

Full Length Research Paper

# Managing physicochemical parameters in compost systems to enhance degradation of petroleum wastes from a sludge dam

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Physical, chemical and biological parameters were optimized during composting to enhance degradation of oil sludge. Mixtures of oil sludge, garden soil, poultry manure and the bulking agents were co-composted in static piles of about 1 m<sup>3</sup> on wooden pallets overlaid with nylon fibre sheets. Temperature, moisture, electrical conductivity (EC), pH, total carbon, total N, heterotrophs and respiration of compost microorganisms were monitored in each pile. Moisture was maintained at between 60 and 70% field capacity. Temperatures reached a mean of 63°C in the compost containing manure and sawdust. The C:N ratio of composts changed significantly during the composting process, reaching 100:41 (sawdust+manure) 100:39 (hay+manure) and 100:31 (woodchips+manure). Respiration of compost organisms rose from 1490 to 3850 CO<sub>2</sub> (µg)/dwt/day in the sawdust+manure compost. Total petroleum hydrocarbons (TPH) decreased by between 52 and 66% in the composts and concentrations of selected polycyclic aromatic hydrocarbons (PAH) by between 78 and 100%. The *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Staphylococcus* species were predominant in all the experiments and all temperature regimes.

**Key words:** Bulking agents, compost bioremediation, crude oil sludge, microorganism, polycyclic aromatic hydrocarbons (PAH), total petroleum hydrocarbons (TPH).

## INTRODUCTION

Large amounts of liquid effluents and solid wastes are generated from different stages in the petroleum refining process. These including waste waters from cleaning processes and storage tank sediments (Shie et al., 2004; Wang et al., 2010; Mandal et al., 2012). The resulting waste is a viscous oily sludge containing high amounts of petroleum derived hydrocarbons, including alkanes and paraffins of 1-40 carbon atoms, cycloalkanes, aromatic compounds with different numbers of benzene rings (US

EPA, 1997; Marin et al., 2006; Pakpahan et al., 2011), asphaltenes and resins (Diallo et al., 2000). Oil sludge from crude oil storage tanks typically consist of sulphides, phenols and heavy metals along with petroleum based hydrocarbons of the aliphatic and aromatic type in very high concentrations. Irrespective of the source, oil sludge contains large amounts of polycyclic aromatic hydrocarbons (PAHs), polychlorinated hydrocarbons and other organic compounds of environmental interest many

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**Abbreviations:** PAHs, Polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyls; TPH, total petroleum hydrocarbons; IRS, infrared spectroscopy; MSA, mineral salts agar; CTAB, cetyltrimethyl ammonium bromide; EDTA, ethylene diamine tetraacetic acid buffer; SDS, sodium dodecyl sulphate; PCR, polymerase chain reaction; PEG, polyethylene glycol; BLAST, basic local alignment search tool; EC, electrical conductivity.

of which are potentially carcinogenic, genotoxic, cytotoxic, mutagenic or of threat to humans (Bojes and Pope, 2007; Kriipsalu et al., 2008; Mandal et al., 2012; Jiang et al., 2013). The environmental impacts due to oil sludge contamination include physical and chemical alteration of natural habitats, lethal and sub-lethal effects on aquatic and terrestrial ecosystems. Thus, oil sludge is a hazardous complex of organic compounds (US EPA 1997; Liu et al., 2010) of environmental concern and should be treated as such.

The treatment and successful disposal of large amounts of oil sludge generated during crude oil refining constitute some of the major challenges faced by oil refineries and petrochemical industries (Srinivasarao et al., 2011). A number of treatment methods have been employed in treating oil sludge including physical, chemical, and biological methods (Mahmoud, 2004, Srinivasarao et al., 2011). These methods, however, have different challenges, which include high cost, production of toxic by-products and lack of available space for landfilling. Landfilling is also faced with the problems of managing leachates, emissions and burrowing animals that periodically expose the wastes (Srinivasarao et al., 2011). Biological methods have been lauded as the most environmentally friendly approaches for treating contaminated sites because they are nature-compatible, reliable, cheaper and easy to adopt compared to physical and chemical methods (Machin-Ramirez et al., 2008). The end products are usually harmless carbon dioxide, water and fatty acids. Oil sludge components, for example, organochlorines and high molecular mass PAHs, however, have continued to be resistant to such treatments, largely due to poor solubility of the sludge matrix in aqueous media. Most biological methods are economically unsound, as they are prone to prolonged periods of treatment, as such may not be suitable for treating some contaminants (Ward et al., 2003). Heavy hydrocarbon components such as high molecular weight PAHs are generally recalcitrant to most bioremediation treatments (Atagana, 2003) due to their low volatility, hydrophobicity and low water solubility which is largely responsible for their low availability to microbial actions hence their persistence in the environment (Kriipsalu et al., 2008; Cameotra and Makker, 2010; Liu et al., 2011).

Compost bioremediation is the application of composting technology in treating contaminants. It is an *ex situ* remediation technology which relies on mixing of organic materials with contaminants (Kriipsalu et al., 2008), a process referred to as co-composting, is becoming popular (Kriipsalu and Nammari, 2010; Srinivasarao et al., 2011). Compost have a rich and diverse microbial population, which could promote degradation of xenobiotic compounds such as polychlorinated biphenyls (PCB) and PAH, high temperatures generated by high metabolic activities of the microbial population, which is conducive for increased solubility of the organic substrate and availability of large amounts of organic substrate to co-metabolize while the

population is adapting the contaminant organic substrate. These characteristics have made composting an attractive option to bioremediation practitioners. Successful aerobic degradation depends on adequate temperature, aeration, and appropriate amounts of moisture (Suler and Finstein, 1997; Steger et al., 2007; Kriipsalu et al., 2008; Rasapoor et al 2009). The temperature profile of a composting system determines the type of microorganisms that would prevail in the compost matrix and subsequently the rate of degradation of organic substrates. The composting process tends to reach thermophilic levels easily thus promoting thermo-tolerant organisms. While this may be advantageous to the composting process, the challenges of enzyme denaturing cannot be overlooked. Hence it is essential to manage the temperature with aeration technics during composting to achieve the desired goals. In composting of contaminant organic substrates, the type and amounts of amendments used and the carbon to nitrogen (C:N) ratio is important, as these influences the degradation of the target compounds.

