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ARTICLE

Biostimulation of Crude Oil Contaminated Soil Using Soybean Waste

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The potential of soybean waste (SBW) in enhancing microbial breakdown of crude oil in soil was studied. The soybean waste contained 5.31% nitrogen and 33.2% crude protein while the soil used for the bioremediation study contained 1.89% organic matter, 1.10% organic carbon, 0.21% nitrogen and 0.08 ppm phosphorus. The textural composition of the soil was 76.58% sand, 8.28% silt, 15.16% clay and the soil was classified as sandy loam. The microbiological analysis revealed that aerobic heterotrophic bacteria counts in oil polluted soil amended with SBW ranged between 1.6×10^8 cfu/g and 8.9×10^8 cfu/g. These counts were higher than those of the unamended soil which ranged from 1.2×10^8 to 3.6×10^8 cfu/g. The crude oil utilizing bacteria counts ranged between 1.2×10^8 cfu/g and 5.3×10^2 cfu/g, while the fungi counts ranged between 2.0×10^2 cfu/g and 9.5×10^2 cfu/g. The crude oil utilizing bacteria identified in soil amended with SBW were species of *Bacillus*, *Acinetobacter*, and *Pseudomonas* and the fungi were species of *Aspergillus, Mucor*, and *Cephalosporium*. Amendment of the soil with SBW raised the exchangeable cations, nitrogen and phosphorus contents of the soil. Crude oil polluted soil amended with SBW also had higher amount of organic carbon than the unamended soil. The extent of crude oil degradation in oil polluted soil amended with 200 g SBW, 400 g SBW and in unamended soil were 54.6, 70.4% and 8.8% respectively after 28 days. The values were significantly different ($P < 0.05$). The results of this study suggest that soybean wastes are good enhancers of crude oil biodegradation in the soil and therefore, can be used in reclaiming crude oil polluted soil.

KEYWORDS: Bioremediation, Crude Oil, Pollution, Waste, Soybean.

1. INTRODUCTION

Soil contamination and its adverse effect on the overall ecosystem is one of the major problems we are facing today.[1] Oil released into the environment is a well-recognized problem in today's world. Oil spills affect many species of plants and animals in the environment, as well as humans.[2] Oil pollution prevents normal oxygen supply and exchange between soil and atmosphere due to the hydrophobic properties of oil.[3]

In Nigeria, most of the terrestrial ecosystem and the shores in oil producing communities are important agricultural land under continuous cultivation. Contact with crude oil results in the damage of the soil condition of this agricultural land, and of microorganisms and plants.[4] Crude oil has a coagulatory effect on soil; it binds the soil particles and hence, reduces aeration. Therefore, seed sown on such soils will fail to germinate. Heavily contaminated soils may remain unproductive for months or years until the oil has been degraded to tolerable levels. Oil has adverse effects on plant growth, which may be root stress, morphological aberration and reduction in biomass. Crude oil contamination of agricultural soils has greatly affected food production particularly in oil producing areas. Also, the use of cross country underground pipelines to convey crude oil and/or refined petroleum products to different parts of Nigeria has led to more frequent cases of farmland contamination through pipe rupture and spillage. Oil contamination in soils results in an imbalance of the carbon to nitrogen ratios. This causes a nitrogen deficiency, which not only retards the growth of agriculturally important microorganisms but even of plants grown on such soils.[5]

Microbial degradation is the major mechanism for the elimination of oil spills from the environment. The ability to actively break down specific fractions of petroleum oil is shown by many microorganisms.[6] As means of reclaiming or remediation of soil polluted with crude oil various technologies have been employed, among which, is the use of soil amendment or additives, such as chicken droppings, and inorganic fertilizer.[7,8] A soil amendment is any material added to a soil to improve its physical properties such as water retention, permeability, water infiltration, drainage, aeration and structure.[9] Certain microbes

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show an increase in population due to the use of petroleum hydrocarbons as nutrients. Such species are commonly being used for the remediation of a contaminated site.[10]

In Nigeria, oil spills occur at an alarming rate and inorganic fertilizers that usually would have been used for the cleanup of the oil spills are expensive and insufficient for agriculture. Therefore, an unconventional alternative, which is cheap and is rich in nitrogen and phosphorus necessary for microbial degradation of oil spills, is sought for. Soybean wastes satisfy these conditions. Besides, soybean wastes are readily available in local food processing houses in Nigeria. The aim of the study was to evaluate the potential of soybean wastes in enhancing the biodegradation of crude oil in the soil. Crude oil degrading microorganisms in the soil were also identified.

