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Bioremediation of Surface Water Contaminated with Hydrocarbons in a Non-oil Producing Area

W. O. Medjor[1*], N. O. Namessan[2], C. A. Wepuaka[3], G. Foshen[1] and A. Suleiman[1]

[1]Department of Chemistry, Taraba State University, Jalingo, Taraba State, Nigeria.
[2]Department of Agronomy, Taraba State University, Jalingo, Taraba State, Nigeria.
[3]Department of Chemistry, School of Science Education, Federal College of Education (Technical), Bichi, Kano State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author WOM designed the study, performed the statistical analysis; wrote the protocol and the first draft of the manuscript. Authors NON, CAW, GF and AS managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The study investigated the microbial degradation, kinetic and physicochemical studies of surface water contaminated with hydrocarbons of common interest (domestic purpose kerosene and diesel) by mixed culture of microorganism at ambient temperature (28-32°C).

Methodology: Four experimental set-ups were arranged of which two served as the control experiments. In this work, microbiological analyses were not carried out but organic supplement used have been reported by earlier investigators to contain heterotrophic bacteria, hydrocarbon degraders and fungi that are capable of utilizing hydrocarbons as carbon substrates. Bioremediation was principally monitored using reduction in TPH and the control experiments. Kinetic study and physicochemical analysis were also carried out.

Place and Duration of Study: Experiments were carried out in the Central Science Laboratory Complex, Taraba State University- Jalingo Nigeria. The study was carried out in a

**Corresponding author: E-mail: weltime.medjor@yahoo.com;*

period of 4 months.
Study Design: In the first experiment, 37.5 g of the organic supplement that served as a source of nutrients and microorganisms was added to surface water simulated with diesel in a plastic container. In a second experiment, the diesel was replaced with domestic purpose kerosene (DPK). In the third and fourth experimental set-ups, the plastic containers had only DPK and diesel respectively without the organic amendment and served as controls. The four experimental set-ups were allowed to stand for 21 days for possible bioremediation.
Results: The results obtained for the diesel medium showed that the native microorganisms had positive response in utilizing hydrocarbon as diesel. The degradation followed a pseudo-first order kinetic with rate constant of 1×10^{2} $mg^{-1}Lhr^{-1}$ and remediation efficiency of 59.14%. Only 23.1% of the physicochemical properties examined at the end of remediation were restored to their initial states and found to be within the W.H.O. standard. For the DPK medium, results showed that the microorganisms present had negative response in utilizing domestic purpose kerosene. At the end of the third week (504 hours) very low remediation efficiency of 39.62% was attained with a rate constant of 1×10^{-3} hr^{-1}. The control experiments showed no appreciable reductions in TPH except for a small decrease by 1.5% for diesel and 2.0% for DKP, this was indicative that bioremediation had occurred in the diesel and DPK microcosms other than the controls.
Conclusion: Bioremediation of surface water contaminated with hydrocarbons using agricultural waste was found not to be very effective as a biological treatment option in a non-oil producing area. The physicochemical properties of the treated surface water samples were grossly impaired and therefore needed post treatments to make them safe for human consumption.

Keywords: Bioremediation; diesel; domestic purpose kerosene; pseudo first order kinetic; agricultural waste; post treatment.

1. INTRODUCTION

Industrialization and extraction of natural resources have resulted in large scale environmental contamination and pollution. Contamination of surface water and air with hazardous and toxic chemical is one of the major problems facing the industrialized world today. Indiscriminate and uncontrolled discharge of industrial and urban waste into the environmental sink has become an issue of major global concern [1-3]. Excess loading of hazardous waste has led to scarcity of clean water and disturbance of oil limiting crop production [4]. The need to remediate contaminated air, water and soils has led to the development of new technologies that emphasize on the destruction of the pollutant rather than the conventional approach of disposal. Bioremediation is the issue of biological interventions of biodiversity for mitigation (and whenever possible, complete elimination) of the noxious effect caused by environmental pollutant in a given site. The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment. Bioremediation uses biological agent, mainly microorganism, e.g. yeast, fungi or bacteria to clean up contaminated surface water [3]. Microorganisms used to perform the function of bioremediation are known as bioremediator. In (Ex situ) contaminants are removed from the original site. (In situ) bioremediation involves the treatment of contaminants where they are located. In this case the microorganism come into direct contact with the dissolved and absorbed contaminant and uses them as substrates for transformation [5]. Compared to other method bioremediation is a more promising and less expensive way for cleaning up contaminated surface water [6]. In bioremediation processes, microorganisms use the contaminant as nutrient or energy sources.

