WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Toxicology Assay and Effects of Native Soap, Poultry Manure and their Combination in Enhancing Bioremediation of Heavy Metals and Crude Oil Polluted Soil

Ikeanumba, Michae Okwudirii[1], Orji, Justina Chibuogwu[2], Nweke, Christian Okechukwu[3], Adieze, Ifechukwu Enyinnaya[4]

[1.] *Biology Department, Alvan Ikoku Federal University of Education. Owerri, Nigeria*
[2.] *Microbiology Department, Federal University of Technology, Owerri, Nigeria*
[3.] *Microbiology Department, Federal University of Technology, Owerri, Nigeria*
[4.] *Microbiology Department, Federal University of Technology, Owerri, Nigeria*

A B S T R A C T

The study assessed the growth response of hydrocarbon-utilizing bacteria (HUB) to varying concentrations of natural soap (NS), poultry droppings (PD), and their combinations over a six-day period. Results obtained showed that lower concentrations (1%) of NS and PD significantly supported bacterial growth compared to higher concentrations (>10%), which demonstrated inhibitory effects. The 1% NS concentration enhanced microbial activity by promoting the production of surface-active compounds such as rhamnolipids, trehalolipids, and surfactin, leading to a reduction in total petroleum hydrocarbon (TPH) levels. Conversely, higher NS concentrations exhibited toxicity, corroborating findings from previous studies. Similarly, 1% PD concentration maximized bacterial growth, while concentrations above 10% showed diminishing effectiveness, potentially due to environmental stressors like heat release and oxygen reduction during breakdown. Combined NS and PD treatments revealed optimal bacterial growth at moderate concentrations (0.5% + 0.5%), with higher combinations (15% + 15%) resulting in a steep decline in HUB activity. The findings underscore the importance of optimizing biostimulation agent concentrations to enhance microbial activity and remediation efficiency. This study recommends the application of 1% biostimulant agents, such as NS and PD, for effective microbial growth and pollutant degradation in crude oil-impacted soils. These results contribute to refining bioremediation strategies, emphasizing the critical role of amendment concentration in achieving sustainable environmental restoration.

Keywords: Hydrocarbon-utilizing bacteria, natural soap, poultry manure, bioremediation, biostimulation, biostimulant and amendment optimization

1. INTRODUCTION

Biostimulation is a method used to enhance the degradation of petroleum hydrocarbons (PHCs) by adding nutrients (such as nitrogen, phosphorus, poultry litter, horse manure, domestic sewage, rice straw biochar, and crop residues) and other supplementary agents like biosurfactants and electron acceptors (e.g., oxygen, chelated Fe(III), nitrates, and sulfate). These additions create a more favorable environment for hydrocarbon-degrading bacterial communities (Coles, Patel, Akinnola, & Helleur, 2009; Gallego et al., 2001; Molina-Barahona, Rodriguez-Vazquez, Hernandez-Velasco, Vega-Jarquin, Zapata-Perez, Mendoza-Cantu, & Albores, 2004). The effectiveness of these components is tied to their ability to either enhance the metabolic activity of the indigenous degrading bacteria or increase the bioavailability of PHCs. Nutrient enrichment, in particular, has been shown to significantly boost the degradation capacity of native microbial communities among various biostimulants (Delille, Coulon, & Pelletier, 2004; Garcia-Blanco, Venosa, Suidan, Lee, Cobanli, & Haines, 2007; Thomassin-Lacroix, Eriksson, Reimer, & Mohn, 2002). For oil spill cleanup, an application of 1–5% nitrogen by weight of the oil, with a nitrogen to phosphorus (N) ratio of 5–10:1, is generally recommended. Theoretically, converting 1 gram of hydrocarbon into microbial biomass requires around 150 mg of nitrogen and 30 mg of phosphorus. Optimal C: N ratios for in situ bioremediation have been explored in various studies, with proposed ratios such as 100:9:2, 100:10:1, 100:10:5, and 250:10:3 being identified as ideal for enhancing hydrocarbon degradation in soil (Zawierucha & Malina, 2011). This study tends to find the effects of different concentrations of native soap, poultry manure and their combination in enhancing bioremediation of heavy metals and crude oil polluted soil.

1.1 Materials and Methods

Source of crude Oil

The crude oil type that was used for this work, was Bonny light crude oil obtained from the Port Harcourt Refinery Company Limited Eleme, in Rivers State. The crude oil was collected in twenty-five liters (25 L) plastic container and stored at room temperature in the laboratory until it when used.

