

Bioremediation of Spent Oil - Contaminated Soil Using Biostimulants

Nna Orji, Chinenye

Chemistry Unit of Applied Mathematics Programme,
Department of Mathematics, National Mathematical Centre
Kaduna-Lokoja Expressway Sheda, Abuja, Nigeria

Email: nenyenna@gmail.com

Received: 2024-06-23

Accepted: 2024-06-30

Published online: 2024-07-05

Abstract

Anthropogenic activities such as those occurring in mechanical workshops have caused environmental pollution. Bioremediation, a biological method that is cost effective, safer and environmentally friendly for reclaiming polluted land can be utilized to degrade the spent oil in soil. This study compared the bioremediation of spent oil-contaminated soil using biostimulants such as organic and inorganic biostimulants and attenuated microcosms within a short period via appropriate standard methods. The initial residual oil content of the microcosm bio-stimulated with various quantities of inorganic fertilizer ranged from 0.022 ± 0.03 to 0.036 ± 0.01 g/g, while at the end of the 42-day incubation period, the residual oil content ranged from 0.013 ± 0.00 to 0.023 ± 0.00 g/g. However, the amount of residual oil in the microcosms biostimulated with organic fertilizer ranged from 0.033 ± 0.01 to 0.024 ± 0.00 g/g at the outset and from 0.010 ± 0.00 to 0.023 ± 0.00 g/g at the end of the process. The mean residual oil content of the attenuated microcosm ranged from 0.056 ± 0.01 g/g at the beginning of the biostimulation period to 0.045 ± 0.00 g/g at the end of the incubation period. The number of colony-forming units of the inherent microbes ranged from 1.7×10^3 to 4.2×10^{10} CFU/g. The results indicate that the biostimulants stimulated the growth of inherent microbes for the biodegradation of spent oil by utilizing the spent oil as the sole source of carbon, thereby remediating the contaminated soil. The results also showed that both organic and inorganic fertilizers can be used as biostimulants, but organic fertilizers add their inherent microbes to contaminated soil, thereby increasing the microbial load of the soil, which enhances its remediation.

Keywords: bioremediation, biostimulant, biostimulation, cold extraction, organic and inorganic fertilizers, spent oil-contaminated soil.

INTRODUCTION

Bioremediation is the process of using microorganisms to degrade (reduce detoxify, mineralize or transform) contaminants in soil, groundwater, sludge, and solids (Azubuike *et al.*, 2016) to harmless compounds. The process involves the maintenance and reproduction of the cells of microorganisms for their survival to break down contaminants by using them as an energy or carbon source and food (Sahota and Sharma 2022). The contaminants that are degraded via bioremediation can be organic or inorganic, such as agrochemicals, chlorinated compounds, dyes, greenhouse gases, heavy metals, hydrocarbons, nuclear waste, plastics, sewage, crude and spent oil, and solvents (Bala *et al.*, 2022, Sonawane *et al.*, 2022, Sales da Silva *et al.*, 2020).

Bioremediation is a cost-effective, safer and environmentally friendly method for removing contaminants (Tripathi *et al.*, 2021) and involves *ex situ* and *in situ* techniques, depending on the cost involved, pollutant type and concentration (Bala *et al.*, 2022). Bioremediation strategies include biocharging, biostimulation, bioaugmentation, bioventing, and natural attenuation (Bala *et al.*, 2022, Zhang *et al.*, 2020). Biostimulation focuses on enhancing the growth of inherent microbes for the degradation of contaminants while ensuring the removal of factors that may hinder the biodegradation of pollutants (Romantschuk *et al.*, 2023, Curiel-Alegre *et al.*, 2022, Goswami *et al.*, 2018). These factors include unfavorable pH and temperature, lack of oxygen, essential nutrients (phosphorus, nitrogen, or carbon), moisture and viable microorganisms (Romantschuk *et al.*, 2023, Mohamed and Mohamed, 2023). Biostimulation is highly efficient, cost effective and eco-friendly (Goswami *et al.*, 2018). The use of biostimulants such as poultry litter (Ogbozige, 2024, Adams *et al.*, 2014), vermicompost (Curiel-Alegre *et al.*, 2022), moss and compost (Mushtaq *et al.*, 2020), water hyacinth compost, spent mushrooms and NPK (Udume *et al.*, 2023), yeast extract (Tumolo *et al.*, 2023), goat droppings (Ogujoifor *et al.*, 2021) and fish waste and goat manure (Awari *et al.*, 2020) have been discussed. Other stimulants include *biochar, oxalic acid, salicylate, NPK, and ammonium sulphate* (Ahmad *et al.*, 2021).

