Co-Composting of PAH-Contaminated Soil With Poultry Manure

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ABSTRACT

Mispah form (FAO: Lithosol) soil contaminated with PAHs was co-composted with poultry manure for nineteen months. The soil was mixed with wood chips to improve aeration and then mixed with poultry manure in a ratio of 4:1. Moisture, temperature, pH, ash content, C:N ratios, and the concentrations of selected PAHs in the compost were monitored monthly. Temperature increased to $60^{O}C$ in the poultry manure compost within three months before decreasing to about $45^{O}C$ and then fluctuating between 35 and $50^{O}C$, while temperature in the control remained about $30^{O}C$ for most of the composting period. PAHs concentrations decreased by between 96% and 100% at the end of the composting period. The 2- and 3- ring PAHs were more easily degraded while the 4- and 5- ring compounds were more recalcitrant, persisting until the end of the composting. The consortia of microorganisms isolated from the compost system included fungi, bacteria and actinomycetes. Microbial activity, monitored by CO_2 evolution correlated with reduction in PAHs concentrations.

INTRODUCTION

Compost bioremediation is carried out by co-composting the contaminated soil with suitable compost materials to effect biodegradation of the contaminant. Previous studies have examined the degradation of organic pollutants in composts (Reid *et al.,* 1999). For example, it has been shown that the microbes present in windrow composts are capable of mineralizing pentachlorophenol (Valo and Slakinoja-Salonen, 1986). Although composting has been used in the remediation of soils contaminated with a number of organic compounds, including PAHs, the use of composting as a bioremediation technology has been given very little attention (Potter *et al.,* 1999). 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 substance than were present in the present study, in spite of the fact that composts have been reported to have good potential for remediation of heavily contaminated sites (Reid *et al*., 1999).

The soil used for this study is a mispah form (FAO: lithosol) contaminated with a wide range of PAHs among which the most prominent are naphthalene 158mg kg^{-1}, anthrecene 72.3mg kg^{-1}, phenanthrene 256 mg kg^{-1}, fluorene 68.3mg kg^{-1}, pyrrole 77.3mg kg^{-1}, pyrene 182.1 mg kg^{-1}, fluoranthene 188.5mg kg^{-1}, chrysene 93.3 mg kg^{-1} and benzo(a)pyrene 68.4 mg kg^{-1}. These high levels of PAHs provided a good opportunity to study and further understand the potentials of composting in soil bioremediation. Earlier, studies (Atagana, 2002; Atagana, 2003) showed that reasonable amounts of higher molecular mass PAHs remained in the soil at the end of 16 weeks and 11 months of pilot-scale and full-scale landfarming respectively. This study intends to exploit the high temperatures prevalent during composting, the high nutrient contents of poultry manure and the heavy microbial load of the compost system in enhancing the biodegrataion of high molecular weight PAHs in the soil system under investigation.

The aim of this study therefore is to investigate the effects of co-composting poultry manure with soil contaminated with different concentrations of polycyclic aromatic hydrocarbons (PAHs), on the degradation of selected PAHs in a static-pile compost system. It is also study the temperature regimes in the compost system and the changes in nutrient composition and moisture occurring therein, during the treatment period. This would help determine the requirements of the compost type and its practical application on large-scale treatment of PAHs-contaminated soils. The remediation target set in this experiment was 1 mg kg^{-1} for the selected PAHs.

MATERIALS AND METHODS

About 2 000kg of PAHs-contaminated mispah form (FAO: lithosol) soil were excavated from the experimental site and transported in clean nylon fibre bags to the laboratory, where it was stored in a cold-room at 4 ^{O} C. Samples of the soil (250g each) were taken to an independent commercial laboratory for determination of the PAHs concentrations by GC/FID. The bulk soil was homogenized in an electric concrete mixer before being used to make the soil compost mixture. Poultry manure and hay for covering the compost heaps were obtained from the University of Natal Experimental Farm, Ukulinga, Pietermaritzburg. Compost heaps were made on slatted, sawn wood pallets overlaid with nylon fibre bags, which allowed passage of air and excess water in and out of the compost heaps.

Homogenized contaminated soil (350 kg) was mixed with wood chips (20cm x 4cm) in a ratio of 1:1 (v/v) and then mixed with poultry manure in ratio of 4:1(contaminated soil + wood chips : compost material) (v/v). The experiments were set up in triplicates. The control was a mixture of contaminated soil and wood chips without poultry manure. The mixtures were placed on a palette and covered with hay for insulation. The experiments were incubated for a total of nineteen months.

A temperature data logger with thermocouples located in the middle of the compost heaps measured temperature hourly. Moisture content was measured weekly and water added as necessary. The pH of the compost mixture was measured at monthly intervals in triplicate by using a standard 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 10g of the mixture in a furnace at 400^{O}C for 6 hours.

Respiration rates of compost-inhabiting microorganisms were determined by the closed jar method (Forster, 1995). Microbial plate counts of samples taken from three different levels (20cm, 35cm, and 50cm) in the compost heaps were done on nutrient agar and represented as colony forming units per gram (cfu g^{-1}). Total organic carbon, extractable phosphorus and total nitrogen were determined by the University of Natal Chemistry Department and KwaZulu-Natal Department of Agriculture, Cedara.

Changes in the concentrations of PAHs were determined by Soxhlet extraction and GC/FID. The GC was a Varian-3800 with argon as the carrier gas and fitted with a 3m capillary column with 0.25µm 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 using Buchanan and Gibbon's Bergey's Manual of Determinative Bacteriology and MacFaddin's Biocemical Tests for Identification of Medical Bacteria. Fungal isolates were identified by microscopic examination with reference to Barnett and Hunter's illustrated genera of imperfect fungi and Raper and Thom's manual of the penicillia.

