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A Case Study of Petroleum Degradation in Different Soil Textural Classes

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Abstract

The effects of different soil textures on biodegradation of petroleum hydrocarbons were investigated during a six-week period. The study employed five soil textural classes commonly found in Port Harcourt metropolis, Nigeria, namely sand, loamy sand, sandy loam, silty clay and clay. Experimental cells containing the different soil textures were contaminated with the same amount of crude oil. The soils were then remediated using agro-technical methods such as biostimulation with nutrients, tilling and watering. Soil properties such as total hydrocarbon content (THC), total heterotrophic bacterial (THB) counts, organic carbon, total nitrogen, pH and moisture content were monitored over time. Bacterial numbers declined significantly in the fine soil textures after petroleum contamination, but were either unaffected or increased significantly in the coarser soil textures. After 6 weeks of similar treatment, the hydrocarbon losses ranged from 42% - 99%; the sandy loam had the highest, while the clay soil had the least THC reduction. The THB counts generally corroborated the THC results. Fold increase in bacterial numbers due to remediation treatment decreased with increasing clay content. The results

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suggest that higher sand than clay content of soil favours faster hydrocarbon bioremediation.

Hydrocarbon biodegradation efficiency increased with silt content among soil groupings such as fine and coarse soils but not necessarily with increasing silt content of soil. Thus, there seems to be cut-off sand and clay contents in soil at which the effect of the silt content becomes significant. These observations merits further investigations with the full range of soil textures.

Keywords: Bacteria count; bioremediation; biostimulation; contaminated soil; soil texture; total hydrocarbon content.

Introduction

Petroleum contamination of soils is a widespread environmental problem linked with crude oil exploration. Bioremediation has gained wide acceptance globally as the most effective treatment technology for organic contaminants. Among bioremediation techniques, biostimulation of indigenous soil microbes through the addition of nutrients, coupled with agro-technical methods such as frequent tilling and watering have proven to be very effective in the attenuation of total petroleum hydrocarbons in soils [1-6]. Moreover, patents have been granted for a number of proprietary techniques to enhance biodegradation of petroleum-hydrocarbons. One of such patents dealt with the use of non-pathogenic, thermophilic bacteria for thermal biodegradation of toxic petroleum hydrocarbons and halogenated organic contaminants [7]. Another employed encapsulation of micro-organisms of the genus, Candida in paraffin wax to form organism-containing wax microshells that can degrade hydrocarbon-based substances [8]. While more recent patents in this area have dealt with biodegradation of petroleum-hydrocarbons in geologic structure such as oil reservoir. These include evaluating, changes in composition of petroleum-hydrocarbons in reservoirs due to biodegradation [9], and biodegradation of trapped hydrocarbons [10]. There is a dearth of information on biodegradation of petroleum hydrocarbons in different soil textures.

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Soil texture is one of the most important physical properties of soils. The relative proportion of the three kinds of soil particles (soil separates) or mineral components of soil – sand, silt and clay, determines it. There are 12 major textural classes as defined by the United States Department of Agriculture (USDA). Soil texture has a major effect on the physical and chemical characteristics of soil, and affects soil behaviour especially retention capacity for moisture and nutrients [11]. Moreover, soil separates influence several other properties/behaviour of soils including aeration, soil organic matter content and decomposition, resistance to pH change and pollutant leaching potential. Thus, different soil textural classes would certainly have a profound influence on the efficiency of hydrocarbon biodegradation. Therefore, this study seeks to understand biodegradation efficiencies in different soil textural classes.