Soil pollution

Uncontaminated soil obtained from the back of Okemini Naval barrack, Rumuolumini, in Obio Akpor Local Government area of Rivers State (this site is not known to be polluted with petroleum hydrocarbon or by any other industrial pollutants), was polluted with crude oil using method adopted by Adieze, Orji, Nwabueze, & Onyeze, (2012).

Isolation of hydrocarbon utilizing bacteria

(i) Enumeration of total heterotrophic microorganisms

Microbial populations in the soil samples were assayed by standard plate count technique. The total aerobic heterotrophic culturable microbial populations present in the soil samples during the study were estimated by spread plate techniques of Pelczar & Chan, (1977).

(ii) Enumeration and isolation of hydrocarbon utilizing species

Enumeration and isolation of hydrocarbon utilizing species was determined by the modified method of Okpokwasili & Amanchukwu, (1988). Characterization of the isolates followed the procedures in the Bergey's manual of determinative bacteriology (Holt, 1994).

Preparation and standardization of bacterial inocula

Hydrocarbon utilizing bacterial species isolates obtained as described above, were inoculated into 10ml sterile normal saline contained in 20 ml sterile test tubes. The suspension was shaken for five minutes to evenly distribute the organisms and then transferred into a sterile 500 ml conical flask containing 190 ml sterile salt broth (Okpokwasili & Amanchukwu, 1988) containing 1% Bonny light crude oil, this mixture was incubated on a rotary shaker (150 rpm) at room temperature of 28±2ºC for five days (Odokuma & Dickson, 2003). The pH of the cultures was monitored and maintained at between 7-7.2 by adjusting the culture with standard phosphate buffer. **(**APHA,1985).

To obtain the inocula, aliquots of the final cultures was centrifuged at 10,000 x g for 20 minutes. The supernatants were discarded and the cell pellets collected and washed twice in 20ml of sterile tap water. After washing, the cell pellets were resuspended in 100 ml sterile distilled water and adjusted to final OD of 0.40 at 660 nm (population between 10^6-10^8 cfu/ml). The suspension was used for toxicity assay for the soil amendments (native soap, poultry droppings and combination of both) used for the work.

Microbiological analysis

Enumeration and isolation of hydrocarbon utilizing species was determined by the modified method of Okpokwasili & Amanchukwu, (1988). Characterization of the isolates followed the procedures in the Bergey's manual of determinative bacteriology (Holt, 1994).

Molecular identification of isolated hydrocarbon utilizing strains

Bacterial genomic DNA extraction

Total DNA isolation and isolates identification were carried out at Nucleometrix Molecular Laboratory, Yengoa, Bayelsa State. The method as described by Saitou & Nei, (1987**),** was adopted for molecular identification of isolates

DNA quantification

The extracted genomic DNA as described above was quantified using the Nanodrop 1000 spectrophotometer.

16S rRNA Amplification

The 16s RRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles.

Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN.

Toxicology assay of different concentrations of amendment on hydrocarbon utilizing bacterial growth

Into three test tubes containing 9.9 ml, 9 ml and 7 ml sterile normal saline contained in 20 ml sterile test tubes and labelled A1n, A2n and A3n respectively. Where A1n represents the test tube containing 9.9 ml, A2n and A3n that of 9 and 7 ml of sterile normal saline respectively. Into the test tubes were added 0.1 g, 1.0 g and 3g of native soap representing 1%, 10% and 30% concentration of the amendment. They were left to stand for 24 hr in order to completely dissolve after which they were shaken for three minutes to obtain a homogenous mixture followed by inoculation with hydrocarbon utilizing bacterial

species isolates obtained as previously described. The setup was prepared in duplicates. The same set up was carried out for other studies which includes poultry manure and combination of both native soap and poultry manure.

.