The aim of this study was to employ and manage the improved metabolic activities offered by the large microbial load, abundant nutrients and high temperatures available in a compost mixture and the enhanced aeration and carbon supplement offered by the bulking agents in improving the degradation of oil sludge.

## MATERIALS AND METHODS

### Oil sludge

Crude oil sludge was obtained from a sludge dam in a petroleum refinery in Durban, South Africa. The sludge contained (mg kg<sup>-1</sup>), TPH 300 592, total organic carbon 395, phenol 633, ammonia 65, chlorides 1, fluorides 1.8, sulphates 21.5, nitrates 23, lead 123, nickel 175, zinc 1445, cadmium 2.8, chromium 165 and copper 105. The sludge was stored at room temperature until required for the experiments.

### Soil

Soil was obtained from an open field, air dried, cleaned and homogenized before use in the experiments. The characteristics are pH 5.45, EC (dS cm<sup>-1</sup>) 2.85, total N 0.08%, total extractable phosphorus 4.75 mg kg<sup>-1</sup>, total organic carbon 5.3 g kg<sup>-1</sup>.

### Poultry manure

Poultry manure was obtained from the University of KwaZulu-Natal experimental farm in Pietermaritzburg.

### Bulking materials

Wood chips measuring between 3 and 5 cm in length were obtained from the yard of a wood-processing factory in Pietermaritzburg, South Africa. Sawdust was obtained from the same wood-processing factory as the woodchips. Hay was obtained from the University of KwaZulu-Natal experimental farm in Pietermaritzburg.

**Table 1.** Experimental design for the compost treatments.

Treatment	Oil sludge + soil	Wood chips	Sawdust	Hay	Poultry manure
1	X				
2	X	X			
3	X		X		
4	X			X	
5	X				X
6	X	X			X
7	X		X		X
8	X			X	X

### Compost experimental design

Sludge was mixed with soil in a ratio of 3:1 (sludge:soil) (w/w). This mixture was then used to formulate eight treatments labelled 1-8. Treatment 1 contained the sludge/soil mixture only and was designated the control. Treatment 2 contained the sludge/soil mixture and woodchips in a ratio of 1:1 (w/v). Treatment 3 contained the sludge/soil mixture and sawdust in a ratio of 1:1 (w/v). Treatment 4 contained the sludge/soil mixture and sawdust in a ratio of 1:1 (w/v). Treatment 5 contained the sludge/soil mixture and poultry manure in a ratio of 3:1 (w/w). Treatment 6 contained the sludge/soil/woodchips mixture and poultry manure in a ratio of 3:1 (w/w). Treatment 7 contained the sludge/soil/sawdust mixture and poultry manure in a ratio of 3:1 (w/w). Treatment 8 contained the sludge/soil/hay mixture and poultry manure in a ratio of 3:1 (w/w) (Table 1). All experiments were placed on wooden pallets (1m<sup>2</sup>), which were covered with nylon fibre sheets and incubated for six months. Each experiment was set up in triplicate. Moisture was maintained at between 60 and 70% field capacity. The compost heaps, except the control were turned with a garden fork once a week for aeration.

### Analytical procedures

Total petroleum hydrocarbons (TPH) was measured by infrared spectroscopy (IRS) using the EPA method 8440 (USEPA, 1997), PAH concentration by GC/MS and respiration of microorganisms was measured by CO<sub>2</sub> evolution by using the closed jar method. Electrical conductivity, pH, moisture and nutrients were measured by standard methods.

### Counts of compost microorganisms

Counts of culturable compost microorganisms were done by the plate count method for viable cells. 5 g of compost was shaken with 45 ml of 0.28% sodium pyrophosphate for 30 min at 10°C and 150 rpm. Dilutions prepared in 0.9% NaCl were spread onto nutrient agar plates to enumerate aerobic heterotrophs. Counts of heterotrophic microorganisms were done in colony forming units (CFU) monthly for the duration of the experiments.

### Isolation and characterisation of microorganisms from compost

The enrichment of organisms was done in mineral salts medium containing per litre KH<sub>2</sub>PO<sub>4</sub> 5g, MgSO<sub>4</sub>. 7H<sub>2</sub>O 5g, NaH<sub>2</sub>PO<sub>4</sub> 5g, NH<sub>4</sub>Cl 5g, NaCl 40g. Trace elements solutions containing per litre distilled water; 15 g FeCl<sub>2</sub>.H<sub>2</sub>O, 90 g NaCl, 19.7 g MnCl<sub>2</sub>. 4H<sub>2</sub>O, 9 g CaCl<sub>2</sub>, 23.8 g CoCl<sub>2</sub>.H<sub>2</sub>O, 0.17 g CuCl<sub>2</sub>.H<sub>2</sub>O, 28.7 g ZnSO<sub>4</sub>, 0.5