Majority of bioremediation studies are limited to particular soil textures. Hence, the paucity of information on the effects of different soil textural classes on biodegradation of petroleum hydrocarbons. Related studies in similar direction include the observation that the rates of degradation of two herbicides, metazachlor and metribuzin depended more on the sand content of soils, while that of another herbicide, metamitron depended on the silt content [12]. While it was suggested that the higher chemical concentration, total organic carbon and specific surface area of silt and clay particles in soil enhanced bioavailability of pyrene sorbed on the particles for degradation by Mycobacterium vanbaalenii PYR-1 [13]. Furthermore, it was observed that carbon mineralisation (using CO_2 respiration method) was significantly higher in fine soil textures (clay loam, loam and silty clay loam) than coarser soil textures (silty loam, loamy sand and sandy loam) [14]. The fine textured soils with clay contents had higher bacterial numbers than the coarse textured soils.

It can be deduced from the foregoing that the influence of soil separates on the degradation of organic contaminants depends on a number of factors. These include contaminant types and speciation, and the bioremediation method employed (whether biostimulation or bioaugmentation – introduction of cultured microorganisms into the contaminated system). To our knowledge, biodegradation of total petroleum hydrocarbons (TPH) in different soil textures

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using biostimulation is rare in the literature. This information is necessary for informed decisions on remediation plans when dealing with different soil textures. Therefore, it was the aim of this study to investigate the response of different soil textural classes to the same level of petroleum contamination and bioremediation treatment. The study also sought to investigate the relative significance of the three soil separates in oil biodegradation.

Materials and methods

Experimental design

Soils with different textural classes were collected from different parts of the study area, Port Harcourt, Nigeria, and experimental cells consisting of mounds of earth (20 cm x 20 cm x 25 cm) constructed from them. The experimental cells were spaced 2.5 m apart and they provided controlled conditions for nutrient concentration, watering and tilling. The cells also prevented excessive run-off of the contaminant. The ambient conditions of the study area include mean annual rainfall of 2,400 mm and monthly relative humidity of 85%; mean daily minimum and maximum temperature of 23°C and 31.5°C, respectively [3]. Thus, the field cells were exposed to the afore-mentioned conditions, as they were located in the open air.

The study employed five treatment cells containing five soil textural classes commonly found in the study area, namely sand, loamy sand, sandy loam, silty clay and clay. The relative proportions of soil separates in the soils are shown subsequently. A sixth cell containing silty clay served as the untreated control. The study sought to compare biodegradation in different soil textures, and hence did not investigate abiotic losses of the contaminant. Hence, each of the soil textures did not have its corresponding control. It should be noted that the use of different soil textures ordinarily suggests variation among many soil properties and make it challenging to be conclusive that observed differences are due to variation in soil textures. However, this study evaluates the relative performance of the same biodegradation treatment in different soil textures in the light of the same variability in properties that would be encountered under field conditions.

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Field procedures

The experimental cells were each contaminated with 0.17 l of Bonny light crude oil. They were then left undisturbed for 3 days to allow for interaction of the contaminant with the soils. Remediation treatment through biostimulation of indigenous microbes commenced after the 3-day period. This entailed application of 15-15-15 NPK fertiliser, tilling and watering. The same quantity of NPK fertiliser (34.4g) was applied to all treatment cells twice during the 6-week study period, 3 days after contamination and after 3 weeks of remediation. Each treatment cell also received 0.26 l of water three times a week and three times tillage per week. Previous studies in the same area have demonstrated the effectiveness of the afore-mentioned levels of fertiliser, water and tillage, especially for silty clay soils [3, 4, 15]. Hence, the same levels were used for all soil types studied.

Analysis

Soil physico-chemical parameters analysed during the study include particle size distribution, pH, moisture content, THC, total nitrogen and total organic carbon, alongside total heterotrophic bacterial (THB) counts. The detailed procedures for the above tests have been described elsewhere [4]. In summary, THC was determined from the absorbance of toluene extract of soil hydrocarbon content, while plate count agar was used for bacterial counts. Data analysis involved simple descriptive and univariate summary statistics such as mean, standard deviation and percentage. The THC was the main index for evaluating biodegradation in the different soil textures. Hence, THC data were subjected to analysis of variance (ANOVA) to compare the variability in hydrocarbon loss in the different soil textures over time.