2. LITERATURE REVIEW

Biostimulation could be perceived as including the introduction of adequate amounts of water, nutrients, and oxygen into the soil, in order to enhance the activity of indigenous microbial degraders or to promote co-metabolism.[11]

Although the diversity of the natural microbial populations often means that the potential for waste remediation exists at polluted sites, factors such as absence of electron acceptors or donors, low nitrogen or phosphorus availability, or a lack of induction of the metabolic pathways responsible for degradation can inhibit waste remediation. In these cases, addition of exogenous nutrients can enhance the degradation of waste, a process known as biostimulation. Biostimulation of *in situ* microbial communities has been used to enhance the degradation of crude oil, tetrachloroethene, diesel fuel and polyaromatic hydrocarbons.[12]

Following an oil pollution, nutrients are rapidly assimilated by soil microorganisms thus depleting the nutrient reserves.[13] Therefore, apart from the environmental problem caused by oil pollution, agronomic and economic aspects are significant. The objective of using amendments is to augment the native fertility status of such soil and to enhance the rate of oil degradation, thus minimizing the contamination of scarce groundwater sources and to improve crop production.[14] The addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process.[15,16] The positive effects of nitrogen amendment by using nitrogenous fertilizer on the microbial activity and/or on petroleum hydrocarbon degradation have been widely demonstrated.[11,12,17,18] In most soil bioremediation studies, inorganic chemical fertilizers have been widely used as biostimulating agent, however, their use is relatively scarce and costly as well as the supply is not sufficient for agriculture due to high demand, let alone for cleaning oil spills.[19] Therefore, the search for cheaper and environmentally friendly options of enhancing the petroleum hydrocarbon degradation through biostimulation has been the focus of research in recent times.[19,20] One of such option is the use of organic wastes derived from plants and animals. Few workers have investigated the potential use of organic wastes from plants such as rice husk and coconut shells,[20] plantain peels, cocoa pod husk,[21] and *Molinga oleifera*. Animal organic wastes like cow dung, pig dung, poultry manure and goat dung[7,18,22] as biostimulating agents in the cleanup of soil contaminated with petroleum hydrocarbons were found to show a positive influence on the petroleum hydrocarbon biodegradation in the polluted environment.

3. MATERIALS AND METHODS

3.1. Collection and Processing of Samples

Bonny (Nigeria) light crude oil was obtained from the Shell Petroleum Development Company of Nigeria Limited (SPDC), Port Harcourt, Nigeria. Soybeans were obtained from the Minna Central Market, Minna, Niger State, Nigeria and processed to obtain the soybean wastes (SBW). The soybean wastes were sun dried for 7 days, ground into fine powder so as to be able to pass through a sieve of 2 mm mesh size and stored in a polythene bag until required. The soil sample used was collected from a farmland located at Bosso, Niger State, Nigeria. The soil sample was sieved with a 2 mm mesh size sieve before use. The microbiological and physicochemical properties of the soybean waste and the soil are presented in Table I.

3.2. Experimental Design and Treatment

Two thousand grammes (2000 g) of soil were introduced into each of 4 experimental pots (EP) with the following treatment options: Experimental pot 1 (EP1) had a soil sample only and served as control 1. EP2 had soil plus 200ml of crude oil (control 2), EP3 had soil plus 200 ml of crude oil and 200 g of soybean waste while EP4 had soil plus 200 ml of crude oil and 400 g of soybean waste. The experiment was set up in duplicates and incubated at room temperature (28 ± 2 °C). The soil samples were analyzed for the following parameters (listed below) at 7 days intervals for a duration of 28 days.

Table I. Microbial and physicochemical properties of soil and soybean waste used.

Parameter	Soil	Soybean waste
Aerobic heterotrophic bacteria	2.7×10^6 cfu/g	1.5×10^6 cfu/g
Crude oil utilizing bacteria	3.2×10^2 cfu/g	2.5×10^2 cfu/g
Fungi	6.0×10^1 cfu/g	3.3×10^1 cfu/g
pH	6.21	8.20
Moisture (%)	9.08	1.89
Nitrogen (%)	0.21	5.31

Cfu/g: Colony forming unit per gramme.