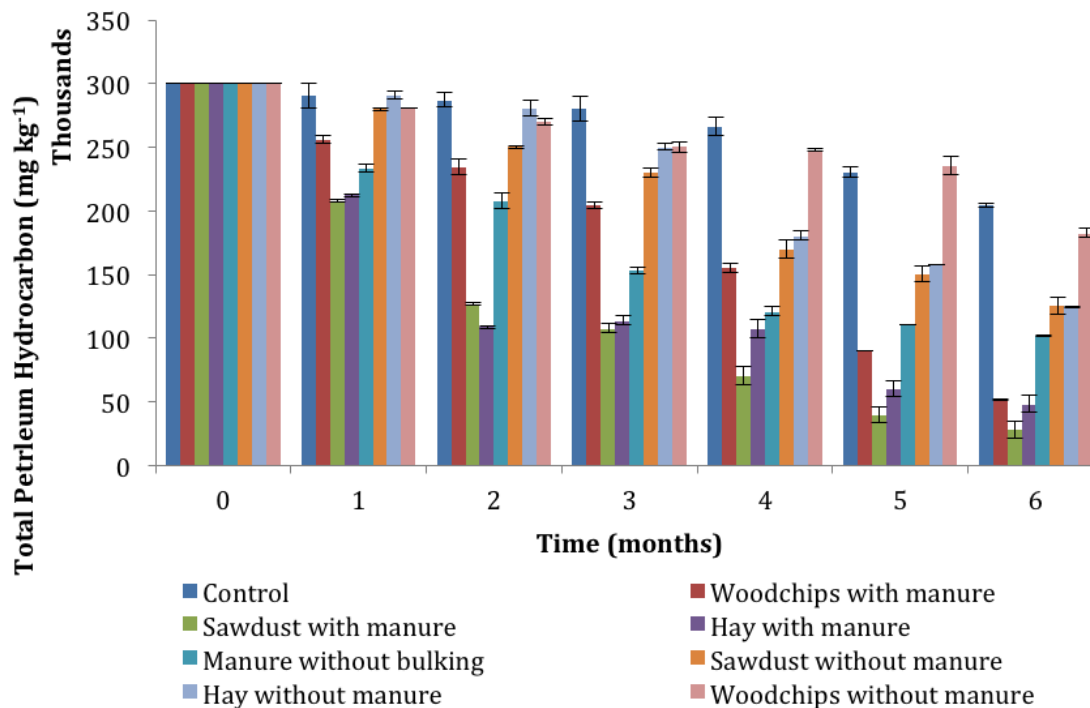
g AlCl<sub>3</sub>, 0.62 g H<sub>3</sub>BO<sub>3</sub>, 0.24 g NiCl<sub>2</sub>.6H<sub>2</sub>O, 10 ml of 10.18 M HCl (32%). About 15 g of homogenised compost was put in 250 ml Erlenmeyer flasks containing 100 ml sterile MSM spiked with 1 ml crude oil. The flasks were stoppered with cotton wool wrapped in aluminium foil and incubated in the dark at 28°C on a rotary shaker at 150 rpm for 21 days. Sub-cultures were made by aseptically transferring 1 ml of the culture into another set of 250 flasks containing 100 ml sterile MSM spiked with 1 ml crude oil, as the only source of carbon for the bacteria growth and incubated for another 21 days at 28°C in a rotary shaker in the dark. The process was repeated for the third time. Oil degrading bacteria were isolated from serial dilutions (10<sup>-8</sup>) of the enrichment culture. Aliquots (0.1 ml) of each of the dilutions 10<sup>-6</sup> to 10<sup>-8</sup> were inoculated onto mineral salts agar (MSA) plates overlaid with 50 µL of oil filter-sterilised through 0.2 µm Millipore filter membrane and incubated for 21-28 days at 28°C and checked daily for bacteria growth to avoid overcrowding of the plates. The enrichment and isolation procedures were adapted from Atagana (2003) and Mashreghi and Marialigeti (2005). Distinct colonies from the MSA plates were inoculated onto nutrient agar plates and incubated for three days at 28°C. Pure colonies were obtained from the selected colonies further purified by serial dilution and streaking to obtain single colonies.

### Genomic DNA extraction of the bacteria isolates

Genomic DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) method. The samples were centrifuged and the pellets were suspended in a solution containing 567 µl tris ethylene diamine tetraacetic acid buffer (tris EDTA), 30 µl 10% sodium dodecyl sulphate (SDS), 3 µl of proteinase K (20 mg/ml) and incubated in a digital dry bath incubator (Accu block™) at 65°C for 1 h. Then 180 µl 5 M NaCl and 80 µl 10% CTAB solutions were added and incubated at 65°C for 10 min. Then 400 µl each of phenol and chloroform were added to each tube. The tubes were centrifuged at 14000 rpm for 15 min and 300 µl of the supernatants was transferred into new sterile Eppendorf tubes and the DNA was precipitated by adding 0.6 volume of cold isopropanol to the tubes. The DNA pellets were collected by spinning at 14000 rpm for 15 min and washing with 200 µl 70% ethanol and spinning at 14000 rpm for 10 min. The DNA pellets were then air-dried and 100 µl TE buffer was added and incubated at 37°C for 60 min to dissolve the DNA pellets. Then 1 µl of RNAase was added to remove bacterial RNA and incubated at 37°C for 60 min. The DNA template was separated by electrophoresis with ethidium bromide (0.1 µg/ml)-stained 1% agarose gel running at 80 V for 60 min, using TAE electrophoresis buffer.

### Polymerease chain reaction (PCR) and sequencing

The PCR was performed using MJ Mini thermal cycler (Bio-Rad,



**Figure 1.** Changes in total petroleum hydrocarbons (TPH) ( $\text{mg kg}^{-1}$ ) during composting. Values are means of three  $\pm$  standard deviation.

Hercules, CA, USA). The PCR products were separated electrophoretically with ethidium bromide ( $0.1 \mu\text{g/ml}$ )-stained 1% agarose gel running at 80 V for 60 min, using TAE electrophoresis buffer. The PCR products ( $20 \mu\text{l}$  each) were cleaned by using  $160 \mu\text{l}$  13% polyethylene glycol (PEG) 8000,  $20 \mu\text{l}$  5 M NaCl solution and  $200 \mu\text{l}$  70% ethanol and sequenced.