Results and discussion

Table 1 – 6 show the physico-chemical properties and bacterial counts in the different soil textural classes. Generally, the values of the major soil properties in the five soil textures were not widely different from one another. Hence, it can be assumed that soil texture would be largely responsible for observed differences. The same level of petroleum contamination (~ 1 wt. %) of the different soil textures resulted in different THC concentrations (Table 1). This depends

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on a number of factors including the particle size distribution of the different soil types, especially as analyses was done on < 2 mm particle sizes while the whole soil mass was contaminated. The THC reduction after 2 and 6 weeks of remediation treatment generally followed the same trend. After 6 weeks of remediation treatments, the sandy loam, which showed the highest THC concentration, still showed the highest percentage THC reduction of ~99%. This was followed closely by the sandy soil with 94% THC reduction, while the loamy sand soil had 78% THC reduction. The soil textures with higher clay contents (silty clay and clay) showed the lowest THC reduction (see Table 1 and Figure 1). The untreated silty clay control had 37% THC loss. This directly compares with 54% THC loss in the treated silty clay soil and provides some idea of hydrocarbon losses due to biodegradation. Two-way ANOVA with replication showed that the THC losses across the different soil textures over time was significant at the 0.00001 probability level.

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Table 1. Total hydrocarbon content of the different soil textures

	Soil composition (%)			Total hydrocarbon content (THC) (mg/kg)				THC loss (%)	
Soil texture	Sand	Silt	Clay	Before contamination	3 days after contamination	2 weeks after remediation	6 weeks after remediation	Remediation period	
								2 weeks	6 weeks
Silty clay control	12.6 ± 0.01	40.6 ± 0.08	46.8 ± 0.09	78 ± 2	16,150 ± 420	14,670 ± 328	10,201 ± 100	9.2	36.8
Sand	87.0 ± 0.3	11 ± 0.2	2.0 ± 0.01	600 ± 10	18,460 ± 100	5,334 ± 60	1,050 ± 50	71.1	94.3
Loamy sand	85.9 ± 0.5	5.3 ± 0.8	8.8 ± 0.2	150 ± 5	22,714 ± 420	7,378 ± 100	4,963 ± 100	67.5	78.2
Sandy loam	74.4 ± 0.01	20.5 ± 0.04	5.1 ± 0.03	150 ± 10	26,460 ± 400	1,600 ± 100	380 ± 20	94.0	98.6
Silty clay	12.6 ± 0.01	40.6 ± 0.08	46.8 ± 0.09	78 ± 2	16,628 ± 150	9,676 ± 150	7,642 ± 50	41.8	54.0
Clay	8.2 ± 0.01	30.5 ± 0.01	61.3 ± 0.03	58 ± 10	20,800 ± 600	16,000 ± 400	12,000 ± 200	23.1	42.3

Results represent mean ± standard deviation of 3 replicates

Table 2. Total heterotrophic bacteria counts of the different soil textures

	Total heterotrophic bacteria count (THB) (x 10^7 CFU/g)				THB fold increase	
Soil texture	Before contamination	3 days after contamination	2 weeks after remediation	6 weeks after remediation	Remediation period	
					2 weeks	6 weeks
Silty clay control	1.05	0.05	0.06	0.10	1.3	2.1
Sand	1.98	1.98	26	55	13.1	27.8
Loamy sand	4.40	4.40	24	52	5.5	11.8
Sandy loam	1.05	10.5	110	290	10.5	27.6
Silty clay	1.05	0.05	0.16	0.23	3.3	4.7
Clay	3.20	0.06	0.11	0.17	1.7	2.7

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Table 3. Total organic carbon content of the different soil textures

