Bioremediation Potential of Oilfield Produced Water in A Crude Oil Contaminated Soil in Nigeria

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ABSTRACT

Bioremediation is application of microorganisms to regain polluted environment; a reliable and safer method for cleanup of crude oil contaminated ecosystem. This research investigated the bioremediation potential of oilfield produced water in a soil. Composite soil samples were contaminated with 270ml (9%) Bonny light crude oil to give crude-polluted soils; uncontaminated soil served as control. Crude oil contaminated soils were treated with equal volumes (100ml) of bacterial culture, produced water, produced water plus bacterial culture, and NPK fertilizer to simulate bioremediation; one untreated soil was left as crude-polluted soil option; soil options incubated for 150 days and analysis done at intervals analysis. Physicochemical parameters and microbial counts fluctuated in the soils throughout the period of experiment. Some physicochemical parameters decreased after pollution with crude oil and in the treated soil options when compared to uncontaminated soil; TOC and THC concentrations increased after crude contamination; TN content were equal in all soil options. Densities of THB and THF reduced after contamination with crude petroleum but increased again in the treated soils; HUB and HUF and percentages increased in crude-polluted soil and in all treated soil options when compared with the control. Percent hydrocarbon reduction were: unpolluted 0.1%, crude polluted 12.8%, bacterial treated 22.9%, produced water treated 23.6%, bacteria+produced water treated 13.5% and fertilizer treated 27.1%. Physicochemical parameters were within permisable limits, and microbial densities were high. Percent hydrocarbon reduction for produced water treated soil option was next highest to fertilizer treated soil option and could be alternative remediation supplement to fertilizer. In conclusion, crude oil contamination of soil and remediation activity decreased some physicochemical parameters and microbial densities, and increased other parameters and counts, and produced water was successful as a treatment application for remediation of crude oil polluted soil.

KEY WORDS: *Potential; composite; supplement; contaminated; physicochemical; microbiological.*

INTRODUCTION

Bioremediation is the act of enhancing natural biodegradation process by use of microbial populations in order to cause an acceleration of the natural degradation of the environment contaminated by petroleum hydrocarbons and chemical spills (Dollah, 2004). But of recent, the use of microorganisms has particularly proved effective (Rosenberg, 1993; Aislabie *et al*., 1998; Atlas and Bartha, 1992; Wardell, 1995; Margesin and Schinner, 2001; Whyte *et al*., 1999; Mohn and Stewart, 2000). Basically, microorganisms play a major role in mopping up oil from crude oil

contaminated environment and in oil exploration (Dollah, 2004). But scientists have discovered an unusual community of microorganisms in the soil and water that can breakdown crude oil into environmental friendly compounds. These microorganisms include bacteria and fungi.

"Produced water" being the main aqueous discharge from oil production platforms comprises of formation water and injected water (Somerville *et. al*, 1987; Wills, 2000). Oil production platforms and onshore oilfields discharge the oily water or produced water into the environments as part of their normal operations (Somerville *et al*., 1987; Wills, 2000). The discharge of produced water is usually done after it has been separated from oil drawn from the reservoir and treated (Girling, 1989; Wills, 2000). Treatments include passing the oily-water through an oil-water separator to reduce the oil content of the final discharged water; addition of water clarifier to remove more oil from the produced water and; biocide application to reduce numbers of sulphate-reducing bacteria and aerobic bacteria to safe level in the wastewater (Read and Blackman, 1980; Obire and Wemedo, 1996; Wills, 2000). Oilfield produced water (wastewater) contains inorganic and organic constituents (Wardley-Smith, 1979; Wemedo *et al*., 2012), hydrocarbon components (Koons *et al.,* 1977; Wemedo *et al*., 2012), and have variations in their chemical composition and behaviour.

Discharge of oilfield wastewater is strictly controlled by legislation because after treatment it still contains traces of oil as well as dissolved inorganic and organic constituents (Koons *et at.*, 1977; *Odeigah et al*., 1997; Wills, 2000) hence its discharge could impact the environment negatively. The permitted level of oil in produced water that can be discharged from an installation is 40ppm, average per month. Some microorganisms are capable of at least partially cleaning the environments polluted with crude oil. Basically, microorganisms play a major role in bioremediation of crude oil contaminated environment (Dollah, 2004); and produced water could be a potential source of these microorganisms (Wemedo *et al*., 2012). Also, chemical constituents of the wastewater may constitute nutrient supplement for naturally occurring microorganisms.