3.3. Enumeration of Microorganisms

Aerobic heterotrophic bacteria and fungi were enumerated by spread inoculating 0.1 ml of the serially diluted sample onto nutrient agar (NA) and Sabouraud dextrose agar (SDA) respectively. The nutrient agar (NA) plates were incubated at 30 °C for 48 hours while the SDA plates were incubated at room temperature (28 ± 2 °C) for 72 hours. Crude oil degrading bacteria (CDB) were enumerated on oil agar, OA (1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4 \cdot 7H_2O$, 1.2 g KH_2PO4, 6.04 g $FeSO_4$,$7H_2O$, 0.1 g NaCl, 20 g agar, 1 percent crude oil in 1000 ml distilled water, pH 7.4). The oil agar plates were incubated at 30 °C for 5 days. The colonies that developed after incubation were counted and expressed as colony forming units per gram (cfu/g) of sample. The bacteria and fungi isolates were subcultured repeatedly on fresh NA and SDA, respectively to obtain pure cultures, which were maintained on agar slants for further characterization and identification.

3.4. Characterization and Identification of Bacteria and Fungi Isolates

The bacterial isolates were characterized using gram staining and biochemical tests including sugar utilization profiles. The isolates were identified by comparing their characteristics with those of known taxa as outlined in Bergey's Manual of Systematic Bacteriology.[23] The fungi isolates were characterized based on macroscopic and microscopic examination[7] and identified using the scheme of Murphy and Philey.[24]

3.5. Determination of pH

The pH of both contaminated and control (uncontaminated) soil was determined using a pH meter (Crison micro pH 2000 Model). The pH was determined by suspending 10 g of the soil sample in 25 ml of distilled water, stirred with a glass rod and mixed well. The pH meter was standardized with buffer solutions of pH = 4 and pH = 7, respectively. The pH of the soil was determined in duplicates.

3.6. Determination of Moisture

The moisture content of soybean waste and soil was determined using the dry weight method. A crucible was dried in an oven at 80 °C for a few minutes, cooled in a desiccator and weighed (W_1). Ten grammes (10 g) of the soil samples were introduced into each crucible (W_2). The crucibles with the samples were dried in an oven at 80 °C until constant weight was reached and quickly transferred to a desiccator to cool and weighed quickly with minimum exposure to the atmosphere (W_3). The loss in weight of the samples during drying is the moisture content. It was calculated using the formula:

$$\text{Moisture contents} = \frac{W_3 - W_1}{W_2 - W_1} \times 100\%$$

3.7. Determination of Nitrogen, Phosphorus and Organic Carbon

The nitrogen content of the soil used for bioremediation, and of the organic wastes was determined using the Kjeldahl method, while the available phosphorus and organic carbon were determined using the methods described by Black[25] and the method outlined by Bossert and Bartha[26] respectively.

4. BIODEGRADATION

4.1. Biodegradation of Crude Oil in Soil

The amount of crude oil degraded in the soil was determined using the weight loss method of Ijah and Ukpe[27] by suspending 10 g of soil in 25 ml of diethyl ether in an Erlenmeyer flask. It was shaken vigorously to extract the oil. The solvent oil mixture was transferred into a preweighed beaker. This was done until all oil was extracted from the soil. The solvent oil mixture was exposed at room temperature overnight to allow the solvent to evaporate completely. The weight of the beaker containing the residual oil was recorded and the percentage of oil degraded (biodeg) was obtained as ratio of the weights of the oil samples,[28]

$$\text{biodeg} = \frac{\text{crude oil (control)} - \text{crude oil (degraded)}}{\text{crude oil (control)}} \times 100\%$$

4.2. Determination of Crude Oil Utilization by Microbial Isolates

The method of Zajic, and Supplisson[29] was followed in this experiment. 5 ml of mineral salt medium was dispensed into each bottle containing 0.05 ml of crude oil.[30] After sterilization at 121 °C for 15 minutes, the medium was allowed to cool before being inoculated with 0.1 ml of nutrient broth of the grown culture of crude oil degrading bacterial isolates. The experiments were incubated at room temperature (28 ± 2 °C) for 21 days. The turbidity, which developed as a result of bacterial growth (if any), was monitored visually at the end of the incubation period and assigned + to + + +, depending on the degree of turbidity.[31]

