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Measuring the effect of co-composting Crude Oil sludge with pig, cow, horse and poultry manures on the degradation of selected Polycyclic Aromatic Hydrocarbons

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Abstract: This study is aimed at measuring the effect of pig, cow, horse and poultry manures on the degradation of selected Polycyclic Aromatics Hydrocarbons present in oil sludge. Four kilograms of soil amended with 1.2 kg of oil sludge was mixed with wood chips in a ratio of 1:2 (w:v) soil mixture: wood chips. The mixture was divided into five parts and four parts were separately mixed with pig, cow, horse or poultry manures in a ratio of 2:1 (w:w) and the fifth portion was used as the control with no manure added. All experiments were incubated for 10 months at room temperature. Compost piles were turned weekly for aeration and moisture level was maintained by adding deionised water enough to prevent the compost from getting dry. Moisture level, pH, temperature, CO_2 evolution and oxygen consumption were measured monthly and the ash content of the compost at the end of experimentation. Highest temperature reached was 27.5°C in all compost heaps, pH ranged from 5.5 to 7.8 and CO_2 evolution was highest in poultry manure at 18.78 µg/dwt/day. Microbial growth and activities were enhanced as indicated by increase in temperature, moisture level, pH value and respiration rate in all the compost piles. Bacteria capable of utilizing PAHs were isolated, purified and characterized by molecular techniques using polymerase chain reaction with specific universal primers and the amplicons were sequenced. Bacteria identified were *Bacillus*, *Arthrobacter* and *Staphylococcus* species. Percentage reduction in PAHs was measured using automated soxhlet extractor with Dichloromethane and gas chromatography/mass spectrometry. Results from PAH concentration measurements showed reduction of between 77% and 99%. Co-composting of contaminated soil with animal manures enhanced the reduction in PAHs.

Introduction

Crude oil is an important mineral resource vital to everyday life (Ward et al. 2003, Das and Chandran 2011). Oil sludge generated during crude oil refining, cleaning of oil storage vessels and waste treatment is a thick, viscous complex mixture of sediment, water, oil and hydrocarbons at varying concentrations (Das and Chandran 2011, Ibuot and Bajhaiya 2013). Oil sludge is mainly composed of alkanes, aromatics, asphaltenes and resin (Diallo et al. 2000, Das and Chandran 2011, Liu et al. 2014). It has high content of aromatic hydrocarbons in the range of 1–40 carbon atoms (US EPA 1997). Oil sludge has been classified by the United States Environmental Protection Agency (US EPA) as a hazardous organic complex (Liu et al. 2010, Ibuot and Bajhaiya 2013). This contaminant enters the environment as a result of human activities, which include deliberate dumping, improper treatments and management, storage, transportation and landfill disposal (Ibuot and Bajhaiya 2013, Liu et al. 2014). This calls for human concern because many of the oil sludge components have been found to be cytotoxic, mutagenic and potentially carcinogenic (Bayoumi 2009, Kumar et al. 2011, Ibuot and Bajhaiya 2013, Ferradji et al. 2014, Zaki et al. 2014). Oil sludge contains volatile organic compounds (VOCs) and semivolatile organic compounds (SVOCs) (PAHs), some of which over the years have been reported as being genotoxic (Bojes and Pope 2007, Ferradji et al. 2014). The environmental impact of oil sludge contamination includes physical and chemical alteration of natural habitats, lethal and sub-lethal toxic effects on aquatics and terrestrial ecosystems (Hassanshahian et al. 2012, Ferradji et al. 2014, Liu et al. 2014, Winquist et al. 2014, Zaki et al. 2014). These effects as well as many others (Liu et al. 2010, Ferradji et al. 2014, Zaki et al. 2014), had led the environmental regulations in many parts of the world to stress on the necessity to decrease emission of volatile organic compounds and polycyclic aromatic hydrocarbon (PAHs), and have placed more restriction on land disposal of oil sludge (Mahmoud 2004). Therefore, treatment and biodegradation of oil sludge is essential and important for the environment.

Generally, oil sludge and its components, particularly the high molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs) (Ibuot and Bajhaiya 2013) are highly recalcitrant under normal conditions. The recalcitrance is attributed to their strong molecular bonds, high molecular weights, hydrophobicity and relative low solubility in water (Liu et al. 2011). The hydrophobic nature of these contaminants results in their partitioning onto soil matrices (Zhao et al. 2005, Das and Chandran 2011) and if disposed without proper treatment, they may become strongly sorbed to the organic fraction of sediments and soils. As a result their degradation becomes slow, as their bioavailability to microbes declines resulting in persistence in the environment (Das and Chandran 2011, Liu et al. 2011, Ferradji et al. 2014). Due to the lower aqueous solubility of the PAHs, they may not serve as sole source of carbon and energy for some soil microbes (Bautista et al. 2009). This is the most important limiting factor in the degradation of oil sludge (Zaki et al. 2014). Hence, their bioremediation in soil-water systems depends strongly on factors such as (1) their desorption rates from the soil surface, (2) the subsequent containment by bulk aqueous phase (3) also, the bioavailability of these compounds to be used as substrates by microorganisms.