In Nigeria, there has been no attention on the useful property of oilfield produced water, in this case, as supplement for bioremediation of crude oil contaminated environment. The information available focussed on effects of the produced water on microorganisms (Obire and Wemedo, 1996; Obire and Amusan, 2002). The objective of this study therefore, was to investigate the application of oilfield produced water as supplement for bioremediation of crude oil contaminated soils; the aim being to determine its usefulness instead of total disregard as mere industrial waste.

MATERIALS AND METHODS
Study area

The study area was Elele-Okiniali Community in Ikwerre Local Government Area of Rivers State, Nigeria; a plain land made up of tropical rainforest vegetation in the Niger Delta area of Southern Nigeria. The oilfield location, owned by Total Fina-Elf Nig. Plc., situate in the area. Oil wells are found spread over the sparse of fallow farmlands used for agricultural purposes by the inhabitants, and there is a flow station. The oilfield is designated "Olo" oilfield location comprising of two sections namely Olo 1 and Olo 2 oilfields about 300m apart. Each section has a number of oil wells, and in addition, the Olo 1 houses the flow station, which serves the entire oilfield location. Sampling was done from four sampling stations as follows: Olo 1: stations 1 and 2; Olo 2: stations 3 and 4. Each station of the Olo 1 and Olo 2 oilfields has an area of 50m^2 with a distance of 50m apart. Station 1 is closest to the flow station about 20m away.

Collection of Produced Water Samples

Freshly treated oilfield produced water samples were collected from the outlet of the separation/treatment plant of Total-Fina-Elf Company Plc oilfield flow station located at Obagi Community in Ogba-Egbema-Ndoni Local Government Area of Rivers State. Sterile sample containers were used to collect the produced water samples. Prior to collection of the produced water, the interior of the nozzle of the outlet valve was flushed by allowing the water to flow to waste for 2 to 3 minutes. After which the sample containers were filled from a gentle stream of the produced water, and the samples were collected freshly-treated before it made contact with the environment, and used for treatment of soil.

Collection of Soil Samples

Soil samples were collected from fallow agricultural lands of the four sampling stations. Surface soil (0 − 15cm depth) was collected using a clean auger borer. To obtain the samples, the auger borer was dug into the soil dawn to the required depth, and the bulked soil samples were put into fresh unused black polythene bags capable of holding the required soil quantity (3kg weight). Duplicated soil samples were obtained from each sampling station, appropriately labeled and immediately transported to the green house for treatment.

Collection of Crude Oil, Diesel Oil and Engine Oil

Crude oil was collected from the "Olo" oilfield flow station from outlet valve of the expedition line with the assistance of the technical staff. To obtain the crude oil, the valve was opened and the crude allowed to gently flow into a newly unused clean plastic container fitted with cork and capable of holding the volume of crude required. The crude was taken to the green house and used to contaminate soils for remediation experiment. Diesel oil and engine oil used during this research were obtained from Total filling station located at Rumuokwuta junction along Ikwerre Road in Port Harcourt.

Preparation and Treatment of Soil Samples

Composite soil was prepared by thoroughly mixing equal quantities of soil samples collected from the four sampling stations. The composite soil was distributed in three kilograms (3kg) weight each into six (6) different fresh unused polythene bags designated Bags A, B, C, D, E and F. The polythene bags were perforated at the bottom to allow excess water gradually drain off as the soil samples were watered in course of the soil incubation. Equal volumes (200ml) of sterile distilled water were used to moisten the soil samples immediately after bulking the soil into the sample bags and at monthly intervals throughout the period of the experiment. Two hundred and seventy milliliters (270ml) or nine percent (9%) of Bonny light crude oil was added to each of the soil separately in the polythene bags to simulate contamination except Bag A, which was left uncontaminated and served as control soil option. The soils and crude oil were thoroughly mixed in the sample bags.

