Biostimulatory Effect Of Processed Sewage Sludge In Bioremediation Of Engine Oil Contaminated Soils

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Abstract: A study was conducted to evaluate the influence of sewage sludge on biodegradation of engine oil in contaminated soil. Soil samples were collected from a mechanics workshop in Sokoto metropolis. The Soil samples were taken to the laboratory for isolation of engine oil degrading bacteria. About 1 g of soil sample was used to inoculate 9 ml of trypticase soy broth and incubated at 28^oC for 24 h. The growth obtained was sub-cultured in mineral salt medium overlaid with crude oil and allowed to stand at 28^oC for 72 h. The culture obtained was then maintained on tryticase soy agar plates at 28^oC for 48 h. A combination of microscopy and biochemical tests was carried out to identify the colonies. The sewage sludge was obtained from sewage collection point located behind Jibril Aminu Hall of Usmanu Danfodiyo University, Sokoto and processed (i.e. dried, grounded and sterilized). A portion of land obtained in a botanical garden was divided into small portions (30 X 30 cm) and the soil was excavated *in-situ* and sterilized in the laboratory. A polythene bag was subsequently used to demarcate between the sterilized soil and the garden soil. The sterilized soil plots were artificially contaminated with equal amount of used engine oil to represent a typical farmland oil spill. The plots were amended with various amount of processed sewage sludge i.e. 200 g, 300 g and 400 g respectively. A pure culture of the bacteria was maintained on trypticase soy broth and was introduced into the sterile amended soil. The plots were watered twice daily for ten days. The degree of biodegradation and heavy metal content were assessed using standard procedures and the results obtained indicate a remarkable reduction in poly aromatic hydrocarbons (PAHs), total petroleum hydrocarbon (TPH) and heavy metal content.

Keywords: Bioremediation, contamination, degrading bacteria, engine oil, poly aromatic hydrocarbons, sewage sludge, total petroleum hydrocarbon.

1 INTRODUCTION

Crude oil is a naturally occurring organic compound mixture that is mostly found below the earth surface (Gordon & Zakpaa, 2015). It is used as fuel and as raw materials in many chemical industries. For example processed crude oil and its related compound are used in manufacturing industries such as paint, plastics and to generate energy (Anderson & Labelle, 2000). Therefore crude oil needs to be transported from drilling point to processing industries. However, transport of petroleum occasionally lead to accidental spills. Oil spillage especially in large volumes can be harmful to wildlife and soil. Anderson & Labelle (2000) reported that, oil spillage can potentially occur for numerous reasons which might include equipment failure, deliberate acts, disaster or human error. Presence of high amount of soil pollution especially caused by oil spillage hinders agricultural activity and endangers life leading to lost in biodiversity.