Various physical, chemical and biological techniques, and combinations of the different techniques have been used in attempt to remediate oil sludge and other hydrophobic organic compounds which contaminated soil and groundwater (Srinivasarao et al. 2011, Udotong et al. 2011, Ferradji et al. 2014). These attempts have not been conclusive; as each technique has its own challenges such as expensive equipment and high energy requirements, generation of toxic by-products that may need further treatment and the lack of available land space for landfill disposal (Liu et al. 2010, Das and Chandran 2011). Besides the lack of land space, landfilling is faced with challenges such as emission of volatile organic compounds, managing leachates, air pollution, and exposure of humans and animals to hazards of oil sludge (Liu et al. 2010, Srinivasarao et al. 2011). Of all the methods, biological methods, the use of microorganisms for the degradation oil sludge (Ibuot and Bajhaiya 2013), stand out as the most environmentally friendly option (Das and Chandran 2011, Chemlal et al. 2012). This is possible as long as a large population of microorganisms that possess the appropriate degrading capabilities is present and the conditions are adequate for their growth and activities (Paulauskiene et al. 2009, Das and Chandran 2011, Chemlal et al. 2012, Liu et al. 2014).

Microbial biodegradation has been known as an effective and inexpensive approach for treating petroleum hydrocarbons (Das and Chandran 2011, Barnabas et al. 2013, Ferradji et al. 2014, Vanishree et al. 2014, Winquist et al. 2014). The organisms involved synthesise enzymes that catalyse the reaction in which these contaminants are degraded to simpler, lower molecular chains and less toxic compounds (fatty acids, CO_2 and H_2O) (Das and Chandran 2011, Hassanshahian et al. 2012, Ferradji et al. 2014, Liu et al. 2014, Winquist et al. 2014). This approach usually relies on adaptivity and subsequent easy proliferation of the indigenous microbes. The initial step in this mechanism is the catabolism of oil sludge by bacteria and fungi, which involves the oxidation of the substrate by oxygenases (Olivera et al. 2009, Das and Chandran 2011, Hassanshahian et al. 2012, Kumar et al. 2011, Liu et al. 2014, Winquist et al. 2014). The use of microorganisms in degrading oil sludge is nature-compatible, reliable, cheaper, and easy to adopt, and the end products are usually harmless compared to physical and chemical methods (Machin-Ramirez et al. 2008, Srinivasarao et al. 2011, Hassanshahian et al. 2012). Although most biological methods are economically unsound, prone to prolonged treatment time and as such they may not be suitable solutions for degrading some contaminants (Ward et al. 2003, Leung 2004). Despite the limitations, biological methods are still being used for the remediation of oil sludge.

Biological techniques for treating contaminated sites are scientifically intense procedures, which must be tailored to specific sites conditions (Boopathy 2000), for an example, the use of composting for the treatment of contaminants is a technique which relies on activities of diverse and adapted successive microbial populations combining the action of both mesophilic and thermophilic organisms, carefully controlled parameters, mixture of nutrients and rich organic materials to improve bioremediation of contaminants (De-qing et al. 2007, Jain et al. 2011, Dadrasnia et al. 2013, Garcia 2013). The microbial activities in compost generate high temperatures, which increases solubility of contaminants and induces microbial co-metabolic activity (Gibb et al. 2001, Sheetal 2012). In compost, there is abundance of nutrients and organic matter that have effect on microbial activities for degradation of contaminants (van Hamme et al. 2003). As the compost matures, the pollutants are degraded, digested, metabolised and transformed into humus as well as inert products by the active microflora within the compost mixture (Wang et al. 2011, Garcia 2013, Singh and Chandra 2014, Vanishree et al. 2014). These features have made composting interesting and to stand out as an advantage among other biological methods in treating contaminants. Most importantly, the large amount of organic substrates for co-metabolism, aeration, moisture content and high temperature are generated during composting by microbial activities in the compost (De-qing et al. 2007, Jain et al. 2011, Sheetal 2012, Dadrasnia et al. 2013). The temperature range in a compost pile determines the presences of mesophilic or thermophilic organisms at the time as well as the rate of substrate co-metabolism and activity. In as much as the high temperature generated is essential in composting, there is a consequence of enzymes denaturing which might be a limiting factor to the biodegradation of contaminants. Hence, it is essential that temperature and other factors such as aeration, nutrients, pH, moisture and microbial activities, which influences the degradation of contaminants be properly managed to achieve optimal oil sludge degradation. Composting could be *ex situ* or *in situ* process and if compared to other methods, the use of composted material and co-composting as bioremediation technique may possibly promote soil sustainability and re-use. The main advantage is waste stabilization and biological reactions occurring during composting convert organic wastes into stable, mainly inorganic forms. These stable inorganic forms may cause little pollution effects if discharged onto land or into water course (Eneji et al. 2006). It is an economically sound, natural process that destroys organic contaminants and the residues obtained are no more harmful (UNIDOI 2003). It is often less expensive and disruption is minimal (Jain et al. 2011). It eliminates waste permanently, eliminates long term liability, and has greater public acceptance, with regulatory encouragement, it can also be coupled with other physical or chemical methods (Idris and Ahmed 2003, Sharma and Mudhoo 2010). Hence, this study has attempted to degrade oil sludge by using different animal manures in co-composting experiments.

