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Full Length Research Paper

Compost bioremediation of hydrocarbon-contaminated soil inoculated with organic manure

Harrison Ifeanyichukwu Atagana

Institute for Science and Technology Education, University of South Africa, Pretoria, South Africa.
E-mail: atagahi@unisa.ac.za.

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Contaminated soil (FAO: Lithosol) containing >380 000 mg kg^{-1} total petroleum hydrocarbons (TPH) was bioremediated by composting. The soil was inoculated with sewage sludge and incubated for 19 months. The soil was mixed in a ratio of 1:1 (v/v) with wood chips. The soil-wood chips mixture was then mixed in a ratio of 4:1 with sewage sludge. Compost heaps were set up in triplicates on wood pallets covered with double layers of nylon straw sheets. Control experiments which contained the contaminated soil and wood chips but without sewage sludge were set up in triplicate. Moisture, temperature, pH, ash content, C:N ratio of the compost mixture and TPH of the soil was monitored monthly. The concentrations of selected hydrocarbons in the contaminated soil were measured monthly during the incubation period. Temperature rose to about 58°C in the sewage sludge compost within two months of incubation, while temperature in the control fluctuated between 15 and 35°C throughout the incubation period. Total petroleum hydrocarbons (TPH) was reduced by 17% in the control experiments and 99% in the sewage sludge compost at the end of the incubation period. The concentrations of most of the selected hydrocarbon components were reduced by up to 100% within the same period. Microbial activities were shown to correlate with the reduction in hydrocarbon contents of the soil.

Key words: Bioremediation, composting, PAHs, sewage sludge, soil.

INTRODUCTION

The use of composting in bioremediation has received little attention (Potter et al*.,* 1999), despite its application in the treatment of soils contaminated with organic compounds for many years. Much of the work on treatment of contaminated soils by composting (Valo and Slakinoja-Salonen, 1986; Potter et al*.,* 1999; Reid et al*.,* 1999) has been done on soils with lower concentrations of the contaminating substances than used in the present study, in spite of the fact that composts have been reported to have potential for remediation of heavily contaminated sites (Reid et al., 1999; Garcia-Gomez et al*.,* 2003; Manios et al*.,* 2006; Marin et al., 2006).

The soil used for this study was a lithosol (FAO) containing >380 000 mg kg^{-1} total petroleum hydrocarbons (TPH). Selected individual hydrocarbons present in the contamination include: phenol 127 mg kg^{-1}, o-cresol 15 mg kg^{-1}, m-cresol 26 mg kg^{-1}, p-cresol 23 mg kg^{-1}, naphthalene 158 mg kg^{-1}, anthrecene 72.3 mg kg^{-1}, phenanthrene 256 mg kg^{-1}, fluorene 68.3 mg kg^{-1}, pyrrole 77.3 mg kg^{-1}, pyrene 182.1 mg kg^{-1}, fluoranthene 188.5 mg kg^{-1}, chrysene 93.3 mg kg^{-1} and benzo (a) pyrene 68.4 mg kg^{-1}. These high concentrations of hydrocarbons, particularly the PAHs provided a good opportunity to study and further understand the potentials of compost bioremediation of organic compounds. Earlier studies showed that higher molecular weight PAHs remained in the soil after 16 weeks and 11 months of pilot-scale and full-scale land farming, respectively (Atagana, 2003; Atagana, 2004a). Earlier composting experiments using hydrocarbon-contaminated soil co-composted with cow manure and mixed vegetable waste showed that more than 90% of the hydrocarbons including some of the recalcitrant components were removed (Atagana et al*.,* 2003). Co-composting hydrocarbon-contaminated soil with poultry manure showed that PAHs could be removed from the soil by composting (Atagana, 2004b). This study intended to exploit the high temperatures achieved during composting, the high nutrient contents and the heavy microbial load of the sewage sludge in enhancing the microbial removal of high concentrations of hydrocar-

bons, particularly those of high molecular weight from the contaminated soil under investigation.

The aim of this study was to investigate the effects of inoculating soil heavily contaminated with a complex mixture of hydrocarbons with sewage sludge on the removal of hydrocarbons in a static-pile compost system. The temperature regimes in the compost systems, the changes in nutrient composition and moisture content during the treatment period were studied. This was meant to determine the requirements of the compost type and its practical application for large-scale treatment of hydrocarbon-contaminated soils. The remediation target set in this experiment was 1 mg kg^{-1} of the selected hydrocarbons and 1000 mg kg^{-1} of total petroleum hydrocarbons (TPH) in the soil. The chosen targets were informed by earlier targets set by government for similar hydrocarbons in soil and partly to the standards used in an earlier experiment (Lees, 1996) dealing with similar hydrocarbons.

MATERIALS AND METHODS

Contaminated soil (FAO: lithosol) containing >380 000 mg kg^{-1} hydrocarbons was mixed with wood chips in a ratio of 1:1 (v/v) to improve aeration and then mixed with sewage sludge in a ratio of 4:1 (contaminated soil + wood chips : sewage sludge) (v/v). Triplicate static pile compost heaps (about 350 kg each) were set up on wooden pallets that were covered with nylon fibre sheet in the open yard. The compost heap was then covered with hay for insulation and incubated for a total of 19 months. The control was a mixture of contaminated soil and wood chips without sewage sludge.