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One of the worst human made environmental spills with over million gallons of crude oil released was reported during the Gulf War in 1991 covering an area of 49 square km (Salam, 1996). This type of spills eventually end up in both aquatic and terrestrial habitat renders a long term threat to living world. Thus, requires urgent attention is required to address this spillage with a cost-effective remediation technique. Bioremediation is an alternative treatment method compared to the physico-chemical clean-up methods as the latter is always accompanied by negative effects. Bioremediation processes involve the use of living organisms to remediate contaminated soil and water by adding additives or improving the availability of microorganisms (Swannell *et al.,* 1996; Nie *et al.*, 2009). Two different approaches are available for bioremediation (*in situ* and *ex situ*). *In situ technique* is based upon treatment of contaminated soil at the site of contamination, while *ex situ* requires the removal of contaminated soil to be treated in another location (Gavrilescu, 2010). In all cases, these techniques depend upon the level of saturation and aeration of the area which will determine whether bioaugumentation or biostimulation will be employed (Vidali, 2001). Bioaugumentation process involves the introduction of foreign microorganisms (i.e. seeding) that have the ability to utilise the crude oil. However, most microorganisms considered for seeding were isolated from previously contaminated sites. Similarly, different species of bacteria that have the potential ability to utilise crude oil were found and isolated in both aquatic and soil environment, as a result introduction of foreign species may render little or no help in treating oil contamination. Moreover literatures have shown that bioaugmentation (seed cultures) has less impact than addition of nutrient to indigenous microflora at the contaminated site (Atlas, 1995; Vidali, 2001). The most widely used bioremediation technique is biostimulation, which utilises the indigenous microflora supplemented with nutrients such as nitrogen, phosphorus and potassium. However, the ability of microorganisms to use crude oil as primary source of carbon depends on chemical and physical properties of the environment concerned as well as the crude oil composition. Hydrocarbon degradation in aquatic environments faces a lot of challenges and shortcomings due to limited availability of nutrients (Atlas, 1995), and it is generally accepted that, nutrient availability is the most common limiting factor (Atlas & Bartha, 1972; Kim *et al.,* 2004). Insufficient nutrient such as nitrogen, phosphorus and potassium generally limit microbial proliferation and result in slow or decline in growth. However, addition of inorganic substance to an oil polluted soil generally speeds up the rate of degradation of crude oil contaminated soil (Ijah *et al.,* 1997; Agamuthu *et al.*, 2013). In view of the cost of fertilizer and its prioritized used in agriculture, an alternative sources of nutrient is therefore required. Study by Ijah *et al.,* (1997) has shown the use of waste product from chicken to achieve high crude oil degradation of 18.7 % and 31.2 %, when crude oil contaminated soil was treated with chicken droppings and fertilizer respectively after 10 weeks. Also, treatment of crude oil contaminated soil with melon shells as nutrient source cut down the contamination level by 30 % when compared with an un-amended soil after 28 days (Abioye *et al.,* 2009). Moreover, study by Umar *et al.* (2012) on the effect of some selected waste in bioremediation of crude oil shows that, bioremediated soil using cow dung and chicken droppings have high removal rate of 41.51 % and 42.08 % respectively, amounting to more than 50 % when compared to control after 3 weeks. This concept reduces the rate of contamination by oil and provides avenue for 3 R's (Reduce, Recycle and Re-used) of waste products requiring less capital. Available literature has shown the efficacy of waste products from animals and plants for use in bioremediation. Therefore, extensive research is required to identify the best alternative waste for bioremediation process. Sewage sludge, though rich in nutrient is faced with sanitary problems and in trying to find the best alternative waste that contains a lot of nutrient for bioremediation with less economic, environmental and health effects (Alvarenga *et al.,* 2015; Peccia & Westerhoff, 2015). Sewage sludge may be considered as substitute due the fact that it mainly contains urine, soapy materials and household waste. Thus, this research is aimed at assessing sewage sludge as a source of nutrient for backing microorganisms to utilize the crude oil (hydrocarbon chain) as the primary source of carbon in bioremediation activity.

2. MATERIALS AND METHDOS

Sample Collection

Contaminated soil sample was collected from an open air automobile mechanic's workshop in Sokoto metropolis; the sample was taken to the laboratory for the isolation of engine oil degrading Bacteria. Sewage sludge was collected from a sewage collection point located behind Jibril Aminu Hall of Usmanu Danfodiyo University, Sokoto and the sewage was processed (i.e. dried, ground to powder and sterilized. Used engine oil was also collected from the mechanics workshop in Sokoto metropolis.

Isolation of crude oil degrading bacteria

Bacteria capable of utilising crude oil as primary source of carbon was isolated using standard protocol as describe in Liu *et al.,* (2010). About 1 g of contaminated soil sample was introduce into a bottle containing 9 ml of trypticase soy broth and incubated at 28^{o}C for 24 h. The growth obtained was sub-cultured in mineral salt medium (containing: 4.0g NH_4Cl, 1.8g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.1g NaCl, 1.2g KH_2PO_4, 0.01g$FeSO_4.7H_2O$ in a litre overlaid with crude oil as source of carbon. The culture was allowed to stand at 28^{o}C for 72 h and shaken to ensure continuous mix between the media and crude oil. The growth obtained was maintained on trypticase soy agar (TSA) at 28^{o}C for 48 h. Pure cultures of the bacteria isolated were established on TSA. Different biochemical tests were conducted using standard protocol as described in Cheesebrough, (2006), and the identity of bacteria was confirmed using charts (Barrow & Feltham, 2004).