Several remediation works have been reported with no or scanty literature on the kinetic study of degradation of hydrocarbon contaminated surface water [6,7]. This study intends to evaluate the effectiveness of bioremediation of hydrocarbons contaminated surface water using organic supplement of agricultural waste with special attention on the kinetics of the biodegradations.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Organic supplement

Poultry, cow and piggery wastes were obtained from poultry and animal farms in Jalingo, Taraba State, North Eastern Nigeria. The wastes were air-dried, ground and thoroughly mixed in 1:1:1 ratio.

2.1.2 Petroleum contaminants

The petroleum contaminants used in the study were diesel and domestic purpose kerosene, obtained from Petroleum Products Marketers in Jalingo, Taraba State. All plastic and glassware utilized were pre-washed with detergent water solution, rinsed with tap water and soaked for 48 hours in 50% HNO_3, then rinsed thoroughly with distilled water and air-dried in the laboratory.

2.2 Sampling Method

Surface water sample was obtained in a pre-sterilized 25 litre container from river Bali, in Bali Local Government Area of Taraba State, North Eastern Nigeria. The sample point is located between latitude 7^{o} 30' to 8^{o} 10' N and longitude 5^{o} 45' to 6^{o} 15' E. The surface water sample was obtained by the grab sampling method along the bank of the flowing river Bali which flows through the Bali main bridge in a 25 litre container as described by [8]. The map of the study area is displayed in Fig. 1.

2.3 Bioremediation Experiments

Four sets experiments were carried out at ambient temperature (28-32°C) following similar procedure of oil application methods that have been reported elsewhere [9-11]. In the first experiment, 37.5 g of the organic supplement (wastes of poultry, piggery and cow dung) that served as a source of nutrients and microorganisms was added to the surface water simulated with diesel in a plastic container. In a second experiment, a second plastic container containing 37.5 g of the organic supplement plus prepared surface water sample contaminated with DPK was arranged. Both experiments were allowed to stand for 21 days for possible bioremediation. In the third and fourth experimental set-ups, the plastic containers had only DPK and diesel respectively without the organic amendment, served as controls and were also allowed to stand for 21 days for possible bioremediation. In this work, microbiological analyses were not carried out but organic supplement used have been reported by earlier investigators to contain heterotrophic bacteria, hydrocarbon degraders and fungi that are capable of utilizing hydrocarbons as carbon substrates [10,12]. These researchers [10,12] recovered a total of 15 bacteria isolates from the total viable count (TVC) plate, of which 6 were hydrocarbon degraders. The genera of indigenous bacteria they identified are: Bacillus spp., Pseudomonas spp., Corynebacterium spp., Flavobacterium spp., Alcaligens spp. and Cellulomonas spp. The fungal isolates obtained include: Candida spp., Aspergillus spp., Detorula spp and Tricoderma spp. Other researchers have also reported that many microorganisms have been found to have the ability to utilize hydrocarbons as sole source of energy and such microorganisms are widely distributed in nature [13]. Bioremediation was principally monitored using reduction in TPH and the control experiments.

2.3.1 Chemical analysis of organic supplement

Chemical analysis was carried out on the organic supplement to ascertain the presence and ratio of essential minerals such as C, N and P.