RESULTS AND DISCUSSION

Changes in pH of The Compost During Composting

The pH of the compost and the control increased in the first four months reaching 8.4 in the compost and 7.6 in the control. The compost pH decreased after the fifth month reaching 6.6 in the ninth month before increasing again to 7.6 in the thirteenth month. The decrease after the thirteenth month was gradual and reached 6.7 at the end of the experiment. The pH of the control followed the same pattern of decrease and reached 6.3 at the end of the experiment. For most of the experiment the pH remained between 6.2 and 7.6, which is well within the recommended range for composting organic materials (Hunter et al., 1981; Kubota and Nakasaki, 1991). The increase in the first four months could be due to the high ammonium content of the poultry manure. The decreases observed in subsequent months are attributed to the degradation of the compost and the hydrocarbons, which resulted in the release of intermediate and final products that probably had lowering effects on the pH of the mixture (Alexander, 1999; Lee and Lee, 2001; Piccolo, 2002).

Changes in The C:N Ratio of The Compost During Composting

A relatively high nitrogen concentration (C:N 25:1 to 35:1) is required to facilitate the effective compost bioremediation (Anderson, 1991; Kubota and Nakasaki, 1991). The C:N ratio in the present study is shown in Tables 1 and 2. The initial C:N ratio of the contaminated soil was 306:1. this ratio was considerably reduced after mixing with wood chips and poultry manure (Table 2). Although the nitrogen content in these C:N ratios are higher than those recommended by Anderson (1991), they are considered adequate for composting organic materials, considering the amount of compost materials used. The C:N ratio in the compost and control changed as the incubation progressed (Table 2). The decrease in nitrogen content in the compost was faster than in the control probably due to higher microbial activity and higher rate of breakdown of the hydrocarbon substrate in the poultry manure compost than in the control.

There was only a slight change in ash component of the compost mixture (4.1 to 4.15) and the control (6.37.6.41), which indicates that there was no significant change in the mineral components of the soil at the end of the experiment.

Microbial population and PAH degradation

The results show that there was a significant difference between the poultry manure compost and the control in the degradation of the PAHs (Fig. 1-9). The poultry manure microflora removed the PAHs faster than the control. Poultry manure has been reported to enhance the degradation of hydrocarbons in soil-compost mixtures (Hill and McCarthy, 1967; Deever and White, 1978). The rapid degradation observed in the compost was expected since poultry manure is rich in carbon and mineral nutrients particularly nitrogen (Kellog-Johnson, 1996). The organisms growing on the nutrients present in the poultry manure were found to readily metabolise the contaminant PAHs in the compost mixture while still growing on the manure. The manure afforded the organisms the opportunity to grow while adapting to the hydrocarbons. It also afforded the organisms the opportunity to produce enzymes that are required to metabolise hydrocarbons in the compost matrix (Sutherland, 1995; Bardos et al., 1996; Diaz et al., 1996). The high microbial load in the compost (7.39E+05) at the start of the composting afforded the population the opportunity to remain high while adapting to and attacking the hydrocarbon substrate.

During the second month of the experiment, counts of heterotrophic microorganisms increased from 7.39E+05 to 3.67E+07 in the compost mixture. During the same period, the largest amounts of reduction in hydrocarbon concentrations were observed (Fig. 1-9). This population increase is probably attributable to the abundance of readily available nutrients in the manure. It can also be attributed to the loss of excess nitrogen as gaseous ammonia (Piccinini *et al.,* 1996). High nitrogen content in soil can be inhibitory to microbial growth, affecting the rate of hydrocarbon degradation (Baker and Herson, 1994; Alexander, 1999). Increase in microbial populations and rapid degradation of some of the hydrocarbons continued after the fourth month probably due to further loss of nitrogen as a result of the increased microbial activity and high temperatures (60^{O}C) reached at the start of the treatment (Piccinini *et al.,* 1996). The population reached a maximum of 4.88E+07 in the eight before it started to decrease, probably as a result of increasing release of ammonia during decomposition of the manure, which eventually became toxic to the microorganisms, explaining the decline in the microbial population to 3.88E+07 in the ninth month. The high temperatures generated by the decomposing manure, 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 eighth month (Fan and Tafuri, 1994). Regular watering when necessary kept the temperature from increasing higher than $60^{O}C$. The 2- and 3- ring PAHs (naphthalene, anthracene, phenanthrene and pyrrole were removed the remediation target of 1 mg kg^{-1} by the third and fourth months (Fig. 1-4) except for fluorene, which persisted until the seventh month (Fig. 5). Degradation of 4- and 5- ring PAHs (pyrene, chrysene, fluoranthene and benzo(a)pyrene became slower from about the eighth month (Fig. 6-9) and residual concentrations of chrysene continued to be above 1 mg kg^{-1} until the sixteenth month (Fig. 7). A decrease in temperature in the poultry manure-contaminated soil mixture in the fifth month from 60 to $45^{O}C$ resulted in an increase in microbial population from 3.7E+07 to 4.12E+07 and subsequently an increase in removal of pyrene, chrysene and fluoranthene (Fig. 6-8). The effect only became evident in benzo(a)pyrene in the sixth month (Fig. 9).

Respiration of compost-inhabiting microorganisms (Fig. 10) and counts of heterotrophic microorganisms showed that microbial activity correlates with hydrocarbon degradation. Although the rapid increase in microbial activity in the first month did not translate into a similar amount of loss in hydrocarbon concentration, the effect of this increase manifested 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 removal of PAHs from the control experiment was similar to the compost system in the first month (Fig.1-9). However, while degradation continued very slowly for the rest of the experimental period in the control, degradation in the compost system increased rapidly from the second month.