#### Basic local alignment search tool (BLASTing) of DNA sequences

The sequences of 16S rDNA region obtained were edited using BioEdit software. The edited sequences were copied in a fasta format. Blasting was done on National Centre for Biotechnology Information (NCBI) website. This was to check and compare the sequences with those on the database (Adeleke et al., 2012).

## RESULTS AND DISCUSSION

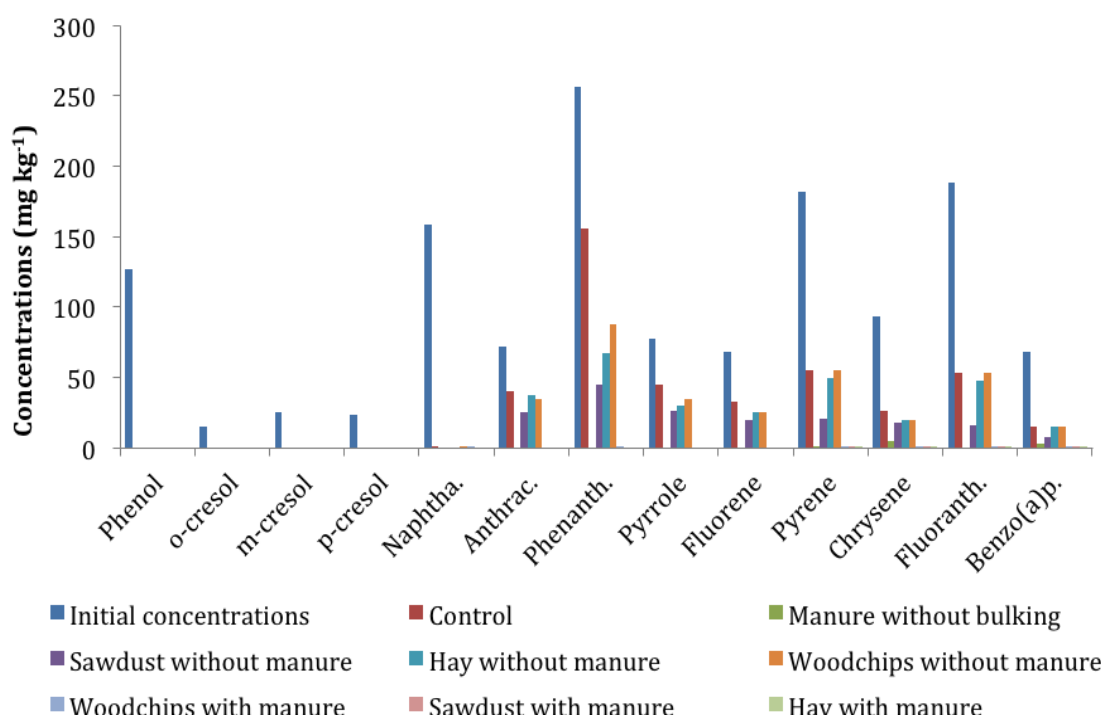
TPH was reduced in all compost experiments including the control during the six months of incubation. Reduction in TPH in the manure amended sawdust compost progressed more rapidly, reaching 30% reduction in the first month of incubation, as compared to 14 and 29% in the manure amended woodchips and hay compost respectively (Figure 1). Reduction in the non-manure experiments with bulking was not significantly different from the control at  $p = 0.05$  at the end of the first month of incubation. After the first month, reduction in the manure amended sawdust and hay composts progressed rapidly

and continued to do so for the rest of the incubation period. However, the manure amended sawdust compost continued to show larger reduction in TPH than the hay compost during the last three months of the composting period. The manure amended woodchips compost was slower in TPH reduction compared to sawdust and hay composts, despite the higher aeration probably due to slow rate of breakdown of wood material used for bulking. Poultry manure clearly enhance the reduction in TPH. This is possibly due to the nutrient composition of the manure, particularly the high nitrogen content, which is believed to have offset the initial nitrogen deficiency in the sludge-compost mixture (Table 2). Microorganisms involved in the metabolism of the carbon substrate present in the compost require adequate amounts of nitrogen, which was low in the sludge-compost. Thus the addition of poultry manure provided additional nitrogen, microorganisms and other nutrients to facilitate the early microbial activity observed in the compost.

The experiment amended with manure but without bulking showed comparable TPH reduction in the first month but gradually became slower probably due to the lack of bulking, which may have hindered aeration. At the end of the composting period, the experiments amended with manure showed the following TPH reduction: Sawdust 91%, hay 84%, woodchips 82% and the experiment amended with manure but no bulking 66%, as compared to the control, which showed a reduction of 32%. It is evident that inoculation of the compost with

**Table 2.** Changes in C:N ratio during composting. Values are means of three.

Composts treatment	Time (month)						
	0	1	2	3	4	5	6
Control	100:3	100:10	100:15	100:18	100:21	100:23	100:25
Woodchips without manure	100:3	100:10	100:15	100:16	100:20	100:21	100:24
Sawdust without manure	100:3	100:10	100:15	100:18	100:20	100:21	100:24
Hay without manure	100:3	100:11	100:12	100:16	100:18	100:22	100:25
Manure without bulking	100:5	100:15	100:20	100:20	100:31	100:28	100:28
Woodchips with manure	100:5	100:10	100:18	100:22	100:25	100:27	100:31
Sawdust with manure	100:23	100:27	100:30	100:37	100:35	100:37	100:41
Hay with manure	100:15	100:25	100:29	100:32	100:35	100:43	100:39

