Journal of Advances in Microbiology

Volume 22, Issue 12, Page 124-132, 2022; Article no.JAMB.101974
ISSN: 2456-7116

Comparative Analysis of the Impact of Crude Oil and Kerosene on Soil Microbiota and the Bio-utilization Potentials of the Indigenous Microorganisms

F. C. Akubuenyi [a*]

[a] *Cross River University of Technology, Calabar, Cross River State, Nigeria.*

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAMB/2022/v22i12733

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/101974

Original Research Article

Received: 25/10/2022
Accepted: 28/12/2022
Published: 31/12/2022

ABSTRACT

This research is focused on evaluating the impact of crude oil and kerosene on soil microbiota. The enumeration of total heterotrophic bacterial count revealed that the soil impacted with crude oil and kerosene yielded low microbial counts ranged from 3.2×10^6 CFU/g, 3.2×10^5 to 2.6×10^3 CFU/g while that of total coliform count obtained from the samples ranges from 2.6×10^5CFU/g to 2.2×10^3 CFU/g. and total fungal count ranged from 2.0×10^2 CFU/g to 1.4×10^2 CFU/g. The genera of bacteria isolated from soils impacted with crude oil and kerosene belong to the genera: *Pseudomonas* spp., *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli, Micrococcus* spp., *Klebsiella* spp., *Corynebacterium and Salmonella* species while *Aspergillus* spp., *Alternaria* spp., *Candidia* spp., *Fusarium* spp., *Trichoderma* spp., *Mucor* spp., *Penicillium* spp., *Cephalosporium* spp., *Rhizopus* spp. are the isolated fungi. The percentage occurrence revealed that *Pseudomonas* spp. and *Escherichia coli* had the highest prevalence of 23.1%, followed by

**Corresponding author: E-mail: felixakubuenyi@gmail.com;*

Staphylococcus spp. (7.7%), *Salmonella* spp. (7.7%), *Bacillus* spp. (7.7%), *Micrococcus* spp. (7.7%) etc. While the fungal isolates had the following prevalence; *Aspergillus* spp. (12.5%), *Penicillium* spp. (12.5%), *Candida* spp. (12.5%), *Mucor* spp. (12.5%), etc. The results obtained indicates that crude oil and kerosene have a negative impact on the microbiota of the soil. The combined effect of the compounds is more harmful than their individual effects. This result implies that farmlands impacted with these products would not be suitable for agricultural purposes since the nitrogen fixing bacteria and other soil enriching microorganisms must have been either killed or inactivated by the toxicity of compounds.

Keywords: Crude oil and kerosene; comparative analysis; bio-utilization; prevalence; soil microbiota.

1. INTRODUCTION

Environmental pollution has been on the rise in the past few decades owing to increased human activities on energy reservoirs, unsafe agricultural practices and rapid industrialization [1]. Amongst the pollutants that are of environmental and public health concerns due to their toxicities are: heavy metals, nuclear wastes, pesticides, greenhouse gases, crude oil and refined petroleum products like kerosene. Environmental pollution associated with petroleum hydrocarbons is one of the world's most common environmental problems [2;3]. Crude oil spillage is one of the most serious environmental problems currently facing the oil producing areas and occurs in large scale in some communities. The oil spillage could be attributed to different causes such as accidental spills, leakage, and vandalization of pipelines and corrosion of pipelines which allow the seepage of crude oil into the environment [4]. The effect of oil spillage on land has become a global issue as land play an important role in the sustenance of man [5]. When land is contaminated, the contaminants change the chemical and biological properties of the soil and are toxic to some soil microorganisms [6;3;7]. The chemical composition of crude oil and kerosene varies significantly and can have diverse effects on different organisms within the ecosystem and these differences are due to variation in concentration levels of the various constituents the contamination changes the physiochemical and biological properties of the soil because the oil may be toxic to some soil microorganisms and plants [8].

