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EFFECT OF ORGANIC WASTE AMENDMENTS ON DEGRADATION OF PAHS IN SOIL USING THERMOPHILLIC COMPOSTING

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ABSTRACT

The feasibility of using various types of organic wastes including pig manure, sewage sludge, and soybean refuse for remediation of soil spiked with phenanthrene, anthracene and pyrene (PAHs) was evaluated through batch-scale composting reactors. The most active degradation of PAHs occurred between day 4 to 30 and maximum removal at the end of composting accounted for 90% of the initial concentrations of the three PAH compounds. Microbial degradation in composting mass would likely be the major factor contributing to removal of PAHs in the soils. Among the three PAHs, degradation of pyrene in the composting mass was relatively slow as indicated by a longer lag period than that of phenanthrene and anthracene. This corresponded well with the high molecular weight and log K_{ow} values of pyrene. The organic amendments were effective in enhancing the degradation of PAHs, and pig manure amendment exhibited a slightly higher removal efficiency than sewage sludge and soybean refuse. A decrease in total organic matter in all treatments indicated that the decomposition process occurred. Toxicity test with cress seed germination was evaluated and no phytotoxicity was noted after 21 days of composting. This preliminary study positively demonstrates the use of composting as a measure to remediate soil contaminated with spiked

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3- and 4-ring PAH compounds and pig manure was recommended as an organic additive for bioremediation of PAH-contaminated soil by composting treatment.

Keywords: Anthracene, composting, degradation, organic wastes, phenanthrene, pyrene.

INTRODUTION

Polycyclic aromatic hydrocarbons (PAHs) are common contaminants in soil, resulting from a variety of natural processes, such as volcanic emissions and forest fires as well as anthropogenic activities including incineration, residential heating, coal burning, coke production and internal combustion engines [1,2]. Since some of the PAH compounds have high mutagenic or carcinogenic potential, the US Environmental Protection Agency has listed 16 PAH compounds as priority pollutants to be monitored in industrial effluents [3, 4].

The remediation of contaminated sites has been attempted using various methods such as solvent washing, air stripping, incineration, composting, electrokinetic remediation, and supercritical extraction [5]. Applicability of these physical, chemical, and biological treatment methods or their combination is critically dependent on soil type, nature and level of contamination, site specifications, and economic feasibility [6]. Bioremediation of contaminated-PAH soils has received increasing attention because of the important role of microorganisms in degrading PAH compounds. As one of the bioremediation methods, composting may provide an accessible, cost-effective alternative for treating PAH-contaminated soils [7]. Composting is a biological process in which microorganisms are responsible for mineralization and humification of organic matter under optimal conditions, such as elevated temperatures (> 50°C), plentiful nutrients, high moisture level, sufficient oxygen, and a suitable pH [8]. The elevated temperature achieved during composting can increase the enzyme kinetics involved in the degradation of PAHs, solubility and mass transfer rates of the contaminants. The aeration provided during aerobic composting provides sufficient oxygen (> 5%) for initiating and completing the breakdown of organic compounds. In addition, the opportunity for cooxidation may be enhanced due to the range of alternative substrates presented in composting mass. There are several studies concerning possible bioremediation of PAH-contaminated soil, sludge, municipal solid waste or waste wood by composting [9-12].

Amendment of PAH-contaminated soil with organic matter, such as manure, sewage sludge, or compost, was reported to enhance PAH degradation [13-15]. Stimulation of microbial activity by supplying organic matter and inorganic nutrients as well as microorganisms to soil is likely the main reason for enhancing the degradation of PAH compounds. However, organic matter in soil contributes to PAH sorption and affects availability to microorganisms for biodegradation [16]. Different types of organic matter are expected to have special effects on PAH removal. In the present composting experiment, three local organic wastes, i.e., pig manure, sewage sludge and soybean refuse, were selected to amend soil artificially contaminated with 3- and 4-ringed PAH compounds. Application of these organic wastes in bioremediation of PAH-contaminated soil may provide means for recycling and reutilization of these organic wastes. On the other hand, some limitations of application of these organic wastes, such as high salinity and metal concentration, may cause adverse effect on degradation of PAHs during composting. It is essential to examine the effect of those organic wastes on composting process as well as PAH degradation for bioremediation purpose. The objectives of this study were to evaluate the efficiency of thermophilic composting for bioremediation of PAH-contaminated soil with three organic wastes, and to study the relationship between removal efficiency and types of organic amendment during compost treatment.