In comparison to earlier studies using the same contaminated soil in which more than 20% of the 4- and 5- ring PAHs persisted at the end of eleven months of treatment (Atagana, 2003), all the PAHs except chrysene were removed to below the remediation target (1 mg kg^{-1}) by the eleventh month in the present experiment. The experiment was extended for six months during which period chrysene was degraded to below the target concentration. However, the experiment was extended for a further three months to achieve complete degradation of the compounds. It was observed that degradation became very slow after the fourth month for the 2- and 3- ring PAHs and after the eighth month for the 4- and 5- ring PAHs and in many cases, traces of the compounds (0.1-0.5 mg kg^{-1}) persisted until the end of the treatment period. This slow degradation is attributed to the very low concentrations of the PAHs remaining in the soil at those times. Eartlier reports have suggested decline in hydrocarbon degradation by soil microorganisms at very low concentrations (Baker and Herson, 1994; Alexander, 1999). This may also be due to incomplete desorption of the sorped hydrocarbon molecules to soil particles.

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.

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 isolates were *Mucor, Rhizopus, Fusarium, Aspergillus, Penicillium and Pleurotus*. By the end of the fourth month, the dominant species were mainly fungi with *Pleurotus*, becoming more prominent. *Fusarium* and *Aspergillus* and *Penicillium* persisted in relatively low amounts but *Mucor* and *Rhizopus* were not evident any more. *Phanerochaete* was also 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, *Pleurotu, Phanerochaete, Fusarium, Pseudomonas and Arthrobacter* were the most persistent species that were identified.

CONCLUSIONS

Although poultry manure has been found to accelerate the composting of garden refuse, its application in bioremediation has not been fully explored. The results in the present experiment have shown that under controlled conditions co-composting of poultry manure with contaminated soil can effectively decontaminate soils heavily contaminated with PAHs. A temperature range of between 35 and 60OC is effective for composting hydrocarbon pollutants in soil with poultry manure.

REFERENCES CITED

Alexander, M., Biodegradation and bioremediation, San Diego, Academic Press (1999).

Anderson, J.G., Treatment of wastes by composting. In Senior, E. (ed), Microbiology of Landfill Sites. Boca Raton, CRC Press Inc., pp. 59-77. (1991).

Atagana, H. I., Bioremediation of creosote contaminated soil: A Pilot-scale landfarming evaluation. *World J. Microbiol and Biotechnol* 19: 571-581(2003).

Atagana, H.I., Bioremediation of creosote-contaminated soil by microbial intervention. A Ph.D. dissertation submitted to the University of Natal, Pietermaritzburg, South Africa (2002.).

Baker, K.H. and D.S., Herson. Bioremediation. McGraw-Hill, Toronto (1994).

Bardos, R.P., Forsythe, S., Westlake, K., The co-treatment of municipal and industrial waste. In M. de Bertoldi, P. Sequi, B. Lemmes and T Papi (eds), The Science of Composting. Blackie Academic and Professional, London. pp. 767-783 (1996).

Barnett, H.L. and Hunter, B.B., Illustrated genera of imperfect fungi. USA, Burgress Publishing, (1972).

Deever, W.R. and White, R.C. 1978. Cited by Wilson, G.B. Sikora, L.J. and Parr, J.F., Composting of chemical industrial wastes prior to land application. In Parr, J.F., Marsh, P.B. and Kla, J.M. (eds), Treatment of hazardous wastes, Noyes Data Corporation, New Jersey, pp. 263-273 (1983).

Diaz, L.F., Savage, G.M. and Golueke, C.G., Stabilization of hazardous wastes through biotreatment. In de Bertoldi, M., Bert, P. and Tiziano, P. (eds), The Science of Composting. Blackie Academic and Professional, London, pp. 1152-1156 (1996).

Eriksson, M., Dalhammar, G. and Borg-Karlson, A.-K., Biological degradation of selected hydrocarbons in an old PAH/creosote contaminated soil from a gas work site. *Appl Microbiol Biotechnol.* 53: 619-626 (2000).

Hill, D.W. and McCarty, P.L., Anaerobic degradation of selected chlorinated pesticides. *J. Water Poll. Cont. Fed.* 39: 1259-1277 (1967).

Hunter, J.V., Finstein, M.S., Suler, D.J and Bopal, R.R. 1981. Cited by Feinstein, M .S., Miller, F.C. and Strom, P.E., Waste treatment composting as a controlled system. In Rhelm, H.J.and Reed, G. (eds), Biotechnology Vol. 8 Microbial degradation. VCH Publishers, NY. 363-398 (1986).

Kubota, H. and Nakasaki, K., Accelerated thermophilic composting of garbage. *BioCycle* 32: 66-68 (1991).

MacFaddin, J.F., Biochemical tests for identification of medical bacteria, Baltimore, (1980).

Piccinini, S., Rossi, L., Bonazzi, G. and Dall= Orso, G., The Emilia-Romaga Experiment in animal manure composting. In M. de Bertoldi, P. Sequi, B. Lemmes and T Papi (eds), The Science of Composting. Blackie Academic and Professional, London. pp. 1275-1280 (1996).

Potter, C.L., Glaser, J.A., Hermann, R. and Dosani, M.A., Remediation of contaminated East River sediment by composting technology. In A. Leeson and B.C. Alleman (eds), Bioremediation technologies for polyclic aromatic hydrocarbon compounds. The fifth international *in situ* and on-site bioremediation symposium. San Diego, California, April 19-22, 1999. Battelle Press, Columbus. pp. 31-36 (1999).

Raper, K.B. and Thom, C., A manual of the penicillia. New York, Hafner Publishing Co., (1968).

Sutherland, J.B., Rafti, F., Khan, A.A. and Cerniglia, C.E., Mechanisms of polycyclic aromatic hydrocarbon degradation. In: Microbial transformation and degradation of toxic organic chemicals (Eds) Young, L.Y. and Cerniglia, C.E. Wiley Liss, New York. pp 269-306 (1995).

