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PHYSICO-CHEMICAL CHARACTERISTICS AND MICROBIAL POPULATION DYNAMICS OF SHEA NUT CAKE POLLUTED SOIL IN NORTHERN GHANA

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Abstract

One hundred and sixty two (162) samples each were collected from shea nut cake polluted and unpolluted (control) soil at three soil depths (0-20 cm, 20-40 cm and 40-60 cm) in Gurugu, Jusonayilli and Kasalgu in the Sanarigu District in the Northern Region of Ghana from September 2009 to July 2010, to compare depth-wise distribution of physico-chemical properties, bacteria and fungi populations in the soil. Soils were sandy-loam, loam and clay for polluted soils in Gurugu, Jusonayilli and Kasalgu and loamy sand, sandy-loam and clay for controls respectively. Moisture, pH, organic carbon and total nitrogen were significantly higher in polluted soils than controls. They were highest in the 0-20 cm depth in Gurugu and Jusonayilli and in the 20-40 cm depth in Kasalgu. Bacteria counts were highest in 0-20 cm depth. Bacteria population was higher during rainy season. Fungi counts were highest in the 0-20 cm depth in Gurugu and Jusonayilli and in the 20-40 cm soil in Kasalgu. Bactria counts were highest in September and fungi in November. Microbial counts were significantly higher in polluted than unpolluted soils. Shea nut cake added organic matter and nutrients to soil which increased bacteria and fungi populations.

Key words: Physico-chemical properties, Shea nut cake, Biodegradation, Selective isolation

Introduction

Shea butter is extracted from the kernels of the shea tree by traditional and agro-food transformation factories producing shea nut cake as a waste product (Hall *et al*., 1996). Large quantities of shea nut cake are generated annually. Iddrisu, (2013) estimated that 60,000 metric tonnes of kernels are consumed every year, generating about 30,300,000 kg of shea nut cake annually. Shea nut cake is high in polyphenolic compounds, including tannins (Hall *et al*., 1996). Tannins are bioactive, easily form complexes with proteins and polysaccharides making shea nut cake toxic and recalcitrant to biodegradation, hence unacceptable to animals as feed though very rich in protein, carbohydrates and fatty acids (Hall *et al*., 1996,). Shea nut cake has thus been described as a waste product of no economic value (Hall *et al*., 1996) and soil is the ultimate recipient in an environmentally unacceptable way (Fig.1).

Fig. 1: Overflow of pit for shea nut cake disposal in Jusonayilli

Soil is a complex ecosystem delimited by physical and chemical properties which determine the type of nutrients and prevailing environmental conditions (Tangjan *et al*., 2009). Nutrients required for microbial growth in soil are inherent in the soil physico-chemical parameters (Cathcart *et al*., 2003). Natural product degrading bacteria are reported to constitute about 1% of the consortium of bacteria in soil increasing in number up to ten fold in polluted soil (Ray, 1994).

Information on soil physico-chemical properties and microbial population dynamics is very essential in bioremediation applications and pollution management. If bacteria are found to be present in the polluted environment and nutrients are lacking, the necessary nutrients and growth conditions are added (fertilization) to increase bacteria population and hasten biodegradation, but if bacteria are found to be absent the appropriate pollutant degrading bacteria are added from external source, either grown from environment already contaminated with such pollutant or genetically engineered (seeding) (Ray, 1994).