MATERIALS AND METHODS

Preparation of Composting Piles

A sandy loam soil (texture: 71% sand, 19% silt and 10% clay) collected from the Kadoorie Farm and Botanic Garden located in Tai Po, Hong Kong, was air-dried at room temperature (25±2°C) for one week. Then the soil was sieved to 2 mm and kept at 4°C in the dark until use. Three PAHs, i.e., phenanthrene, anthracene, and pyrene (Sigma Chemical Co.), were dissolved in methanol/acetone (2:1 v/v) and then spiked into soil at each 50 mg kg^{-1} (w/w dry weight) and thoroughly mixed. The methanol and acetone in the mixture were allowed to evaporate and the spiked soil was incubated for one month at room temperature (25±2°C) as artificial aging. Afterwards, this PAH-spiked soil was separately amended with sewage sludge (SS), soybean refuse (SB), and pig manure (SP) using sawdust as a bulking agent to obtain a C/N ratio of about 30. The ratios of soil, organic wastes and sawdust (w/w dry wt) were 3 : 0.14 : 0.86 for SS, 3 : 0.29 : 0.71 for SP, and 3 : 0.05 : 0.95 for SB. Four kilograms ± 0.1 kg of the mixtures were prepared and composted in bench-scale composting reactors connected with an on-line computer control system to ensure a supply of sufficient air (0.4 L min^{-1} kg^{-1}) to the composting mass, and to monitor the changes in temperature during the composting period. There were duplicate samples for each treatment. About 100 g of sample was removed from each reactor on days 0, 4, 7, 14, 21, 28, 35, and 49 for chemical and biological testing.

Analytical Procedures

Total organic carbon was determined with Walkley-Black method while total nitrogen and phosphorus was extracted by Kjeldahl digestion, and the contents of NH_4-N determined by indophenol blue method and soluble PO_4-P by the molybdenum blue method [17]. Fresh samples were oven-dried at 105°C for 24 h for measuring the moisture content. Total organic matter was measured by loss of weight on ignition of oven-dried samples at 550°C for 16 h. pH and electrical conductivity (EC) were determined using aqueous extract of air-dried samples at 1:5 solid : deionized water (DIW, w/v) using an Orion 920 ISE pH meter and an Orion 160 conductivity meter, respectively.

Enumeration of colony-forming units (CFU) of bacteria and fungi was carried out by plate spreading method [18]. A suspension of 1g sample in 10 ml physiological sodium chloride solution was prepared and decimal dilutions were incubated at 30°C on nutrient agar for bacteria, and on rose bengal agar for fungi. Incubation time was 2 days for bacteria, and 7 days for fungi.

Seed germination and root length tests were performed on water extracts obtained by shaking fresh samples at 1:2 solid:distilled water (w/v) for 1 hour. Ten cress (*Lepidium sativum, L.*) seeds were placed in each petri-dish containing 6 ml of the extract and incubated at 25°C in the dark for 72 h. Treatments were evaluated by counting the number of germinated seeds and the length of root radicals. The responses were calculated by a germination index obtained by multiplying the percent germination by the percent root growth as related to control [19].

The contents of PAH compounds were determined using the method described by Loser and Ulbricht [12]. About 5 g of sample were Soxhlet extracted with 150 ml acetone/dichloromethane (DCM) for 16 hours following by an alkaline hydrolysis step to extract PAHs sorbed onto the organic matrix. The extract was concentrated in a rotary evaporator under reduced pressure to near dryness, and then dissolved in 1 ml hexane before clean-up. A Florisil cartridge with copper granules on the top was set up for clean-up of the extract which was concentrated to 2 ml. The extracts were analyzed by gas chromatography/mass spectroscopy (GC/MS) according to the USEPA methods (8270C). The GC column was a HP-5MS fused silica capillary column (30 mm x 0.25 mm, 0.25 μm film thickness, Supelco), and the column outlet was located in the MS ion source. Helium was used as the GC carrier gas. Following injection (1μl), the GC column was held at 50°C for 1 min and the temperature-programmed to 300°C at 4°C/min. The MS was operated in the selected ion monitoring (SIM) mode. Identification of the target compounds was based on their GC retention time relative to those of the internal standards. The minimum detectable level was approximately 0.01 μg g^{-1}.

Statistical Analysis

All data were analyzed by a Statistical Analysis for Science (SAS) package through an IBM compatible personal computer. The means and standard deviations of duplicate treatments were calculated. The Least Significant Difference test was used to compare the means of all different treatments within the same sampling interval for all parameters [20].