2.4 Kinetics of Bioremediation

Changes in total petroleum hydrocarbon (TPH) with time were monitored on weekly basis in the diesel and DPK microcosms for 4 weeks following similar methods adopted by [14,9]. 50 mL of the diesel contaminated surface water that had undergone bioremediation was taken in a 250 mL separating funnel. To this 10 mL of n-hexane was added and shaken manually for 2 minutes. The separating funnel was clamped on a retort stand and left for 20 minutes with the stopper removed. The water layer was drained off and the n-hexane layer was read off by T-60 UV/Visible spectrophotometer (2007 model) at a wavelength of 350 nm. A standard calibration curve was first constructed. A standard concentration of 1000 mg/L of diesel was prepared by dissolving 1 mL of diesel in 1000 mL volumetric flask and made up to mark using n-hexane. This solution was the standard stock. Working standards of 0, 10, 20, 40, 60, 80, 100 mg/L were prepared from the standard stock. The extraction and analysis was also repeated for domestic purpose kerosene contaminated surface water samples at wavelength of 310 nm.

2.5 Determinations of Physicochemical Parameters of Uncontaminated, Contaminated and Treated Groundwater Samples

Physicochemical properties of uncontaminated, contaminated and treated groundwater samples were investigated. pH was determined using a digital Jenway pH model 3505. Turbidity of surface water sample was determined using HI 93414 model turbidimeter. Dissolved oxygen of surface water sample was determined using a model DO-5509 dissolved oxygen meter. Total dissolved solids (TDS) were determined using TDS meter. Electrical Conductivity of the surface water sample was measured using a digital Jenway conductivity meter model DDS 307. Inorganic phosphate, ammonia, nitrate, total alkalinity were determined using standard procedures described by [8]. The salinity values of the surface water samples were determined using a salinity meter. Surface water sample was digested using the standard method proposed by [15] and heavy metals were analysed by Atomic Absorption Spectrophotometer (AAS) following procedures adopted by [16].

3. RESULTS AND DISCUSSION

3.1 Bioremediation

The results of the total petroleum hydrocarbon (TPH) left at the end of the bioremediation for the two contaminants of common interest (diesel and domestic purpose kerosene) are displayed on Table 1 and the profile represented in Fig. 2.

The result of the analysis of the organic supplement used in the study is displayed on Table 2.