Bacteria and fungi population dynamics of polluted soils, and the occurrences of changes in soil properties due to the presence of such pollutants as sewage, agricultural waste, some agro-industrial wastes and petroleum hydrocarbon have been extensively researched and documented (Abdul Rahman *et al*, 1986; Zwolinski *et al*, 1988,; Ali *et al*, 1989; Ray 1994; Mazzafera *et al*, 2002; Hemida, 2005; Hamzah *et al*, 2010). Cement contaminated soil for example has been reported to increase soil pH and decreased microbial populations (Hemida , 2005), while hydrocarbon pollution has been observed to lower the pH towards acidity and increases microbial population and diversity (Truong Son, 2005; Hamzah *et al*, 2010). Earlier research also observed that hydrocarbon and agro-waste polluted soils have higher bacterial and fungal population than unpolluted soils and have also reported bacteria and fungal populations at different soil depths, locations, seasons and in relation to various physico-chemical properties of soil and pollutant contamination levels. Such results have been effectively used to improve crop yield and in bioremediation applications (Hamzah

et al, 2010; Truong Son, 2005; Laukova *et al*, 2002; Ebuehi *et al*, 2005; Adesemoye *et al*, 2006; Bahig *et al*, 2008; Hamida *et al,* 2005 and Zwolinski *et al*, 1989; Kennedy *et al* 2005; Tangjan *et al* 2009; Shukla, *et al* 1989; Arunachalam, *et al* 1997; Dkhar 1983; Smith *et al* 1994; Adeduntan & Adeniyl, 2009). However, the physico-chemical properties and microbial population dynamics of shea nut cake polluted soil as a basis for further research into bioremediation application has not been investigated, even though soil is the ultimate recipient of the industrial waste. This study was designed to investigate the physico-chemical characteristics and bacterial and fungal populations of shea nut cake polluted soil in Northern Ghana.

Materials and methods

Soil sampling

One hundred and sixty two (162) samples were taken from shea nut cake contaminated soil and one hundred and sixty two (162) from soil with no history of shea nut cake contamination were collected from Gurugu, Jusonayilli and Kasalgu in the Tamale Metropolis of the Northern Region of Ghana from September 2010 to July 2011. The samples were taken at random at alternate months from three soil depths, 0-20 cm, 20-40 cm and 40-60cm into sterile polyethylene bags using graduated steel soil auger and stored at 4^{o}C. The samples were sieved through a sieve of 1.7 mm pore diameter to remove large objects and cultured for bacteria and fungi colony forming units (cfu) within 24h. A portion of the sieved soil was used for the assessment of the physical and chemical properties.

Soil analysis for physico-chemical characteristics

Fresh soil samples were examined physically and colour noted. Soil texture was determined by the method described by Berry *et al* (2007). Soil moisture content was determined by the gravimetric method described by Hausembuiller (1975). The Potassium dichromate volumetric method described by Walkley & Black, (1934) was used to determine the organic carbon content. Total Nitrogen was determined by the Kjeldahl Nitrogen TC WI 2003(E) method described by Janssen & Koopmann (2005). The pH of the soil samples was measured with a pH meter, Gallenkamp Model 640 and combination electrodes (a set of glass electrode and reference electrode), (Type No. PHM-110-010Y). All chemicals, solutions and calibration standards of analar grade were from BDH Chemical Supply Ltd. Poole, UK. All tests were carried out in triplicates and results expressed as mean.

Enumeration of soil microorganisms

Standard microbiological procedures for determining total soil microbial numbers, described by Truong Son (2005) were adapted for bacteria and fungi. Serial dilution of sample in sterile normal saline (0.9%NaCl) and spread plate techniques were used. Nutrient agar plates, pH 7.0, were supplemented with Nystatin U.S.P. 100,000 I.U. from Biomedicine S.P.R.L, Brussels, Belgium as anti-fungal. Inoculated plates were incubated aerobically at 25^{o}C for 24 hours and up to 72 hours for bacteria growth. For fungi, Potato Dextrose Agar plates with Benzylpenicilin from Letap Pharmaceuticals Ltd, Accra, Ghana as antibacterial agent were inoculated and incubated at 25^{o}C aerobically for up to 5days observing daily for growth. Inoculation was done in triplicates. Plates with 30 to 300 colonies were selected, counted and average of total population for the three plates expressed as colony forming units per gram dry weight of soil.