Spent oil, also referred to as used/waste engine oil, is a lubricating oil that has served adequately in engine and is no longer useful for its initial purpose because it is considered contaminated with different impurities (Nna Orji *et al.*, 2018a). These impurities include heavy metals (Kashif *et al.*, 2018, Nna Orji *et al.*, 2018b), aliphatic hydrocarbons, mono- and polyaromatic hydrocarbons (PAHs) (Nna Orji 2019), and chemical additives (Nwite and Alu 2015). Hence, during the service of cars, generators, etc., spent oil is withdrawn from engines, dumped indiscriminately on the soil, and they find their way into water drainages and farmlands, which is a common practice in motor and generator mechanics (Azorji *et al.*, 2022). Thus, these harmful components of the spent oil are released into the environment, polluting plants, water bodies, farmlands and humans. This causes a reduction in the water holding capacity, aeration properties and pH of soils (Nwite and Alu 2015). Heavy metals in the environment cause cardiovascular, kidney and liver issues; skin, neuron and nephron toxicity; anemia; and tremor and increases the risk of cancer, which results in death (Mitra *et al.*, 2022), while PAHs are highly toxic, mutagenic, teratogenic, immune-toxicogenic and carcinogenic, damaging the DNA structure and causing mutations (Patel *et al.*, 2020) in all living organisms.

Spent oil in the soil can reduce the growth and productivity of plants (da Silva Correa *et al.*, 2022, Huang *et al.*, 2019) and cause damage, such as leaf rolls, chlorosis, growth inhibition, root tip browning, and plant death (Alori *et al.*, 2022).

Therefore, there is a need to bioremediate soil contaminated with spent oil to reclaim land for farm and other purposes using biostimulation, an environmentally friendly method to avoid further contamination of the environment. This study compared the bioremediation of spent oil-contaminated soil using organic and inorganic

biostimulants and attenuated microcosms within a short period of 42 days. Further long-term studies are needed to validate the results obtained in this study.

Soil Sampling

Soil was sampled from a mechanic workshop located at the Jude Garage in the Gwagwalada Area Council using a soil auger and stored in a dark-colored sterile polyethylene bag to prevent direct sunlight. The soil was labeled accordingly, transported immediately to the laboratory and stored inside a refrigerator at $4\pm 1^{\circ}\text{C}$ immediately. Goat droppings were sampled from a local livestock farm located within the Small-Sheda community at Sheda Abuja, which is opposite the National Mathematical Centre, while fertilizer was purchased from the Gwagwalada market.

Processing of Samples

The soil samples were sieved through a 2 mm pore size mesh to remove large debris. The goat droppings were air-dried in a drying room, pulverized, sieved through a 2 mm sieve and stored in a labeled container.

Biostimulation using organic and inorganic fertilizer

Microcosm experiments were performed in 250 ml Erlenmeyer flasks. Approximately 40 g of the contaminated soil sample was weighed into each of 14 conical flasks. Then, NPK and goat droppings were added in different quantities and microcosms labeled accordingly. Microcosms F_1 and Gd_1 contained 15 g of NPK and 15 g of goat dropping, respectively; F_2 and Gd_2 contained 30 g of NPK and 30 g of goat dropping, respectively; and F_3 and Gd_3 contained 45 g of NPK and 45 g of goat dropping, respectively. Moreover, control microcosms labelled C, which contained no biostimulant was used. These microcosms were duplicated to yield 14 microcosms.

Approximately 10 ml of sterile distilled water was added to each flask and swirled to mix the contents of the flask properly. Thereafter, the flasks were incubated at $30\pm 2^{\circ}\text{C}$. The addition of water was repeated every week to ensure adequate moisture content for microbial growth (*Ogujoifor et al.*, 2021). The residual oil content of the soils was determined via extraction, evaporation and gravimetric methods every 7 days for 42 days to determine the extent of oil loss in each microcosm experiment (Nna Orji, 2019).

Sampling

Thorough mixing and the addition of distilled water for adequate microbial growth were ensured. Periodic sampling from each flask was carried out after 0, 7, 14, 21, 28, 35 and 42 days of incubation to measure the residual oil after biodegradation by the inherent microorganisms following biostimulation with NPK and goat droppings.

Approximately 1 g of soil sample was collected from the flask after proper mixing. This process was performed for individual microcosms.

Extraction

The samples were analyzed for the extent of biodegradation of the spent oil via a cold extraction method (Nna Orji, 2019) using dichloromethane as the solvent.

METHOD OF DATA ANALYSIS

Analysis of variance (ANOVA) was used to determine if the relationships between treatment conditions were statistically significant ($p < 0.05$) at various time points during the experiments. Tukey and Dunnett's multiple comparisons test at $\alpha = 0.05$ simultaneous confidence level was used for this analysis and the results were generated using the GraphPad Prism 7 Statistical Software ® Program.