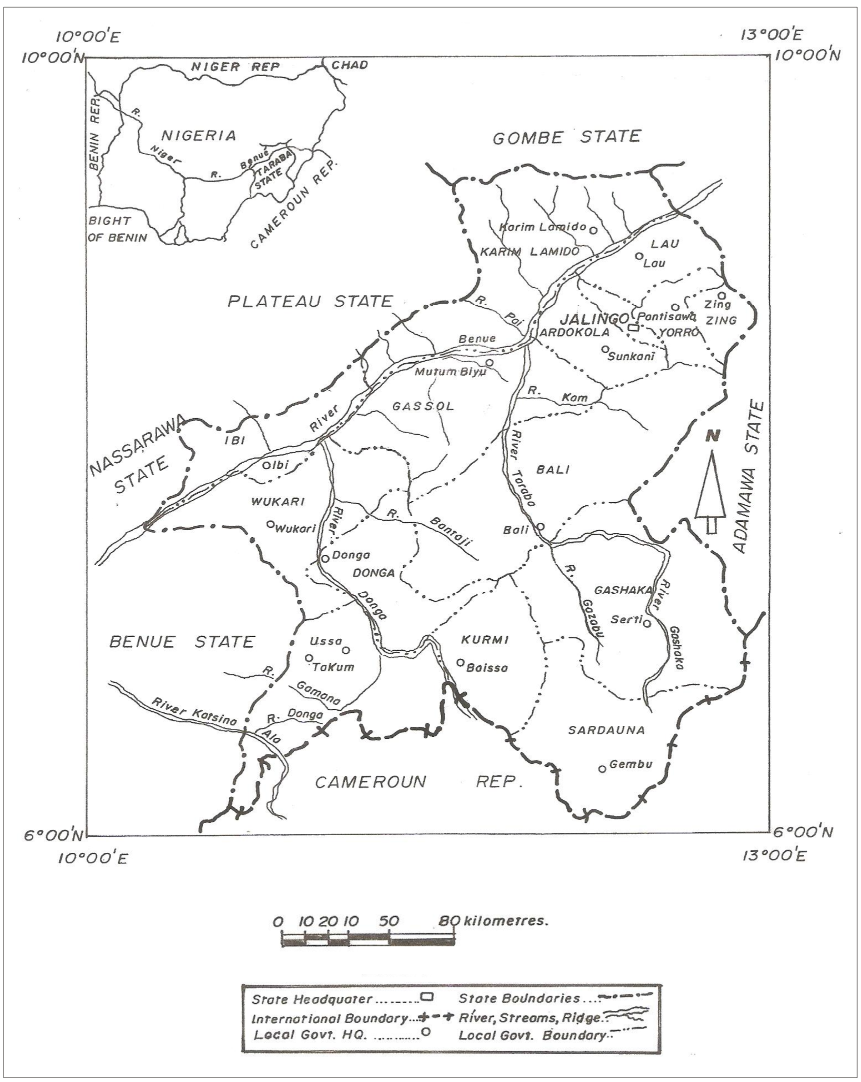

Fig. 1. A map of Bali Local Government Area in Taraba State showing the study area

Table 1. Data for progress profile of TPH as diesel and domestic purpose Kerosene versus time in bioremediation method

Time/hours	TPH left as diesel(mg/L) $[A]_t$	% remediation in diesel medium	TPH left as DPK(mg/L) $[A]_t$	% remediation in DPK medium
0	6291.20±10.7	0.00	5373.23±10.65	0.00
168	4530.60±6.57	27.99	2037.30±6.50	38.92
336	2895.80±6.77	53.97	2127.20±6.77	36.22
504	2684.50±6.75	57.32	2013.70±6.80	39.62
672	2570.10±6.72	59.14	2292.50±6.72	31.27

Chemical analysis had shown that the organic supplement used for the experiment was very rich in nitrogen, organic carbon and phosphorus as required by microorganisms for growth and reproduction (Table 2). The adjustment of carbon/nitrogen/phosphorus ratios by addition of nitrogen and phosphorus in the form of N-P-K fertilizer simulated the biodegradation of crude oil and individual hydrocarbon has been reported [17,18]. The addition of organic amendment was meant to accelerate biodegradation by providing the necessary support and stimulation for native microbes to quickly multiply and produce enzymes to speed up the metabolic process of biodegradation.

Table 2. Data for chemical analysis of organic supplement

Parameters	
pH	7.90±0.01
Nitrogen (mg/kg)	0.51±0.001
Phosphorus (mg/kg)	0.11±0.005
Organic Carbon (%)	7.53±0.03
Organic Matter (%)	12.98±0.01

The progress of the TPH as diesel and DPK versus time in the bioremediation process is shown in Fig. 2.

The profile showed that the total petroleum hydrocarbon as diesel and DPK decreased with time. This assertion supported the findings of other investigators who reported similar results [10]. Earlier investigators [9,10,12] have reported that mixed waste of piggery, cow dung and poultry have the potential of biostimulating native microorganisms in groundwater obtained from an oil producing area thereby enhancing the degradation of hydrocarbons. The highest remediation efficiencies of 59.14 % and 39.62 % were obtained at the end of the 4th and 3rd week for the diesel and DPK contaminants respectively. These results were low compared with those obtained by [10] who used groundwater and organic supplement whose source was from an oil producing region of the Niger-Delta of Nigeria and got high values of percent remediation of 82.35% on the 4th week for diesel and 81.30% on the 3rd week for DPK. The plausible explanation for these wide variations could be that the microorganisms in the groundwater were from an oil producing area and were not under stress being familiar with the contaminants in their environment [19,20]. The results also showed that the microorganisms in the DPK medium were probably more affected by the devastating poisoning effect of DPK with poor remediation efficiency of 39.62% in comparison with the moderate removal efficiency of 59.14% obtained with the diesel contaminant. The observed trend is corroborated by the works of other investigators which showed that when DPK is present in water, becomes more soluble and as a result release more volatile toxic components which lead to poor growth of the microbes [21-23]. It was also possible that the observed reduction in TPH considered as remediation in both the DPK and diesel mediums were partly due to colloidal complexation of the contaminants by the organic amendment. The controls used in the experiments showed a reduction in TPH by 2% for the DPK medium and 1.5% for the diesel medium (Table 3). These reductions in TPH were not very significant and were indicative that bioremediation had occurred in the non-control mediums where remarkable reductions in TPH were observed (Table 1). Earlier investigators that worked with groundwater contaminated with hydrocarbons using organic supplement from an oil producing area have also reported similar results [10,12].