Soil texture	Total organic carbon (%)			
	Before contamination	3 days after contamination	2 weeks after remediation	6 weeks after remediation
Silty clay control	0.36 ± 0.01	0.32 ± 0.002	0.26 ± 0.16	0.12 ± 0.02
Sand	0.33 ± 0.02	0.30 ± 0.03	0.38 ± 0.04	0.20 ± 0.01
Loamy sand	0.30 ± 0.01	0.32 ± 0.02	0.36 ± 0.03	0.30 ± 0.02
Sandy loam	0.24 ± 0.02	0.33 ± 0.08	0.31 ± 0.05	0.29 ± 0.02
Silty clay	0.36 ± 0.01	0.38 ± 0.05	0.32 ± 0.02	0.25 ± 0.03
Clay	0.30 ± 0.04	0.48 ± 0.06	0.42 ± 0.06	0.36 ± 0.02

Results represent mean ± standard deviation of 3 replicates

Table 4. Total nitrogen content of the different soil textures

Soil texture	Total nitrogen (%)			
	Before contamination	3 days after contamination	2 weeks after remediation	6 weeks after remediation
Silty clay control	0.58 ± 0.05	0.38 ± 0.14	0.33 ± 0.05	0.31 ± 0.06
Sand	0.14 ± 0.03	0.18 ± 0.01	0.05 ± 0.001	0.04 ± 0.005
Loamy sand	0.14 ± 0.002	0.20 ± 0.02	0.06 ± 0.02	0.09 ± 0.02
Sandy loam	0.12 ± 0.01	0.16 ± 0.01	0.03 ± 0.02	0.04 ± 0.001
Silty clay	0.58 ± 0.05	0.21 ± 0.02	0.06 ± 0.01	0.04 ± 0.01
Clay	0.49 ± 0.08	0.25 ± 0.03	0.09 ± 0.06	0.06 ± 0.02

Results represent mean ± standard deviation of 3 replicates

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Table 5. Soil pH of the different textural classes

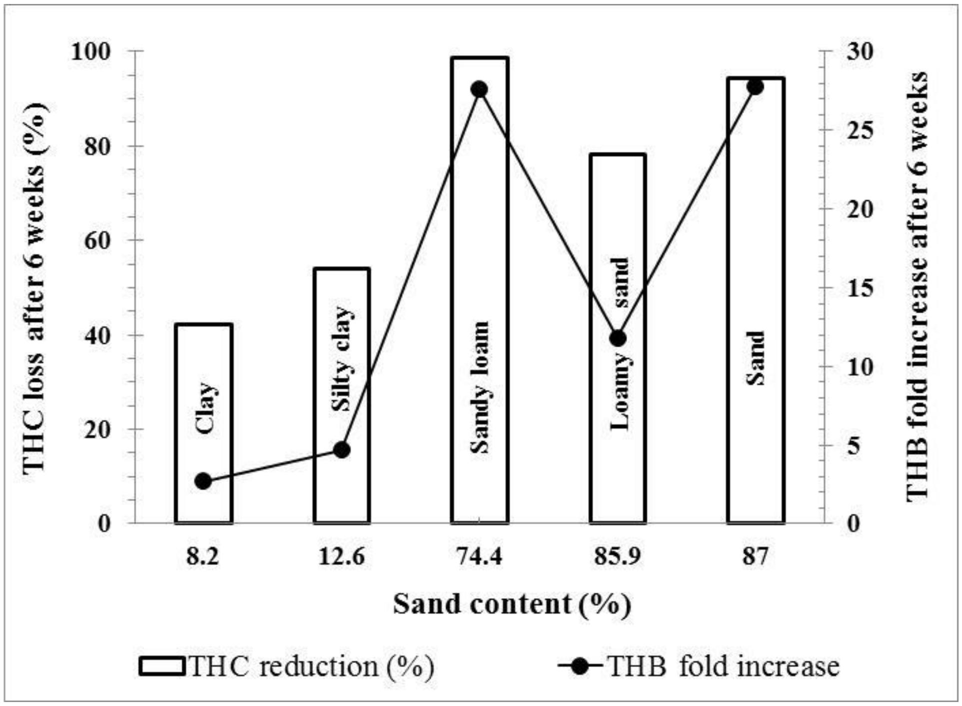
Soil texture	pH (1:5)			
	Before contamination	3 days after contamination	2 weeks after remediation	6 weeks after remediation
Silty clay control	5.70 ± 0.20	6.90 ± 0.20	6.44 ± 0.30	6.44 ± 0.30
Sand	6.70 ± 0.10	6.85 ± 0.10	7.10 ± 0.20	5.10 ± 0.20
Loamy sand	6.30 ± 0.20	6.10 ± 0.30	6.10 ± 0.10	5.40 ± 0.10
Sandy loam	7.70 ± 0.20	8.10 ± 0.02	6.22 ± 0.80	6.22 ± 0.80
Silty clay	5.70 ± 0.20	7.10 ± 0.40	5.40 ± 0.10	6.40 ± 0.10
Clay	4.80 ± 0.50	5.00 ± 0.02	5.01 ± 0.20	4.80 ± 0.20