After contamination of the soils with crude oil, various treatment applications (100ml volume each) were introduced onto each sample and thoroughly stirred except Bag B, which was left untreated after crude contamination and taken as crude contaminated soil option. Bag C was treated with bacteria broth culture obtained by adding 100ml of produced water into 900ml of sterile nutrient broth (1:10 dilution) and incubated for 24 hours. This treatment was designated as bacteria-treated crude contaminated soil option. Bag D was treated with freshly-collected oilfield produced water and taken as produced water-treated crude contaminated soil option. Bag E was treated with bacteria broth culture and produced water (50:50v/v) and taken as bacteria-produced water treated crude contaminated soil option. While bag F was treated with NPK fertilizer solution, diluted with sterile de-ionized water (1:10v/v) and designated as fertilizer treated crude contaminated soil option. The soil options were incubated for 150 days (remediation period) and samples taken at intervals of Day 1, Day 14, 30 days, 90 days and 150 days for physicochemical

analysis and at additional intervals of 7 days and 60 days for microbiological analysis. Day 1 sample was taken from the freshly-collected composite soils of each soil option before moistening with sterile de-ionized water. Moistening of soil with sterile distilled water was done monthly throughout the period of remediation.

Physicochemistry of soil samples

Physicochemistry of soil samples involved analysis of pH, electrical conductivity, total hydrocarbon content, percent total organic carbon, percent total nitrogen, available phosphorus, and exchangeable potassium. Measurement of pH was done using a pH meter (Model No 7 Corning); the electrode was first stabilized in distilled water and immediately put into the sample to obtain the reading at stabilization. Electrical conductivity of the samples was determined using a Jenway 4020 conductivity meter; the electrode was immersed into the sample, allowing the meter to stabilize and results recorded. Toluene extraction method was used for measurement of total hydrocarbon content (Odu *et al*., 1985); and the extract was read directly at 420nm using Spectronic 20. Hydrocarbon concentrations were calculated by multiplying with the appropriate dilution factor and results expressed as parts per million (ppm). Percent total organic carbon was determined by the wet combustion (titration) method of Walkley and Black (1934); results obtained by calculation. The macro-Kjeldals method of Jackson (1962) was employed for measuremant of total nitrogen. Ammonium-nitrogen (NH_4-N) in the distillate was determined by titration and then percent nitrogen content was calculated. Available phosphorus was analyzed using the ascorbic acid molybdate (blue color) method of Bray and Kurtz, 1945; Murphy and Riley, 1962; after mixing the sample with the reagents, absorbance of the solution was read on a spectrophotometer (Spectronic 20, Milton Roy Company, NY) at 882 wavelengths, and the amount of phosphorus in the sample determined by reading from the standard curve previously prepared. Exchangeable K^+ was determined by Air-Natural Gas Flame method using flame photometer and the concentration of the extract determined from the meter readings and calibration curve; calculation was done to obtain the exchangeable potasssium values.

Microbiology of soil samples

Microbiology of soil samples involved enumeration and isolation of bacteria and fungi (total heterotrophic and hydrocarbon-utilizing) from control and treated soil options as well as analysis of percent hydrocarbon-utilizing bacteria and fungi. Enumeration of bacteria and fungi from soil samples were performed using the ten-fold serial dilution method of Harrigan and McCance (1990) and Ofunne (1999) to obtain up to 10^{-3} dilutions. Finally, 0.1ml (aliquots) of appropriate dilutions was spread plated in duplicate, using a sterile bent glass rod, onto the surface of fresh dried sterile nutrient agar medium (for bacteria), saborourd dextrose agar medium (for fungi), and oil agar medium (for hydrocarbon-utilizing bacteria and fungi) in Petri dishes, and incubated at temperature of 30 ± 2^0C for 24 - 48 hours or 3 - 7 days (in case of hydrocarbon-utilizing bacteria and fungi), after which plates that had significant growth were counted and the colonies recorded.

RESULTS

Ranges of physicochemical parameters and microbial populations in control, crude oil contaminated, bacteria treated-, produced water treated-, bacteria plus produced water treated- and fertilizer treated- crude oil contaminated soil options throughout the period of experiment are shown in Tables 1 and 2 respectively.

Mean pH values and THF counts were higher in control soil and lower in the other soil options. Concentration of phosphorus was slightly higher in control soil, decreased in other soil options and increased again in fertilizer treated soil. Mean data of THC and %TOC were lower in

control soil and increased in other soil options; %TN values were equal in control and other soil options but increased slightly in fertilizer treated soil. Mean EC values were high in control soil, decreased in crude contaminated soil but increased in other soil options and decreased to lowest value in fertilizer treated soil option. Mean potassium concentration was high in control soil, decreased in crude contaminated soil option but increased in the other soil options above the control soil. Mean THB counts were high in control soil, reduced in the other soil options except in bacteria-treated soil option where it peaked. Mean HUB, %HUB, HUF and %HUF were lowest in control soil and increased in the other soil options.

