CHAPTER FOUR

4.0 Results and Discussion

4.1 Results of biostimulation studies

4.1.1 Physicochemical properties of soil and organic wastes

Table 4.1 shows the physicochemical properties of soil and the organic wastes used for bioremediation studies. The soil sample had low nitrogen content (0.4%) while brewery spent grain (BSG) recorded appreciable nitrogen content (1.02%) compared to banana skin (BS) (0.4%) and spent mushroom compost (SMC) (0.5%). The pH of the soil used for the bioremediation was slightly acidic in nature at pH 6.12.

Table 4.1 Physicochemical Properties of Soil and Organic Wastes Used for Bioremediation

		Organic wastes			
Parameter	Soil	BSG	BS	SMC	Oil
pH	6.12±0.23	6.66±0.49	7.04±0.29	5.64±0.25	-
Nitrogen (%)	0.4±0.02	1.02±0.1	0.4±0.01	0.5±0.03	1.82±0.6
Phosphorus (mg/kg)	21.8±1.5	20.6±2.0	21.2±1.4	22.5±1.8	0.25±.1
Organic C (%)	10.3±1.1	10.9±0.91	10.5±1.3	10.2±1.1	28.4±5.1
Moisture (%)	7.0±0.3	71.84±3.5	38.5±2.86	62.3±4.12	-
C : N	25.7±6.3	10.7±2.1	26.3±4.4	20.4±2.0	15.6±2.3
Sand (%)	37.5±2.6	-	-	-	-
Silt (%)	18.75±1.95	-	-	-	-
Clay (%)	43.75±2.75	-	-	-	-
Texture	Sandy clay	-	-	-	-

BSG: Brewery spent grain, BS: Banana skin, SMC: Spent mushroom compost

The soil used for bioremediation had C:N ratio of 25.7, this is a low C:N ratio for effective biodegradation of oil in the soil, hence the need for addition of organic wastes as a source of nutrients (N and P). Roling et al., (2002) reported stimulated biodegradation of hydrocarbon in soil amended with 2.5 g of N per kilogram which gives C:N ratio greater than 300. BSG had the highest N content among the three organic wastes used, this is one of the most important limiting nutrient for effective bioremediation to take place (Okoh, 2006; Kim et al., 2005). The moisture contents of BSG was higher than those of other organic wastes, this might enable the BSG to harbor some important microorganisms that will contribute positively to the biodegradation of oil in the soil (Abioye et al., 2009a). The pH of SMC was slightly acidic, the reason for this might be because it was used to grow fungi (mushroom) which grow better in an acidic environment, therefore the initial substrate of SMC might be slightly acidic in nature. The particle size analysis of the soil showed that the soil texture was sandy clay.

4.1.2 Biodegradation of used lubricating oil (15% oil pollution)

Total petroleum hydrocarbon was determined in this study for the three different percentages of oil pollution (5%, 10% and 15%) and two different percentages (5% & 10%) of organic wastes rather than individual petroleum components because used oil is highly variable and have altered structure due to combustion process and temperature (Tauscher, 1988; Adesodun and Mbagwu, 2008).

The percentages of biodegradation of used lubricating oil in soil contaminated with 15% (w/w) used lubricating oil and amended with 5% and 10% different organic wastes throughout the study period are shown in Fig. 4.1 and 4.2, respectively. The percentage biodegradation of the used oil was very low within the first 28 days of study. At the end of

28 days in soil amended with 10% organic wastes, there was 17.1%, 24% and 5.4% TPH degradation in soil amended with BSG, BS, and SMC, respectively, (Fig. 4.1). Whereas in soil amended with 5% organic wastes, 6.9%, 10.5% and 5.2% oil degradation were recorded in BS, BSG and SMC amended soil, respectively, within the same period of time (Fig. 4.2). The reason for the low percentage of oil degradation within the first 28 days might be attributed to the toxicity of the oil on the microbial flora of the soil, due to high concentration of the oil which might likely had negative effects on the biodegradative activities of the microbial population in the contaminated soil. This initial trend of low biodegradation due to high oil concentration has been reported by different authors (Rahman, et al., 2002; Ijah and Antai, 2003b) who argued that high concentration of hydrocarbon can be inhibitory at the initial stage to the indigenous microorganisms in the soil.

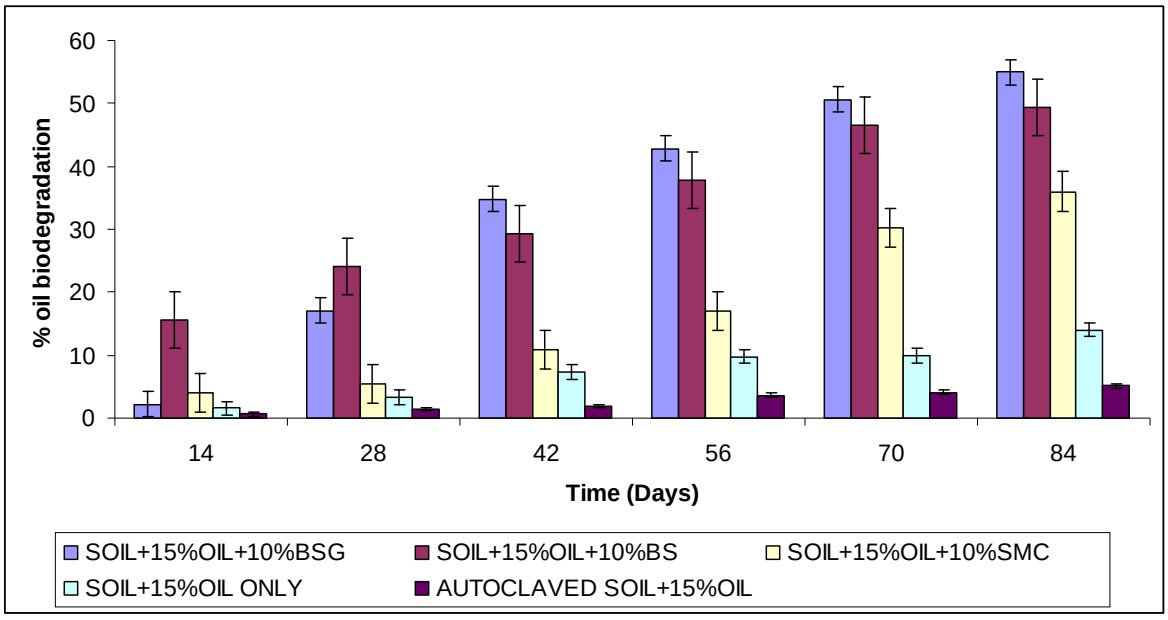


Figure 4.1 Percentage biodegradation of petroleum hydrocarbon in soil contaminated with 15% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

At the end of 84 days, oil contaminated soil amended with 10% and 5% BSG recorded 55% and 38% biodegradation, whereas the treatment amended with 10% and 5% BS recorded 49% and 33% biodegradation while those of oil contaminated with 10% and 5% SMC recorded 36% and 29% oil biodegradation respectively. This results is in contrast with the results of Ijah and Antai, (2003a) who reported extensive biodegradation of crude oil in soil polluted with 10% and 20% crude oil within the period of three to six months. The differences observed in these results might be due to the different oil used in the study, in this study used lubricating oil was utilized which have altered structures due to combustion process in automobiles (Taucher, 1988), it also contains some quantity of heavy metals which might be toxic to microorganisms in the soil thereby hindering their biodegradative activities.

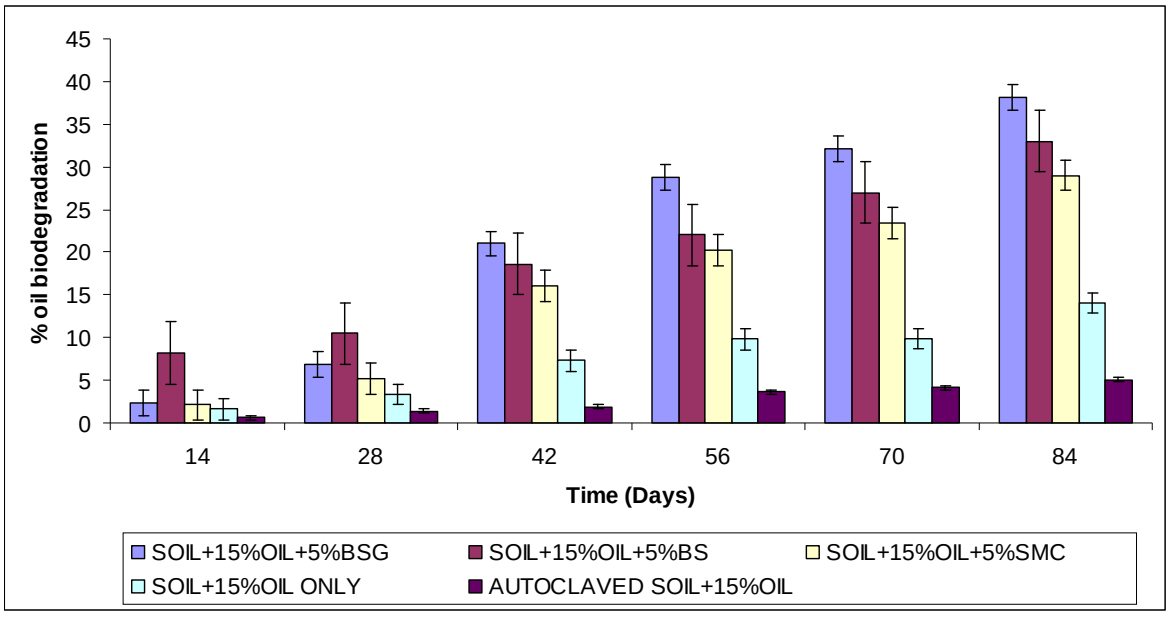


Figure 4.2 Percentage biodegradation of petroleum hydrocarbon in soil contaminated with 15% used lubricating oil and amended with 5% organic wastes. Bars indicates standard error (n = 3).

Also, from the results of 15% oil pollution, it was observed that soil amended with 10% organic wastes recorded higher percentage of oil biodegradation than those amended with 5% organic wastes. The reason for the observed results might be due to differences in the nutrient contents of these two percentages of organic wastes in stimulating the indigenous microorganisms. This is in contrast to the findings of Chaineau, et al., (2005) who reported that low nutrient addition to a crude oil contaminated soi recorded 15% increase in hydrocarbon assimilation by microorganisms in the contaminated soil, compared to the treatments with high nutrient amendments. The reason for the difference in this results and that of Chaineau et al., (2005) might be due to differences in the nutrient amendments, in this study organic wastes were used which did not pose any toxicity to the soil microorganisms while in their studies, mixtures of different inorganic salts were used. It might also be due to differences in the type of oil used for the studies, while used lubricating oil was used in this study, crude oil was used by Chaineau et al., (2005). This finding is supported by the report of Lau et al., (2003) who stated that addition of organic wastes such as spent mushroom compost to oil contaminated soil do reduce toxicity of oil to the soil microorganisms, through its buffering effects on the microbial flora.

Statistical analysis showed a significant difference at $P<0.05$ between the amended soil and the unamended polluted soil in all the treatments, thus proving the positive contribution of organic wastes to the biodegradation of used lubricating oil in the soil. Similar results was obtained by Adesodun and Mbagwu, (2008) and Abioye et al., (2009a) who recorded significant differences between the soil amended with melon shell, cow dung and poultry manure and those of unamended crude oil and spent lubricating oil polluted soil. However, there was no significant difference among the treatments amended with BSG, BS and SMC, though SMC recorded the least biodegradation percentage (36%) at the

end of 84 days compared to those treatments with BSG and BS (Fig. 4.1). The reason for this might be due to low pH of the treatment with SMC throughout the 84 days of the study. Low pH has been described by various authors as one of the conditions that do affects the growth and biodegradative activities of bacteria in soil (Atlas, 1988, Verstraete et al., 1976, Okoh, 2006).

The effectiveness of each amendment was determined by calculating the net percentage loss of used lubricating oil in the contaminated soil as shown in Tables 4.2 and 4.3. The highest net percentage oil loss was observed at 84 days in soil amended with 10% and 5% BSG (41% and 24.1%). The highest net percentage oil loss recorded in 10% and 5% BS treated soil were 36.8% and 19%, while those of SMC were 22% and 15%, respectively. From the results BSG treated soil recorded the highest net percentage oil loss compared to those of BS and SMC, this might be due to appreciable concentration of N present in BSG compared to BS and SMC. Nitrogen is known as one of the limiting nutrient necessary for biodegradation of organic pollutants in soil (Ijah and Safiyanu, 1997; Barahona et al., 2004).

Table 4.2 Net Percentage Loss of Total Petroleum Hydrocarbon in Soil contaminated with 15% used lubricating oil and amended with 10% organic wastes

	Time (Days)					
Treatment	14	28	42	56	70	84
A	13.8±2.1	20.8±2.4	21.8±2.8	27.9±3.2	36.8±2.3	35.9±4.2
B	0.5±0.1	13.7±2.1	27.5±3.2	32.8±4.1	40.8±5.4	41.0±3.8
C	2.5±0.7	2.0±0.8	3.6±1.2	7.2±1.7	20.4±2.3	22.7±2.7

A = Soil+15%Oil+BS, B = Soil+15%Oil+BSG, C = Soil+15%Oil+SMC

Net % loss = % loss in TPH of oil polluted soil amended with organic wastes – % loss in TPH of unamended polluted soil.

Table 4.3 Net Percentage Loss of Total Petroleum Hydrocarbon in Soil contaminated with 15% used lubricating oil and amended with 5% organic wastes

	Time (Days)					
Treatment	14	28	42	56	70	84
A	6.6±1.8	7.2±1.8	11.3±1.4	12.4±3.8	17.1±3.3	19.0±4.2
B	0.7±0.2	3.6±1.3	13.7±3.4	18.9±3.8	22.2±3.8	24.1±2.9
C	0.5±0.1	1.9±1.1	8.8±1.3	10.4±2.5	13.5±3.1	15.0±3.7

A = Soil+15%Oil+BS, B = Soil+15%Oil+BSG, C = Soil+15%Oil+SMC
Net % loss = % loss in TPH of oil polluted soil amended with organic wastes – % loss in TPH of unamended polluted soil.

4.1.3 Biodegradation of used lubricating oil (10% oil pollution)

Figures 4.3 and 4.4 shows the level of biodegradation of used lubricating oil in soil contaminated with 10% (w/w) used lubricating oil and amended with 5% and 10% organic wastes. The results show rapid reduction in the total petroleum hydrocarbons within the first 14 days of study in all the treatment amended with 10% organic wastes compared to that of 5% organic waste amendment which recorded low percentage of oil loss within the same period of time. At the end of 14 days there was 51%, 56% and 35% loss of oil in soil amended with 10% BSG, BS and SMC (Fig. 4.3), respectively, while 21%, 23% and 19% biodegradation were recorded in the soil treatment amended with 5% BSG, BS and SMC (Fig. 4.4).

The rapid biodegradation observed in the first 14 days in soil amended with 10% organic wastes might be due to bioavalability of the oil to the hydrocarbon degrading bacteria in the soil which rapidly support their metabolic activities. Jorgensen et al., (2000) also reported similar results with 70% oil biodegradation within the first month in soil contaminated with lubricating oil and composted with bark chips in a biopile. This was also supported by the report of Martin et al., (2007) who recorded 63% TPH loss within 15 days in soil contaminated with weathered petroleum hydrocarbon and amended with coffee beans. Bossert and Bartha (1984) reported that when oil is applied to soil at the rate of 0.5 to 10% (w/w), extensive biodegradation of the oil components occur within the first three months of oil application.

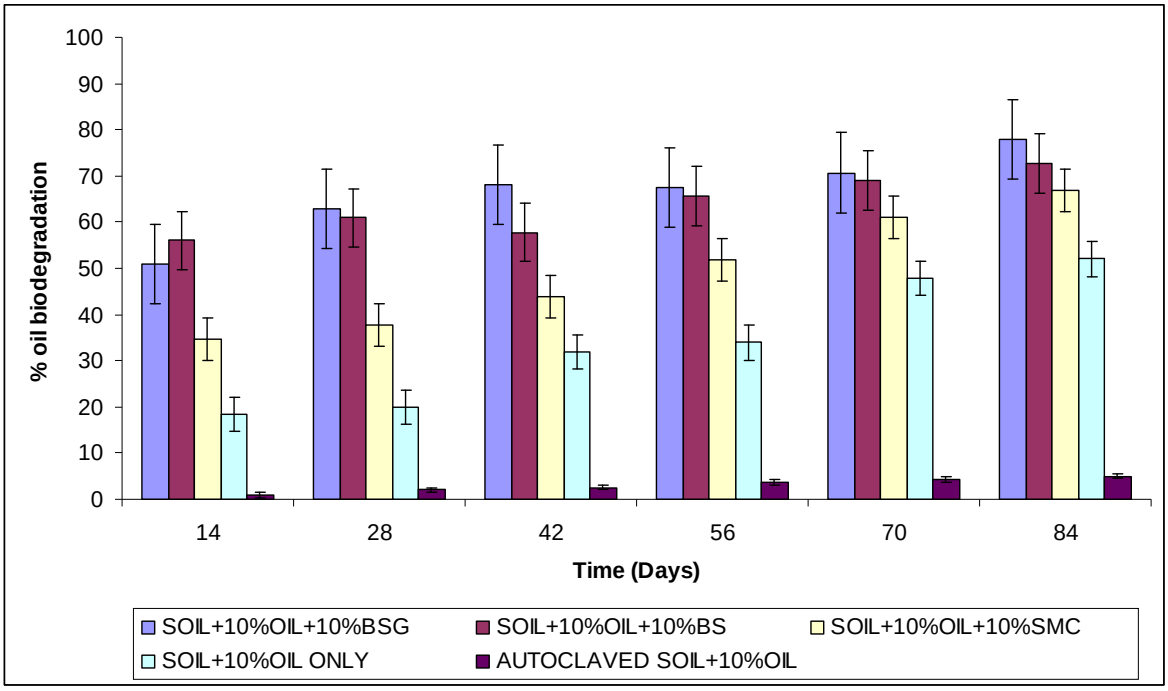


Figure 4.3 Percentage biodegradation of petroleum hydrocarbon in soil contaminated with 10% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

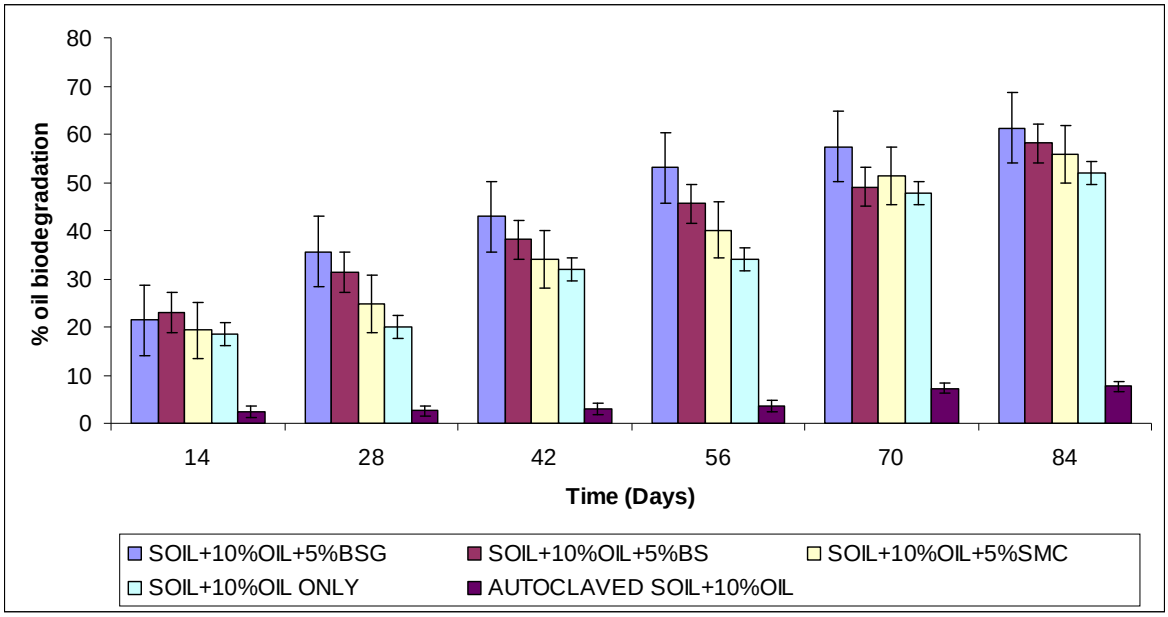


Figure 4.4 Percentage biodegradation of petroleum hydrocarbon in soil contaminated with 10% used lubricating oil and amended with 5% organic wastes. Bars indicates standard error (n = 3).

In this study at the end of 84 days, polluted soil amended with 10% and 5% BSG showed the highest percentage of oil biodegradation compared to other organic wastes amendments. 78% and 61% oil loss were recorded in 10% and 5% BSG amended soil respectively, whereas 10% and 5% BS and SMC treatments recorded 73%, 58% and 67%, 56% oil biodegradation, respectively (Figures 4.3 and 4.4). High percentage biodegradation recorded in BSG amended soil might be due to ability of this organic waste to contribute to increase oxygen diffusion and mineral nutrients availability as earlier observed by different authors as some of the characteristics of residues from grain (Elektorowicz, 1994; Piehler et al., 1999). It might as well contribute to the carbon source quality and act as mechanical support surface for bacterial adsorption to the oil (Piehler et al., 1999). BSG like other organic wastes possibly improves the soil physicochemical characteristics to speed up microbial adaptation in the oil contaminated soil (Jorgensen et al., 2000).

In addition, the effectiveness of BSG in enhancing biodegradation of used lubricating oil might be due to presence of relevant microbial population (Jorgensen et al., 2000) in the organic waste which might contribute to hydrocarbon mineralization in addition to the indigenous microbial population in the contaminated soil.

The net percentage loss of TPH in soil amended with 5% and 10% organic wastes are shown in Tables 4.4 and 4.5. Soil amended with BSG shows the highest percentage oil loss throughout the study period (except in 14^{th} day) in both soil amended with 5% and 10% organic wastes. However SMC amended soil recorded the least net percentage oil loss throughout the 84 days in both 5% and 10% organic wastes amendments. This is in sharp contrast with the findings of Chiu et al., (2009) who reported 54% net percentage TPH loss in oil contaminated soil amended with SMC. The differences in the two results might be

due to differences in the composition of SMC used for the study, also it might be due to the fact that freshly contaminated soil with used lubricating oil was used in this study while Chiu et al., (2009) utilized contaminated soil from an industrial area. There is every possibility that microorganisms present in the soil that they used had already adapted to the oil contaminated soil environment, therefore the enhancement with SMC stimulated their activities faster than the freshly contaminated soil in this study.

There was no significant difference at $P<0.05$ in the net percentage oil loss in soil treated with 10% organic wastes. However, significant difference was recorded between the soil treated with 5% BSG, BS and SMC at $P<0.05$.

Table 4.4 Net Percentage Loss of Total Petroleum Hydrocarbon in Soil contaminated with 10% used lubricating oil and amended with 10% organic wastes

	Time (Days)					
Treatment	14	28	42	56	70	84
A	38±3.1	41±1.8	26±2.1	32±0.2	26±1.3	21±0.6
B	33±2.8	43±2.5	36±3.8	37±2.2	31±2.9	26±1.8
C	17±1.7	18±1.2	12±1.5	28±0.7	18±1.3	15±2.1

A = Soil+10%Oil+BS, B = Soil+10%Oil+BSG, C = Soil+10%Oil+SMC

Net % loss = % loss in TPH of oil polluted soil amended with organic wastes – % loss in TPH of unamended polluted soil.

Table 4.5 Net Percentage Loss of Total Petroleum Hydrocarbon in Soil contaminated with 10% used lubricating oil and amended with 5% organic wastes

	Time (Days)					
Treatment	14	28	42	56	70	84
A	4.6±1.3	11.4±3.1	6.1±1.5	11.7±3.6	1.3±0.8	6.1±1.7
B	3.0±1.1	15.7±2.8	10.9±4.1	19.2±2.8	9.6±2.7	9.3±1.5
C	0.9±0.3	4.8±1.4	2.0±1.1	6.2±2.9	3.5±1.6	3.8±1.2

A = Soil+10%Oil+BS, B = Soil+10%Oil+BSG, C = Soil+10%Oil+SMC

Net % loss = % loss in TPH of oil polluted soil amended with organic wastes – % loss in TPH of unamended polluted soil.

4.1.4 Biodegradation of used lubricating oil (5% oil pollution)

The percentage of oil biodegradation in soil polluted with 5% used lubricating oil and amended with 10% and 5% organic wastes are shown in Figures 4.5 and 4.6, respectively. The results revealed rapid and high biodegradation of the used lubricating oil at the end of 84 days, soil amended with 10% organic wastes recorded the highest percentage of oil biodegradation compared to all the treatments amended with 5% organic wastes. Percentage of biodegradation was also appreciably high (64.4%) in the unamended soil but significantly lower than amended soil (Fig. 4.6). The reason for this relatively high and progressive biodegradation in all the soil contaminated with 5% used lubricating oil might be due to low concentration of oil in the soil. This concentration might not pose serious callenges to the metabolic activities of soil microrganisms coupled with the organic wastes amendments which likely supplied nutrient to the microbial population present in the contaminated soil thereby enabling them to degrade almost completely the oil contaminant.

These results are in agreement with the findings of Rahman et al., (2002) who reported increase in the rate of biodegradation of crude oil, as the concentration of oil reduced.

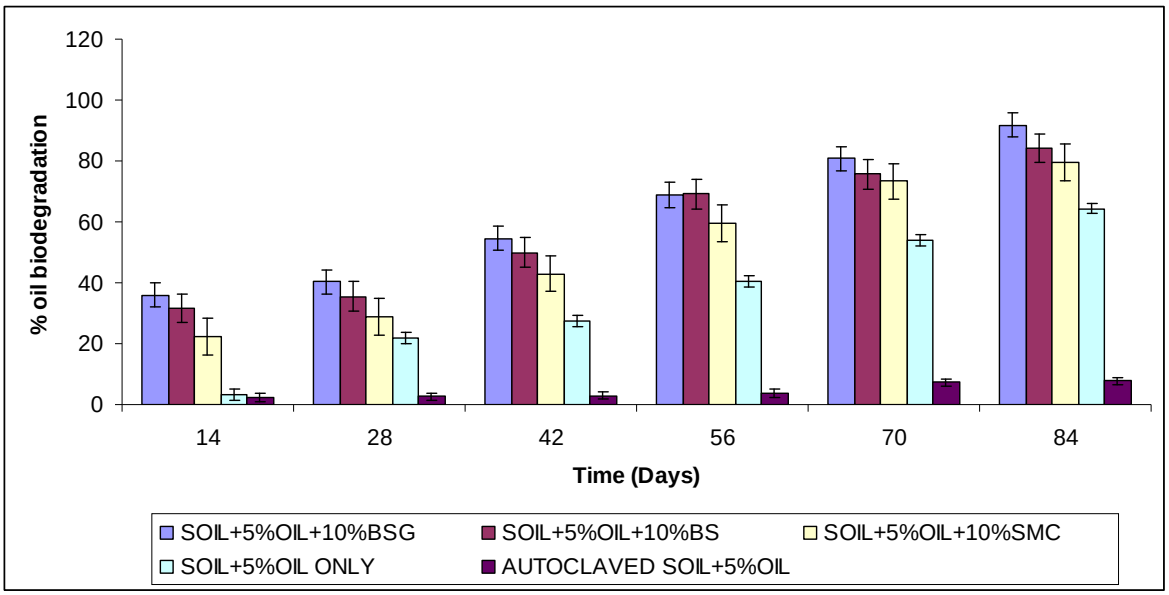


Figure 4.5 Percentage biodegradation of petroleum hydrocarbon in soil contaminated with 5% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

Soil amended with 10% and 5% BSG recorded 91.8% and 76.3% oil biodegradation, respectively, followed by 84.2% and 71.2% oil degradation in soil treated with 10% and 5% BS while 79.5% and 67.8% degradation was recorded in soil amended with 10% and 5% SMC respectively. The results are in contrast with the findings of Adesodun and Mbagwu (2008) who reported 30% and 41.6% biodegradation of spent lubricating oil in soil contaminated with 5% spent lubricating oil and amended with cow dung and pig wastes within the period of three months. The differences in these results might be due to different composition of used lubricating oil utilized for the studies, or differences in the organic wastes used. It might be due to differences in the soil composition used for the

studies. BSG amended soil recorded highest percentage biodegradation (91.8% and 76.3%) in both treatments with 10% and 5% BSG. This might be due to high N and P contents present in BSG. N and P are known as one of the most important nutrients needed by hydrocarbon utilizing bacteria to carry out effective and efficient biodegradative activities of xenobiotics in the soil environment (Frederic et al., 2005; Kim et al., 2005; Okoh, 2006)

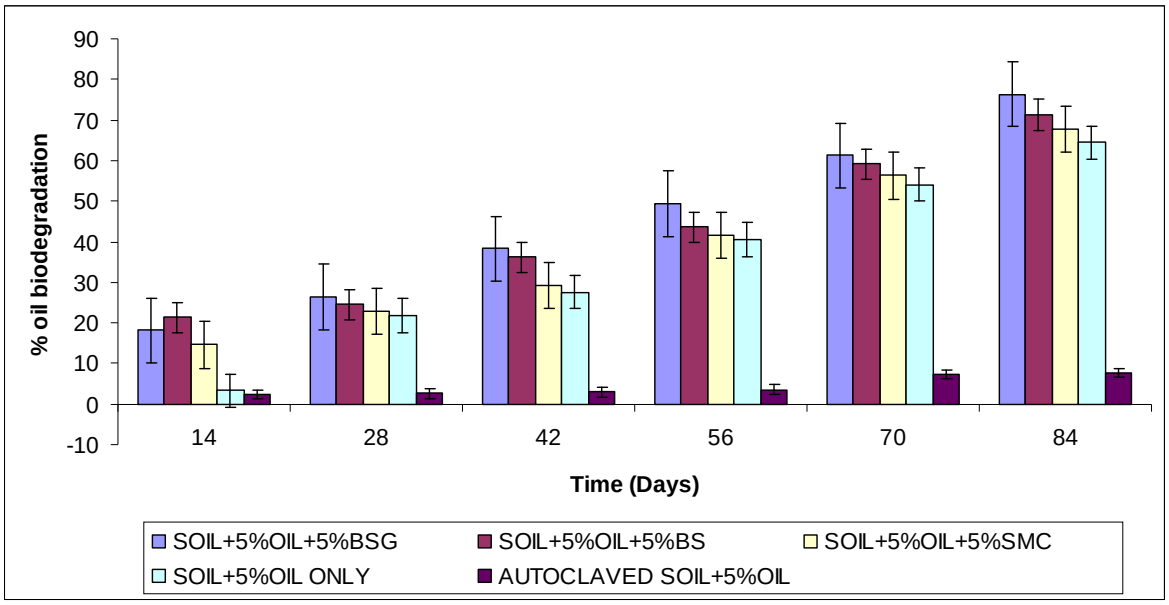


Figure 4.6 Percentage biodegradation of petroleum hydrocarbon in soil contaminated with 5% used lubricating oil and amended with 5% organic wastes. Bars indicates standard error (n = 3).

7.7% of the degradation might be due to non biological factors such as evaporation or photodegradation. This was recorded in the autoclaved contaminated soil treated with 0.5% sodium azide. This was in sharp contrast to the findings of Palmroth et al., (2002) who recorded as high as 70% diesel oil loss within 28 days of study in sodium azide treated soil. The differences in these results might be because poisoned control in this study was an autoclaved soil mixed with 0.5% sodium azide, whereas, Palmroth et al., (2002) used only

0.5% sodium azide without autoclaving the soil, thus the sodium azide possibly could not completely sterilize the soil.

Tables 4.6 and 4.7 show the net percentage loss of TPH in soil treated with 10% and 5% of different organic wastes. Soil treated with BSG recorded the highest net percentage loss throughout the study period, this is similar to the results obtained with 10% and 15% oil pollution. However, no significant difference was recorded at $P<0.05$ between the net percentage loss of all the treatmnets amended with organic wastes. This is in contrast with the findings of Adesodun and Mbagwu (2008) who reported significant difference between the net percentage oil loss of contaminated soil treated with cow dung and pig wastes within three months period. The differences might be due to differences in the physicochemical properties of soil and different organic wastes used for the studies.

Table 4.6 Net Percentage Loss of Total Petroleum Hydrocarbon in Soil contaminated with 5% used lubricating oil and amended with 10% organic wastes

	Time (Days)					
Treatment	14	28	42	56	70	84
A	23.4±2.4	13.7±1.8	22.4±2.3	28.7±3.20	21.6±1.9	19.8±2.2
B	32.8±3.1	18.6±2.3	27.0±2.4	28.3±1.3	26.7±3.4	27.4±2.8
C	19.1±2.7	7.2±1.2	15.4±1.5	19.2±1.9	19.2±2.1	15.2±1.7

A = Soil+5%Oil+BS, B = Soil+5%Oil+BSG, C = Soil+5%Oil+SMC

Net % loss = % loss in TPH of oil polluted soil amended with organic wastes – % loss in TPH of unamended polluted soil (i.e. biotic control).

Table 4.7 Net Percentage Loss of Total Petroleum Hydrocarbon in Soil contaminated with 5% used lubricating oil and amended with 5% organic wastes

	Time (Days)					
Treatment	14	28	42	56	70	84
A	18.0±3.6	3.2±0.6	8.7±1.3	3.1±1.1	5.0±1.4	6.8±1.3
B	14.9±2.3	4.6±0.9	10.8±3.1	8.9±2.6	7.2±1.49	11.9±1.7
C	11.3±1.7	1.1±0.7	1.7±0.6	1.0±0.3	2.2±1.1	3.4±1.2

A = Soil+5%Oil+BS, B = Soil+5%Oil+BSG, C = Soil+5%Oil+SMC
Net % loss = % loss in TPH of oil polluted soil amended with organic wastes – % loss in TPH of unamended polluted soil.

4.1.5 Biodegradation rate constant and half life of used oil in soil contaminated with 15%, 10% and 5% used lubricating oil

First order kinetics model of Yeung et al., (1997) was used to determine the rate of biodegradation of used lubricating oil in the various treatments. Table 4.8 shows the biodegradation rate constant (k) and half life ($t_{1/2}$) for the soil contaminated with 15%, 10% and 5% used lubricating oil amended with 10% (different) organic wastes. Data for the sampling periods were combined before this model could be used. Soil amended with BS in 15% oil pollution shows the highest biodegradation rate of 0.0556 day^{-1} and half life of 12.46 days, the biodegradation rates and half life of soil amended with BSG and SMC are 0.0479 day^{-1} , half life of 14.47 days and 0.0216 day^{-1}, half life of 32.11 days, respectively. High biodegradation rate recorded in BS amended soil above that of BSG might be due to initial rapid loss of used lubricating oil in the first 28 days of study in BS amended soil than those of BSG and SMC amended soil. This is however different from the results of Adesodun and Mbagwu (2008), who reported the highest biodegradation rate in oil-

contaminated soil amended with pig wastes, which had the highest percentage of biodegradation throughout the study period.

Table 4.8 Biodegradation rate and half life of hydrocarbon in oil polluted soil amended with 10% organic wastes

Treatment	Biodegradation constant (k) day^{-1}	Half life ($t_{1/2}$) (days)
Soil + 15% Oil + BS	0.0556	12.46
Soil + 15% Oil + BSG	0.0479	14.47
Soil + 15% Oil + SMC	0.0216	32.11
Soil + 15% Oil	0.0092	75.06
Autoclaved Soil+15% Oil	0.0033	211.78
Soil + 10% Oil + BS	0.3016	2.30
Soil + 10% Oil + BSG	0.3163	2.19
Soil + 10% Oil + SMC	0.2189	3.17
Soil + 10% Oil	0.1604	4.32
Autoclaved Soil+10% Oil	0.0051	135.91
Soil + 5% Oil + BS	0.4010	1.73
Soil + 5% Oil + BSG	0.4361	1.59
Soil + 5% Oil + SMC	0.3100	2.24
Soil + 5% Oil	0.1886	3.68
Autoclaved Soil+5% Oil	0.0079	87.74

The half life (time it will take for half of the hydrocarbon to degrade) is a function of biodegradation rate constant, hence the soil amended with BS in 15% oil pollution recorded the least time (half life) of 12.46 days.

In soil contaminated with 10% and 5% used lubricating oil, treatments with 10% BSG amendment recorded the highest biodegradation rates of 0.3163 day^{-1}, and 0.4361 day^{-1} respectively and half life of 2.19 days in 10% pollution and 1.59 days in 5% pollution respectively. The result shows significant relationships between the rate of biodegradation and concentration of oil in the contaminated soil. As seen in these results, higher biodegradation rates were recorded in soil contaminated with 5% oil compared to 15% oil pollution. The low biodegradation rate and subsequent high half life in soil contaminated with 15% oil could be attributed to reduction in the activity of soil microbes in this oil pollution level (Adesodun and Mbagwu, 2008). Bossert and Bartha (1984) stated that sensitivity of soil microflora to petroleum hydrocarbons is a factor of quantity and quality of oil spilled and previous exposure of the native soil microbes to oil. Schaefer and Juliane (2007) also concluded that bioremediation is a useful method of soil remediation if pollutant concentrations are moderate.

