EFFECTS OF BIOREMEDIATION ON PHYSICOCHEMICAL PROPERTIES AND HEAVY METAL CONTENT OF GROUNDWATER CONTAMINATED WITH DIESEL USING MIXED CULTURE MICROORGANISMS

BY

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ABSTRACT

Bioremediation of groundwater contaminated with diesel by a mixed culture of microorganisms was investigated. The results obtained showed that the microorganisms; total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB) and total fungi (TF) had a positive response in utilizing total petroleum hydrocarbon (TPH) as diesel. The order of hydrocarbon utilization was: THUB>TC>THB with mean growth rate constants of 5.95×10^{-3} $hour^{-1}$, 3.97×10^{-3} $hour^{-1}$ and 2.98×10^{-3} $hour^{-1}$ respectively. A total of 10 bacterial isolates were recovered from the total viable count (TVC) plate, of which 6 were hydrocarbon degraders. The genera of the indigenous bacteria identified were: Bacillus spp., Pseudomonas spp., Corynebacterium spp., Flavobacterium spp., Alcaligens spp., Aeromonas spp., Cellulomonas spp., Chromobacterium spp., Serratia spp., and Proteus spp. The four fungal isolates obtained included: Candida spp., Aspergillus spp., Detorula spp. and Tricoderma spp. Notable bioremediation markers: chemical oxygen demand (COD) were significantly reduced by 97.98%; biochemical oxygen demand at day-5 (BOD_5) by 7.58 %; TPH as diesel by 91.50% and alkalinity increased by 163.64%. In conclusion, while there were appreciable improvements in some physiochemical properties and found within the W.H.O Standard, others needed post-treatment to make the groundwater safe for domestic and agricultural uses.

Keywords: **Bioremediation; Physicochemical properties; Groundwater; Diesel.**

INTRODUCTION

The rapid increase in the world population has resulted in increased demand for hydrocarbon products such as diesel, kerosene, petrol, pesticides and other industrial chemicals. Although many of these chemicals are utilized or destroyed, a percentage is released into the air, water and soil representing a potential environmental hazard (Stephen *et al.*, 2011). Diesel pollution is on the increase in Nigeria as well as in other developing countries (Stephen *et al.*, 2011). Groundwater and rivers constitute the main sources of water supplies that humans use (for drinking, cooking, cleaning, industry and agriculture).

These sources of water supplies are polluted by natural geological sources, pesticides, industrial discharge from various processing industries and oil spillage during oil exploration and exploitations or accidental discharge. Oil renders the environment unsightly and constitutes a potential threat to humans, animals and vegetation (Edewor *et al.*, 2004). Spills in freshwater environments have been shown to adversely affect the

diversity and abundance of the aquatic macro-invertebrate community, with the observed effects associated with oil sorption and substrate coating (Poulton *et al.*, 1998). A thick layer of oil inhibits the metabolism of plants and suffocates them. Destruction of plants affects the whole food web and decreases the natural habitats of numerous species.

The dependence of humans on oil around the world that leads to inevitable spillages, has called for more studies into oil pollution problems (Jain *et al.*, 2011). Several bioremediation works on soil and water using microorganisms have been reported (Venosa *et al.*, 1996; van Hamme *et al.*, 2003; Piskonen and Itävaara, 2004). No much attention has been paid to their effects on physicochemical properties and heavy content. This work is aimed at investigating the effects of bioremediation on physicochemical properties and heavy content of groundwater contaminated with diesel.

MATERIALS AND METHODS

Materials

The petroleum contaminant used in the study was diesel. It was obtained from Petroleum Products Marketers in Agbor, Delta State, Nigeria. All plastic and glass wares 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.

Sampling Method

Groundwater sample was obtained in a pre-sterilized 10 litre container from the tap of a borehole located in Delta State University, Abraka, Nigeria. In the early hours of the morning, the borehole tap was allowed to run for about 2-3 minutes to eliminate impurities and then snap-shot samples were taken at once by grab sampling (Ademoroti, 1996).

Organic Supplement

Poultry, cow and piggery wastes were obtained from poultry and animal farms in Agbor. The wastes were air-dried, grounded and thoroughly mixed in 1:1:1 ratio.

Preparation of Sample for Bioremediation Experiment

Pollution was simulated in the laboratory by contaminating groundwater sample with diesel. A plastic container was filled with 900 cm^3 of the groundwater sample. 100 cm^3 of diesel was added to the groundwater sample in the plastic container and stirred thoroughly using a magnetic stirrer for 10 minutes to obtain 10% contamination.