1.2 Results

Table 1: Biochemical characterization and probable identity of hydrocarbon utilizing bacterial (HUB) isolates

S/N	organisms	GS	Shp	Pg	Es	Mt	Ct	Ox	VP	MR	Ur	O/F	Ci	In
M1	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M2	*Acinetobacter* spp.	-	rod	w	-	-	+	-	-	-	-	-	+	+
M3	*Enterobacter* spp.	+	rod	w	-	+	+	+	-	+	+	+	+	-
M4	*Alcaligenes* spp.	-	rod	c	-	+	+	+	-	+	+	+	+	-
M5	*Erwinia* spp.	-	rod	y	-	+	-	-	+	-	-	+	+	-
M6	*Bacillus* spp.	-	rod	c	+	+	+	+	-	-	+	-	-	-
M7	*Micrococcus* spp.	+	cocci	y	-	-	+	-	-	+	-	+	-	-
M9	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M10	*Erwinia* spp.	-	rod	y	-	+	-	-	+	-	-	+	+	-
M11	*Micrococcus* spp.	+	cocci	y	-	-	+	-	-	+	-	+	-	-
M12	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M13	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M14	*Acinetobacter* spp.	-	rod	w	-	-	+	-	-	-	-	-	+	+
M15	*Acinetobacter* spp.	-	rod	w	-	-	+	-	-	-	-	-	+	+
M16	*Alcaligenes* spp.	-	rod	c	-	+	+	+	-	+	+	+	+	-
M17	*Bacillus* spp.	-	rod	c	+	+	+	+	-	-	+	-	-	-
M18	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M19	*Alcaligenes* spp.	-	rod	c	-	+	+	+	-	+	+	+	+	-
M20	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M22	*Erwinia* spp.	-	rod	y	-	+	-	-	+	-	-	+	+	-

Key: Es. – Endospore; Gs. − Gram stain; Shp. − Shape; Pg. − Pigmentation; Mt. − Mortality; Ct. − Catalase test; Ox. − Oxidase; Vp. − Voges-proskauer; MR. − Methyl Red; Ur. − Urease; O/F. − Oxidation/Fermentation; Ci. − Citrate utilization; In. − Indole; C − Cream; W− White; Y − Yellow; plm − Pleomorphic shape, sph − Spherical shape

Table 2: Molecular equivalent of biochemical identified hydrocarbon utilizing bacteria (HUB) isolates

S/N	Probable identity of isolates from biochemical tests	Molecular identity of isolate
1	*Pseudomonas* spp.	*Pseudomonas xiamenensis*
2	*Acinetobacter* spp.	*Acinetobacter baumanii*
3	*Alcaligens* spp.	*Alcaligenes cloacae*
4	*Enterobacter* spp.	*Enterobacter cloacae*
5	*Erwinia* spp.	*Pantoa dispersa*

6	*Bacillus* spp.	*Lysinibacillus fusiformis*
7	*Micrococcus* spp.	*Kocuria palus*

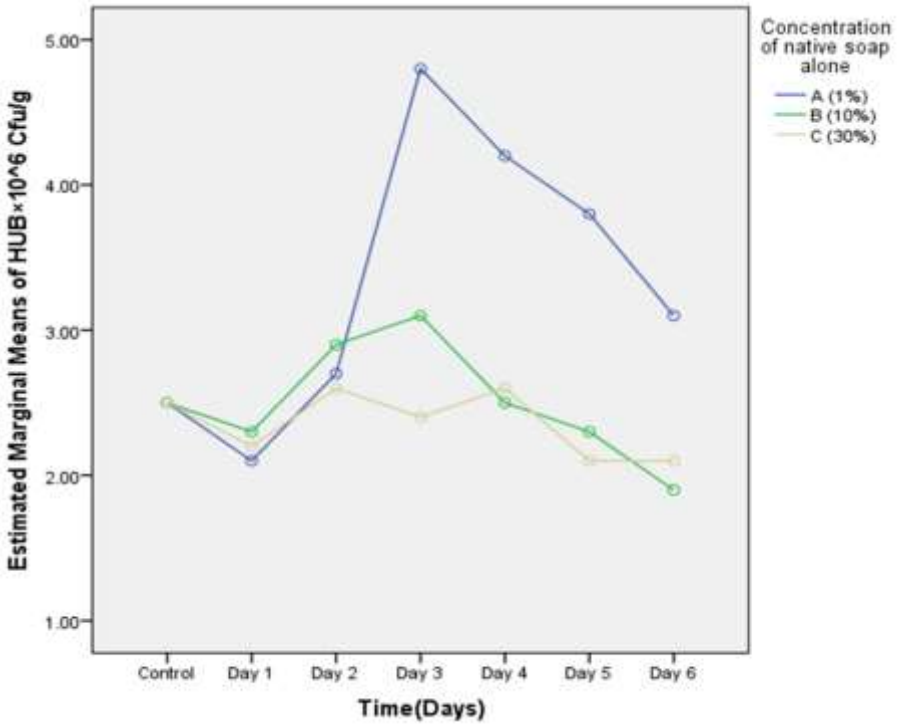

Fig. 1 -Growth response of hydrocarbon utilizing bacterial (HUB × 10^6 Cfu/g) in different concentrations of native soap solution