Contamination of soil by crude oil could lead to reduced microbial density and activities. Soil conditions of agricultural land, microorganisms as well as plants are damaged or altered by any contact with crude oil [9]. Excess oil in soil limits the availability of nitrogen [10]. Soils that are polluted with petroleum hydrocarbons (PHCs) and associated products are different from unpolluted soils due to changes in their biological as well as physicochemical properties. Petroleum hydrocarbon may interfere with the plant-fungus relationship by altering the soil environment so that movement of diffusible chemical signals such as auxins is prevented. Soil biological activity, including soil microbial biomass, is influenced by a range of physiochemical, environmental parameters and perturbations. Therefore, soil microbial activity may be used to assess disturbed soil [11]. Biologically and biochemically mediated processes in soils are of utmost importance to ecosystem functions. There is a huge diversity of organisms belonging to different taxonomic and physiologic groups that interact at different levels within the community in soil biota [12;13;14].

In this biota, soil microorganisms constitute a source and are the driving force behind many soil processes, including the transformation of organic matter, nutrient release, transformation of carbon, nitrogen, phosphorus and sulphur, degradation of xenobiotic compounds, the formation of soil physical structure and enhanced nutrient uptake by plants [12]. They degrade organic pollutants by using them as their carbon and energy source. And more than 200 species of bacteria, fungi, and even algae are capable of degrading hydrocarbons because of their ubiquitous nature. Onwurah et al. [15], reported that *Pseudomonas, Micrococcus* and *Bacillus* can metabolize the toxic components of crude oil, leading to degradation. Crude oil and refined petroleum products pollute our farmlands and aquatic environment thereby hindering the production of crops growth and aquatic lives and may negatively affect the activities of soil microorganisms responsible for the good crop yield and soil fertility. Crude oil and refined petroleum products may also affect the agricultural productivity of ecosystems by causing dislocation in the biogeochemical cycle where microorganisms participate. Due to the need for sufficient information in this area, this study was designed to analyze the comparative toxicity of crude oil and kerosene on soil microbiota.

2. MATERIALS AND METHODS

2.1 Sample collection

Soil samples were collected with the aid of a sterilized auger from Cross River University of Technology Campus, Calabar agricultural farmland. The Bonny light crude oil was collected from EPZ depot, Calabar, Cross River State, and kerosene was collected from Nigeria National Petroleum Corporation (NNPC) station, Calabar, Cross River State in a sterilized McCartney bottle. All the samples collected were taken to Microbiology laboratory for further investigation.

2.2 Microbiological Analysis

2.2.1 Enumeration of total heterotrophic bacterial (THB) counts

The total heterotrophic bacterial loads of soil samples were determined using pour plate technique as described by Cheesbrough [16]. Serial dilution (10^1-10^{10}) was done, from the soil samples. Exactly one millimeter (1ml) was taken from each selected dilution (10^2, 10^4, 10^6) into sterile petri dishes molten nutrient agar at 37^oC for 24 hours. Thereafter, plates with colony growth were counted and expressed in colony forming unit.

2.2.2 Determination total coliform counts

The total coliform bacterial loads of soil samples were determined using pour plate technique as described by Cheesbrough [16]. Serial dilution (10^1-10^{10}) was done, from the soil samples. Exactly one millimeter (1ml) was taken from each selected dilution (10^2, 10^4, 10^6) into sterile petri dishes with molten MacConkey agar at 37^oC for 24 hours. Thereafter, plates with colony growth were counted and expressed in colony forming unit.

2.3 Screening for hydrocarbon utilizing bacteria

The hydrocarbon utilizing potential of sample was carried out under aerobic conditions following the method adopted by Chikere and Ekwuabu [17]. A loop full of 24 hours old culture of each hydrocarbon utilizing bacteria were inoculated into BH (Bushnell-Hass) broth containing 1% (v/v) crude oil. Biodegradation was recorded with the discolouration of Dichlorophenolindophenol (DCPIP) oxidation reduction reagent after 14 days of incubation at 30°C.

2.3.1 Biochemical characterization and identification of bacterial isolates

Pure isolates from the corresponding agar slants were characterized and identified using morphological, biochemical and physiological characterization as described by Cheesbrough [16].