A temperature data logger with thermocouples located in the middle of the compost heaps was used to monitor changes in temperature. The moisture content of the compost was measured weekly by using the method described by Forster (1995a) and water was added as required. The pH of the aqueous extract of the compost mixture was measured at monthly intervals in triplicate with a pH meter (Crison Micro pH 2000^{TM}). The ash content of the compost mixture was determined at the start and at the end of the experiment by heating 10 g of the mixture in a furnace at 400°C for 6 h.

CO_2 evolution measured by using the closed jar method (Alef, 1995) at room temperature was used to estimate microbial activity. Microbial plate counts of samples taken from three different levels (20, 35 and 50 cm) in the compost heaps were carried out on nutrient agar and the data represented as colony forming units per gram (cfu g^{-1}). Total organic carbon was determined by a conductometric method (Forster, 1995b) extractable phosphorus by the Bray-1-P test (Recommended Chemical Soil Test Procedures No221, University of Missouri Agricultural Experiment Station, Columbia, Missouri 1998) and total nitrogen was determined by soil digestion method (Forster, 1995c).

Total petroleum hydrocarbon (TPH) was determined by infrared using US EPA Method No. 8440 (1996) and absorbance was determined with a Nicolet Avatar 320 IR Spectrophotometer at wave numbers between 2760 and 3070 cm^{-1} and an integration value for the absorbance peak was automatically generated. Changes in the concentrations of selected hydrocarbons were determined by Soxhlet extraction and GC/FID analysis. The GC was a Varian-3800 with argon as the carrier gas and fitted with a 3 m capillary column with 0.25 µm film thickness. Two temperature programmes were used (Eriksson et al., 2000) in order to obtain good separation and quantification of the more volatile compounds.

Identification of bacterial isolates was done by biochemical tests (Holt, 1994; MacFaddin, 1980). Fungal isolates were identified by microscopic examination with reference to Barnett and Hunter (1972), Raper and Thom (1968).

Analysis of variance (ANOVA) was done to determine the level of significance at $p < 0.05$ between the results obtained at each period of the composting process. All statistical analysis was performed using PlotIT software.

RESULTS AND DISCUSSION

Changes in pH and temperature during composting

The pH of the sewage sludge compost and the control increased from 7.0 and 6.9 to 8.1 and 7.7, respectively within four months of incubation and then fluctuated between 6.2 and 7.7, 7.1 and 6.7, respectively (Figure 1). The pH ranges observed in these experiments are well within the recommended range for composting organic materials (Feinstein et al., 1986; Kubota and Nakasaki, 1991; Marin et al., 2006). The high increases in the first four months could be from high metabolic activities possibly resulting in the production of intermediate metabolites in the compost systems. The decreases observed in subsequent months are attributed to the degradation of the compost and the hydrocarbons, which may have resulted in the release of acidic intermediate and final products that probably lowered pH of the mixture (Alexander, 1999; Eweis et al., 1999) (Figure 1).

The temperature of the control experiment, which ranged between 12 and 30°C fluctuated with the daily diurnal air temperatures, which ranged between 17 and 33°C during the experimental period (Figure 2). Temperatures in the sewage sludge compost rose to 58°C in the second month of incubation and remained relatively stable until it started to decrease at the end of the third month reaching 30°C. Temperatures became relatively stable after the seventh month, fluctuating between 29 and 40°C for the remainder of the experiment (Figure 2). The large increase in temperatures in the first two months was due to the high initial microbial load, 1.28 x 10^7 in the sewage sludge, which resulted in high metabolic activities (Figure 3). However, the high temperature became inhibitory to continued microbial growth after two months, resulting in a decrease in microbial activity (Figure 3). A subsequent decrease in temperature in the fourth month (Figure 2) resulted in increase in microbial activity in the following month. This phenomenon has been previously reported by Potter et al. (1999) (Figures 2 and 3).

Changes in the C:N ratio of the compost during composting

The sewage sludge and the contaminated soil had initial C:N ratios of 9:1 and 306:1, respectively. The high C:N ration of the soil is attributed to the TPH content (>380 000 mg kg^{-1}) of the soil, which will naturally inhibit microbial growth (Baker and Herson, 1994: Alexander,