This study was to measure the effect of co-composting oil sludge with pig, cow, horse and poultry manures on the reduction in Polycyclic Aromatic Hydrocarbons concentrations present in oil sludge under laboratory conditions.

Materials and methods

Soil

Garden soil was air-dried and analysed to determine the soil type, organic carbon content, total nitrogen content, total phosphorus content (C:N:P), soil pH and water holding capacity (WHC). The concentrated acid digestion method (CADM) with Induction Coupled Plasma (ICP-OES Optima 4300 DV, Perkin Elmer, Waltham, MA, USA) was used to determine metals as well as elements present in soil sample. The C:N:P analysis on all samples was conducted by waterlab (Pty) Ltd. The procedures followed were standard methods for C:N:P analysis (US EPA 1984, Stevenson and Cole 1999).

Oil sludge

Oil sludge from a refinery was characterized by automated soxhlet extraction with Dichloromethane with gas chromatography/mass spectrometry (GC/MS) and concentrated acid digestion method (CADM) with Induction Coupled Plasma (ICP-OES Optima 4300 DV, Perkin Elmer, Waltham, MA, USA) was used to determine metals as well as elements present in oil sludge sample.

Animal manures

Cow, pig, horse, and poultry manures were collected from University of Pretoria, Onderstepoort, Pretoria, South Africa. The manures were characterized for organic carbon content, total nitrogen content, total phosphorus content. The C, N and P analysis on all samples was conducted at Waterlab (Pty) Ltd by using standard methods. (US EPA 1984, Stevenson and Cole 1999).

Bulking agent

The bulking agent used to enhance aeration was wood-chips of 8–10 cm × 0.3–0.5 cm.

Chemicals

Tetrachloromethane (CCl_4, 99.55%, molar mass 153.8236 g/mol, density 1.594 g/ml) was purchased from Merck South Africa and used to dissolve the oil sludge before mixing with soil.

Preparation of compost mixture

One thousand two hundred grams of crude oil waste sludge was dissolved in 400 ml of CCl_4. Then the mixture was added to 4 kg of soil and air dried at room temperature to evaporate excess CCl_4 after which 100 g was analysed for selected PAHs as shown in Table 1. Wood chips were mixed with the amended soil in a ratio of 1:2 (w:v) soil: wood chips. The mixture was divided into five parts and four parts were separately mixed with either pig, cow, horse or poultry manures in a ratio of 2:1 (w:w). The fifth portion was used as the control with no manure added. All treatments including the control were incubated in PVC troughs measuring 22cm (diameter) × 9.2 cm (Depth) at room temperature for a period of ten months. All treatments were replicated three times.

Temperature changes were monitored by placing thermometer in the middle of the compost heaps. Moisture content of the compost was measured by using the method adopted from Forster (1995), and water was added to the compost pile when necessary to maintain moisture level between 50% and 80% not allowing it to get dried. The pH was measured monthly using the aqueous extract of the compost mixture with a pH meter (Crison Micro pH 2000™). The ash content of the compost mixture was determined at the initial stage and at the end of composting experiment by heating 10 g of the compost mixture in a furnace at 400°C for 6 hr. Carbon dioxide evolution was measured by using the closed jar method adopted from Alef (1995), at room temperature, which was used to monitor microbial activities. Organisms present in composts were isolated and characterised.

Isolation of oil degrading bacteria from enrichment cultures

Identification of bacteria capable of degrading PAHs was carried out using the gram-reaction test and molecular techniques. The gram-reaction test was done to ascertain morphological characterisation and purity of the colonies before proceeding to do molecular identification, which includes DNA extraction, Polymerease chain reaction (PCR) and sequencing.

Determination of Polycyclic Aromatic Hydrocarbon (PAH) reduction during composting

In the present study, interest was on PAHs present in crude oil waste sludge. Therefore, extraction of PAHs from compost samples was performed using automated soxhlet extraction an EPA method 3541 (Stewart 1989, Lopez-Avila 1991). The PAHs present in the extracts were quantified by GC/MS using US EPA 8270 (Bobak 2010). The stock standard was restek cat No 8270-1 which contains semivolatile mix. It was purchased from Sigma Aldrich, South Africa. The concentration of the stock standard was 1000 ppm and it was used to prepare the calibration standards of 10 ppm, 30 ppm and 50 ppm. Working standard solution was prepared from the surrogate standard using dichloromethane. Calculation of the required concentrations was based on the chemical formula:

$$C_1V_1 = C_2V_2$$

where C_1 = Concentration of stock solution, C_2 = Concentration to be made, V_1 = Volume to be determined, V_2 = Volume required.