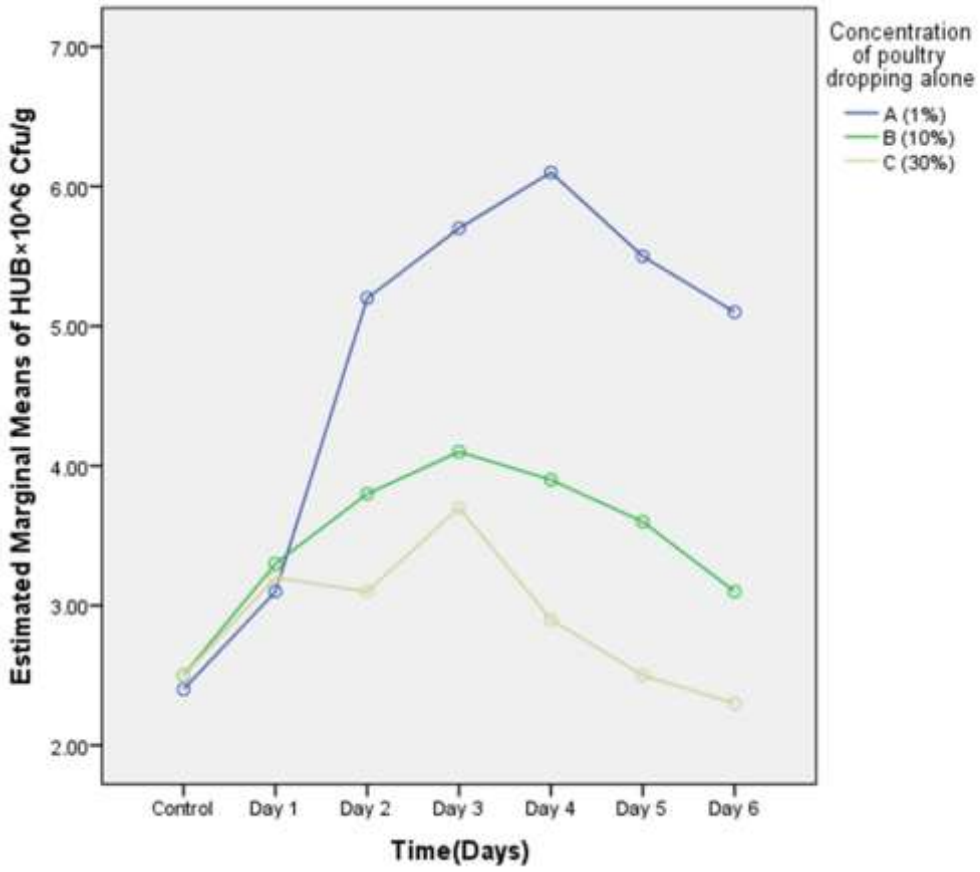

Figure 2: Growth response of Hydrocarbon utilizing Bacteria (HUB × 106 Cfu/g) in different concentrations of poultry dropping