Bioremediation Experiment

Two sets of experiments were carried out at ambient temperature (28-32 °C) following similar procedures of oil application method that has been reported elsewhere (Venosa *et al.*, 1996). In the first experiment, the prepared groundwater sample contaminated with diesel was placed in a plastic container. To this sample was added 25 g of the organic supplement that served as source of microorganisms and nutrients. In the second experiment, a second plastic container containing prepared groundwater sample contaminated with diesel was left without organic supplement and served as a control. Bioremediation was allowed to progress in both set-ups for 42 days. Bioremediation indicators monitored included: changes in total petroleum hydrocarbon (TPH), chemical oxygen demand (COD), biochemical oxygen demand (BOD) and alkalinity. Total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB), total fungi (TF) counts were enumerated; isolated, identified, and also monitored. Isolation and enumeration of heterotrophic, hydrocarbon utilizing bacteria and fungi was carried out using the spread plate technique as described by Alpha (1998). Fungal isolates were identified using the method described by

Harrigan and McCane, (1976). Characterizations of isolates [Gram staining reaction, Citrate utilization test, Motility test, Spore staining, Oxidase test, Catalase test, Methyl red/Voges-Proskauer (Mrvp), Indole production test, Oxidation and fermentation of sugars and Urease hydrolysis] were carried out adopting methods described by Stewart and Beswick (1977) and Cruickshank *et al.*, (1975). The growth rate during the exponential phase was expressed in terms of the mean growth rate constant (k) using the formula: $n = (\log N_t - \log N_o)/0.301$ and $k = n/t$ where N_o = population number at lag phase; N_t = population number at log phase, n = the number of generations in time t and t = time to attain the log phase. The mean generation time (g) is the reciprocal of the mean growth rate constant, $g = 1/k$ (Prescott, *et al.*, 2008).

Determinations of physicochemical parameters of uncontaminated, contaminated and treated groundwater samples

Physicochemical properties of uncontaminated, contaminated and treated groundwater samples were determined. pH was determined using a digital Jenway pH model 3505. Turbidity of groundwater sample was determined using HI 93414 model turbidimeter. Dissolved oxygen of groundwater sample was determined using a model DO-5509 dissolved oxygen meter. Total dissolved solids (TDS) were determined using TDS meter. Electrical conductivity of the groundwater sample was measured using a digital Jenway conductivity meter model DDS 307. Chemical oxygen demand (COD), biochemical oxygen demand at day-5 (BOD_5), total suspended solids (TSS), inorganic phosphate, ammonia, nitrate, total alkalinity were determined using standard procedures described by Ademoroti, (1996). The salinity values of the groundwater samples were determined using conductivity values extrapolated from a curve of known salinity concentrations of sodium chloride plotted against corresponding conductivity readings. Total solid (TS), was taken as the sum of TDS and TSS. For determination of heavy metal content, groundwater samples were digested and analyzed by Atomic Absorption Spectrophotometer (AAS) following procedures described by Ademoroti, (1996). TPH analyses were carried out using T- 60 UV/Visible spectrophotometer (2007 model, United Kingdom) at wavelength of 350 nm. Biogenic factor (TPH contributions from the organic supplement) was neglected and therefore total hydrocarbon content (THC) was considered as total petroleum hydrocarbon (TPH).

Quality Assurance

Analytical grade hexane was used in extracting hydrocarbon from water and in preparing working standards used in constructing calibration curves. Reagent blanks (analyte-free water analyzed with the samples) were used to correct any absorption of light by hexane.

Statistical treatments

Samples were prepared in replicates of three to provide data for statistical treatment. Standard deviation was used to crosscheck precision. Blank runs were also conducted to reduce the occurrences of determinate errors. SPSS software was used for the analysis of variance.

RESULTS AND DISCUSSION

The bioremediation results at the end of the 42-day of the experiment showed that indigenous microbes had a positive response in utilizing hydrocarbon. There was a TPH reduction of 91.5% in the first experimental set-up. No appreciable reduction in TPH (only 2.5%) was observed in the control experiment and this drop in TPH may be due to evaporation. The results of the microbiological analysis in the experimental set-up containing the organic supplement showed that a total of 10 bacterial isolates were recovered from the

total viable count (TVC) plate, of which 6 were hydrocarbon degraders. The genera of the following indigenous bacteria were identified: *Bacillus* spp., *Pseudomonas* spp., *Corynebacterium* spp., *Flavobacterium* spp., *Alcaligens* spp., *Aeromonas* spp., *Cellulomonas* spp., *Chromobacterium* spp., *Serratia* spp. *and Proteus* spp. The fungal isolates obtained include: *Candida* spp., *Aspergillus* spp., *Detorula* spp. *and Tricoderma* spp. The mathematics of growth for the total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB) and total fungi (TF) population is given in Table 1.