Data analysis

Physical properties (sand, silt and clay), organic carbon and nitrogen were expressed as percentage. Soil pH was expressed as absolute values. Percentage moisture was expressed as log . Bacterial and fungal populations were expressed as log of colony forming units (cfu). Data was subjected to Analysis of Variant (ANOVA) using GenStat (Release 10.3DE (PC/Windows Vista) 04 VSN International ltd (Rotamsted Experimental Station) Genstat Co. U.K) to determine significant difference. Significantly different means were separated using the least significant difference (l.s.d) at 5% method. Regression analysis was used to determine the correlation between physic-chemical properties and microbial population. $P< 0.05$ was denoted positive correlation.

Results

Physico-chemical properties of soil

Results of the textural characteristics of the studied soils are shown in Table 1. Soil in Gurugu was sandy loam, Jusonayilli was loam and Kasalgu was clay for shea nut cake polluted soils. Unpolluted soils were sandy loamy sand, loam and clay for Gurugu, Jusonayilli and Kasalgu respectively.

Table 1: Soil texture characteristics of shea nut cake polluted and unpolluted soils

Soil Particle	GURUGU		JUSONAYILLI		KASALGU	
	Control	Polluted	Control	Polluted	Control	Polluted
Sand	80%**)**	80%	75%	**45%**	**30%**	25%
Silt	15%	10%	20%	**45%**	30%	20%
Clay	**5%**	10%	5%	**10%**	40%	55%
Texture class	Loamy sand	Sandy loam	Sandy loam	**Loam**	Clay	Clay

ANOVA P-value = 0 l.s.d. (0.389); location and soil types compared not significantly (P>1) different; Location, soil type and texture were significantly different (P<0.001)

Moisture content results are shown in Table 2. Moisture contents were higher in shea nut cake polluted soils than their respective controls, with Jusonayilli recording the highest in shea nut cake polluted soil and Kasalgu in unpolluted soils. Soil moisture distribution of the study sites for the months sampled are shown in Table 3. Moisture contents were highest in September in all sites. Of the three sites Kasalgu recorded the highest total moisture content. No significant difference was observed in Gurugu and Jusonayilli in July and November.

Table 2: Moisture content (mean of %) location, by soil type and soil depth

	(a) Soil with no history of shea nut cake pollution			(b) Shea nut cake polluted soil		
SOIL DEPTH	**GURUGU**	**JUSONAYILLI**	**KASALGU**	**GURUGU**	**JUSONAYILLI**	**KASALGU**
0-20 cm	1.92	1.86	2.27	3.15	3.5	3.09
20-40 cm	1.85	1.8	2.47	3.1	3.37	3.18
40-60 cm	1.83	1.82	2.49	2.99	3.18	3.08
Mean	1.87	1.83	2.41	3.08	3.35	3.12
l.s.d	0.006					

P-value of soil type compared < 0.001

Table 3: Moisture content by month and location (log of $\%g^{-1}$)

Month	Gurugu	Jusonayilli	Kasalgu
Jan	2.34701a	2.48683b	2.76568cd
Mar	2.29591a	2.47951b	2.54134cd
May	2.37628a	2.58623b	2.80496cd

Jul	2.55118a*	2.56191a*	2.81168cd
Sep	2.64516a	2.78517b	2.84259cd
Nov	2.63131a*	2.63999a*	2.80831cd

l.s.d 0.015371

***Mean values with the same letter across the row are not significantly different from each other**.

Soil pH values by location, soil type and soil depth are indicated in Table 4. Shea nut cake polluted soils generally recorded higher pH than unpolluted soil and highest in the top 0-20cm soil depth, except Kasalgu where there were no significant differences across soil depth**.** The highest pH was recorded in Jusonayilli in polluted soil and Kasalgu in unpolluted soil.

Organic carbon contents of the soils sampled are given in table 4. Organic carbon contents were significantly higher in shea nut cake polluted soils than unpolluted soils. Highest values were observed in the top 0-20 cm soil depth in Gurugu and Jusonayilli and in the 20-40 cm depth in Kasalgu.