The analysis of the semivolatile compounds present in the sample extracts were carried out with the GC/MS Agilent 7860GC system and 5975C MSD, equipped with a 7683B autosampler (Weavers et al. 2006, Smith and Lynam 2009). The sampler syringe was 5.0 µl and splitless injection was 1.0 µl. The carrier gas used was helium 30 cm/s and at a constant flow rate of 1 ml/min. The inlet, splitless, 260°C, purge flow was 50 ml/min at 0.5 min and gas saver was at 80ml/min at 3 min. Inlet liner was the deactivated dual taper direct connect. The column was Agilent HP-5ms ultra-inert 30 m × 0.25 mm × 0.25 µm film thickness. The oven program was started at 40°C for 1minute to 100°C (15°C/min), 10°C/min to 210°C (1 min), 5°C/min to 310°C and it was held for 8min. The detection was MSD source at 300°C, quadrupole at 180°C, transfer line at 290°C, scan range 45 to 450 amu. PAHs were identified by retention times matching to standards concentration. The value of the chromatogram was quantified using peak area integration. All reagents used were analytical grade and were used without further purifications.

Results and discussion

The characteristics of the garden soil used and the C:N:P ratios of the animal manures used in this study are shown in Tables 1 and 3 respectively. The soil sample was a sandy loan and contained some trace elements as shown by its characteristics, nutrients necessary to support plant growth and a good microbial population as well as their activities (Table 1) (Mann 2008). The animal manures were rich in C:N:P and their ratios were in substantial amounts which were necessary to stimulate microbial growth and activities in compost pile (Table 3).

Determination of Polycyclic Aromatic Hydrocarbon (PAH) reduction during composting

The characteristics of the oil sludge before composting (Table 2) showed that 16 PAHs of both low and high molecular weight hydrocarbons ranges were present in substantial quantities with a few metals such as Zn (8.33 mg kg^{-1}), Fe (46.35 mg kg^{-1}) and Mg (22.37 mg kg^{-1}). The hazardous PAHs components present in the oil sludge included acenaphthene, acenaphtylene, pyrene chrysene, benzo[a] fluoranthene, benzo[a]pyrene, indenol (1,2,3-cd) pyrene, among others (Table 2) (Shaoping and Laishi 2011). They are a family of compounds that have the potential to cause cancer (Bayoumi 2009, Kumar et al. 2011, Shaoping and Laishi 2011, Ibuot and Bajhaiya 2013, Ferradji et al. 2014, Zaki et al. 2014). These PAHs do not act directly as carcinogens but react in the body together to form PAH epoxides that are active carcinogenic agents (IARC 2007, CONCAWE report 2001, 2005, Bayoumi 2009). However, many components of oil sludge were not detected because they were below the detection limits (<0.01mg/kg) by the GC/MS used.

The results obtained from GC/MS analysis showed that the reduction in the selected PAHs ranged between 77 and

Table 1. Characteristics of garden soil used for the experiment

Soil parameter	Sand [% wt]	Silt [% wt]	Clay [% wt]	Texture	pH (H_2O)	Total organic carbon in %[mg/l]	Total organic N [mg/l]	Total P [mg/l]	Cr [mg kg^{-1}]	Pb [mg kg^{-1}]	Ni [mg kg^{-1}]	Cu [mg kg^{-1}]	Zn [mg kg^{-1}]	Mn [mg kg^{-1}]	Fe [mg kg^{-1}]	Co [mg kg^{-1}]	Mg [mg kg^{-1}]	Dry matter content [% DM]	Moisture content [% MC]
Characteristics [Conc]	61.3	21.3	9.3	sandy loam	5.56	1.02	20	4.4	121.7	31.91	10.13	38.08	9.65	92.38	67.04	2.45	22.37	90.48	9.52

Table 2. Concentration of the selected PAHs present in oil sludge (mg/kg). Values are mean of three actual values ± standard error

Selected PAH	Naphthalene	1-methyl naphthalene	2-methyl naphthalene	Acenaphtylene	Acenaphthene	Fluorene	Anthracene	Phenanthrene	Fluoranthene	Pyrene	Chrysene	Benzo[a] anthracene	Benzo[b] fluoranthene	Benzo[k] fluoranthene	Benzo[a]pyrene	Indenol (1,2,3-cd) pyrene
Initial conc. (mgkg^{-1})	95.32± 0.03	205.81±10.14	195.70±0.01	5.05±0.1	7.94±0.96	23.11±0.96	40.44±0.69	1.44±0.49	1.44±0.46	10.83±1.33	44.77±4.07	1.44±0.59	21.66±2.1	2.17±0.6	7.22±1.12	5.78±1.38

Table 3. Characteristics of animal manures used for the experiment in % and mg/L

Animal manures	Total organic C [%]	Total N [mg/L]	Total P [mg/L]
Poultry	49.2	277	254
Cow	54.9	109	46
Horse	52.7	81	50
Pig	50.6	904	252