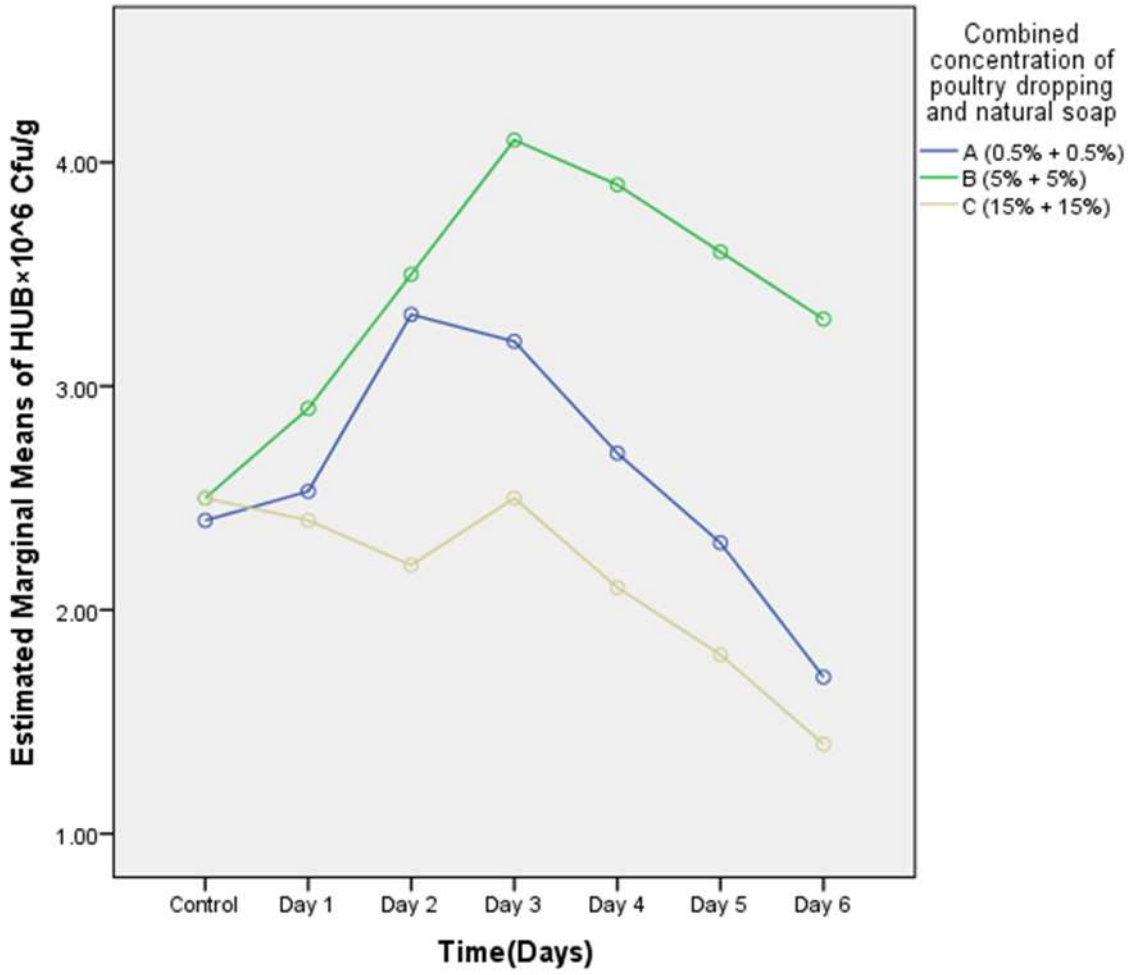

Figure 3: Growth response of Hydrocarbon utilizing Bacteria (HUB × 106 Cfu/g) in different concentrations of combined native soap and poultry droppings

1.3 Discussion

The response of hydrocarbon utilizing bacterial in different concentrations of native soap solution was assessed for a period of six days. The result suggests that lower concentration (1%) of native soap supported the growth of the test organisms more significantly than higher concentrations greater than 10%. This is in agreement with the previous work of Olajuyigbe, Adeoye-Isijola, & Adedayo, (2017); whose study on Black soap concluded that the minimum inhibitory concentration for *Klebsiella pneumoniae* and *Enterococcus faecalis* ranged between 0.125 mg/mL and 2 mg/mL, *Staphylococcus aureus* (0.25–4) mg/mL, *Escherichia coli* (0.125–4) mg/mL This means that higher concentration of the soap results in the death of the studied bacteria. Also the study by Wemedo, Amadi, Nedie, & Olaolu, (2018), agreed that the high concentration of soap leads to higher significant mortality rate in the study organisms. Also the previous study by Anoliefo, Ikhajiagbe, Okoye, & Omoregie, (2016). Anoliefo et al. (2019), found that 1% w/v local soap-in-water caused an increase in rise of surface-active chemicals following the application of soap treatments, including rhamnolipids, trehalolipids, sophorolipids, emulsan, liposan, and surfactin which led to increase in the polluted soil's microbial consortia resulting to a general decrease in the soil's total petroleum hydrocarbon (TPH) concentrations.