The results of the kinetics of the two bioremediation experiments were pseudo-first order and first order whose plots are depicted in Figs. 3 and 4 for the diesel and DPK contaminants respectively. The regression coefficients were found to be between 0.759 – 0.998 considering points of best fits. The outcome of the results adequately fitted into the first and pseudo first kinetic data of model. In $([A]_o/[A]_t$ versus t, time.

The kinetics of bioremediation for the diesel and DPK contaminants followed pseudo-first order and first order with rate constants of $1x10^2$ $mg^{-1}Lhr^{-1}$ and $1x10^{-3}hr^{-1}$ respectively.

The results of physicochemical parameters of unpolluted, polluted and treated surface water samples in the DPK and diesel broths are given in Table 4. In the diesel medium, bioremediation process was found to be very effective in the removal of cadmium with remediation efficiency of 84.7%; there was low remediation efficiency for nickel (9.43%) and chromium (4.08%). The process was also effective in the reduction of nitrate and phosphate level with remediation efficiencies of 53.5% and 23.5% respectively. It was found to be ineffective in improving the levels of turbidity, alkalinity, chloride, ammonia and total dissolved solids. Out of thirteen (13) physicochemical parameters analysed only four of the properties (salinity, nitrate, alkalinity and selenium) were found to be within the W.H.O. [24] standard after remediation. This accounted for 30.7% of the physicochemical properties.

Table 3. Reduction in TPH in the control experiments

Control	TPH (mg/L) at initial time 0 hour before bioremediation	TPH (mg/L) left at final time 672 hours after bioremediation	% removal of TPH
Diesel	6291.20±10.7	6196.83±10.65	1.5
DPK	5373.23±0.00	5265.77±0.00	2.0

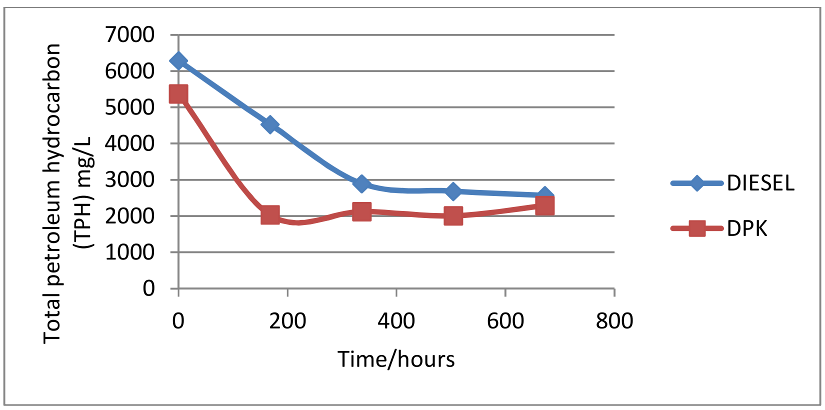

Fig. 2. Progress Profile of TPH as Diesel and DPK versus Time in Bioremediation Method