Figure 1*: Shows the bioremediation site and plots with polythene bag*

Bioremediation

Bioremediation process was setup using protocol reported by Agamuthu *et al.,* (2013). A portion of land, obtained in the botanical garden was sub-divided into small portions of known dimensions (30 X 30 cm), as shown in Fig 1. The soil was excavated and sterilized in the laboratory by autoclaving. Then a demarcation was made between the sterilized soil and the garden soil using polythene bag. A deliberate contamination of the sterilize soil plots was done with equal amount of used engine oil to stimulate a typical farmland oil spill.

Figure 2*: Shows the bioremediation site and amending soil with the processed sewage sludge.*

Different amounts of processed sewage sludge with 200 g, 300 g, 400 g and control, were used to amend the engine oil contaminated soil plots (Fig 2). Pure culture of the bacterial isolate *Pseudomonas aeruginosa* was introduced into the plot to carry out the bioremediation process. The plots were constantly watered twice daily and left for ten days.

Analysis of bioremediated Soil

Heavy metals content of the bioremediated soil was analysed spectrophotometrically following pre-extraction of cations with dithionite as described by Osuji & Onojake (2004). The total petroleum hydrocarbon test and polycyclic aromatic hydrocarbon were carried out using standard protocols SW

EPA 846 and ASTM D-5765 as described in Schwab *et al.*, (1999). Residual oil was extracted from remediated soil and processed (cleaned) before subjecting to gas chromatography and mass spectrometry (GCMS).

3. RESULTS AND DISCUSSION

Microorganisms have diverse metabolic pathways which give them the ability to survive different conditions (Dorota & Andrzej, 2012). Microorganisms isolated in this work were mostly dominated by *Pseudomonas species;* this might be connected with its vast ability to utilize wide range of carbon source (Nwadinigwe & Onyeidu, 2012). The Bacteria isolated and identified includes; *Pseudomonas aeruginosa, Bacillus subtilis* and *Bacillus licheniformis.* Sewage sludge is believed to be nutrient rich which if processed can be utilized effectively as a source of growth facilitator for microorganisms to enable them sustain and carry out their metabolic activities. Sewage sludge is generally composed of urine, soapy material and household waste and other suspended particles that may eventually settle down under the gutter. These wastes are mostly laden with products that serve as source of nitrogen, phosphorus and potassium. Dorota & Andrzej (2012) reported that, *Pseudomonas aeroginosa* has the ability to produce enzymes that will degrade heavy metals and utilize the hydrocarbon as a source of energy.

***Table 1**: Heavy metal contents of engine oil contaminated soil seeded with various amount of sewage sludge.*

Heavy metals	Concentration (mg/kg)			
	200g	300g	400g	Control (0g)
Cobalt	6.29	7.01	6.53	8.33
Cadmium	0.71	0.51	0.62	1.01
Chromium	15.9	15.4	16.5	19.5
Magnesium	101.3	99.5	100.4	115.8

The results shows that all contaminated soil samples contain high amount of heavy metals as proposed by Osuji & Onojake (2004) in crude oil contaminated soil. The heavy metals content varied with different amount of sewage sludge (Fig 3), and contained mostly cobalt, cadmium, chromium and magnesium (Table 1). The heavy metals associated with the engine oil contaminate the soil and this was detected by analyzing the engine oil used during the contamination process. In addition to this, sewage sludge also contains trace of heavy metals, but the content is 80 % less than the engine oil used.

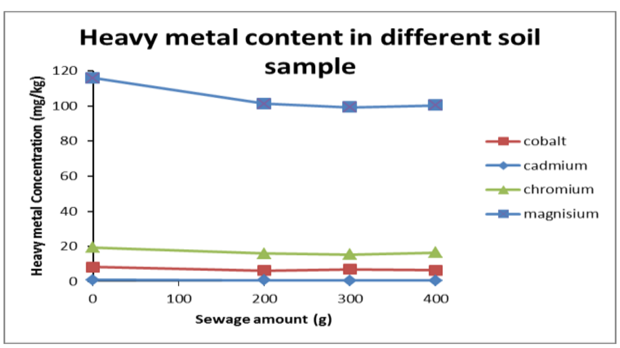

***Figure 3**: Shows the heavy metal content of the bioremediated soil seeded with different amount of sewage.*

The result also reveals high reduction in polycyclic aromatic hydrocarbons (Fig 5) and total petroleum hydrocarbon (Fig 4) of the bioremediated soil seeded with different amounts of sewage sludge (Table 2). The results showed that, sewage sludge increase the rate of biodegradation process of the engine oil.