Results represent mean ± standard deviation of 3 replicates

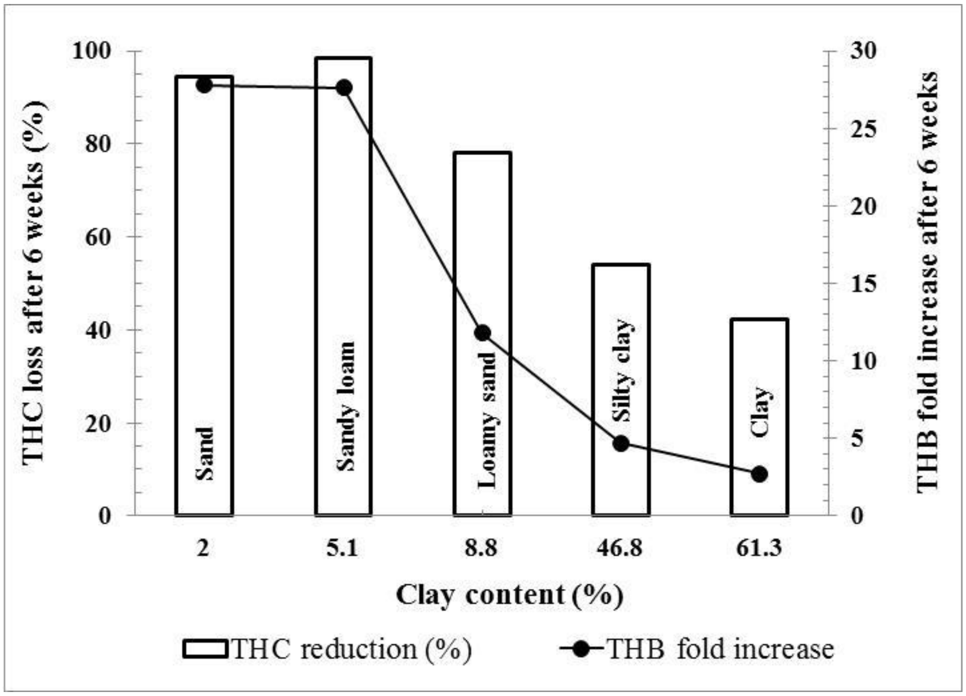
Table 6. Moisture content of the different soil textures

Soil texture	Moisture content (%)			
	Before contamination	3 days after contamination	2 weeks after remediation	6 weeks after remediation
Silty clay control	23 ± 1.0	13 ± 2.0	11 ± 1.0	16 ± 0.40
Sand	24 ± 1.0	14 ± 2.0	12 ± 1.0	16 ± 0.20
Loamy sand	23 ± 0.2	12 ± 1.0	17 ± 2.0	15 ± 0.01
Sandy loam	20 ± 0.2	14 ± 2.0	15 ± 1.0	16 ± 0.70
Silty clay	23 ± 0.2	16 ± 1.0	16 ± 1.0	15 ± 0.60
Clay	18 ± 1.0	13 ± 2.0	14 ± 0.2	15 ± 0.50