2.3.2 Determination total fungal count

The total fungal loads of soil samples were determined using spread plate technique. Serial dilution (10^1-10^{10}) was done, from the soil samples. Exactly one millimeter (1ml) was taken from each selected dilution (10^2, 10^4, 10^6) into sterile petri dishes with molten Potato dextrose agar at 37^oC for 72 hours Thereafter, plates with colony growth were counted and expressed in colony forming unit.

2.3.3 Identification of fungal isolates

The colonial morphologies of the fungal isolates on Sabouraud Dextrose Agar were observed for colour and type of growth following microscopic identification as described by Murray et al. [18]. This was done by preparing a wet mount using lactophenol cotton blue to observe the microscopic characteristics of the fungi such as type of hyphae (whether septate or non-septate). A drop of Lactophenol blue was placed on a clean microscope slide, with the aid of an inoculating needle, a small portion of growth midway between the colony center and edge was gently removed and placed on the dropped Lactophenol blue on the slide. With two sterile dissecting needles, the fungus was gently teased apart so that it is thinly spread out in the Lactophenol. After which a coverslip was placed on the edge of the Lactophenol and slowly lowered. Then placed under the microscope for examination. Both microscopic and macroscopic features of the fugal isolates were matched based on the mycological atlas for fungal identification [18].

2.4 Screening for hydrocarbon utilizing fungi

In order to screen for hydrocarbon utilizing fungi, the fugal isolates were transferred into plates of Rose-Bengal Chloramphenicol (RBC) agar and Bushnell-Hass (mineral salt) agar supplemented with 0.05% (v/v) streptomycin. Sterile Whatman filter papers were soaked in medium containing crude oil and were aseptically placed into the lids of inoculated Bushnell-Haas agar plates; this technique is called the vapour phase transfer [19]. After the inoculation procedures, RBC agar plates and Bushnell-Haas agar were incubated at 30ºC for 7days and 14 days respectively and isolates counted and recorded.

3. RESULTS

The enumeration of the samples analyzed had different microbial loads. The total heterotrophic bacterial count of the samples from different locations revealed that the crude oil and kerosene has effect on the soil microbiota as the soil samples without crude oil and kerosine yielded high bacterial count ($3.7x10^9$CFU/ml) when compared to soil samples impacted with crude oil and kerosine ($2.6x10^3$CFU/ml) as shown in Table 1.

The total coliform count of the samples showed that the soil samples impacted with crude oil and kerosene yielded low coliform count ($2.4x10^3$CFU/ml) when compared to the control ($3.2x10^7$CFU/ml) as displayed in Table 2.

The fugal screening also revealed that the Crude oil and kerosene had a negative impact on fugal loads as the soil without Crude oil and kerosene had higher fungal load ($2.2x10^4$ CFU/ml) compared to the soil samples impacted with Crude oil and kerosine ($1.4x10^2$ CFU/ml) as seen in Table 3.

Biochemical characterization and identification processes revealed as shown in Table 4 that *Salmonella* species, *Micrococcus* species, *Bacillus* species, *Staphylococcus* species, *Escherichia coli, Klebsiella* species etc. were the suspected bacterial isolates observed in the samples.

The degradative potentials of hydrocarbons by bacterial isolates are presented in Table 5.

The prevalence of the bacterial isolates revealed that *Pseudomonas* species and *Escherichia* coli had the highest number of occurrences followed by *Streptococcu*s specie, *Bacillus* specie etc. as displayed in Table 6.

The total fungal screening (Table 7) revealed that *Aspergillus* species, *Mucor* species, *Penicillium* species etc. were the fungal isolate obtained.

The degradative potentials of hydrocarbon utilizing fungi isolates are presented in Table 8.

The prevalence of fungal isolates showed that the fungal species present in this study, *Aspergillus* species, *Penicillium* species, *Mucor* species, *Candida* species etc as displayed in Table 9.