5. RESULTS AND DISCUSSION

The counts of aerobic heterotrophic bacteria (AHB) was 1.5×10^8 cfu/g while that of the soil was 2.7×10^8 cfu/g. Crude oil utilizing bacteria and fungi counts were 2.5×10^2 cfu/g and 3.3×10^1 cfu/g in soybean waste and 3.2×10^2 cfu/g and 6.0×10^1 cfu/g in soil, respectively. Species of *Bacillus, Pseudomonas, Micrococcus* and *Acinetobacter* were identified. It was observed that species of *Bacillus* were more frequently isolated than *Pseudomonas* and *Micrococcus*. Fungi were isolated and identified as species of *Aspergillus, Cephalosporium* and *Mucor*. The pH of the soybean waste was 8.20 while that of the soil was 6.21.

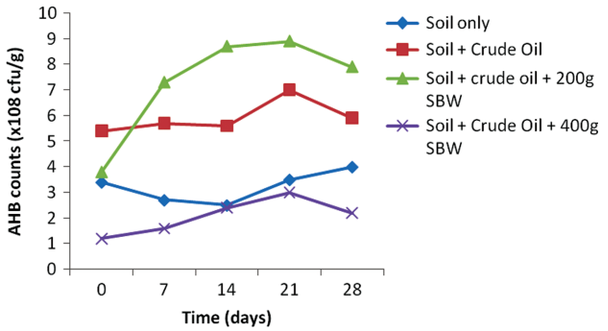

Fig. 1. Aerobic heterotrophic bacteria (AHB) counts in crude oil polluted soil amended with soybean waste (SBW).

The soybean waste had a moisture content of 1.89% while that of the soil was 9.08%. The nitrogen content of the soybean waste was 5.31% while that of the soil was 0.21% (Table I).

Soil amended with soybean waste (SBW) had higher counts of aerobic heterotrophic bacteria AHB (Fig. 1), crude oil utilizing bacteria CUB (Fig. 2) and fungi (Fig. 3) than the unamended soil. CUB counts for oil polluted (unamended) soil and for amended polluted soils were slightly higher than for the unpolluted control soil. It was observed that crude oil inhibited the proliferation of fungi in the soil (Fig. 3). The inhibition was overcome by the amendment of the polluted soil with SBW. The results revealed that fungi counts in oil polluted soil amended with either 200 g or 400 g SBW were significantly ($P < 0.05$) higher than those of the unpolluted control soil and the amended oil polluted soil.

The results in the present study indicate an increase in the counts of aerobic heterotrophic bacteria and fungi in soil amended with soybean waste. This agrees with the findings of Abioye et al.,[16] who recorded similar results. The counts of hydrocarbon utilizing microorganisms in the soil amended with organic wastes were appreciably higher as compared to those of unamended and poisoned control soil. The reason for the higher counts of bacteria in amended soil might be the result of the presence of appreciable quantities of nitrogen and phosphorus in the organic

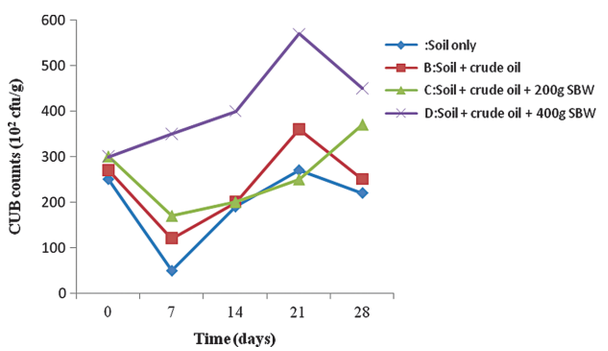

Fig. 2. Crude oil utilizing bacterial (CUB) counts in crude oil polluted soil amended with soybean waste (SBW).

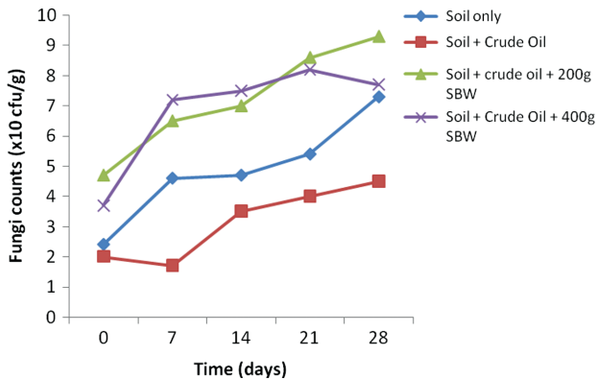

Fig. 3. Fungi counts in crude oil polluted soil amended with soybean waste (SWB).