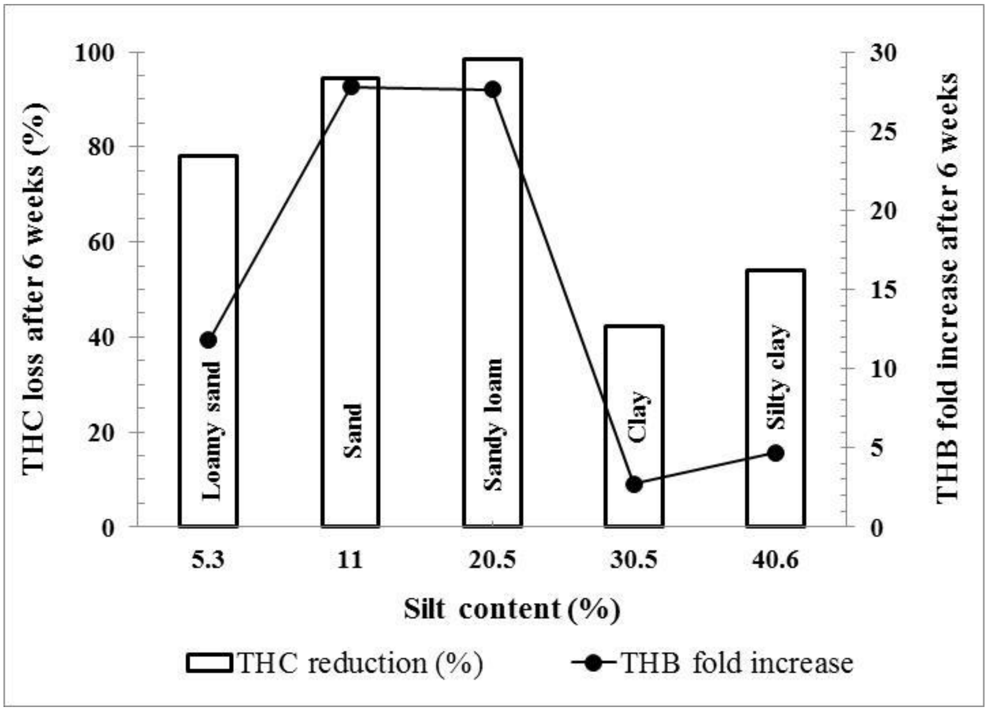
Results represent mean ± standard deviation of 3 replicates


THC loss after 6 weeks (%)
100
80
60
40
20
0
THB fold increase after 6 weeks
30
25
20
15
10
5
0
Clay
Silty clay
Sandy loam
Loamy sand
Sand
8.2
12.6
74.4
85.9
87
Sand content (%)
THC reduction (%)
THB fold increase

(a)


THC loss after 6 weeks (%)
100
80
60
40
20
0
THB fold increase after 6 weeks
30
25
20
15
10
5
0
Sand
Sandy loam
Loamy sand
Silty clay
Clay
2
5.1
8.8
46.8
61.3
Clay content (%)
THC reduction (%)
THB fold increase

(b)



(c)

Figure 1. Effects of (a) sand, (b) clay, and (c) silt contents on hydrocarbon loss and bacterial numbers after 6 weeks of bioremediation treatment

The effects of the three soil separates on THC reduction is highlighted in Figure 1. There is no clear trend in THC reduction with sand content (Figure 1a). However, percent THC reduction apparently decreased with increasing clay content, albeit with an abnormally between the sandy and loamy sand soils (Figure 1b). It can be seen from Figure 1c that although contaminant loss did not necessarily increase with increasing silt content of soil, it did increase among soil groupings. In other words, THC loss increased with increasing silt content among the coarser soil textures with high sand proportions (i.e. sand, loamy sand and sandy loam). The same applies to the fine soil textures as the silty clay showed higher THC loss than the clay. The former had higher silt content than the latter. The THC results corroborate observations on higher oil removal efficiency in soils with high proportion of sand rather than clay [16]. They are also similar to previous observations on the dependence of metamitron degradation on the silt content of soil [12]. They however differ from the observation of that fine soil textures favoured carbon mineralisation than coarser soil textures [14].