***Table 2**: Total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) content of bioremediated Soil seeded with different amount of sewage sludge.*

Amended Soil Sample (g)	Concentration (mg/kg)	
	TPH	PAH
Control (engine oil)	15558	106
Soil amended with 200g of sewage	1432	59.6
Soil amended with 300g of sewage	1556	68.4
Soil amended with 400g of sewage	1605	82.4

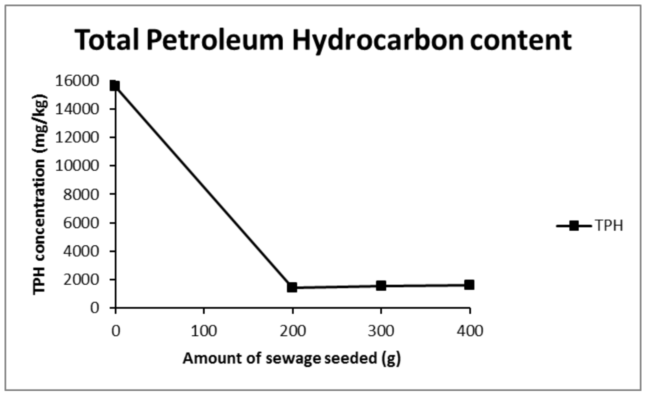

***Figure 4**: Shows the total petroleum hydrocarbon contain of the soil seeded with different amount of sewage sludge.*

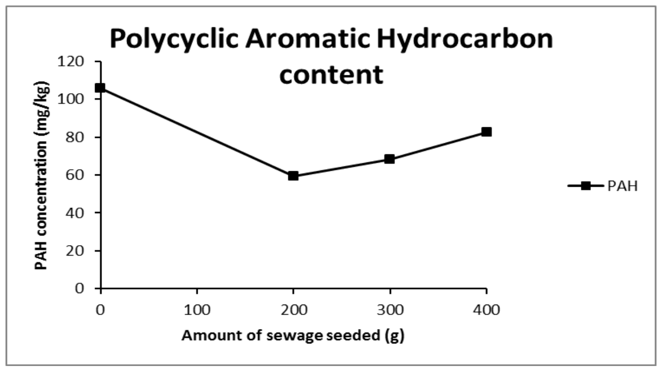

***Figure 5**: Shows the polycyclic aromatic hydrocarbon contain of the soil seeded with different amount of sewage sludge.*

From the result shown (Table 2) there is high rate of degradation which account for over 50 % of the polycyclic aromatic hydrocarbon and more than 90 % reduction in total petroleum hydrocarbon. This trend was observed in all the plots seeded with different amount of sewage sludge ($P < 0.05$). Though there is a slight variation with the plot containing 200 g of sewage sludge having higher degradation when compared with 300 g. The deviation might be connected with the amount of heavy metal content which hinders the growth of the bacteria (Faulwetter *et al.,* 2009). Microbial activity might be hindered or even tempered with in the presence of heavy metal elements such as cobalt, cadmium and chromium, which have the ability to inhibit enzymatic activity and slow or shut down the metabolic and growth activity of cells (Faulwetter *et al.,* 2009). Further experiment involving different microorganisms especially, those known to utilize both heavy metals and engine oil may further optimize the use of sewage sludge in bioremediation. The result is in agreement with a research conducted by Nwadinigwe & Onyeidu (2012) that studied bioremediation of crude oil polluted soil using bacteria and poultry manure monitored by Soya beans productions. It was discovered that bioaugmentation with bacteria and biostimulation with poultry manure gives an effective remediation process.

4. CONCLUSION

Based on the results, sewage sludge has significant effect in providing essential nutrient for bacteria to grow and utilize hydrocarbon, although high amount of heavy metal was observed. The metals might have come either from the sewage or the crude oil, thus further optimization and refinement of this simplistic, cost effective and environmentally sustainable model with potentials to control the rampant and localized oil spillages from industrial, household discharges, local vending and indiscriminate disposals by automobile mechanics in the developing world.

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