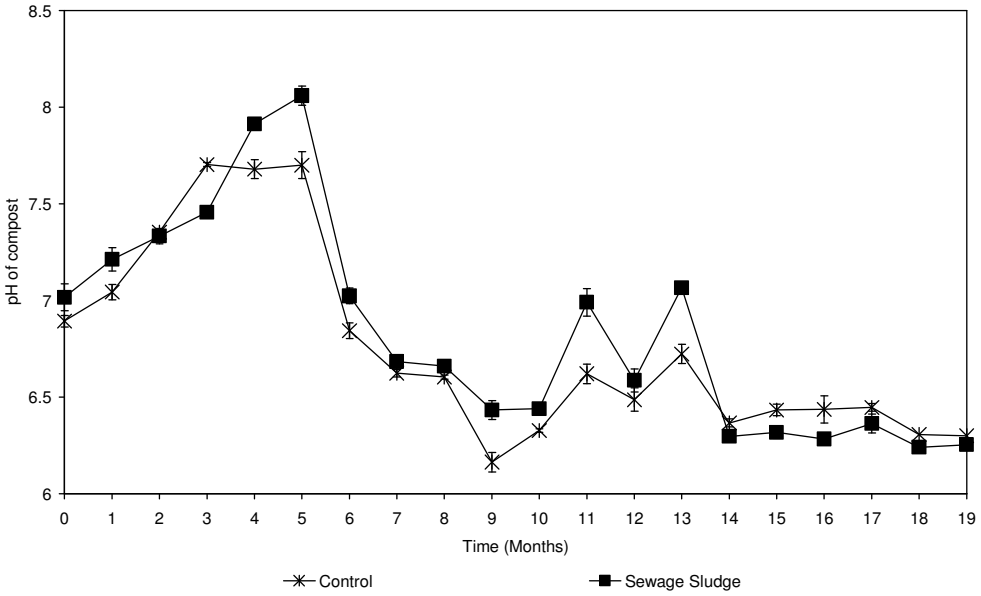

Figure 1. Changes in the pH of compost during composting. Values are means of three replicates ± 1 Standard Error.

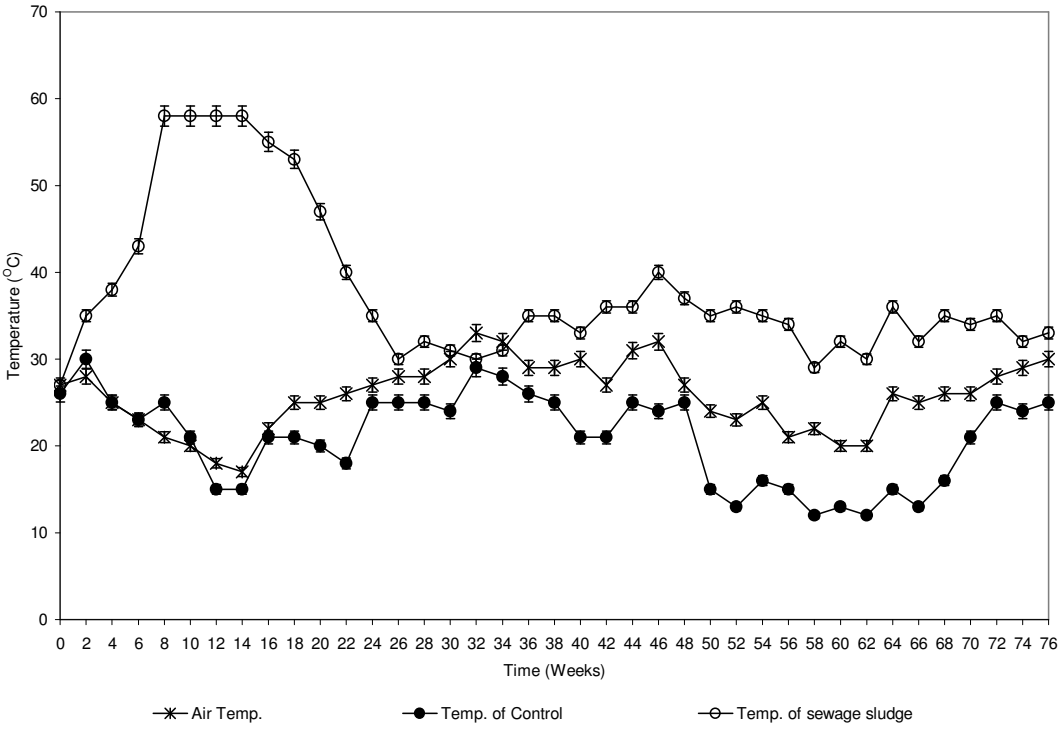

Figure 2. Changes in temperature of the compost during composting. Values are means of three replicates ± 1 Standard Error.

1999). The C:N ratio of the sewage sludge compost mixtures was 23:1 (Table 1) at the start of the composting. Although this nitrogen content is higher than those recommended for effective compost bioremediation (between 25:1 to 35:1) (Anderson, 1991; Kubota and Nakasaki, 1991), they were considered adequate for this experiment, considering the level of contamination in the soil and the results obtained in earlier experiment in