Valo, R. and Salkinoja-Salonen, M., Bioremediation of chlorophenol-contaminated land. *Appl. Microbiol. Biotechnol.* 25: 68-75 (1986).

Table 1. C:N ratios of contaminated soil and poultry manure before mixing. Values are means of three ± 1 standard Deviation.

Compost materials before mixing	**C (g kg^{-1})**	**N (g kg^{-1})**	**C:N**
Contaminated soil	24.53 x 10	0.08 x 10	306:1
Poultry manure	36.03 x10	3.63 x 10	10:1

Table 2. Changes in C:N ratios of compost mixtureduring incubation. Values are means of three ± 1 Standard Deviation.

Compost-soil mixture	**0 Time**	**6 Months**	**12 Months**	**18 Months**
Contaminated soil (Control)	306:1	326:1	322:1	289:1
Poultry Manure Compost	2:1	3:1	5:1	8:1

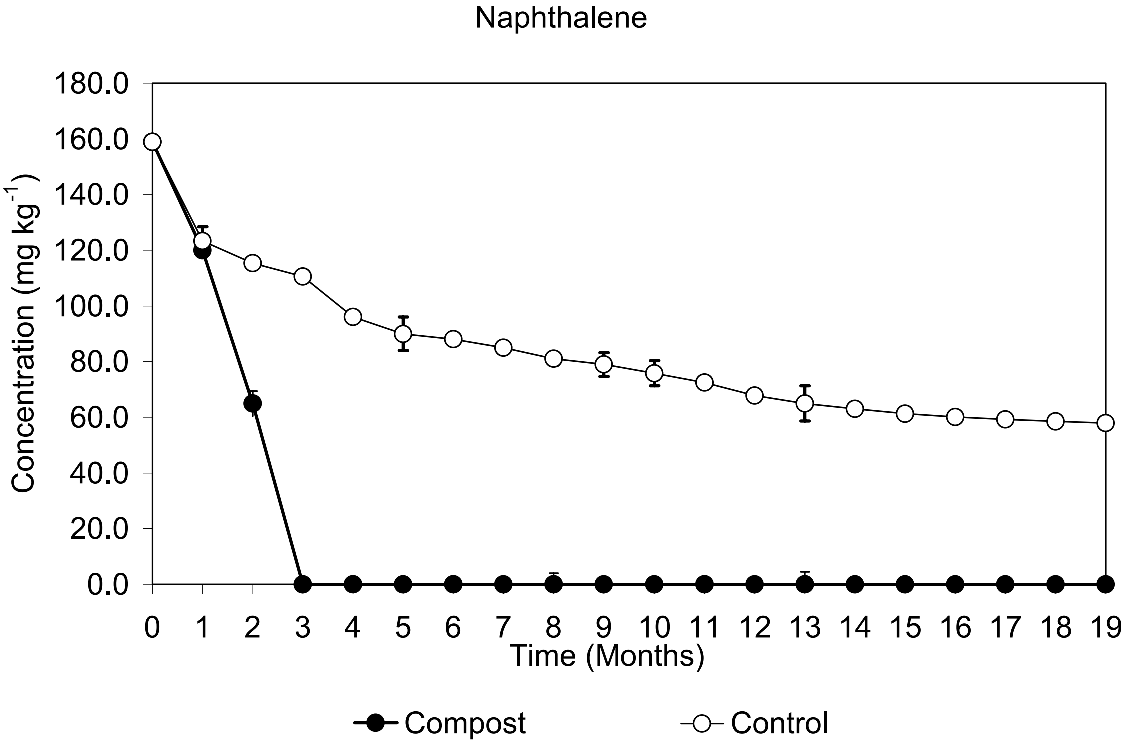

Figure 1. Changes in concentration of naphthalene during composting. Values are means of three ± 1 Standard Error.

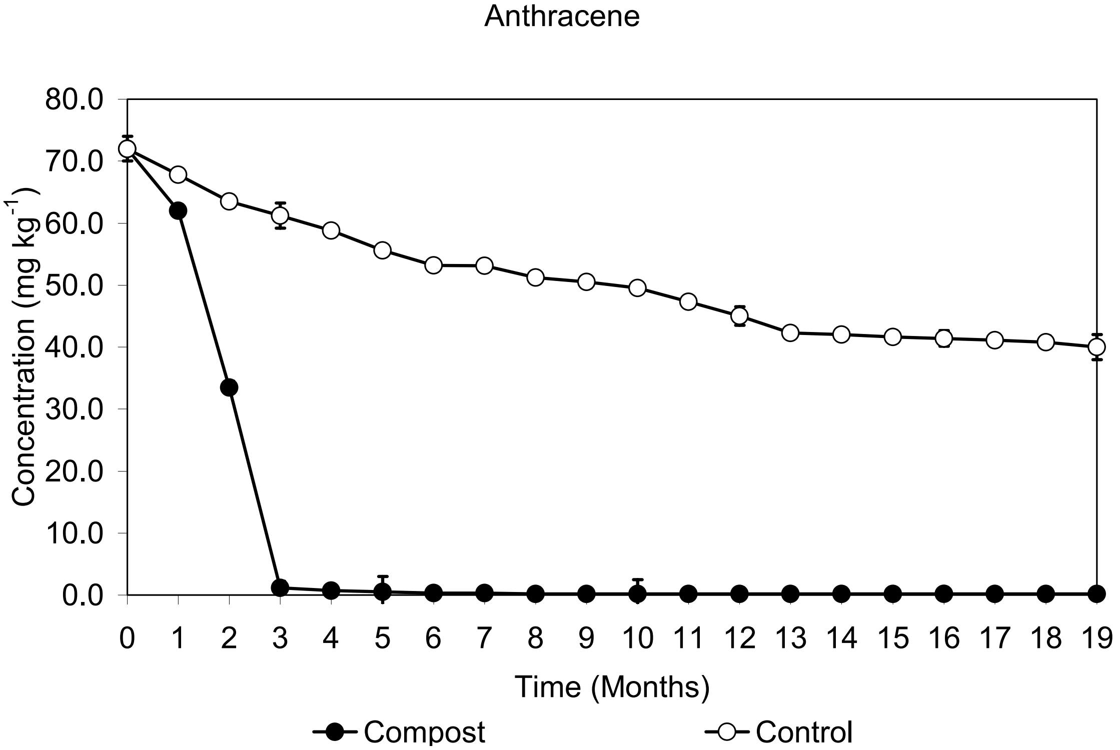

Figure 2. Changes in concentration of anthracene during composting. Values are means of three ± 1 Standard Error