wastes, especially the high nitrogen content in the SBW, which are necessary nutrients for microbial biodegradative activities. One of the criteria that were considered in choosing materials for oil spill remediation is the nutrient status of the materials and the presence of hydrocarbon degrading microorganisms in them.[32]

The microbial isolates were able to utilize crude oil as sole source of carbon and energy. The crude oil utilizing bacteria identified were species of *Pseudomonas*, *Bacillus*, *Micrococcus* and *Acinetobacter* (Table II). These bacteria have been implicated in crude oil degradation by other investigators.[27, 33–35] *Bacillus* sp. and *Aspergillus* sp. were more abundant than other bacteria. This is probably due to the fact that *Bacillus* form spores, which help the organisms to survive harsh conditions such as the sun-drying process used in processing the SBW utilized in the present work. The following fungi species were identified in the soybean waste; *Aspergillus niger, Mucor mucedo* and *Cephalosporium acremonium*. These organisms have been isolated from bioremediation materials such as periwinkle shells, poultry manure and groundnut shells and identified as crude oil utilizers.[35–38]

The rates of biodegradation of crude oil in soil amended with soybean waste (SBW) increased gradually with time throughout the period of the study (Fig. 4). It was observed

Table II. Utilization of crude oil by microbial isolates.

Microorganism	Growth of microorganisms in crude oil medium after 21 days
Bacillus subtilis	+++
Bacillus megaterium	++
Bacillus coagulans	++
Pseudomonas aeruginosa	+++
Micrococcus kristinae	+
Acinetobacter calcoaceticus	++
Aspergillus niger	+++
Cephalosporium acremonium	+
Mucor mucedo	++

+++: Maximum growth, ++: moderate growth, +: minimal growth.

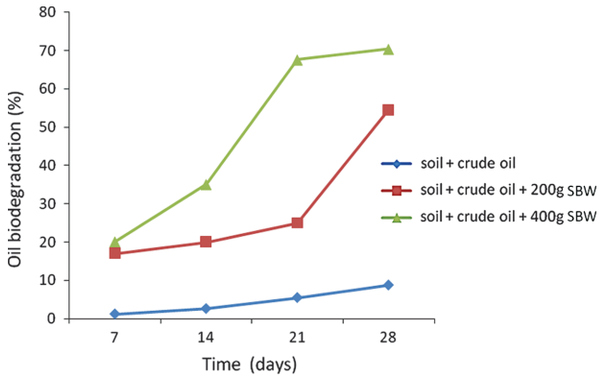

Fig. 4. Crude oil loss in oil polluted soil amended with soybean waste (SBW).

that after 28 days 70.4% of crude oil was degraded in soil amended with 400 g of SBW as compared to 8.8% biodegradation in unamended soil. Similarly, 54.6% of crude oil was degraded in soil amended with 200 g of SBW.

Biostimulation studies revealed that the rates of oil breakdown in oil polluted soil amended with soybean waste (SBW) increased with time. In unamended polluted soil, 8.8% of the oil was biodegraded after 28 days whereas in polluted soil amended with soybean waste, 54.6% (for polluted soil amended with 200 g SBW) and 70.4% (for polluted soil amended with 400 g SBW), of the oil was degraded within the same period of time. Statistical analysis using the analysis of variance (ANOVA) indicated that the values were significantly different ($P < 0.05$). Thus, 400 g of SBW amendment enhanced the crude oil biodegradation more than the 200 g SBW (Fig. 4). This may be due to the high nitrogen (5.31%) and protein (33.2%) content of the soybean waste, which might have been easily released into the soil to favour the growth of crude oil utilizing bacteria in the amended soil. Abioye et al.[16] and Agbor et al.[21] also reported similar findings when they identified the high nitrogen content in brewery spent grain, melon shell and cocoa pod/plantain peels, respectively as one of the most important nutrient for effective bioremediation to take place. 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. It promotes sufficient aeration for the indigenous bacteria present in the soil, thereby enhancing their metabolic activities in the contaminated soil. It might as well be due to the ability of these organic wastes to neutralize the toxic effects of the oil on the microbial population by rapid improvement of the soil physicochemical properties.[39]

The results revealed that the pH of unpolluted soil ranged between 4.19 and 7.10 while that of unamended oil polluted soil ranged between 3.05 and 6.68 after 28 days (Table III). The pH-values of the soil amended with 200 g

Table III. pH of crude oil polluted soil amended with soybean waste (SBW).

	pH values			
Time (days)	A	B	C	D
0	4.19	3.05	5.52	5.75
14	7.00	6.60	6.39	6.07
28	7.10	6.68	6.30	6.66

Key: A = Soil only, B = Soil + crude oil, C = Soil + crude oil + 200 g SBW, D = Soil + crude oil + 400 g SBW.