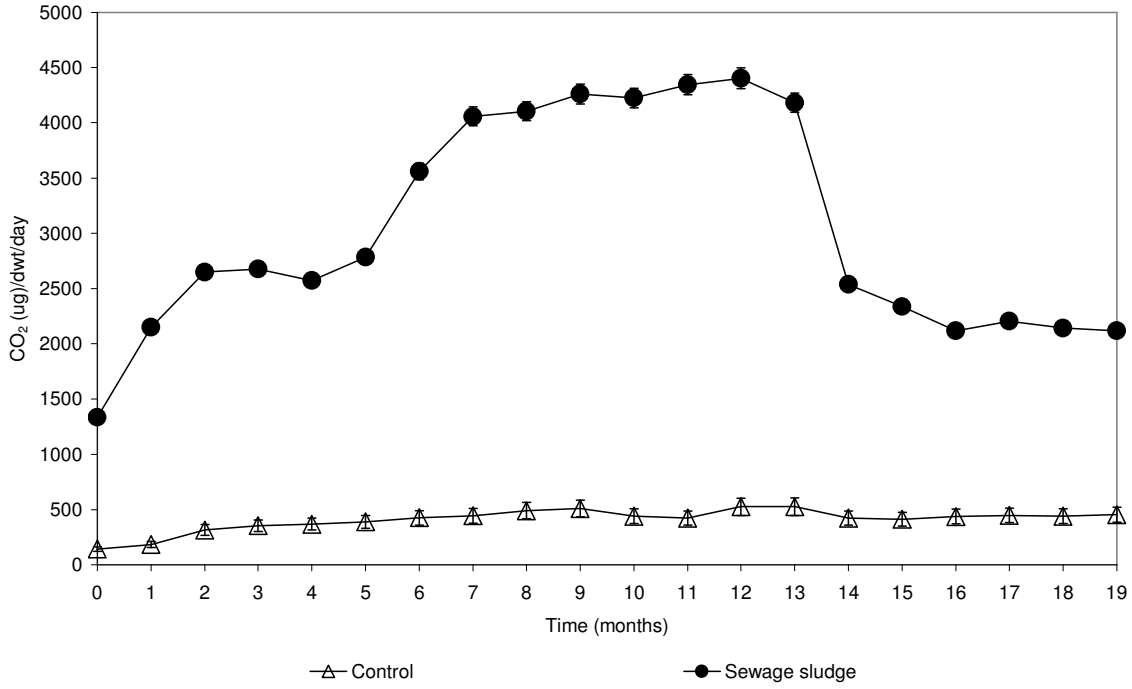

Figure 3. Changes in the respiration rates of compost inhabiting microorganisms during composting. Values are means of three replicates ± 1 Standard Error.

Table 1. Changes in the C:N ratios of compost mixture during incubation.

Treatment	0	6 Months	12 Months	18 Months
Contaminated soil (control experiment)	306:1	326:1	322:1	289:1
Sewage sludge compost	23:1	15:1	15:1	17: 1

Values are means of three ± 1 Standard Deviation.

which up to 90% of the total hydrocarbons were removed (Atagana, 2003; Atagana, 2004a). The C:N ratio in the compost and control changed as the incubation progressed (Table 1). The decrease in nitrogen content in the sewage sludge compost was faster than in the control probably due to higher microbial activity and higher rate of breakdown of the hydrocarbon substrates in the compost systems.

Ash components of the compost mixtures changed slightly from 4.62 to 4.60 in the sewage sludge compost and 6.37 to 6.41 in the control, which indicates that there was no significant change in the mineral components of the soil at the end of the experiment (Table 1).

Changes in total petroleum hydrocarbon (TPH) content and microbial populations

The changes in TPH content of the sewage sludge compost were significantly different at $p<0.05$ from the control experiment (Figure 4). In the first two months, decreases in TPH levels were 67.8% in the sewage sludge compost and 10% in the control. Sewage sludge has been reported to enhance the degradation of hydrocarbons in soil-compost mixtures (Hill and McCarthy, 1967; Wilson et al., 1983). The rapid degradation of hydrocarbons in the compost system was expected since sewage sludge is rich in nutrients and has high microbial population (Combs et al., 2001; Schmitt and Rehn, 2002). At the end of the incubation period TPH had decreased by 17% in the control experiment and 99.8% in the sewage sludge. The organisms growing on the nutrients present in the compost system readily metabolise the contaminant hydrocarbons in the compost mixture while still growing on the sludge. This deduction was made, as isolates from the sewage sludge could not establish easily on the same contaminated soil in the absence of the sewage sludge (results not shown). The organisms, while growing on the sludge substrate, probably produced enzymes that were used in metabolising the hydrocarbons in the compost matrix (Sutherland et al., 1995; Bardos et al., 1996; Diaz et al., 1996). The high microbial load in the compost (Figure 5) at the start of the composting afforded the population the opportunity to remain high while adapting