Table 4.9 shows the biodegradation rate constant (k) and half life ($t_{1/2}$) for the soil contaminated with 15%, 10% and 5% used lubricating oil and amended with 5% organic wastes. Soil amended with BSG recorded the highest biodegradation rates and least half life in soil contaminated with 15%, 10% and 5% used lubricating oil. The biodegradation rates and half life of BSG amended soil are 0.0342 day^{-1} and 20.26 days, 0.3005 day^{-1} and 2.31 days, 0.4035 day^{-1} and 1.72 days in 15%, 10% and 5% oil pollution respectively; this was almost three times higher than those of unamended soil. The high biodegradation rates from the first order kinetics recorded by BSG amended soil in all the pollution level may be due

to its high N and P and its buffering effects in the oil contaminated soil over those of BS and SMC.

Table 4.9 Biodegradation rate and half life of hydrocarbon in oil polluted soil amended with 5% organic wastes

Treatment	Biodegradation constant (k) day^{-1}	Half life ($t_{1/2}$) (days)
Soil + 15% Oil + BS	0.0318	21.79
Soil + 15% Oil + BSG	0.0342	20.26
Soil + 15% Oil + SMC	0.0198	35.00
Soil + 15% Oil	0.0092	75.06
Autoclaved Soil+15% Oil	0.0033	211.78
Soil + 10% Oil + BS	0.2862	2.42
Soil + 10% Oil + BSG	0.3005	2.31
Soil + 10% Oil + SMC	0.1987	3.49
Soil + 10% Oil	0.1604	4.32
Autoclaved Soil+15% Oil	0.0051	135.91
Soil + 5% Oil + BS	0.3696	1.87
Soil + 5% Oil + BSG	0.4035	1.72
Soil + 5% Oil + SMC	0.2856	2.43
Soil + 5% Oil	0.1886	3.68
Autoclaved Soil+5% Oil	0.0079	87.74

Therefore the performance of BSG over BS and SMC can possibly be attributed to its C:N ratio. The result is similar to those of Namkoong et al., (2002) who reported high

biodegradation rates in soil amended with sewage sludge that had appreciable quantity of N and P, within 30 days of study.

Comparison of the first order degradation rate kinetics of soil amended with 10% and 5% organic wastes shows higher biodegradation rates in all the treatments amended with 10% organic wastes compared to those of 5% organic wastes amendments. The results might be due to low nutrient quantity that microbial population had access to in 5% organic wastes amendments compared to those of 10% organic waste amendments which probably provided more nutrients to the microbial communities in the oil contaminated soil, which in turn enhance their activities in degrading the oil. This result is similar to the findings of Namkoong et al., (2002) who reported higher biodegradation rate constant and low half life in diesel contaminated soil and amended with 1:0.3 sewage sludge compared to those amended with 1:0.1 under the same conditions. However, this result is in contrast with the findings of several authors who reported that excessive nutrient concentration do inhibit biodegradation activity. For example excess NPK was reported to have negative effects on the level of hydrocarbon biodegradation (Oudot et al., 1998; Chaineau et al., 2005; Challian et al., 2006). The difference exhibited in this study might be because organic wastes were used as source of nutrient in this study which does not pose any toxicity problem to the soil microbial population.

4.1.6 Microbial counts in soil contaminated with 15% used lubricating oil

The counts of aerobic heterotrophic bacteria (AHB) in soil contaminated with 15% used lubricating oil and amended with 10% BSG ranged between 46.0 $x10^6$ CFU/g and 195.0 $x10^6$ CFU/g while that of soil amended with BS and SMC ranged from 32.0 x 10^6 CFU/g to 111.0 x 10^6 CFU/g and 10 x 10^6 CFU/g to 54.0 x 10^6 CFU/g respectively (Fig. 4.7). The

unamended control soil had the count of AHB ranging between 3.5 x 10^6 CFU/g and 22.0x 10^6CFU/g. The counts of AHB recorded in soil polluted with 15% used lubricating oil was lower than those isolated in soil contaminated with 10% crude oil and amended with poultry manure and NPK fertilizer by Ijah et al., (2008). The reason for these different results might be attributed to differences in the concentration of oil used and differences in the organic wastes utilized in the two studies. Increase in counts of AHB was observed on 28 days with decrease in count between 42 and 70 days. This fluctuation might be due to decrease in the concentration of nutrient between 42 and 70 days compared to that of 28 days. The reason might as well be due to bioavailability of the oil and to the bacteria at 28th and 84th days, respectively.

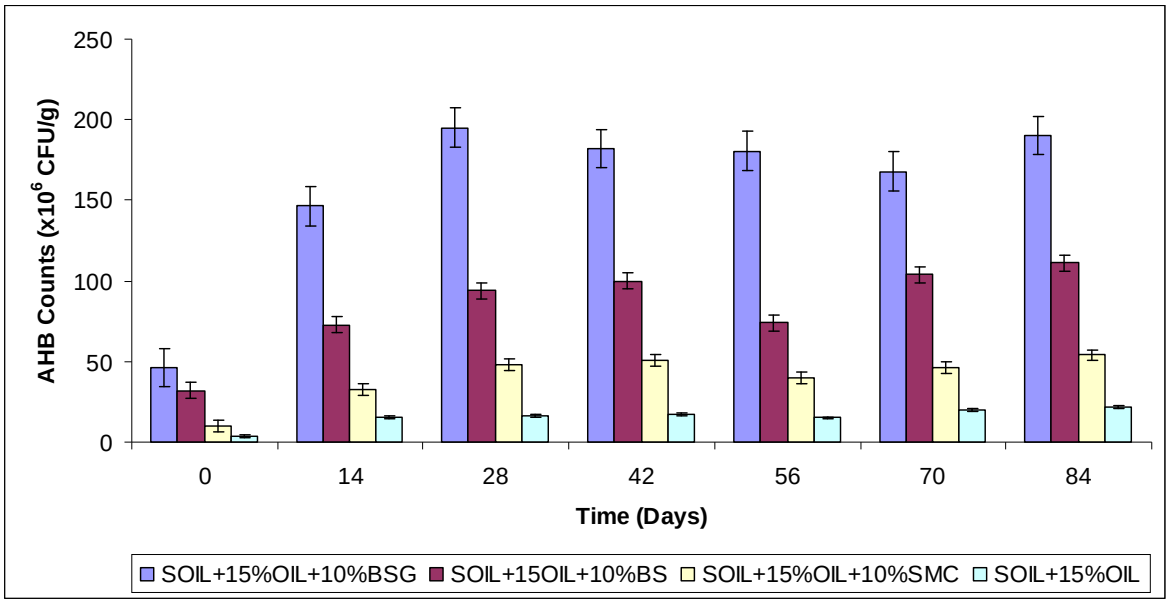


Figure 4.7 Counts of aerobic heterotrophic bacterial (AHB) population in soil contaminated with 15% used lubricating oil amended with 10% organic wastes. Bars indicates standard error (n = 3).

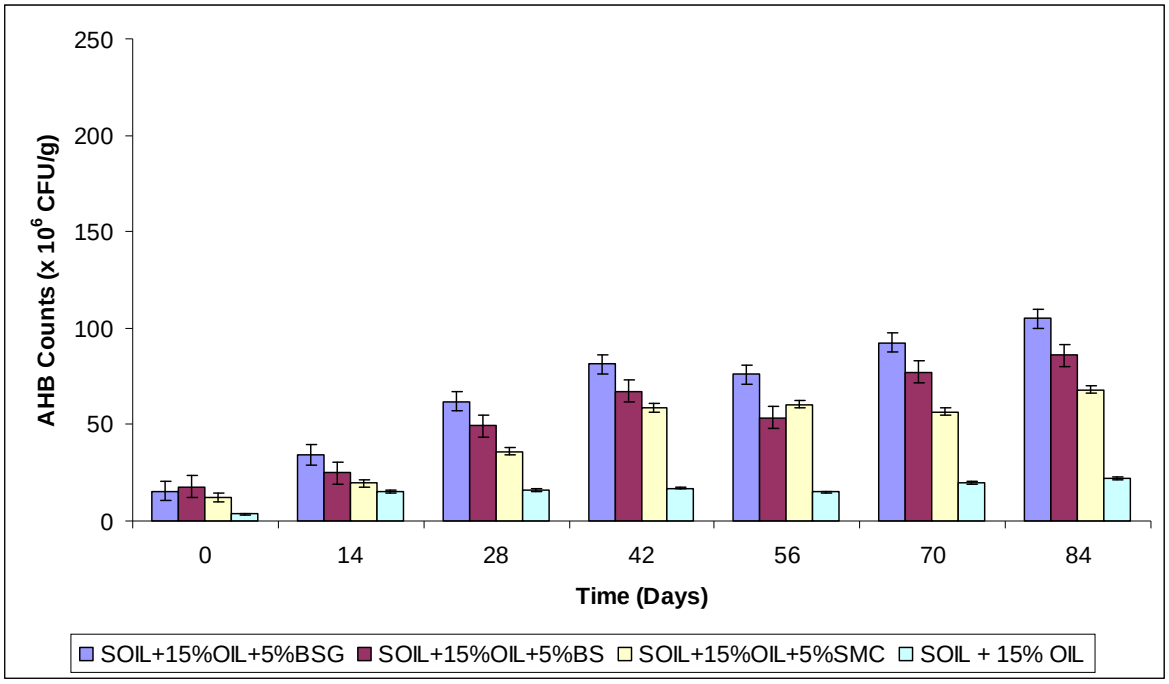


Figure 4.8 Counts of aerobic heterotrophic bacterial (AHB) population in soil contaminated with 15% used lubricating oil amended with 5% organic wastes. Bars indicates standard error (n = 3).

AHB counts in soil amended with 5% organic wastes were lower than those of 10% organic wastes amendments. BSG amended soil recorded the highest AHB counts (Figure 4.8). The reason for low AHB counts in soil amended with 5% organic wastes compared to those amended with 10% organic wastes might be due to the presence and supply of more nutrients to the microbial flora in soil amended with 10% organic wastes than those amended with 5% organic wastes. These results is similar to the findings of several authors who argued that supply of more organic nutrients do stimulate the proliferations of bacteria in contaminated soil (Jorgensen, et al., 2000; Bento et al., 2005; Ijah, et al., 2008; Abioye et al., 2009b). Statistical analysis shows significant difference between the AHB count in soil amended with BSG and other treatments, whereas there was no significant difference between the soil amended with BS and SMC at confidence level of $P<0.05$. Count of hydrocarbon utilizing bacteria (HUB) was also higher in oil contaminated soil amended

with 10% organic wastes (Fig.4.9). The count of HUB in soil amended with 10% BSG was significantly higher than those amended with BS and SMC. HUB count in BSG amended soil ranged from 24.0 x 10^5 CFU/g to 210.0 x 10^5 CFU/g while those amended with BS and SMC ranged from 15.0 x 10^5 CFU/g to 167 x 10^5 CFU/g and 3.0 x 10^5 CFU/g and 38.0 x 10^5 CFU/g respectively. However, the HUB count in unamended control soil was extremely (2.0 x 10^5 CFU/g to 14.0 x 10^5 CFU/g) lower than those amended with organic wastes.

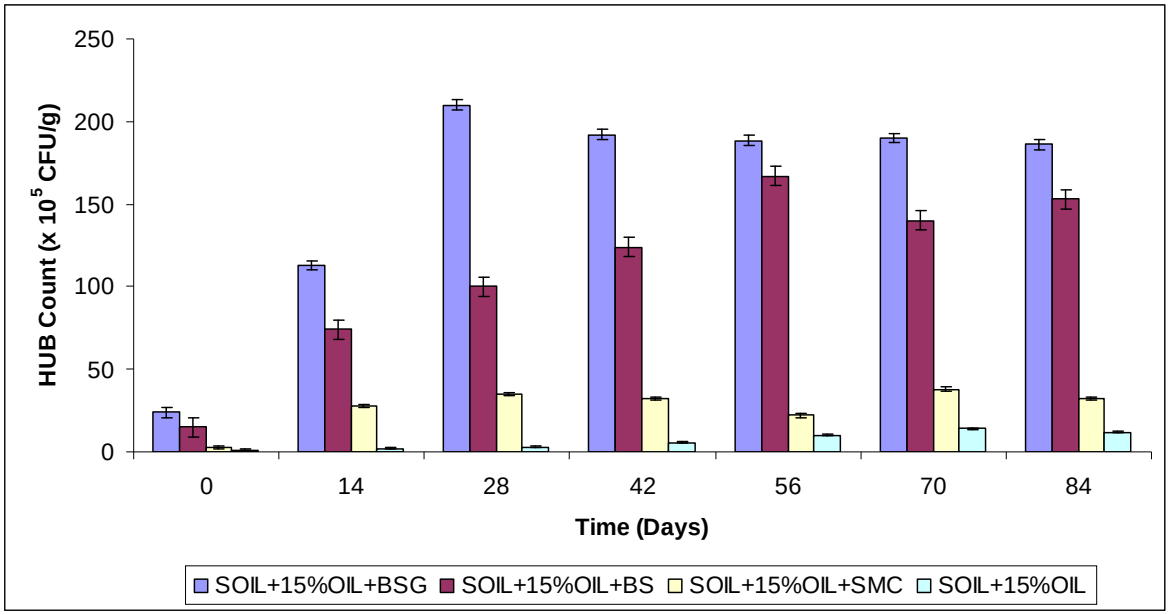


Figure 4.9 Counts of hydrocarbon utilizing bacterial (HUB) population in soil contaminated with 15% used lubricating oil amended with 10% organic wastes. Bars indicates standard error (n = 3).

Figure 4.10 shows the counts of HUB in all the treatments with 5% organic wastes. BSG amended soil shows higher number of HUB compared to those of BS and SMC. Count of HUB in soil amended with BSG was significantly different from those of soil amended with SMC and unamended polluted soil, but no significant difference between HUB counts

in soil amended with BSG and BS at P<0.05 confident level in both treatments amended with 10% and 5% organic wastes. However, in the soil treatment with autoclaved soil poisoned with sodium azide, the count of AHB and HUB was nil throughout the experimental period.

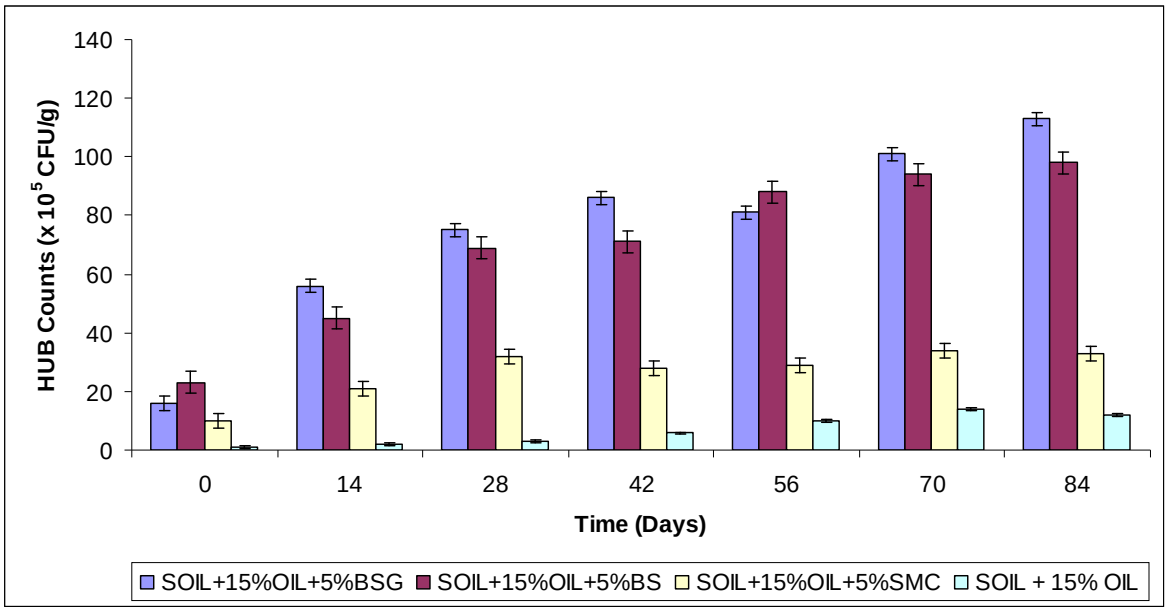


Figure 4.10 Counts of hydrocarbon utilizing bacterial (HUB) population in soil contaminated with 15% used lubricating oil amended with 5% organic wastes. Bars indicates standard error (n = 3).

The higher counts of AHB and HUB recorded in all the organic wastes amended soil compared to the unamended polluted soil might be due to the ability of these organic wastes (mostly BSG that recorded higher counts) to neutralize the toxic effects of the oil on the microbial population by rapid improvement of the soil physicochemical properties (Jorgensen et al., 2000). The organic wastes might help in improving the soil aeration which consequently favoured the growth of the isolated bacterial species which are solely aerobic in nature.

4.1.7 Microbial counts in soil contaminated with 10% used lubricating oil

Figures 4.11 and 4.12 show the counts of aerobic heterotrophic bacteria (AHB) in soil contaminated with 10% used lubricating oil and amended with 10% and 5% organic wastes. AHB counts in 10% BSG amended soil ranged between 18.1 x10^7 CFU/g and 60.0 x10^7 CFU/g while that of soil amended with 10% BS and 10% SMC ranged from 15.3 x10^7 CFU/g to 37.0 x10^7 CFU/g and 10.1 x10^7 CFU/g to 25.3 x10^7 CFU/g respectively.

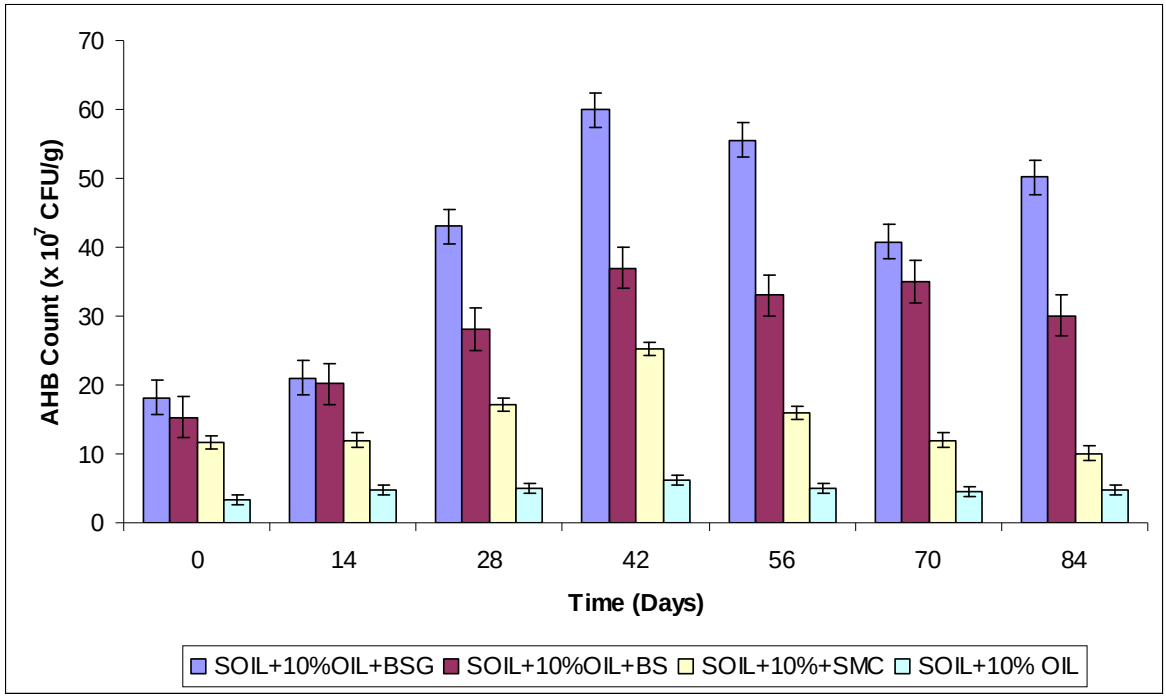


Figure 4.11 Counts of aerobic heterotrophic bacterial (AHB) population in soil contaminated with 10% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

AHB counts in 5% organic wastes amendments shows higher counts of AHB in all the treatments amended with organic wastes compared to the unamended soil in the range BSG > BS > SMC throughout the 84 days of study with BSG recording the highest count between 28 and 84 days. The low counts of bacteria recorded in unamended polluted soil compared with polluted soil amended with different organic wastes may be attributed to

toxicity effects of the oil on the bacterial population in the unamended polluted soil. It might also be attributed to lack of adequate nutrient in the unamended soil. Similar results has been reported by Lee et al., (2003) and Okoh, (2006) who argued that introduction of nutrient into contaminated soil stimulates microbial growth and enhance the rate of hydrocarbon degradation. AHB increased progressively throughout the 84 days, this might be due to bioavailability of the oil contaminant to the bacterial population which srved as nutrients for them.

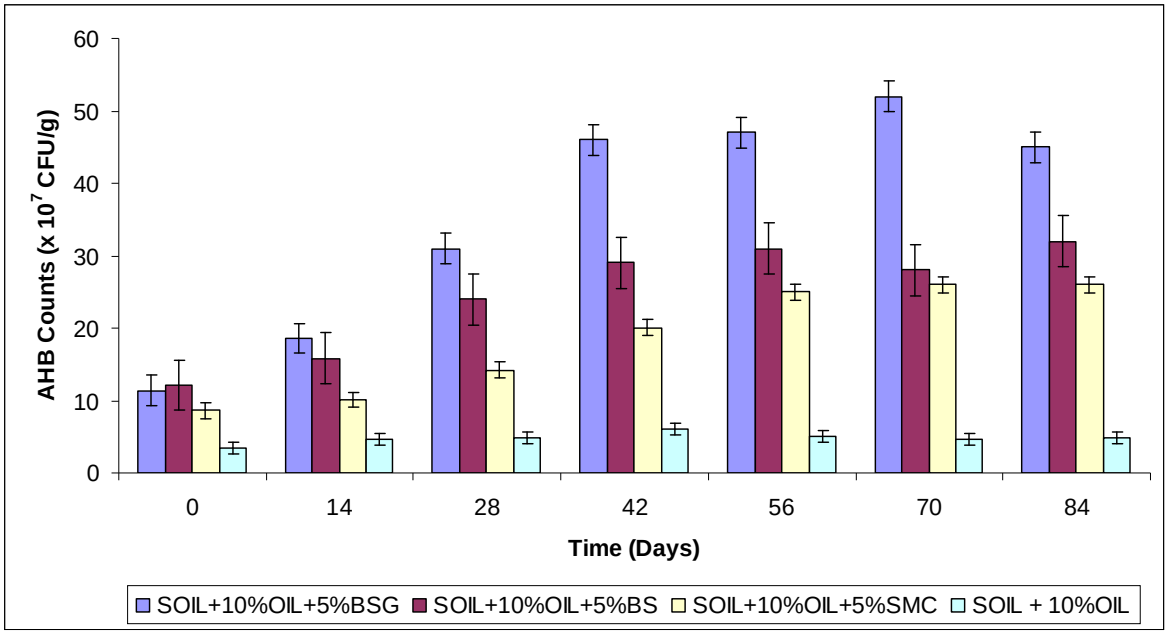


Figure 4.12 Counts of aerobic heterotrophic bacterial (AHB) population in soil contaminated with 10% used lubricating oil and amended with 5% organic wastes. Bars indicates standard error (n = 3).

The unamended control soil had the count of AHB ranging between 3.4 x10^7 CFU/g and 5.0 x 10^7 CFU/g. There was significant difference at $P<0.05$ between AHB count of soil amended with BSG and those of SMC and unamended polluted soil whereas no significant difference was observed between the AHB counts of BS and BSG. The count of

hydrocarbon utilizing bacteria (HUB) was also higher in oil contaminated soil amended with different organic wastes (Figures 4.13 and 4.14). The count of HUB in soil amended with 10% BSG was higher than those amended with 10% BS and SMC. HUB count in 10% BSG amended soil ranged from 10.2 x10^6 CFU/g to 80.5 x10^6 CFU/g while those amended with 10% BS and SMC ranged from 8.4 x10^6 CFU/g to 52.0 x10^6 CFU/g and 11.5 x10^6 CFU/g and 32.4 x10^6 CFU/g respectively.

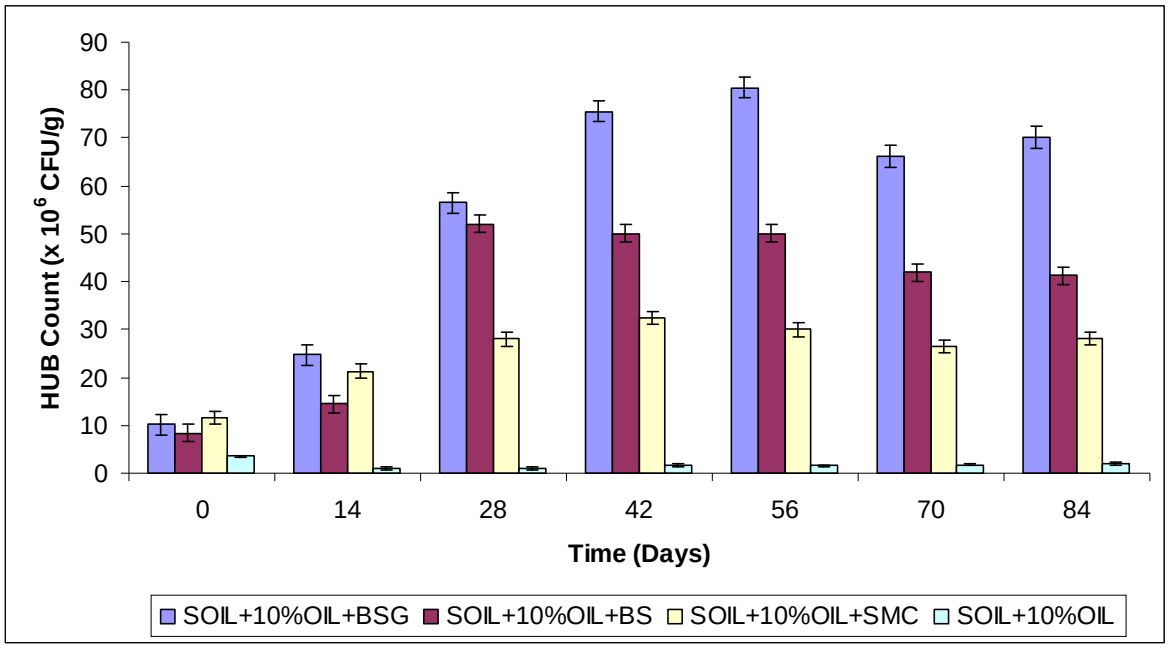


Figure 4.13 Counts of hydrocarbon utilizing bacterial (HUB) population in soil contaminated with 10% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

HUB counts in soil amended with 5% organic wastes were lower than those amended with 10% organic wastes (Figure 4.14). However, BSG amended soil recorded the highest HUB compared to BS and SMC amendments in soil amended with 5% organic wastes. The HUB count in unamended control soil was (1.0 x10^6 CFU/g to 3.5 x10^6 CFU/g) lower than those amended with organic wastes (Fig. 4.15). Counts of HUB in soil amended with BSG was

statistically different from those of other treatments at P<0.05 confidence level. However, in the soil treatment with autoclaved soil poisoned with sodium azide, the count of AHB and HUB was nil (data not shown) throughout the experimental period. This was in contrast to the findings of Palmroth et al., (2002) who recorded growth of bacteria in their poisoned controlled soil. The differences might be due to differences in the microbial ecology of the soil used for the two experiments. It might also be because, Palmroth et al., (2002) only used sodium azide for the poisoned control, while in this study combination of sodium azide and autoclave was employed to achieved complete sterilization of the soil.

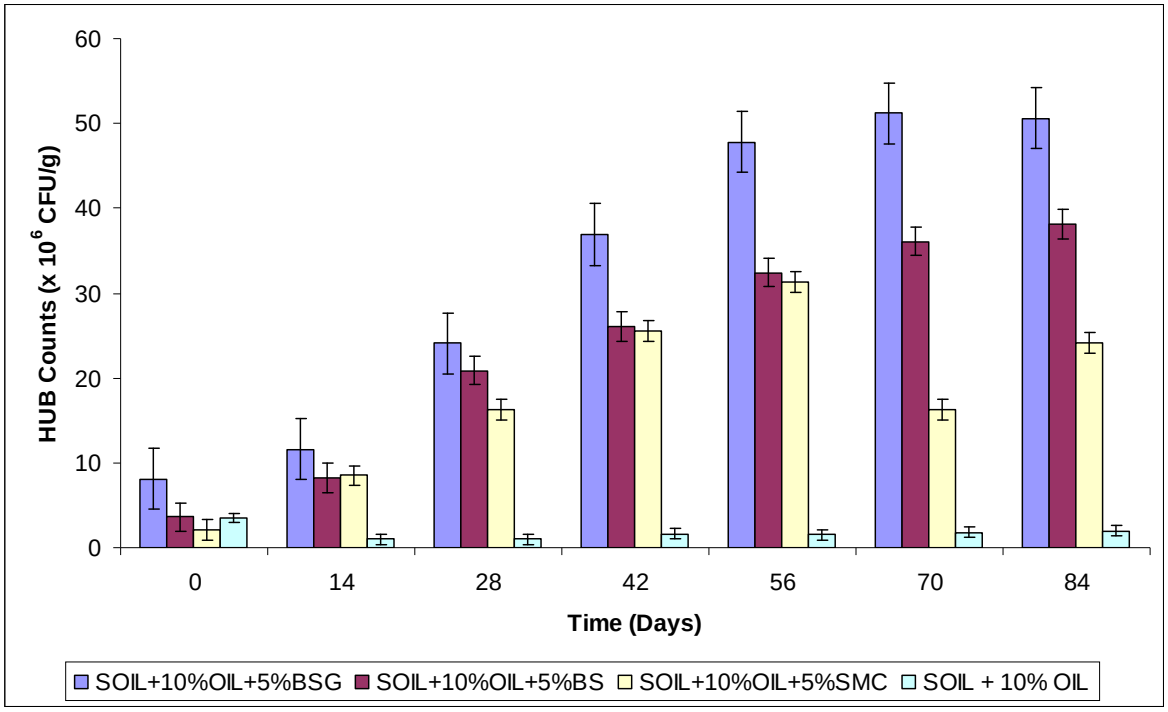


Figure 4.14 Counts of hydrocarbon utilizing bacterial (HUB) population in soil contaminated with 10% used lubricating oil and amended with 5% organic wastes. Bars indicates standard error (n = 3).

Higher counts of AHB and HUB demonstrated by soil amended with 10% organic wastes compared to those of 5% amendments might be due to the quantity of organic wastes added which probably provides more nutrients to the soil bacteria than those amended with 5%

organic wastes. This result is supported by the findings of Bento et al., (2005) and Barahona et al., (2004) who recorded higher counts of bacteria in long beach soil contaminated with diesel oil and in diesel contaminated soil amended with corn and crop residues.

4.1.8 Microbial counts in soil contaminated with 5% used lubricating oil

Counts of aerobic heterotrophic bacteria (AHB) in soil contaminated with 5% used lubricating oil and amended with 10% BSG ranged between 51.2 $x10^7$ CFU/g and 126.0 $x10^7$ CFU/g while that of soil amended with 10% BS and SMC ranged from 50.8 $x10^7$ CFU/g to 127.0 $x10^7$ CFU/g and 15.5 $x10^7$ CFU/g to 48.0 $x10^7$ CFU/g respectively, between 0 and 84 days (Figure 4.15).

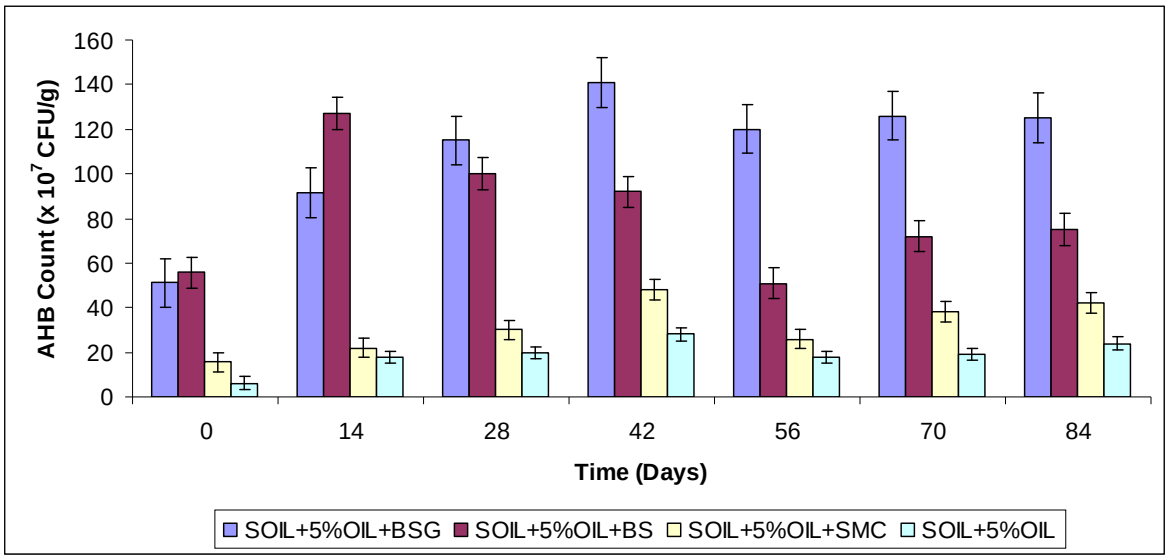


Figure 4.15 Counts of aerobic heterotrophic bacterial (AHB) population in soil contaminated with 5% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

Figure 4.16 also shows the counts of AHB in soil amended with 5% organic wastes. BSG amended soil recorded the highest count of 92 x 10^7 CFU/g at the end of 84 days compared to those of BS and SMC amended soil, but the counts in 5% organic wastes amendments was much lower than those of 10% organic wastes amendments. The unamended control soil had the count of AHB ranging between 6.2 x10^7 CFU/g and 28.0 x10^7 CFU/g. No significant difference was recorded between AHB counts of soil amended with organic wastes, however there was significant difference between the counts of AHB in soil amended with organic wastes and unamended polluted soil at $P<0.05$ confidence level. Count of hydrocarbon utilizing bacteria (HUB) in soil contaminated with 5% used lubricating oil and amended with 10% and 5% organic wastes are shown in Figures 4.17 and 4.18.

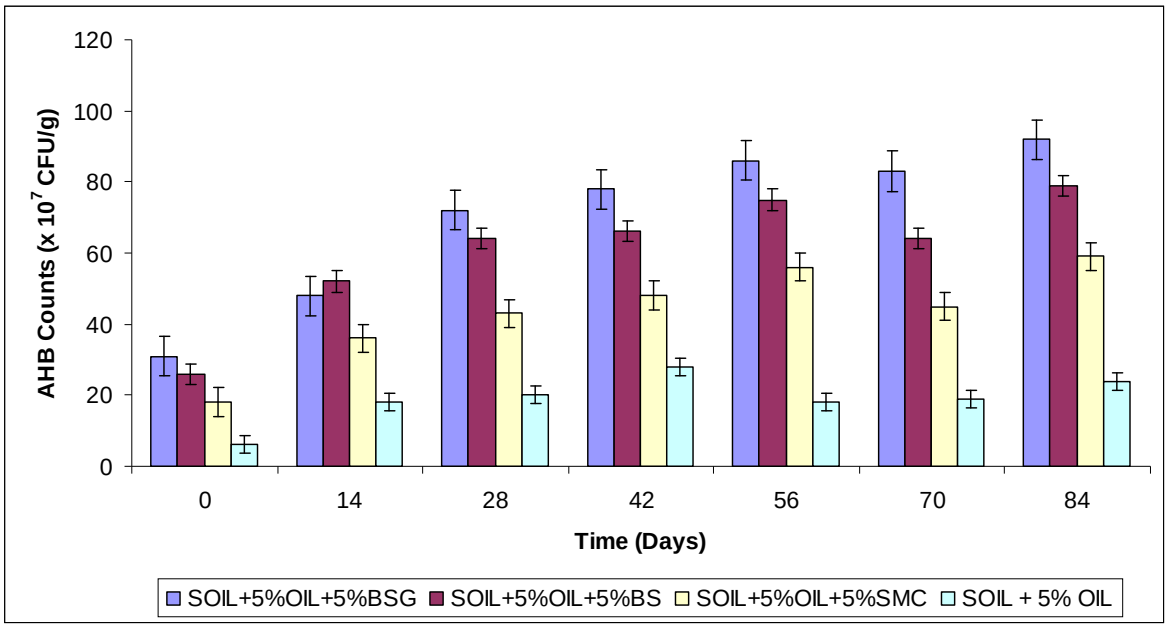


Figure 4.16 Counts of aerobic heterotrophic bacterial (AHB) population in soil contaminated with 5% used lubricating oil and amended with 5% organic wastes. Bars indicates standard error (n = 3).