Table 4: soil pH

	(a) Soil with no history of shea nut cake pollution			**(b) Shea nut cake polluted soil**		
SOIL DEPTH	**GURUGU**	**JUSONAYILLI**	**KASALGU**	**GURUGU**	**JUSONAYILLI**	**KASALGU**
0-20 cm	4.01	5.03	5.7	6.73	8.98	5.7
20-40 cm	4.01	5	6	6.02	8.49	6
40-60 cm	4	4.99	5.6	6.01	8.01	5.7
Mean	4.01	5.01	5.77	6.25	8.49	5.8

l.s.d **0.157**

P-value of soil type compared < 0.001

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Table 5: Organic carbon content (mean of %)

	(a) Soil with no history of shea nut cake pollution			**(b) Shea nut cake polluted soil**		
SOIL DEPTH	**GURUGU**	**JUSONAYILLI**	**KASALGU**	**GURUGU**	**JUSONAYILLI**	**KASALGU**
0-20 cm	1.98	0.88	0.73	3.06	3.64	0.83
20-40 cm	1.47	0.59	1.19	2.21	1.73	1.66
40-60 cm	0.94	0.33	0.47	1.08	1.02	0.62
Mean	1.46	0.6	0.8	2.12	2.13	1.04

l.s.d **0.241**

P-value of soil type compared < 0.001

Total nitrogen contents of the soils studied are given in Table 5. Soil nitrogen values were significantly higher in shea nut cake polluted soils than unpolluted soils. Higher values were recorded in the top soil than deeper soils in Gurugu and Jusonayilli and in the sub-soil (20-40 cm depth) in Kasalgu for both shea nut cake polluted and unpolluted soils.

TABLE 6: Total nitrogen content (mean of %)

(A) SOIL WITH NO HISTORY OF SHEA NUT CAKE POLLUTION				(B) SHEA NUT CAKE POLLUTED SOIL		
SOIL DEPTH	**GURUGU**	**JUSONAYILLI**	**KASALGU**	**GURUGU**	**JUSONAYILLI**	**KASALGU**
0-20 cm	0.16	0.07	0.07	0.29	0.31	0.08
20-40 cm	0.14	0.05	0.11	0.2	0.15	0.16
40-60 cm	0.08	0.03	0.04	0.09	0.03	0.06
Mean	0.13	0.05	0.07	0.19	0.16	0.1
l.s.d	**0.005**					

P-value of soil type compared < 0.00

Enumeration of soil microorganisms

Table 7 gives the bacteria population of soils by location and soil depth. Bacteria counts were significantly higher in shea nut cake polluted soil than unpolluted soil and were highest in the 0-20 cm depth in both shea nut cake polluted and unpolluted soils, decreasing with increasing soil depth in all the three sites studied.

TABLE 7: Bacteria population (mean of colony forming units per gram of soil dry weight) by location, soil type and depth

Soil with no history of shea nut cake pollution			(b) Shea nut cake polluted soil		
SOIL DEPTH	**GURUGU**	**JUSONAYILLI**	**KASALGU**	**GURUGU**	**JUSONAYILLI**	**KASALGU**
0-20 cm	7.6	7.63	6.56	7.91	7.94	6.86
20-40 cm	6.57	6.58	5.54	6.8	6.78	6.73
40-60 cm	5.53	5.55	5.5	5.8	5.74	5.66
Mean	6.57	6.59	5.87	6.84	6.82	6.42

l.s.d 0.0044; P-value of soil type compared < 0.001

Results of fungi colony forming units are given in Table 8. Fungal populations are generally higher in shea nut cake polluted soils than unpolluted soils. Highest fungal counts were recorded in shea nut cake polluted soil in Gurugu. Table 9 represents total bacterial counts for the three sites over the months sampled. Bacteria populations were highest in September in all three sites with no significant difference between Gurugu and Jusonayilli.