DISCUSSION

Values of pH were within acidic range and fluctuated within this range in all the soil options. Statistically, there was no significant difference (p>0.05) between the pH of control soil and treated crude contaminated soil options, which can be explained that the variations in pH were insignificant. pH values observed at intervals of incubation increased with increasing days of incubation in two soil options and slightly decreased in three options. There was significant difference (P<0.05) between the periods of incubation. Mean pH values were slightly higher in control soils than crude contaminated treated soil options; pH values were not altered in any significant way. Values of electrical conductivity (μ$/cm) in the control, crude contaminated and treated crude contaminated soil options were higher in some samples and low in the other samples; values were high at beginning, decreased with increasing time of incubation and increased again towards the end of the experiment, only crude contaminated soil option had lower values. EC values showed no significant difference (p>0.05) in the soil options, and time of incubation. Fertilizer treated crude contaminated soil option had lower mean EC values than control option while the other treatment options had increased EC values above that of control soil option.

Total hydrocarbon content reduced with time in all the soil options and traces of hydrocarbon were observed in uncontaminated soil samples; uncontaminated soils could have received the traces of oil from crude oil vapour arising from the wellheads and other operations, which falls back to the soil periodically. Percent hydrocarbon reduction of crude oil at the end of experiment showed that fertilizer-treated soil had the highest reduction, followed by produced water-treated soil, bacteria treated soil option, bacteria plus produced water treated soil option; crude contaminated soil option, and the lowest was control soil which suggested that the study area had not been exposed to contamination by crude petroleum. THC values showed significant difference (P<0.05) between the soil options but no significant difference (P>0.05) between the time intervals of incubation. Produced water increased bioremediation activities in the soil and could be recommended as the cheapest nutrient supplement for bioremediation of petroleum contaminated soil. Percent total organic carbon were high in all the soil options and decreased with time; control soil option had lowest %TOC values throughout the remediation period, while the other soil options recorded higher values than the control. %TOC values showed no significant difference (P<0.05) between the soil options but significant difference occurred between the time intervals of incubation. Mean values showed that addition of crude oil to the soils increased total organic carbon content above that of the control soil option, which was maintained throughout remediation.

Percent total nitrogen were generally low, less than 1% in all cases; control soil samples had higher %TN values at the end of remediation than the other soil options. Mean %TN values were lower in control soil but equal in all the treatment options. Remediation activities slightly increased

soil nitrogen. %TN values showed no significant difference between the soil options but significant difference (P<0.001) occurred between the time intervals of incubation. Phosphorus values increase with time of experiment in all the soil options; mean values were slightly higher in control than in the other soils. Phosphorus values showed no significant difference between the soil options but significant difference (P<0.05) existed between time intervals of incubation. Remediation activity did not influence phosphorus concentration of the soils. Potassium values general decreased from the beginning to the middle of the experiment and increased again towards the end of experiment; mean values were slightly higher in crude contaminated-treated soils than in control soil but lower in crude contaminated soil. Potassium values showed no significant difference between the soil options but significant difference (P<0.001) was observed between the time intervals of incubation. Remediation did not change potassium concentration of soil.

Counts of heterotrophic bacteria and fungi fluctuated in the soil options and days of incubation throughout the period of experiment; counts did not follow any particular trend but generally decreased at day 14 and increased again in most soil options. Mean counts were higher in control than crude contaminated and treated crude contaminated soil options; only crude plus bacteria treated-crude contaminated soil had higher bacterial counts than control soil. Statistically, THB counts showed no significant difference between the soil options and the time intervals of remediation but significant difference (P<0.05) existed in THF counts between time intervals of remediation and no significant difference between the soil options. Produced water contains bacteria (Wemedo *et all*., 2009, 2012) and inorganic constituents (Koons *et at*., 1977; Odeigah *et al*., 1997; Wemedo and Obire, 1996; Wills, 2000; Wemedo *et all*., 2012).