RESULTS AND DISCUSSION

The biodegradation results are presented in Fig. 1. The figure reveals that the initial mean concentration of spent oil in the contaminated soil at the outset of the experiment ranged from 0.024 ± 0.00 g/g to 0.056 ± 0.01 g/g. At the end of the biostimulation period, the mean concentration ranged from 0.010 ± 0.00 to 0.045 ± 0.00 g/g. For the biostimulated microcosms, the residual oil obtained prior to the initiation of biostimulation ranged from 0.022 ± 0.00 g/g of F₃ to 0.036 ± 0.00 g/g of F₁.

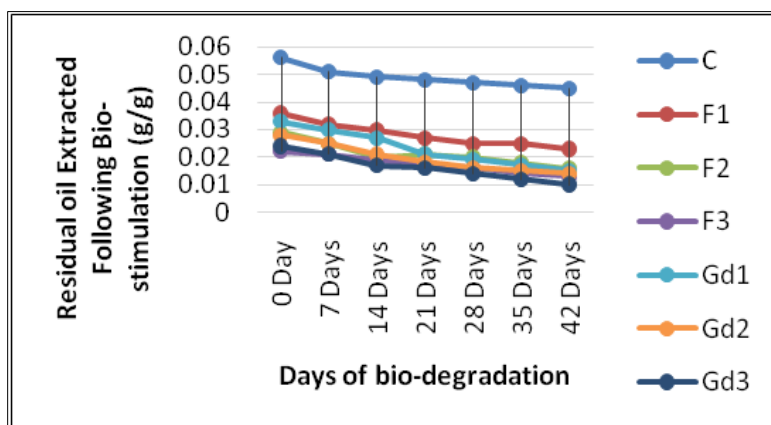


Figure 1: Biodegradation of oil following biodegradation with biostimulants

After 7 days of biodegradation following the biostimulation process using inorganic (NPK fertilizer) or organic (goat droppings) stimulants on oil-contaminated soil in the microcosm experiment, a range of 0.021 ± 0.00 g/g of F₃ to 0.032 ± 0.00 g/g of F₁ was obtained. After the 14th day of biostimulation, the amount of residual oil extracted ranged from 0.017 ± 0.00 g/g of Gd₃ to 0.030 ± 0.00 g/g of F₁.

The amount of residual oil obtained after 21 days of biodegradation ranged from 0.016 ± 0.00 g/g of Gd₃ to 0.027 ± 0.00 g/g of F₁, while 0.014 ± 0.00 g/g of Gd₃ to 0.025 ± 0.00 g/g of F₁ after 28 days of biostimulation. After 35 days of biostimulation, 0.012 ± 0.00 g/g of Gd₃ decreased to 0.025 ± 0.00 g/g of F₁, while 0.010 ± 0.00 g/g of Gd₃ decreased to 0.023 ± 0.00 g/g of F₁ after 42 days. The residual oil in the control microcosms decreased from 0.056 ± 0.002 to 0.0451 ± 0.00 g/g after 42 days of incubation. The Attenuated microcosms performed significantly worse than the biostimulated microcosms due to reduced levels of nitrogen and phosphorous in the contaminated soil (Robichaud *et al.*, 2019, Okoli *et al.*, (2015), which limited the growth of the inherent microbes.

Figure 2 shows the colony forming units (CFUs) of serially diluted incubated soil following biostimulation with inorganic and organic fertilizers. As shown in the figure, the greatest number of colony-forming units was 4.2×10^{10} CFU/g from the microcosm biostimulated with 45 g of goat dropping, while the least was 2.5×10^4 CFU/g from the attenuated microcosm.

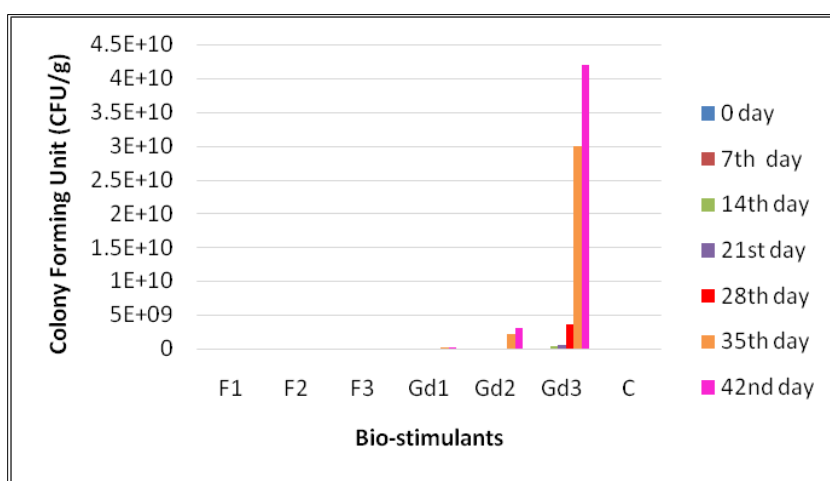


Fig 2: Colony forming units (CFUs) of serially diluted soil incubated following biostimulation with inorganic and organic fertilizers