99.99% in all manure amended compost piles over a period of ten months' incubation. The initial concentrations of the PAHs for the 2 to 6 rings detected were between 1.44 mg kg^{-1} to 205.81 mg kg^{-1} before the co-composting process of oil sludge. Reduction in the control experiment was not significantly different from the manure amended compost piles at the end of the experiment. This could be as a result of the fungi action because fungi invaded the control experiment. The percentage reduction in the concentration of the selected PAHs was highest in the pig manure amended co-compost, followed by cow and horse manure amended co-compost; the reduction in PAHs for poultry manure amended co-compost was the least. The low percentage reduction observed in poultry manure amended co-compost compared to the other treatments could possibly be due to the rapid increase in temperature in the poultry manure amended co-compost, which may have affected microbial growth and activities (Fig. 1). This is also possibly because the high nitrogen content of the poultry manure provides a rich environment for microorganisms to grow rapidly and cause increase in microbial activities subsequently in temperature. As such the decrease in temperature affected the reduction in the concentration of the selected PAHs in poultry manure amended co-compost as high temperature enhances PAHs bioavailability and biodegradability.

Percentage reductions in selected PAHs obtained in this study are shown as follows: Poultry- (naphthalene 97.86 percent, 1-methyl-naphthalene 97.86 percent, 2-methyl-naphthalene 99.02 percent, fluorene 99.68 percent, anthracene 98.00 percent, pyrene percent 92.86 percent, chrysene 96.63 percent, benzo[b]fluoranthene 87.42 percent); Horse-(naphthalene 98.41 percent, 1-methyl-naphthalene 99.87 percent, 2-methyl--naphthalene 99.92 percent, fluorene 99.83 percent, anthracene 99.93 percent, pyrene 99.66 percent, chrysene 99.82 percent, benzo[b]fluoranthene 99.40 percent); Cow-(naphthalene 99.49 percent, 1-methyl-naphthalene 99.96 percent, 2-methyl--naphthalene 99.98 percent, fluorene 99.94 percent, anthracene 99.95 percent, pyrene 99.72 percent, chrysene 99.89 percent, benzo[b]fluoranthene 99.86 percent); Pig-(naphthalene 99.65 percent, 1-methyl-naphthalene 99.97 percent, 2-methyl--naphthalene 99.99 percent, fluorene 99.96 percent, anthracene 99.97 percent, pyrene 99.88 percent, chrysene 99.93 percent, benzo[b]fluoranthene 99.82 percent). The results are the calculation of the remaining PAH concentration in this analysis using the mean value of three duplicates for each sample. They detailed results for each PAH extracted and analysed using GC/MS which were identified and quantified. The results obtained from the compost piles have shown that composting can be used to degrade PAHs present in oil sludge (Whyte 1997, Bengtsson et al. 1998, Bastieans 2000, Jose et al. 2006, Meintanis et al. 2006).

Changes in temperature during composting

There was an increase in temperatures in all the composts piles amended with manure except the control experiment, which contained no manure. Poultry manure amended compost pile showed a higher temperature than other manure-amended piles. This, as earlier explained could be a result of the high nitrogen content of the poultry manure, which may have stimulated microbial growth and activities. The initial decrease in temperature in cow manure compost may be due to the fact that the compost retained much more water than other compost piles. During composting, the temperature of the compost fluctuated in all the experiments, with cow manure compost showing the lowest temperature (Fig. 1). The fluctuating temperature of the compost indicates that the level of microbial activities in all compost piles was enhanced. The temperature in the control experiment remained low ranging between 22 and 23°C during the treatment period. It may be due to the relatively low microbial activity, which was a result of lower nutrient and low organic matter content compared to the ones with manure amendments. This increase in temperature was anticipated to positively affect the biodegradation of hydrocarbons within the compost mixtures, as high temperatures affect solubility of the organic compounds and enhance their bioavailability and biodegradability (Leahy and Colwell 1990). Low temperatures, on the other hand, impede microbial growth and proliferation (Potter et al. 1999) and reduce the bioavailability and consequently biodegradation of the target contaminants under normal circumstances (Gibb et al. 2001). This is usually due to decrease in enzymatic activities, as high temperature

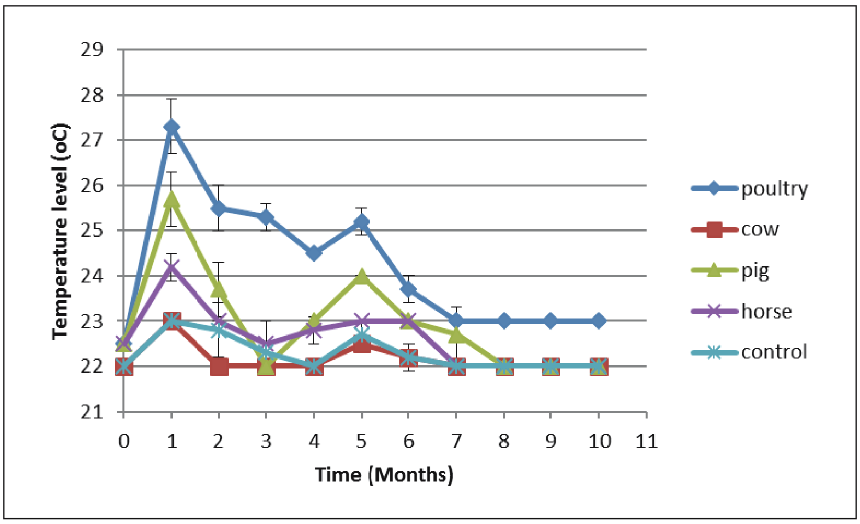

Fig. 1. The temperature of the composts during incubation of the co-composting of the contaminated soil. Values are mean of three ± standard error for the compost piles