RESULTS AND DISCUSSION

Changes in Chemical Properties

Selected physicochemical properties of sewage sludge, soybean refuse, pig manure, sawdust, and soil pertaining to the composting are shown in Table 1. The different chemical properties of materials in the composting mass could influence the biodegradation process at some extent during composting. The low content of total organic matter (2.31%) in soil made it necessary to amend with other organic matter for providing available nutrients for microorganisms. The enriched nitrogen and phosphorus of three organic wastes is expected to enhance the microbial activity during composting process. The three organic wastes contained comparable nutrients, but soybean refuse contained the highest organic matter. Furthermore, organic wastes contained a high number of total microorganisms up to 10^8 CFU g^{-1} that provided more potential active PAH-degrading microorganisms.

The changes in chemical parameters, including temperature, pH, and total organic matter are shown in Figure 1 for delineating the composting process and evaluating the effects of different organic wastes on the composting process. All treatments entered thermophilic phase within a day and maintained a temperature between 40 to 50°C for about one month, and then cooled down to 25°C till the end of composting. In general, temperature in the thermophilic range is shown to greatly accelerate decomposition of organic matter. Furthermore, high temperature can likely soften up soil organic matter matrix [21], which will speed up the PAH release from soil for microbial degradation. However, too high a temperature can inhibit PAH biodegradation due to thermal inhibition for most microbial growth [22]. The range of temperature (<55°C) in the thermophilic phase is suitable for PAH-degradation by most thermophilic microorganisms [10]. The treatment with soybean refuse amendment had the highest temperature among the three organic materials used. However, there was no significant difference in temperature changes among the three treatments during composting.

The pH of all treatments decreased throughout the composting period (Fig. 1b). It may be due to the organic acid formation during the decomposition of organic matter, volatilization of ammonia, and the nitrification at the later stage of the composting process [23]. Pig manure addition (SP) exhibited a significantly higher pH level throughout the composting process compared to sewage sludge (SS) and soybean refuse (SB). The optimum pH condition for composting is usually between 6 and 8, which is also the optimum for PAH degradation [24, 25]. In this study, the composting mass of soil amended with three different organic waste materials maintained an optimum pH condition for PAH degradation during the composting process.

Organic matter is oxidized or degraded during composting and the net loss of organic matter can reflect the decomposition activity. The loss of organic matter in SP treatment was about 16.5% that was significantly higher ($p<0.05$) than those of SS (11.5%) and SB (13%) treatments (Fig. 1c). Although total organic matter in pig manure was the lowest among the three organic wastes, it might consist of more easily degradable organic matter than SS and SB, which would likely be beneficial to decomposition of PAHs in the composting mass.

Changes in Microbial Population

The bacterial population of SP, SB and SS treatments increased with the onset of the thermophilic phase, reached the maximum at day 7 for SP and SB and 14 for SS, and then decreased gradually until the end of the composting period (Fig. 2). PAH-contaminated soil with sewage sludge amendment (SS) had the highest population count of bacteria reaching 10^9 CFU g^{-1}. However, this treatment did not show a corresponding higher loss of organic matter during composting process. Although the initial population of bacteria in the control PAH-contaminated soil with no organic amendment was relatively lower than the other treatments, it still reached 10^8 CFU g^{-1} at day 14, which may be explained by the degradation of anthracene and phenanthrene that occurred in the control soil. Kastner et al. [26] reported that the amount of microflora is not the limiting factor for degradation of PAH with up to four rings, because of the ubiquitous presence of a potent and versatile mineralizing microflora in PAH-contaminated soils.

The fungal CFUs during the first 14 days showed small variation in all treatments with an average of 10^5 CFU g^{-1} because their growth was inhibited at high temperature (> 45°C) (Figure 2) [8]. Then fungal population increased to a value above 10^6 CFU g^{-1} by 21 days. Fungi usually utilize complex organic materials as substrates in the later stage of composting process [27]. Since the most active stage of PAH degradation occurred in the first 28 days, fungi might have contributed little to PAH degradation than bacteria did during composting. Although the bacterial and fungal counts in the present study were at acceptable levels in PAH-contaminated soils with organic matter amendments, they were not sensitive indicators for PAH degradation in the composting process. The result concurred with those obtained by Potter et al. [10], who reported that rate of removal of PAHs from soil during the first 4 weeks of compost treatment did not correlate with reactor biomass concentration.