SBW ranged from 5.52 to 6.39 and that of soil amended with 400 g SBW ranged from 5.75 to 6.66 over the same period. The pH of the polluted soil amended with soybean wastes fell within the acidic range, while that of amended soil wasless acidic than that of the unamended soil. This may have been one of the conditions that increased the rate of biodegradation of crude oil in amended soil since crude oil degrading microbes grow and utilize hydrocarbons better at near neutral to alkaline pH-values. The acidic pH must be due to accumulation of acidic metabolites in the soil resulting from biodegradation of the oil pollutant.[28]

Figure 5 shows the organic carbon (OC) level of crude oil polluted soil amended with soybean waste. The organic carbon levels of the oil polluted soil and the oil polluted soil amended with SBW were considerably higher than that of the unpolluted control soil between zero and 14 days of study. It was observed however, that after 14 days, the organic carbon content of the unpolluted control soil increased greatly while that of the contaminated soil amended with 400 g SBW decreased sharply. Other treatments maintained a slight increase in the organic carbon level from 14 to 28 days. A high concentration of organic carbon is an indication of soil contamination. This was deduced from the fact that the values of OC increased after contamination with crude oil. The higher concentrations of OC in the control suggested a relatively slow degradation of the crude oil, probably due to the poisoning

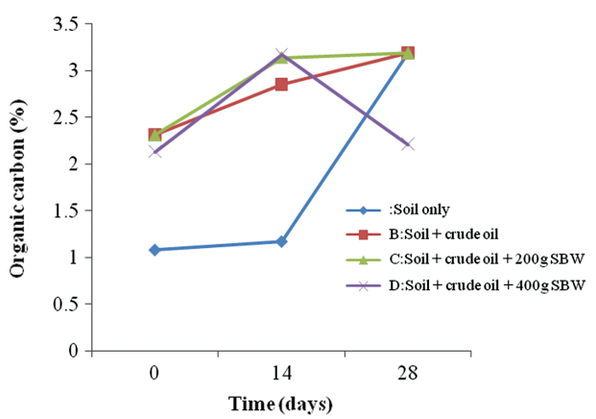

Fig. 5. Organic carbon of crude oil polluted soil amended with soybean waste (SBW).

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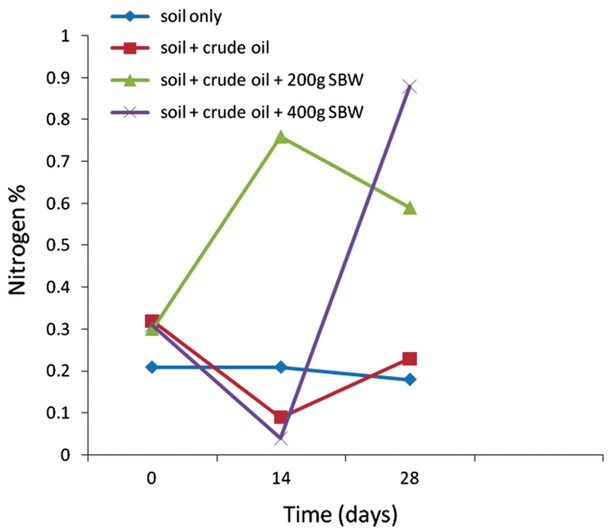

Fig. 6. Nitrogen content of crude oil polluted soil amended with soybean waste (SBW).

of the microbes and the presence of long chain hydrocarbons and/or lack of essential nutrient elements. This observation is in line with the report of Jidere et al.[36] that bioremediation of crude oil in a natural ecosystem is relatively slow.