The count of HUB in soil amended with 10% BSG was about 300% to 400% higher than those amended with 10% BS and SMC (Fig. 4.1). HUB count in 10% BSG amended soil ranged from 47.0 $x10^6$ CFU/g to 146.0 $x10^6$ CFU/g while those amended with 10% BS and SMC ranged from 42 $x10^6$ CFU/g to 120 $x10^6$ CFU/g and 12.0 $x10^6$ CFU/g and 51.0 $x10^6$ CFU/g respectively within the 84 days of study. Counts of HUB in soil amended with 5% organic wastes is similar to those of 10% organic wastes amendments in the sense that BSG treated soil recorded higher HUB counts throughout the study period. However, the HUB count in unamended control soil was (3.0 $x10^6$ CFU/g to 15.0 $x10^6$ CFU/g), lower than those amended with organic wastes. HUB counts in soil amended with BSG as in 10% and 15% pollution level was significantly different from those of soil amended with SMC and unamended polluted soil, but not significantly different from soil amended with BS at $P<0.05$ confidence level. Higher counts of AHB and HUB recorded in soil contaminated with 5% used lubricating oil compared to the counts in 10% and 15% oil might be attributed to low concentration of oil in these treatments. This is because high percentage of oil in contaminated soil might be poisonous to some of the indigenous microbial population, thereby killing or inhibiting their growth. This finding was reported by Ijah and Antai, (2003b) who argued that oil concentration above 100μg/g of soil is considered toxic to microbial growth in the contaminated soil.

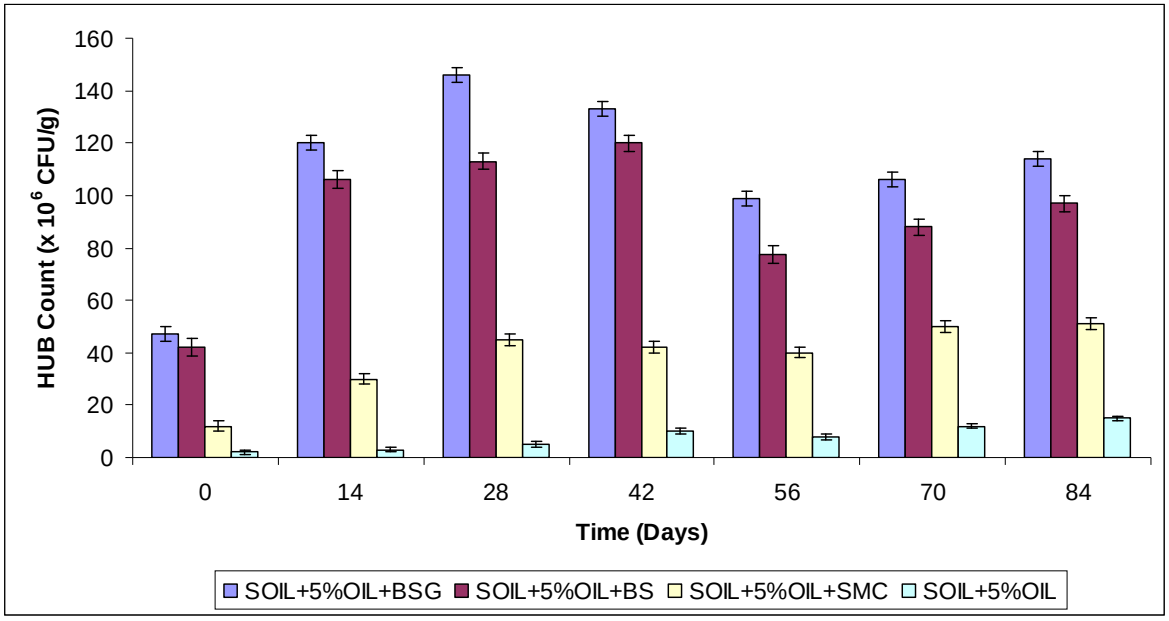


Figure 4.17 Counts of hydrocarbon utilizing bacterial (HUB) population in soil contaminated with 5% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

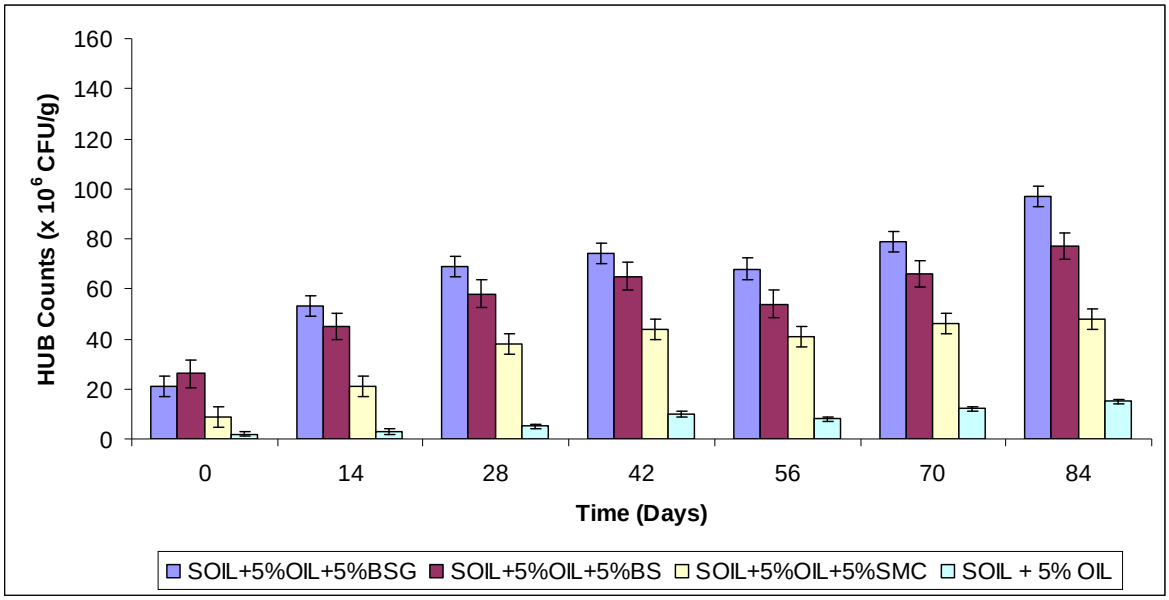


Figure 4.18 Counts of hydrocarbon utilizing bacterial (HUB) population in soil contaminated with 5% used lubricating oil and amended with 5% organic wastes. Bars indicates standard error (n = 3).

The counts of hydrocarbon utilizing bacteria (HUB) in all the soil amended with organic wastes was appreciably higher compared to that of unamended and autoclaved control soil in the three different oil pollution level (15%, 10%, 5%) considered in this study. These counts are comparable to those of Ijah and Antai (2003), who observed counts of hydrocarbon degraders in oil polluted soil to be $x10^6$ CFU/g but lower than those obtained by Antai and Mgbomo (1989) whose counts of HUB in hydrocarbon contaminated soil was $x10^8$ CFU/g. This may be due to differences in microbial ecology of the soil or characteristics of the experimental soils. The differences can also be attributed to the type of oil used for the studies which was crude oil whereas used lubricating oil was used in this study which contained some heavy metals which might likely impedes bacterial proliferations. However, soil contaminated with 5% used lubricating oil recorded more HUB and AHB than 10% and 15% oil pollution, the reason might be due to limited concentration of oil in this treatment which does not pose more toxicity to the bacteria present in the soil, this reason was supported by the findings of Rahman et al., (2002) who recorded appreciable increase in the number of bacteria as oil concentration decreased from 10% to 1% in contaminated soil. The reason for higher counts of bacteria in amended soil may be as a result of presence of appreciable quantities of nitrogen and phosphorus in the organic wastes, especially high nitrogen content in BSG (Table 4.1) which are necessary nutrient for bacterial biodegradative activities (Nakasaki et al., 1992; Ijah and Antai, 2003a; Joo et al., 2001; 2007; Adesodun and Mbagwu, 2008). The reason for increased biodegradation of oil in amended soil as compared to the unamended soil might also be due to the presence of organic wastes in the soil which helps to loosen the compactness of the soil making sufficient aeration available for the indigenous bacteria present in the soil, thereby enhancing their biodegradative activities of the oil from the soil.

The hydrocarbon utilizing bacteria (HUB) isolated from the used oil contaminated soil were identified as species *Acinetobacter, Micrococcus, Pseudomonas aeruginosa, Nocardia, Bacillus megaterium, Bacillus sp.* and *Corynebacterium.* These bacterial species had been implicated in hydrocarbon degradation by different authors (Ijah, 1998; Ahn et al., 1999; Van Hamme et al., 2003; Bento et al., 2005; Das and Mukherjee, 2007; Martin et al., 2007). *Bacillus* sp. grew extensively on the oil agar better than other isolates; this might be due to the presence of efficient hydrocarbon degradative enzymes systems and the presence of catabolic genes involved in hydrocarbon degradation in the bacterial species (Kyung-Hwa et al., 2006; Majid et al., 2008).

4.1.9 CO_2 evolution in soil contaminated with 15% 10% and 5% used lubricating oil

Tables 4.10 to 4.12 show the results of the amount of CO_2 liberated from soil treated with 15%, 10% and 5% (w/w) used lubricating oil and amended with 10% and 5% different organic wastes. In 15% oil pollution, the cumulative CO_2 production in all samples increased gradually to the last day of sampling. Lower amount of CO_2 (35.76 mg g^{-1}) was liberated in unamended oil polluted soil at the end of 28 days compared to those of oil polluted amended soil where 49.43 mg g^{-1}, 50.6 mg g^{-1} and 51.55 mg g^{-1} CO_2 were liberated in soil amended with BS, BSG and SMC respectively and 39 mg g^{-1}, 43.01 mg g^{-1} and 37.4 mg g^{-1} CO_2 were liberated in soil amended with 5% BS, BSG and SMC respectively (Table 4.10). There was no significant difference between the quantities of CO_2 liberated in soil amended with organic wastes, however significant difference was recorded between those of amended and unamended polluted soil at $P<0.05$ confidence level. This result is in agreement with the findings of Namkoong et al., (2002) who reported increase in concentration of CO_2 evolved in diesel contaminated soil amended with

compost and sewage sludge at the ratio of 1:0.5 compared to those amended with 1:0.1 and unamended polluted soil.

Table 4.10 Concentration of CO_2 (mg g^{-1}) in soil treated with 15% used lubricating oil and amended with organic wastes

Incubation	Treatments			
Period (days)	A	B	C	D
10% organic wastes amendments				
7	44.37 ± 0.34	42.17 ±1.5	32.71 ± 1.41	1.43 ± 0.47
14	45.69 ± 2.04	47.0 ± 1.46	44.22 ± 0.38	13.97 ± 0.47
21	48.62 ± 3.63	51.48 ± 1.43	50.31 ± 1.43	28.5 ± 0.93
28	49.43 ±3.04	50.6 ± 1.92	51.55 ± 0.67	35.76 ± 1.87
5% organic wastes amendments				
7	26.41±3.56	32.31±4.12	22.52±2.31	1.43±0.47
14	28.72±1.98	33.10±5.21	25.61±4.13	13.97±0.47
21	33.32±3.87	38.28±5.56	29.87±3.21	28.5±0.93
28	39.00±4.56	43.01±5.41	37.4±2.11	35.76±1.87

A = Soil + 15%Oil+ BS, B = Soil + 15%Oil + BSG, C = Soil + 15%Oil + SMC, D = Soil + 15%Oil

In 10% oil pollution at the end of the study period a relatively lower amount of CO_2 (28mg) was liberated in unamended oil polluted soil compared to those of oil polluted amended soil

where 52.73 mg g^{-1}, 53.17 mg g^{-1} and 49.13 mg g^{-1} CO_2 were liberated in soil amended with 10% BS, BSG and SMC (Table 4.11), respectively. The concentration of CO_2 evolved in soil amended with 5% organic wastes was lower than those recorded in soil amended with 10% organic wastes. 43.72 mg g^{-1}, 48.1 mg g^{-1} and 40.1 mg g^{-1} CO_2 were recorded in soil amended with 5% BS, BSG and SMC respectively after 28 days (Table 4.11). The differences in the amount of CO_2 liberated in all the treatment were not different significantly at $P<0.05$.

Table 4.11 Concentration of CO_2 (mg g^{-1}) in soil treated with 10% used lubricating oil and amended with organic wastes

Incubation	Treatments			
Period (days)	A	B	C	D
10% organic wastes amendments				
7	12.83 ± 0.92	12.1 ± 2.1	13.13 ± 1.99	8.69 ± 0.78
14	36.08 ± 2.68	34.83 ± 3.1	33.22 ± 0.66	18.26 ± 0.93
21	48.99 ± 0.67	48.77 ± 0.34	43.78 ± 0.22	25.0 ± 0.47
28	52.73 ± 0.7	53.17 ± 0.83	49.13 ± 0.46	28.0 ± 0.47
5% organic wastes amendments				
7	14.21±1.32	10.13±1.20	8.56±2.10	8.69±0.78
14	22.71±3.65	23.68±2.18	20.19±2.45	18.26±0.93
21	36.21±6.10	39.24±4.32	31.21±2.31	25.00±0.47
28	43.72±3.21	48.10±1.87	40.10±1.98	28.00±0.47

A = Soil + 10% Oil+ BS, B = Soil + 10% Oil + BSG, C = Soil + 10% Oil + SMC, D = Soil + 10% Oil

Table 4.12 shows the results of the amount of CO_2 liberated from soil treated with 5% (w/w) used lubricating oil and amended with 10% and 5% organic wastes. The CO_2 production in all samples increased gradually to the last day of sampling. 36 mg g^{-1} was liberated in unamended oil polluted soil while 54.13 mg g^{-1}, 57.12 mg g^{-1} and 47.92 mg g^{-1} CO_2 were liberated in soil amended with 10% BS, BSG and SMC respectively and 46.23 mg g^{-1}, 50.84 mg and 44.81 mg g^{-1} CO_2 were recorded after 28 days in soil amended with 5% BS, BSG and SMC respectively (Table 4.12). The differences in the amount of CO_2 liberated in all the treatment were not different significantly at $P<0.05$.

Table 4.12 Concentration of CO_2 (mg g^{-1}) in soil treated with 5% used lubricating oil and amended with organic wastes

Incubation	Treatments			
Period (days)	A	B	C	D
10% organic wastes amendments				
7	23.01 ± 2.12	18.34 ± 1.31	14.87 ± 2.04	12.67 ± 2.28
14	33.05 ± 2.32	32.86 ± 2.61	27.62 ± 1.60	15.87 ± 2.43
21	51.72 ± 3.85	52.56 ± 4.14	39.45 ± 1.22	26.54 ± 3.02
28	54.13 ± 2.7	57.12 ± 2.73	47.92 ± 2.76	36.09 ± 2.77
5% organic wastes amendments				
7	15.23±1.43	18.41±1.32	11.01±0.98	12.67±2.28
14	27.5±2.61	26.56±1.78	23.14±2.67	15.87±2.43
21	39.96±2.56	41.32±3.23	33.18±2.32	26.54±3.02
28	46.23±2.76	50.84±3.56	44.81±5.43	36.09±2.77

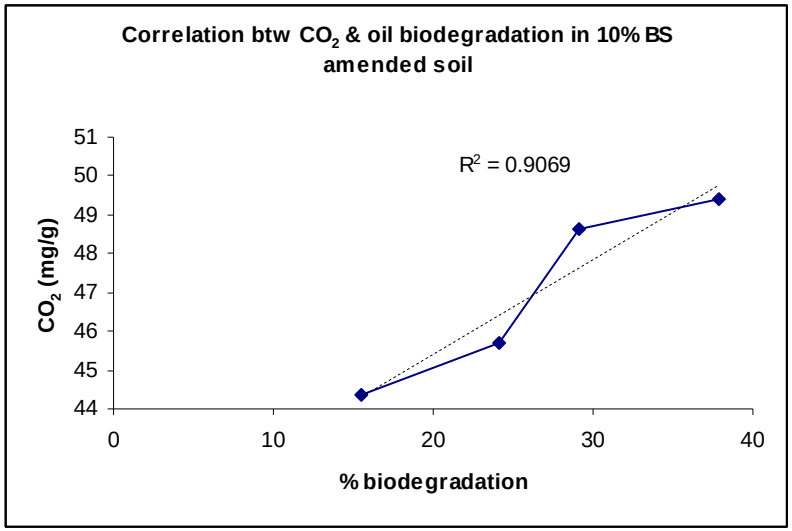
A = Soil + 5% Oil+ BS, B = Soil + 5% Oil + BSG, C = Soil + 5% Oil + SMC, D = Soil + 5% Oil

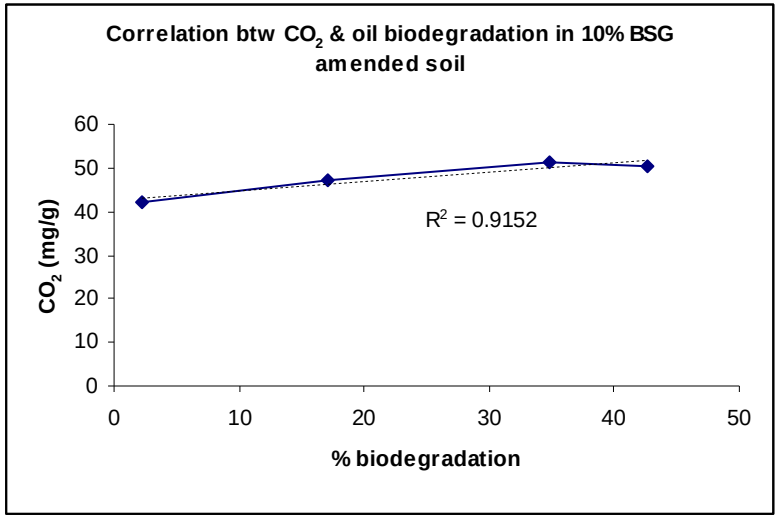
Increased in microbial activity in this study, as measured by CO_2 evolution indicates that the microorganisms in the organic wastes amended soil are metabolically active, which possibly contributed to the increased rates of oil biodegradation in the amended soil. This support the findings of several authors (Namkoong et al., 2002; Ijah and Antai, 2003b; Bento et al., 2005). The high amount of CO_2 liberated in soil amended with organic wastes in soil contaminated with 15%, 10% and 5% used lubricating oil is an indication of high utilization of hydrocarbon fractions as a source of carbon and energy by microbial community than that of unamended polluted soil.

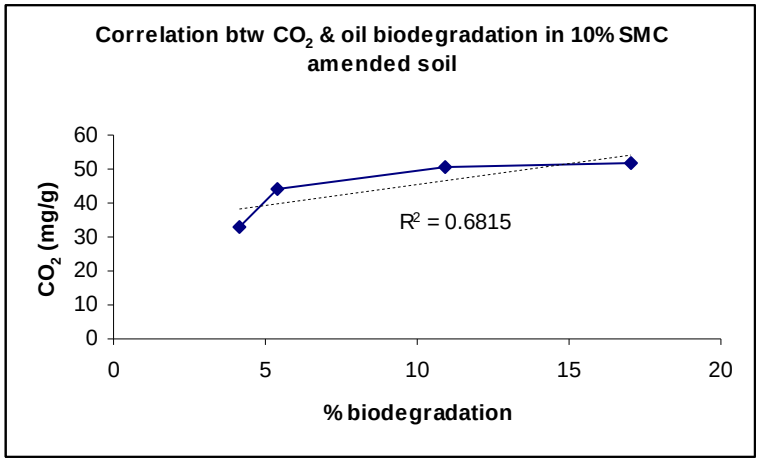
4.1.10 Correlation between CO_2 evolution and oil biodegradation

The relationships between the oil biodegradation and concentration of CO_2 evolved in soil contaminated with 15%, 10% and 5% used lubricating oil and amended with 10% and 5% different organic wastes are shown in Figures 4.19 to 4.21. The results of oil biodegradation in soil contaminated with used lubricating oil and amended with 10% organic wastes shows strong positive linear correlation with evolution of CO_2 in soil amended with organic wastes and unamended soil, while that of BS amended soil shows weak correlation in 10% oil pollution. Unamended polluted soil shows better correlation (R^2 = 0.97) than those of BS amended soil in 10% oil pollution. This might be due to different factors, such as non-availability of the organic compounds to the bacteria community in BS amended soil. BSG amended soil shows strong correlation (R^2 = 0.9 and above) in all the different concentrations of oil pollution (5%, 10% & 15%). This might be due to presence of appreciable N concentration in BSG. The positive linear correlation between CO_2 evolution and oil biodegradation recorded in most of the treatments can be attributed to the increase in microbial activities in all the treatments which implies that

most of the oil breakdown in the contaminated soil can be attributed to microbial degradation due to appreciable release of CO_2 during the process of oil breakdown. These results agrees with the findings of several authors (Ijah and Antai, 2003b; Roling et al., 2004; Aluyor and Ori-Jesu, 2009; Morais and Tornisielo, 2009 and Abioye, et al., 2010), who reported positive linear correlation of oil biodegradation with high evolution of CO_2 in oil contaminated.







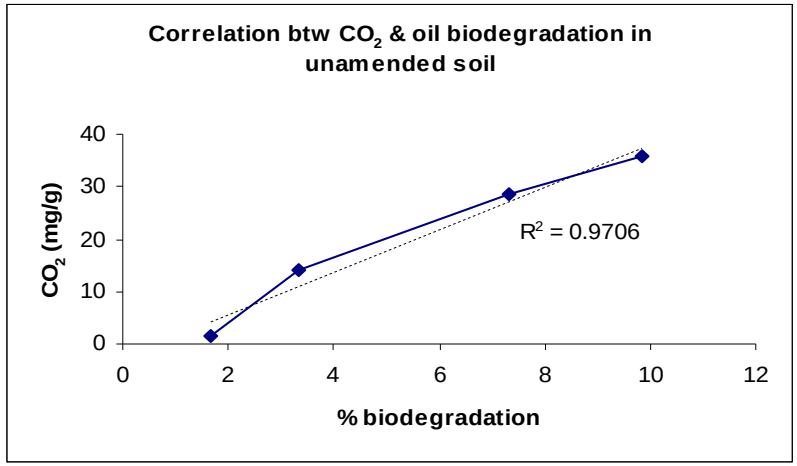


Figure 4.19 Correlation between CO_2 evolution and oil biodegradation in 15% oil pollution

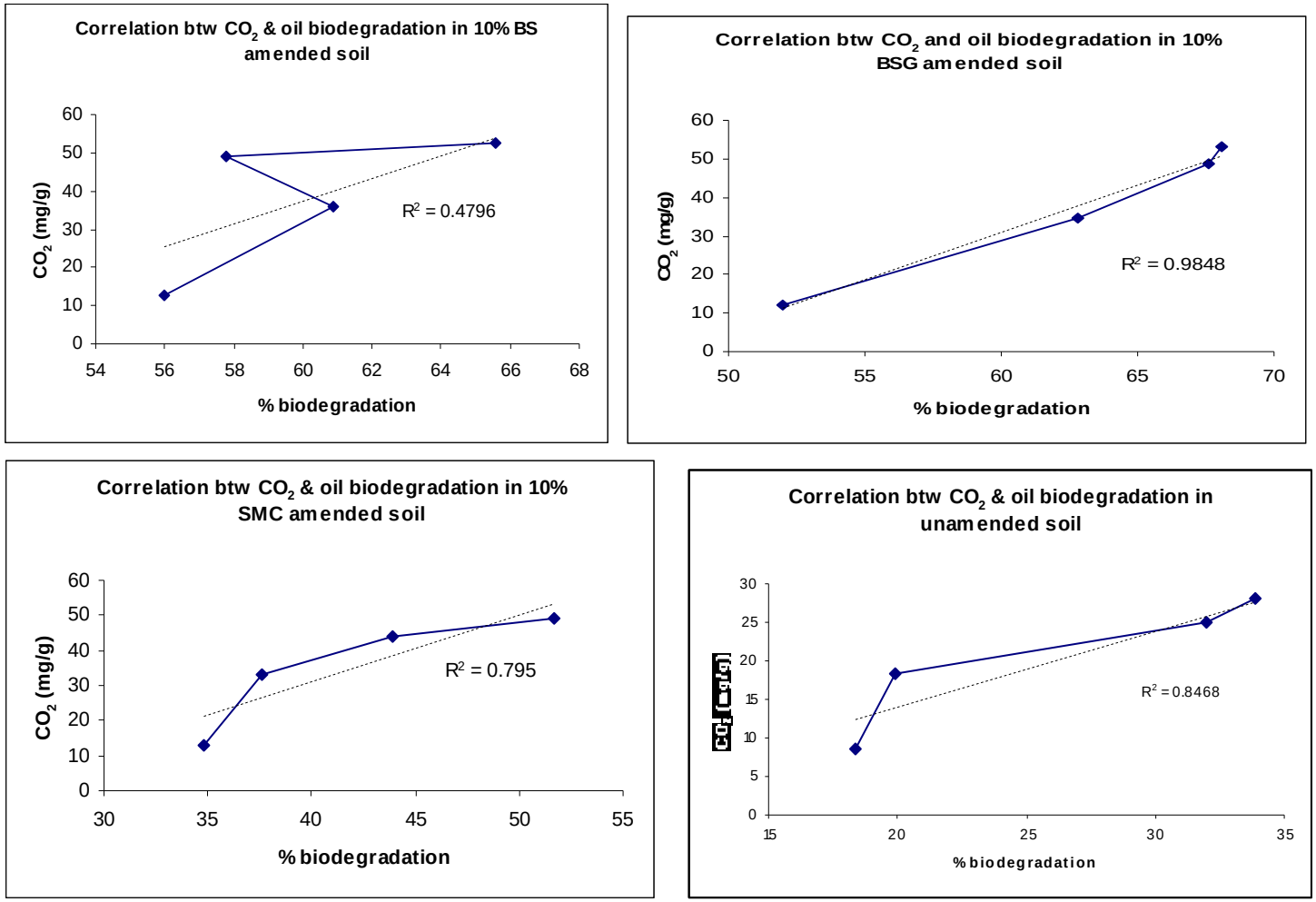


Figure 4.20 Correlation between CO_2 evolution and oil biodegradation in 10% oil pollution

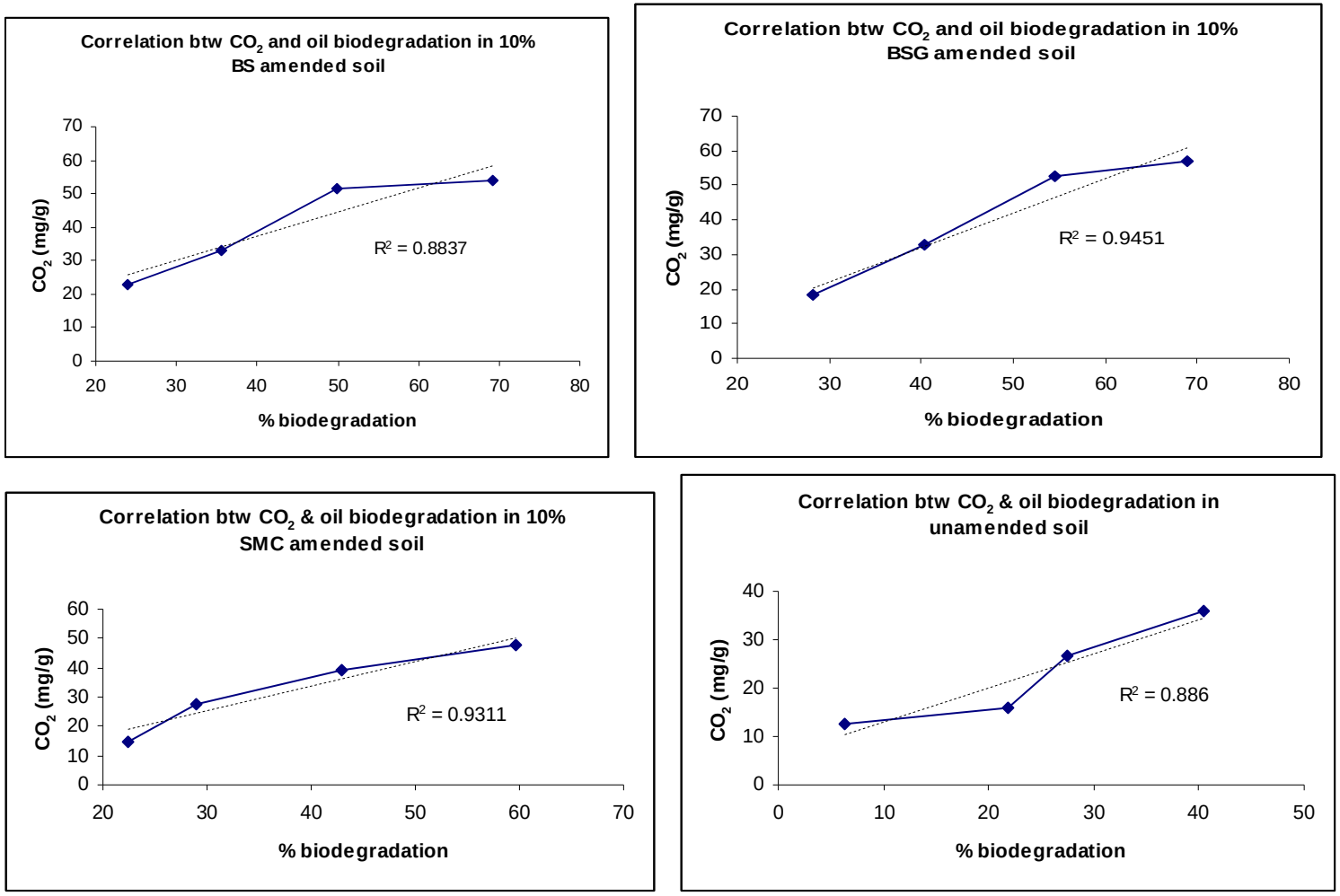


Figure 4.21 Correlation between CO_2 evolution and oil biodegradation in 5% oil pollution

4.1.11 pH of soil contaminated with 5%, 10% and 15% used lubricating oil

The pH of the soil used for bioremediation studies was 6.12 (Table 4.1). The pH for the various treatment of the soil contaminated with 15%, 10% and 5% used lubricating oil and amended with 10% and 5% organic wastes varied greatly from slightly acidic to neutral pH throughout the study period. Figures 4.22 to 4.27 shows the pH for soil contaminated with 15%, 10% and 5% used lubricating oil and amended with 10 and 5% organic wastes respectively.

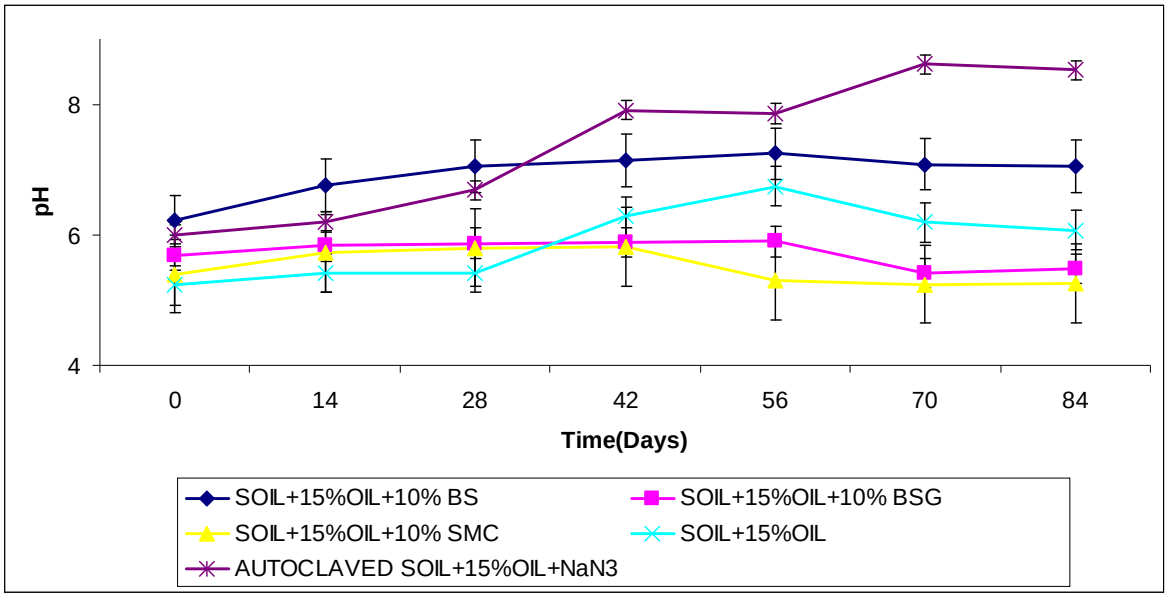


Figure 4.22 pH of soil contaminated with 15% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

Addition of organic wastes mostly BS and BSG raised the pH of the soil from 6.12 to as high as 8.34 in soil treated with 5 and 10% organic wastes. Soil amended with SMC recorded low pH of 5.02; this might be because the SMC (which was used to grow mushroom) was acidic in nature because fungi grow better in slightly acidic medium. This might probably account for low biodegradation of oil in soil amended with SMC.

Biodegradation of oil is always favoured by neutral or slight alkaline pH (Ijah and Antai, 2003a; Okoh, 2006). The results obtained confirm an earlier finding by Ijah et al., (2008) that addition of chicken droppings to crude oil contaminated soil produced a buffering effect on the soil by raising the pH of the soil from 6.75 to 7.76. The drop in pH between 42 and 84 days might be due to accumulation of secondary metabolites (which are slightly acidic in nature) resulting from microbial degradation of oil.

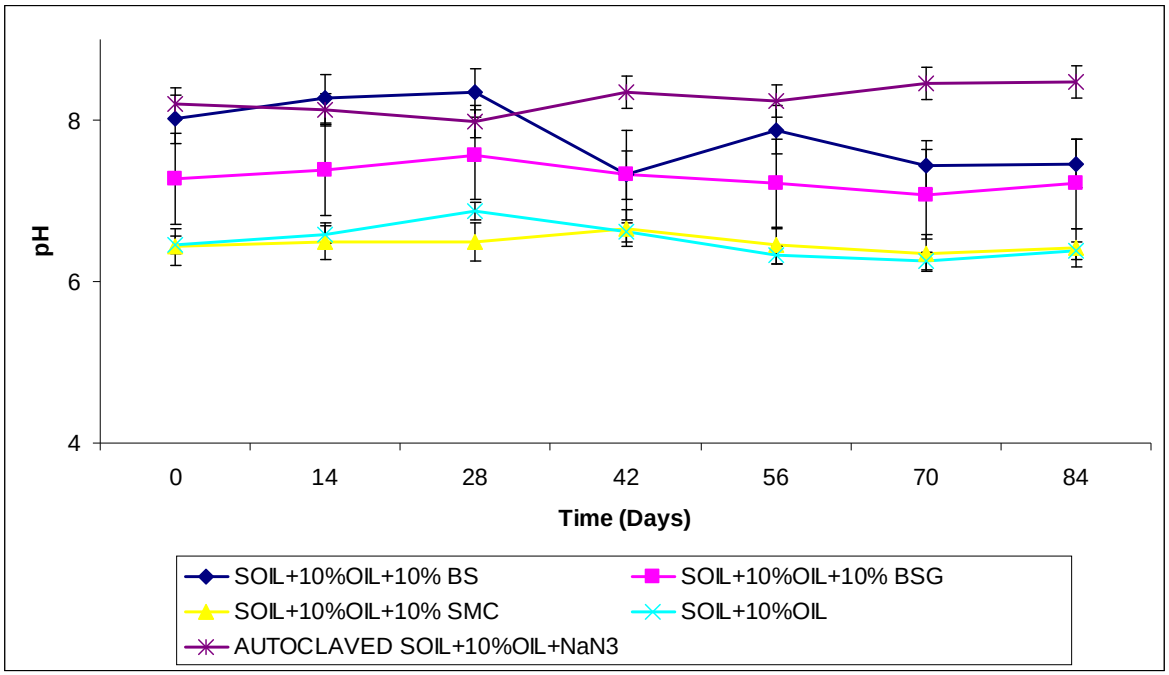


Figure 4.23 pH of soil contaminated with 10% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

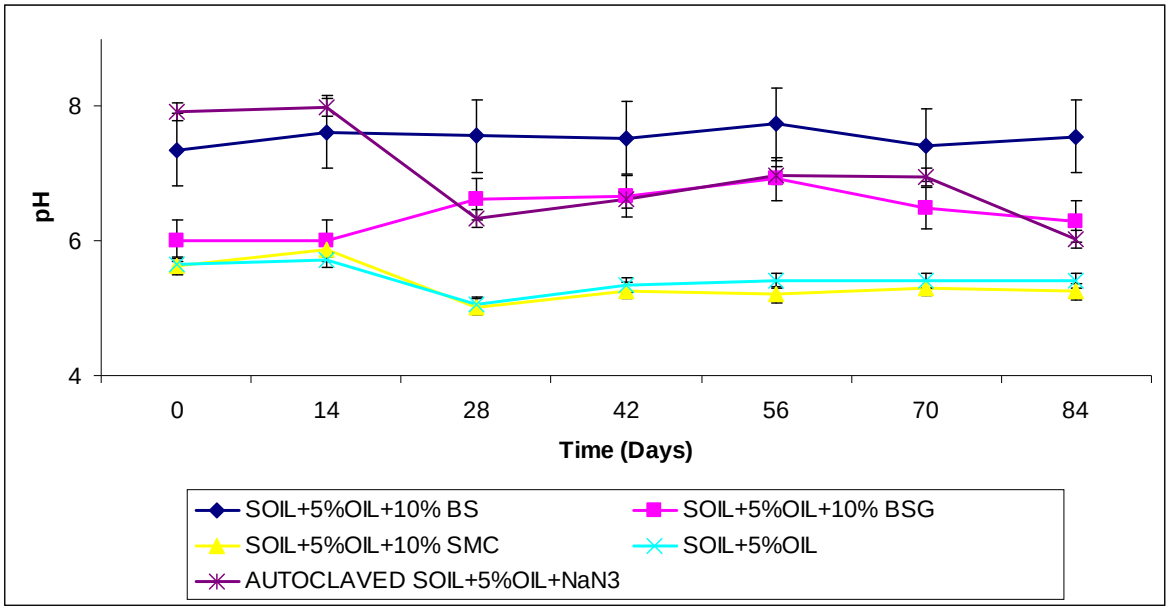


Figure 4.24 pH of soil contaminated with 5% used lubricating oil and amended with 10% organic wastes. Bars indicates standard error (n = 3).