The growth response of hydrocarbon utilizing bacterial (HUB) species in different concentrations of poultry dropping (poultry manure) was assessed. It was observed that the treatment with 1% poultry dropping concentration resulted in a significant increase in bacterial species growth. The results indicate that the 1% concentration of poultry droppings is the most effective treatment for increase in bacterial species growth. The 10% concentration also shows a beneficial effect but is less effective than the 1% concentration. The 30% concentration has the least effect, indicating that higher concentrations might not proportionally increase efficacy and could potentially have adverse effects. These observations are in contrast with the previous works of Zhang, Sun, Wang, Peng, Wang, Lin, Yang, Hua, & Wu, (2023). They observed that high concentrations of chicken manure resulted in distinct microbial profiles. Also Jin, H., Zhang, Yan, Yang, Fang, Li, Shao, Wang , Yue, Wang , Cheng, Shi, & Qin, (2022); Yang, Ashworth, DeBruyn, Willett, Durso, Cook, & Owens, (2019); Acosta-Martínez, and Harmel, (2019) all studied the impact of poultry manure application on soil microbial communities and found that high concentrations of poultry manure altered the complexity and structure of both bacterial and fungal networks, enhancing the abundance of certain keystone taxa which play crucial roles in nutrient cycling. Also the study by Okafor, Orji, Agu, Awah, Okeke, Okafor, & Okoro, (2016) contrasted the

findings of this work. They studied the impact of poultry droppings in the bioremediation of crude oil-polluted soil was evaluated. Different concentrations of the poultry droppings (10%, 30%, and 50%) were also studied and the result obtained from this study shows that the microbial growth rate increased as the concentration of the poultry droppings increased. The reason for the contrast in observed results could be among other factors due to concentration of the pollutants at which this study was carried. At 10% crude oil with heavy metals pollution, 30% percent poultry manure could be detrimental to the growth of HUB due to heat released to the environment from its breakdown which might result in additional stress on the bacteria resulting to their death.

The growth response of hydrocarbon utilizing bacteria (HUB in different combined concentrations of natural soap (NS) and poultry droppings (PD) over six days, shows that all groups showed a decline, the study shows that the combination (0.5% + 0.5%) to most significantly support the growth of HUB followed by (5% +5%) combination, with the highest concentration (15% + 15%) showing the steepest drop. Although the analysis of the growth response of hydrocarbon-utilizing bacteria (HUB) in various combined concentrations of natural soap (NS) and poultry droppings (PD) is limited, the findings of Akpokodje & Hilary (2019) align with this observation. In their study, *"Bioremediation of Hydrocarbon Contaminated Soil: Assessment of Compost Manure and Organic Soap,"* they reported that the growth and activity of hydrocarbon-degrading bacteria significantly increased when low to moderate concentrations of poultry manure were combined with natural soap.

Conclusion

The results from the study shows that amendment with lower concentrations of native soap, poultry droppings (1% and 10%) are more effective in supporting the growth of tested bacterial species. Higher concentration (30%) showed a less effectiveness in the support of the growth of tested bacterial species, this is likely due to potential toxicity or its inhibitory effects. This highlights the importance of optimizing the concentration of biostimulation agents (amendments) such as native soap, poultry dropping or their combined concentrations prior to application in a pollutant impacted soil for effective remediation processes.

Recommendation

This study tends to suggest that for a more effective bioremediation process through the application of organic amendment, application of 1% biostimulator agents such native soap and poultry manure to a crude oil impacted site will give a more significant microbial growth yield followed by 10% than higher concentration of 30% which showed a less effectiveness in the support of the growth stimulation.

Acknowledgements

This work has been supported by Tertiary Education Trust Fund (TETFund). Special thanks to Professor Orji, Justina Chibuogwu , Professor Nweke, Christain Okechukwu and Professor Adieze, Ifechukwu Enyinnaya for their invaluable guidance and supervision throughout the study. Their expertise and encouragement were pivotal to the successful completion of this work..

References

Adieze, I. E., Orji, J. C., Nwabueze, R. N., & Onyeze, G. O. C. (2012). Hydrocarbon stress response of four tropical plants in weathered crude oil contaminated soil in microcosms. *Inter. J. of Environ. Studies*, *69*:490-500.

American Public Health Association (1985). Standard methods for the examination of water and waste water (10th edn.). Washington, DC: American Public Health Association.

Akpokodje, O. & Hilary, U. (2019). Bioremediation of Hydrocarbon Contaminated Soil: Assessment of Compost Manure and Organic Soap. *ransactions on Engineering and Computing Sciences*, *7*:13–22.

