2-Methylphenol | 10.833 | 95-57-8 | 0.00 |
| 3-Methylphenol | 11.428 | 108-39-4 | 0.00 |
| 4-Methylphenol | 11.706 | 106-44-5 | 0.00 |
| 2-Nitrophenol | 12.215 | 88-75-5 | 0.82 |
| 3-Nitrophenol | 12.489 | 554-84-7 | 0.00 |
| 4-Nitrophenol | 12.713 | 100.02-7 | 0.00 |
| Cyclohexyl-4,6-dinitrophenol | 14.252 | 131-89-5 | 0.00 |
| 2,3-Dichlorophenol | 14.457 | 576-24-9 | 0.00 |
| 2,4-Dichlorophenol | 14.785 | 120-83-2 | 1.03 |
| 2,5-Dichlorophenol | 15.369 | 583-78-8 | 1.13 |
| 3,4-Dichlorophenol | 15.521 | 95-77-2 | 0.00 |
| 3,5-Dichlorophenol | 15.859 | 591-35-5 | 1.75 |
| 4-Chloro-2-methylphenol | 16.274 | 1570-64-5 | 0.00 |
| 2-Chloro-5-methylphenol | 16.618 | 615-74-7 | 0.00 |
| 2,3-Dimethylphenol | 17.153 | 526-75-0 | 0.00 |
| 2,4-Dinitrophenol | 17.471 | 95-87-4 | 0.00 |
| 2,5-Dimethylphenol | 17.862 | 95-87-4 | 0.00 |
| Benzoate | 18.331 | 532-32-1 | 0.00 |
| Catechol | 18.515 | 120-80-9 | 0.00 |
| Muconic acid | 18.872 | 3588-17-8 | 0.00 |
| TOTAL | | | 05.73 |

Figure 4: GC-MS results for phenol biodegradation on day 10

The concentration of 2000mg/l was observed to be quite toxic to the consortium and the individual isolates. After 24 hours, about 75 % of the initial bacterial population were lost. Most of the previously mentioned studies on phenol toxicity to bacteria had concentrations ranging between 100 and 1000mg/l (Goudar *et al*., 2000; Al Hashemi, 2015). In a review, Al Hashemi *et al.* (2014) reported that above 1300mg/l, no phenol degradation was observed. It also reported that the lag phase of the bacteria

species increased with increase in initial phenol concentration for concentration less than 1300mg/l. The ability of about 25% of the initial bacterial population to survive after 48 hours on exposure to 2000mg/l shows that the consortium to be hyper tolerant to phenol. These findings are not consistent with reports of Goudar *et al*. (2000). In a study of the degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species, Hasan and Jabeen, (2015) reported the ability of these bacteria to survive and grow in phenol concentrations of up to 2174mg/l and 2190 mg/l respectively.

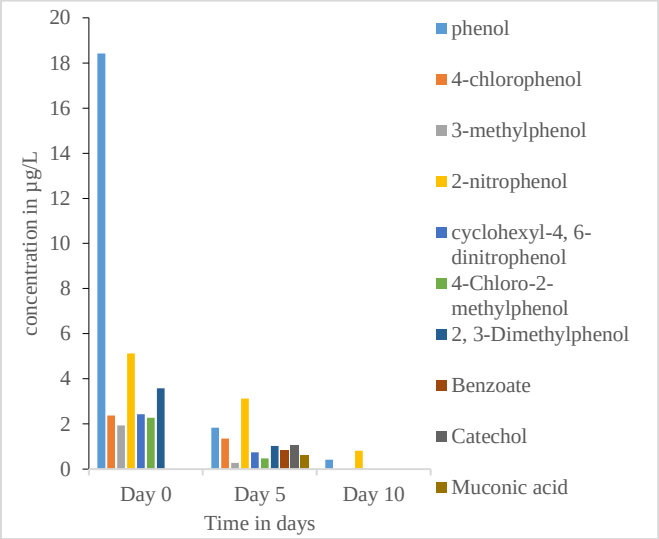



Figure 5: Biodegradation of phenol and phenolic compounds over a ten-day period.

Recently, in isolation, characterization and growth kinetics of phenol, a hyper tolerant bacteria from sewage-fed aquaculture system, Nandi *et al*. (2019) isolated *Pseudomonas*, *Acinetobacter* and *Bacillus* capable of growing in phenol concentration of 1500mg/l. This is consistent with the findings in this study where the organisms were able to survive in concentration above 1300mg/l.

The ability of the organisms to withstand high phenol concentrations can be attributed to adaptation to phenol utilization resulting from their prior exposure to phenol as the sole source of carbon. Banerjee and Ghoshal, (2017) reported that microorganisms are able to tolerate several toxic organic compound through different ways of adaptive mechanisms including changes in their fatty acid composition of membrane lipids. Gao *et al.* (2008) stated that extracellular polymeric substances (EPS) on cell surfaces are vital for microbial metabolism and also formation and stability of biofilms.

## V. Conclusion

Treatment of phenol tainted industrial effluents have been identified by the present study to be effective using pre-exposed, exogenous microorganisms bio-mined from crude oil impacted sites in the Niger Delta region of Nigeria. The study also revealed that the organisms were not significantly affected by the phenol toxicity and needed no immobilization techniques making them a veritable tool for the cleanup of phenol. The biodegradation efficiency of the consortia of the organisms also showed a significant reduction in the phenol residues. There is need for Government agencies to support indigenous studies in the niche mining research as a pivotal approach to harnessing the high value microbiota as agents for the biodegradation or biomineralization of phenol in Nigeria.

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