Table 1: Data for Mathematics of growth for the Total Heterotrophic Bacteria (THB), Total Hydrocarbon Utilizing Bacteria (THUB) and Total Fungi (TF) population in diesel medium

Type	Population number at lag phase (N_o) (CFU/mL)	Population number at log phase (N_t) (CFU/mL)	n	k ($hour^{-1}$)	g (hour/generation)
THB	3.50×10^5	6.10×10^5	1	1.48×10^{-3}	336
THUB	9.40×10^3	2.80×10^4	2	3.96×10^{-3}	168
TF	1.30×10^4	9.30×10^4	2	1.98×10^{-3}	252

The order of hydrocarbon utilization was: THUB >TF>THB with mean growth rate constants of 5.95×10^{-3} $hour^{-1}$, 3.97×10^{-3} $hour^{-1}$ and 2.98×10^{-3} $hourr^{-1}$ respectively. The THUB population had the shortest mean generation time of 168 hour/generation followed by the TF population with a mean generation time of 252 hour/generation and the THB population had the longest mean generation time of 336 hour/generation. Generation times varied markedly with the species of microorganism and the environmental conditions as shown in this work and corroborated by the findings of earlier investigators (Prescott, *et al.*, 2008). Analysis of variance (ANOVA) of the results of the populations of the total hydrocarbon utilizing bacteria (THUB), total heterotrophic bacteria (THB) and total fungi (TF) used in the study showed significant difference at $p < 0.05$. This statistical finding supported the fact that the microorganisms had different utilization capabilities for the diesel as a source of carbon for food as also shown by the mathematics of growth in Table 1. The increase in the microbial population during the bioremediation process except for the control microcosm was also an indication of their presence and abilities to utilize diesel as carbon and energy sources. The results of physicochemical properties and heavy metal content of groundwater samples before and after contamination, after bioremediation and W.H.O. Standard are presented in Table 2.

Table 2: Mean results ± standard deviations from triplicate analysis of physicochemical properties and heavy metal content of groundwater samples before and after contamination, after bioremediation and W.H.O. Standard.

Parameters	Before contamination (groundwater sample)	After contamination (diesel + groundwater ample)	After bioremediation	W.H.O. Standard
pH	8.60±0.060	9.50±0.000	8.30±0.006	9.20
Electrical Conductivity (µS/cm)	100.00±0.010	100±0.000	2450±8.07	1200
Turbidity (NTU)	0.20±0.000	856 ± 2.34	1030.00±16.169	5.00
Alkalinity (mg/L)	148.00±0.01	132.00±0.000	216.00±4.450	100
Dissolved Oxygen (mg/L)	7.29±0.133	3.55±0.000	5.04±0.133	15
BOD_5 (mg/L)	2.80±0.133	7.39±0.066	6.83±0.005	7.5
COD (mg/L)	524.00± 0.010	270±0.01	5.46±0.01	10
COD/ BOD_5	187.14	36.54	0.80	-
% Reduction in COD			97.98	
Salinity (mg/L)	103.19±0.000	103.19±0.000	1111.19±34.931	250
Nitrate (mg/L)	148.00±3.440	16.00±0.01	100.00±2.000	50.00
Phosphate (mg/L)	0.28±0.001	0.16±0.003	0.64±0.0006	0.02
Ammonia (mg/L)	0.53±0.001	0.89 ±0.005	6.50±0.02	0.5
Total Dissolved Solids (mg/L)	115.00±8.086	170.00±0.000	1030.00±16.169	500
Total Suspended Solids (mg/L)	1.089±0.003	1.383±0.024	6.903±0.005	10.00
Total Solid (mg/L)	116.09±7.76	171.38±5.55	1036.90±2.67	1500
Cadmium (mg/l)	0.21±0.001	0.45±0.0004	2.90±0.005	0.001
Chromium (mg/L)	0.10±0.0001	0.60 ±0.006	0.44±0.001	0.05
Nickel (mg/L)	0.62±0.0003	0.59±0.001	7.60±0.010	0.02
Lead (mg/L)	BDL	BDL	BDL	0.05
Mercury (mg/L)	0.37 ±0.001	0.42±0.001	1.14±0.0006	0.02
Vanadium mg/L)	BDL	BDL	BDL	0.05
Selenium (mg/L)	BDL	BDL	BDL	0.05
TPH (mg/L)	26.00±0.010	5199.26±3.770	411.42±0.248	10.00