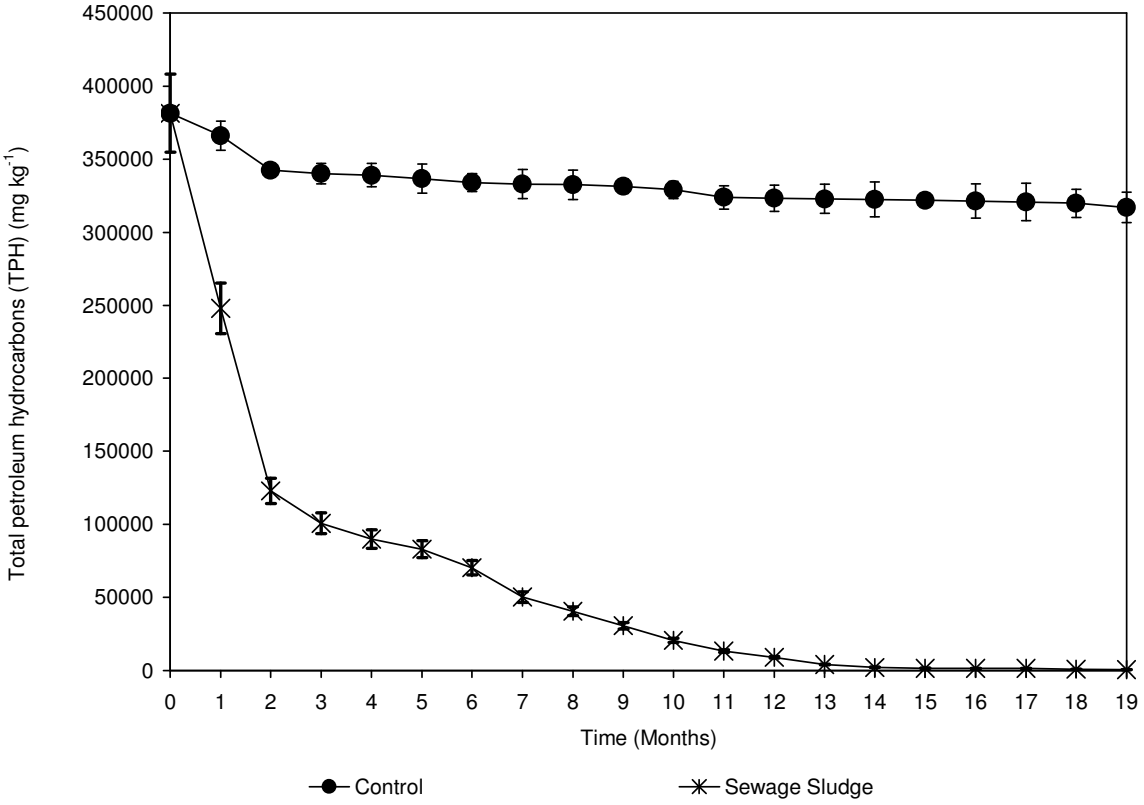

Figure 4. Monthly reduction in total petroleum hydrocarbons (TPH) during composting. Values are means of three replicates ± 1 Standard Error.

to and attacking the hydrocarbon substrate.

During the second month of the experiment, counts of heterotrophic micro-organisms increased from 1.28 x 10^7 to 1.86 x 10^7 in the sewage sludge, probably due to the ready supply of available nutrients in the sludge, and 3.78 x 10^4 to 7.15 x 10^4 in the control (Figure 5). During the same period, the largest amounts of reduction in TPH content were observed (Figure 4).

Increases in microbial populations and rapid reductions in hydrocarbon content continued after the fourth month probably due to the decrease in temperature as mentioned earlier, and a decrease in nitrogen levels caused by the initial high microbial activity (Piccinini et al., 1996; Atagana, 2004b). The populations reached a maximum of 3.3 x 10^7 in sewage sludge compost in the fourth month and 7.6 x 10^4 in the control experiment in the eleventh month before decreasing (Figure 5), probably as a result of the large decrease in the concentration of hydrocarbons and the nutrient base. The high temperatures generated in the compost (Figure 2), which resulted in the increased loss of water from the soil-compost matrix may also have resulted in the decrease in microbial population after the sixth month (Fan and Tafuri, 1994). Regular watering when necessary kept the temperature from increasing higher than 58°C in the compost system (Figure 5).

Changes in the concentrations of selected hydrocarbons

The tested phenols (phenol, o-cresol, m-cresol and p-cresol) and the 2- and 3- ring PAHs (naphthalene, anthracene, phenanthrene, fluorene and pyrrole) were removed below the remediation target of 1 mg kg^{-1} by between the third and ninth month of incubation (Figures 6 and 7). The removal of the 4- and 5- ring PAHs (pyrene, chrysene, fluoranthene and benzo (a) pyrene) became slower from about the eighth month, and residual concentrations of chrysene continued to be above 1 mg kg^{-1} until the sixteenth month (Figure 8). A decrease in temperature in the compost mixtures in the fifth month (Figure 2) resulted in an increase in microbial populations (Figure 5) with a subsequent increase in the removal of pyrene, chrysene and fluoranthene. The increased decrease in concentration only became obvious for benezo (a) pyrene by the seventh month (Figure 8). Apart from the phenolics and naphthalene, most components persisted to the end of the incubation period in the control (Figures 6, 7 and 8).

Respiration of micro-organisms in the compost systems (Figure 3) and counts of heterotrophic microorganisms (Figure 5) shows that microbial activity correlated with changes in TPH and selected hydrocarbons levels. The