Removal of PAHs

Removal efficiency of three PAHs from soil amended with different solid organic wastes during composting process is shown in Figure 3. The reason for selecting anthracene, phenanthrene and pyrene as the target PAH compounds in this study was because of their high occurrence in contaminated soils as well as their relatively high aqueous solubility compared with other PAHs, especially, phenanthrene. With the occurrence of an initial lag period, concentrations of solvent-extractable anthracene, phenanthrene, and pyrene in SS, SB, and SP treatments were significantly reduced during the first 20 days of composting and reached the lowest levels of < 10% of initial spiked concentrations after 30 days. The highest activity of degradation was noted between 4 to 30 days of composting.

The degradation of PAHs was linear as would be expected for a substrate with low water solubility and a limited rate of mass transfer [28]. To have a clear understanding of the extent of removal for each PAH in the composting mass, a plot of logarithm of the PAHs remaining as a function of time is shown in Figure 4. During the period of most active removal of PAHs (within the first 28 days), the relationships can be described by first-order kinetics with an initial lag period. The kinetic rate constant (*k*) of each type of PAH in the composting mass during the period of the most active removal was calculated using the following equation [29].

$$k = \frac{\mathrm{Ln}(C_t - C_0)}{t - c}$$

where C_0 = the concentration of the PAH compound at day 0, C_t = the concentration of the corresponding PAH compound at day 28, t = 28 days and c = the lag time. The lag time was 7 days for phenanthrene and anthracene and 14 days for pyrene. The *k* value of phenanthrene, anthracene and pyrene in the treatment SP, SB and SS are given in Table 2. The k values indicate that degradation of both pyrene and phenanthrene was slightly better in soil with pig manure amendment.

The quick reduction in concentration of 3-ringed compounds (anthracene and phenanthrene) in this experiment is likely due to the existence of bacteria capable of using these compounds as sole source of carbon and energy. However, disappearance of pyrene could be partly attributed to pyrene-degradative microorganisms, or partly due to cometabolism by the diverse populations of compost microbes [30]. Raymond et al. [31] reported that mixed cultures of microorganisms may result in enhanced PAH metabolism through utilization of intermediate biotransformation products from one microorganism which served as substrates for catabolic metabolism by other microorganisms. Finstein et al.

[32] reported that 79% of pyrene was reduced during composting mixture of sewage sludge and various organic compounds.

PAH-contaminated soil with pig manure amendment showed the highest activity of degradation of phenanthrene as indicated by larger kinetic rate constant (k) (Figure 4). No significant difference was noted between SB and SS treatment with the exception that a lower degradation of pyrene in SS. Although the control PAH-contaminated soil with no organic amendment showed the lowest removal of PAHs among all the treatments, a removal percentage of 90%, 40% and 10% of initial concentrations for phenanthrene, anthracene, and pyrene, respectively, was observed. The loss of PAHs can be explained by the vaporization loss, organic matter sorption, and/or degradation activity of indigenous microorganisms in soil. The vaporization loss was neglected because there were undetectable PAHs in the exhaust air from all composting reactors. Wild et al. [33] reported that volatilisation of PAH loss from soil is likely to be important for naphthalene, but not for the higher ringed compounds. There is possibility for PAHs to be sorbed onto the organic matrix, which may render some fraction of PAHs unavailable for organic extraction. Loser and Ulbricht [12] included alkaline hydrolysis in the traditional Soxhlet extraction in order to set free the proportion sorbed onto the organic matter in a PAH-contaminated wood. They concluded that the disappearance of PAHs was mainly due to biodegradation and less than 5% was due to sorption onto organic soil matrix. Wischmann and Steinhart [34] observed that soil amended with compost helped to enhance elimination of all PAH compounds monitored in soil. The significant losses of these PAH compounds by biodegradation were verified with an alternative analysis procedure including a saponification step that can free the proportions sorbed to the organic soil matrix. Although sorption of PAHs into organic matrix is difficult to assess in the long term, PAHs with three and four rings are slightly susceptible to adsorption loss during composting process. Therefore, microbial degradation in soil would likely be the major factor contributing to removal of PAHs in the soils in the present study. Compared to the control soil, amendment with pig manure, soybean refuse and sewage sludge significantly enhanced the biodegradation of PAHs.