Table 1. Total heterotrophic bacterial (THB) count of soil samples impacted with Crude oil and kerosine respectively and soil samples without Crude oil and kerosene (control)

Sample Code	samples	THB counts (CFU/ml)
SC	Soil + Crude oil	3.2×10^5
SK	Soil + Kerosine	3.0×10^6
SCK	Soil + crude oil+ Kerosine	2.6×10^3
SS	Soil (control)	$3.\ 7 \times 10^9$

Table 2. Total Coliform (TC) count of soil samples impacted with crude oil and kerosene respectively and soil samples without Crude oil and kerosene (control)

Sample Code	Samples	TC Counts (CFU/ml)
SC	Soil + Crude oil	2.2×10^5
SK	Soil + Kerosine	2.6×10^5
SCK	Soil + crude oil+ Kerosine	2.4×10^3
SS	Soil (control)	$3.\ 2 \times 10^7$

Table 3. Total Fungal (TF) count of soil samples impacted with Crude oil and kerosine respectively and soil samples without crude oil and kerosene (control)

Sample Code	Samples	TF count (CFU/ml)
SC	Soil + Crude oil	2.0×10^2
SK	Soil + Kerosine	1.8×10^2
SCK	Soil + crude oil+ Kerosine	1.4×10^2
SS	Soil (control)	2.2×10^4

Table 4. Biochemical characterization and identification of bacterial isolates

Morphological Characteristics	Cell shape	Gram reaction	Oxidase	Catalase	Citrate	Indole	Methyl red	Vogues Proskaeur	Glucose	Lactose	Sucrose	TSI slant	TSI butt	Gas	H_2S	Suspected organisms
Circular, pinkish	Cocci in clusters	-	+	+	-	-	+	-	-	-	+	Y	Y	+	-	*Staphylococcus* species
Creamy, circular, rough	Long rods	+	+	+	-	-	-	-	-	-	-	R	Y	+	-	*Bacillus* species
Circular, transparent, Opaque	Short rods	-	+	+	-	+	+	+	-	-	+	R	Y	+	+	*Corynebacterium* species
Spreading, colorless, rough	Rods in chains	-	-	+	+	-	-	-	+	-	+	Y	Y	+	-	*Pseudomonas* species
Colorless, rod-like	Tiny rods	-	+	+	+	-	-	-	-	+	+	Y	Y	+	-	*Klebsiella* species
Creamy, long, smooth	Rods in single	-	-	+	-	+	+	-	-	+	-	Y	Y	-	-	*Escherichia coli*
Circular, creamy, curve-shaped	Cocci	+	+	+	-	+	-	-	-	+	-	R	Y	-	-	*Micrococcus* species
Convex, curve-shaped	Rods	-	+	+	+	-	+	+	+	+	-	Y	R	-	+	*Salmonella* species
Creamy, long, smooth	Rods	-	+	+	-	+	+	-	-	+	-	Y	Y	-	-	*Escherichia coli*
Circular, raised, rough	Cocci	-	-	+	-	+	+	-	-	+	-	Y	Y	+	-	*Streptococcus* species
Spreading, rough, colorless	Rods in chains	-	+	+	+	-	-	-	+	-	+	Y	Y	+	-	*Pseudomonas* species
Creamy, long, smooth	Rods	-	+	-	+	+	+	-	-	+	-	Y	Y	-	-	*Escherichia coli*
Spreading, rough, colorless	Rods in chains	-	+	+	+	-	+	-	-	-	+	Y	Y	+	-	*Pseudomonas* species

Table 5. Hydrocarbon utilization bacteria

S/N	Organisms	Dedradative screening
1	*Pseudomonas* spp	+
2	*Streptococcus* spp	-
3	*Salmonella* spp	-
4	*Bacillus* spp	-
5	*Escherichia* coli	+
6	*Micrococcus* spp	-
7	*Klebsiella* spp	+
8	*Staphylococcus* spp	-
9	*Corynebacterium* spp	-

Table 6. The prevalence of bacterial isolates obtained from this study

Organisms	Frequency	Percentage (%)
Pseudomonas spp	3	23.1
Streptococcus spp	1	7.7
Salmonella spp	1	7.7
Bacillus spp	1	7.7
Escherichia coli	3	23.1
Micrococcus spp	1	7.7
Klebsiella spp	1	7.7
Staphylococcus spp	1	7.7
Corynebacterium spp	1	7.7
Total	13	100