The nitrogen content of the oil polluted soil amended with 200 g SBW was much higher than that of the unpolluted control soil. The same was observed for the oil contaminated soil as well as for the oil contaminated soil amended with 400 g SBW (Fig. 6). The results could be due to the fact that the nitrogen content of the soil has not been used by the microorganisms in the soil for the oil biodegradation process as much as in other treatments. Nitrogen is an important nutrient for microbial degradation of organic pollutants such as crude oil.[16, 31, 37] However, after 28 days, the oil polluted soil which received 400 g SBW amendment recorded the highest level of nitrogen probably due to the activities of nitrogen fixing bacteria in the soil and the nitrogen content of the SBW. Abioye et al.[31] made similar observations. The implication of this finding is that higher amounts of SBW may be required to remediate crude oil polluted soil.

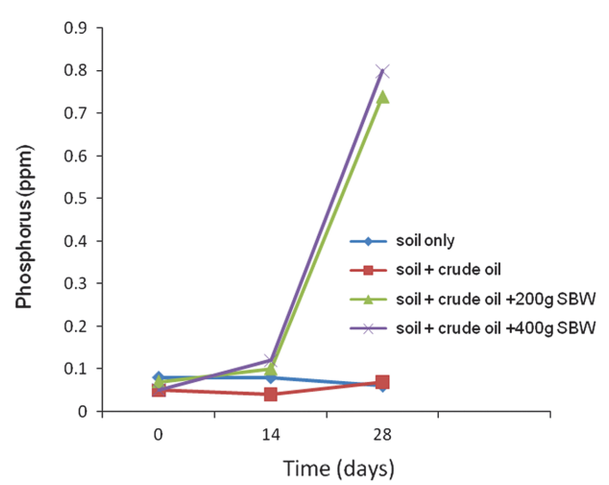

Fig. 7. Phosphorus content of crude oil polluted soil amended with soybean waste (SBW).

The phosphorus content of the soil in all treatments stayed low up to 28 days with the exception of the oil polluted soils amended with 200 g and 400 g SBW, which exhibited 0.7 ppm and 0.8 ppm of phosphorus, respectively after 28 days (Fig. 7). Agarry et al.[37] reported that crude oil biodegradation depended on phosphorus availability.

6. CONCLUSION

In the present study, soybean wastes increased the biodegradation of crude oil in soil, which implies that microbial degradation of crude oil has been enhanced. This enhancement could probably be due to the mineral nutrients and the crude oil degrading microorganisms present in the soybean wastes. In addition, the SBW raised the soil pH making it less acidic which could favour most crude oil degrading microorganisms. The various bacterial isolates degraded the crude oil at varying rates. Soybean wastes are good materials for reclaiming crude oil polluted soil. Their use in reclaiming soil polluted with oil will also solve the problem of waste disposal in the Nigerian environment. Soybean waste could be combined with other bioremediation material for a more effective bioremediation activity.

References and Notes

1. A. F. Umar, F. Tahir, M. Larkin, O. M. Oyawoye, B. L. Musa, M. B. Yerima, and E. B. Agbo, *Adv. Microbiol.* 2, 587 **(2012)**.
2. M. B. Yakubu, *A. J. Biotechnol.* 6, 2821 **(2007)**.
3. R. M. Atlas and R. Bartha, Microbial Ecology: Fundamental and Applications, 3rd edn., Addison-Westley Publishing Company **(1977)**, Reading.
4. C. I. Onuoha, A. E. Arinze, and A. E. Ataga, *Global J. on Agricultural Sci.* 2, 1596 **(2003)**.
5. M. B. Yerima, S. E. Agina, A. A. Zuru, K. Venil, H. M. Maishanu, A. L. Shinkafi, and Z. A. Kashim, *J. Sustainable Dev. Environ. Protect.* 1, 69 **(2011)**.
6. M. D. Makut and P. Ishaya, *A. J. Micro. Res.* 4, 1698 **(2010)**.
7. U. J. J. Ijah and S. P. Antai, *The Environ.* 23, 89 **(2003)**.
8. U. J. J. Ijah, H. Safiyanu, and O. P. Abioye, *Science World J.* 3, 63 **(2008)**.
9. J. G. Davis and C. R. Wilson, *Horticulture* 7, 235 **(2005)**.
10. S. Lotfinasabasl, V. R. Gunale, and N. S. Rajkumar, *Bioscience Discovery* 3, 186 **(2012)**.
11. R. G. Kanissery and G. K. Sims, *Applied Environ. Soil Sci.* 1 **(2011)**.
12. L. Cosgrove, P. L. McGeechan, P. S. Handley, and G. D. Robson, *Appl. Environ. Microbiol.* 76, 810 **(2010)**.
13. K. S. M. Rahman, I. M. Banat, J. Thahira, T. Thayumanavan, and P. Lakshmanaperumalsamy, *Bioresour. Technol.* 81, 25 **(2002)**.
14. A. Amadi, Effects of petroleum hydrocarbon on the ecology of soil microbial species and performance of maize and cassava, Ph.D. Thesis, University of Ibadan, Nigeria **(1990)**, p. 286.
15. R. Margesin, M. Hammerle, and D. Tscherko, *Microbiol. Ecol.* 53, 259 **(2007)**.
16. O. P. Abioye, A. Abdul Aziz, and P. Agamuthu, *Malaysian J. Sci.* 28, 127 **(2009)**.