Key: BDL= Below detectable level

pH is an important quality parameter which indicates the aesthetic quality of water such as taste but has no serious health significance . pH affects the degree of ionization of toxic substances such as ammonia (Stirling, 1985). The groundwater sample obtained at the end of bioremediation process had a pH of 8.3. This pH was within the permissible limit of 6.5 - 9.5 set by W.H.O. (1996) for quality drinking water /agricultural uses but slightly lower than that of the uncontaminated groundwater sample (pH = 8.6). Electrical conductivity indicates the presence of dissolved solids and contaminant especially electrolytes but does not give information about specific chemical component (Esry and Habicht, 1986). The electrical conductivity value for the uncontaminated and contaminated groundwater samples was 100 µS/cm. The electrical conductivity value of the treated diesel contaminated groundwater sample was 2450 µS/cm (Table 2). This extremely

high value was due to exogenous inputs of salts/ions from the animal wastes, coupled with the removal of substantive amount of hydrocarbons which previously provided non-polar environment for ion-reduced mobility. The resultant effect was increased ionic mobility, velocity and high electrical conductivity. Increased electrical conductivity value indicated that remediation had occurred although the efficiency of remediation could not meet up with the required standard for electrical conductivity (1000 µS/cm) set for quality drinking water in Nigeria (W.H.O. 1996).

The turbidity of diesel simulated groundwater sample was 856 NTU. This value was found to be astronomically high and an indicator that the prepared groundwater sample was extremely polluted compared to 100 - 275 NTU turbidity values of for highly polluted water reported by Rump, (1999). This exceptionally high value of turbidity must be connected with the heavy organic load from the organic supplement used in the experiment and indicated high load of colloidal matter and by implication high concentrations of solids. The turbidity value (1030 NTU) from the bioremediation work was far higher than the permissible turbidity limit of 5 NTU recommended by W.H.O. (1996). The uncontaminated groundwater sample had an alkalinity concentration of 148 mg/L while that of the diesel simulated groundwater sample was 132 mg/L as shown in Table 2. The decrease in the alkalinity value recorded after contamination was likely due to chemical interaction between the acidic components of the contaminant and the carbonates or hydroxyl ions present in the groundwater sample. The alkalinity value for groundwater sample obtained at the end of the bioremediation process was 216 mg/L (Table 2). The increase in the alkalinity (163.64%) for the groundwater sample obtained at the end of the remediation process implied biodegradation; as alkalinity is the best indicator for measuring microbial wastes during biodegradation. The treated groundwater sample had its alkalinity level above the 100 mg/L desirable level recommended for quality drinking water in Nigeria (W.H.O. 1996). The dissolved oxygen value of the uncontaminated groundwater sample was 7.30 mg/L while that of the diesel simulated groundwater sample had its value as 3.55 mg/L. The drop in the dissolved oxygen value after the groundwater sample was simulated with diesel indicated pollution. The dissolved oxygen value for the treated diesel contaminated groundwater sample was 5.04 mg/L (Table 2). The subsequent increase in dissolved oxygen at the end of the bioremediation process was a strong indication that remediation had occurred. The value of dissolved oxygen at the end of remediation was only slightly above the threshold of 5.0 mg/L for dissolved oxygen reported by Cruise and Miller, (1994) for drinking water and could be more than 5.0 mg/L for agricultural purposes. Dissolved oxygen requirement of less than 6 mg/L is reported for high quality drinking water by (Rump, 1999). According to USEPA (1999) classification (dissolved oxygen < 2 mg/L) could be fatal to most aquatic species while 5 mg/L - 6.0 mg/L are sufficient for the aquatic species, the latter condition was satisfied. The dissolved oxygen of the treated diesel contaminated groundwater was within the 15 mg/L recommended by (W.H.O. 1996). The uncontaminated groundwater sample had a BOD_5 value of 2.80 mg/L while that of the diesel simulated groundwater sample was 7.39 mg/L (Table 2). The rise in BOD_5 after contamination showed that the groundwater sample was polluted. The BOD_5 of the treated contaminated groundwater was 6.83 mg/L and accounted for 7.58% reduction in BOD. This drop corroborated the fact that bioremediation had taken place and is supported by the findings of earlier investigators (Yavuz and Koparal, 2006; Saien and Nejati, 2007). The treated contaminated