The THB counts in Table 2 show that 3 days after contamination there was significant decline in bacterial numbers in the fine soil textures (silty clay and clay). However, there was no negative effect of oil contamination in the coarser soil textures (sand and loamy sand). Interestingly, there was a ten-fold increase in bacterial numbers in the sandy loam soil in the aftermath of oil contamination. The higher specific surface area of silts and clays in the fine soil textures probably enhanced availability of the contaminant sorbed on the soil particles to microbes leading to greater toxicity [13]. Further, better aeration of the coarse soil textures would enhance microbial survival compared to the fine soil textures since oxygen is usually a limiting nutrient in oil-contaminated soils. The THB counts generally followed the same trend as the THC with the sandy loam and sandy soils recording 28-fold increase in bacterial numbers and the clayey soil recording 3-fold increase after 6 weeks (Figure 1). Figure 1 also shows that the effects of the soil separates on bacterial numbers are similar to those of the THC. However, there is a clear trend on the effect of clay content on bacterial numbers. Fold increase in bacterial numbers due to remediation treatment decreased with increasing clay content of soil (Figure 1b). The higher increase in bacterial numbers in the coarser soils is probably due to their ease of tillage when moist, which provides better aeration compared to the finer soils. Beside better aeration, the coarser soils might also easily allow for contaminant loss by volatilisation and deep percolation. The higher bacterial counts in the coarser soils can also be rationalised with the hypothesis that coarser soils exhibit larger pores that are unsaturated at most matric potentials, in which water is held in pore corners as isolated water films. These isolated water films then provide opportunities for increased bacterial diversity [17, 18].

The other soil properties (organic carbon, total nitrogen, pH and moisture content) used to support the THC and THB counts did not show any marked effect on hydrocarbon degradation in the different soil textures. These properties mainly corroborate the contaminant attenuation recorded, especially the decreases in organic carbon and soil pH over time (Tables 3 and 5). Moreover, the values of the soil properties over time across the different soil textures did not have a very wide range as expected of different soil types (Table 3 – 6). Thus, observed differences in performance can be largely attributed to variation in soil textures. Albeit, it is understood that other soil properties not considered here might also have some measure of influence on the performance of the different soils.

Conclusions

The findings of this study suggest that oil-contaminated coarser soils are more amenable to bioremediation through biostimulation than finer soils. The results suggest that higher sand than clay content of soil favours faster hydrocarbon bioremediation, especially in the study area. Fold increase in bacterial numbers due to remediation treatment decreased with increasing clay content. It is thought that the coarser soils provide better aerated microhabitats for bacteria survivability and activity during crude oil contamination as well as in the course of remediation treatments. Hydrocarbon biodegradation efficiency increased with silt content among soil groupings such as fine and coarse soils but not necessarily with increasing silt content of soil. Thus, there seems to be cut-off sand and clay contents in soil at which the effect of the silt content becomes significant - a conjecture that merits further investigations with the full range of soil textures.

The results show that among the five soil textures encountered in the study area, the relative ease of oil bioremediation through biostimulation is in the order, sandy loam > sand > loamy sand > silty clay > clay. This demonstrates the relative performance of the different soil textures with regard to their utility in 'land farming' / treatment of hazardous wastes. For instance, mixing of soils with petroleum sludge for better treatment of the hazardous waste. The data generated in this work provides a valuable starting point for treatability studies on different soil textures in terms of different levels of treatment applications required.

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