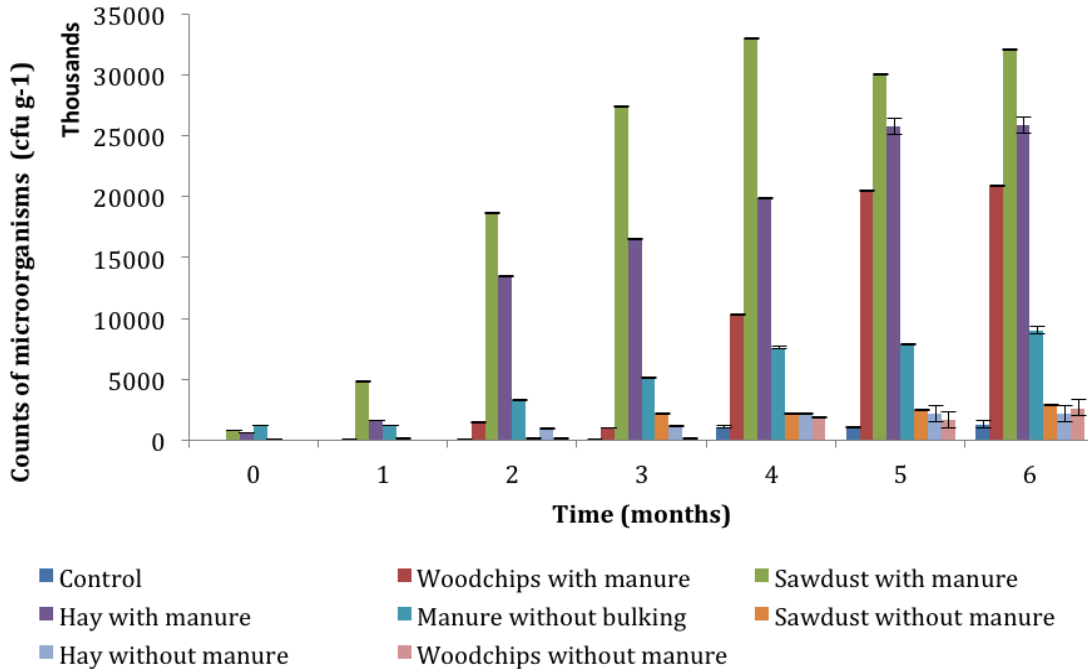
**Figure 2.** Final concentrations ( $\text{mg kg}^{-1}$ ) of PAHs in compost. Values are means of three  $\pm$  standard deviation.

poultry manure enhanced the rate of oil sludge degradation in the compost systems. The experiments containing bulking materials but no manure showed a reduction of between 39 and 58%. This is an indication that the bulking materials enhanced aeration, which was vital for the oxidation of the compounds present in the oil sludge. The bulking enhanced aeration, helped to maintain aerobic degradation of the sludge. However, the physical and chemical properties of the bulking agent played a role in enhancing degradation. The small particulate materials of the sawdust and hay, which provided large surface area for microbial attack degraded more rapidly compared to the woodchips. This microbial

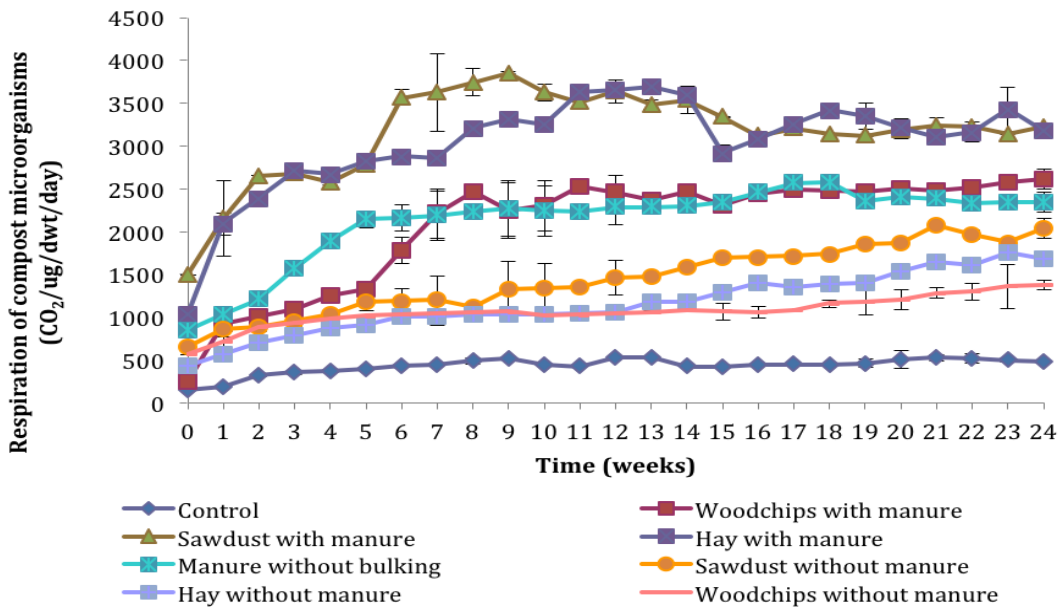
degradation of the bulking agent offered additional nutrient that allowed the co-metabolisation of the sludge.

Results of analyses of selected PAHs showed that the phenols and the low molecular weight PAHs were removed in all the experiments. However, the high molecular weight PAHs continued to remain till the end of the incubation period in the experiments that did not contain manure and also in the control experiments (Figure 2). This again is a clear indication that the presence of the manure was vital for the degradation of the oil sludge components.

The level of reduction in TPH and PAH concentrations in the experiments correlates with the level of microbial



**Figure 3.** Counts of heterotrophic microorganisms (CFU g<sup>-1</sup>) in compost. Values are means of three ± standard deviation.