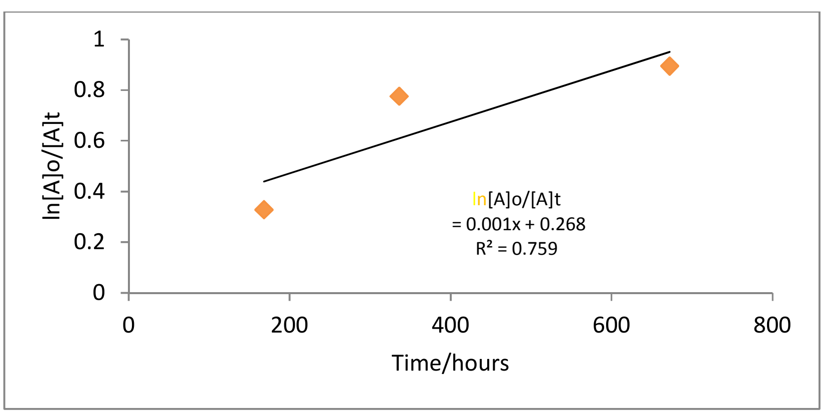

Fig. 3. Pseudo – first order plot for diesel in Bioremediation method

Table 4. Mean results±Standard Deviations from triplicate Analysis of Physicochemical Properties of Surface Water Samples before and after contamination, after Bioremediation and W.H.O. Standard

Parameters	Before contamination surface water sample	After contamination Diesel + surface water sample	After bioremediation diesel medium	After contamination DPK + surface water sample	After bioremediation DPK medium	W.H.O. Standard
pH	8.60±0.060	9.5±0.060	8.3±0.060	9.4±0.000	7.5±0.000	9.20
Turbidity (NTU)	20.50±0.000	144.00±0.000	450±0.008	720.00±0.008	450.00±15.169	5.00
Alkalinity (mg/L)	66.00±0.01	80.00±0.005	570±6.720	540.00±6.720	540.00±0.000	100
Salinity as chloride (mg/L)	0.80±0.000	0.4±0.000	0.4±0.000	0.4±0.000	0.40±0.000	250
Nitrate (mg/L)	1.68±0.044	2.37±0.000	1.10±0.000	1.51±0.022	3.30±0.001	50.00
Phosphate (mg/L)	0.14±0.001	0.17±0.000	0.13±0.000	0.19±0.008	0.14±0.0005	0.02
Ammonia (mg/L)	0.80±0.001	0.61±0.005	0.94±0.000	0.01 ±0.0001	0.58±0.01	0.5
Total Dissolved Solids (mg/L)	105.00±8.086	120.00±0.000	1300.00±15.169	140.00±0.000	760.00±1.000	500
Cadmium (mg/l)	3.15±0.001	53.00±0.000	8.10±0.000	12.00±0.001	0.60±0.01	0.001
Chromium (mg/L)	0.05±0.0001	0.98±0.001	0.94±0.001	0.01±0.0001	1.10±0.006	0.05
Nickel (mg/L)	1.29±0.0003	7.74±0.001	7.01±0.001	2.30±0.0005	6.33±0.01	0.02
Lead (mg/L)	1.25±0.001	3.95±0.001	4.80±0.001	1.10±0.001	5.20±0.006	0.05
Mercury (mg/L)	0.31 ±0.001	1.44±0.001	1.52±0.001	1.26±0.001	0.80±0.0001	0.02
Vanadium (mg/L)	Beyond detectable level (BDL)	BDL	BDL	BDL	BDL	0.05
Selenium (mg/L)	0.01±0.000	0.16±0.0005	0.04±0.0005	0.12±0.000	0.13±0.000	0.05
Arsenic (mg/L)	BDL	BDL	BDL	BDL	BDL	
TPH (mg/L)	-	6291.20±10.7	2570.10±6.72	5373.23±3.21	2292.50±6.89	10.00