The colony forming units of the microcosms biostimulated with inorganic fertilizer ranged from 2×10^3 to 7×10^5 CFU/g, while those of the organic fertilizer microcosms ranged from 3.7×10^4 to 4.2×10^{10} . The attenuated microcosm concentration ranged from 1.7×10^3 to 2.5×10^4 CFU/g. From the figure, the least performing microcosm was the attenuated microcosm since there was nothing that enhanced the growth of the inherent microorganisms for the utilization of the oil contaminant as the sole carbon source (Robichaud *et al.*, 2019). The microcosms biostimulated with 45 g of goat waste performed significantly better than the rest of the microcosms. This is due to the inherent nutrients, high organic matter content and presence of microorganisms in goat droppings, which enhance the growth of microbes in the soil for the sole use of oil as a

food, carbon and energy source (Ogujoifor *et al.*, 2021, Benchouk *et al.*, 2017). Hence, degrading the oil contaminant and reducing the residual oil extracted following the biostimulation process

From the ANOVA, it was observed that the interaction between the quantity of stimulant and days of biostimulation affected the residual oil obtained after extraction following biostimulation, which caused a variation of 0.98% with $p < 0.001$ and was considered extremely significant. The number of days of biostimulation affected the amount of residual oil obtained, accounting for 14.14% of the total variance, with a p value < 0.001 , and the effect was considered extremely significant, as observed by Awari *et al.* (2020). Additionally, the quantity of stimulant affected the residual oil extracted from the soil following biostimulation, causing 84.64% of the total variance, with a p value < 0.001 , which was considered extremely significant. Generally, the quantities of stimulants used for the biostimulation of inherent microorganisms affected the biostimulation process (Ahmad *et al.*, 2021) better than the number of days during which biodegradation occurs, thereby causing a positive effect on the biodegradation of oil contaminants, which led to a reduction in the quantity of oil extracted. The effectiveness of bioremediation was due to the inherent nutrients and time allowed for degradation to occur (Nwankwegu *et al.*, 2016)

Multiple comparisons between the residual oil extracted from the control microcosms and the biostimulated microcosms differed significantly on all days of extraction following biostimulation with the stimulants. The residual oils extracted from the individual biostimulated microcosms following biostimulation at 0, 7, 14, 28, 35 and 42 days were all significantly lower than those extracted from the control microcosms, with $p = 0.0001$ at $\alpha = 0.05$. This indicated that the stimulation was effective, as the residual oil obtained from these microcosms differed appreciably from and was lower than that of the attenuated microcosms due to little or no nitrogen, phosphorus or organic matter (Swapna *et al.*, 2021).

The control microcosm had a significant effect on the biodegradation of the contaminant throughout the incubation period at $p < 0.001$. The amount of residual oil extracted after 28, 35 and 42 days was significantly lower than that extracted after 7 days of incubation. Those extracted after 35 and 42 days were significantly lower than those extracted after 14 days of incubation ($p = 0.004$ and $p < 0.001$, respectively). The residual oils, 0.048 ± 0.01 and 0.047 ± 0.01 g/g, extracted after 21 and 28 days, respectively, were significantly greater than the 0.045 ± 0.00 g/g extracted after 42 days of incubation. This is due to the incubation period allowed for bioremediation (Nwankwegu *et al.*, 2016) and for the replication of the colony forming units of the inherent microbes, which ranged from 1.7×10^3 to 2.5×10^4 CFU/g during the incubation period of 42 days.

The enhancement of the biodegradation process using NPK fertilizer was significant after every 14 days of residual oil extraction following biostimulation. The ANOVA results showed that the different quantities of NPK fertilizer needed time to dissolve and add their respective nutrients to the soil to stimulate the inherent

microorganisms to biodegrade the oil contaminants in the soil. From the analysis, it could be significantly better to allow for at least 14 days of biostimulation before every extraction from the microcosms biostimulated with NPK, as this could give significant results than when the residual oil was extracted every 7 days, especially for microcosms biostimulated with 45 g of NPK.