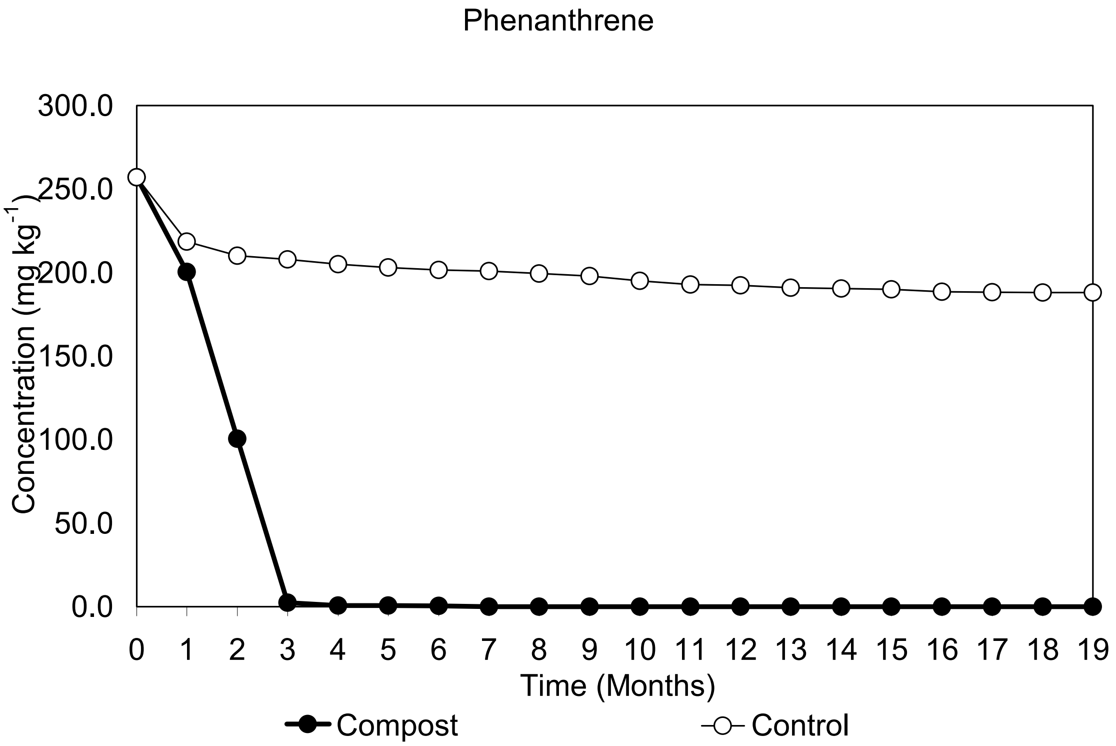

Figure 3. Changes in concentration of phenanthrene during composting. Values are means of three ± 1 Standard Error

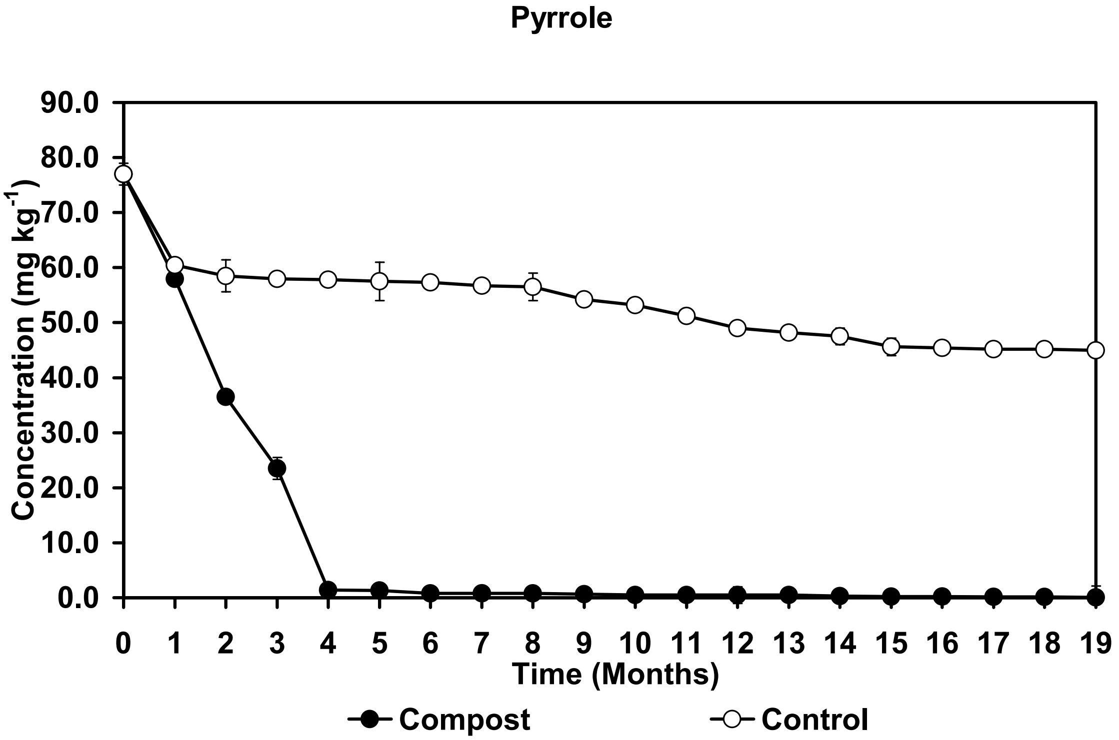

Figure 4. Changes in concentration of pyrrole during composting. Values are means of three ± 1 Standard Error