**Figure 4.** Respiration (CO<sub>2</sub>/μg/dwt/day) of compost microorganisms during composting. Values are means of three ± standard deviation.

activities in the compost. The sawdust + manure compost showed a much higher microbial activity, as shown in the counts of heterotrophic organisms (Figure 3) and the respiration of compost organisms (Figure 4). Although, there was no significant difference between the

respiration of organisms in the hay and sawdust composts, microbial counts were consistently higher in the sawdust than in the hay. These high levels of microbial activities observed in the composts are attributed to the initial elevated microbial load supplied by the poultry manure and

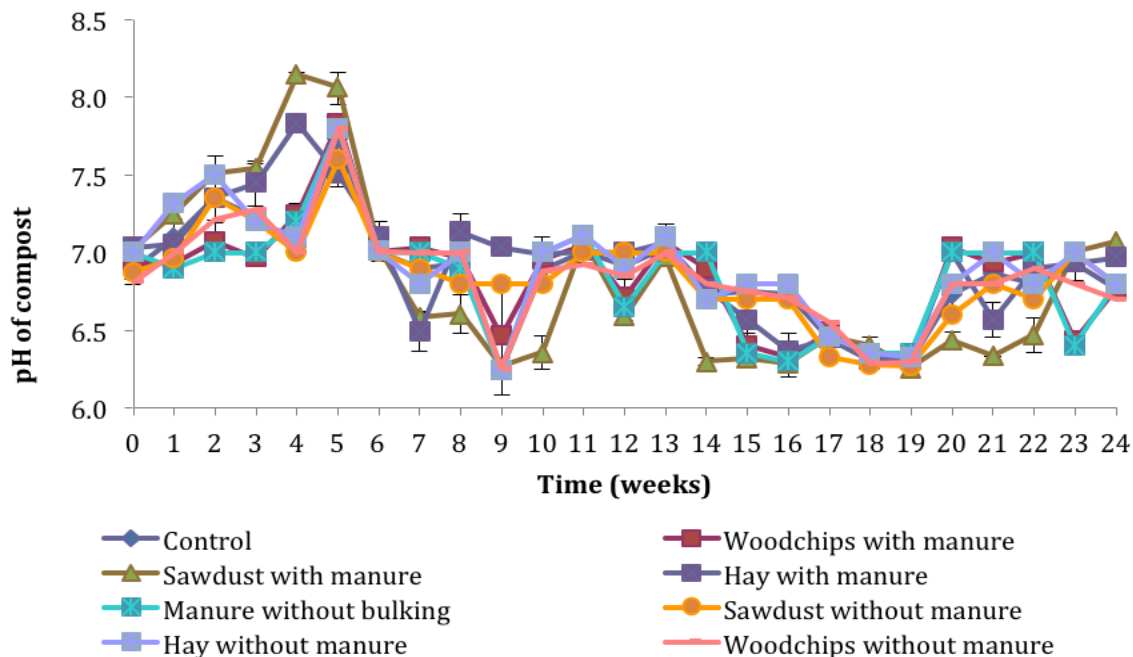


Figure 5. pH of compost during incubation. Values are means of three  $\pm$  standard deviation.

the aeration, which was supplied by turning the compost heaps weekly. This can be seen from the low levels of activities in the control experiments, which showed low microbial activities throughout the incubation period.

The initial high levels of TPH was expected to impede the activity of compost microorganisms but this did not seem to happen probably due to the diluting effect of the bulking materials and the amount of soil used in the preparation of the compost. This can also be attributed to the available carbon and nutrients provided by the compost system as well as the initial large population of microorganisms. Compost systems have been reported to enhance the degradation of a number xenobiotic compounds including hydrocarbon compounds (Giles et al., 2001; Atagana, 2003). However the reduction of TPH by compost organisms at the levels used in this experiment is remarkable and worth investigating further.

The counts of compost inhabiting microorganisms (Figure 3) and the results of the respiration of microorganisms (Figure 4) supports the large amounts of reduction in TPH. Although relatively lower microbial activities were shown by the woodchip compost when compared with the sawdust and hay experiments, reduction in TPH in the woodchip compost is comparable with the latter two. This may be due to the type of organisms present in the woodchip compost, which may consist of lignin degrading organisms that are known to adapt readily to hydrocarbon substrates (D'Annibale et al., 2006; Marin et al., 2006; Atagana, 2009). The pH of the compost systems rose sharply in the first four weeks of incubation reaching 8.1 in the sawdust compost and 7.8 in the hay and woodchips in the fifth week (Figure 5).

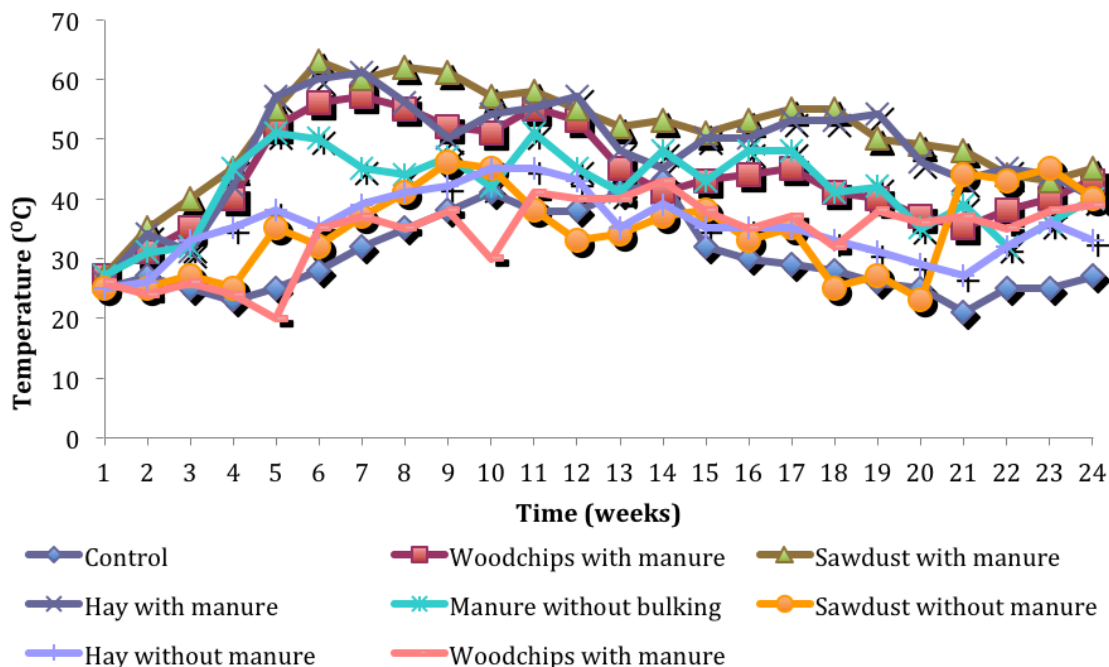
The pH lowered to below 7 in most experiments and remained largely below 6.5 for the rest of the experimental period. The drop in pH to slightly acidic range is an indication of the possible production acidic metabolites. However, this did not affect the activities of compost microorganisms. It is assumed that the majority of the microorganisms present are acid tolerant, as some of these are known to be hydrocarbon and lignin degraders.