Densities of hydrocarbon-utilizing bacteria and percent hydrocarbon-utilizing bacteria greatly fluctuated in all soil options reaching their peak at different days and showed no partcular trend in variations throughout the period of experiment. Mean values were generally higher in the other soil options than in control option, which showed that addition of crude oil to the soils and the treatment options increased HUB populations, and %HUB. However, HUB counts showed no significant difference between soil options but significant difference (P<0.05) existed during time intervals of remediation. Percent bacterial hydrocarbon-utilizers were approximately 1% or greater than 1% in all the soil options. Percent hydrocarbon-utilizers showed no significant difference between the soil options and time intervals of remediation. Counts of hydrocarbon-utilizing fungi and percent hydrocarbon-utilizing fungi fluctuated in the soil options and days of experiment, and did not follow any particular trend throughout the period of experiment. Mean HUF counts and their percentages were higher in other options than in control option. HUF counts showed no significant difference between the soil options but significance difference (P<0.05) existed between the time intervals of remediation. Percent hydrocarbon-utilizing fungi was highest at day 14; there was no significant difference between the soil options but significant difference (P<0.05) existed between time intervals of remediation.

Occurrence of hydrocarbon-utilizing bacteria and fungi with certain levels of percent hydrocarbon-utilizers recorded in the uncontaminated soil samples indicated that hydrocarbon-utilizing microorganisms occurred naturally in the soil of the study area especially being area with oil exploration activity. However, mean percent hydrocarbon-utilizers were far greater in crude contaminated soil option and treated soil options than in the control soil. Generally, the greater the values of percent hydrocarbon-utilizers (above %1) the heavier the concentrations of crude oil in the soil. Contamination of soil with crude oil and treatments applied increased hydrocarbon-utilizing microbes and their percentages far above the indiginous bacterial hydrocarbon-utilizers of the control soil option. Atlas (1981) reported that petroleum degrading microorganisms occur in

nature and increased in response to crude oil contamination; however, during recovery after spillage, the numbers of hydrocarbon-utilizers return, at most sites, to background levels as the oil disappeared due to biodegradative removal.

CONCLUSION

The study noted that oilfield wastewater can be useful for clean up of crude oil contaminated soil, and the use of oilfield wastewater as nutrient supplement and as a source of microorganisms for bioremediation of crude oil-contaminated soil had proved successful. The potential of oilfield wastewater for bioremediation activities is because it contains inorganic constituents (Somerville *et al*., 1987; Obire and Wemedo, 1996, 2002; Obire and Amusan, 2003; Wemedo *et al*., 2012) and high microbial load such as bacteria and fungi (Wemedo *et al*., 2009, 2012). Produced water-treated soil had the next highest percent reduction of hydrocarbon to fertilizer-treated soil. Bioremediation activity of oilfield produced water and other treatment options increased some physicochemical parameters and decreased others; bacteria and fungi also responded differently to bioremediation in the soil options but their numbers were somewhat high.

REFERENCES

Aislabie, J., M. Mcleod, and R. Fraser (1998). Potential for Bioremediation of hydrocarbon in soil from the Ross Dependency, Antarctica. Appl. Microbiol. Biotechnol., 49:210 – 214.

Atlas, R. M.(1981). Microbial degradation of petroleum hydrocarbons: an environmental Perspective. Microbiological Review, 45(1): 180 – 209.

Atlas, R. M. and Bartha, R. (1992). Hydrocarbon Biodegradation and Oil Spills Bioremediation. Adv. Micrbiol. Ecol. 12:257-338.

Bray, R. H. and Kurtz, L. T. (1945). Determination of Total Organic Carbon and Available Forms of Phosphorus in Soils. Soil Science, 59:39 – 45.

Dollah, S. A. (2004). Bioremediation of Petroleum Hydrocarbon Polluted Soil: Potentials, Principles and applications. J. Environ. Mgt. Educ. 1(2):171-180.

Girling, A. E. (1989). Acute and chronic toxicity of produced water from North Sea Oil production platform to the Calanoid copepod. *Acartia tonsa.* Bull. Environ. Contam. Toxicol. 43:280-286.

Jackson, M. L. (1962). Soil Chemistry Analysis. Prentice Hall, New York.

Koons, C. B., C. D. Mcauliffe and F. T. Weiss (1977). Environmental Aspects of produced waters from oil and gas extraction operations in offshore and coastal waters. J. Petrol. Technol. 29:723-729.

Mohn, W. W. and Stewart, G. R. (2000). Limiting factors for hydrocarbon bioremediation at low temperature Arctic soils. Soil Biol. Biochem. 32:1161-1172.

Harrigan, W. F. and M. E. McCane (1990). Laboratory Methods In Food and Dairy Microbiology. 8th Edition. Academic Press, London. Pp 136 – 138.