Table 8: Bacteria population by month and location (log of cfug^{-1}soil dry weight)

Month	Gurugu	Jusonayilli	Kasalgu
Jan	6.649a	6.655b	6.093cd
Mar	6.588a	6.615b	6.070cd
May	6.686a	6.669b	6.111cd
Jul	6.723a	6.713b	6.156cd
Sep	6.796a*	6.796a*	6.225cd

Nov	6.777a	6.773b	6.195cd

l.s.d.=0.002216

*Mean values with the same letter across the row are not significantly different from each other.

Fungal populations across soil depths in the two soil types studied are given in Table 9. Counts were higher in shea nut cake polluted soils than unpolluted soils.

Table 9: Fungi population by location, soil type and depth

	Soil with no history of shea nut cake pollution			**(b) Shea nut cake polluted soil**		
SOIL DEPTH	**GURUGU**	**JUSONAYILLI**	**KASALGU**	**GURUGU**	**JUSONAYILLI**	**KASALGU**
0-20 cm	3.44	3.43	3.4	3.72	3.63	3.61
20-40 cm	3.42	3.42	3.43	3.62	3.57	3.76
40-60 cm	3.4	3.41	3.39	3.63	3.47	3.54
Mean	3.42	3.42	3.41	3.66	3.56	3.64
l.s.d	0.004248					

P-value of soil type compared < 0.001

Fungal total counts for the three study sites over the months sampled are given in Table 10. Fungal counts were highest in November in all the sites studied, with no significant difference in Gurugu and Kasalgu.

Table 10: Fungal population by month and location

Month	Gurugu	Jusonayilli	Kasalgu
Jan	3.522a	3.463b	3.514c
Mar	3.503a*	3.437b	3.499a*d
May	3.532a	3.482b	3.513cd
Jul	3.550a	3.500b	3.520cd
Sep	3.552a	3.514b	3.535cd
Nov	3.575a	3.537b	3.551cd

(l,s.d=0.004248)

***Mean values with the same letter across the row are not significantly different**

Table 11: SUMMARY OF REGRESSION ANALYSIS: Correlation between bacteria and fungi cfu and soil physico-chemical properties. KEY: A (Jusonayilli); B (Gurugu); C (Kasalgu)

Moisture against bacteria

SITE	**B**	**R^2**	**P Value**	**Deviation from zero**
A	0.1051 ± 0.1200	0.4339	0.5422	Not Significant
B	1.098 ± 0.5097	0.8229	0.2766	Not Significant
C	-0.8587 ± 1.744	0.1952	0.7087	Not Significant

Moisture against fungi				
A	-0.001924 ± 0.001225	0.7114	0.361	Not Significant
B	0.01383 ± 0.006734	0.8084	0.2884	Not Significant
C	**0.05578 ± 0.003924**	**0.9951**	**0.0447**	**Significant**
pH against Bacteria				
A	-3.412 ± 62.99	0.002925	0.9656	Not Significant
B	**67.38 ± 0.8936**	**0.9998**	**0.0084**	**Significant**
C	-33.26 ± 111.5	0.08168	0.8155	Not Significant
pH against Fungi				
A	0.8974 ± 0.08808	0.9905	0.0623	Not Significant
B	**0.8556 ± 0.02732**	**0.999**	**0.0203**	**Significant**
C	3.332 ± 0.3261	0.9905	0.0621	Not Significant
Organic Carbon against bacteria				
A	2.028 ± 20.24	0.009947	0.9364	Not Significant
B	19.34 ± 9.420	0.8082	0.2886	Not Significant
C	-12.70 ± 37.18	0.1045	0.7905	Not Significant
Organic Carbon against Fungi				
A	0.2816 ± 0.07245	0.9379	0.1603	Not Significant
B	0.2434 ± 0.1242	0.7933	0.3004	Not Significant
C	**1.129 ± 0.06583**	**0.9966**	**0.0371**	**Significant**
Total Nitrogen against Bacteria				
A	-45.91 ± 763.5	0.003604	0.9618	Not Significant
B	231.9 ± 152.8	0.6974	0.3708	Not Significant
C	-142.1 ± 426.0	0.1001	0.7951	Not Significant
Total Nitrogen Against Fungi				
A	10.89 ± 1.003	0.9916	0.0585	Not Significant
B	2.910 ± 1.995	0.6802	0.3827	Not Significant
C	**12.89 ± 0.8454**	**0.9957**	**0.0417**	**Significant**