There was a significant reduction in the quantity of residual oil extracted from microcosms biostimulated with 15 g of NPK as the days of biodegradation progressed, as shown in Fig. 1. The residual oils extracted after 21, 28, 35 and 42 days were significantly lower than those extracted after 14 days, while those extracted after 35 and 42 days were significantly lower than those extracted after 21 days at $p=.03$ and $p=.0002$. However, there was no significant difference among the 0.025 ± 0.00 , 0.025 ± 0.00 and 0.023 ± 0.00 g/g residual oils extracted after 28, 35 and 42 days of incubation, respectively. This implies that significant enhancement of the inherent microbes did not occur during the 28-42 day of incubation period and may be due to the lack of nutrient release from NPK as a result of total consumption of the nitrogen content of the fertilizer. However, from Fig 2, the microbes grew from 2×10^3 CFU/g at 0 day to 9×10^4 CFU/g at 42 day.

The amount of residual oil extracted from days 7 to 42 following biostimulation with 30 g of NPK was significantly lower than that extracted during the beginning of the biodegradation process ($p<0.001$). The growth of the microbes were significantly enhanced by 30 kg of NPK after 21-42 days of incubation, as the amount of residual oil extracted on these days was significantly lower than that extracted after 14 days of incubation. Moreover, the residual oil, 0.021 ± 0.00 g/g, extracted after 21 days was significantly greater than the 0.018 ± 0.00 and 0.016 ± 0.00 g/g extracted after 35 and 42 days of incubation, respectively. However, the values of 0.016 ± 0.00 g/g extracted after 42 days were significantly lower than those of 0.020 ± 0.00 and 0.018 ± 0.00 g/g extracted after 28 and 35 days, with $p=.000$ and $p=.02$, respectively. This indicates that 30 kg of NPK was able to release nitrogen up to the last day of the incubation period and enhanced the growth of the inherent microorganisms from 3.7×10^5 CFU/g during the period of biostimulation.

The mean residual oil content of 0.019 ± 0.00 to 0.013 ± 0.00 g/g extracted from 14-42 days of biodegradation following biostimulation with 45 kg of NPK was significantly lower than that of 0.022 ± 0.03 g/g extracted after 0 days of incubation. Thus, the growth of the microbes increased, which was followed by a decrease in the amount of residual oil. The microorganisms grew from 5×10^4 on the 14th day to 7×10^5 CFU/g after 42 days of incubation. This indicates that the inherent microbes grew as they utilized the oil contaminant as a sole carbon source for their growth and survival in the contaminated environment. The amount of residual oil extracted after 21-42 days of incubation was significantly lower than that extracted after the 7th day of incubation. The residual oil content of 0.019 ± 0.00 g/g extracted after 14 days was significantly greater than that of 0.016 ± 0.00 , 0.014 ± 0.00 and 0.013 ± 0.00 g/g extracted after 28, 35 and 42 days of incubation, respectively. The quantity of residual oil (0.017 ± 0.00

g/g) extracted after 21 days was significantly greater than 0.014 ± 0.00 and 0.013 ± 0.00 g/g extracted after 35 and 42 days, respectively. The residual oil, 0.013 ± 0.00 g/g, extracted after 42 days was significantly lower than the 0.016 ± 0.00 g/g extracted after 28 days, but there was no significant difference between the 0.014 ± 0.00 g/g extracted after 35 days and the 0.013 ± 0.00 g/g extracted after 42 days of incubation. This was revealed in the growth of the inherent microorganisms from 7×10^3 at 28 days to 7×10^5 CFU/g after 42 days of incubation.

As shown in Fig. 1, the residual oils extracted after 7, 14, 21, 28, 35 and 42-day incubation period in Gd₁ were significantly lower than the 0.033 ± 0.01 g/g extracted at 0 days. Hence, there was an enhancement of the biodegradation process by the inherent microbes as the microbes increased from 3.7×10^4 at 0 days to 1.6×10^8 CFU/g after 42 days. The residual oils obtained after 14, 21, 28, 35 and 42 days were significantly lower than those extracted after 7 days of incubation. Moreover, extractions after 28, 35 and 42 days of biostimulation were significantly lower and thus better enhanced the growth of the microbes than those after 21 days of biostimulation. The performance of the stimulant at the 21st to 42nd days of biostimulation significantly improved the stimulation for biodegradation every 14 days until the end of the biostimulation. This could be attributed to the organic matter content and microorganisms inherent in the goat droppings that were introduced into the soil, which improved the early stage of the biodegradation process (Ogujoifor *et al.*, 2021, Benchouk *et al.*, 2017). This same effect was observed for the 30 g goat dropping treatment. However, the inherent microorganisms grew from 2×10^5 to 3×10^9 CFU/g following biostimulation with 30 g of goat droppings for the entire incubation period of 42 days. ANOVA revealed that throughout the duration of biodegradation, the microcosms with 30 g of goat dropping performed significantly better than those containing 15 g of goat dropping. This can be correlated with the quantities of residual oil, 0.029 ± 0.00 g/g, extracted at 0 days to 0.016 ± 0.00 g/g at 42 days of incubation following biostimulation with 30 g of goat dropping. At 0 and 42 days of incubation, residual oils of 0.036 ± 0.01 and 0.023 ± 0.00 g/g, respectively were extracted following biostimulation with 15 g of goat droppings. Moreover, the range of colony-forming units of the inherent microbes, 2×10^5 to 3×10^9 CFU/g, in the microcosms biostimulated with 30 g of goat droppings was significantly greater than 3.7×10^4 to 1.6×10^8 CFU/g in microcosms biostimulated with 15 g of goat droppings. Generally, the quantity of organic matter in 30 g of goat droppings was greater than that in 15 g of goat droppings (Ogujoifor *et al.*, 2021).