17. S. B. Akinde and O. Obire, *World J. Microbiol. Biotechnol.* 24, 1999 **(2008)**.
18. S. E. Agarry, C. N. Owabor, and R. O. Yusuf, *Bioremediation J.* 14, 135 **(2010)**.
19. B. Y. Danjuma, S. Abdulsalam, and A. D. I. Sulaiman, *Int. J. Emerging Trends in Eng. & Dev.* 2, 478 **(2012)**.
20. R. O. Nyankanga, R. N. Onwonga, F. S. Wekesa, D. Nakimbugwe, D. Masinde, and J. Mugisha, *J. Agric. Sci.* 4, 223 **(2012)**.
21. R. B. Agbor, I. A. Ekpo, U. U. Udofia, E. C. Okpako, and E. B. Ekanem, *Arch. Appl. Sci. Res.* 4, 1372 **(2012)**.
22. N. R. Krieg and J. G. Holt, Bergey's Manual of Systemic Bacteriology, Williams and Wilkins Ltd., Baltimore **(1994)**.
23. C. J. Alexopoulus and C. W. Mims, Introductory Mycology, 3rd edn., Wiley, New York **(1979)**.
24. J. Murphy and J. A. Philey, *J. Anal. Chem.* 27, 31 **(1962)**.
25. C. A. Black, Method of Soil Analysis II, American Society of Agronomy, Madison **(1965)**, pp. 573–590.
26. I. Bossert and R. Bartha, The fate of petroleum in soil ecosystem. In: Petroleum Microbiology, edited by R. M. Atlas, Macmillan Publishing Company, New York **(1984)**, pp. 435–473.
27. U. J. J. Ijah and L. I. Ukpe, *Waste Manag.* 12, 55 **(1992)**.
28. O. Kokub, M. Shafeeq, Z. M. Khalid, and K. A. Malik, Isolation, screening and characterization of biosurfactant producing bacteria, edited by K. A. Malik, S. H. M. Nagui, and M. I. H. Afeem, *Proceedings of International Symposium of Biotechnology or Energy*, NIAB/NIBGE, Faibal, Pakistan, December **(1989)**, pp. 221–232.
29. E. Zajic and B. Supplisson, *Biotechnol. Bioeng.* 14, 331 **(1972)**.
30. U. J. J. Ijah and M. Ndana, *The Environ.* 23, 249 **(2003)**.
31. O. P. Abioye, P. Agamuthu, and A. R. AbdulAziz, *Biotechnol. Res. Int. 2012* 1 **(2012)**.
32. S. P. Antai and E. Mgbomo *Micro. Lett.* 40, 137 **(1989)**.
33. O. P. Abioye and U. J. J. Ijah, *BioTechnol.: An Indian Journal* 6, 115 **(2012)**.
34. V. I. Ibekwe, K. C. Ubochi, and E. U. Ezeji, *Environ. Sci. Technol.* 29, 7 **(2006)**.
35. D. Damisa, T. S. Oyegoke, U. J. J. Ijah, N. U. Adabara, J. D. Bala, and R. Abdulsalam, *Int. J. Applied Biology and Pharm. Technol.* 4, 136 **(2013)**.
36. C. M. Jidere and F. O. R. Akamigbo, *Agro-Science J. Trop. Agric., Food, Environ. and Extension* 8, 24 **(2009)**.
37. S. E. Agarry and L. A. Jimoda, *J. Environ. Earth Sci.* 3, 51 **(2013)**.

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