promotes thermophilic organism to synthesise enzymes that catalyse degradation reaction (Michel 1996, Madigan et al. 1997, Niehaus et al. 1999, Pernilla et al. 2007).

Changes in moisture content during compost

The moisture levels in these experiments were observed to be relatively stable at about 50% from the first month of incubation to the eighth month. This is because the experiments were covered with sparsely perforated plastic sheeting to retain the moisture. The moisture levels in the control experiments were stable at about 44% within the same period (Fig. 2). Earlier reports have suggested that water contents of between 50% and 80% enhanced the biodegradation of organic contaminants in soil (Alexander 1999). Microbial growth and activities are normally maintained at their optimum at these moisture levels and the degradation of the target contaminants is enhanced. Therefore moisture is necessary not only to meet the physiological requirements of microorganism. It is also needed for the transportation of nutrients, metabolic by-products within and outside the microorganisms and for their activities. This enhanced the reduction in concentration of the hydrocarbon contaminants.

Changes in pH during composting

The pH in poultry, cow, pig, horse and control experiments increased from 5.9–7.9, 5.8–7.6, 5.6–7.8, 5.6–7.7 and 5.6–6.8 respectively. There was a sharp pH decrease after the fifth month in all treatment, and then a slight increase was observed in the control, cow and horse compost pile. The poultry compost pile had a sharp increase to 7.7 while the pig decreased on the seventh month. It eventually became stable with little fluctuation during the remaining composting period (Fig. 3). Biodegradation of organic contaminants has been reported to be faster at neutral or near neutral pH (Fu and Alexander 1992). However, neutral pH or near neutral are more favourable to bacteria while fungi are known to be tolerant of acidic pH conditions (Al-Daher et al. 1998). The pH values were within the recommended pH range for composting organic compounds (van Hamme et al. 2003). The increase in pH of the compost pile may be due to high content of ammonia

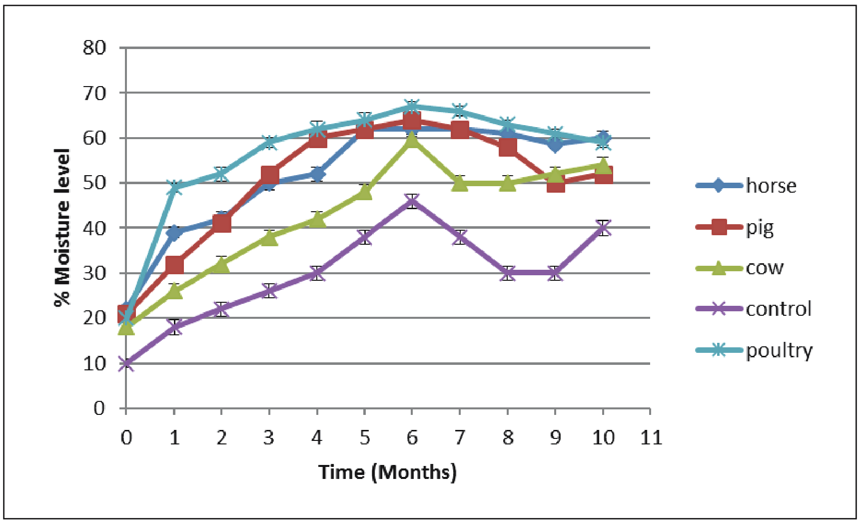

Fig. 2. The moisture level of the composts during the incubation of the co-composting of the contaminated soils. Values are mean of three ± standard error for the compost piles