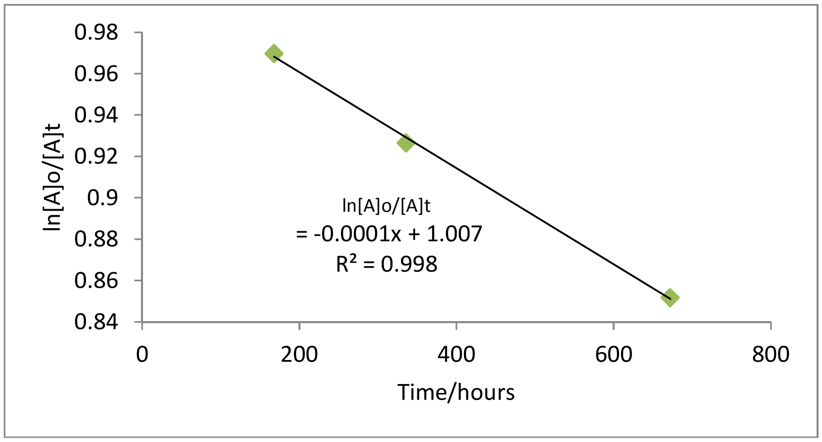

Fig. 4. First order plot for DPK in Bioremediation method

The results of physicochemical properties of surface water samples before and after contamination, after bioremediation and W.H.O. Standard are presented in Table 4.

In the DPK medium, bioremediation was also found to be very effective in the removal of cadmium from the surface water contaminated with DPK with remediation efficiency of 95%. There was low remediation efficiency for mercury (36.50%) and phosphate (26.30%). The process was also found to be ineffective in the removal of turbidity, alkalinity, ammonium, total dissolved solids, vanadium, selenium, lead, nickel and chromium. After remediation only three (3) out of the fifteen (15) physicochemical properties analysed were within the W.H.O. [24] standard for drinking water. This represented 23.1% of the physicochemical properties.

4. CONCLUSION

In conclusion, the organic supplement of piggery, poultry and cow dung used in the study was capable of biostimulating the native microorganisms in the surface water for enhanced bioremediation only to an extent. Moderate and low remediation was attained in the diesel and DPK mediums respectively. Invariably microorganisms with high degradation potential such as the hydrocarbon utilizing bacteria were absent being a non - oil producing area or sparingly present and adversely affected by the contaminants leading to the low efficiency. The study has clearly established that microorganisms with potential of degrading hydrocarbons were more readily available and thrived better in oil producing areas compared to non - oil producing areas although it is generally reported that such microorganisms are widely distributed in nature [13]. The treated samples of the diesel and DPK contaminated surface water needed post treatment to make them fit for domestic and agricultural uses since most of the physicochemical properties particularly in the DPK medium that were highly impaired. It is recommended that the treated surface water should undergo further chemical treatments to reduce the concentration of diesel or DPK to an acceptable level thereby making the surface water safe for domestic and agricultural uses. Surface water bodies in non-oil producing regions must be properly protected against pollution or reduce the tendency of occurrence to the barest minimum since natural attenuation would likely occur at a very slow pace and enhanced bioremediation by application organic amendment such as agricultural waste does not seem to be a practical solution. Further research should be carried out using enhanced bioremediation via bioaugumentation on surface water contaminated with hydrocarbons in such areas.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gupta R, Mahapatara H. Microbial biomass. An economical alternative for removal of heavy metals from waste water. Indian Journal of Experimental Biology. 2003;41945966.