Margesin, R. and Schinner, F. (2001). Bioremediation (Natural Attenuation and Biostimulation) of Diesel-oil Contaminated Soil in an Alpine Glacier Sking Area. Appl. Environ. Microbiol. 67(7):3127-3133.

Murphy, J. and Riley, J. P. (1962). A modified single solution method for the determination of phosphorus in natural waters. Analytical Chemistry. 27: 31 – 36.

Obire, O. and Amusan, F. O. (2003). The Environmental Impact of Oilfield Formation Water on a Freshwater sream in Nigeria. J. Appl. Sci. Environ. Mgt. 7(1): 61-66.

Obire, O. and Wemedo, S. A. (1996). The effect of oilfield wastewater on the microbial populations of a soil in Nigeria. Niger Delta Biologia. 1(1):77 – 85.

Obire, O. and Wemedo, S. A. (2002). Mycoflora of a Kerosene-polluted Soil in Nigeria. J. Appl. Sci. Environ. Mgt. 6(1):14-17.

Odeigah, P. G. C., Nurudeen, O. and Amund, O. O. (1997). Genotoxicity of oilfield wastewater in Nigeria. Hereditas, 126:161 – 167.

Odu, C. T. I., Esuruoso, O. F., Nwaboshi, L. G. and Ogunwale, J. A. (1985). Environmental study of the Nigerian Agip Oil companyoperational area. Soil and Fresh Water Vegetation. Union Grafts Publics. Milan, pp 22 – 25.

Ofunne, J. L. (1999). Bacteriological Examination of Clinical Sp[ecimens. Achugo Publications, Ama J. K. Recreation Park, Owerri, Nigeria.

Read, A. D. and Blackman, R. A. (1980). Oily water discharges. Regulatory, Technical and Science Publishers Ltd, London.

S. A. Wemedo and O. .Obire (2012). Acute Toxicity Test of Oilfield Wastewater on Bacterial Community of a Soil in Nigeria. Res. J. Environ. Toxicol., 6(1): 25-32.

Somerville, H. J., Benneth, D., Davenport, J. N., Holt, M. S., Lynes, A., Mahieu, A., Mcccurt, B., Parker, J. G., Stephenson, R. R., Watkinson, R. J, and Wilkinson, T. G. (1987). Environmental effect of produced water from North Sea Oil Operations. Mar. Pollut. Bull. 18(10): 549 – 558.

Rosenberg, E. (1993). Microorganisms to combat pollution, Boston, Khuwer Academic Publishers. Pp. 64 72.

Wardell, L. J. (1995). Potential for bioremediation of fuel contaminated soil in Antarctica. J. of Soil Contam. 4:111-121.

Walkley, A. and Blackman, I. A. (1934). A critical examination of a rapid method for determining organic carbon in soils – effect of variation in digestion conditions and on inorganic soil constituents. Soil Science. 63:251-264.

Wardley-Smith, J. (1983). The control of oil pollution, Graham and Trotman Ltd., London, pp 37–44.

Wemedo, S. A., N. P. Akani and C. E. Eke (2009). Fungi in an oilfield wastewater in Nigeria. Asian J. Biological Sciences. 2(2): 54-57.

Wills, J. (2000). The effects of discharges of produced waters. Ekologickeskaya Vahkta Sakhulina (Sakhalina Environment Watch) 25th May, Sakhalina, pp 1-5.

Whyte, L. G., Bourbomiere, L., Bellerose, C., Inniss, W. E. and Greer, C. W. (1999). Bioremediation assessment of hydrocarbon-contaminated soils from the High Arctic. Bioremed. J. 3:69-79.

Table 1: Ranges and means±SD of physicochemical parameters of control, crude-contaminated and treated crude-contaminated soil options during bioremediation