After 42 days of biodegradation, 45 kg of goat manure significantly enhanced the growth of inherent microbes from 1.2×10^7 at 0 days to 4.2×10^{10} CFU/g due to the increase in organic matter and nutrients from the biostimulant (Ogujoifor *et al.*, 2021). The growth of these microorganisms, as shown in Fig. 2, was significantly greater than that of the other microorganisms and enabled the use of the oil contaminant as the sole carbon source. This caused a reduction in the residual oil extracted and was significantly lower than that of all the other quantities of biostimulants on the 42nd day of biodegradation (Awari *et al.*, 2020). The amount of residual oil extracted from

microcosms biostimulated with 45 g of goat droppings was significantly lower after 42 days of incubation. However, there was no significant difference between the biostimulation of 45 g of goat droppings and 45 g of NPK after 0, 7, 14, 21, 28 and 35 days of incubation. According to the ANOVA results, there was no significant difference in the performance of the 45 g of NPK and 30 g of goat droppings, but the 30 g of goat droppings performed better than the 30 and 15 g of NPK and 15 g of goat droppings after the entire incubation period of 42 days.

CONCLUSION

In this study, organic and inorganic fertilizers enhanced the growth of inherent microbes for the degradation of oil in contaminated soils. However, organic fertilizer better enhanced the remediation of contaminated soil than did inorganic fertilizer due to the prevailing high organic content of the goat droppings and the inherent presence of microorganisms. The attenuated microcosms indicated that time is needed for remediation to occur and without biostimulants there will be much delay in the resuscitation and growth of the inherent microbes for the sole use of spent oil as food, energy and carbon sources. This process prolongs the reduction and degradation of the spent oil in contaminated soils.

References

- Adams, G., Tawari-Fufeyin, P., and Ehinomen, I. (2014). Bioremediation of spent oil contaminated soils using poultry litter. *Research Journal in Engineering and Applied Science*; 3(2) 124-130.
- Ahmad M., Wang, P., Li J., Wang, R., Duan, L., Luo, X., Irfan, M., Peng, Z., Yin, L., Li, W. (2021). Impacts of Biostimulants on Pyrene Degradation, Prokaryotic Community Compositions, and Functions. *Environmental Pollution*; 289, pp 117863. <https://doi.org/10.1016/j.envpol.2021.117863>
- Alori, E.T., Gabasawa, A.I., Elenwo, C.E., and Agbeyegbe, O.O. (2022). Bioremediation Techniques as Affected by Limiting Factors in Soil Environment. *Frontiers in Soil Science*; 2. <https://doi.org/10.3389/fsoil.2022.937186>
- Awari, V.G., Ogbonna, D.N. and Nrior, R.R. (2020). Biostimulation Approach in Bioremediation of Crude Oil Contaminated Soil Using Fish Waste and Goat Manure. *Microbiology Research Journal International*; 30(1): 33-46. <https://doi.org/10.9734/mrji/2020/v30i130188>
- Azorji, J.N., Okechukwu, R.U., Udebuani, A.C. and Duru, C.M. (2022). An Appraisal of the Volume, Potential Use and Disposal Methods of Spent Engine Oil Generated in Disposal Methods of Spent Engine Oil Generated in Imo State, Southeast Nigeria *Journal of Environmental Treatment Techniques* 2022, Volume 10, Issue 3, Pages: 228-234
- Azubuikwe, C.C., Chikere, C.B. and Okpokwasili, G.C. (2016). Bioremediation Techniques—Classification Based on Site of Application: Principles, Advantages, Limitations and Prospects. *World J Microbiol/Biotechnol.* 2016; 32(11): 180.