The soil's pH increased in all the treatments after contamination with used lubricating oil and addition of organic wastes, it later decreased between 14 and 28 days SMC treated soil and 56 and 70 days in BS and BSG treatments. The reason for the decrease in pH of the contaminated between 14 and 56 days might be due to extensive microbial degradation of oil within this period with the production of some acidic radicals which probably lowered the pH. This result is similar to the findings of Tisdale and Nelson (1975) and Al-Saleh and Obuekwe, 2005 who observed and reported that the decrease in pH during remediation may have resulted from the production of acid radicals through the process of nitrification of the applied nutrients.

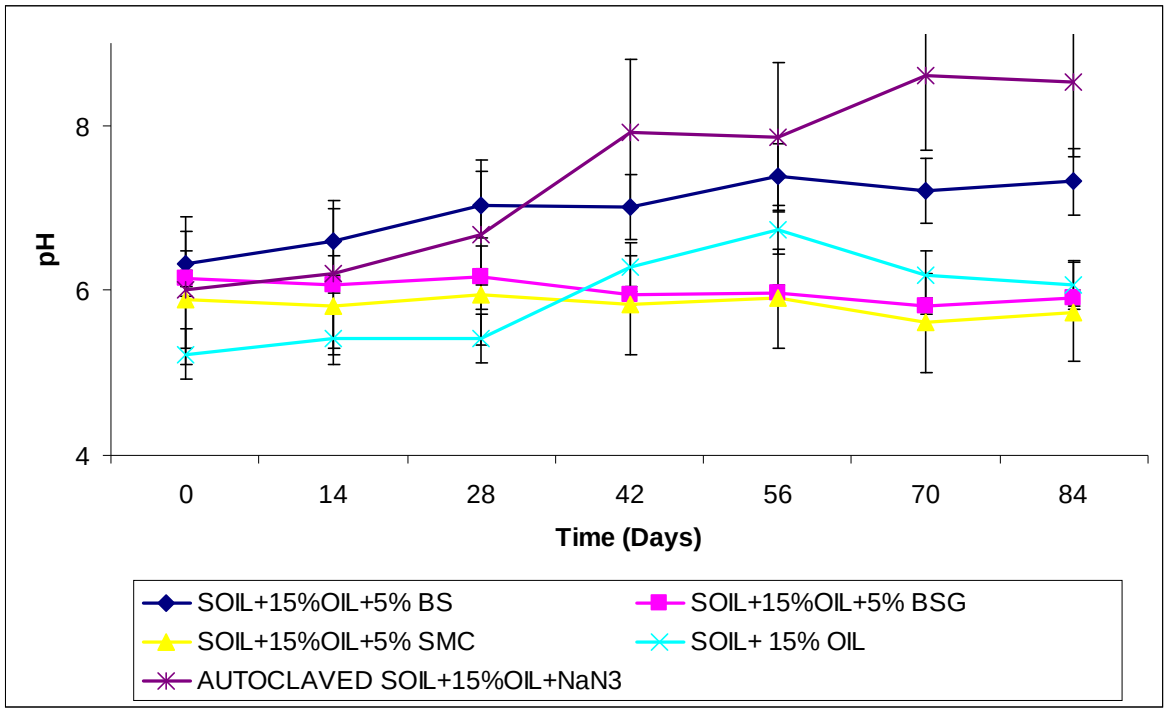


Figure 4.25 pH of soil contaminated with 15% used lubricating oil and amended with 5% organic wastes. Bars indicates standard error (n = 3).

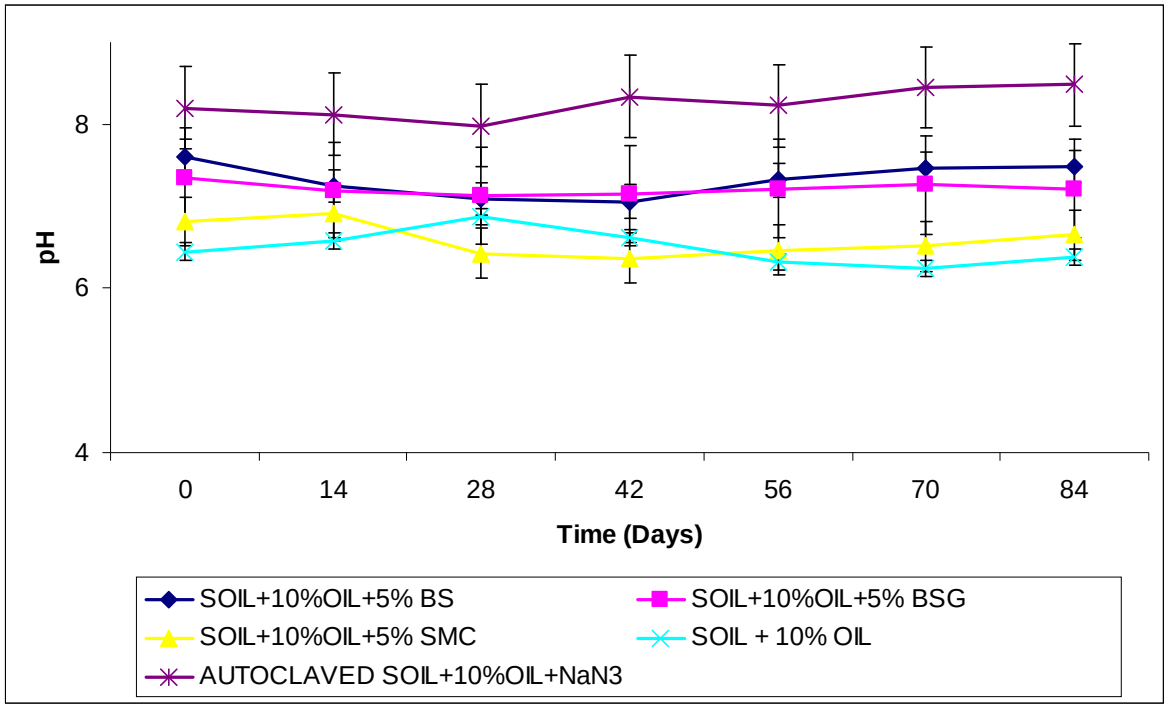


Figure 4.26 pH of soil contaminated with 10% used lubricating oil and amended with 5% organic wastes. Bars indicates standard error (n = 3).

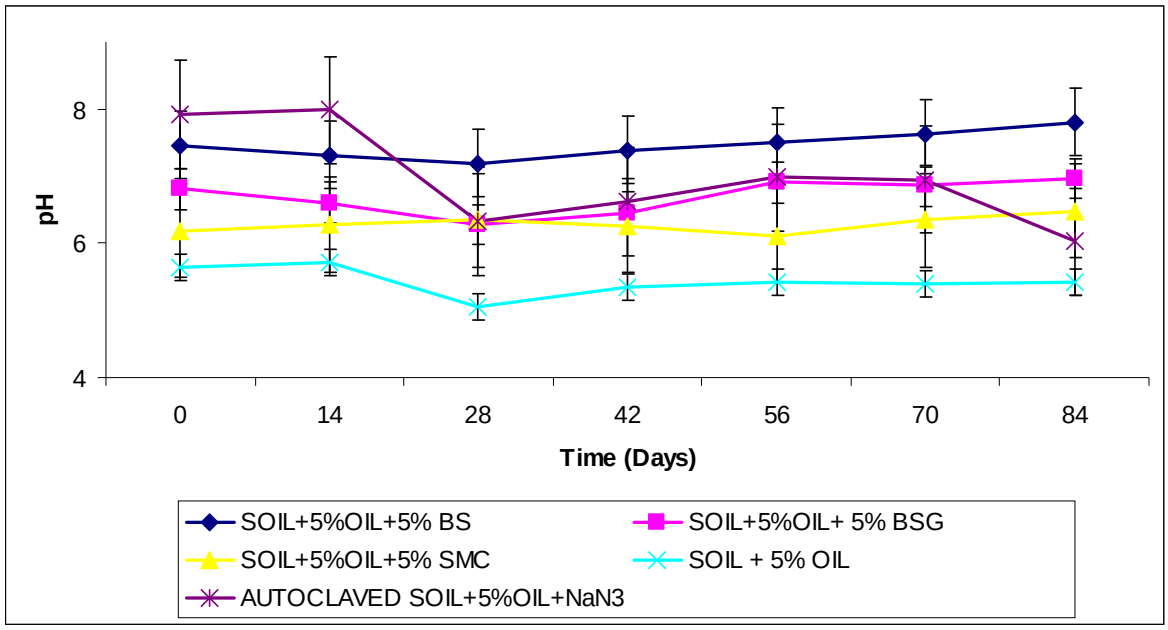


Figure 4.27 pH of soil contaminated with 5% used lubricating oil and amended with 5% organic wastes

4.1.12 Germination toxicity test

Lettuce (*Lactuca sativa*) is an important agricultural crop and it is fairly sensitive to toxic chemicals (mostly petroleum contaminants), which led to its wide spread use for toxicity tests (Banks and Schults, 2005; Oleszczuk, 2008). The results of germination toxicity test with lettuce for soil amended with 10% and 5% organic wastes are shown in Tables 4.13. The results revealed 100% germination in remediated soil contaminated with 5% used lubricating oil and amended with BSG over the period of 84 days whereas 40% and 30% germination rates were recorded in soil contaminated with 15% oil and amended with 10% and 5% BSG, respectively. 100% germination was recorded in uncontaminated control soil, while only 20% and 0% were recorded in poisoned controlled soil in soil contaminated with 5% and 10% lubricating oil respectively. The result shows positive correlation between loss of oil in the contaminated soil and seed germination, it also revealed that

remediation of soil contaminated with high concentration of petroleum hydrocarbons needs a longer period of time possibly with increased quantity of organic wastes amendment to be completely restored into a state suitable for agricultural purposes. The results are in agreement with the findings of Banks and Schultz (2005) and Millioli et al., (2009) who recorded decrease in number of germinated seeds with increasing quantities of petroleum concentration in the soil.

Table 4.13 Seed germination (%) toxicity test

Percentage of Oil pollution	Treatments					
	A	B	C	D	E	F
10% organic wastes amendment						
5	80±6.0	100	80±6.0	40±6.0	20±0	100
10	70±10	80±6.0	60±0	40±6.0	0	100
15	40±5.8	40±6.0	20±0	10±0	0	100
5% organic wastes amendment						
5	70±0	100	70±10	40±6.0	20±0	100
10	50±10	60±0	40±10	40±6.0	0	100
15	30±0	30±10	10±0	10±0	0	100

A = Soil + Oil+ BS, **B** = Soil + Oil + BSG, **C** = Soil + Oil + SMC, **D** = Soil + Oil, **E =** Autoclaved soil + Oil + NaN_3, **F** = Uncontaminated soil

Table 4.14 shows the results of seed germination index in soil contaminated with 15%, 10% and 5% used lubricating oil and amended with different organic wastes. Germination index (GI) of soil treated with BSG recorded the highest germination index (83.33%, 40% & 30%) in all the treatments with 5% and 10% organic wastes amendments, this result further proved the effectiveness of BSG in enhancing biodegradation of hydrocarbon in oil

contaminated soil. The results are similar to the finding of Barahona, et al., 2005 and Oleszczuk, (2008), who reported that composting reduce phytotoxicity of diesel oil and wastewater sludge to the germination of *Lepidium sativum* after composting the sludge for 76 days. However, the GI of unamended polluted soil and the amended soil contaminated with 15% used lubricating oil was very low, signifying low biodegradation of oil in these treatments. The negative effect of hydrocarbons on the germination index may be attributed to their inherent toxicity or to the perturbations they cause in soil and plants due to their hydrophobic properties (Adam and Duncan, 2002; Ogboghodo et al., 2004). Hydrocarbons may coat roots, preventing or reducing gas and water exchange and nutrient absorption; they may also enter the seeds and alter the metabolic reactions or kill the embryo by direct, acute toxicity; after penetrating the plant tissues, hydrocarbons damage cell membranes and reduce the metabolic transport and respiration rate (Adam and Duncan, 2002; Labud et al., 2007). But, a more likely reason for the inhibitory effect of hydrocarbons on germination is its physical water repellent property. The film of hydrocarbons around the seeds may act as a physical barrier, preventing or reducing both water and oxygen from entering the seeds. This would inhibit the germination response (Adam and Duncan, 2002).

Table 4.14 Seed germination toxicity index (%)

Percentage of Oil pollution	Germination toxicity index (%)				
	A	B	C	D	E
10% organic wastes amendment					
5	40.00	83.33	33.34	13.33	3.27
10	23.33	40.00	20.00	10.00	0.00
15	6.53	13.33	5.00	1.65	0.00
5% organic wastes amendment					
5	29.17	83.33	35.00	13.33	3.27
10	16.67	30.00	13.33	10.00	0.00
15	4.90	10.00	2.50	1.65	0.00

A = Soil + Oil+ BS, **B** = Soil + Oil + BSG, **C** = Soil + Oil + SMC, **D** = Soil + Oil, **E** = Autoclaved soil + Oil + NaN_3

4.2 Results of biostimulation studies conducted for 12 months at the experimental site exposed to sunlight and rainfall

The biostimulation study under a natural condition was conducted at the experimental site exposed to sunlight and rainfall (though covered with net) for 12 months (April, 2009 to April, 2010). Temperature ranges between 28^0C – 36^0C throughout the study period. Due to positive effectiveness of 10% organic wastes amendment under laboratory condition, only 10% organic wastes amendments was used for the simulated natural condition.

4.2.1 Biodegradation of used lubricating oil

Soil samples were taken from each treatment every three months to determine the percentage of oil biodegradation for the period of 12 months. Figures 4.28 to 4.30 shows the percentage of oil biodegradation in soil contaminated with 15%, 10% and 5% used lubricating oil and amended with different organic wastes.

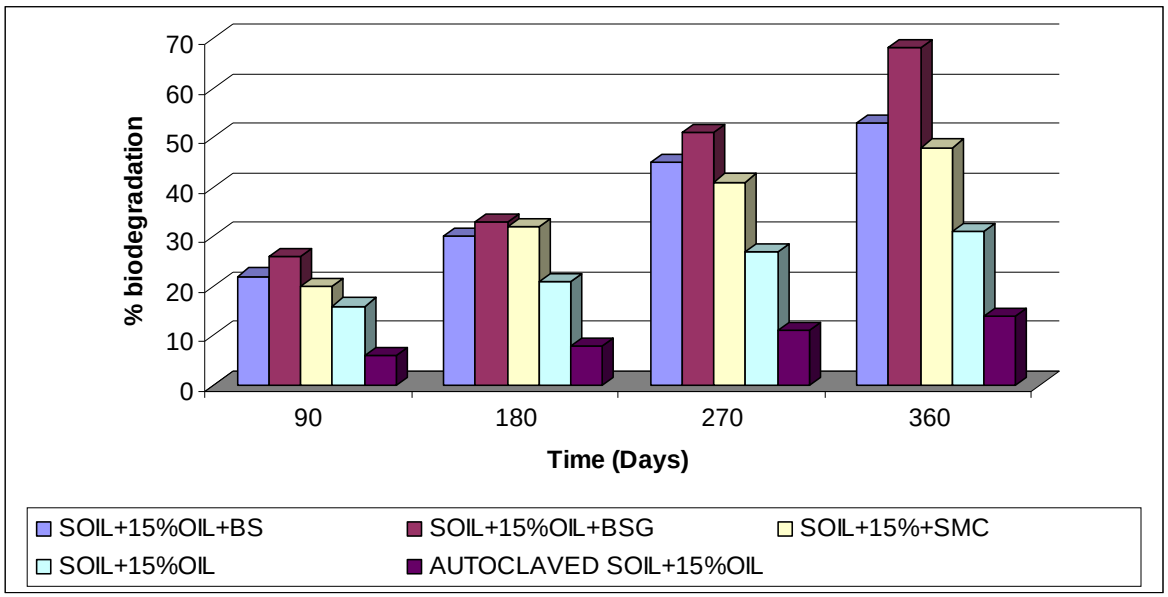


Figure 4.28 Percentage biodegradation of TPH in soil contaminated with 15% used lubricating oil

The percentage of oil biodegradation in soil contaminated with 15% oil at the end of 12 months in soil amended with BS, BSG and SMC are 53%, 68% and 48%, respectively

(Figure 4.28). The percentage of biodegradation recorded in 15% oil treatment under simulated natural condition was higher than the percentage biodegradation recorded under laboratory condition. BSG treated soil recorded the highest biodegradation (68%). The reason for this might be due to longer bioremediation time of 12 months in this condition. It might also be due to favourable environmental conditions of sunlight which possibly had positive influence on the rate of oil biodegradation. Temperature plays an important roles in biodegradation of petroleum hydrocarbons, firstly by its direct effect on the chemistry of the pollutants, and secondly on its effect on the physiology and diversity of the microorganisms (Venosa and Zhu, 2003; Okoh, 2006). Temperature is also known to affect the rate of biodegradation and play a significant role in controlling the nature and extent of microbial hydrocarbon metabolism (Rowland et al., 2000; Frederic, 2005).

Figure 4.29 and 4.30 shows the percentage of oil biodegradation in 10% and 5% oil pollution respectively.

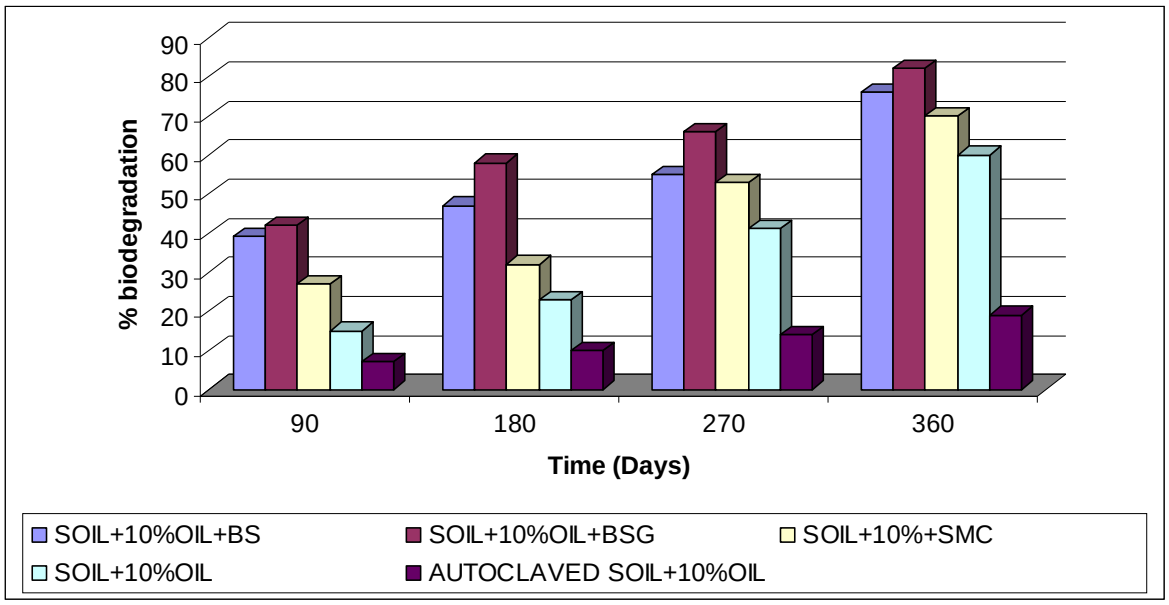


Figure 4.29 Percentage biodegradation of TPH in soil contaminated with 10% used lubricating oil

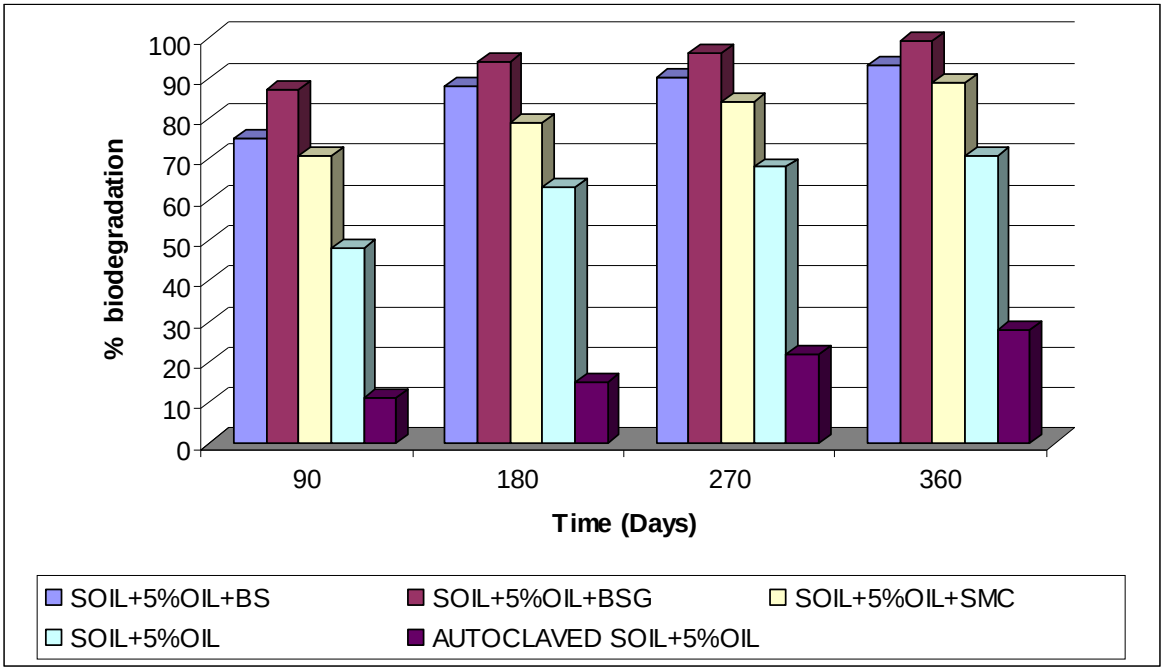


Figure 4.30 Percentage biodegradation of TPH in soil contaminated with 5% used lubricating oil

At the end of 12 months, soil contaminated with 5% used lubricating oil recorded extensive biodegradation of 93%, 99.4% and 89% in soil amended with BS, BSG and SMC, respectively while the unamended soil recorded 71% biodegradation. In soil contaminated with 10% oil, 76%, 82% and 70% oil biodegradation were recorded at the end of 12 months. The extensive oil biodegradation recorded in all the treatments under the simulated natural condition compared to those under laboratory condition can be due to various environmental conditions such as rainfall and temperature (the temperature ranged between 28 and 37^0 C) which possibly stimulated the activities of the microorganisms in the soil. As reported by various authors temperature plays a significant role in controlling the nature and extent of microbial hydrocarbon metabolism (Nedwell, 1999; Frederic, 2005). Temperature affects the rate of biodegradation, as well as the physical nature and chemical composition of hydrocarbons (Whyte et al., 1998; Rowland et al., 2000). Another reason

for the increased oil loss in all the treatments might be due to evaporation and photodegradation of part of the used lubrication oil, this was reflected in the percentage oil loss in the poisoned control treatment of 12%, 19% and 28% in 5%, 10% and 15% oil contamination, respectively.

4.2.2 Biodegradation of hydrocarbon fractions

Biodegradation of hydrocarbon fractions present in the used lubricating oil was determined at three months intervals for the period of 12 months to determine the extent of biodegradation of different hydrocarbon fractions using GC/FID. The hydrocarbon fractions were divided into four fractions which are: $C_7 – C_9$, $C_{10} – C_{14}$, $C_{15} – C_{28}$ and $C_{29} – C_{36}$ (Palmer, 1993; Alberdi et al., 2001).

4.2.2.1 Biodegradation of $C_7 – C_9$ fractions in used lubricating oil

Table 4.15 shows the extent of biodegradation of $C_7 – C_9$ hydrocarbon fractions in soil contaminated with 5%, 10% and 15% used lubricating oil and amended with different organic wastes for the period of 12 months. The results of soil contaminated with 5% used lubricating oil revealed complete biodegradation (below the detection limit) of the petroleum fractions from the initial concentration of 88 mg/kg within the period of three months in soil amended with BS, BSG and SMC whereas, it takes six months before the $C_7 – C_9$ fractions was degraded below the detection limit in unamended and sterile polluted soil. In soil contaminated with 10% used lubricating oil, the petroleum fraction ($C_7 – C_9$) was degraded below the detection limit within the first six months in soil amended with BS and SMC, whereas in BSG amended soil the time for degradation below detection limit was within the first three months of studies (proving the effectiveness of BSG compared to

other organic wastes). The degradation of $C_7 – C_9$ below detection limit in unamended and sterile soil polluted with 10% used lubricating oil takes nine months. The results of soil contaminated with 15% used lubricating oil shows complete degradation of the hydrocarbon fraction $C_7 – C_9$ in BSG amended soil within the first six months whereas degradation below detection level was achieved within nine months in soil amended with BS, SMC and unamended polluted soil, while those of sterile soil was achieved in the 12 month. The rapid biodegradation of hydrocarbons fraction between $C_7 – C_9$ in all the different treatments to the levels below the detection limits might be due to the volatility of some of this fractions and the ease of breakdown of these fractions (due to their simple molecular structures) by bacteria present in the contaminated soil than branched isomers (iso-butane and iso-pentane) (Pallasser, 2000; George et al., 2002; Wenger et al., 2002).

Table 4.15 Concentration (mg/kg) of $C_7 – C_9$ fractions in soil contaminated with 5%, 10% and 15% used lubricating oil.

	Time (months)				
Treatment	0	3	6	9	12
Soil+5%oil+BS	88	ND	ND	ND	ND
Soil+5%oil+BSG	88	ND	ND	ND	ND
Soil+5%oil+SMC	88	ND	ND	ND	ND
Soil+5% oil only	88	58	ND	ND	ND
Sterile soil+5%oil	88	67	ND	ND	ND
Soil+10%oil+BS	136	61	ND	ND	ND
Soil+10%oil+BSG	136	ND	ND	ND	ND
Soil+10%oil+SMC	136	52	ND	ND	ND
Soil+10% oil only	136	87	58	ND	ND
Sterile soil+10%oil	136	92	61	ND	ND
Soil+15%oil+BS	206	145	98	ND	ND
Soil+15%oil+BSG	206	63	ND	ND	ND
Soil+15%oil+SMC	206	148	86	ND	ND
Soil+15% oil only	206	174	109	ND	ND
Sterile soil+15%oil	206	185	123	67	ND

ND: Not detected at lowest detection limit of 50 mg/kg

The soil amended with organic wastes, mostly BSG shows better and faster degradation of these fractions, the reason for this might be due to its positive effects on hydrocarbon degrading bacteria which enhance their multiplication thereby speedy up the rate of hydrocarbon degradation. This is similar to the study by Ijah and Antai (2003b), who reported complete degradation of C_7 to C_{12} fractions within three months in soil contaminated with 10% crude oil.

4.2.2.2 Biodegradation of $C_{10} – C_{14}$ fractions in used lubricating oil

Table 4.16 shows the results of biodegradation of $C_{10} – C_{14}$ hydrocarbon fractions in used lubricating oil-contaminated soil after 12 months of biostimulation studies with three different organic wastes. 5% oil-contaminated soil amended with BS, BSG and SMC recorded complete degradation of the hydrocarbon fractions below the detection limit within six months compared to those of $C_7 – C_9$ fractions which took only nine months for the complete degradation in amended soil. There was no complete degradation of the fraction ($C_{10} – C_{14}$) in the sterile polluted soil throughout the 12 months period, while complete degradation was achieved within the nine month in the unamended polluted soil. Soil contaminated with 10% used lubricating oil recorded oil biodegradation below detection limit in soil amended with BSG within the nine month whereas that of BS amended soil extended to the 12 month while complete degradation below detection limit was not achieved in soil amended with SMC, unamended and sterile polluted soil throughout the 12 months period. The sterile polluted soil at 12 month has residual $C_{10} – C_{14}$ fractions of 114 mg/kg.

Table 4.16 Concentration (mg/kg) of $C_{10} - C_{14}$ fractions in soil contaminated with 5%, 10% and 15% used lubricating oil.

	Time (months)				
Treatment	0	3	6	9	12
Soil+5%oil+BS	139	83	ND	ND	ND
Soil+5%oil+BSG	139	62	ND	ND	ND
Soil+5%oil+SMC	139	91	ND	ND	ND
Soil+5% oil only	139	106	67	ND	ND
Sterile soil+5%oil	139	118	82	64	58
Soil+10%oil+BS	184	139	103	78	ND
Soil+10%oil+BSG	184	117	92	ND	ND
Soil+10%oil+SMC	184	144	112	92	59
Soil+10% oil only	184	156	128	103	64
Sterile soil+10%oil	184	172	154	133	114
Soil+15%oil+BS	242	186	127	76	ND
Soil+15%oil+BSG	242	164	109	61	ND
Soil+15%oil+SMC	242	191	138	92	67
Soil+15% oil only	242	190	156	117	95
Sterile soil+15%oil	242	217	189	153	135

ND: Not detected at lowest detection limit of 50mg/kg

In soil contaminated with 15% used lubricating oil, complete biodegradation below detection level was only achieved in soil amended with BS and BSG at the 12^{th} month while complete degradation was not achieved in contaminated soil amended with SMC and those of unamended and sterile polluted soil throughout the 12 months study period (Table 4.16). The rapid biodegradation of $C_{10} - C_{14}$ fractions has been reported to be among the most rapidly biodegraded components of oil, although they are also susceptible to removal

by extensive water washing. Empirically, the first sign of biodegradation are usually n-alkane in the C_{10} to C_{13} range, which probably reflects an optimal carbon number with increasing enthalpy of reaction and decreasing water solubility as the alkane carbon number increases (Palmer, 1993; Masterson, et al., 2001).

The results, like those of $C_7 - C_9$ reveal the effectiveness of BSG to effect complete degradation of $C_{10} - C_{14}$ fractions in all the different level of pollution, this still pointed out its ability to stimulate the indigenous bacteria in degrading the hydrocarbon fractions due to its nutrient composition. The results is similar to those reported by Ijah and Antai, 2003 who discovered that C_{14} fraction was completely degraded in soil contaminated with 10% crude oil within the period of 12 months. Chang et al., (2010) also reported substantial degradation of C_{10} to C_{16} hydrocarbon fraction in aged petroleum hydrocarbon-contaminated soil.

4.2.2.3 Biodegradation of $C_{15} - C_{28}$ fractions in used lubricating oil

The results of biodegradation of $C_{15} - C_{28}$ hydrocarbon fractions in the soil contaminated with 5%, 10% and 15% used lubricating oil and amended with different organic wastes are shown in Table 4.17. The results shows that the hydrocarbon fractions $C_{15} - C_{28}$ were not degraded below the detection limit in all the treatments, however the degree of biodegradation varies greatly based on the percentage of oil pollution and organic wastes amendments. The reason for incomplete biodegradation of these hydrocarbon fractions below detection limit might be due to their complex structural nomenclature, which always posed some significant difficulty to hydrocarbon utilizing bacteria in their complete biodegradation (Peters and Moldowan, 1993). In soil contaminated with 5% oil, BSG amended soil recorded highest biodegradation of $C_{15} - C_{28}$ hydrocarbon fractions from the

initial concentration of 3810 mg/kg to 296 mg/kg after 12 months of study. The unamended polluted soil recorded reduction in the hydrocarbon fraction from 3810 mg/kg to 966 mg/kg after 12 months period of study (Table 4.17). Studies with soil contaminated with 10% and 15% oil pollution also revealed BSG amended soil as the best treatment where the oil fractions were reduced from 8150 mg/kg to 676 mg/kg in 10% pollution and from 11341 mg/kg to 1260 mg/kg in 15% oil pollution. The unamended polluted soil and sterile polluted soil recorded very low biodegradation of the $C_{15} - C_{28}$ fractions throughout the 12 months period in soil contaminated with 10 and 15% used lubricating oil. The increase in the biodegradation of $C_{15} - C_{28}$ fractions in soil amended with organic wastes might be due to nutrient composition of the organic wastes especially BSG. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants, especially nitrogen, phosphorus and in some cases iron (Okoh, 2006). Depending on the nature of the impacted environment, some of these nutrients could become limiting, hence the additions of nutrients are necessary to enhance the biodegradation of oil pollutants (Choi et al., 2002; Kim et al., 2005).

Table 4.17 Concentration (mg/kg) of $C_{15} – C_{28}$ fractions in soil contaminated with 5%, 10% and 15% used lubricating oil.

Treatment	Time (months)				
	0	3	6	9	12
Soil+5%oil+BS	3810	2122	1760	1322	968
Soil+5%oil+BSG	3810	1235	348	321	296
Soil+5%oil+SMC	3810	2231	1750	1428	974
Soil+5% oil only	3810	3601	3510	2161	966
Sterile soil+5%oil	3810	3783	3598	2879	1190
Soil+10%oil+BS	8150	6210	4900	3281	759
Soil+10%oil+BSG	8150	4271	715	691	676
Soil+10%oil+SMC	8150	7012	5100	3301	1630
Soil+10% oil only	8150	7854	7220	7014	6810
Sterile soil+10%oil	8150	8043	7830	7692	7410
Soil+15%oil+BS	11341	10213	7160	6589	5950
Soil+15%oil+BSG	11341	5874	1620	1501	1260
Soil+15%oil+SMC	11341	9531	8534	7840	6670
Soil+15% oil only	11341	11012	10600	10650	9890
Sterile soil+15%oil	11341	10890	9780	10350	10400

4.2.2.4 Biodegradation of $C_{29} – C_{36}$ fractions in used lubricating oil

Results of biodegradation of $C_{29} – C_{36}$ hydrocarbon fractions in soil contaminated with 5%, 10% and 15% used lubricating oil within the period of 12 months are shown in Table 4.18. The results of the study revealed that these fractions of petroleum hydrocarbons were not properly degraded in all the treatments with the exception of BSG amended soil where over 90% of the $C_{29} – C_{36}$ hydrocarbon fractions were degraded within the 12 month period. The partial degradation of these hydrocarbon fractions has been reported by different authors

that they are not easily degraded by microorganisms in the soil because they are hydrophobic solids at physiological temperatures (Bartha and Atlas, 1977; Alberdi et al., 2001; George et al., 2002). In soil contaminated with 5% used lubricating oil, soil amended with BSG recorded reduction in the concentration of $C_{29} - C_{36}$ from 2643 mg/kg to 221 mg/kg in 12 months, while those amended with BS and SMC were reduced to 766 mg/kg and 800 mg/kg respectively, whereas in unamended soil and sterile contaminated soil, the biodegradation of the hydrocarbon fractions was minimal (reduction from 2643 mg/kg to 1231 mg/kg and 1790 mg/kg, respectively). Soil contaminated with 10% oil recorded reduction in the concentration of these fractions from 5350 mg/kg to 491 mg/kg, 647 mg/kg and 1080 mg/kg, respectively. The reason for low biodegradation of these hydrocarbon fractions might also be attributed to the fact that during biodegradation of hydrocarbon in soil or sediments, low molecular weight fractions are known to be degraded first by microorganisms before degrading the higher molecular weight petroleum fractions (Borressen et al., 2003; Coulon et al., 2004; Sanscartier et al., 2009).

Table 4.18 Concentration (mg/kg) of C_{29} – C_{36} fractions in soil contaminated with 5%, 10% and 15% used lubricating oil.

	Time (months)				
Treatment	0	3	6	9	12
Soil+5%oil+BS	2643	1651	1030	956	766
Soil+5%oil+BSG	2643	1150	278	243	221
Soil+5%oil+SMC	2643	2371	1090	978	800
Soil+5% oil only	2643	2367	2480	1823	1231
Sterile soil+5%oil	2643	2567	2500	2353	1790
Soil+10%oil+BS	5350	4622	3480	1956	647
Soil+10%oil+BSG	5350	2300	520	501	491
Soil+10%oil+SMC	5350	4612	3810	2281	1080
Soil+10% oil only	5350	5002	4390	3813	2762
Sterile soil+10%oil	5350	5191	4719	4225	3891
Soil+15%oil+BS	6871	5814	5140	4756	4520
Soil+15%oil+BSG	6871	3031	919	872	800
Soil+15%oil+SMC	6871	6207	5870	5188	4840
Soil+15% oil only	6871	6752	6350	6213	6130
Sterile soil+15%oil	6871	6692	6310	6241	6160

Therefore, in this study possibly the low molecular weight fractions were first degraded by indigenous microorganisms before the higher molecular weight, thus, accounting for the low biodegradation of the higher molecular fractions in the range of C_{29} to C_{36}.