Anoliefo, G. O., Ikhajiagbe, B., Okoye, P. C., & Omoregie, O. (2016). Utilizing local soap-derived biosurfactant for degradation of petroleum hydrocarbon polluted soils: sustainable remediation in focus. *Annals of Science, 1:43-51*.

Coles, C. A., Patel, T. R., Akinnola, A. P. & Helleur, R. J. (2009). Influence of bulking agents, fertilizers and bacteria on the removal of diesel from a newfoundland soil. *Soil Sediment Contam. 18 383–396.*

Delille, D., Coulon, F. & Pelletier, E. (2004). Effects of temperature warming during a bioremediation study of natural and nutrient-amended hydrocarbon-contaminated sub-Antarctic soils. *Cold Reg. Sci. Technol.* 40 61–70.

Garcia-Blanco, S., Venosa, A. D., Suidan, M. T., Lee K., Cobanli S. & Haines J. R. (2007). Biostimulation for the treatment of an oil-contaminated coastal salt marsh. *Biodegradation* 18:1–15

Holt, J.G. (1994) Bergey's manual of determinative bacteriology. 9th Edition, Lippincott Williams and Wilkins, Baltimore.

Jin, H., Zhang, D., Yan, Y., Yang, C., Fang, B., Li, X., Shao, Y., Wang, H., Yue, J., Wang, Y., Cheng, H., Shi, Y & Qin, F. (2022). Short-term application of chicken manure under different nitrogen rates alters structure and co-occurrence pattern but not diversity of soil microbial community in wheat field. *Frontiers in Microbiol.13*, 975571.

Molina-Barahona, L., Rodriguez-Vazquez, R., Hernandez-Velasco, M., Vega-Jarquin, C., Zapata-Perez, O., Mendoza-Cantu, A., & Albores, A. (2004). Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. *Appl. Soil Ecol. 27 165–175.*

Odokuma, L. O., & Dickson, A. A. (2003). Bioremediation of a crude oil polluted tropical rain forest soil. *Global J. of Environ. Sci.2:29-40.*

Okafor, U. C., Orji, M. U., Agu, K. C., Awah, N. S., Okeke, B. C., Okafor, O. I., & Okoro, N. C. (2016). Bioremediation of crude oil-polluted soil using broiler-chicken droppings. *Journal of Applied and Environmental Microbiology*, *4*(4), 75-84.

Okpokwasili, G. C. & Amanchukwu, S.C. (1988). Petroleum hydrocarbon degradation by *Candida* species. *Environ. Int*., 14: 243-247.

Olajuyigbe, O., Adeoye-Isijola, M. & Adedayo, O. (2017). A comparison of the antibacterial activity of some African black soaps and medicated soaps commonly used for the treatment of bacteria-infected wound. *J. of Medicinal Plants for Econ. Development*, *1*:*1-8.*

Pelczar, M. J. & Chan, E.C. 1977. Laboratory Exercises in Microbiology, 4th edition, McGraw Hill, Inc

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biol. and evol.4*:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Thomassin-Lacroix, E. J. M., Eriksson, M., Reimer, K. J. & Mohn, W. W. (2002). Biostimulation and bioaugmentation for on-site treatment of weathered diesel fuel in Arctic soil. *Appl. Microbiol. Biotechnol.* 59 551–556

Wemedo, S., Amadi, A., Nedie, P. & Olaolu, J.O. (2018). Comparison of antimicrobial activity of locally produced soaps and conventional medicated soaps on bacterial isolates from skin and wound. *World J. of Pharm. and Med. Res. 4:16-20*

Yang, Y., Ashworth, A. J., DeBruyn, J. M., Willett, C., Durso, L. M., Cook, K., Moore Jr, P.A., & Owens, P. R. (2019). Soil bacterial biodiversity is driven by long-term pasture management, poultry litter, and cattle manure inputs. *Peer J*, *7*, e7839.

Zawierucha, I., & Malina, G. (2011). Bioremediation of contaminated soils: effects of bioaugmentation and biostimulation on enhancing biodegradation of oil hydrocarbons. *Bioaugment., biostimulat. and biocontrol*, 187-201.

Zhang, D., Sun, J., Wang, D., Peng, S., Wang, Y., Lin, X., Yang, X., Hua, Q., & Wu, P. (2023). Comparison of bacterial and fungal communities structure and dynamics during chicken manure and pig manure composting. *Environ. Sci. and Pollut.Res.30*:94347-94360.