Physicochemical Parameters	Control (No Crude)	Crude only	Crude + Bacteria	Crude + Produced water	Crude + Bacteria + Produced water	Crude + Fertilizer
pH_w (1:2:5)	5.20 - 6.50 (5.80±0.47)	4.70 - 6.20 (5.58±0.68)	4.80 - 6.20 (5.64±0.64)	4.90 - 6.30 (5.78±0.60)	4.90 - 6.20 (5.72±0.56)	4.50 - 6.30 (5.68±0.72)
Electrical Conductivity (μS/cm)	110 - 260 (158±61)	80 - 201 (140±54)	90 - 290 (199±85)	90 - 290 (215±87)	85 - 380 (242±95)	60 - 130 (96±29)
Total Hydrocarbon content (mg kg^{-1})	2.45 - 10.15 (6.58±3)	3375.29 - 4460.41 (3975.42±40)	2443.07 - 4381.88 (3394.55±84)	2378.77 - 4376.99 (3159.31±23)	3214.56 - 4354.13 (3585.60±20)	2485.93 - 4783.40 (4020.10±95)
Percent Total Organic Carbon	0.52 - 1.58 (0.94±0.4)	1.46 - 3.43 (2.14±0.5)	1.38 - 3.19 (2.04±0.7)	1.61 - 2.74 (1.93±0.5)	1.57 - 2.61 (2.00±0.5)	1.76 - 3.12 (2.12±0.6)
Percent Total Nitrogen	0.04 - 0.11 (0.07±0.03)	0.06 - 0.10 (0.07±0.02)	0.06 - 0.09 (0.07±0.02)	0.06 - 0.08 (0.07±0.01)	0.07 - 0.08 (0.07±0.05)	0.07 - 0.09 (0.08±0.01)
Available Phosphorus (mg kg^{-1})	79.60 - 85.76 (81.56±2.5)	73.58 - 86.70 (80.50±5.4)	76.54 - 84.36 (79.89±3.1)	67.25 - 83.89 (74.31±7.0)	63.49 - 79.20 (72.71±6.9)	71.14 - 92.79 (80.52±9.7)
Exchangeable potassium (meq 100g^{-1})	0.16 - 0.29 (0.21±0.05)	0.14 - 0.26 (0.19±0.06)	0.17 - 0.37 (0.23±0.08)	0.16 - 0.28 (0.22±0.06)	0.16 - 0.29 (0.23±0.06)	0.18 - 0.34 (0.25±0.06)

Mean values in parenthesis

Table 2: Ranges and means±SD of microbial populations of control, crude-contaminated and treated crude-contaminated soil options during bioremediation

Microbial Populations	Control (No Crude)	Crude only	Crude + Bacteria	Crude + Produced water	Crude + Bacteria + Produced water	Crude + Fertilizer
Total Heterotrophic Bacteria $(X10^5 CFU\ G^{-1})$	4.0 - 13.5 (8.4±4.0)	0.85 - 19.4 (5.3±6.6)	4.9 - 25.7 (10.8±8.6)	1.15 - 10.8 (5.6±3.4)	1.45 - 28.8 (7.7±10.0)	1.19 - 18.4 (6.6±6.2)
Hydrocarbon-utilizing Bacteria $(X10^4 CFU\ G^{-1})$	0.8 - 8.0 (2.6±2.5)	0.8 - 29.3 (5.9±10.5)	0.3 - 19.2 (6.5±6.4)	1.4 - 11.1 (7.8±8.6)	0.9 - 29.8 (12.3±12.5)	0.8 - 28.0 (8.0±9.7)
% Hydrocarbon-utilizing Bacteria	1.0 - 5.9 (3.1±1.9)	0.7 - 88.8 (26.1±37)	1.1 - 34.9 (9.6±12)	2.1 - 96.5 (23.5±34)	0.4 - 80.5 (20.0±28)	2.9 - 23.2 (11.7±7.6)
Total Heterotrophic fungi $(X 10^5 CFU\ G^{-1})$	1.9 - 55 (25.9±26)	0.4 - 38 (9.3±13)	0.5 - 54 (13.6±22)	0.4 - 68 (16.5±25)	0.4 - 36 (9.2±14)	0.4 - 44 (11. 0±17)
Hydrocarbon-utilizing Fungi $(X 10^3 CFU\ G^{-1})$	0.1 - 2.7 (1.1±1.1)	0.4 - 3.1 (1.6±0.9)	0.3 - 3.5 (1.5±1.2)	0.1 - 2.9 (1.4±1.1)	0.2 - 2.1 (1.0±0.7)	0.3 - 2.0 (1.2±0.7)
% Hydrocarbon-utilizing Fungi	2.5 - 31.6 (7.7±11)	1.2 - 450 (85.8±161)	6.5 - 133 (51.6±44)	4.0 - 275 (53.1±99)	5.3 - 325 (60.6±118)	4.0 - 275 (66.1±95)

Mean values in parenthesis