The results of soil contaminated with 15% used lubricating oil shows rapid degradation of C_{29} – C_{36} fractions in soil amended with BSG from 6871 mg/kg to 800 mg/kg within 12 months, whereas low biodegradation was recorded in all other treatments at the end of 12 month (Table 4.18). The reason for the low degradation of these fractions in all the

treatments with 15% oil might be due to high concentration of oil in the soil, as this is known to inhibit the growth of microorganisms with suitable enzyme systems (Teschener and Wehner, 1985).

4.2.2.5 Biodegradation of PAHs in used lubricating oil

Table 4.19 shows the concentration of different polycyclic aromatic hydrocarbons (PAH) present in the used lubricating oil employed in the bioremediation studies. Fluorene, phenanthrene, anthracene, fluoranthene and pyrene are the main PAH present in the used lubricating oil.

Table 4.19 PAHs concentration in used lubricating oil

PAH	Concentration (mg/L)
Fluorene	20.00
Phenanthrene	77.00
Anthracene	36.00
Fluoranthene	18.00
Pyrene	24.00

Table 4.20 shows the results of biodegradation of different PAHs within the period of 12 months. The results revealed degradation of fluorine below the detection limit of 0.5 mg/kg in all the treatments and at all pollution levels whereas complete degradation of phenanthrene and anthracene was only achieved in soil amended with organic wastes, while the two PAHs were not completely degraded in unamended and sterile polluted soil. In soil contaminated with 15% used lubricating oil, only soil amended with BSG recorded complete degradation of fluoranthene and pyrene below the detection limit after 12 months

of study, while other treatments did not records complete degradation of fluoranthene and pyrene after 12 month period. The reason for complete degradation of PAHs recorded in soil treated with organic wastes might be due to the soil texture improvement provided by the organic wastes which possibly increased oxygen transfer to the bacteria present in the contaminated soil. The organic wastes might also enhance their abilities to breakdown the PAHs in the contaminated soil. It has been observed that the addition of straw, compost, manure, etc. helps to enhance degradation by improving soil texture, oxygen transfer, and providing energy to the microbial population (Haritash and Kaushik, 2009). Lau, et al., (2003) observed that the addition of SMC to PAHs contaminated soil reduced toxicity, added enzymes, microorganisms and nutrients for the microorganisms involved in degradation of PAHs. Also loss of PAHs recorded in the sterile polluted soil might be due to different processes such as volatilization, adsorption, photolysis or chemical degradation which are known to contribute to PAHs degradation in contaminated soil (Haritash and Kaushik, 2009).

Table 4.20 PAHs concentration in soil contaminated with 5%, 10% and 15% used lubricating oil after 12 months remediation

	PAHs concentration (mg/kg)									
	Flu		Phe		Ant		Fth		Pyr	
Treatment	**0**	**12**	**0**	**12**	**0**	**12**	**0**	**12**	**0**	**12**
Soil + 5% oil + BS	6.0	ND	13	ND	6.3	ND	4	ND	5.4	ND
Soil + 5% oil + BSG	6.0	ND	13	ND	6.3	ND	4	ND	5.4	ND
Soil + 5% oil + SMC	6.0	ND	13	ND	6.3	ND	4	ND	5.4	ND
Soil + 5% oil only	6.0	ND	13	ND	6.3	ND	4	ND	5.4	ND
Sterile soil + 5% oil	6.0	ND	13	ND	6.3	ND	4	ND	5.4	1.8
Soil + 10% oil + BS	8.5	ND	16.2	ND	9.6	ND	5.8	ND	6.7	ND
Soil + 10% oil + BSG	8.5	ND	16.2	ND	9.6	ND	5.8	ND	6.7	ND
Soil + 10% oil + SMC	8.5	ND	16.2	ND	9.6	ND	5.8	ND	6.7	1.6
Soil + 10% oil only	8.5	ND	16.2	0.9	9.6	0.8	5.8	0.8	6.7	1.8
Sterile soil + 10% oil	8.5	1.7	16.2	1.5	9.6	1.9	5.8	1.6	6.7	2.1
Soil + 15% oil + BS	10	ND	19.4	ND	11.8	ND	6.9	0.6	9.6	2.1
Soil + 15% oil + BSG	10	ND	19.4	ND	11.8	ND	6.9	ND	9.6	ND
Soil + 15% oil + SMC	10	ND	19.4	ND	11.8	ND	6.9	1.2	9.6	2.2
Soil + 15% oil only	10	ND	19.4	0.9	11.8	0.7	6.9	1.1	9.6	2.3
Sterile soil + 15% oil	10	3.2	19.4	4.1	11.8	1.1	6.9	2.6	9.6	4.3

ND: Not detected at lowest detection limit of 0.5 mg/kg, FLU: Fluorene, PHE: Phenanthrene, ANT: Anthracene, FTH: Fluoranthene, PYR: Pyrene

4.2.3 Microbial counts in used lubricating oil contaminated soil

The counts of HUB in the soil contaminated with 15%, 10% and 5% used lubricating oil in the natural environment are shown in Figures 4.31 to 4.33. Counts of HUB in soil polluted with 15% used lubricating oil ranged from 1 x 10^5 CFU/g to 216 x 10^5 CFU/g (Fig. 4.31), while HUB counts in 10% oil pollution ranged from 1 x 10^6 CFU/g to 103 x 10^6 CFU/g (Fig. 4.32) whereas the counts in 5% oil pollution ranged from 2 x 10^6 CFU/g to 131 x 10^5 CFU/g (Fig. 4.33) at the end of the 12 months study period. Soil amended with BSG recorded the highest counts of HUB in all the oil pollution level compared to all other treatments. There was significant difference between BSG, SMC, unamended and autoclaved treated soil at $P<0.05$ confidence level. However no significant difference was recorded between BSG and BS treatment.

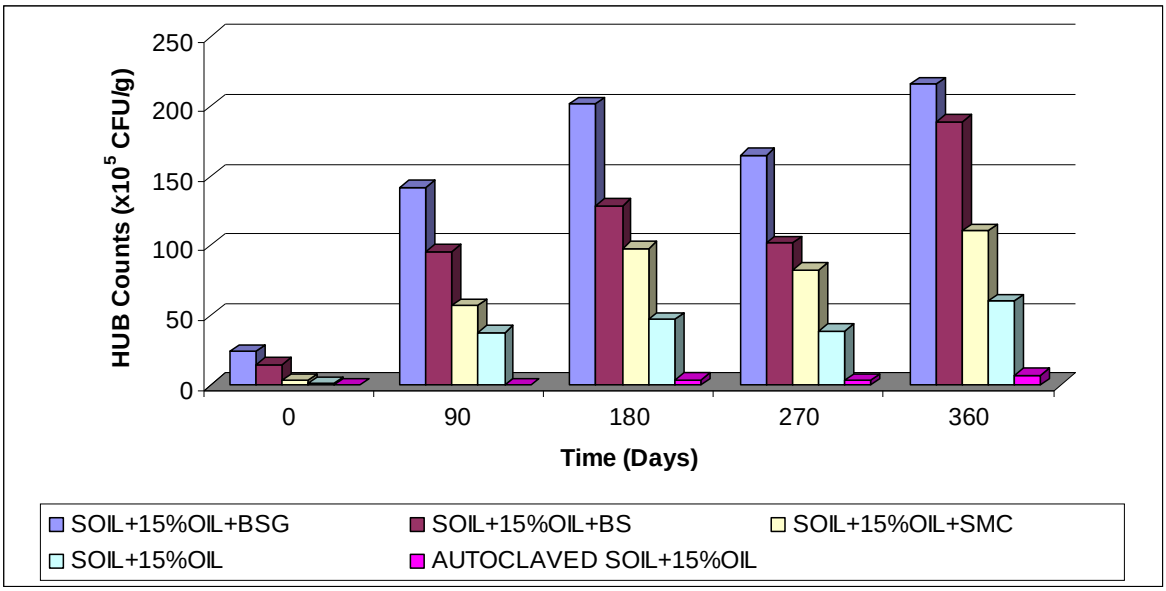


Figure 4.31 Counts of HUB in soil contaminated with 15% used lubricating oil

The counts of HUB recorded in all the three level of pollution was higher than the counts recorded in the study carried out under the laboratory conditions in all the organic wastes

amended soil, though the counts was low in the ninth months in all the treatments, the reason for this might be due to low level of rainfall characterized with dried season and high temperature (36^0C) experienced during this period. The counts of HUB in all the treatments correlate positively to the rate of biodegradation of hydrocarbon in the oil contaminated soil, thus suggesting that majority of the oil loss was as a result of microbial degradation. This is similar to the findings of Amund et al., (1993) and Ijah and Antai, (2003a) who reported extensive biodegradation of hydrocarbons in crude oil-contaminated soil by different species of hydrocarbon degrading bacteria in a field study.

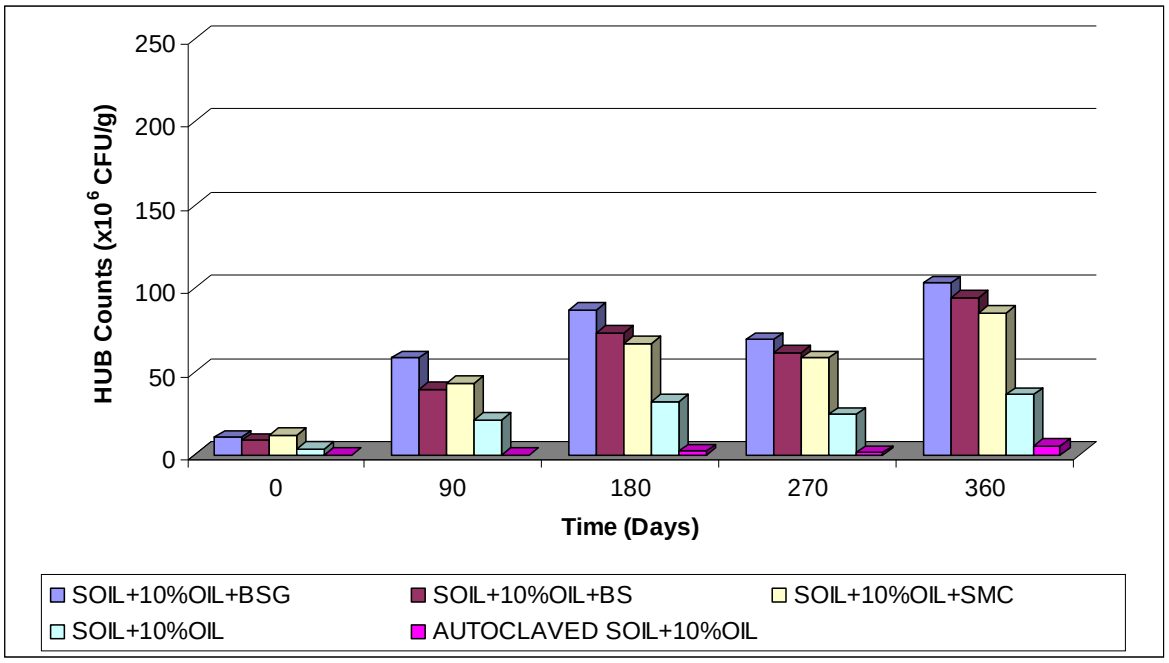


Figure 4.32 Counts of HUB in soil contaminated with 10% used lubricating oil

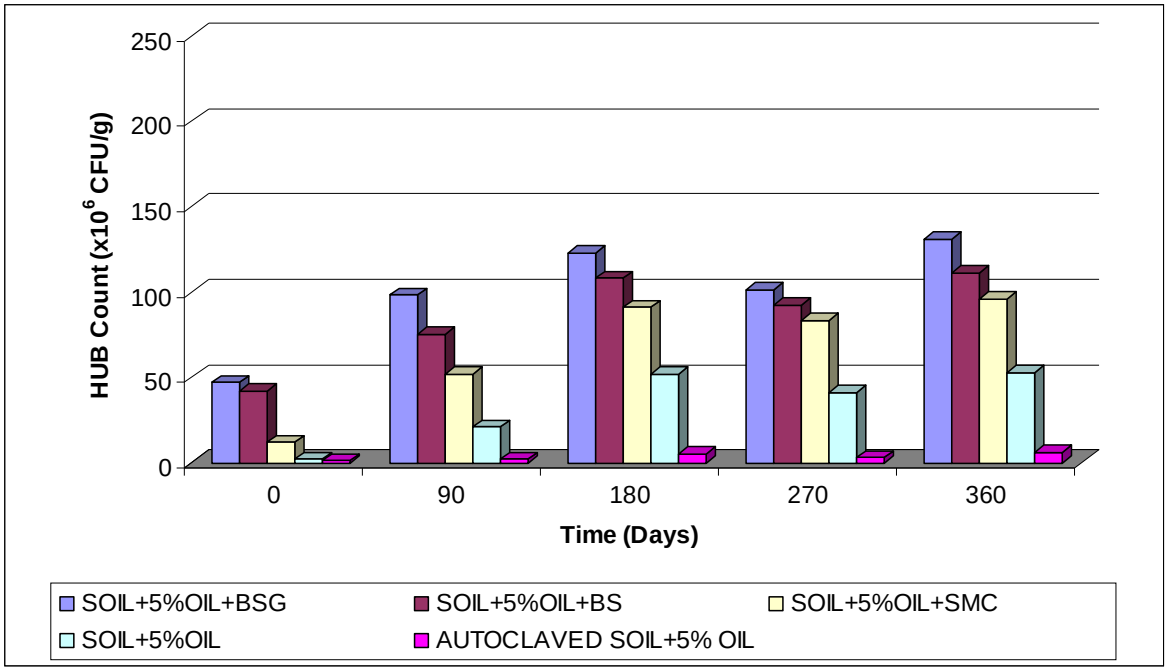


Figure 4.33 Counts of HUB in soil contaminated with 5% used lubricating oil

4.3 Results of phytoremediation studies with *Jatropha curcas* carried out under laboratory conditions

4.3.1 Response of plants to the oil

The appearance of the *Jatropha* plants in response to different concentration of used lubricating oil was monitored throughout the 180 days of the experiment. No plant death was recorded in all the treatments of soil contaminated with 1% used lubricating oil; however some of the plants showed signs of phytotoxicity such as yellowing of leaves and stunted growth compared with control. These phytotoxicity signs might be as a result of stress on the plants caused by the presence of the oil in the soil. The signs are in line with the findings of Vouillamoz and Milke (2009) who reported reduced growth rate in rye grass planted on diesel contaminated soil. Plants in soil contaminated with 2.5% used lubricating oil showed high symptoms of phytotoxicity with death of at least one Jatropha plant recorded in each treatment. These results show that Jatropha plants can tolerate minimum degree of exposure to hydrocarbons. The pictures of the Jatropha plants are as shown in Plate 4.1.

A

B

C

D

Plate 4.1 Pictures of *Jatropha curcas* – A: Jatropha amended with BSG**; B:** Jatropha amended with SMC; **C:** Phytotoxicity effect of oil on Jatropha; **D:** Control plant.

4.3.2 Loss of used lubricating oil in soil contaminated with 2.5% and 1% oil

The percentage loss of waste lubricating oil in soil treatment contaminated with 2.5% and 1% oil are shown in Figures 4.33 and 4.34. The percentage loss of waste lubricating oil at the end of 180 days in soil contaminated with 2.5% and 1% oil ranged from 11.6 – 89.6% and 14.8 – 96.6%, respectively in all the different treatments. Contaminated soil treated with BSG recorded the maximum loss of oil (89.6% and 96.6%) in 180 days followed by soil treated with BS (82.1% and 90.1%) in 2.5% and 1% contaminated soil, respectively. The contaminated soil containing only Jatropha plant, without organic wastes treatment recorded 56.6% and 67.6% oil loss while control soil without Jatropha plant showed 36.9% and 51% oil loss in 2.5% and 1% contaminated soil, respectively at the end of 180 days. 11.6% and 14.2% oil loss in soil contaminated with 2.5% and 1% oil may be due to non biological factors like evaporation; this was recorded in autoclaved soil treated with sodium azide after 180 days. High loss of oil in soil treated with BSG and Jatropha plants may be due to the presence of appreciable nitrogen (1.02%) contents in BSG (Table 4.1). This was recorded also in the previous works, where soil amended with BSG recorded (67 – 78%) loss of used lubricating oil in soil (Abioye et al., 2009b, 2010). It was also noticed that Jatropha plant amended with BSG grows better and taller (about 20% than other treatments) with lots of fibrous roots than other treatments in the experimental set up. The result is similar to that of Palmroth et al., (2002), who recorded 60% loss of diesel fuel in 30 days in diesel contaminated soil planted with pine tree and amended with NPK fertilizer, and also related to the findings of Dominguez-Rosado and Pichtel (2005) who recorded 67% degradation of used motor oil in oil contaminated soil planted with sunflower and mustard plants. Statistical analysis showed that there was no significant difference between the soil treated with BS, BSG and SMC at ($P<0.05$), whereas significant difference was

observed between the soil treated with different organic wastes, soil with only Jatropha plants and soil without Jatropha plants. These results indicated that addition of organic wastes into the contaminated soil planted with Jatropha increased the loss of oil in the soil by almost 30%; this is in line with the findings of Vouillamoz and Milke (2009), who observed that compost addition combined with phytoremediation, increases the rate of removal of diesel fuel in soil. Similar results was also reported by Dominguez-Rosado and Pichel (2004) who recorded 67% used motor oil degradation with sunflower and mustard, and with addition of NPK fertilizer, the oil was completely removed.

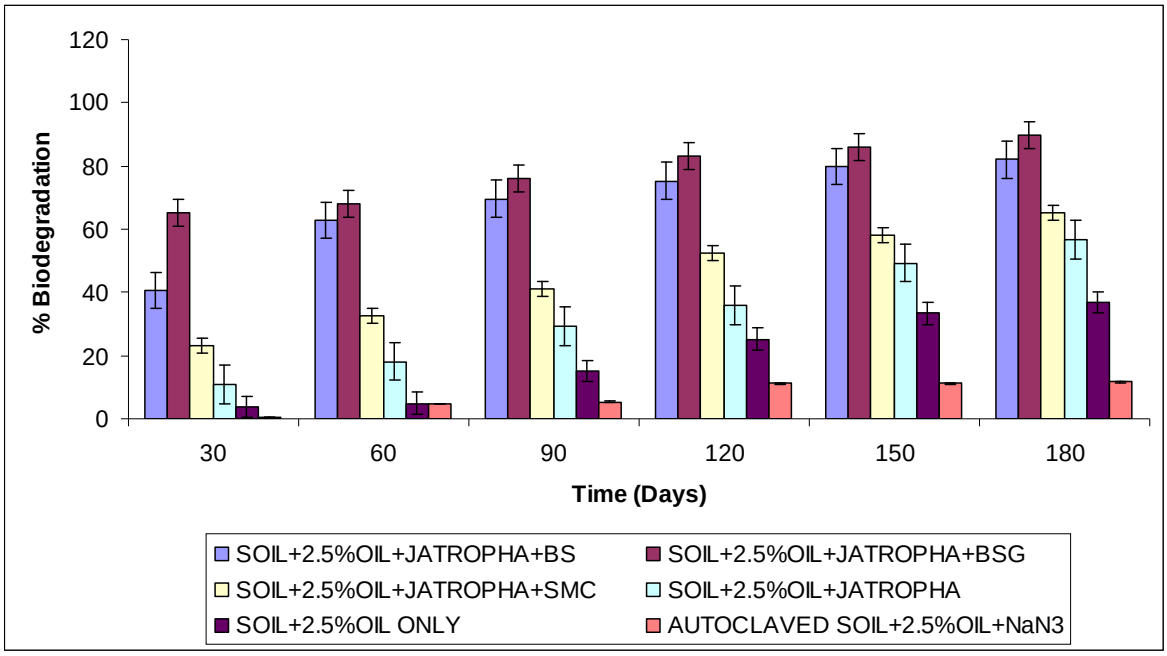


Figure 4.34 Percentage biodegradation of used lubricating oil in soil contaminated with 2.5% used lubricating oil. Bars indicates standard error (n = 3).

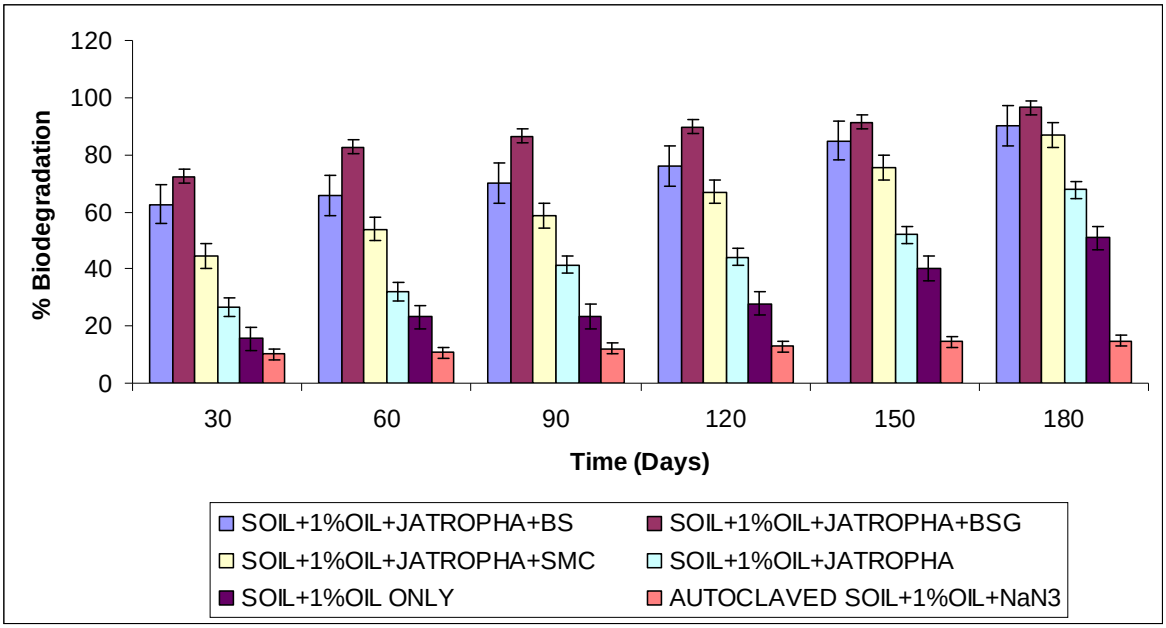


Figure 4.35 Percentage biodegradation of used lubricating oil in soil contaminated with 1% used lubricating oil. Bars indicates standard error (n = 3).

4.3.3 Uptake of oil by Jatropha

Jatropha roots of different treatment were Soxhlet extracted to determine if there was phytoaccumulation of hydrocarbons in the plant root. GC/MS analysis of the extract did not show presence of hydrocarbons in all the treatments. This is in sharp contrast with the results of Palmroth et al., (2002), who observed an uptake of diesel oil by grass root. The differences might be due to different plants and or oil used for the studies; it might as well be due to differences in the weather conditions. Palmroth et al., (2002) work was conducted in a cold temperate zone of Finland while this study was conducted in the tropical zone (Malaysia). The study agrees with the findings of Chaineau et al., (1997) who did not observe uptake of hydrocarbons by maize root. However, the result is similar to that of Santosh et al., (2009), who observed that application of organic amendments stabilizes the As, Cr and Zn in heavy metals contaminated soil and reduced their uptake by plant tissues.

The result suggests that the mechanism of hydrocarbons removal by the Jatropha plants may be via rhizodegradation which has been well documented (Abhilash et al., 2009; Gerhardt et al., 2009). Also, the removal of the oil may be as a result of root exudates produced by the *Jatropha* plant which enhance the activities of soil microorganisms in mineralizing the oil in the soil. This is supported by the findings of different authors, who stated that flavonoids and other compounds released by roots can stimulate growth and activity of hydrocarbon degrading bacteria (Leigh et al., 2002; Thoma et al., 2003; Corgie et al., 2004; Chaundry et al., 2005; Leigh et al., 2006). In addition, root growth and death are known to promote soil aeration which can enhance oxidative degradation of organic contaminants (Leigh et al., 2002; Kuiper et al., 2004).

4.3.4 Bacterial counts

Aerobic heterotrophic bacterial (AHB) counts in Jatropha remediated soil ranged from 31 x 10^7 CFU/g to 169 x 10^7 CFU/g in all the treatments amended with organic wastes, while the unamended treatments recorded low counts of AHB which ranged from 10 x 10^7 CFU/g to 73 x 10^7 CFU/g. However, the AHB count was about 10% higher in soil amended with BSG than that of BS and about 20 to 35% than that of SMC (Figures 4.36 and 4.37). The counts of hydrocarbon utilizing bacteria (HUB) in soil contaminated with 2.5% and 1% waste lubricating oil are shown in Figures 4.38 and 4.39. Contaminated soil treated with BSG and Jatropha remediation shows high counts of HUB (240 x 10^5 CFU/g and 193 x 10^5 CFU/g) in both soil contaminated with 2.5% and 1% oil respectively. This is similar to the findings of Ijah and Antai (2003b), whereas the treatment with only Jatropha plant without organic wastes amendments recorded low counts of HUB (48 x 10^5 CFU/g and 45 x 10^5 CFU/g) in 2.5% and 1% pollution respectively. The reason for the increase in counts of

HUB in contaminated soil amended with organic wastes might be due to the presence of nutrients in the organic wastes especially nitrogen and phosphorus that enhanced the multiplication of bacteria in the soil. The HUB isolated from the contaminated soil were identified as species of *Pseudomonas, Bacillus megaterium, Micrococcus* and *Corynebacterium*. These bacterial species has been implicated in hydrocarbon degradation by different authors (Ahn et al., 1999; Van Hamme et al., 2003; Bento et al., 2005). These bacterial species together with root exudates of Jatropha plants possibly helped in the removal of used lubricating oil from the soil (Corgie et al., 2004).

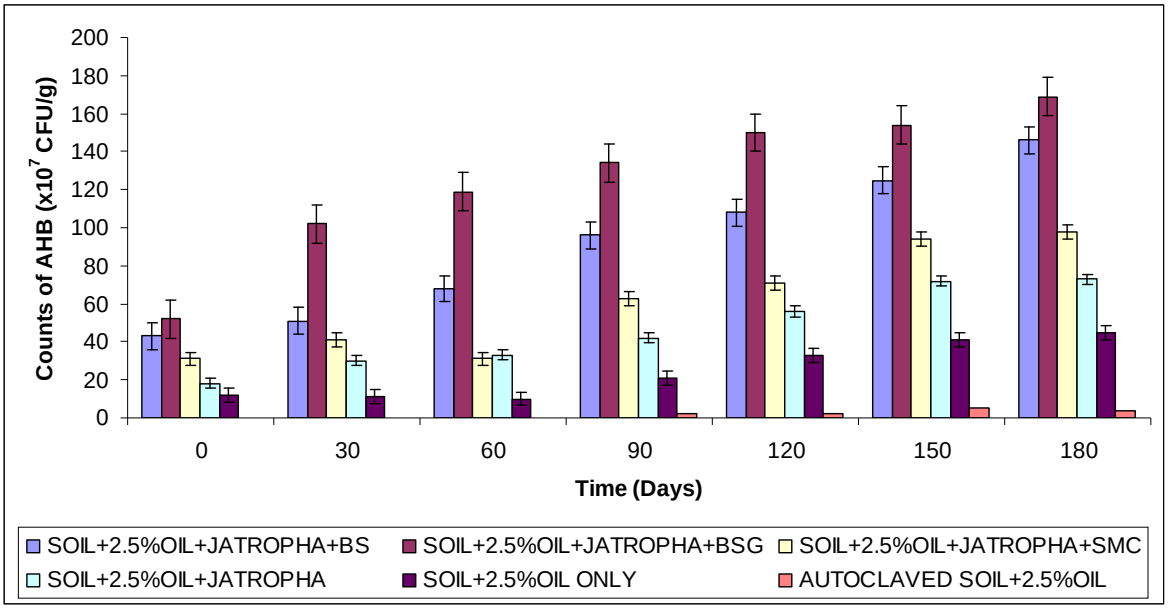


Figure 4.36 Counts of aerobic heterotrophic bacteria in soil contaminated with 2.5% used lubricating oil. Bars indicates standard error (n = 3).

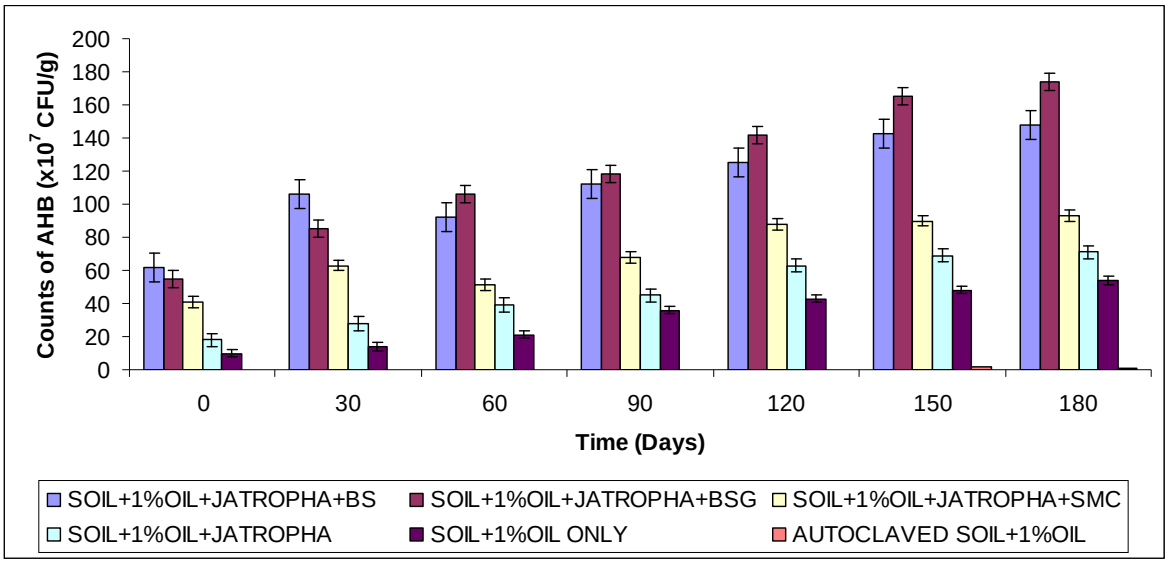


Figure 4.37 Counts of aerobic heterotrophic bacteria in soil contaminated with 1% used lubricating oil. Bars indicates standard error (n = 3).

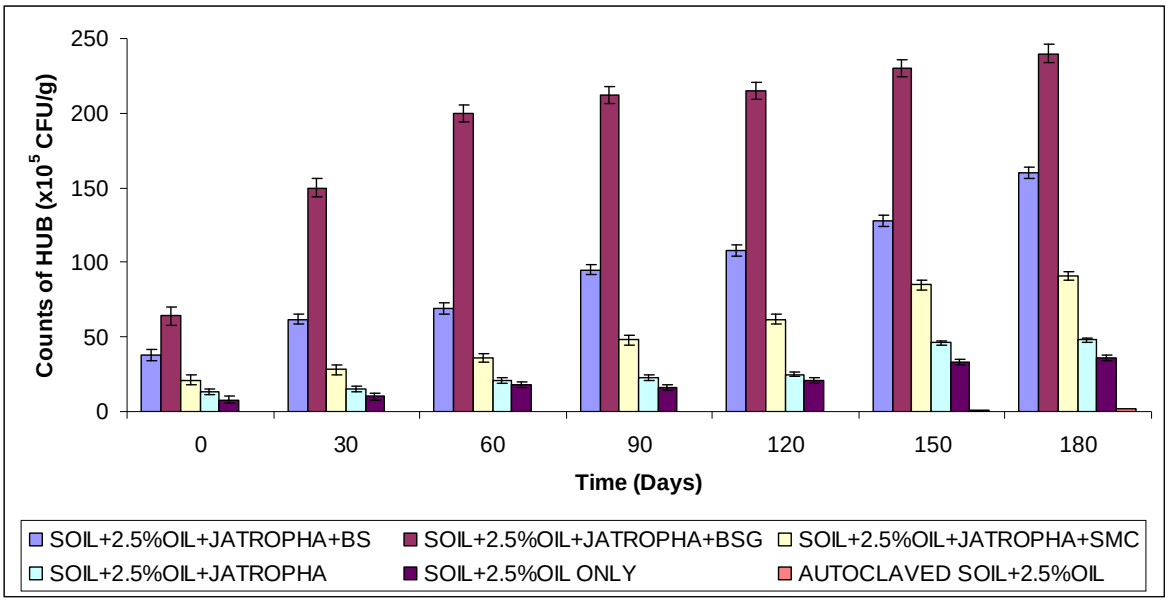


Figure 4.38 Counts of hydrocarbon utilizing bacteria in soil contaminated with 2.5% used lubricating oil. Bars indicates standard error (n = 3).

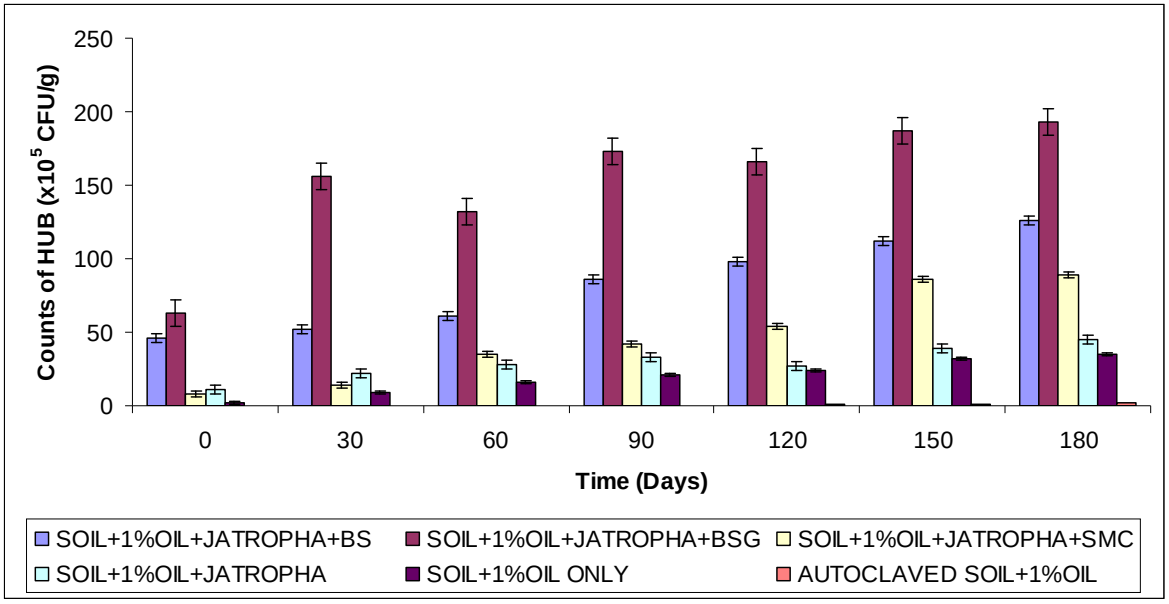


Figure 4.39 Counts of hydrocarbon utilizing bacteria in soil contaminated with 1% used lubricating oil. Bars indicates standard error (n = 3).

4.3.5 Uptake of Heavy metals by *Jatropha curcas*

Table 4.21 shows the heavy metals concentration in used lubricating oil, soil used for phytoremediation and oil polluted soil before phytoremediation experiment. Zinc, iron and lead are the major metals detected in the lubricating oil used for the study; these metals have been reported by different authors in used lubricating oil (Whisman et al., 1974; Kuokkanem et al., 2001; Boughton and Horvath, 2004; Adesodun and Mbagwu, 2008). Concentration of zinc in the oil was more than those of lead and iron, however the soil used for the phytoremediation contained 76.34 mg/kg of iron compared to 10.3 mg/L present in the used lubricating oil, it also contained 0.02 mg/kg of Zn and <0.05 mg/kg of Pb, compared to 86.05 mg/L and 0.2 mg/L of Zn and Pb respectively present in the used lubricating oil. This is an indication that accumulation of Fe in the plant parts might be from the Fe present in the soil used for the study while any accumulation of Zn and Pb may

likely come from those present in the used lubricating oil utilized for the phytoremediation study. The residual metal concentration in soil of different treatments after 180 days of remediation is shown in Table 4.22

Table 4.21 Heavy metal concentrations of used lubricating oil, unpolluted soil (used for phytoremediation) and soil contaminated with 1% and 2.5% oil before remediation.

	Heavy metals (mg/kg)		
Substrate	Fe	Zn	Pb
Used lubricating oil*	10.29	86.05	0.20
Soil (unpolluted)	76.34	0.02	<0.05
Soil+1% oil	77.02	32.15	0.09
Soil+2.5% oil	79.43	38.32	0.12

*- mg/l

Portion of the Jatropha roots, stems and leaves from different soil treatments were dried at 60 ^{0}C for 3 days, ground and 0.5 g digested with mixture of acids were analyzed with ICP-OES to determine the accumulation of metals from the oil and soil. Appreciable quantities of Fe, Zn and little quantity of Pb were detected to have accumulated in the root of Jatropha after 180 days of study. Fe accumulation in the root of the Jatropha plant in soil contaminated with 2.5% and 1% used lubricating oil ranged from 9.94 mg/kg to 26.34 mg/kg and 14.61 mg/kg to 23.40 mg/kg, respectively in different treatment as shown in Table 4.23.