The present experimental results demonstrate that it is feasible to co-compost organic wastes with PAH contaminated soil for the removal of phenanthrene, anthracene, and pyrene and the removal rate during the first 30 days of composting was between 1.0 to 1.5 $\mu g\ g^{-1}$ per day (Figure 3). However, the degradation rate of PAHs may be lower for aged contaminated soils because PAHs may be more strongly bound in organic matter. It is reported that the longer the exposure to the PAHs, the greater their incorporation into the organic matrix and the more recalcitrant they become [30]. Potter et al. [10] who studied composting of field PAH-contaminated soil for 12 weeks with organic waste amendment, reported that concentrations of PAHs with two to four rings in their molecular structures decreased significantly until contaminant concentration plateaus appeared by the eighth week. The plateaus may indicate a fraction of PAHs that remains inaccessible to microorganisms for biological degradation. In this study, the contact time of spiked PAH compounds to soil before composting was only one month. Therefore, it is necessary to examine aging effect on PAH degradation during composting, which will be addressed in our further study.

Physicochemical Biodegradability Relationship

The physical and chemical characteristics of PAHs play an important role for their persistence and bioavailability to microorganisms in the environment because mass transfer from sorption sites or crystalline surfaces of PAHs to an aqueous phase is essential for biodegradation [1, 16]. This can be demonstrated by comparing the degradation kinetic rate of the PAHs in this experiment with various known properties of each PAH such as aqueous solubility, molecular weight, and octanol/water partition coefficient (K_{ow}). Table 2 shows the apparent relationship between the rate constants (k) and selected physical and chemical characteristics of the three PAH compounds.

The higher water solubility of phenanthrene (1.29 mg l^{-1}) as compared to anthracene (0.07mg l^{-1}) (Table 2) contributed to the faster biodegradation of phenanthrene. The relatively higher solubility of phenanthrene also makes it one of most toxic PAH compounds in the environments [30]. Although the water solubility of pyrene (0.14 mg l^{-1}) was higher than the anthracene (0.07 mg l^{-1}), the 3-ringed anthracene was biodegraded faster than the 4-ringed pyrene. Therefore, biodegradation of PAH compounds was also affected by other physical and chemical characteristics of PAHs. High temperature will typically increase the solubility and mass transfer rate of the contaminants, thereby making them more available for degradation. This is considered the reason why thermophilic composting treatment is more rapid for biodegradation of PAHs than that of mesophilic treatment [7].

Anthracene and phenanthrene of 3-ringed aromatic structure have similar molecular weight but their biodegradation rates were different in the present experiment. Although the angular ring arrangement of phenanthrene is considered thermodynamically more stable than the linear arrangement of anthracene, the so-called ‘bay region’ due to angular arrangement might have favored enzyme attack on phenanthrene rather than the linear anthracene [35].

In general, the higher the molecular weight of the PAHs, the greater its resistance to microbial degradation [36]. Degradation of pyrene showed a relatively lower degradation rate than the other two PAHs. However, with an increase in composting time, the concentrations of pyrene decreased significantly to about 10% of the initial concentration. It may be due to the cometabolic metabolism with 3-ringed PAH compounds that resulted in an increase in the removal of pyrene after 7 days of composting. A similar result was also reported by Carmichael and Pfaender [37] that phenanthrene and chrysene were mineralized to approximately the same extent in a contaminated soil, although both had different physical and chemical characteristics.

K_{ow} is often used as an indicator for affinity of a given compound to soil. Because microorganisms can mainly degrade dissolved PAHs, the slow desorption of PAHs from compost mass to water phase might be a major cause limiting biodegradation [38-40]. The larger Log K_{ow} of pyrene indicated that a higher affinity of pyrene for solid organic matter than the other two PAH compounds. It is generally believed that sorption of PAHs onto the organic carbon matrix reduces the biodegradability of PAHs, because PAH compounds are immobilized by adsorption onto organic matter [30]. This may also contribute to the lower degradation rate of pyrene.

Toxicity Evaluation

At the beginning of composting, organic matter amendment caused a slight inhibition of Cress seed germination as compared to the control with distilled water only. Composting process usually remedies phytotoxicity caused by organic compounds and low molecular weight organic acids through aerobic decomposition [42]. Therefore, the adverse effect of three organic wastes on seed germination was recovered gradually with an increase in composting time.

The initial GI values in all composting samples were between 20 to 40%. After 21 days of composting, GI reached 100% and still increased to about 150% until the end of composting. This indicates that the extra nutrients in soils following organic matter amendments stimulated the growth of cress seeds under the present conditions [43]. The variation in GI of PAH-contaminated soil with/without organic matter amendment did not show a good correlation with the removal of PAHs. Our results concurred well with those reported by

Potter et al. [10] that toxicity of PAH-contaminated soils did not correlate well with PAH concentrations in the soil. Nevertheless, other tests on plant and animals will be carried out to ensure the safety of soil compost for land application.