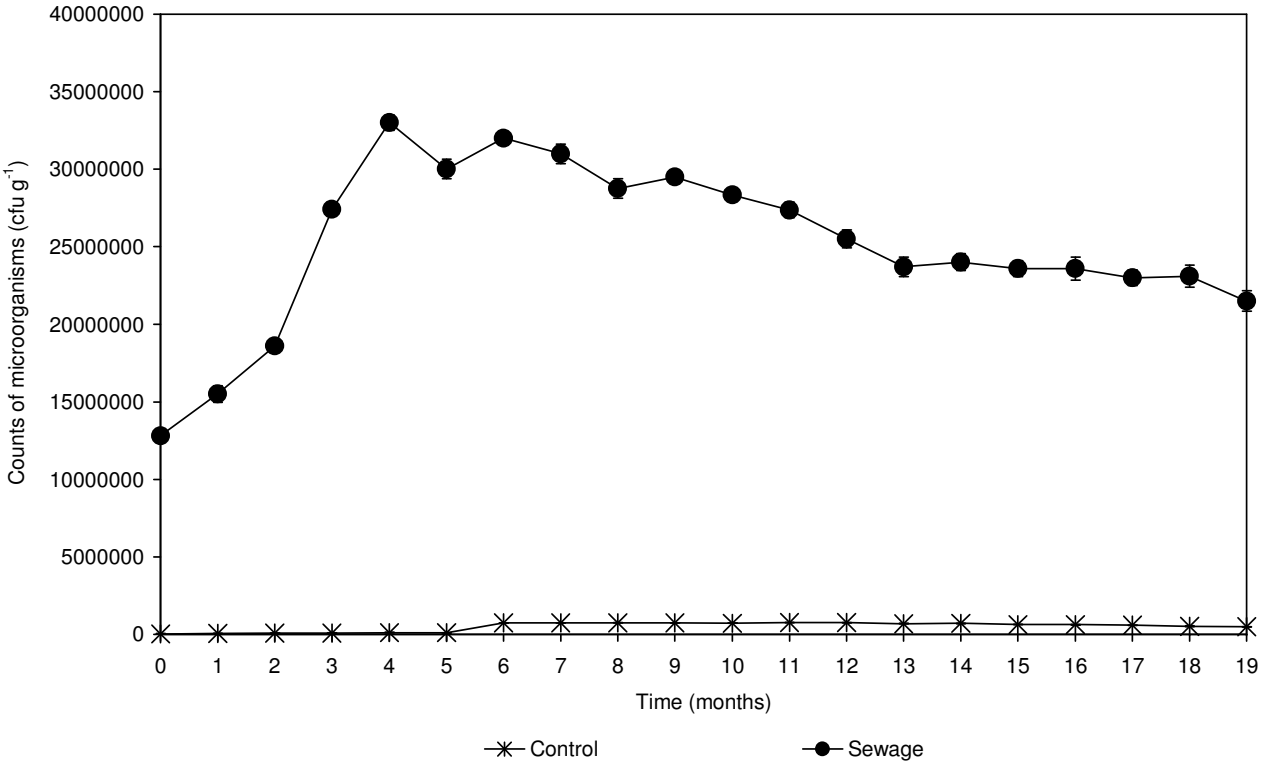

Figure 5. Counts of microorganisms in the compost during composting. Values are means of three replicates ± 1 Standard Error.

effect of the rapid increases in microbial activity in the first month manifested in higher decreases in concentrations in the second month. This delayed effect is attributed to the time used by the organisms to adapt to the hydrocarbon medium and also other factors such as high initial nitrogen content and high temperatures as discussed earlier.

The rate of removal of hydrocarbons from the control experiment and the sewage sludge compost system showed a similar trend in the first month (Figures 6, 7 and 8). However, while removal continued very slowly for the rest of the experimental period in the control experiment, it increased rapidly from the second month in the compost system.

Compared to earlier studies using a similar contaminated soil in which more than 20% of the 4- and 5- ring PAHs persisted at the end of eleven months of treatment (Atagana, 2004a), all the tested components were removed below the remediation target (1 mg kg^{-1}) by the end of the incubation period.

Contrary to expectations, high nitrogen content and high temperatures had limited effects on the microbial load and degradation capacity of the compost probably due to the reasons discussed earlier (Figures 8).

At the start of composting, a mixed population of bacteria and fungi dominated the compost. The dominant bacteria isolates were *Pseudomonas* sp., *Bacillus* sp., *Rhodococcus* sp. and *Mycobacterium* sp. among others that were not readily identified. The dominant fungal species were *Mucor, Rhizopus, Fusarium, Aspergillus, Penicillium* and *Pleurotus*. By the end of the fourth month, the dominant species were mainly fungi with *Pleurotus* sp., becoming more prominent. *Fusarium, Aspergillus* and *Penicillium* persisted in relatively low amounts at the end of incubation. *Mucor* and *Rhizopus* were not evident in the compost at the end. *Phanerochaete* was isolated after the fourth month. Three *Pseudomonas* species and A*rthrobacter* sp. were isolated after the fourth month. Although, there was a dynamic change in the microbial population of the compost system during the composting period, *Pleurotus, Phanerochaete, Fusarium, Pseudomonas and Arthrobacter* were the most persistent genera that were identified.

Conclusions

Although, sewage sludge accelerated the composting of garden refuse, their application in compost bioremediation has not been fully explored. The results in the present experiment shows that under controlled conditions inoculation of compost containing hydrocarbon-contaminated soil with sewage sludge can effectively accelerate the removal of such contaminants from the soil matrix.