Table 4.22 Residual metal concentration in soil remediated with *J. curcas* under laboratory condition after 180 days

	Heavy metals (mg/Kg)	
Substrate	Fe	Zn
Soil+2.5% oil + BS + Jatropha	64.32	33.67
Soil+2.5% oil + BSG + Jatropha	61.78	31.45
Soil+2.5% oil + SMC + Jatropha	63.65	33.76
Soil+2.5% oil + Jatropha	66.56	34.23
Soil+1% oil + BS + Jatropha	45.97	26.69
Soil+1% oil + BSG + Jatropha	42.67	26.20
Soil+1% oil + SMC + Jatropha	45.23	27.43
Soil+1% oil + Jatropha	47.83	28.12
Soil without oil + Jatropha	64.32	ND

The result is in contrast with the findings of Palmroth et al., (2006), who reported that there was no accumulation of heavy metals in the plant tissue in soil contaminated with weathered hydrocarbons and heavy metals and amended with NPK and biowaste compost. The differences observed in the results might be due to different contaminated soil used, in this study; freshly contaminated soil was used while in the study of Palmroth et al., (2006) weathered hydrocarbon contaminated soil was used, the differences can also be attributed to differences in the plants used for the studies, while in this study *Jatropha curcas* was used, they used pine and poplar for their studies.

Table 4.23 Heavy metal contents in root of *Jatropha curcas* in soil contaminated with 2.5% and 1% used lubricating oil

Treatment	Heavy metals (mg/Kg)		
	Fe	Zn	Pb
Soil + 2.5% oil + BS + Jatropha	12.14	8.45	0.01
Soil + 2.5% oil + BSG + Jatropha	19.47	6.21	0.01
Soil + 2.5% oil + SMC + Jatropha	26.34	4.14	0.02
Soil + 2.5% oil + Jatropha	9.94	6.43	ND
Soil + 1% oil + BS + Jatropha	21.10	6.24	ND
Soil + 1% oil + BSG + Jatropha	23.40	7.83	0.02
Soil + 1% oil + SMC + Jatropha	19.57	6.31	0.01
Soil + 1% oil + Jatropha	21.28	7.02	0.01
Soil without oil + Jatropha	14.61	ND	ND

ND: Not detected

Appreciable quantity of Zn was also detected in the root of Jatropha of different treatment, the quantity of Zn accumulated in the root of the plant ranged from 4.14 mg/kg to 8.45 mg/kg and 6.24 mg/kg to 7.83 mg/kg in soil contaminated with 2.5% and 1% used lubricating oil, respectively (Table 4.23). Accumulation of Pb in the root of Jatropha plant was minimal for both 2.5% and 1% oil pollution; this might be due to low quantity of Pb present in both used lubricating oil and the soil used for the phytoremediation study. Accumulation of Fe was higher (23.4 mg/kg) in 1% oil contaminated oil treated with *Jatropha* and BSG than that of 2.5% oil contamination (19.47 mg/kg). This might be due to better growth recorded in 1% oil contamination than that of 2.5% oil contamination.

Translocation of Fe and Zn from the root of *Jatropha* plant to the stem and leaves was recorded in all the treatments with used lubricating oil, whereas little quantity of Pb (0.01 mg/kg) was only detected in the stem of Jatropha in treatment amended with SMC. The quantity of Fe detected in the stem of Jatropha plant ranges between 2.46 mg/kg and 9.74 mg/kg in soil treated with 2.5% oil and between 2.46 mg/kg and 6.12 mg/kg in soil treated with 1% oil (Table 4.24) Accumulation of Fe in the leaves were very minimal with the highest quantity been 3.06 mg/kg in the soil treated with 1% used lubricating oil and amended with BSG (Table 4.25).

Table 4.24 Heavy metal contents in stem of *Jatropha curcas* in soil contaminated with 2.5% and 1% used lubricating oil

Treatment	Heavy metals (mg/Kg)		
	Fe	Zn	Pb
Soil + 2.5% oil + BS + Jatropha	8.15	3.04	ND
Soil + 2.5% oil + BSG + Jatropha	9.74	2.45	ND
Soil + 2.5% oil + SMC + Jatropha	4.18	2.63	0.01
Soil + 2.5% oil + Jatropha	3.04	1.81	ND
Soil + 1% oil + BS + Jatropha	5.35	2.83	ND
Soil + 1% oil + BSG + Jatropha	6.12	3.10	ND
Soil + 1% oil + SMC + Jatropha	3.83	1.83	0.01
Soil + 1% oil + Jatropha	3.16	1.02	ND
Soil without oil + Jatropha	2.46	ND	ND

ND: Not detected

Bioaccumulation of Zn in the stem and leaves of *Jatropha* plants varies greatly based on different organic wastes amendment and the percentage of oil pollution with soil contaminated with 1% used lubricating oil and amended with BSG recorded the highest accumulation of Zn (3.10 mg/kg) in the stem of *Jatropha* whereas the highest accumulation of Zn (1.34 mg/kg) in leaves of Jatropha was recorded in 2.5% oil contamination amended with BSG. No accumulation of Pb was recorded in the leaves of Jatropha in all the treatments (Table 4.25). Soil amended with BSG recorded the highest accumulation of Fe and Zn in *Jatropha* roots, stems and leaves. This might be due to better N concentrations present in BSG than BS and SMC.

Table 4.25 Heavy metal contents in leaves of *Jatropha curcas* in soil contaminated with 2.5% and 1% used lubricating oil

Treatment	Heavy metals (mg/Kg)		
	Fe	Zn	Pb
Soil + 2.5% oil + BS + Jatropha	1.13	0.18	ND
Soil + 2.5% oil + BSG + Jatropha	2.92	1.34	ND
Soil + 2.5% oil + SMC + Jatropha	1.43	0.24	ND
Soil + 2.5% oil + Jatropha	1.75	0.45	ND
Soil + 1% oil + BS + Jatropha	1.91	0.36	ND
Soil + 1% oil + BSG + Jatropha	3.06	0.74	ND
Soil + 1% oil + SMC + Jatropha	2.10	0.31	ND
Soil + 1% oil + Jatropha	1.93	0.28	ND
Soil without oil + Jatropha	0.62	ND	ND

ND: Not detected

From the results of the bioaccumulation of Fe, Zn and Pb in Jatropha tissues describe above, it shows clearly the ability of *Jatropha curcas* to accumulate heavy metals (Fe, Zn & Pb) in the different plant tissues, accumulation of these metals were more pronounced in the Jatropha treated plants amended with organic wastes compared to the treatment with only Jatropha without organic wastes amendments. The organic wastes might have contributed positively to the ability of the plant and to the bioavailability of these metals in the polluted soil thereby enhancing the capacity of the plant to uptake the metals into the different plants parts. These results are similar to the results of Santosh et al., (2009) who reported appreciable accumulation of Zn, Cr and As in the root stem and leaves of *Jatropha curcas* in soil contaminated with different concentration of Zn, Cr and As and amended with dairy sludge and bio-fertilizer. However, it contradicts the findings of Tordoff et al., (2000) and Walker et al., (2004) who reported that organic amendments of soil contaminated with metals always decrease the bioavailability of the metal in the soil. The differences in the results might be because the metals in the contaminated soil in this study are from used lubricating oil whereas the metals in the findings of Tordoff et al., (2000) came from direct contamination of soil with the salts of these metals. Jatropha root accumulated higher percentage of Zn and Fe than other parts of the plant; this implies that Jatropha root can be an important sink for bioavailable Zn and Fe. This suggests that possibly Fe and Zn were co-transported in Jatropha plant and thus share the same transport mechanisms. Pb concentration in this study was primarily localized in the root of *Jatropha curcas* with only 0.01 mg/kg detected in the stem only in soil amended with SMC, the reason for this might be due to low concentration of Pb in the contaminated soil or might be due to lack of transport mechanisms for Pb in Jatropha plants. This agrees with the findings of Blaylock et al. (1997) who noted that Pb translocation from roots to shoot is very slow

4.3.6 Rate of metal uptake by *Jatropha curcas* under laboratory condition

The results in Table 4.26 revealed that uptake rate of Fe and Zn by Jatropha within the period of six month study was higher in soil contaminated by 1% used lubricating oil than those of soil contaminated by 2.5% oil. However, soil amended with organic wastes recorded higher rate of Fe and Zn uptake in all the treatments compared to unamended soil. The reason for higher metal uptake in soil contaminated with 1% oil might be due to the fact that plants in the soil polluted with 1% oil did not experience much stress due to low level of oil contamination compared to those in 2.5% oil pollution, hence they are able to grow better and uptake the metal at higher rate than those in 2.5% oil pollution. The results in Table 4.27 also still point to the fact that soil amended with BSG recorded higher rate of Fe and Zn uptakes in both level (2.5% and 1%) of oil pollution. The reason for this higher uptake rate shown by this treatment can be attributed to the rate of plant growth in this treatment which was much taller and better than plants in other treatments.

Table 4.26 Rate constant of uptake of Fe and Zn by *J. curcas* studied under laboratory condition

	Rate of uptake ($month^{-1}$)	
Treatment	Fe	Zn
Soil + 1% oil + BS + Jatropha	0.046	0.031
Soil + 1% oil + BSG + Jatropha	0.058	0.034
Soil + 1% oil + SMC + Jatropha	0.048	0.026
Soil + 1% oil + Jatropha	0.039	0.022
Soil + 2.5% oil + BS + Jatropha	0.035	0.022
Soil + 2.5% oil + BSG + Jatropha	0.042	0.033
Soil + 2.5% oil + SMC + Jatropha	0.037	0.021
Soil + 2.5% oil + Jatropha	0.029	0.019
Soil without oil + Jatropha	0.034	0.000

4.3.7 Bioconcentration and translocation factors of metals in *J. curcas*

Table 4.27 shows the bioconcentration factor (BCF) and translocation factor (TF) of Zn in the Jatropha plant. The highest BCF was recorded in soil polluted with 1% oil and amended with BSG; while the highest TF in stem was recorded in soil treated with 2.5% oil and amended with SMC. The highest TF in leaves was observed in 2.5% oil pollution amended with BSG. There was a significant difference between the TF of Zn in the stem and TF in the leaves of Jatropha at $P< 0.05$ significant level.

Table 4.27 Bioconcentration factor (BCF) and translocation factor (TF) of Zinc in Jatropha remediated soil

Treatment	Zinc (Zn)		
	BCF	TF (in stem)	TF (in leaves)
Soil + 2.5% oil + BS + Jatropha	0.3045	0.3598	0.0213
Soil + 2.5% oil + BSG + Jatropha	0.2609	0.3945	0.2158
Soil + 2.5% oil + SMC + Jatropha	0.1829	0.6353	0.0580
Soil + 2.5% oil + Jatropha	0.2268	0.2815	0.1087
Soil + 1% oil + BS + Jatropha	0.2933	0.4535	0.0577
Soil + 1% oil + BSG + Jatropha	0.3630	0.3959	0.0945
Soil + 1% oil + SMC + Jatropha	0.2628	0.2900	0.0491
Soil + 1% oil + Jatropha	0.2588	0.1453	0.0399
Soil without oil + Jatropha	0.0000	0.0000	0.0000

Table 4.28 shows the bioconcentration factor (BCF) and translocation factor (TF) of Fe in the Jatropha plant. The highest BCF was recorded in soil polluted with 1% oil and amended with BSG as it was in BCF of Zn; while the highest TF in stem was recorded in soil treated with 2.5% oil and amended with BS. Highest TF in leaves was observed in 2.5% oil pollution planted with Jatropha without organic waste amendments. There was no significant difference between the TF of Fe in the stem and TF in the leaves of Jatropha at $P< 0.05$ significant level.

Bioconcentration factor (BCF) is the capacity of metal accumulation in relation with plant biomass (Santosh et al., 2009). The BCF for Zn and Fe was higher in all the treatment amended with organic wastes (except BF in 2,5% oil pollution amended with SMC)

compared to those without organic wastes amendment, this might be because the organic waste provided nutrient for the plant growth that produces high plant biomass thereby encouraging bioaccumulation of the metal in the plant parts more than those of unamended treatments. This results disagree with the finding of Santosh et al.. (2009) who discovered that Jatropha plants without organic amendments accumulates more Zn, As and Cr than those amended with organic wastes, the differences in these results might be because in their study they contaminated the soil with salts of these metals while in this study the soil was contaminated with used lubricating oil which happens to contain metal contaminants. The translocation factors recorded in this study was lower than that of Adesodun et al., (2010) who recorded translocation factor of Zn greater than 1 in soil contaminated with Zn and remediated with sunflower. The differences recorded in the two results might be due to different plants used for the studies.

Table 4.28 Bioconcentration factor (BCF) and translocation factor (TF) of Iron in Jatropha remediated soil

Treatment	Iron (Fe)		
	BCF	TF (in stem)	TF (in leaves)
Soil + 2.5% oil + BS + Jatropha	0.2697	0.6713	0.0931
Soil + 2.5% oil + BSG + Jatropha	0.4045	0.5002	0.1500
Soil + 2.5% oil + SMC + Jatropha	0.4022	0.1587	0.0543
Soil + 2.5% oil + Jatropha	0.1854	0.3058	0.1761
Soil + 1% oil + BS + Jatropha	0.4881	0.2536	0.0905
Soil + 1% oil + BSG + Jatropha	0.5389	0.2615	0.1308
Soil + 1% oil + SMC + Jatropha	0.4218	0.1957	0.1073
Soil + 1% oil + Jatropha	0.4362	0.1485	0.0907
Soil without oil + Jatropha	0.2227	0.1684	0.0424

4.3.8 pH of soil in Jatropha remediation soil under laboratory condition

The pH of Jatropha remediation in soil contaminated with 2.5% and 1% used lubricating oil is shown in Figures 4.40 and 4.41. The pH of the soil varies greatly from slightly alkaline to slightly acidic in all the treatments. pH of soil amended with BSG were more slightly acidic than other treatments; this might be because the plants in BSG amended soil grows better than other treatments and the root produced more exudates which are slightly acidic in nature. It may also be as a results of high metabolic activities of the rhizosphere microorganisms which produced a slightly acidic end products. The drop in pH of soil contaminated with 2.5% oil and amended with BSG to acidic pH (5.6) from 60 days till the end of 180 days might be due to high microbial activities in this treatment compared to the treatment with 1% oil pollution. Microorganisms are known to produce acidic radicals

during biodegradation of organic compounds. This might be responsible for the low pH in this treatment.

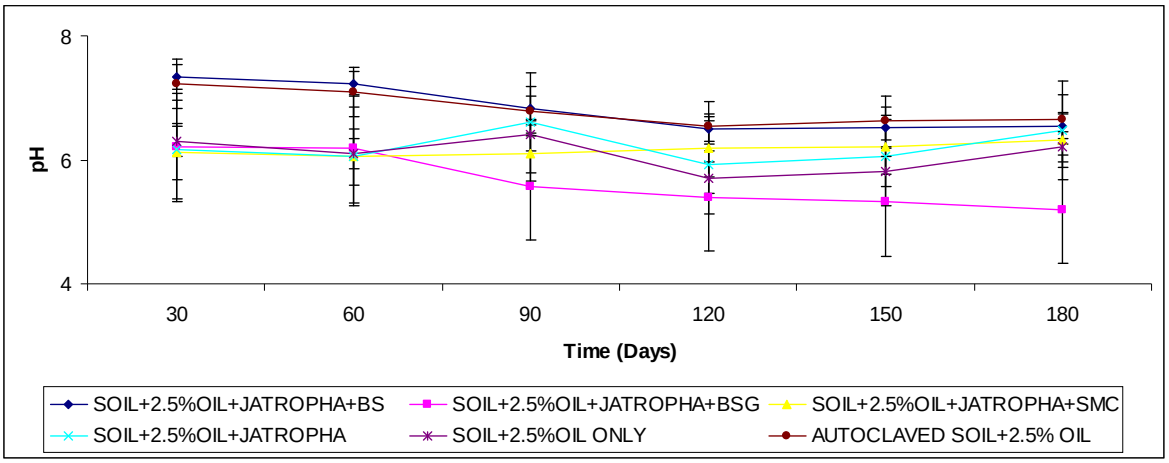


Figure 4.40 pH of soil contaminated with 2.5% used lubricating oil remediated with Jatropha. Bars indicates standard error (n = 3).

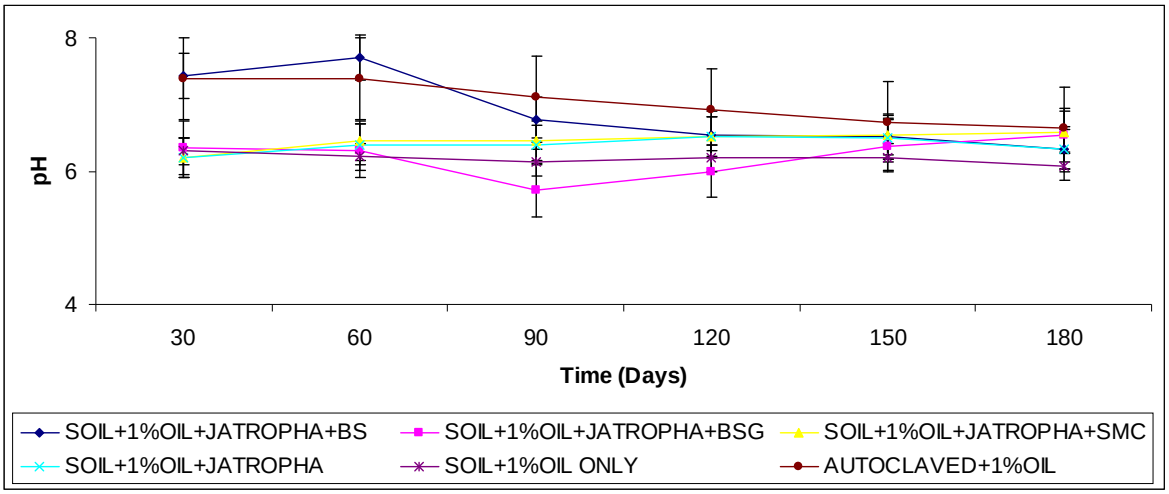


Figure 4.41 pH of soil contaminated with 1% used lubricating oil remediated with Jatropha. Bars indicates standard error (n = 3).

4.4 Results of phytoremediation studies with *Jatropha curcas* exposed to sunlight and rainfall

4.4.1 Response of plants to the oil

The appearances of *J. curcas* exposed to sunlight and rainfall was better than those studied under laboratory condition (28 ± 2^0C). No death of the plant was recorded in both soil contaminated with 2.5% and 1% oil pollution throughout the 180 days of the experiment. The reason for this better growth and appearance of the plant might be due to the fact that the plant had enough sunlight to carry out the photosynthetic activities compared to those maintained under laboratory condition. The plants in oil contaminated soil and amended with organic wastes grows better than the unamended plants, the reason might be due to the presence of organic wastes. Organic amendments have been reported to reduce heavy metal toxicity to plants by complexing metals (O'Dell et al., 2007; Pichtel and Bradway, 2008).

4.4.2 Loss of used lubricating oil in soil contaminated with 2.5% and 1% oil

Loss of used lubricating oil in soil contaminated with 2.5% and 1% oil are shown in Figures 4.42 and 4.43. At the end of 180 days, 2.9 – 82.8% and 6.51 – 85.2% oil loss were recorded in soil contaminated with 2.5% and 1% used lubricating oil, respectively in all the different treatments. Contaminated soil treated with BSG recorded the highest loss of oil (82.8% and 85.2%) in 180 days followed by soil treated with SMC (77.8% and 79.9%) in 2.5% and 1% contaminated soil, respectively. The results are in complete contrast with the phytoremediation set up under laboratory condition with the same plant, where the highest percentage of oil degradation was 89.6% and 96.59% in 2.5% and 1% oil pollution. The treatment amended with SMC was better than that of BS amended soil, whereas under laboratory conditions the soil amended with BS recorded higher loss of oil than those of

SMC amendment. The reason for these different results might be as a result of changes in the climatic conditions in which plant in the SMC amended treatment grows better than those of BS amendment, this observation has been well documented by various authors (Euliss et al., 2008; Dowling and Doty, 2009). The contaminated soil containing only Jatropha plant, without organic wastes treatment recorded 60.5% and 63.4% oil loss while control soil without Jatropha plant showed 50.4% and 55.2% oil loss in 2.5% and 1% contaminated soil, respectively at the end of 180 days. 14.8% and 18.3% oil loss in soil contaminated with 2.5% and 1% oil may be due to non biological factors like evaporation; this was recorded in autoclaved soil treated with sodium azide after 180 days. High loss of oil in soil treated with Jatropha plants and BSG or SMC may be due to the fact that BSG and SMC had the capacity to positively enhance the growth of Jatropha (due to presence of some nutrients in these organic wastes) with numerous fibrous roots which in turn had some rhizospheric effects on biodegradation of used lubricating oil in the soil. The result is in agreement with that of Palmroth et al., (2002), who recorded 60% loss of diesel fuel in 30 days in diesel contaminated soil planted with pine tree and amended with NPK fertilizer. Statistical analysis showed that there was no significant difference between the soil treated with BS, BSG and SMC at ($P<0.05$), whereas significant difference was observed between the soil treated with different organic wastes, soil with only Jatropha plants and soil without Jatropha plants. Sharp decrease in the percentage of biodegradation was recorded on the 120^{th} day of the study in all the treatments compare to what was recorded on the 90^{th} day. This might be as a result of dry climatic condition (36^{0}C) within this month (June to July, 2009), it was noticed that there was no rainfall for number of days during this particular period of the study, hence it slow down the microbial activities in the soil. These results indicated that addition of organic wastes into the contaminated soil planted with Jatropha

increased the loss of oil in the soil by about 22%; this is in line with the findings of Vouillamoz and Milke (2009), who observed that compost addition combined with phytoremediation, increases the rate of removal of diesel fuel in soil.

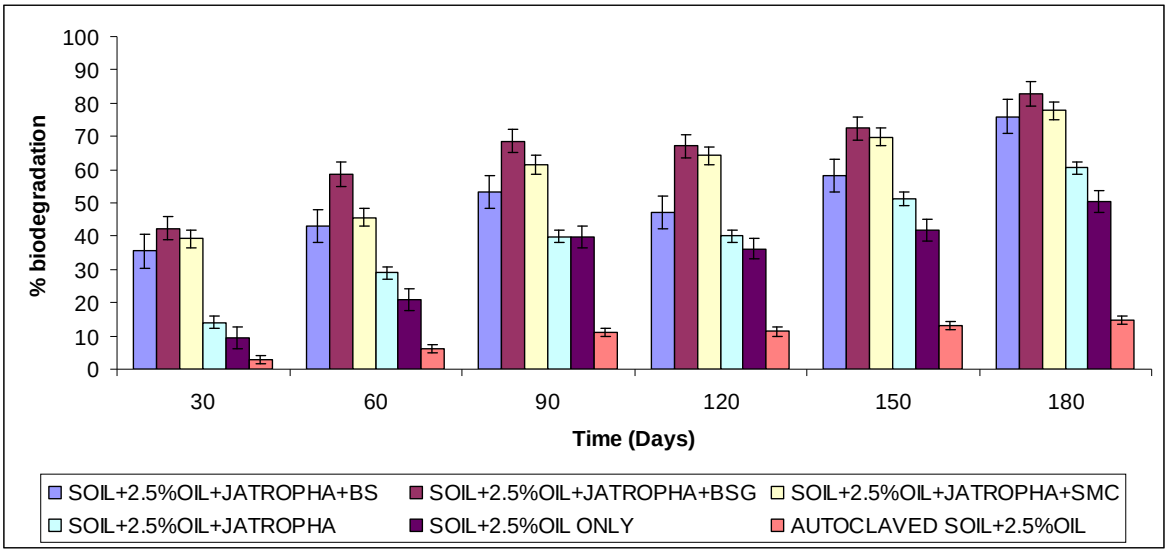


Figure 4.42 Percentage biodegradation of used lubricating oil in soil contaminated with 2.5% oil. Bars indicates standard error (n = 3).

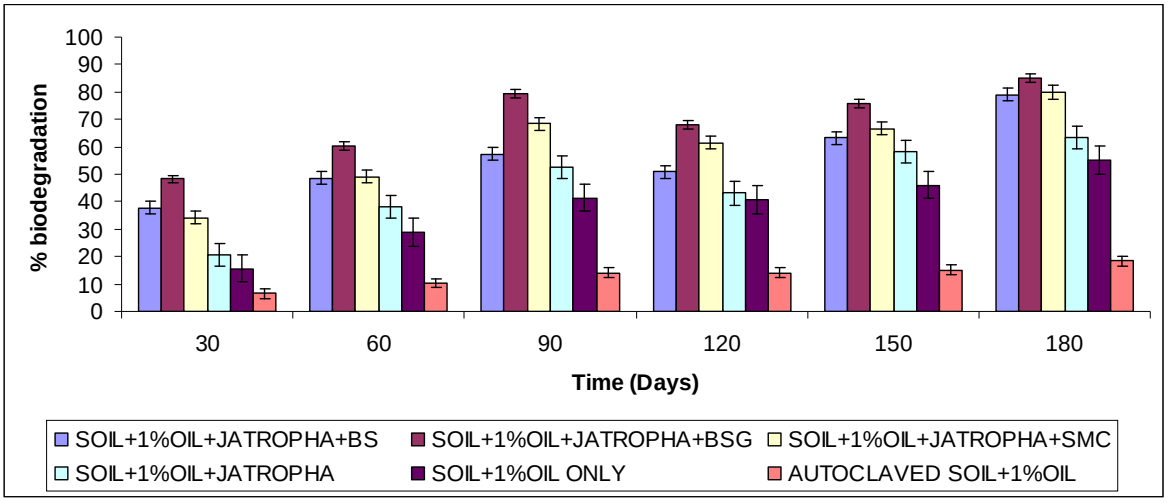


Figure 4.43 Percentage biodegradation of used lubricating oil in soil contaminated with 1% oil. Bars indicates standard error (n = 3).

4.4.3 Uptake of oil by Jatropha

Soxhlet extracts of Jatropha roots and stems were analyzed with GC/MS to determine the phytoaccumulation of hydrocarbons in the plant roots. The extract did not show presence of hydrocarbons in all the treatments, the result was not different from those of Jatropha plants under laboratory conditions. This is also in contrast with the results of Palmroth et al., (2002), who observed an uptake of diesel oil by grass root. The reason for this might be due to differences in the hydrocarbon source used for the two studies; diesel oil was used by Palmroth et al., (2002) which contains light hydrocarbons compared to heavy hydrocarbons in lubricating oil used in this study. However, the result is similar to that of Santosh et al., (2009), who observed that application of organic amendments stabilizes the As, Cr and Zn in heavy metals contaminated soil and reduced their uptake by plant tissues. The result suggests that the mechanism of hydrocarbons removal by the Jatropha plants may be the same as those studied under laboratory condition i.e. via rhizodegradation which has been well documented (Abhilash, et al., 2009; Gerhardt, et al., 2009). Also, the removal of the oil might be as a result of root exudates produced by the Jatropha plant which enhance the activities of soil microorganisms in mineralizing the oil in the soil.

4.4.4 Bacterial counts

Counts of aerobic heterotrophic bacterial (AHB) in soil contaminated with 2.5% and 1% used lubricating oil in Jatropha remediated soil exposed to sunlight and rainfall ranged from 4×10^7 CFU/g to 124×10^7 CFU/g and 32×10^7 CFU/g to 132×10^7 CFU/g (Figures 4.44 and 4.45), respectively in all the treatments amended with organic wastes, while the unamended treatments recorded low counts of AHB which ranged from 2×10^7 CFU/g to 66×10^7 CFU/g in 2.5% oil pollution and 9×10^7 CFU/g to 61×10^7 CFU/g in 1% oil

pollution. The AHB count was about higher in soil amended with BSG than those of BS and SMC (Figures 4.44 and 4.45).

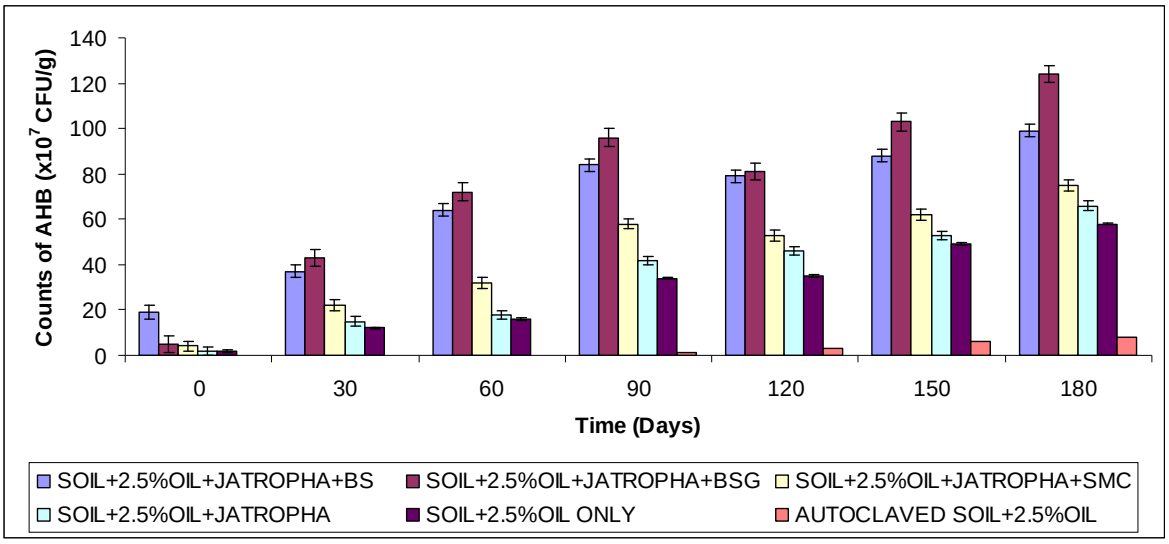


Figure 4.44 Counts of aerobic heterotrophic bacteria in soil contaminated with 2.5% used lubricating oil. Bars indicates standard error (n = 3).

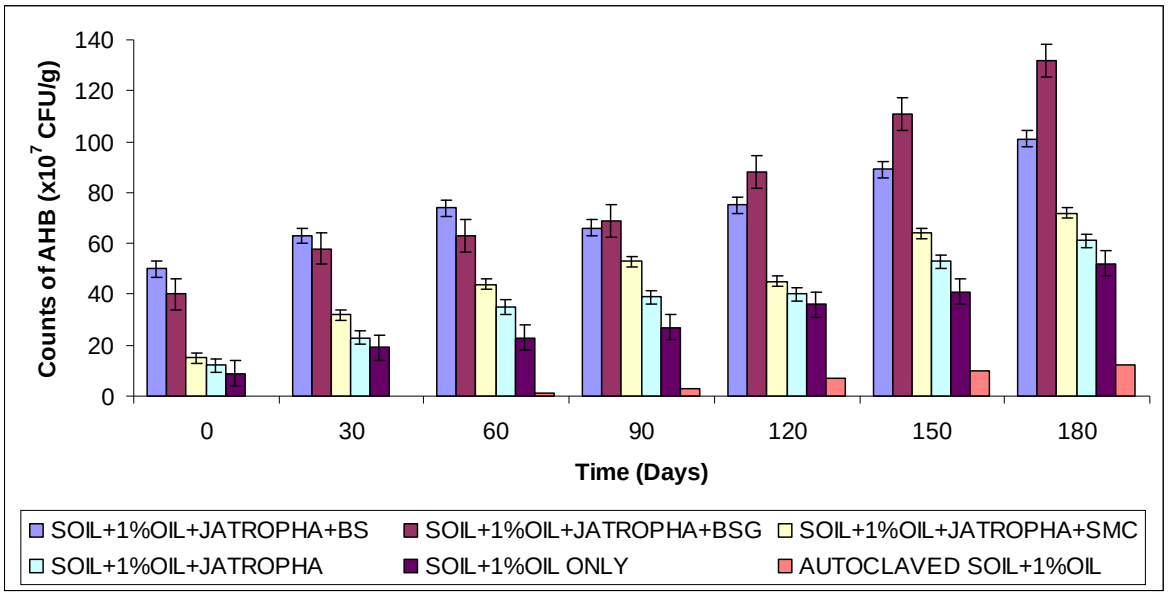


Fig. 4.45 Counts of aerobic heterotrophic bacteria in soil contaminated with 1% used lubricating oil. Bars indicates standard error (n = 3).

The counts of hydrocarbon utilizing bacteria (HUB) in soil contaminated with 2.5% and 1% waste lubricating oil are shown in Figures 4.46 and 4.47. Soil treated with BSG and *Jatropha* shows counts of HUB ranging between 240 x 10^5 CFU/g and 118 x 10^5 CFU/g in soil contaminated with 2.5% and 1% oil, respectively. The count of HUB in this study was similar to the counts recorded in the study under laboratory condition, however it was noticed that the counts of HUB and AHB in soil amended with BS and BSG dropped drastically in the 120^{th} day compare to the count recorded in the previous sampling period, this observation was also noticed in the percentage loss of used lubricating within the same period. The reason for this might be due to a sudden change in the climatic condition during which there was a dry spell with temperature of 36^0C. The treatment with only Jatropha plant without organic wastes amendments recorded low counts of HUB (81 x 10^5 CFU/g and 58 x 10^5 CFU/g) in 2.5% and 1% pollution respectively. The reason for the increase in counts of HUB in contaminated soil amended with organic wastes might be due to the presence of nutrients in the organic wastes especially nitrogen and phosphorus that enhanced the multiplication of bacteria in the soil. The HUB isolated from the contaminated soil were similar to those isolated from the study under laboratory condition, the reason might be probably because the soil used for the two studies are from the same source. The bacteria were identified as species of *Pseudomonas, Bacillus subtilis, Micrococcus*. These bacterial species has been implicated in hydrocarbon degradation by different authors (Ahn et al., 1999; Van Hamme et al., 2003; Ijah and Antai, 2003a; Bento, et al., 2005). These bacterial species together with root exudates of Jatropha plants might possibly contributed to the removal of used lubricating oil from the soil.

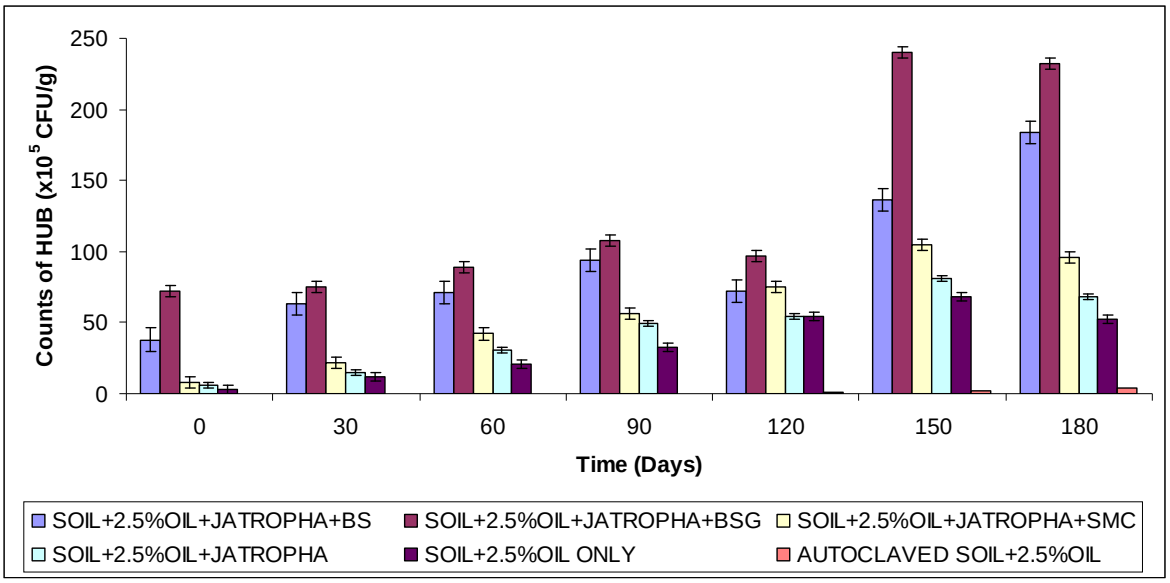


Figure 4.46 Counts of hydrocarbon utilizing bacteria in soil contaminated with 2.5% used lubricating oil. Bars indicates standard error (n = 3).