groundwater sample had its BOD_5 value within the safe limit of (7.5 mg/L) recommended for quality drinking water by W.H.O. (1996). The chemical oxygen demand (COD) value of the uncontaminated groundwater sample was 524 mg/L and that of the diesel simulated groundwater sample was 270 mg/L (Table 2). The COD being a measure of the total oxidizable organic matter is expected to increase on addition of the contaminant. The observed COD drop for diesel simulated groundwater sample was possibly due to some aromatic hydrocarbons, straight-chain aliphatic and nitrogenous compounds present which were not readily oxidizable. The COD value of the treated diesel contaminated groundwater sample was 5.46 mg/L and represented 97.98% reduction in COD. The COD/BOD ratio of the treated diesel contaminated groundwater sample was 0.81 and relatively very low compared with value obtained for the diesel contaminated groundwater sample (36.54). Low COD/BOD ratio shows low concentration of oxidizable organic matter and high ratio indicates high levels of oxidizable organic matter in water/wastewater (Neilson, 2002). The significant reduction in COD in the treated diesel contaminated groundwater sample indicated that effective bioremediation had taken place and its value was within the 10 mg/L safe permissible limit for quality drinking water set by W.H.O. (1996). Salinity was determined as chloride. The chloride ion concentration in the uncontaminated and contaminated groundwater samples was 103.19 mg/L. This suggested that there was no contribution of chloride ions from the diesel as contaminant. The salinity of the treated diesel ccontaminated groundwater sample had its value as 1111.19 mg/L (Table 2). This concentration was far above the recommended standard (100 mg/L - 250 mg/L) for chloride ions in drinking water by W.H.O. (1996). Chloride in small concentrations are not harmful to humans in drinking water, and with some adaptation, the human body can tolerate water with as much as 200 mg/L chloride ion. However, above a concentration of 250 mg/L chloride, the water may taste salty (Hauser, 2001). The contribution of salts from the animal waste may have led to the high salinity value for the treated groundwater sample. The uncontaminated groundwater sample had nitrate concentration of 148 mg/L while that of diesel simulated groundwater sample was 16 mg/L (Table 2). The treated diesel contaminated groundwater sample had a nitrate value of 100 mg/L and was within the maximum permissible level of 250 mg/L recommended for quality drinking water by W.H.O. (1996). The phosphate concentration in the uncontaminated groundwater sample was 0.28 mg/L and that of diesel simulated groundwater sample had a concentration of 0.16 mg/L (Table 2). The decrease in the phosphate level in the diesel simulated groundwater sample may be due as chemical interaction between the phosphate and some other components from the contaminant. The treated contaminated groundwater sample had its phosphate concentration as 0.23 mg/L. The subsequent increase in the phosphate concentration was due to contributions of the organic supplement used in the bioremediation process. The phosphate concentration was above the 0.02 mg/L maximum acceptable limit set by W.H.O. (1996) and therefore increases the tendency of troublesome algae bloom in water (Esry and Habicht, 1991). However, appreciable increase in phosphate may be used as a source of nutrient and mineral enrichment for water needed for agricultural purposes. The concentration of nitrogen as ammonia in the uncontaminated groundwater sample was 0.53 mg/L while that of diesel contaminated groundwater sample was 0.89 mg/L. The increase was due to the contribution of nitrogen from components of diesel. The subsequent elevated