Changes in C:N ratio of the compost matrix reflects the reduction in TPH (Table 2). The ratio increased with time as the TPH lowered. This can only be attributed to the lowering of carbon source due to the degradation activities. The changes in electrical conductivity (EC) were not significant (Table 3). This is an indication that the system was stable during the composting period. Stable electrical conditions, when conducive for microbial activities promote microbial growth and subsequently enhance degradation of substrates. This is also associated with the relatively stable pH observed after week five during the incubation period.

The high thermophilic temperatures (63°C) recorded during the incubation period (Figure 6) is an indication of high metabolic activities. The drop in temperature to about 35°C for more than half of the incubation period could be attributed to aeration an activity, which was carried out by turning of the compost heaps and the regular watering. This lowering of temperature was probably instrumental to the high and stable microbial population observed in the compost matrix.

Some of the bacteria identified in the compost include the following: *Arthrobacter* spp., *Bacillus aryabhatai*,





**Figure 6.** Changes in temperature (°C) of compost. Values are means of three  $\pm$  standard deviation.

**Table 3.** Electrical conductivity (dS cm<sup>-1</sup>) of compost system during incubation. Values are means of three.

Composts treatment	Time (month)						
	0	1	2	3	4	5	6
Control	0.90	0.91	0.95	1.50	1.00	0.92	1.10
Woodchips without manure	0.93	0.93	0.93	0.95	1.50	1.10	1.20
Sawdust without manure	0.95	09.96	1.05	1.80	1.60	1.20	1.40
Hay without manure	0.93	0.95	1.01	1.80	1.50	1.30	1.80
Manure without bulking	1.68	1.90	2.10	2.20	2.10	2.25	2.50
Woodchips with manure	0.95	1.20	1.25	2.50	2.7	2.55	2.75
Sawdust with manure	2.15	2.20	2.35	2.50	2.65	2.80	3.15
Hay with manure	2.40	2.25	2.35	2.75	2.80	2.80	3.20

*Bacillus Licheniformis*., *Bacillus subtilis*, *Bacillus* spp., *Brevibacterium frigoritolerans*, *Corynebacterium* spp., *Geobacillus* spp., *Norcadia* spp., *Paenibacillus* spp., *Pseudomonas* spp., *Ralstonia* spp., *Rodococcus* spp., *Staphylococcus* spp., *Staphylococcus* spp., *Staphylococcus saprophyticus*, *Streptomuyces* spp., *Thermobifida* spp., *Thermomonospora* spp., *Variovorax* spp.

Although, *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Staphylococcus* species were dominant in all the treatments at all temperature regimes during the period of incubation, all other genera were present in all the treatments at all times except for *Thermobifida* spp., *Thermomonospora* spp., *Geobacillus* spp. and *Norcadia* spp., which were not encountered in the two months of

incubation. While the absence of *Thermobifida* and *Thermomonospora* in the first two months could be justified, as they are thermophiles and the temperature of the composts only reached thermophilic stages in the second month of incubation, the absence and appearance of *Geobacillus* and *Norcadia* at the later stages of the incubation could not be explained. *Thermobifida* and *Thermomonospora* were not present in the control experiments and this could be attributed to the temperature of the control experiments which did not exceed the mesophilic range at any time during the experiment. The abundance of these organisms in most of the compost piles including the control is an indication of the ecology of the genera, being predominantly soil borne. The organisms identified in this study are known



to inhabit hydrocarbon contaminated soils and utilise oil as sole carbon source (Das and Mukherjee, 2007; Bayoumi, 2009; Koukkou et al., 2009).

## Conclusions

The results from these experiments have shown that the different parameters operating in the compost affect the overall functioning of the compost system and consequently the degradation of the hydrocarbon compound present. While the bulking materials, woodchips, sawdust and hay aided initial aeration in the compost, they also provided additional substrate and nutrient for the organisms to grow on, which in turn enhanced the overall degradation of the high concentrations of oil sludge present in the compost matrix within reasonable timeframe. The results have further showed that sawdust can compost faster than hay and woodchips to provide the necessary supplementary carbon substrate that is required by the microbial population to co-metabolise the sludge hydrocarbons. The results suggest that despite the rapid increase in temperature the population of the organisms present in the compost system continued to increase and additional organisms added over time. Aeration through periodic turning of the compost and watering proved to be advantageous in keeping the temperature stable and maintaining a viable population of microorganisms.