CONCLUSIONS

The present study demonstrated that it is feasible to utilize organic wastes for co-composting soil spiked with phenanthrene, anthracene and pyrene. The PAH removal rate in soil with organic waste amendment was significantly higher than that of the control soil. Up to 90% of initial spiked PAHs were removed within the first 30 days of composting for soil receiving organic waste amendments. PAHs with 3-ringed structure (phenanthrene and anthracene) were removed more effectively than 4-ringed PAHs (pyrene) under the present composting conditions. The high molecular weight and Log K_{ow} of pyrene accounted for its low degradation rate. From the degradation results, it can be indirectly concluded that availability of PAHs did not appear to be significantly reduced by the adsorption of these three PAH compounds onto the freshly added organic wastes. Among the three kinds of organic wastes used, pig manure amendment demonstrated a slightly higher efficiency in biodegradation of organic matter and PAH compounds over other two organic wastes. Further experiments are required to study effects of aging and soil types and to examine the mechanisms of enhancing degradation. Nevertheless, this study indicates that remediation of PAH-contaminated soil through thermophilic composting looks promising.

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Captions of Figures

Figure 1 Changes in temperature (a), pH (b), and total organic matter (c) during composting of PAH-contaminated soil with organic wastes. (SS = sewage sludge, SB = soybean refuse, SP = pig manure, respectively).

Figure 2 Changes in the populations (CFUs) of bacteria (a) and fungi (b) during composting of PAH-contaminated soil with organic wastes. (SS = sewage sludge, SB = soybean refuse, SP = pig manure, respectively).

Figure 3 The percentage removal of phenanthrene (a), anthracene (b) and pyrene (c) during composting of PAH-contaminated soil with organic wastes. (SS = sewage sludge, SB = soybean refuse, SP = pig manure, respectively).

Figure 4 Logarithmic transmission of the data from Figure 3.

Figure 5 Changes in the seed germination (a), root length (b), and germination index (c) during composting of PAH-contaminated soil with organic wastes. (SS = sewage sludge, SB = soybean refuse, SP = pig manure, respectively).

Table 1 Selected physicochemical properties of soil, sewage sludge, pig manure, soybean refuse and sawdust

Item	Soil	Sewage sludge	Pig manure	Soybean refuse	Sawdust
pH (solid:water = 1:5)	7.00	7.45	6.95	7.11	5.55
	(0.03)[a]	(0.06)	(0.08)	(0.11)	(0.07)
EC (dS m^{-1}) (solid:water = 1:5)	0.15	1.86	1.40	0.52	0.02
	(0.01)	(0.23)	(0.11)	(0.02)	(0.00)
Moisture content (%)	4.86	82.5	43.3	47.9	8.12
	(0.55)	(1.32)	(2.45)	(0.67)	(0.35)
Total organic matter (% of dry weight)	2.31	79.7	71.2	90.5	99.5
	(0.2)	(2.33)	(2.11)	(1.25)	(0.01)
Total carbon (% of dry weight)	1.39	38.8	33.2	42.0	46.5
	(0.06)	(1.40)	(1.06)	(0.77)	(2.78)
Total nitrogen (% of dry weight)	0.17	6.88	3.03	10.0	0.22
	(0.01)	(0.45)	(0.12)	(0.52)	(0.00)
Total phosphorus (% of dry weight)	0.11	1.94	1.72	ND	ND
	(0.00)	(0.06)	(0.01)		
Total microorganisms (CFU g^{-1})	10^6	10^8	10^8	10^7	ND

[a] Standard deviation of triplicates.

ND: not determined.

Table 2 Relations between rate constants (k) of PAH degradation in SP, SS and SB treatments and formula, aqueous solubility, molecular weight, and K_{ow} of PAH compounds

	Characteristics[a]				Rate constants (k day^{-1})		
	Formula	Water solubility (mg l^{-1})	Molecular weight	LogK_{ow}	SP	SS	SB
Phenanthrene	$C_{14}H_{10}$	1.29	178	4.57	-0.23	-0.19	-0.17
Anthracene	$C_{14}H_{10}$	0.07	178	4.54	-0.17	-0.17	-0.16
Pyrene	$C_{16}H_{10}$	0.14	202	5.18	-0.22	-0.16	-0.20

SS = sewage sludge, SB = soybean refuse, SP = pig manure, respectively.