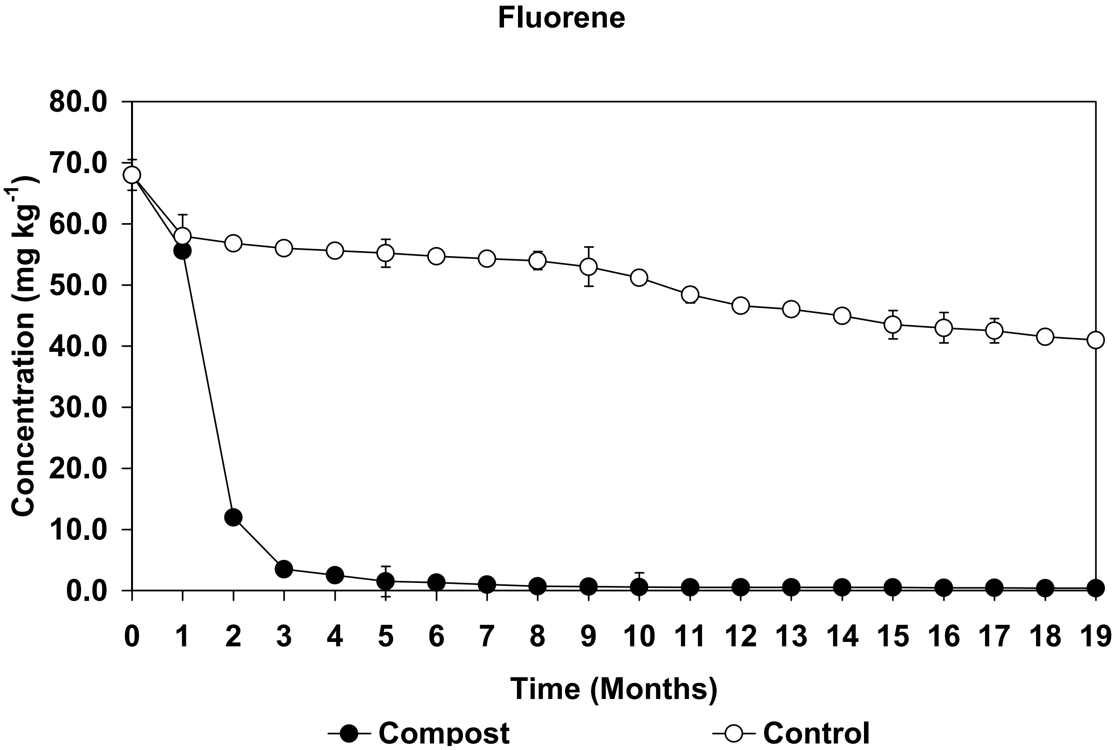

Figure 5. Changes in concentration of fluorene during composting. Values are means of three ± 1 Standard Error

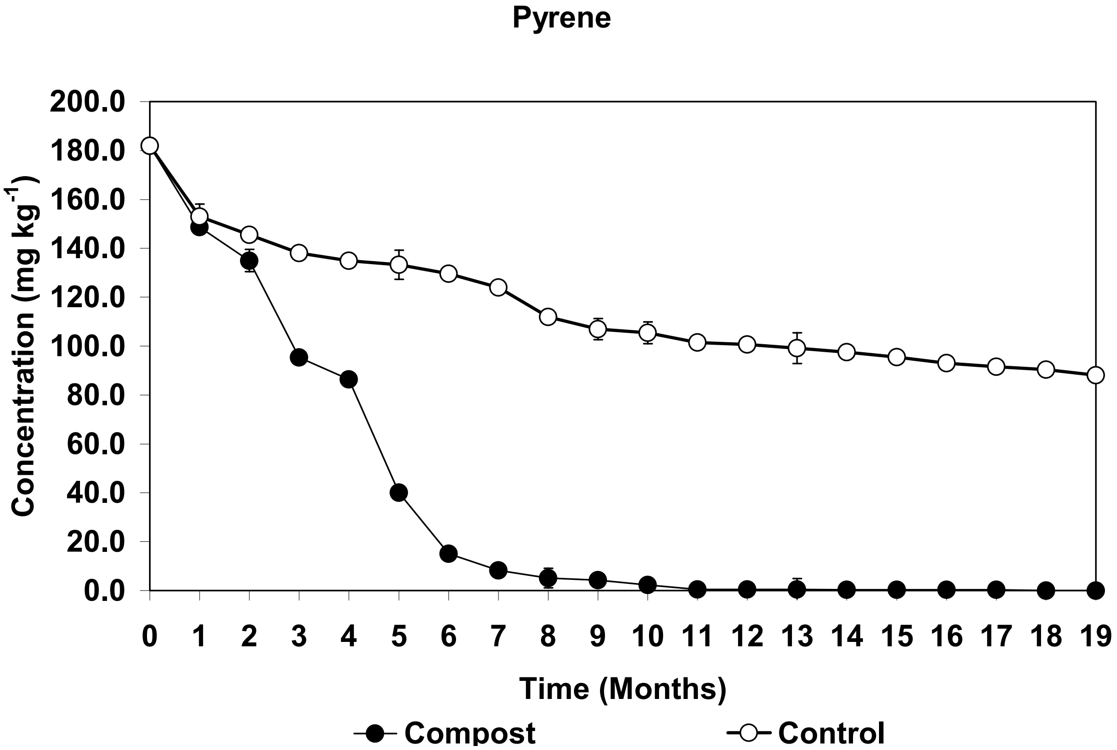

Figure 6. Changes in concentration of pyrene during composting. Values are means of three ± 1 Standard Error