## REFERENCES

- Adeleke RA, Cloete TE, Khasa D (2012). Culturable microorganisms associated with Sishen iron ore and their potential roles in biobeneficiation. *World J. Microbiol Biotechnol.* 28:1057-1070.
- Atagana HI (2003). Bioremediation of Creosote-Contaminated Soil: A Pilot-Scale Landfarming Evaluation. *World J. Microbiol. Biotechnol.* 19:571-581.
- Atagana HI (2009). Biodegradation of PAH by fungi in contaminated soil containing cadmium and nickel ions. *Afr J. Biotechnol.* 8(21):5780-5789.
- Bayoumi RA (2009). Bacterial bioremediation of polycyclic aromatic hydrocarbons in heavy oil contaminated soil. *J. Appl. Sci. Res.* 5(2):197-211.
- Bojes HK, Pope PG (2007). Characterization of EPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in tank bottom solids and associated contaminated soils at oil exploration and production sites in Texas. *Regul. Toxicol. Pharmacol.* 47(3):288-295.
- Cameotra SS, Makkar RS (2010). Biosurfactant-enhanced bioremediation of hydrophobic pollutants. *Pure Appl. Chem.* 82(1):97-116.
- D'Annibale A, Rosetto F, Leonardi V, Federici F, Petruccioli M (2006). Role of autochthonous filamentous fungi in bioremediation of a soil historically contaminated with aromatic hydrocarbons. *Appl. Environ. Microbiol.* 72(1):28-36.
- Das K, Mukherjee AK (2007) Differential utilization of pyrene as the sole source of carbon by *Bacillus subtilis* and *Pseudomonas aeruginosa* strains: role of biosurfactants in enhancing bioavailability. *J. Appl. Microbiol.* 102:195-203.
- Diallo M, Cagin T, Faulon JL, Goddard WA (2000). Thermodynamic properties of asphaltene: a predictive approach based on computer assisted structure elucidated and atomistic simulations. In: Asphaltene and asphalts II (eds TF Yen and GV Chilingarian), developments in petroleum science 40B, Elsevier Amsterdam. pp. 103-127.
- Giles, WR, Kriel JR, Stewart JR (2001). Characterisation and bioremediation of a weathered oil sludge. *Environ. Geosci.* 8(2):110-122.
- Jiang GC, Xie SX, Chen M, Wang RS, Li ZY, Mao H, Zeng LF (2013). A Study on Oil Sludge Fueling Treatment and Its Mechanism in Field Operations. *Pet. Sci. Technol.* 31(2):174-184.
- Koukkou AI, Kallimains A, Kavakiotis K, Perisynakis A, Sproer C, Pukall R, Drains C (2009). *Arthrobacter phenanthrenivorans* sp. Nov., to accommodate the phenanthrene-degrading bacterium *Arthrobacter* sp. Strain sphe3. *Int. J. Syst. Evol. Microbiol.* 59(2):275-279.
- Kriipsalu M, Nammari D (2010). Monitoring of biopile composting of oily sludge. *Waste Manag. Res.* 28:395-403.
- Kriipsalu M, Marques M, Hogland W, Nammari DR (2008). Fate of polycyclic aromatic hydrocarbons during composting of oil sludge. *Environ. Technol.* 29(1):43-53.
- Liu T, Hou J, Zuo Y, Bi S, Jing J (2011). Isolation and characterization of a biosurfactant producing bacterium from Daqing oil-contaminated site. *Afr. J. Microbiol. Res.* 5(21):3509-3514.
- Liu W, Luo Y, Teng Y, Li Z, Ma L Q (2010). Bioremediation of oily sludge-contaminated soil by stimulating indigenous microbes. *Environ Geochem Health.* 32:23-29.
- Machin-Ramirez C, Okoh AI, Morales DK, Mayolo-Delouis M, Quintero R, Trejo-Hernandez (2008). Slurry-phase biodegradation of weathered oily sludge waste. *Chemosphere* 70:737-744.
- Mahmoud S (2004). Novel technology for sustainable petroleum oily sludge management: Bio-neutralization by indigenous fungal-bacterial co-cultures. A Masters Degree submitted to the Department of Building, Civil and Environmental Engineering at Concordia University, Montreal, Quebec, Canada.
- Mandal AK, Sarma PM, Jeyaseelan CP, Channashettar VA, Singh B, Lal B, Datta J, (2012). Large-scale bioremediation of petroleum hydrocarbon contaminated waste at Indian oil refineries: case studies. *Int. J. Life Sci. Pharm. Res.* 2(4):L114-L128.
- Marin JA, Moremo JL, Hernandez T, Garcia C (2006). Bioremediation by composting of heavy oil refinery sludge in semiarid conditions. *Biodegradation* 17:251-261.
- Mashreghi M, Marialigeti K (2005). Characterization of bacteria degradation petroleum derivatives isolated from contaminated soil and water. *J. Sci. Islam. Repub. Iran* 16(4):317-320.
- Pakphan EN, Isa MH, Kutty SRM (2011). Polycyclic Aromatic Hydrocarbons in Petroleum Sludge Cake: Extraction and Origin - a Case Study. *Int. J. Appl. Sci. Technol.* 1 (5):201-207.
- Shie J, Lin J, Chang C, Wu C, Lee D, Chang C, Chen Y (2004). Oxidative thermal treatment of oil sludge at low heating rates. *Energy Fuel* 18:1272-1281.
- Srinivasarao-Naik B, Mishra IM, Bhattacharya SD (2011). Biodegradation of total petroleum hydrocarbons from oily sludge. *Bioremed. J.* 15(3):140-147.
- Steger K, Javis A, Vasara T, Romantschuk T, Sundh I (2007). Effects of differing temperature management on development of actinobacteria populations during composting. *Res. Microbiol.* 158:617-624.
- Suler DJ, Finstein MS (1997). Effect of temperature, aeration, and moisture on CO<sub>2</sub> formation in bench-scale, continuously thermophilic composting of solid waste. *Appl. Environ. Microbiol.* 33(2):345-350.
- USEPA (1997). Total recoverable petroleum hydrocarbons by infrared spectrophotometry. EPA method No. 8440.
- Wang J, Yin J, Ge L, Shao J, Zheng J (2010). Characterization of oil sludge from two oil fields in China. *Energy Fuel* 24:973-978.
- Ward W, Singh A, van Hamme J (2003). Accelerated biodegradation of petroleum hydrocarbon waste. *J. Ind. Microbiol. Biotechnol.* 30:260.