Table 7. Characterization and identification of fungal isolates

Morphological characteristics	Microscopic examination	Suspected organisms
Yellow-green, blue-green, grey-green, filamentous growths that turn black sporulation	Long septate hyphae with swollen conidiopore	*Aspergillus* spp.
Green with raised rough surface colonies	Septate and branch brush like conidial head condiophore	*Penicillium* spp
White wooly growth that turns darker as it sporulates	Non septate hyphae with straight Sporangiophores spherical spores	*Mucor* spp.
Olivaceous-black, grewish colour on plate	Multicelled, matalae with phialide	*Alternaria* spp
Yellowish green on plate	Branch phialides with chlamydosphores	*Trichoderma* spp.
Whitish on petri dish	Multicelled-metalae with pseudohyphae form	*Candida* spp.
Grey colour on plate	Conidia bearing phialide	Cephalosporium spp.
Shiny velvet black fluffy growth	Curve septate hyphae with conidia	*Curvularia* spp.
Whitish felt mycelium	Branched conidiophores, smooth and rough conidia in pairs and chain	*Fusarium* spp.

Table 8. Hydrocarbon utilization fungi

S/N	Organisms	Degradative screening
1	*Aspergillus* spp	+
2	*Penicillium* spp	+
3	*Mucor* spp	+
4	*Aiternaria* spp	-
5	*Trichodermma* spp	-
6	*Candida* spp	+
7	*Cephalosporium* spp	+
8	*Curvularia* spp	-
9	*Fusarium* spp	+

Table 9. Percentage of occurrence of fungal isolate

Organisms	Frequency	Percentage (%)
Aspergillus spp	1	12.5
Penicillium spp	1	12.5
Mucor spp	1	12.5
Aiternaria spp	1	12.5
Trichodermma spp	1	12.5
Candida spp	1	12.5
Cephalosporium spp	1	12.5
Curvularia spp	1	12.5
Total	8	100

4. DISCUSSION

The results obtained showed that soil samples impacted with crude oil and kerosene yielded low microbial population and diversity when compared to soil samples without kerosine and crude oil. The low microbial population obtained from soil impacted with crude oil and kerosene could be traced to the toxicity of crude oil and kerosene. This is in agreement with the reports of Mona et al. [20], who stated that excessive levels of heavy metals (hydrocarbons) can be damaging to organisms. The result corroborates the findings of Akubuenyi [21] which reported that the total heterotrophic bacterial and fungal counts of soil samples impacted with engine oil decreased when compared to unimpacted soil. Also, the reports of Shabir et al. [22], clearly stated that spills of crude oil and other hydrocarbons can lead to a significant decline in quality of soil and make it unfit for use. In a related study, Nyoyoko et al. [23] reported that higher concentration of crude oil has an adverse effect on fungi diversity while enhancing the population of fewer fungi.

The ability of some microorganisms to survive the impact of these hydrocarbons (Crude oil and Kerosene) could be traced to the fact that those microbes developed the physiological ability to use petroleum products as a source of carbon and energy. This corroborates the reports of Vinothini et al. [24], who opined that refined petroleum supply only carbon and energy source to resident microbes while crude oil supplies in addition to carbon and energy, mineral nutrients such as nitrogen, sulphur. These nutrients stimulate the growth of the hydrocarbon utilizing microorganisms, thereby enhancing their potentials for application in bioremediation. Raghad et al. [25] while working on the effect of petroleum hydrocarbons contamination on soil microorganisms and biodeterioration deposited that soil contamination with hydrocarbons significantly increased the count hydrocarbon utilizing bacteria.