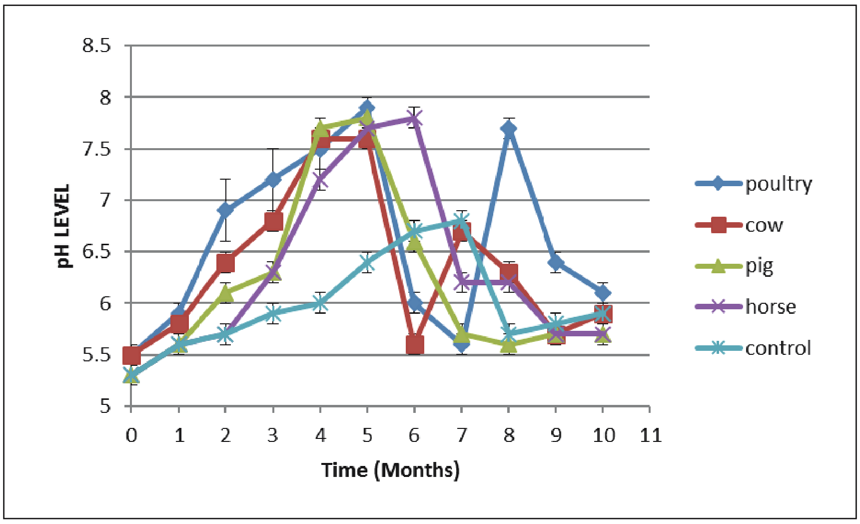

Fig. 3. The pH of the composts during the period of incubation of the co-composting of the contaminated soil. Values are mean of three ± standard error for the compost piles

from the manures. The decrease observed after the fifth month may be due to the degradation of compost materials and the petroleum hydrocarbon. The decrease may be due to the release of intermediates and other products that have low pH effect on the mixture according to Fava and Piccolo, (2002) and Lee et al. (2007).

Changes in ash content during composting

Ash content of the compost mixture showed that there were no significant difference from the initial soil-compost mixture and that of the end of the composting period (Table 4). Therefore, there were no significant changes in the mineral components of the soil at the end of the composting period. This further agrees that composting process does not alter the soil components after treatment.

Changes in CO_2 evolution of compost piles during composting organisms

The respiration rate increased between 0.5 to 18.78 µg/dwt/day (dwt = dry mass weight) in the first six months of composting in all the experiments while the control experiments were stable from the fourth month. Carbon dioxide evolution was highest in poultry amended manure at 18.78 µg/dwt/day. The increase observed in respiration experiment showed that there was an increase in the microbial activities in the compost pile mixture (Fig. 4). This may be due to the utilization of substrates (nutrients and hydrocarbons) in the compost pile mixture by microorganisms. Carbon dioxide emission increased as the treatment proceeded and as compost piles were turned for aeration. This is the effect of oxygen consumption for the growth and activities of the degrading microorganisms. The respiration rate of the soil microorganism decreased slightly in the fourth month as horse and control compost pile were stable during this period. This indicates the reduction in the microbial population by succession of mesophilic to thermophilic and availability of the target contaminants in the compost piles system. This also indicates that co-metabolic activities of the microorganisms have contributed to enhance the reduction of the concentration of hydrocarbon contents of oil sludge in the compost pile mixture. The control set up which had no manures showed increase in the respiration experiment. This may be due to fungi growth observed in the control compost pile system. The decrease in the respiration rate observed towards the end of the treatment process may be due to the decrease in carbon from the oil-sludge components. The lack of carbon in compost system may have reduced the population of the degrading microorganisms present in the compost pile mixture. This is possible because carbon is the source of energy. Respiration experiments have been used to study the aerobic biodegradation of contaminants in contaminated soils (Mahmoud, 2004). In this study, soil respiration experiments were helpful to quantify the effects of the nutrients and microorganisms from the animal manures as well as those from the soil in the bioremediation of the oil sludge. Nutrients encouraged microbial growth

Table 4. Ash mass (g) initial stage and end-of-the composting period (10 months). Values are mean of three ± standard error for the compost piles

Soil-compost mixture	Initial	End
Poultry	4.03± 0.21	4.08± 0.16
Cow	4.01± 0.19	4.01± 0.20
Pig	3.77± 0.15	3.78± 0.14
Horse	3.34± 0.04	3.36± 0.04
Control	4.04± 0.33	4.07± 0.32

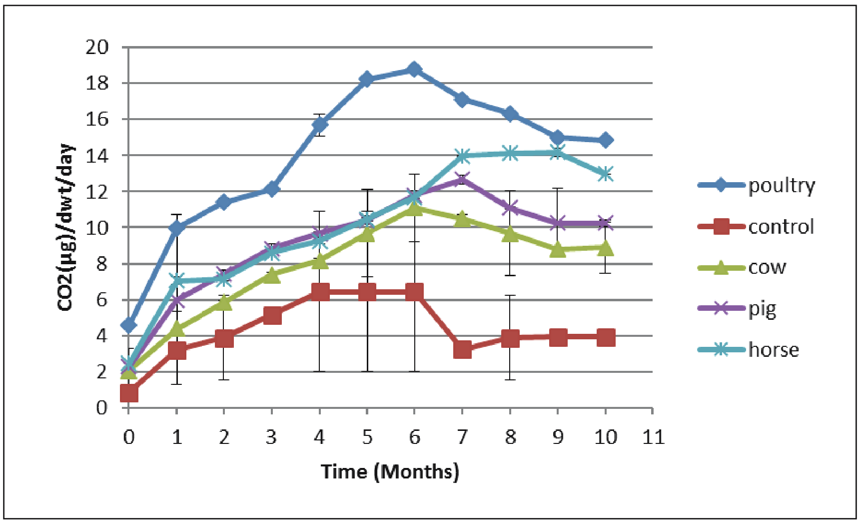

Fig. 4. The respiration rate of soil microorganisms in the composts during the incubation of the co-composting of the contaminated soils. Values are mean of three actual values ± standard error for the compost piles (dwt = dry mass weight)