concentration of ammonia (6.50 mg/L) for the treated diesel contaminated groundwater sample was possibly as a resultant conversion of nitrates into NH_3 (Nitrogen from the organic supplement) by the denitrifying bacteria called denitrificans, e.g. *Pseudomonas* and *Achromobacter* (Lee, 2005). This assertion is supported by the findings of (Rump, 1999), where it was reported that water with ammonia level between 0.3 mg/L - 1.00 mg/L is contaminated and contains putrefying bacteria. The ammonia concentration of the treated diesel contaminated groundwater sample was far higher than (0.5 mg/L) recommended limit for quality drinking water allowed by W.H.O. (1996). The concentration of the total dissolved solids (TDS) in the uncontaminated groundwater sample was 115 mg/L while that of the diesel contaminated groundwater sample was 170 mg/L. The treated diesel contaminated groundwater sample had a value of 1030 mg/L for total dissolved solids. The high TDS value was as a result of the heavy organic load of the animal waste. The removal of hydrocarbons from the system eradicated the non-polar environment thereby increased the solubilization of the solutes present thus increased the TDS value. The TDS value was far above the 500 mg/L set by W.H.O. (1996). The total suspended solids (TSS) level for the uncontaminated groundwater sample was 1.089 mg/L while that of the diesel contaminated groundwater sample was 1.38 mg/L. The introduction of diesel into the groundwater sample made the medium to have a non-polar hydrophobic environment and decreased solubility for solutes present may be mostly responsible for the high value of TSS in comparison with the value for the uncontaminated groundwater sample. The treated diesel contaminated groundwater sample had TSS value of 6.90 mg/L. This value of the total suspended solids of the treated diesel contaminated groundwater was lower than the 10 mg/L set by W.H.O. (1996). Total solid of the uncontaminated groundwater sample was 116.09 mg/L. After simulation of the groundwater sample with diesel, the total solids concentration was 171.38 mg/L suggesting that there was contribution of total solid from the diesel as contaminant. Total solids after remediation were significantly high as result of contribution from the organic supplement. The uncontaminated groundwater sample had the following heavy metal ion concentrations Ni = 0.62 mg/L, Hg = 0.37 mg/L, Cd = 0.21 mg/L and Cr = 0.10 mg/L. Vanadium, lead and selenium were beyond the level of detection. The diesel contaminated groundwater had heavy metal ion concentrations of: Ni = 0.59 mg/L, Hg = 0.42 mg/L, Cd = 0.45 mg/L and Cr = 0.60 mg/L. Vanadium, lead and selenium were also beyond the level of detection. There was a slight reduction in the concentration of nickel after contaminating the groundwater sample with diesel that may be due complexation that rendered it in less available form. The concentrations of Cd and Hg were found to increase in the diesel contaminated groundwater sample. It was suggested that the increase in the levels of cadmium and mercury in the diesel contaminated groundwater sample can be attributed to refinery additives. Lead metal was not detected in diesel contaminated groundwater sample. Lead metal must have undergone complexation with the diesel rendering it to some chemical form that was less available for detection. The treated diesel contaminated metal ion concentrations of Ni = 7.60 mg/L, Hg = 1.14 mg/L, Cd = 2.90 mg/L and Cr = 0.44 mg/L. Vanadium, lead and selenium were beyond the level of detection (Table 2). Except for vanadium, lead and selenium which were beyond the level of detection, other heavy metals in the study had their metal ion concentrations higher than the maximum permissible limits allowed by W.H.O. (1996). The treated diesel contaminated groundwater sample showed significant increase in concentrations of

the heavy metals due to the contributions from the animal wastes. Animal waste is also considered as manure. Composts and manure have been reported in literature as important non-point sources of metal pollutants such as Cd, Cu, Ni, Pb, and Zn and As (Alloway, 1995). The bioremediation process was not capable of removing heavy metal ions in solution except for chromium that had its metal ion concentration reduced by 26.67%. Bioremediation of organic contaminants is easier than that of inorganic contaminants such as heavy metals because the heavy metals are not biodegradable and microorganisms can only change the speciation of metal contaminants (Pascale and Raina, 2000). The reference total petroleum hydrocarbon (TPH) as diesel in the uncontaminated groundwater sample was 26 mg/L. The treated diesel contaminated groundwater sample had total petroleum hydrocarbon removal efficiency of 91.50%. This significant reduction in TPH was due to the microorganisms which utilized the contaminant as growth nutrients. In the control experiment that had no organic supplement no appreciable reduction was observed because these microorganisms were absent or not in significant population. This finding was supported by the earlier work of Amadi, (1992) who reported that high population sizes suggest increase in hydrocarbon assimilation. At the end of the bioremediation, the concentration of total petroleum hydrocarbon as diesel was 411.42 mg/L. This value was far higher than the reference TPH and the 10 mg/L for quality drinking water set by W.H.O. (1996).

In conclusion, high bioremediation efficiency was attained and some of the physiochemical properties such as pH, dissolved oxygen (DO), total suspended solids, total solids and nitrate of the treated sample showed appreciable improvement and were found within the W.H.O Standard. Other physicochemical parameters (alkalinity, salinity, turbidity, electrical conductivity, phosphate, ammonia, total dissolved solids, heavy metals and TPH) were impaired, compromising quality and therefore the need for post treatment before the treated diesel contaminated groundwater becomes safe for domestic and agricultural uses.

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