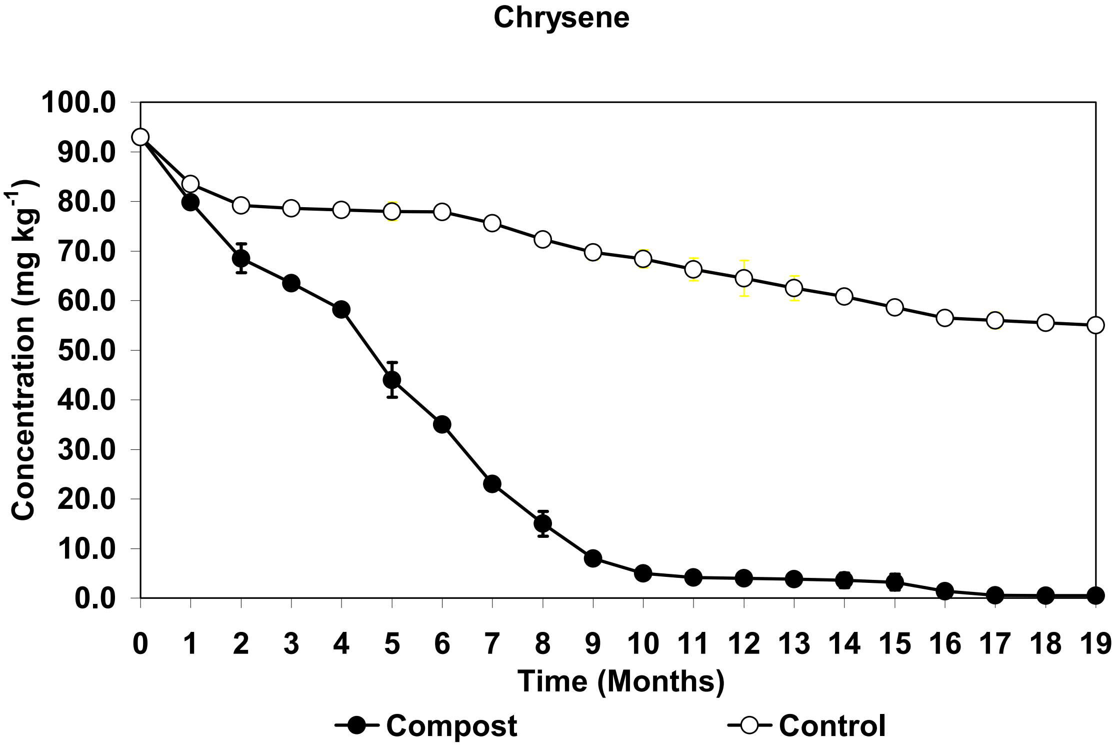

Figure 7. Changes in concentration of chrysene during composting. Values are means of three ± 1 Standard Error

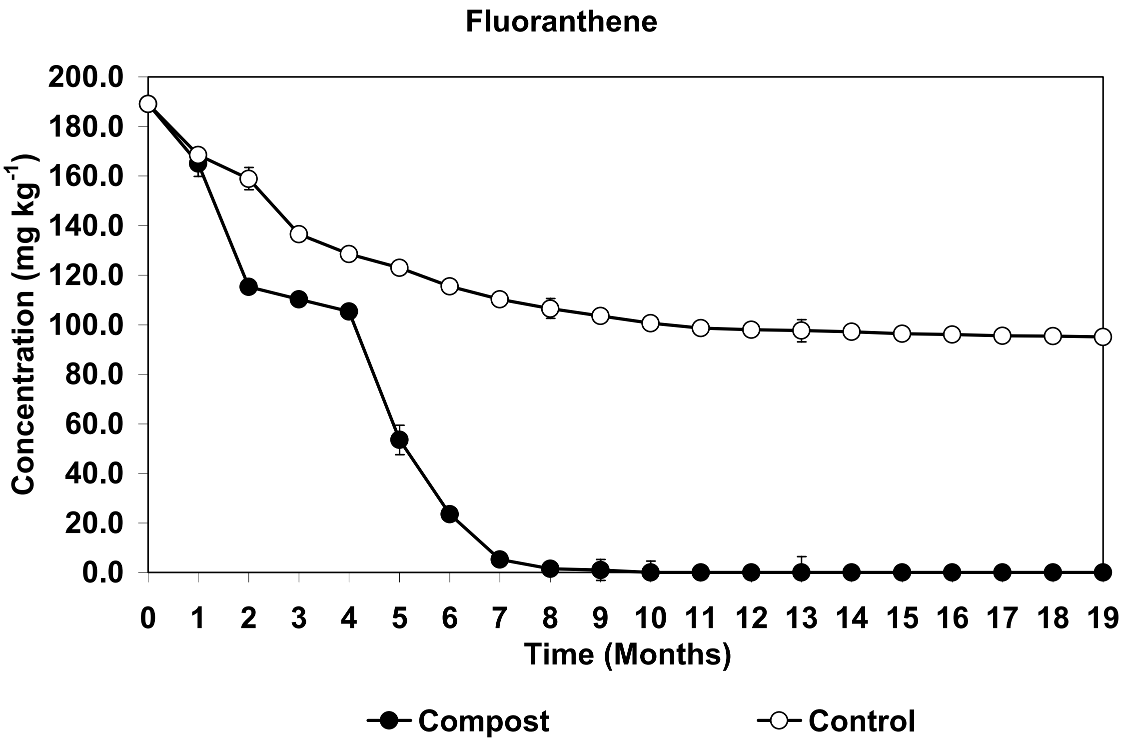

Figure 8. Changes in concentration of fluoranthene during composting. Values are means of three ± 1 Standard Error