- <https://doi.org/10.1007/s11274-016-2137-x>
- Bala, S.; Garg, D.; Thirumalesh, B.V.; Sharma, M.; Sridhar, K.; Inbaraj, B.S.; Tripathi, M. (2022). Recent Strategies for Bioremediation of Emerging Pollutants: A Review for a Green and Sustainable Environment. *Toxics*, 10, p 484.
<https://doi.org/10.3390/toxics10080484>
- Curiel-Alegre, S., B., Rumbo, C., Khan, A.H.A., Tamayo-Ramos J.A., Rad, C., Gallego, J.L.R. and Barros, R. (2022). Evaluation of Biostimulation, Bioaugmentation, and Organic Amendments Application on the Bioremediation of Recalcitrant Hydrocarbons of Soil. *Chemosphere*; 307, pp. 135638.
<https://doi.org/10.1016/j.chemosphere.2022.135638>.
- Da Silva Correa, H., Blum, C.T., Galvão, F. et al. (2022). Effects of Oil Contamination on Plant Growth and Development: A Review. *Environ SciPollut Res* 29, pp 43501–43515 <https://doi.org/10.1007/s11356-022-19939-9>
- Goswami M., Chakraborty P., Mukherjee K., et al. (2018). Bioaugmentation and Biostimulation: A Potential Strategy for Environmental Remediation. *J Microbiol Exp.* 2018; 6(5): 223-231. <https://doi.org/10.15406/jmen.2018.06.00219>
- Huang Y., Pan H., Wang Q., Ge Y., Liu W., and Christie P., (2019). *Enrichment of the Soil Microbial Community In The Bioremediation of a Petroleum-Contaminated Soil Amended With Rice Straw or Sawdust.* *Chemosphere.* 224, 265-271.
<https://doi.org/10.1016/j.chemosphere.2019.02.148>
- Kashif, S., A Zaheer, A., Arooj, F., and Farooq, Z. (2018). Comparison of heavy metals in Fresh and Used Engine oil, *Petroleum Science and Technology*; 36:(18), 1478-1481, <https://doi.org/10.1080/10916466.2018.1496105>
- Mitra, S., Chakraborty, A.J., Tareq, A.M., Emran, T.B., Nainu, F., Khusro, A., Idris, A. M., Khandaker, M.I., Osman, H., Alhumaydhi, F.A., Simal-Gandara, J. (2022). Impact of Heavy Metals on the Environment and Human Health: Novel Therapeutic Insights to Counter the Toxicity. *Journal of King Saud University – Science*; 34(3), 101865. <https://doi.org/10.1016/j.jksus.2022.101865>
- Mohamed, B.A., and Mohamed, S. (2023). Biobutanol Production from Agricultural Wastes. In: *Valorization of Wastes for Sustainable Development*, Elsevier, pp. 181-200, <https://doi.org/10.1016/B978-0-323-95417-4.00008-1>
- Mushtaq, M.U., Iqbal, A., Nawaz, I. et al. (2020). Enhanced uptake of Cd, Cr, and Cu in *Catharanthus roseus* (L.) G.Don by *Bacillus cereus*: Application of Moss and Compost to Reduce metal Availability. *Environ SciPollut Res* 27, pp 39807–39818.
<https://doi.org/10.1007/s11356-020-08839-5>
- Nna Orji, C., Abdulrahman, F.W., Isu, N.R. (2018a). Pollution Status of Heavy Metals in Spent Oil Contaminated Soil in Gwagwalada. *Asian Journal of Applied Chemistry Research* 1(2): 1-10, 2018. <https://doi.org/10.9734/AJACR/2018/42122>
- Nna Orji, C., Abdulrahman, F.W., Isu, N.R. (2018b). Assessment of Heavy Metal Pollution in Soil from an Automobile Mechanic Workshop in Abuja. *Asian Journal of Environment & Ecology* 6(1): 1-14, 2018.