The biochemical and identification processes revealed that the probable bacterial organisms present in the samples. They were found to include the following genera: *Pseudomonas species, Bacillus species, Staphylococcus* *species, Streptococcus* species*, Escherichia coli, Micrococcus* species*, Klebsiella* species and *Corynebacterium and Salmonella species.* While *Aspergillus species, Alternaria* species*, Candida* species*, Trichoderma* species*, Fusarium* species, *Mucor* species*, Penicillium* species*, Cephalosporium* species *Curvularia* species were the genera of fungi present in the sample. This result is in agreement with the reports of previous studies [26], were a total of twelve fungal isolates belonging to the genera; *Aspergillus species, Alternaria* species*, Candidia* species*, Fusarium* species*, Trichoderma* species*, Mucor* species*, Penicillium* species*, Cephalosporium* species*, Rhizopus species, Rhodoturula* species *Curvularia* species*, Cladosporium* specie and nine bacterial isolates belonging to the genera *Pseudomonas species, Bacillus species, Staphylococcus species, Streptococcus* species*, Escherichia coli, Micrococcus* species*, Klebsiella* species and *Corynebacterium and Salmonella* species, were isolated in the Niger Delta region. Akubuenyi [2] also obtained similar microorganisms from the study of the impact of engine oil on soil microbial community around mechanic workshops. Nyoyoko et al. [23] and Sarmadi et al. [27] in separate studies isolated and identified similar microorganisms.

Petroleum contamination of soil is particularly a serious problem because of the impact it has on soil functioning, and on the whole ecosystem. Agricultural soils, which are continually exploited to produce food and fodder, are particularly sensitive to contamination, as agricultural soils generally display poor resilience that is, they are incapable of recovering from any type of aggression, and contamination. This is in agreement with the position of Sarmadi et al. [27] that the kerosene contamination of soil leads to reduction of optimum moisture content of the soil. Research has shown that kerosene contamination increases cohesion, decreased internal strength of soils, and caused reduction in the permeability of soil [27]. The effect of crude oil and kerosene brought about alterations to soil functioning which reduces soil fertility and its consequent low crop yield.

5. CONCLUSION

Crude oil and kerosene contain substances that impact negatively on the growth of microbiota of soil origin. The combined effect of the compounds is more harmful than their individual effects. Because soil microorganisms are major contributors of soil fertility, then farmlands impacted with these products would not be suitable for agricultural purposes since the nitrogen fixing bacteria and other soil enriching microorganisms must have been either killed or inactivated by the toxicity of compounds. Hence, crude oil and kerosene should not be emptied into farmlands without proper treatment.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Hadia-e-Fatima Ahmed A. Heavy metal pollution- A mini view. Journal of Bacteriology and Mycology. 2018;6(3): 179-181.
2. Benal T, Shivani K, Pagre RL, Chitnis S. Study of prevailing of deuteromycetous fungi on the petro-polluted soil, International Research Journal of Biological Sciences. 2014; 3(11):28-31.
3. Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P, Yu H. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A Perspective Analysis. Frontiers in Microbiology. 2018;9: 145-156.
4. Wang C, Liu X, Guo J, Lv Y, Li Y. Biodegradation of marine oil spill residues using aboriginal bacterial consortium based on Penglai 19-3 oil spill accident. China. Ecotoxicology and Environmental Safety. 2018;159.
5. Abii TA, Nwosu PC. The effect of oil-spillage on the soil of Eleme in Rivers State of the Niger Delta area of Nigeria. Research Journal of Environmental Sciences. 2009;3(3): 316-320.
6. Hentati OR, Lachhab R, Ayadi M, Ksibi M. Toxicity assessment for petroleum-contaminated soil using terrestrial invertebrates and plant bioassays, Environmental Monitoring and Assessessment, 2013;185:2989-2998.
7. Udeani CKT, Obroh AA, Okwuosa NC, Achukwu UP, Azubike N. Isolation of bacteria from mechanic workshops soil environment contaminated with used engine oil. African Journal of Biotechnology. 2009;8(22):6301-6303.
8. Minai-Tehrani D, Herfatmanesh A. Biodegradation of aliphatic and aromatic

fractions of heavy crude oil-contaminated soil: A pilot study. Bioremediation Journal. 2007;11(2): 71-87.