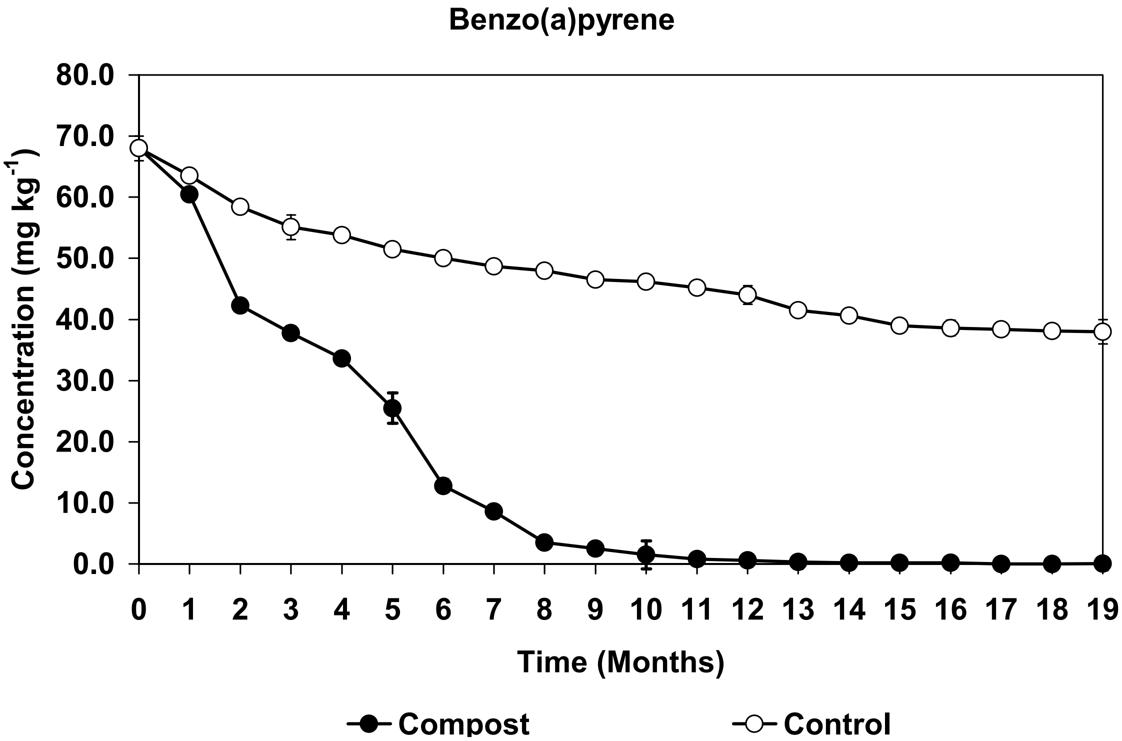

Figure 9. Changes in concentration of benzo(a)pyrene during composting. Values are means of three ± 1 Standard Error

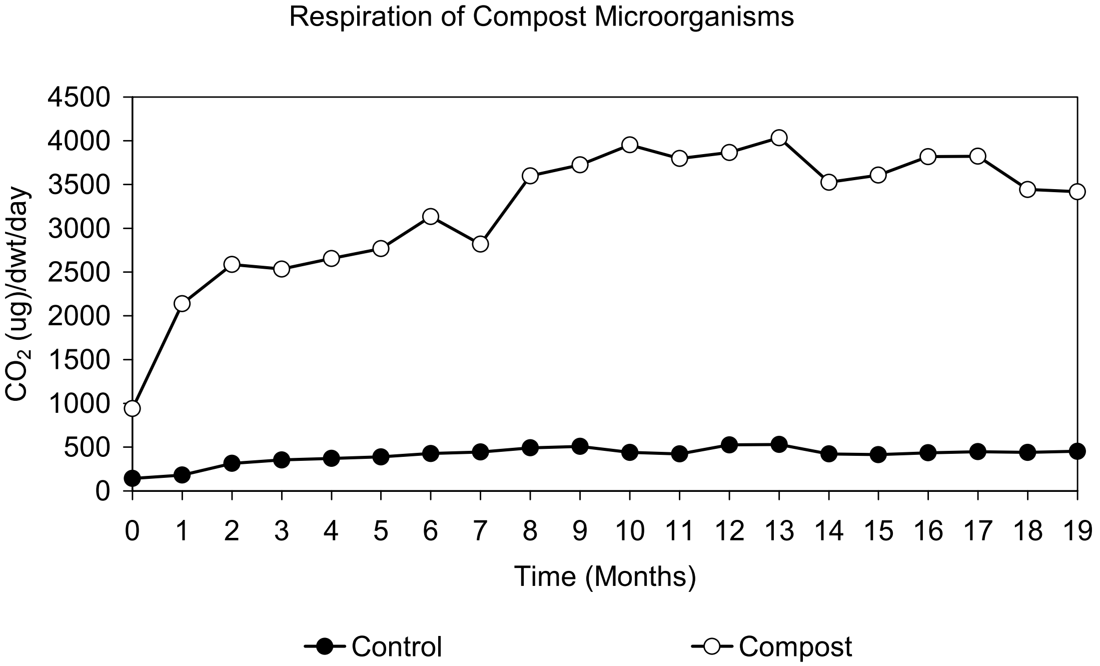

Figure 10. Measurements of respiration of compost inhabiting microorganisms during incubation. Values are means of three ± 1 Standard Error.