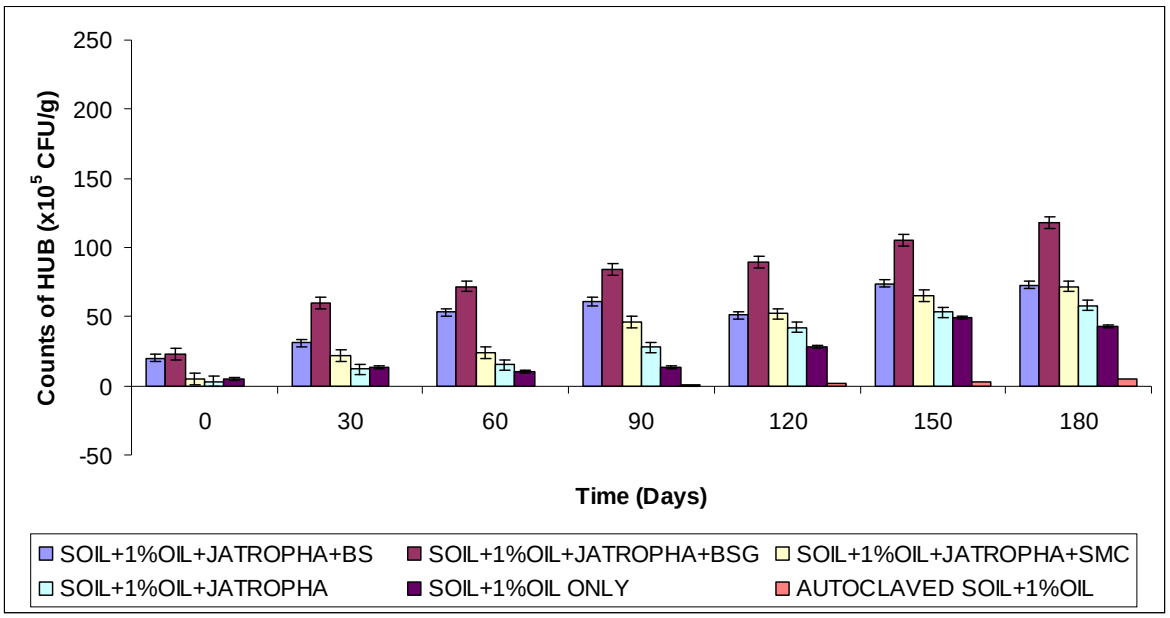


Figure 4.47 Counts of hydrocarbon utilizing bacteria in soil contaminated with 1% used lubricating oil. Bars indicates standard error (n = 3).

4.4.5 Uptake of Heavy metals by *J. curcas*

The residual metal concentration in soil of different treatment after 180 days of remediation is shown in Table 4.29. The results revealed that appreciable accumulation of Fe and Zn and little quantity of Pb in the roots, and stems of Jatropha. Unlike the Jatropha remediation set-up under laboratory condition, no accumulation of Fe and Zn was detected in the leaves of the Jatropha remediated set up exposed to sunlight and rainfall. The reason for this differences in the two results might be that these two metals (Fe & Zn) were localized only at the root and stem of the plant due to differences in the climatic conditions, or the reason might be that some of the metal translocated to the leaf region of the plant were metabolized or volatilized into the environment through the stomata on the leaves which is one of the mechanisms plant employed in phytoremediation (Ma and Burken 2002). This reason might as well be responsible for lower accumulation of Fe and Zn in both the root and stem of the Jatropha plant in this study. This is in sharp contrast with the findings of Santosh et al., (2009) who recorded accumulation of Zn in root, stem and leaves of Jatropha. The reason for the differences in the results might be due to changes and differences in the climatic conditions of the two experimental sites.

Table 4.29 Residual metal concentration in soil remediated with *J. curcas* under simulated natural condition after 180 days

	Heavy metals (mg/Kg)	
Substrate	Fe	Zn
Soil+1% oil + BS + Jatropha	42.52	25.72
Soil+1% oil + BSG + Jatropha	38.21	23.80
Soil+1% oil + SMC + Jatropha	41.34	24.12
Soil+1% oil + Jatropha	45.23	28.34
Soil + 1% oil only	61.45	30.12
Soil+2.5% oil + BS + Jatropha	60.31	30.34
Soil+2.5% oil + BSG + Jatropha	55.48	28.36
Soil+2.5% oil + SMC + Jatropha	61.02	31.41
Soil+2.5% oil + Jatropha	63.12	35.30
Soil + 2.5% oil only	68.41	36.52
Soil without oil + Jatropha	61.23	ND

Appreciable quantities of Fe, Zn and little quantity of Pb were detected in the root of Jatropha after 180 days of study. The Fe accumulation in the root of the Jatropha plant in soil contaminated with 2.5% and 1% used lubricating oil ranged from 6.56 mg/kg to 14.83 mg/kg and 5.10 mg/kg to 14.03 mg/kg, respectively in different treatment as shown in Table 4.30. The quantity of Fe accumulated in the root of Jatropha in this study was lower than the amount accumulated in the Jatropha root of the set up under laboratory conditions. The reason for this might be because most of the Fe quantities were translocated to the

above ground parts of the plant where some were either metabolized or volatilized into the environment through the stomata on the leaves or through the stem (Ma and Burken, 2002). Zn was also detected in the root of Jatropha of different treatment, the quantity of Zn accumulated in the root of the plant ranged from 6.83 mg/kg to 8.10 mg/kg and 5.10 mg/kg to 8.20 mg/kg in soil contaminated with 2.5% and 1% used lubricating oil, respectively (Table 4.30). This is in contrast to the findings of Walker et al., (2004) and Kumar et al., (2008) who reported that addition of biosludge and dairy sludge reduces the bioavailability of Zn to Jatropha root in soil contaminated with mixture of heavy metals. The reason for the differences in the two results might be attributed to difference in soil type used or may be due to differences in the source of Zn pollution in the contaminated soil. Accumulation of Pb in the root of Jatropha plant was detected at minimal concentration only in the root of Jatropha plant with BS and BSG amendment in 2.5% oil pollution and only BS amendment in 1% oil pollution, this might be due to low quantity of Pb present in both used lubricating oil and the soil used for the phytoremediation study.

Table 4.30 Heavy metal contents in root of *J. curcas* in soil contaminated with 2.5% and 1% used lubricating oil

Treatment	Heavy metals (mg/Kg)		
	Fe	Zn	Pb
Soil + 2.5% oil + BS + Jatropha	10.31	6.83	0.01
Soil + 2.5% oil + BSG + Jatropha	14.83	8.10	0.01
Soil + 2.5% oil + SMC + Jatropha	8.93	7.12	ND
Soil + 2.5% oil + Jatropha	6.56	6.96	ND
Soil + 1% oil + BS + Jatropha	6.18	5.10	0.01
Soil + 1% oil + BSG + Jatropha	14.03	8.20	ND
Soil + 1% oil + SMC + Jatropha	7.11	6.72	ND
Soil + 1% oil + Jatropha	5.10	6.06	ND
Soil without oil + Jatropha	11.31	ND	ND

ND: Not detected

Translocation of metals (Fe & Zn) from the root of Jatropha plant to the stem was recorded in all the treatments with used lubricating oil, whereas little quantity (0.01 mg/kg of Pb) was detected in the stem of Jatropha in treatment amended with SMC and BS. The quantity of Fe detected in the stem of Jatropha plant ranges between 4.20 mg/kg and 8.33 mg/kg in soil treated with 2.5% oil and between 3.04 mg/kg and 4.83 mg/kg in soil treated with 1% oil (Table 4.31). Accumulation of Zn in the stem of Jatropha plants varies greatly based on different organic wastes amendment and the percentage of oil pollution. Soil contaminated with 1% used lubricating oil and amended with BSG recorded the highest accumulation of Zn (7.05 mg/kg) in the stem of Jatropha whereas lowest accumulation of Zn (2.01 mg/kg)

in the stem of Jatropha was recorded in soil contaminated with 1% used lubricating oil and amended with BS. The reasons for this variation might be due to bioavailability of the metals in the different treatments as proposed by Tordoff et al., (2000) and Walker et al., (2004) that role of organic amendments such as fermented compost, which contains a high proportion of humid organic matter decreases the bioavailability of heavy metals in soil. Also, the reason might be attributed to the potential of BSG in stimulating better growth of the Jatropha plant in oil contaminated soil, thereby enhancing the root to accumulate more Zn which was further translocated to the stem.

Table 4.31 Heavy metal contents in stem of *J. curcas* in soil contaminated with 2.5% and 1% used lubricating oil

Treatment	Heavy metals (mg/Kg)		
	Fe	Zn	Pb
Soil + 2.5% oil + BS + Jatropha	6.15	4.28	ND
Soil + 2.5% oil + BSG + Jatropha	8.33	7.03	ND
Soil + 2.5% oil + SMC + Jatropha	5.14	6.32	0.01
Soil + 2.5% oil + Jatropha	4.20	3.01	ND
Soil + 1% oil + BS + Jatropha	3.12	2.01	0.01
Soil + 1% oil + BSG + Jatropha	4.83	7.05	ND
Soil + 1% oil + SMC + Jatropha	4.15	3.18	ND
Soil + 1% oil + Jatropha	3.04	4.21	ND
Soil without oil + Jatropha	5.23	ND	ND

ND: Not detected

4.4.6 Rate of metal uptake by *J. curcas* under natural condition

Table 4.32 shows that rate of uptake of Fe and Zn by Jatropha within the period of six month study like those of treatment studied under laboratory condition was higher in soil contaminated by 1% used lubricating oil than those of soil contaminated by 2.5% oil with higher uptake rate shown in the treatment amended with organic wastes. However, unlike the Jatropha studied under laboratory condition, the rate of Fe and Zn uptake in the study under natural condition was higher (between 0.014 to 0.077 $month^{-1}$) in all the treatments compared to those of the study under laboratory condition. The reason for this higher rate of metal uptake in the Jatropha plants exposed to sunlight and rainfall throughout the study period might be due to the favourable condition of growth (i.e. sunlight and rainfall) that the plants were exposed to which promote their growth better than those of plants under laboratory condition, hence the plants were able to uptake the metal at higher rate. The results in Table 4.36 also shows that soil amended with BSG recorded higher rate of Fe and Zn uptakes in both level (2.5% and 1%) of oil pollution just like the study under laboratory condition. The reason for this higher rate of uptake of Fe and Zn shown by this treatment can as well be attributed to the rate of growth of plant in this treatment which was much taller and better than plants in other treatments, which possibly promote better translocation of the metals in the plant tissues.

Table 4.32 Rate of uptake of Fe and Zn by *J. curcas* studied under natural condition

	Rate of uptake ($month^{-1}$)	
Treatment	Fe	Zn
Soil + 1% oil + BS + Jatropha	0.059	0.037
Soil + 1% oil + BSG + Jatropha	0.077	0.050
Soil + 1% oil + SMC + Jatropha	0.063	0.048
Soil + 1% oil + Jatropha	0.048	0.021
Soil + 2.5% oil + BS + Jatropha	0.046	0.039
Soil + 2.5% oil + BSG + Jatropha	0.060	0.050
Soil + 2.5% oil + SMC + Jatropha	0.044	0.033
Soil + 2.5% oil + Jatropha	0.038	0.014
Soil without oil + Jatropha	0.042	ND

4.4.7 Bioconcentration and translocation factors of metals in *J. curcas*

Table 4.33 shows the bioconcentration factor (BCF) and translocation factor (TF) of Zn in the Jatropha plant. The highest BCF (0.4743) was recorded in soil polluted with 1% oil and amended with BSG, while the highest TF in stem was recorded in soil treated with 2.5% oil and amended with SMC, this result is similar with the BCF and TF recorded in the study under laboratory conditions.

Table 4.33 Bioconcentration factor (BCF) and translocation factor (TF) of Zn in Jatropha remediated soil

Treatment	Zinc (Zn)	
	BCF	TF (in stem)
Soil + 2.5% oil + BS + Jatropha	0.2899	0.6266
Soil + 2.5% oil + BSG + Jatropha	0.3948	0.8679
Soil + 2.5% oil + SMC	0.3507	0.8876
Soil +2.5% oil + Jatropha	0.2602	0.4325
Soil + 1% oil + BS + Jatropha	0.2212	0.3941
Soil + 1% oil + BSG + Jatropha	0.4743	0.8597
Soil + 1% oil + SMC + Jatropha	0.3079	0.4732
Soil + 1% oil + Jatropha	0.3194	0.6947
Soil without oil + Jatropha	0.0000	0.0000

Table 4.34 shows the bioconcentration factor (BCF) and translocation factor (TF) of Fe in the Jatropha plant. The highest BCF was recorded in soil polluted with 1% oil and amended with BSG as it was in BCF of Zn; while the highest TF (0.6402) in stem was recorded in soil treated with 2.5% oil and Jatropha plant without organic amendment.

The BCF was high in all the treatment amended with organic wastes (except the treatment without oil plus Jatropha) compared to those without organic wastes amendment, this might be because the organic waste provided nutrient for the plant growth that produces high plant biomass thereby encouraging bioaccumulation of the metal in the plant parts more than those of unamended treatments. Another reason for this might be because the organic waste used in this study help to make the metal contaminants available in the soil by

loosening the soil compartment (the soil used is sandy clay soil) for the root of Jatropha to absorb, whereas in the unamended soil the metal contaminant possibly has been adsorbed unto the clay soil thereby making the uptake by Jatropha root difficult. This results disagree with the findings of Santosh et al., (2009) and Kumar et al., (2008) who discovered that Jatropha plants without organic amendments accumulates more Zn, As and Cr than those amended with organic wastes, the differences in these results might be because in their study they contaminated the soil with salts of these metals while in this study the soil was contaminated with used lubricating oil which happen to contained metal contaminants.

Table 4.34 Bioconcentration factor (BCF) and translocation factor (TF) of Fe in Jatropha remediated soil

Treatment	Iron (Fe)	
	BCF	TF (in stem)
Soil + 2.5% oil + BS + Jatropha	0.2072	0.5965
Soil + 2.5% oil + BSG + Jatropha	0.2916	0.5617
Soil + 2.5% oil + SMC + Jatropha	0.1771	0.5756
Soil +2.5% oil + Jatropha	0.1355	0.6402
Soil + 1% oil + BS + Jatropha	0.1538	0.5048
Soil + 1% oil + BSG + Jatropha	0.3119	0.3443
Soil + 1% oil + SMC + Jatropha	0.1862	0.5837
Soil + 1% oil + Jatropha	0.1346	0.5961
Soil without oil + Jatropha	0.2167	0.4624

4.4.8 pH of soil in Jatropha remediation soil exposed to sunlight and rainfall

The pH of Jatropha remediations in soil contaminated with 2.5% and 1% used lubricating oil are shown in Figures 4.48 and 4.49. The pH of the soil varies greatly from slightly alkaline to slightly acidic in all the treatments. This is in line with the findings of Okoh et al., (2006) and Ijah et al., (2008) who reported variation in pH of soil contaminated with petroleum hydrocarbons over a period of time. pH of soil amended with BSG were more slightly acidic than other treatments just like the pH of the set up under laboratory condition; this might be because the plants in BSG amended soil grows better than other treatments and the root produced more exudates which are slightly acidic in nature (Gerhardt et al., 2009). It may also be as results of high metabolic activities of the rhizosphere microorganisms which produced slightly acidic metabolic end products.

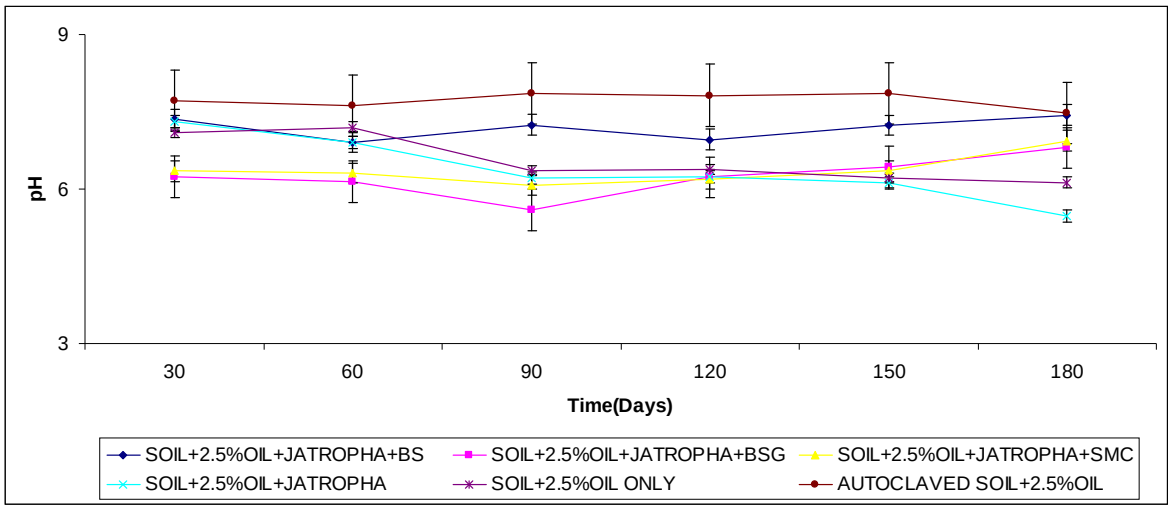


Figure 4.48 pH of soil contaminated with 2.5% used lubricating oil remediated with Jatropha. Bars indicates standard error (n = 3).

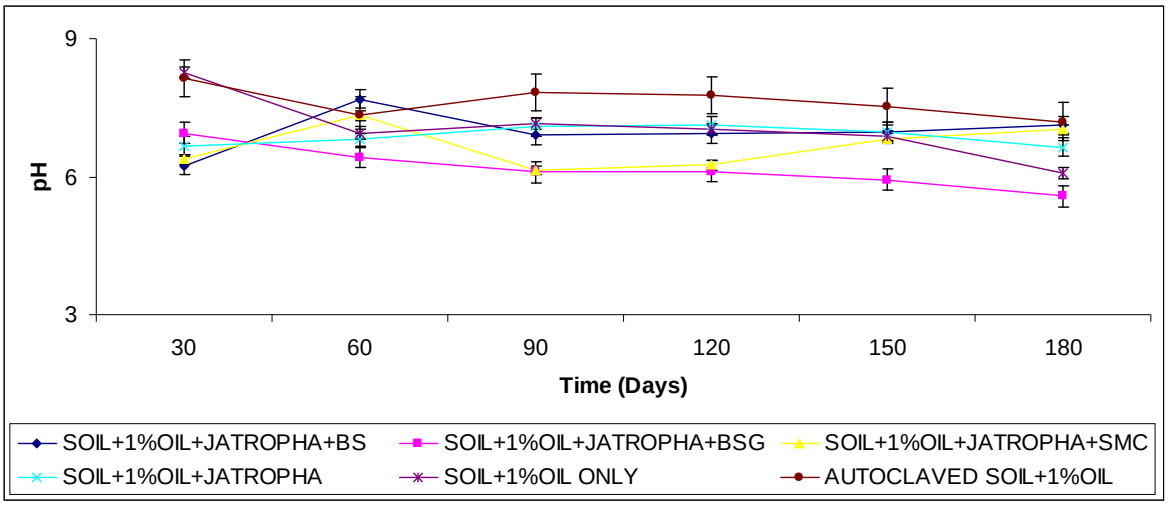


Figure 4.49 pH of soil contaminated with 1% used lubricating oil remediated with Jatropha. Bars indicates standard error (n = 3).

4.5 Results of phytoremediation with *Hibiscus cannabinus* exposed to sunlight and rainfall

The phytoremediation study with *H. cannabinus* was not conducted under laboratory condition due to the nature of the plant which requires abundant sunlight to survive. The trial set-up under laboratory condition did not survive, hence the studies was conducted at the natural experimental site (roof top of IPS, Universiti Malaya) where the plants were exposed to sunlight and rainfall. Also, unlike the study with Jatropha which was conducted for 180 days, studies with *H. cannabinus* was conducted for 90 days because the plant grows fast and flowered within this period. The pictures of *H. cannabinus* is shown in Plate 4.2

Plate 4.2 *H. cannabinus* used for phytoremediation of soil contaminated with used lubricating oil

4.5.1 Loss of used lubricating oil in soil

The percentage biodegradation of used lubricating oil in soil contaminated with 2.5% oil and 1% oil are shown in Figures 4.48 and 4.49. Percentage biodegradation at the end of 90 days in soil contaminated with 2.5% and 1% oil ranged from 2.9% to 86.4% and 6.5% to 91.8% in all the treatments, respectively. Contaminated soil treated with BSG as a source of nutrient for Hibiscus recorded the highest loss of oil (86.4% and 91.8%) in 2.5% and 1% contaminated soil, respectively; while soil treated with SMC only shows 66.1% and 67.1% oil loss in 2.5% and 1% contaminated soil at the end of 90 days respectively. However, the contaminated soil containing only Hibiscus plant without organic waste amendment recorded 52% and 58% oil biodegradation, while control soil without Hibiscus plant recorded 39.8% and 41.3% oil loss in 2.5% and 1% pollution at the end of 90 days. 11.1% and 14.1% oil loss in soil contaminated with 2.5% and 1% oil may be due to non-biological factors like evaporation, photodegradation etc. this was recorded in autoclaved soil treated with sodium azide after 90 days. The percentage of oil biodegradation in all the treatments amended with organic wastes were not significantly different at $P < 0.05$ significant level, but significant difference was recorded between the treatment amended with organic wastes and those without organic wastes, thus establishing the fact that organic wastes positively contributed to the degradation of the oil from the soil.

High percentage loss of oil in soil treated with organic wastes (BSG, BS, and SMC) and Hibiscus might be due to the presence of appreciable quantities of nutrients (N & P) in the organic wastes which possibly enhanced the growth of bacteria present at the rhizosphere of the plants. It may also be due to the fact that addition of organic wastes to the contaminated soil before planting of Hibiscus helps to loosen the compactness of the soil making sufficient aeration available for the indigenous bacteria present in the soil, thereby

enhancing their biodegradative activities of the oil from the soil. The results are similar to the results obtained in the earlier studies with *Jatropha curcas* in which contaminated soil treated with BSG and Jatropha recorded 96.6% oil biodegradation after 180 days (Agamuthu et al., 2010). The result is also in agreement with the findings of Palmroth et al., (2002) who recorded 60% loss of diesel fuel in 30 days in diesel-contaminated soil planted with pine tree and amended with NPK fertilizer. The results revealed that addition of organic wastes into the contaminated soil planted with Hibiscus rapidly enhanced both the growth of *Hibiscus cannabinus* and biodegradation of oil in the soil. This is in agreement with the findings of Vouillamoz and Milke (2009) who observed that compost addition combined with phytoremediation increases the rate of removal of diesel fuel in soil.

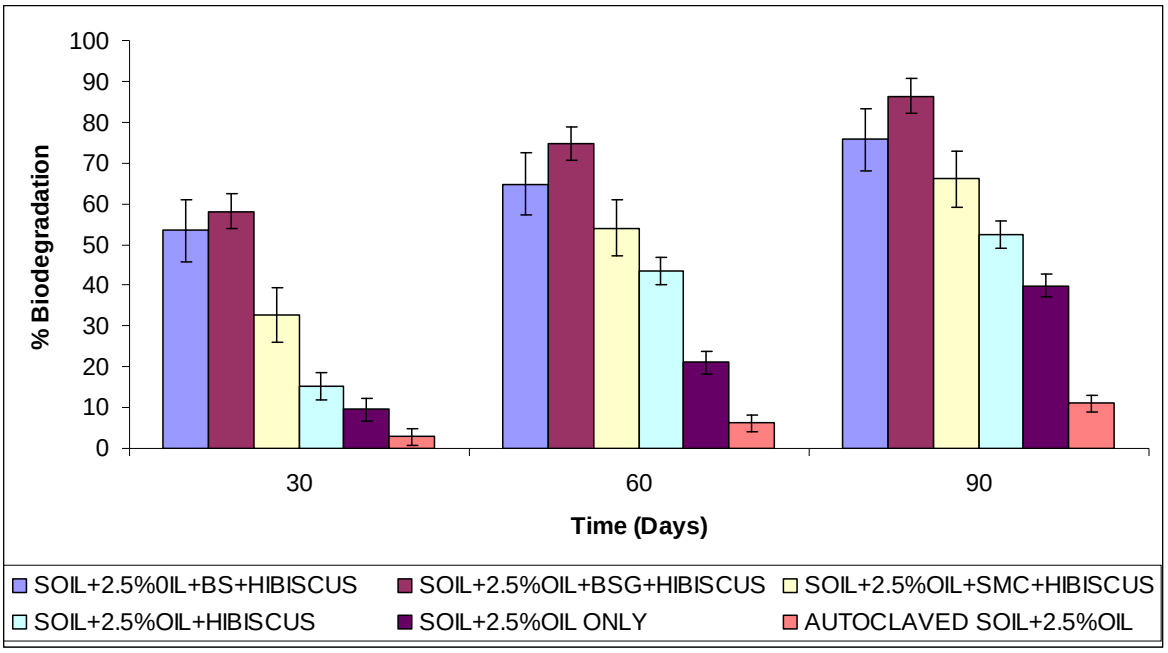


Figure 4.50 Percentage biodegradation of used lubricating oil in soil contaminated with 2.5% oil and remediated with *Hibiscus*. Bars indicates standard error (n = 3).

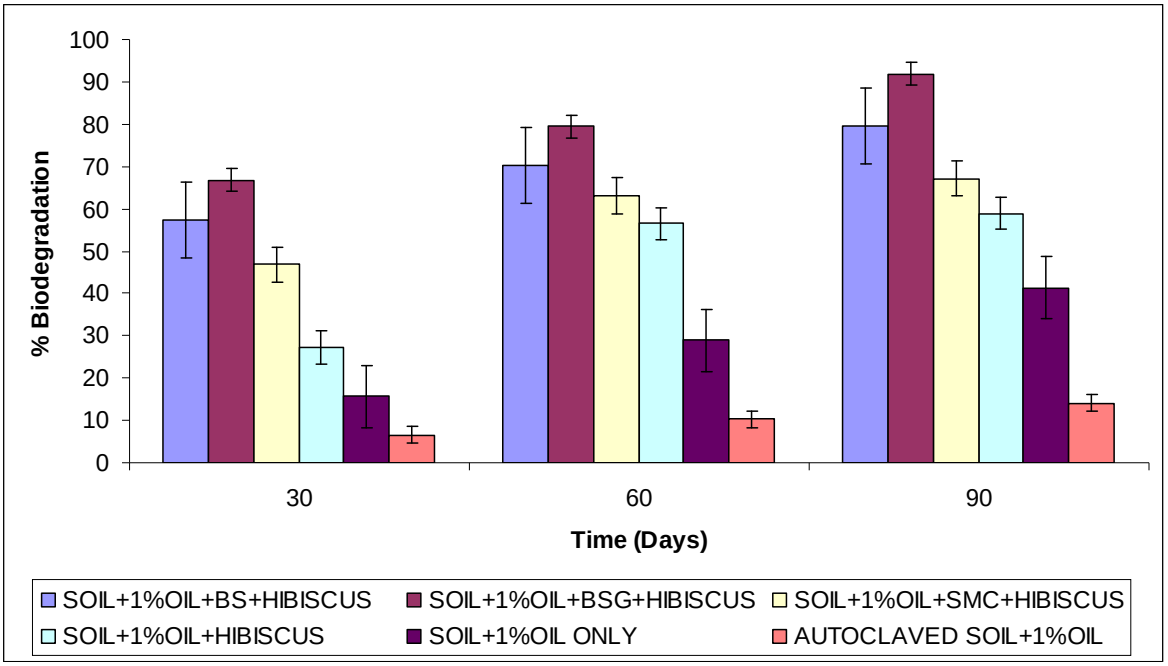


Figure 4.51 Percentage biodegradation of used lubricating oil in soil contaminated with 1% oil and remediated with *Hibiscus*. Bars indicates standard error (n = 3).

4.5.2 Bacterial counts

Four different hydrocarbon utilizing bacteria (HUB) were identified (*Pseudomonas aeruginosa*, *Bacillus* sp., *Micrococcus sp.* and *Acinetobacter* sp.) from the different treatment. These bacterial species is the same as those isolated from *Jatropha* remediated soil, because the soil used for the phytoremediation are from the same source. These bacterial species might possibly contribute to the degradation of used lubricating oil at the rhizosphere region of the plant due to their increased number in the rhizosphere region. These bacterial species are among the microorganisms listed by Miller and Litsky (1976) as microorganisms possessing abilities to degrade petroleum fractions. The counts of aerobic heterotrophic bacteria (AHB) in the Hibiscus remediated soil ranged from 12 x 10^7 CFU/g to 230 x 10^7 CFU/g and 20 x 10^7 CFU/g to 250 x 10^7 CFU/g in soil contaminated with 2.5% and 1% oil respectively in all the treatments (Figures 4.52 and 4.53).

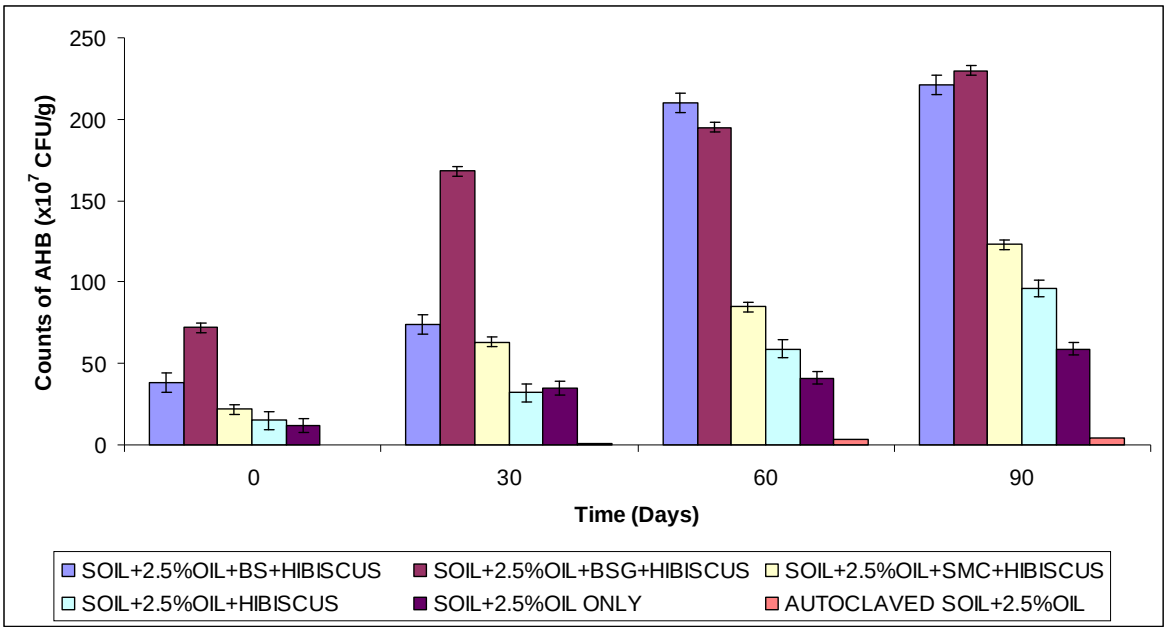


Figure 4.52 Counts of aerobic heterotrophic bacteria in soil contaminated with 2.5% used lubricating oil. Bars indicates standard error (n = 3).

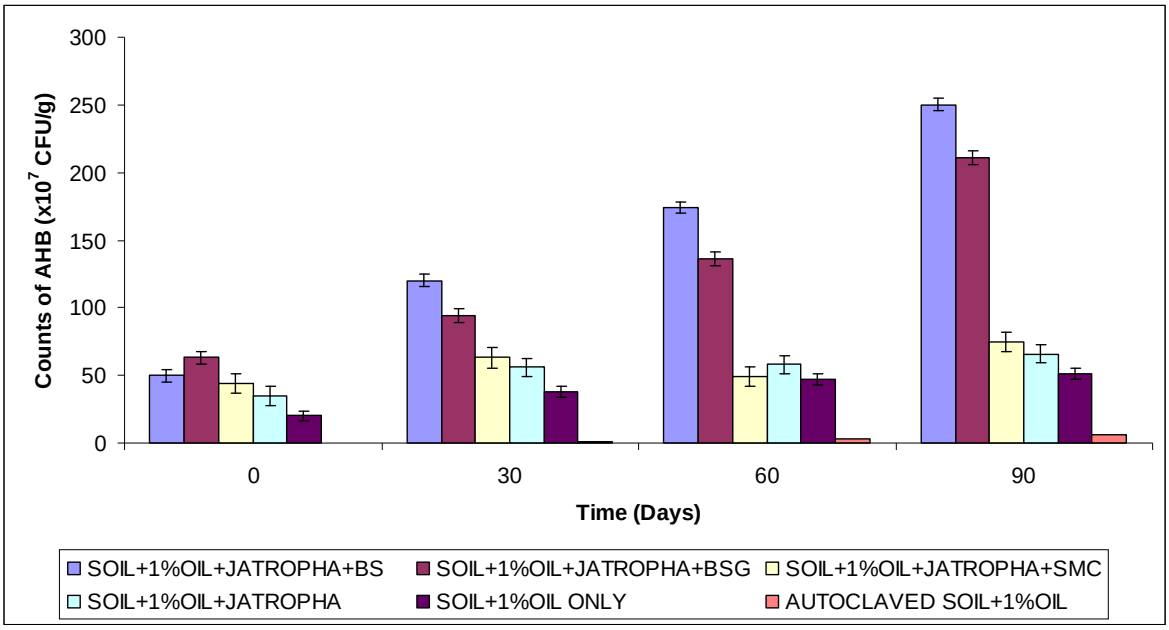


Figure 4.53 Counts of aerobic heterotrophic bacteria in soil contaminated with 1% used lubricating oil. Bars indicates standard error (n = 3).

The counts of hydrocarbon utilizing bacteria (HUB) in 2.5% and 1% contaminated soil are shown in Figures 4.54 and 4.55. The counts of HUB in all the treatments ranged from 12 x 10^5 CFU/g to 101 x 10^5 CFU/g in 2.5% pollution and 8 x 10^5 CFU/g to 84 x 10^5 CFU/g. Soil treated with BSG and *Hibiscus cannabinus* recorded high counts of HUB (101 x 10^5 CFU/g and 84 x 10^5 CFU/g) in both soil contaminated with 2.5% and 1% oil respectively at the end of 90 days, this is similar to the previous study where it was reported that BSG enhanced the multiplication of HUB better than BS and SMC (Abioye et al., 2010). However, treatment with *Hibiscus cannabinus* alone recorded low counts of HUB compared with those amended with organic wastes (43 x 10^5 CFU/g and 38 x 10^5 CFU/g) in 2.5% and 1% oil pollution. This is similar to the results of initial findings with *Jatropha curcas* (Agamuthu et al., 2010).

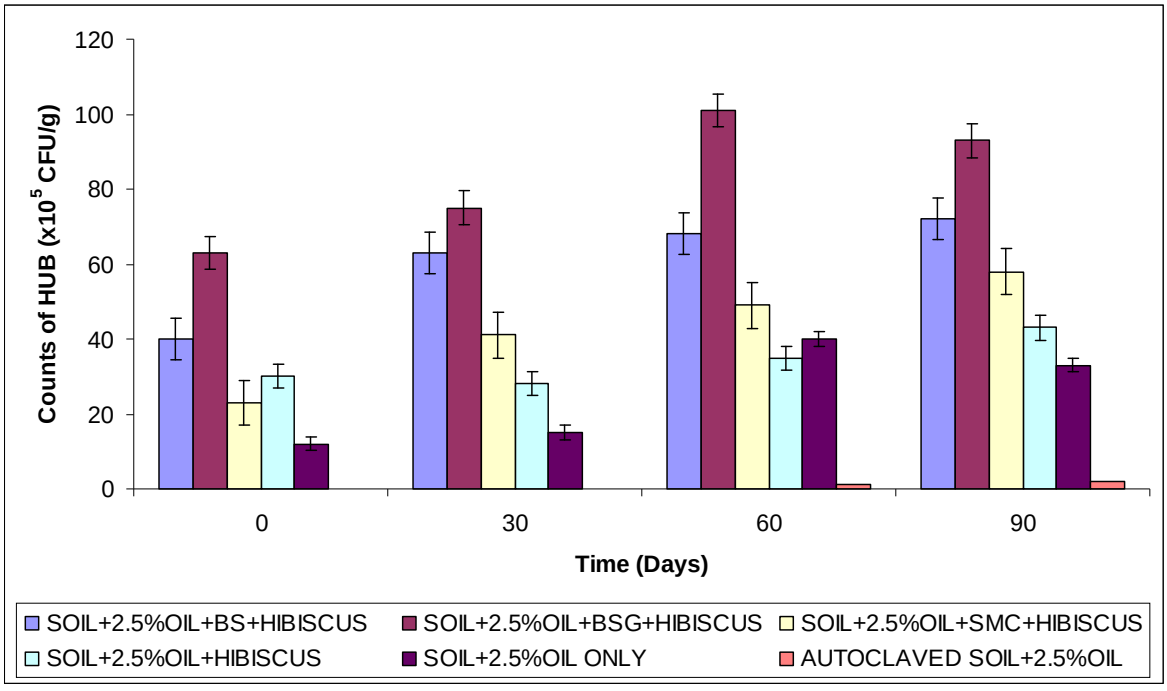


Figure 4.54 Counts of hydrocarbon utilizing bacteria in soil contaminated with 2.5% used lubricating oil. Bars indicates standard error (n = 3).