9. Onuoha SC, Victor O, Uraku AJ, Uchedu DO. Biodegradation potentials of hydrogen degraders from waste-lubricating Oil-spilled soils in Ebonyi State, Nigeria. International Journal of Agriculture and Biology. 2012; 13(4): 586-590.
10. John RC, Akpan MM, Essien JP, Ikpe DL. Variation in Rhizosphere Microbiological Properties of Tropical Legumes Grown on Oil Contaminated Wetland Utisoil. Nigerian Journal of Microbiology. 2010; 24(1):2081-2087.
11. Labud V, Garcia C, Hernander T. Effect of hydrocarbon pollution on the microbial properties of sandy and a clay soil. Chemosphere. 2007;66: 1863–1871.
12. Lopes AR, Faria C, Prieto-Fernández A, Trasar-Cepeda C, Manaia CM, Nunes OC. Comparative study of the microbial diversity of bulk paddy soil of two rice fields Subjected to organic and conventional farming. Soil Biology and Biochemistry. 2012;43(1):115-125.
13. Dombrowski N, Donaho JA, Gutierrez T, Seitz KW, Teske AP, Baker BJ. Reconstructing metabolic pathways of hydrocarbon-degrading bacteria from the Deep-water Horizon oil spill. Nature Microbiology. 2016;1:16057.
14. Dvorak P, Nikel P, Damborský J, de Lorenzo V. Bioremediation 3.0: engineering pollutant-removing bacteria in the times of systemic biology. Biotechnol Advances. 2017; 35:845–866.
15. Onwurah INE, Ogugua VN, Onyike NB, Ochonogor AE, Otitoju OF. Crude oil spills in the environment, effects and some innovative clean-up biotechnologies. Int. J. Environ. Res. 2007;1(4) 307-320.
16. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, United Kingdom. 2006;62-70.
17. Chikere C, Ekwuabu C. Culture-Dependent Characterization of Hydrocarbon Utilizing Bacteria in Selected Crude Oil-Impacted Sites in Bodo, Ogoni land, Nigeria. African Journal of Environmental Science and Technology. 2014;8401-406.
18. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, (Ed.). Manual of clinical microbiology, 8th edition. ASM Press, Washington, DC; 2003.
19. Chikere CB, Azubuike CC. Catechol-2,3-dioxygenase screening in putative hydrocarbon utilizing bacteria. Int. Res. J. Microbiol. 2013;4 (1): 1-6.
20. Mona SZ, Nadia E, Hamman AM, Shalaby SI. Aquatic bioremediation of metals. Life Science Journal. 2014;11(4):66-72.
21. Akubuenyi FC. Determination of the influence of used engine oil on soil microbial community around mechanic workshops. International Journal of Biotech Trends and Technology. 2019; 9(4):52-58
22. Shabir G, Afzal FM, Anwar R, Tahseen ZK. Biodegradation of kerosene in soil by a mixed bacterial culture under different nutrient conditions. International Journal of Biodeterioration Biodegradation. 2008;61: 161.
23. Nyoyoko VA, Anyanwu C, Christopher MA. Evaluation of the effect of crude oil and kerosene on soil microbial population. International Journal of Applied and Natural Sciences. 2022; 3:117-138.
24. Vinothini C, Sudhakar S, Ravikumar R. Biodegradation of petroleum and crude oil by *Pseudomonas putida* and *Bacillus cereus*. International Journal of Current Microbiology and Applied Sciences. 2015; 41:318-329
25. Raghad NH, Mehjin AA. Effect of petroleum hydrocarbons contamination on soil microorganisms and biodegradation. Raf. J. Sci. 2019;28(1);13-22.
26. Nseabasi NO, Antai SP. Toxic effect of kerosene contamination on the survival of bacteria and fungi species in soil from Niger delta. International Research Journal of Microbiology. 2012;3(12): 382-387.
27. Sarmadi MS, Zohrevand P, Rezaee M. Effect of kerosene contamination on the physical and mechanical properties of sandy soil. Inno. Infrast. Solut. 2019;4:7.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/101974