Discussion

Analysis of varience of soil texture by location and soil type (Table 1) showed significant difference ($P<0.001$). The destribution of soil bacteria is controlled by a number of environmental factors such as moisture content, pH and soil organic matter (Kennedy et al., 2005). Higher moisture content in shea nut cake polluted soil than unpolluted soil in Table 2 observed in the study is in agreement with Cathcart *et al* (2003) who reported that soil high in organic matter has increased water holding capacity. Higher moisture content in the rainy season (May-October) in Table 3 is consistent with Tanjang *et al*., 2009 who reported seasonal variation in soil moisture content with highest figures for summer. Soil pH is influenced by the nature and degree of pollution. Hydrocarbon-

polluted soils have been reported to have acidic pH (Hamzah *et al.,* 2010). Shea nut cake increases soil pH as seen in higher pH values in shea nut cake polluted soil than unpolluted soil (Table 4). The results agree with previous reports of Berry *et al.,* (2007) which associate higher pH values with soil rich in organic matter. Tangjan *et al.,* (2009) also reported higher pH in soil high in organic matter.

Higher concentrations of organic carbon in the 0-20cm depth in samples from Jusonayilli and Gurugu (Table 4) are supported by Cathcart *et al*., 2003 who reported that on level grounds organic matter is more concentrated in the top soil than deeper depths. Highest concentration of organic carbon in the 20-40 cm soil depth in samples from Kasalgu (Table 4) is consistent with Tangjang *et al*, (2009), who reported that top soil of sloppy lands tends to record lower organic matter content due to erosion losses from the hill slope during heavy rains. High organic carbon in the deeper soils in Gurugu is in agreement with Tangjang *et al*, (2009) who observed high concentration of organic matter in deeper depths of loamy sand soil, which they attributed to higher rate of infiltration. Higher concentration of nitrogen in shea nut cake polluted soil than unpolluted soil (Table 5) is in agreement with Cathcart *et al* (2003), who associated the concentration of nitrogen in soil with soil organic matter content, which increases as the organic matter increases. Previous research reported that bacteria are naturally present in soil, rapidly increasing in number in hydrocarbon polluted soil (Ray, 1994; Truong Son, 2005). In the current study, the mean of log of bacteria colony forming units g^{-1} soil dry weight in Shea Nut Cake contaminated soil ranged from 5.66 to 7.94 and in uncontaminated soil from 5.50 to 7.63 (Table 7) indicating higher bacteria population in contaminated soils than uncontaminated soils. In another report, Ebuehi *et al*., 2005 noted 1.22×10^{8} cfu g^{-1} soil dry weight and 3.0×10^{4} cfu g^{-1} soil dry weight for total heterotrophic and hydrocarbon utilizing bacteria from hydrocarbon contaminated and uncontaminated soils respectively. Higher bacterial count in the 0-20 cm depth is in agreement with previous reports of Bahiq *et al*., (2008) who observed higher bacterial populations in the surface soil than the deeper depths. Significant seasonal variations observed in bacteria populations in all three sites with higher bacteria counts in the rainy season than the dry season and highest in September (Table 8) are in agreement with Jha *et al.,* (1992) who recorded peak in bacteria population during rainy season, which they attributed to favourable soil moisture and temperature. Lower counts in dry season could be due to low moisture stress as observed by Tanjang *et al*., 2009 in winter. Highest fungi population in the 20 – 40cm depth soil in Kasalgu than top 0 -20cm depth observed in our study (Table 9) is in agreement with Tangjang *et al.,* (2009), which they attributed to run – off losses of fungal propagules with plant residue from the hill slope. Highest fungal counts in November (Table 10) could be due to the absence of running water of rains and are supported by Tanjang *et al*., 2009 who reported highest fungal populations in the post-rainy season month. Higher bacterial count than fungal counts in the study are consistent with Tangjang *et al*, 2009, who observed higher bacterial counts than fungi due to slower growth of fungi than bacteria. Negative soil moisture correlation results with bacteria in all sites are in agreement w*ith* Tangjang *et al* (2009), who in a previous study on native Traditional Agroforestry soil reported negative correlations of moisture with bacteria and fungi.