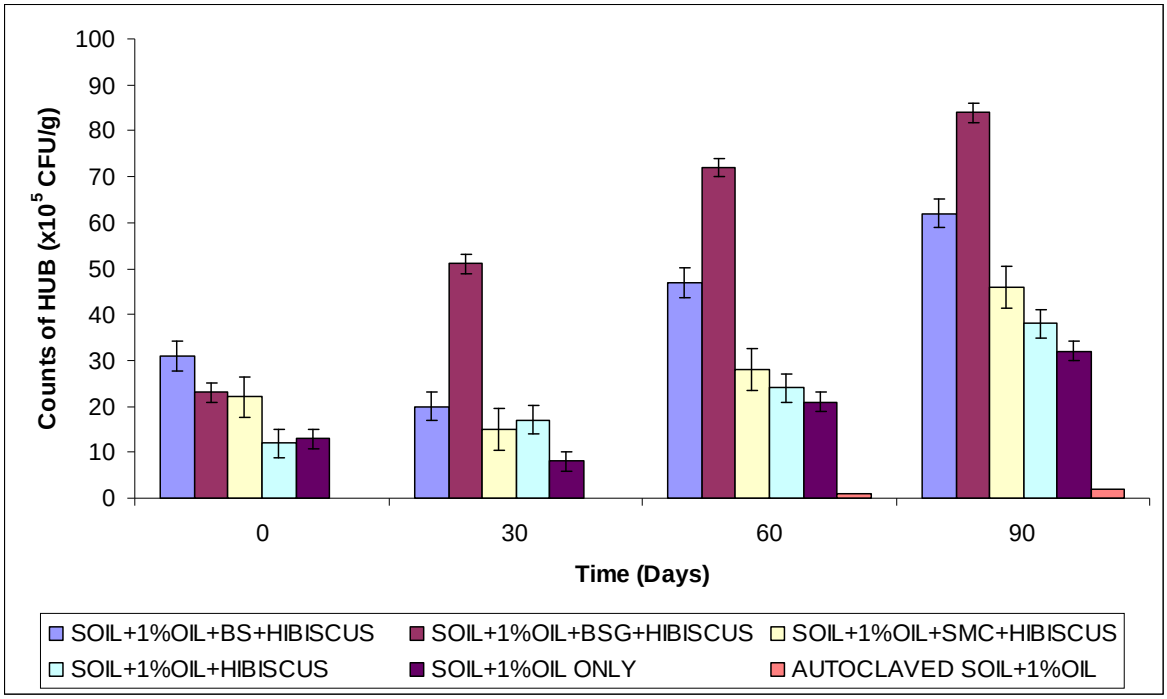


Figure 4.55 Counts of hydrocarbon utilizing bacteria in soil contaminated with 1% used lubricating oil. Bars indicates standard error (n = 3).

4.5.3 Uptake of oil and metals by *H. cannabinus*

The results of oil uptake by *H. cannabinus* was similar to the results obtained with *J. curcas* remediation in which the root of Jatropha did not accumulate any traces of the hydrocarbon in all the treatments, possibly that is why there was no report of *H. cannabinus* found as plant used for remediation of hydrocarbon compared to its usage for heavy metal remediation. The results suggest that the mechanisms of hydrocarbon removal by *H. cannabinus* may be rhizodegradation or by the microorganisms present at the rhizosphere of the plant whose activities might have been enhanced by the added organic wastes or by the root exudates produced by *H. cannabinus*. This conformed with the findings of (EPA 2000b) and (Hutchinson et al., 2003) who reported that uptake of hydrocarbons into plants, although possible, is not expected in great quantities given the compounds' chemical

properties, including high molecular weights, relatively low solubilities in water, and hydrophobic nature. These results pointed out to the fact that degradation of used lubricating oil in the soil might be through the activities of rhizosphere microorganisms. Many times more microorganisms are generally found in the plant rhizosphere than in unplanted soil, which suggests that hydrocarbon degradation could be enhanced by the presence of vegetation (Hutchinson et al., 2003). Numerous researchers have established that the primary mechanism for the disappearance of both petroleum hydrocarbons and PAHs is rhizodegradation (EPA 2000b; Hutchinson et al., 2003). There is some indication that the presence of hydrocarbons may even encourage the proliferation of hydrocarbon-degrading microorganisms (Hutchinson, 2003). Table 4.35 shows the residual metal concentration in soil of different treatment after 90 days of remediation

Table 4.35 Residual metal concentration in soil remediated with *H. cannabinus* under natural condition after 90 days

Substrate	Heavy metals (mg/Kg) Fe	Zn
Soil+1% oil + BS + Hibiscus	46.29	25.21
Soil+1% oil + BSG + Hibiscus	48.72	23.15
Soil+1% oil + SMC + Hibiscus	51.23	25.51
Soil+1% oil + Hibiscus	57.34	27.65
Soil + 1% oil only	62.3	30.12
Soil+2.5% oil + BS + Hibiscus	57.37	29.12
Soil+2.5% oil + BSG + Hibiscus	59.45	28.32
Soil+2.5% oil + SMC + Hibiscus	61.71	28.73
Soil+2.5% oil + Hibiscus	69.43	34.06
Soil + 2.5% oil only	72.23	34.87
Soil without plant + Hibiscus	73.45	36.12

The results of heavy metals uptake by *H. cannabinus* revealed appreciable accumulation of Fe and Zn in the root and stem of Hibiscus, while no metal accumulation was detected in the leaves of all the treatments. Fe accumulation in the root of the plant in soil contaminated with 2.5% and 1% used lubricating oil ranged from 12.58 mg/kg to 47.02 mg/kg and 10.58 mg/kg to 38.37 mg/kg, respectively (Table 4.36). Soil amended with BS and planted with *Hibiscus* accumulated higher concentration of Fe (47.02 mg/kg and 22.67 mg/kg) in 2.5% and 1% oil pollution, respectively. This Fe accumulation was higher than those of BSG and SMC treated soil. This observation is in sharp contrast with the results obtained in *Jatropha* experiment where BSG amended soil accumulated higher Fe. The

reason for this might be due to differences in the physiological systems of the two plants, in which case BS was more suitable for the growth and uptake of Fe in *Hibiscus* plant.

Table 4.36 Heavy metal concentration in root of *H. cannabinus* in soil contaminated with 2.5% and 1% used lubricating oil.

Treatment	Heavy metals (mg/Kg)		
	Fe	Zn	Pb
Soil + 2.5% oil + BS + Hibiscus	47.02	1.00	0.01
Soil + 2.5% oil + BSG + Hibiscus	12.58	1.48	ND
Soil + 2.5% oil + SMC + Hibiscus	13.20	0.97	ND
Soil + 2.5% oil + Hibiscus	16.01	0.32	ND
Soil + 1% oil + BS + Hibiscus	22.67	0.35	ND
Soil + 1% oil + BSG + Hibiscus	10.58	0.91	ND
Soil + 1% oil + SMC + Hibiscus	15.17	0.89	ND
Soil + 1% oil + Hibiscus	38.37	0.37	0.01
Soil without oil + Hibiscus	29.87	ND	ND

ND: Not detected

Fe accumulation in the stem of *H. cannabinus* ranged from 1.26 mg/kg to 2.37 mg/kg and 1.16 mg/kg to 1.46 mg/kg in 2.5% and 1% oil pollution (Tables 4.37). Traces of Pb was only detected in the root of Hibiscus in 2.5% oil pollution amended with BS and that of 1% oil pollution without organic wastes amendment. However no Pb was detected in the stem of all the treatments. The results revealed the ability of *H. cannabinus* to accumulate Fe in the root and translocate this metal into the stem of the plant. Accumulation of Zn in the root

of *H. cannabinus* ranges from 0.32 mg/kg to 1.48 mg/kg in soil treated with 2.5% oil and from 0.35 mg/kg to 0.91 mg/kg in soil treated with 1% oil. Zn concentration in the stem of *H. cannabinus* was higher than the accumulation in the root; it ranged from 0.32 mg/kg to 1.64 mg/kg in soil contaminated with 2.5% oil and from 0.27 mg/kg to 1.43 mg/kg in soil treated with 1% oil.

Table 4.37 Heavy metal concentration in stem of *H. cannabinus* in soil contaminated with 2.5% and 1% used lubricating oil.

Treatment	Heavy metals (mg/Kg)		
	Fe	Zn	Pb
Soil + 2.5% oil + BS + Hibiscus	1.33	0.47	ND
Soil + 2.5% oil + BSG + Hibiscus	1.63	1.64	ND
Soil + 2.5% oil + SMC + Hibiscus	2.37	0.32	ND
Soil + 2.5% oil + Hibiscus	1.45	0.53	ND
Soil + 1% oil + BS + Hibiscus	1.45	0.47	ND
Soil + 1% oil + BSG + Hibiscus	1.16	1.43	ND
Soil + 1% oil + SMC + Hibiscus	1.32	0.27	ND
Soil + 1% oil + Hibiscus	1.46	0.37	ND
Soil without oil + Hibiscus	1.26	ND	ND

ND: Not detected

The results of metal accumulation is similar to the study conducted by Hassinen et al., (2009) who reported accumulation of Zn and Fe in the root and shoot of hybrid aspen in the first year of planting on a metal contaminated site. Addition of organic wastes to the contaminated soil in addition to planting of *H. cannabinus* also promoted better biomass

yield as well as better accumulation of Zn and Fe, this might be due to nutrients in the organic wastes which enhanced the growth of *H. cannabinus* with lots of fibrous roots. The results is in line with the findings of Mun et al., (2008) who reported higher bioaccumulation of Pb in the root and stem of *H. cannabinus*, in their studies, the authors discovered higher accumulation of Pb in the root and stem when fertilizer was added to one of the treatment. However, the results was in contrast to that of (Tordoff et al. 2000; Walker et al. 2004 and Santosh et al., 2009) who reported that application of dairy sludge to soil contaminated with metal and metalloid significantly reduced the uptake of As, Cr and Zn by *J. curcas*. The differences in the two results might be due to different plant and organic wastes used for the studies or it may be due to different environmental factors and ecology of the soil used for the phytoremediation studies.

4.5.4 Rate of metal uptake by *H. cannabinus*

Table 4.38 shows the uptake rate of Fe and Zn by *H. cannabinus* within the period of study (3 months). The rate of uptake of Fe and Zn in all the treatments ranged between 0.018 to 0.108 $month^{-1}$ and 0.039 to 0.109 $month^{-1}$, respectively. The rate of uptake of Fe and Zn within 3 months of study was relatively higher than the rate of uptake recorded in study with *J. curcas* (6 months) in all the treatments. The reason for this higher rate of metal uptake might be due to the differences in the plant physiological systems and also may be because *H. cannabinus* grows faster and taller than Jatropha, attaining the height of 140 cm within three months compared to that of Jatropha of 90 cm in six months period. Unlike the studies with *J. curcas*, higher rate of Fe and Zn uptake was recorded in soil contaminated with 2.5% used lubricating oil. Higher rate of metal uptake was also recorded in all the treatments amended with BSG and BS. This may be due to the potential of the organic

wastes in enhancing the growth of *H. cannabinus* which also directly promotes the rate of uptake of Fe and Zn in the amended treatments compared to the unamended treatments.

Table 4.38 Rate of uptake of Fe and Zn by *H. cannabinus*

	Rate of uptake ($month^{-1}$)	
Treatment	Fe	Zn
Soil + 1% oil + BS + Hibiscus	0.089	0.081
Soil + 1% oil + BSG + Hibiscus	0.072	0.109
Soil + 1% oil + SMC + Hibiscus	0.055	0.077
Soil + 1% oil + Hibiscus	0.018	0.050
Soil + 2.5% oil + BS + Hibiscus	0.108	0.092
Soil + 2.5% oil + BSG + Hibiscus	0.097	0.101
Soil + 2.5% oil + SMC + Hibiscus	0.084	0.096
Soil + 2.5% oil + Hibiscus	0.045	0.039
Soil without oil + Hibiscus	0.075	0.000

4.5.5 Bioconcentration and translocation factors of metals in *H. cannabinus*

Table 4.39 shows the bioconcentration factor (BCF) and translocation factor (TF) of Zn in the *H. cannabinus*. The highest BCF (0.0814) was recorded in soil polluted with 2.5% oil and amended with BSG, while the highest TF in stem was recorded in soil treated with 2.5% oil without organic waste amendment, the result is complete contrast with the BCF and TF recorded in the phytoremediation study with *J. curcas*, but agrees with the findings of Santosh et al., (2009) who reported high TF in soil without organic wastes amendments.

Table 4.39 Bioconcentration factor (BCF) and translocation factor (TF) of Zn in Hibiscus remediated soil

Treatment	Zinc (Zn)	
	BCF	TF (in stem)
Soil + 2.5% oil + BS + Hibiscus	0.0634	1.4300
Soil + 2.5% oil + BSG+ Hibiscus	0.0814	1.1081
Soil + 2.5% oil + SMC+ Hibiscus	0.0336	0.3278
Soil +2.5% oil + Hibiscus	0.0222	1.6563
Soil + 1% oil + BS + Hibiscus	0.0256	1.3352
Soil + 1% oil + BSG + Hibiscus	0.0727	1.5766
Soil + 1% oil + SMC + Hibiscus	0.0361	0.3034
Soil + 1% oil + Hibiscus	0.0229	1.0109
Soil without oil + Hibiscus	0.0000	0.0000

Table 4.40 shows the bioconcentration factor (BCF) and translocation factor (TF) of Fe in the *H. cannabinus* plant. The highest BCF (0.6588) was recorded in soil polluted with 1% oil without organic waste amendment; while the highest TF (0.1795) in stem was recorded in soil treated with 2.5% oil and *Hibiscus* plant amended with SMC.

There was no significant difference between the BCF of Hibiscus remediated soil amended with organic wastes and those without organic wastes amendments. The reason for this might possibly be that the Hibiscus plants in soil amended with organic wastes were able to stabilized some of the metals through phytodegradation mechanism; hence the bioaccumulated metals were minimal in the plant tissues.

Table 4.40 Bioconcentration factor (BCF) and translocation factor (TF) of Fe in Hibiscus remediated soil

Treatment	Iron (Fe)	
	BCF	TF (in stem)
Soil + 2.5% oil + BS + Hibiscus	0.6087	0.0283
Soil + 2.5% oil + BSG+ Hibiscus	0.1789	0.1296
Soil + 2.5% oil + SMC +Hibiscus	0.1960	0.1795
Soil +2.5% oil + Hibiscus	0.2198	0.0906
Soil + 1% oil + BS + Hibiscus	0.3994	0.0653
Soil + 1% oil + BSG + Hibiscus	0.1942	0.1096
Soil + 1% oil + SMC + Hibiscus	0.2727	0.0870
Soil + 1% oil + SMC + Hibiscus	0.6588	0.0381
Soil without oil + Hibiscus	0.3919	0.0422

4.5.6 pH of soil in *H. cannabinus* remediation

Figures 4.56 and 4.57 shows the pH of soil in Hibiscus remediated soil contaminated with 2.5% and 1% used lubricating oil. The pH of the soil varies greatly from slightly alkaline to slightly acidic in all the treatments. The pH of soil amended with BSG were slightly acidic (as low as 5.30) than other treatments just like the pH of Jatropha remediation. This might be as well because the plants in BSG amended soil grows better than other treatments and the root produced more exudates which are slightly acidic in nature. It may also be as a result of high metabolic activities of the rhizosphere microorganisms which produced slightly acidic metabolic end products.

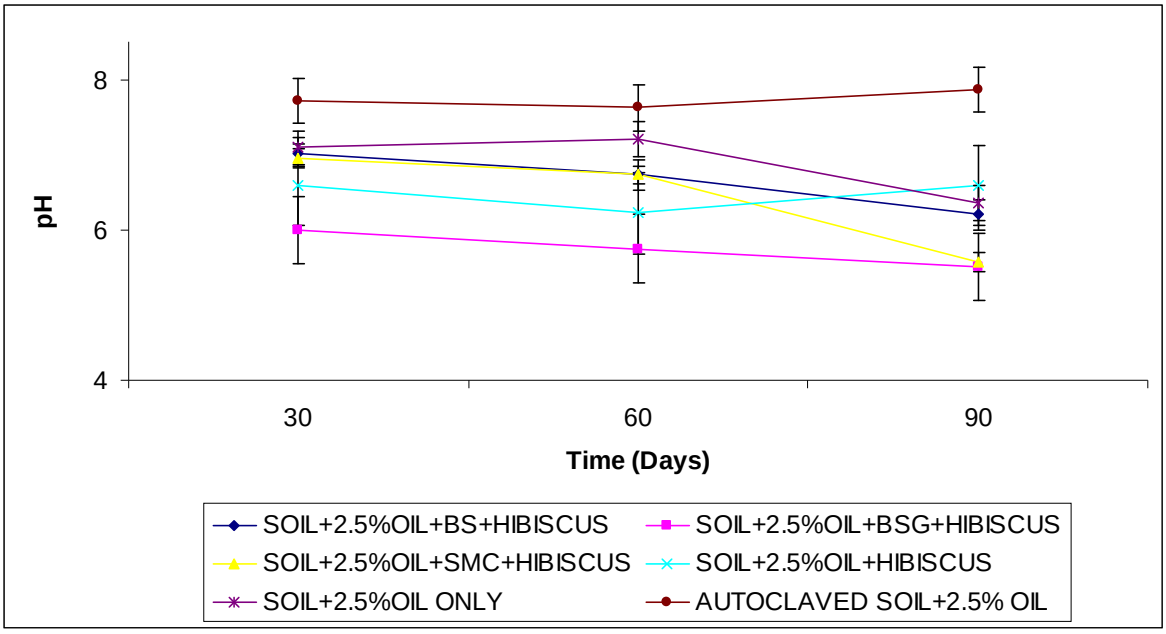


Figure 4.56 pH of soil contaminated with 2.5% used lubricating oil remediated with *H. cannabinus.* Bars indicates standard error (n = 3).

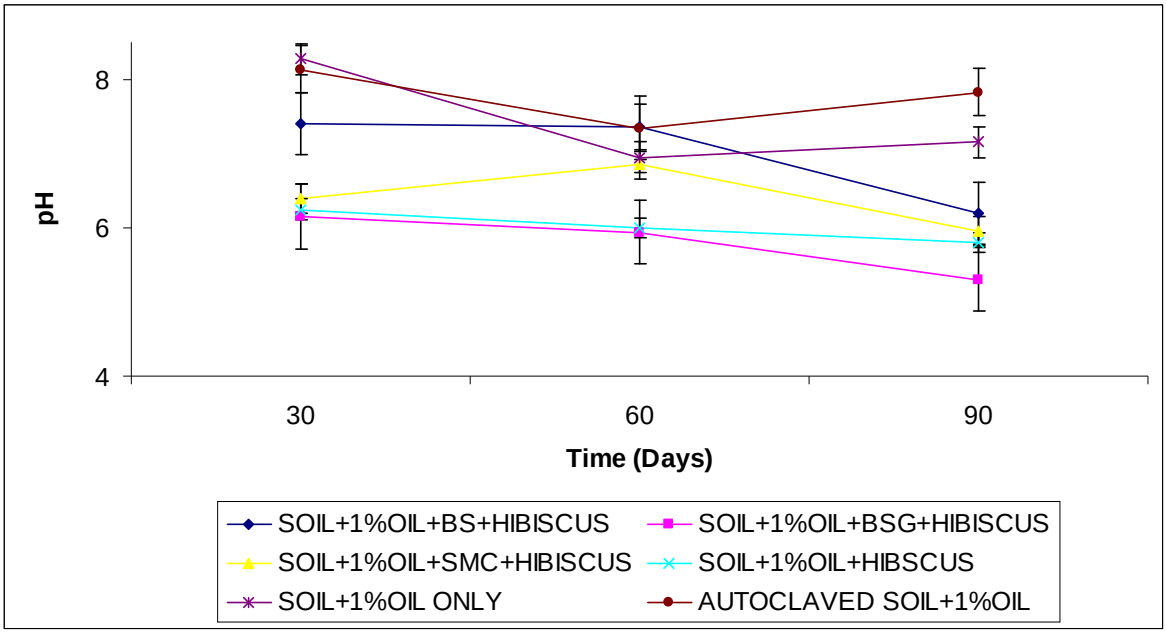


Figure 4.57 pH of soil contaminated with 1% used lubricating oil remediated with *H. cannabinus.* Bars indicates standard error (n = 3).

4.6 Results of biodegradation test with bacteria and yeast isolated from oil contaminated soil

Based on rapid growth on oil agar and in test tubes of mineral salt medium containing used lubricating oil as the only carbon and energy source, four microbial isolates (two bacterial and two yeast species isolated from used lubricating oil contaminated soil) were selected out of the sixteen microbial isolates (ten bacteria and six yeasts) for the biodegradation studies. The four microbial isolates were identified as species of *Micrococcus luteus, Pseudomonas aeruginosa, Trichosporon mucoides* and *Candida tropicalis.* These four microorganisms have been previously implicated in biodegradation of petroleum hydrocarbons by various authors (Ijah, 1998, Zhang, et al., 2005, Zahra, et al., 2006, Kayode-Isola, et al., 2008). The results of the percentage of oil biodegradation by the different microbial isolates are shown in Table 4.41. Compared to the percentage of biodegradation in control flask without microbial inoculation, there was appreciable loss of oil in the flasks inoculated with different microbial isolates. There was rapid biodegradation of oil within the first 7 days of incubation with flask inoculated with *Candida tropicalis* recording 27.8% biodegradation in the seventh day compared to the 1% recorded in uninoculated flask. At the end of the 28 days *Candida tropicalis* recorded the highest percentage of oil biodegradation (40.6%) followed closely by *Trichosporon mucoides* (38%), *Micrococcus luteus* (36.8%) and *Pseudomonas aeruginosa* (33.8%). Out of the four microbial isolates *Candida tropicalis* shows higher percentage of oil biodegradation than those of bacterial isolates. The reason for this might be the ability of yeast cells to withstand the toxic effects produced by the oil more than the bacteria; also the reason may be due to the presence of effective degradative enzymes systems in the yeast isolates.

Table 4.41 Percentage of used lubricating oil biodegradation by microbial isolates

Microbial Isolates (days)	Oil biodegradation (%) 7	14	21	28
Pseudomonas aeruginosa	26.4±2.1	28.2±1.8	29.6±3.4	33.8±4.2
Micrococcus luteus	23.8±1.9	32.6±5.1	35.0±2.6	36.8±3.7
Trichosporon mucoides	24.6±3.2	36.2±3.8	38.4±5.1	38.0±4.5
Candida tropicalis	27.8±1.5	36.4±2.7	39.0±3.8	40.6±2.6
Control	1.0±0.8	1.8±1.1	2.4±1.8	2.6±0.9

These results are supported by the findings of Walker et al., (1978) who found that *Candida* degraded South Louisiana crude oil more extensively than bacteria *Pseudomonas* and Coryneforms. *Candida tropicalis* recorded the highest percentage (40.6%) of oil breakdown compared to other isolates studied, this result is similar to that of Ijah (1998) who reported 68.9% crude oil degradation by *Candida tropicalis* in 16 days. Also Palittapongarnpim et al., (1998) reported that *Candida tropicalis* degraded 87.3% of total petroleum hydrocarbon within 7 days of incubation in medium containing crude oil as the sole source of carbon. The difference in results in the percentage of oil biodegradation might probably due to different oil used for the studies, used lubricating oil contains other contaminants like heavy metals which probably inhibits the growth of the organisms and subsequently reduce the rate of oil biodegradation compared to the results of the two different authors above. *Pseudomonas aeruginosa* degraded the least percentage of oil (33.8%) at the end of the 28 days compared to other isolates. This might possibly due to non production of biosurfactants by the isolated strains. It is reported that *Pseudomonas aeruginosa* strains

that produced biosurfactants degrade hydrocarbon faster and better than non biosurfactants producing strains (Song et al., 2006).

4.6.1 Biodegradation of hydrocarbon fractions in used lubricating oil

Biodegradation of hydrocarbons fractions present in the used lubricating oil was determined at seven days intervals for the period of 28 days to determine the extent of biodegradation of different hydrocarbon fractions using GC/FID. The hydrocarbon fractions were divided into four fractions which are: $C_7 – C_9$, $C_{10} – C_{14}$, $C_{15} – C_{28}$ and $C_{29} – C_{36}$.

Table 4.42 shows the biodegradation of $C_7 – C_9$ hydrocarbon fractions by the four microbial isolates tested for their ability to degrade used lubricating oil within the period of 28 days. The results shows that all the flasks inoculated with different microbial isolates recorded complete biodegradation of $C_7 – C_9$ fractions below the detection limit in the 28 days compared to the uninoculated control flask. The reason for the complete loss of these hydrocarbon fractions might be due partly to volatilization and because they are short chain hydrocarbons, their degradation might not pose serious challenge to the microbial isolates used for this study. This result is similar to the findings of (Ijah, 1998; Pallasser, 2000; George et al., 2002) who recorded complete degradation of these hydrocarbon fractions (from crude oil) in flasks inoculated with three different microbial isolates within the period of four days.

Table 4.42 Biodegradation of $C_7 - C_9$ hydrocarbon fractions by microbial isolates

Microbial Isolates (days)	Concentration (mg/kg) 7	14	21	28
Pseudomonas aeruginosa	98	85	67	ND
Micrococcus luteus	94	73	64	ND
Trichosporon mucoides	96	71	60	ND
Candida tropicalis	88	65	56	ND
Control	107	96	84	78

ND: Not detected at lowest detection limit of 50 mg/kg

The results of biodegradation of $C_{10} - C_{14}$ hydrocarbon fractions revealed complete degradation of these fractions below the detection level in flask inoculated with *Candida tropicalis* and *Trichosporon mucoides* at the end of 28 days of incubation; however degradation below detection limits was not achieved in the flasks inoculated with *Pseudomonas aeruginosa* and *Micrococcus luteus* (Table 4.43). The efficiency of oil biodegradation demonstrated by *Candida tropicalis* and *Trichosporon mucoides* might be due to their abilities to withstand the inhibitory component of the hydrocarbon fractions or probably due to the fact that the organisms possess an efficient degradative enzyme systems which enable them to degrade the hydrocarbon fractions below the detection limit. This has been supported by different authors (Ijah, 1998; Zhang et al., 2005; Zahra et al., 2006; Kayode-Isola et al., 2008) who argued that these two microorganisms (*Candida tropicalis* and *Trichosporon mucoides)* were able to degrade C_{10} to C_{14} hydrocarbon fractions because of their efficient degradative enzyme systems..

Table 4.43 Biodegradation of $C_{10} - C_{14}$ hydrocarbon fractions by microbial isolates

Microbial Isolates (days)	Concentration (mg/kg)			
	7	14	21	28
Pseudomonas aeruginosa	105	91	83	67
Micrococcus luteus	111	93	76	59
Trichosporon mucoides	96	71	64	ND
Candida tropicalis	83	68	53	ND
Control	125	116	109	102

ND: Not detected at lowest detection limit of 50 mg/kg

Tables 4.44 and 4.45 shows the results of biodegradation of $C_{15} - C_{28}$ and $C_{29} - C_{36}$ hydrocarbon fractions within the period of 28 days by different microbial isolates. The results revealed partial degradation of both hydrocarbon fractions, however the flask inoculated with *Candida tropicalis* and *Trichosporon mucoides* recorded the highest degradation of $C_{15} - C_{28}$ and $C_{29} - C_{36}$, respectively. The partial degradation recorded in these fractions of hydrocarbons by the microbial isolates might be due to the complex structural arrangements of these hydrocarbon fractions which possibly made them less susceptible to microbial degradation (Alberdi et al., 2001; George et al., 2002).

Table 4.44 Biodegradation of $C_{15} – C_{28}$ hydrocarbon fractions by microbial isolates

Microbial Isolates (days)	Concentration (mg/kg)			
	7	14	21	28
Pseudomonas aeruginosa	651	622	591	550
Micrococcus luteus	620	586	573	564
Trichosporon mucoides	623	608	583	526
Candida tropicalis	601	582	541	493
Control	675	667	651	648

Table 4.46 Biodegradation of $C_{29} – C_{36}$ hydrocarbon fractions by microbial isolates

Microbial Isolates (days)	Concentration (mg/kg)			
	7	14	21	28
Pseudomonas aeruginosa	495	481	462	451
Micrococcus luteus	496	478	466	444
Trichosporon mucoides	492	480	462	426
Candida tropicalis	486	463	458	432
Control	503	495	486	478

4.7 Comparison of biostimulation and phytoremediation of used lubricating oil contaminated soil

Though different oil percentages were used for bioremediation (5%, 10% & 15%) and phytoremediation (1% & 2.5%), because none of the plants survived when planted in soil contaminated with 5% used lubricating oil-contaminated soil in the pilot studies. The results recorded in both biostimulation with organic wastes and phytoremediation revealed that phytoremediation is more suitable for remediation of soil contaminated with low concentration of oil (1% to 2.5% w/w) whereas, biostimulation through the use of organic wastes amendments will be more suitable for remediation of soil contaminated with high (between 5 – 10%) concentration of oil as it was observed from the results of biostimulation with organic wastes amendments. The results is in support of the reports by various authors, who argued that phytoremediation is useful only for remediation of soil contaminated by low concentration of contaminants in soil or water body (Sung, et al., 2001; Aken, 2008; Dowling and Doty, 2009). This is because plants are sensitive to high concentration of hydrocarbons contamination.

4.8 Comparison of Jatropha and Hibiscus phytoremediation results

From the results of phytoremediation studies with *Jatropha curcas* and *Hibiscus cannabinus* for the period of 180 days and 90 days, respectively. *Hibiscus cannabinus* remediated soil recorded the highest rate of oil biodegradation compared to those of *Jatropha curcas* remediated soil. After 180 days, total percentage of oil loss recorded in Jatropha remediated soil were 85.2% and 82.8% in soil contaminated with 1% and 2.5% oil, respectively. 91.8% and 86.4% oil loss were recorded in soil contaminated with 1% and 2.5% used lubricating oil, respectively and remediated with *Hibiscus* plant after 90 days.

From these results, it can be deduced that Hibiscus is suitable for short term remediation of oil polluted because it grows to maturity faster than Jatropha plant but don't develop into a shrub compared to Jatropha. Jatropha on the other hand will be good for long term remediation of oil contaminated soil because of its potential to grow and develop into a shrub which can survive for several years.

4.9 General Discussion

The overall objectives of this study was to enhance biodegradation of used lubricating oil in soil through the use of organic wastes amendments (biostimulation) and two different plants (phytoremediation), and to compare the results of these enhancements under laboratory and natural conditions. Three organic wastes (BS, BSG and SMC) used for the amendments contained appreciable quantities of nitrogen and phosphorus which are limiting nutrients for microorganisms to effectively degrade organic pollutants in soil, water and sediments.

For the bioremediation studies, soil contaminated with different percentages of used lubricating oil (5%, 10% and 15%) were studied for the period of 84 days under laboratory condition and twelve months under natural condition. Of all the organic wastes amendments, BSG demonstrated the best potential in enhancing the biodegradation of used lubricating oil in soil compared to BS and SMC in all the oil pollution levels. BSG enhanced the degradation of the oil by 55%, 78% and 92% in soil contaminated with 15%, 10% and 5% used lubricating oil, respectively. The reasons for this potential exhibited by BSG might be attributed to its high N contents compared to other organic wastes used for the study. High N and P content has been reported by various authors as the main nutrients

that enables different organic wastes to enhance biodegradation of hydrocarbons in oil contaminated soil (Ijah and Antai, 2003; Bento et al., 2005; Adesodun and Mbagwu, 2008; Abioye et al., 2010). The potential of organic wastes used in remediation of oil contaminated soil to enhance biodegradation of used lubricating oil was in the following order BSG > BS > SMC, in most of the treatments.

Biodegradation of used lubricating oil proceeded rapidly in soil contaminated with 5% (w/w) used lubricating oil and reached 92% biodegradation in 84 days whereas in soil contaminated with 10% oil the percentage biodegradation was 78% at the end of 84 days while soil contaminated with 15% oil recorded 55% at the end of 84 days. These results clearly show that percentage of oil concentration in a contaminated soil determines the rate of biodegradation by the indigenous microorganisms. The reason for this might be due to negative effects that high concentration of oil usually has on the microbiota of any contaminated soil. High concentration of hydrocarbon in soil is known to affect its biodegradability through its inhibitory effects on microorganisms (Rahman et al., 2002). This finding was in agreement with the findings of Ijah and Antai, (2003b) who reported that biodegradation of crude oil at concentration of 30% and 40% was very low compared to those of 10% oil pollution.

Two different percentages of organic wastes (5% and 10%) were also tested on the three pollution levels (15%, 10% & 5%) in order to determine the percentage of organic wastes amendment that will best enhance the biodegradation of used lubricating oil in contaminated soil. The results of the study revealed that used lubricating oil contaminated soil amended with 10% organic wastes recorded the highest percentage of oil

biodegradation in all the different organic wastes used for enhancing oil biodegradation in soil compared to the percentage of oil biodegradation recorded with 5% organic wastes amendment. The reason for these results might be that, the higher the quantity of organic wastes used, the more the nutrient supplied to the indigenous microbial population which possibly increased their population which in-turn increases the rate and percentage of oil biodegradation in 10% organic wastes amendments (Frederic, 2005; Kim et al., 2005; Okoh, 2006). Another reason for this result might be attributed to the potential of 10% organic waste amendments to improve the aeration and the texture of the soil compared with those amended with 5% organic wastes (Elektorowicz, 1994; Piehler et., 1999) Studies by various authors have shown that improved aeration in oil contaminated soil tends to increase the rate of oil biodegradation in an oil contaminated soil (Jorgensen, 2000).

Oil contaminated soil amended with BSG still proved to be the best of the organic waste amendments in simulated natural condition studied to determine the level of biodegradation of different hydrocarbon fractions from C_7 to C_{36} and the concentration of polyaromatic hydrocarbons at the end of 12 months study. These results showed clearly that BSG can play a significant positive role in enhancing remediation of soil contaminated with petroleum hydrocarbons within the shortest possible time.

Phytoremediation of used lubricating oil-contaminated soil was also studied using two types of plants namely: *Jatropha curcas* and *Hibiscus cannabinus.* The *J. curcas* was studied both under laboratory condition and natural condition, exposed to rainfall and sunlight. Jatropha plant demonstrated good tolerance to the oil concentrations (2.5% and 1% oil) throughout the period of study (180 days); this might be due to the hardiness nature of the plant which enables it to survive under harsh environmental conditions. The root of

the plants did not accumulate the oil from the results of GC/MS of the root extracts; this result signifies that the mechanism of oil degradation by the plant is likely to be through rhizodegradation mechanisms. This is supported by the increased number of bacteria present at the rhizosphere region of the plant. Many times more microorganisms are generally found in the plant rhizosphere than in unplanted soil, which suggests that hydrocarbon degradation could be enhanced by the presence of vegetation (Hutchinson, 2003). Numerous researchers have established that the primary mechanism for the disappearance of both petroleum hydrocarbons and PAHs is rhizodegradation (EPA 2000b; Hutchinson, et al., 2003). Though there was no traces of oil accumulation in the root of Jatropha plant, the increased biodegradation of the oil at the rhizosphere region of the plant might as well be due to production of root exudates by the plant, some of which are proteinous in nature, which possibly contributed to the biodegradative activities of bacterial species present in this region of plant.

Though, Jatropha plant did not accumulate oil in its root, there was appreciable accumulation of Fe and Zn (present in the soil and used lubricating oil) in the plant root and most of them were translocated to the shoot region of the plant. These results indicate *Jatropha curcas* as a potential plant for remediation of soil contaminated by heavy metals. This finding corroborates the reports of different authors who had demonstrated the ability of *J. curcas* to remediate soil contaminated with Zn, Pb, Cd, As and Cr through its bioaccumulation potential (Mangkoedihardjo and Surahmaida, 2008; Jamil et al., 2009; Santosh et al., 2009).

Comparison of the results of Jatropha remediation under lab condition and those conducted under natural conditions shows that percentage of oil loss under lab condition was higher

(96.6%) than those of natural condition (85.2%). This is similar to the observation of Gerhardt et al., (2009) who explained that the reason for this might be due to plant stress factors such as variation in temperature, nutrients, precipitation and presence of insects which are not present in laboratory studies; these stress factors can pose a significant challenge for field application.

The results of phytoremediation with *H. cannabinus* was similar to those of *J. curcas* because no accumulation of oil was detected form the root extract of *H. cannabinus* and there was appreciable accumulation of Fe and Zn both in the root and shoot of the plant. However, the rate of oil degradation in *H. cannabinus* was higher than those of Jatropha plant. In six months studies with Jatropha plant 85.2% and 82.8% oil losses were recorded in soil contaminated with 1% and 2.5% oil respectively, while in three months studies with *H. cannabinus* 86.4% and 91.8% oil loss was recorded in soil contaminated with 1% and 2.5% oil respectively. This high percentage of oil loss recorded in *H. cannabinus* remediated soil might be due to its higher rate of growth than those of Jatropha plant. The higher growth rate of *H. cannabinus* resulted in abundant of fibrous root by the plant which probably contributed positively to higher rate of oil biodegradation in the contaminated soil via rhizodegradation mechanism.

Addition of 5% organic wastes (BS, BSG and SMC) to the *Jatropha* and *Hibiscus* phytoremediation studies, positively enhanced biodegradation of used lubricating oil and bioaccumulation of Fe in the contaminated soil. BSG was more effective in *Jatropha* studies while BS was more effective in the studies with *H. cannabinus.* The differences in the activities of these organic wastes in both plants might be due to differences in the physiological systems of the plant with BSG more suitable for *Jatropha* and BS more suitable for *Hibiscus.*

The frames of the study covered the biostimulation of soil contaminated with different concentrations (5%, 10% and 15% w/w) of used lubricating oil and amended with organic wastes (BS, BSG and SMC). It also includes phytoremediation with *Jatropha curcas* and *Hibiscus cannabinus* at 1% and 2.5% used lubricating oil concentrations. Both biostimulation and phytoremediation were studied under laboratory and natural conditions. However, possible limitations of the work are; the study was limited to the 1%, 2.5%, 5%, 10% and 15% concentrations of used lubricating oil. Lower or higher concentration of used lubricating oil might possibly give different results than what was obtained in this study. Also, only three organic wastes (BS, BSG and SMC) were used, other available organic wastes might as well give different results.