[a]Data from Bjseth [44] (1983).

Figure 1

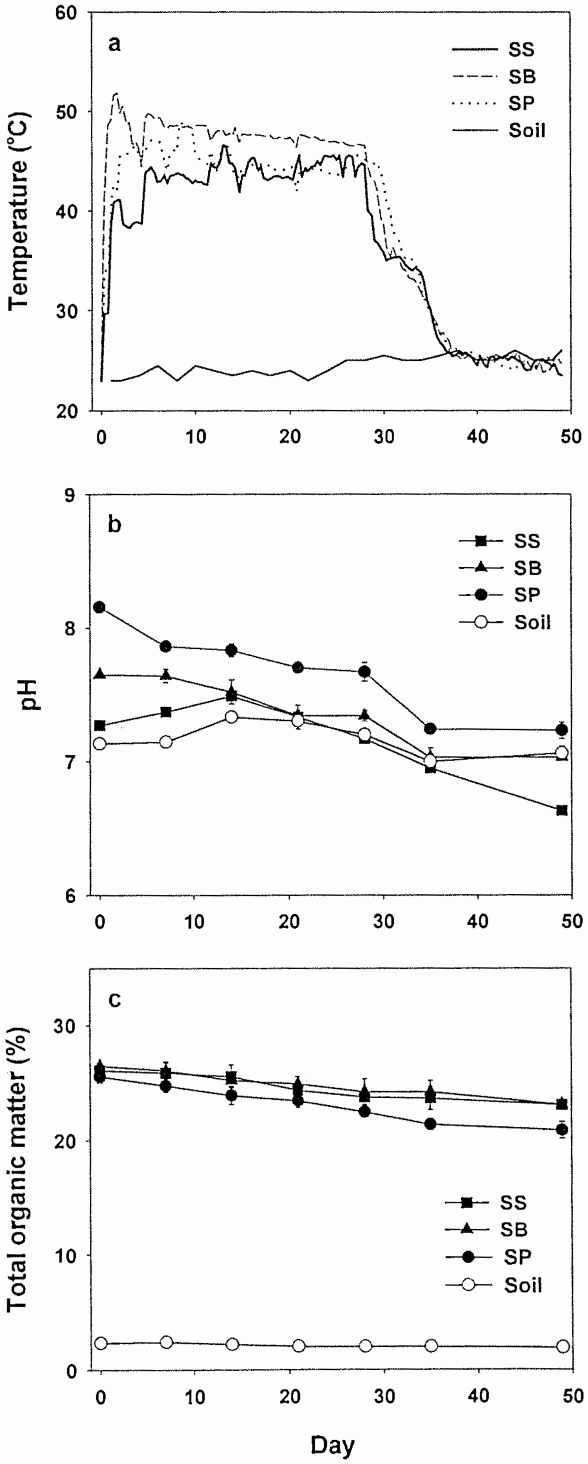

a
Temperature (°C)
60
50
40
30
20
0
10
20
30
40
50
SS
SB
SP
Soil
b
pH
9
8
7
6
0
10
20
30
40
50
SS
SB
SP
Soil
c
Total organic matter (%)
30
20
10
0
0
10
20
30
40
50
SS
SB
SP
Soil
Day