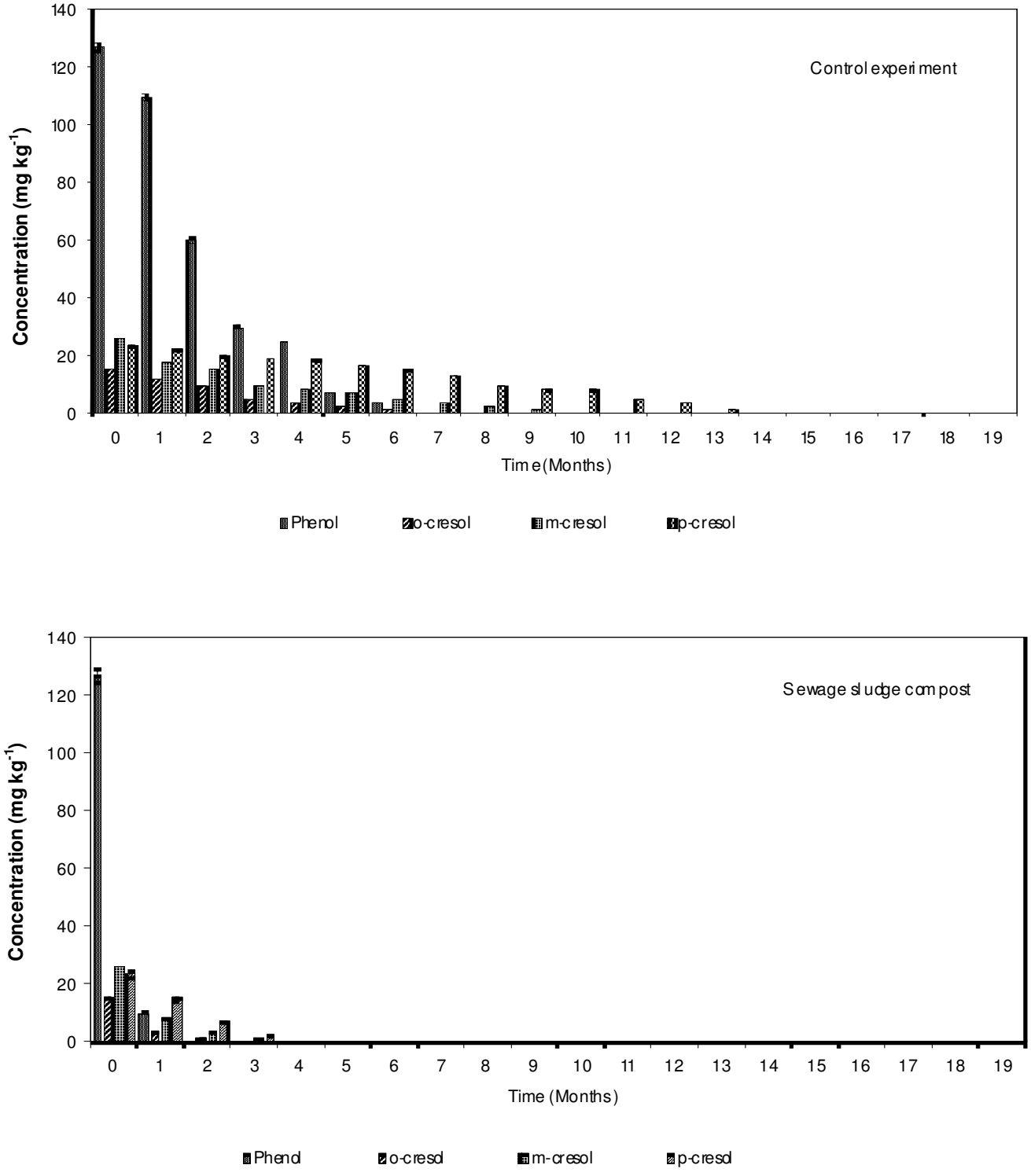

Figure 6. Changes in the concentrations of selected hydrocarbons (phenols) during composting. Values are means of three replicates ± 1 Standard Error.