and enhanced the utilization of hydrocarbons in soil compost pile. The results obtained from the characterized garden soil sample used in this treatment test showed that trace elements were present in the soil. The trace elements included copper, zinc, iron, chromium, lead, nickel, manganese, cobalt, and magnesium. Many of these metallic elements play essential role in the function of microorganisms. However, excess of these essential trace metal elements and non-essential trace metal elements can be toxic to microorganisms. In addition, aeration by turning the compost piles at intervals enhanced microbial growth and activities. The increase in microbial activities was reflected as an increase in respiration rate. The moisture level, pH, temperature and carbon dioxide evolution results obtained in this study showed that microbial activities and breakdown of the organic hydrocarbons substrates present in the compost pile systems were enhanced.

The results obtained from the compost piles have shown that composting can be used to degrade PAHs present in oil sludge (Ouyang et al. 2005, Jose et al. 2006, Meintanis et al. 2006). Parameters measured in all compost piles attributed to the degradation of PAHs. The increase in temperature of the compost treatments indicates that the level of microbial activities in all composting piles was encouraged. In the co--composting piles with horse, pig, poultry and cow manure it was observed that microbial activities were responsible for the increase in temperature. This increase in temperature also affects the biodegradation of hydrocarbons within the compost soil mixtures. At increased temperature, microorganisms break down the hydrocarbon because of increase in the solubility of the target contaminants (Gibb et al. 2001). The pH values were within the recommended pH range for composting organic compounds (near neutral, Fig. 3) (van Hamme et al. 2003). The increase in pH of the compost pile may be due to high content of ammonia from the manures. Increased pH was more favourable to microorganisms for their activities. The results obtained from the respiration experiments showed that there was an increase in the microbial activities in the composting pile mixture (Fig. 4). This may be due to the utilization of substrates (nutrients and hydrocarbons) which had positive effect on the degradation of PAHs by microorganisms. The initial concentration of PAHs obtained of the spiked enrichment culture before incubation, the results obtained from the samples from enrichment culture, and subcultured media after incubation showed that microorganisms were able to degrade PAHs, suggesting the existence of active bacterial consortia in this period (Katsivela et al. 2003). These active bacteria isolated included both gram-positive as well as gram-negative, and gram-positive bacteria were dominant in all the compost treatments. A good amplification of products was obtained during the PCR reaction. The primers amplified 1500 base pairs band of the 16S rDNA gene fragment using 3 µl of the DNA template for each sample. The homology sequence of the 16S rDNA of these isolates showed that they belong to 3 different clades namely *Firmicutes*, *Proteobacteria* and *Actinobacteria.* Their genera included the following: *Bacillus spp., Arthrobacter spp, Brevibacterium frigoritolerans*, *Variovorax spp., Bacillus subtilis strain, Bacillus Licheniforms strain, Staphylococcus Succinus strain, Staphlococcus spp.*, *Staphylococcus saprophyticus*, *Paenibacillus spp., Bacillus circulan strain, Bacillus pumilus* and *Arthrobacter globiformis, Bacillus aryabhattai strain, Paenibacillus lautus strain, Ralstonia spp.* and *Geobacillus spp.*

Conclusion

From the results obtained in this study, it can be concluded that a wide variety of bacteria identified are responsible for the degradation of the oil refinery sludge components in the compost piles. This is possible since these bacteria can adapt, grow and survive in such compost systems, they may potentially degrade the oil sludge components. The degradation of oil sludge components is done by bacteria consortia through the production of enzymes, biosurfactants and using the hydrocarbons as source of carbon and energy to survive. Furthermore, the biosurfactant produced by these bacteria are capable of enhancing the solubility of PAHs present in oil sludge co-compost media. As biosurfactants enhanced the solubility of PAHs, biodegradation rate of petroleum hydrocarbons (PAHs) increased in the media. Hence, there was 77 to 99 percent reduction of the PAHs as observed from the results obtained. Biosurfactants can also increase the cell surface hydrophobicity of the biosurfactant-producing strain that results in a high uptake of PAHs. This also means that as the cell surface hydrophobicity increased, there was bioavailability of PAHs in aqueous phase which made it easier for microorganisms to degrade organic contaminants (PAHs). This also helped to achieve 77 to 99 percent reduction of the PAHs as obtained in this study. Therefore, co-composting with animal manures may be suitable for practical field application for effective *in situ* and *ex-situ* bioremediation of oil sludge. It has been noted that co-composting process is an effective and controlled technology (with attributes such as nutrients, temperature, moisture, large population of microbes) for the degradation oil sludge. The results further agreed that composting process does not alter the soil components after treatment as shown in Table 4. At the end of the process, the residual products are not hazardous to the environment which is one of the advantages of composting process (UNIDOI 2003). However, co-composting with animal manures could be efficiently used for bioremediation of oil sludge polluted soils.

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