- Nwankwegu, A.S., Orji, M.U., Onwosi, C.O. (2016). Studies on Organic and in-Organic Biostimulants in Bioremediation of Diesel-Contaminated Arable Soil. *Chemosphere*; 162: pp.148–156
<https://doi.org/10.1016/j.chemosphere.2016.07.074>
- Nwite, J.N. and Alu, M.O. (2015). Effect of Different Levels of Spent Engine Oil on Soil Properties, Grain Yield Of Maize and its Heavy Metal Uptake in Abakaliki, South Eastern Nigeria. *Journal of Soil Science and Environmental Management*; Vol. 5(4), pp. 44-51, <https://doi.org/10.5897/JSSEM12.070>
- Ogbozige, F.J. (2024). Modelling Kinetics Of Selected Organic Amendments on Biostimulation of Crude Oil Contaminated Soil. *Bayero Journal Of Engineering And Technology*; 19(1):1-11
- Ogujoifor, I.F., Machido, D.A., & AttaH. I. (2021). Effect of the Application of Goat Dung on the Bioremediation of Polluted Soil by Hydrocarbon Degrading Bacteria: a Microcosm Study. *Fudma Journal of Sciences*, 5(1), pp 278-287.
<https://doi.org/10.33003/fjs-2021-0501-566>
- Okoli, C.P., Adewuyi, G.O., Zhang, Q., Zhu, G., Wang, C., and Guo, Q. (2015). Aqueous scavenging of Polycyclic Aromatic Hydrocarbons Using Epichlorohydrin, 1, 6-HexamethyleneDiisocyanate and 4 4-EthyleneDiphenylDiisocyanate Modified Starch: Pollution Remediation Approach. *Arabian Journal of Chemistry*; 22:1519–1528.
- Patel, A.B., Shaikh. S. Jain, K., R., Desai, C., Madamwar, D. (2020). Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. *Front Microbiol.*; 11: 562813. <https://doi.org/10.3389/fmicb.2020.562813>
- Robichaud, K., Lebeau, M., Martineau, S., and Amyot, M. (2019). Bioremediation of Engine-oil Contaminated Soil Using Local Residual Organic Matter. *Peer J*.7: e7389. <https://doi.org/10.7717/peerj.7389>
- Romantschuk, M., Lahti-Leikas, K., Kontro, M., Galitskaya, P., Talvenmäki, H., Simpanen, S., Allen, J. A., and Sinkkonen, A. (2023). Biostimulation, Bioremediation, Biodegradation, in Situ, Bacteria, Organic Contaminants. *Frontiers in Microbiology*; 4: 1258148.
<https://doi.org/10.3389/fmicb.2023.1258148>
- Sahota, N.K. and Sharma, R. (2022). Bioremediation: Harnessing Natural Forces for Solid Waste Management. In: Baskar, C., Ramakrishna, S., Baskar, S., Sharma, R., Chinnappan, A., Sehrawat, R. (eds) *Handbook of Solid Waste Management*. Springer, Singapore. https://doi.org/10.1007/978-981-16-4230-2_107
- Sales da Silva I.G, Gomes de Almeida F.C, Padilha da Rocha e Silva N.M, Casazza A.A, Converti A, Sarubbo L.A. (2020). *Soil bioremediation: Overview of technologies and trends. Energies* 13:4664. <https://doi.org/10.3390/en13184664>
- Sonawane, J.M.; Rai, A.K.; Sharma, M.; Tripathi, M.; Prasad, R. (2022). Microbial Biofilms: Recent Advances and Progress in Environmental Bioremediation. *Sci. Total Environ.*, 824, 153843.

- Swapna, A.A., RajaniVijayamma, R. and Radha, D.S. (2021). Effects Of Spent Engine Oil On Soil Characteristics And Selected Phytochemicals In Amaranthus Hybridus. <https://doi.org/10.21203/rs.3.rs-939651/v1>
- Thenmozhi R, Arumugam K, Nagasathya A, Thajuddin N, Paneerselvam A. Studies on Mycoremediation of Used Engine Oil Contaminated Soil Samples. *Advances in Applied Science Research*. 2013; 4(2): 110- 118.
- Tripathi, M.; Singh, D.N.; Prasad, N.; Gaur, R. (2021). Advanced Bioremediation Strategies for Mitigation of Chromium and Organics Pollution in Tannery. In *Rhizobiont in Bioremediation of Hazardous Waste*; Kumar, V., Prasad, R., Kumar, M., Eds.; Springer: Singapore, pp. 195–215.
- Tumolo M, De Paola D, Uricchio VF, Ancona V. (2023). Biostimulation Effect of Different Amendments on Cr(Vi) Recovering Microbial Community. *N Biotechnol*; 78: pp. 29-41. <https://doi.org/10.1016/j.nbt.2023.09.007>
- Udume, O. A., Abu, G. O., Stanley, H. O., Vincent-Akpu, I. F., Momoh, Y., Eze, M. O. (2023). Biostimulation of Petroleum-Contaminated Soil Using Organic and Inorganic Amendments. *Plants (Basel)*. Jan 17; 12(3):431. <https://doi.org/10.3390/plants12030431>
- [Zhang](#),c. [Daoji Wu](#), and [Huixue Ren](#)(2020) Bioremediation of Oil Contaminated Soil Using Agricultural Wastes via Microbial Consortium. *Sci Rep*. 2020; 10:9188. <https://doi.org/10.1038/s41598-020-66169-5>