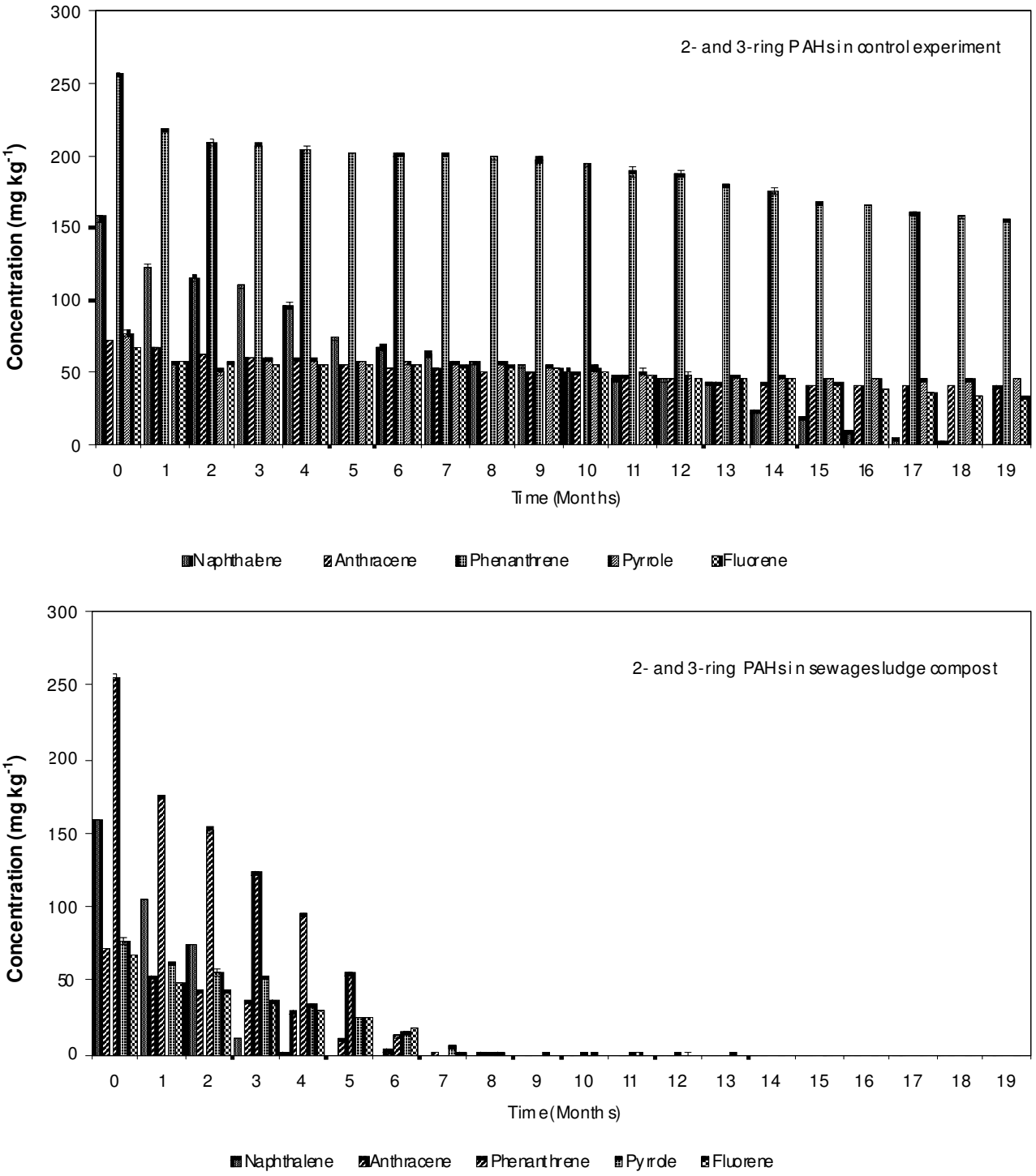

Figure 7. Changes in the concentrations of selected hydrocarbons (2- and 3- ring PAHs) in the compost during composting. Values are means of three replicates ± 1 Standard Error.

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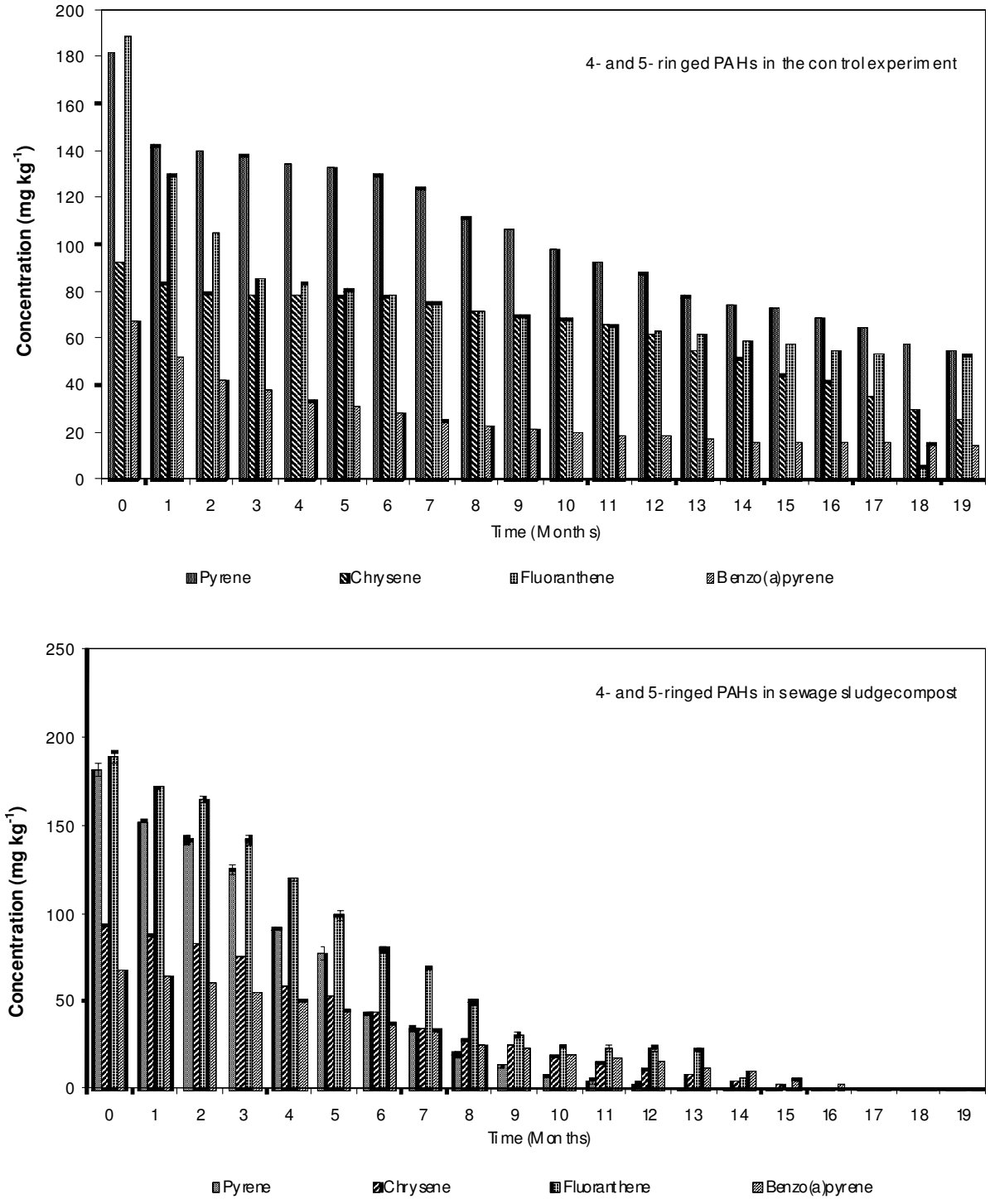

Figure 8. Changes in the concentrations of selected hydrocarbons (4- and 5- ring PAHs) in the compost during composting. Values are means of three ± 1 Standard Error.

maritzburg, South Africa; and Department of Soil Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

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