Negative correlation of pH with both bacteria and fungi in Jusonayilli and Gurugu in present study (Table 11) are also in agreement w*ith* Tangjang *et al* (2009), who reported negative correlations of pH with both bacteria and fungi. Tangjang *et al*, 2009 in the same study however observed significant positive correlations between carbon and nitrogen for bacteria and fungi which is the contrary in the current study. This could be that carbon and nitrogen in shea nut cake contaminated soil on level grounds of Jusonayilli and Gurugu are in abundance and not limiting factors as occur in native soil whose nitrogen and carbon are depleted faster than replenished. However positive correlation results of nitrogen with fungi in Kasalgu agrees with Tangjang *et al*, 2009 and could be due to run-off losses of shea nut cake from the hill slope during rains.

Conclusion

The study observed that bacteria and fungi were naturally present in shea nut cake polluted soil.

Shea nut cake increased soil pH towards alkalinity. Shea nut cake added organic matter and nutrients to the soil, which increased bacteria and fungi populations in shea nut cake polluted soil.

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References

Bahig A. E., Aly E. A., Khaled A. A., Amel K. A. (2008). Isolation, characterization and EcoI application of bacterial population from agricultural soil at Sohag Province, Egypt. Malaysian j. Microb. 4(2): 42-50.

Cathcart J. (2003). Soil Organic Matter. Agriculture and rural development, AESA Soil Quality Program Resources Sciences Branch, Alberta Agriculture and Food, Alberta ca.FS2001-1SQ

Ebuehi O. A. T., Abibo I. B., Shekwolo P.D., Sigismund K. I., Adoki A., Okoro I. C. (2005). Remediation of crude oil contaminated soil by enhanced natural attenuation technique. J. Appl. Sci. Environ. Mgt. 9:103-106.

Hall J.B., Aebischer D.P., Tomlison H.F.,Osei-Amaning E., Hindle J.R. (1996). Vitellariaparadoxa. A Monograph. School of Agricultural and Forest Sciences. University of Wales, Bangor, **8:**105pp

Iddrisu A. (2013). Biochemical and Microbiological Analysis of Shea nut cake: A waste product from Shea butter processing. MPhil Thesis, KNUST, Kumasi. African Journal of Biotechnology

Jha D.K., Sharma G. D. and Mishra R. R. (1992). Soil microbial population numbers and enzyme activities in relation to altitudes and forest degradation. Soil Biol. Biochem. 24: 761-767

Ray G. (1994). Bioremediation and its application to Exxon Valdez Oil Spill in Alaska. Ray's Environmental Science Web Site

Tangyang S, Arunachalam K, Arunachalam A and Shukla A.K. (2009). Microbial population dynamics of soil under traditional agroforestry systems in Northeast India. Res. J. Soil Biol. 1:1

Truong Son Pham (2005). Hydrocarbon degrading bacteria- in the search for potential species. TIN TÚ`C Tin Tú`c/Tin nôibô/ Chi tiêt.