2. Hernande A, Mellado RP and Martinez JL. Metal accumulation and vanadium-induced multi-drug resistance by environmental isolates of Escherichia Herdman and Enterobacter cloacae. Applied and Environmental Microbiology. 1998;044317-4320.
3. Strong, Burges JE. Treatment methods for wine related and distillery wastewaters. Review Bioremediation Journals. 2008; 127087.
4. Kamaludeen Ramasamy K, Sara Purwin Banu. Bioremediation of metals microbial processes and technique in Environmental Bioremediation technologies edited by Singh SN and Tripath, RD (Springer Publication NY) 173-187 Hunt, and Eccess (1986), Chemistry reaction structure and properties (second edition); 2006.
5. Bouwer, Zehnder J. Comparison of bioaugmentation and biostimulation surface water and land Contamination University of Edinburgh, Scotland; 2009.
6. Piskonen R, Itavaara M. Evaluation of Chemical Pre-treatment of contaminated soil for improved PAH Bioremediation. Applied Microbiology Biotechnology. 2004;65:627.
7. van Hamme JD, Singh A, Ward OP. Recent advances in petroleum microbiology. Microbiology and Molecular Biology Reviews. 2003;67(4):503–549.
8. Ademoroti CMA. Standard Methods of Treatment of Water and Wastewaters. Fodulex Press Ltd., Ibadan; 1996.
9. Medjor WO, Akpoveta OV. Kinetic studies of bioremediation of hydrocarbon contaminated groundwater. Research Journal of chemical Sciences. 2012;2(1): 38–44.
10. Medjor WO, Egharevba F, Akpoveta OV, Wepuaka CA, Jatto EO. Effects of bioremediation on physicochemical properties and heavy metal content of groundwater contaminated with diesel using mixed culture microorganisms. Mambilla: Journal of Sciences and the Environment. A publication of Faculty of Science, Taraba State University, Jalingo, Nigeria. 2013(a);1:96-106.
11. Venosa AD. Oil spill bioremediation on coastal shorelines: a critique. In: S.K. Sikdar & R.I. Irvine (Eds.), Bioremediation: Principles and Practice. Vol. III. Bioremediation Technologies. Technomic, Lancaster, PA. 1998;259-301.
12. Medjor WO, Egharevba F, Akpoveta OV, Namessan NO, Jatto EO. Biodegradation and Kinetic studies of groundwater contaminated with domestic purpose kerosene using microorganisms from agricultural waste. Mambilla: Journal of Sciences and the Environment. A publication of Faculty of Science, Taraba State University, Jalingo, Nigeria. 2013(b); 1:120-129.
13. Atlas RM. Microbial degradation of petroleum hydrocarbons: An environmental perspective. Microbiological Reviews. 1981;45:180-209.
14. Macgill for Water/ Wastewater Analysis Nigerian Institute for Rubber Research. (NIFOR) Benin City, Edo State, Nigeria; 2000.
15. American Water Works Association, AWWA Standard Methods for the Examination of Water and Wastewater, 16th edition; 1985.
16. Caroline M, Luis R, Juan S. A Comparison of two digestion methods for the analysis of heavy metals by flame atomic absorption spectroscopy, University of Valencia, Spain; 2007.
17. Okpokwasili GC, Oton NS. Comparative applications of bioreactor and shake flask systems in the laboratory treatment of oily sludge. International Journal of Environment and Waste Management. (2006);1(1):49-60.
18. Zhu X, Venosa AD, Suidan MT, Lee K. Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands. Report under a contract with Office of Research and Development, U.S Environmental Protection Agency; 2001.
19. Mishra S, Jyot J, Kuhad RC, Lal B. Evaluation of Inoculums Addition To Stimulate In Situ Bioremediation of Oily-Sludge-Contaminated-Soil. Appl. Environ. Microbiol. 2001;67:1675.
20. Prescott LM, Harley JP, Klein DA. Microbiology, fifth edition. McGraw-Hill Publication. 2002;106-107.
21. Agbonlahor DE. Petroleum Hydrocarbon Degrading capabilities of Microbial Isolates from Ripe Pawpaw Fruit. Nigerian Annals of Natural Sciences. 2004;5(1).

22. Migashihara T, State A, Simidu U. An MPN method for the enumeration of Marine hydrocarbon degrading bacteria. Bulletin of Japanese Society of Scientific Fisheries. 1978;10(44):1127-1134.
23. Nuzzi R. Effects of water soluble extracts of oil on phytoplankton. In: Proceedings of Joint Conference on Prevention and control of oil spills, American Petroleum Institute, Washington, D.C. 1973;809-813.
24. W.H.O. World Health Organizaion; 1991.

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