Figure 2

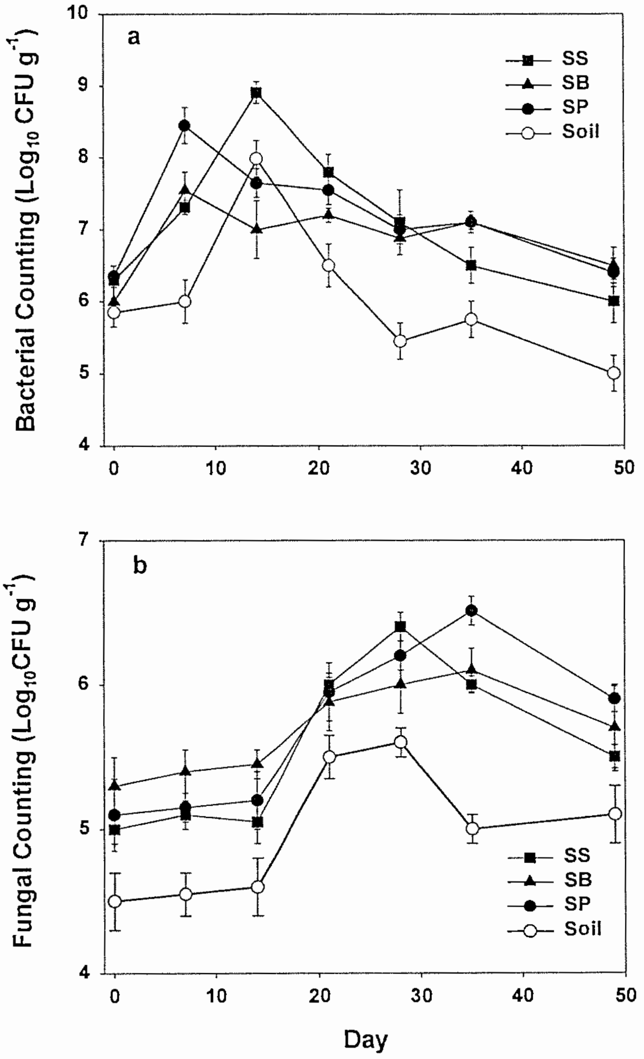

a
Bacterial Counting ($\log_{10}$ CFU g^{-1})
10
9
8
7
6
5
4
0
10
20
30
40
50
SS
SB
SP
Soil
b
Fungal Counting ($\log_{10}$CFU g^{-1})
7
6
5
4
0
10
20
30
40
50
SS
SB
SP
Soil
Day

Figure 3

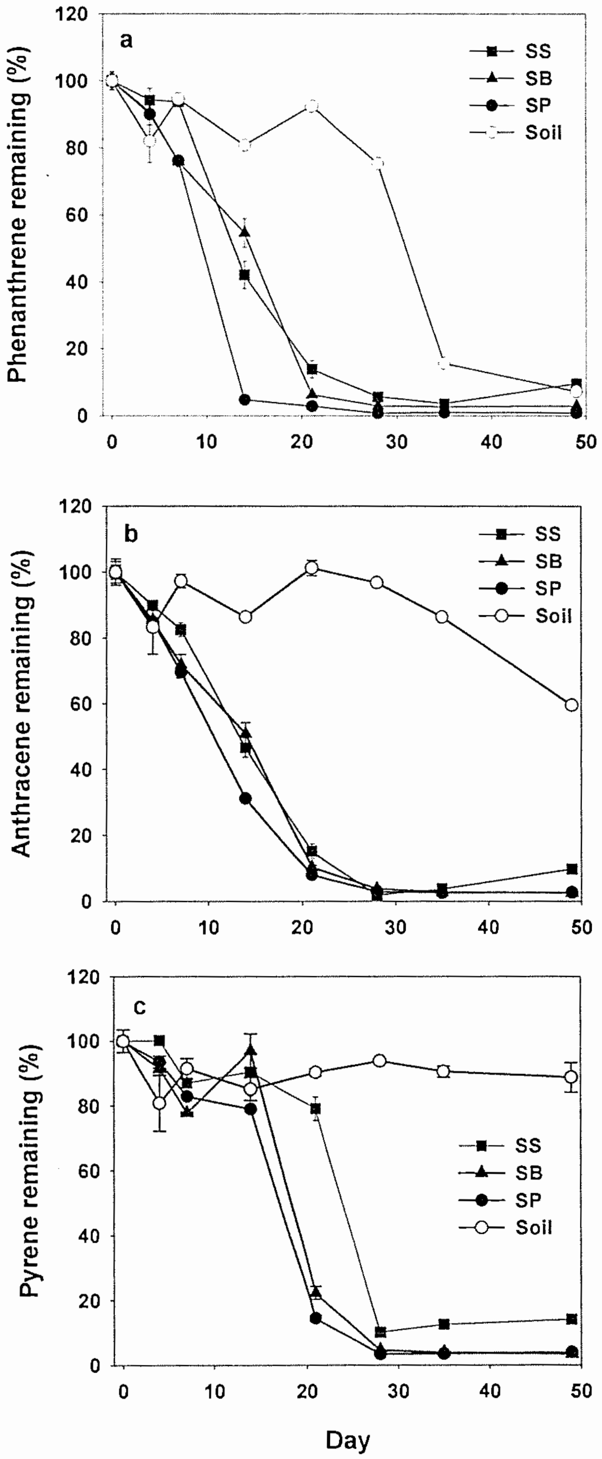

a
Phenanthrene remaining (%)
120
100
80
60
40
20
0
0
10
20
30
40
50
SS
SB
SP
Soil
b
Anthracene remaining (%)
c
Pyrene remaining (%)
Day

Figure 4

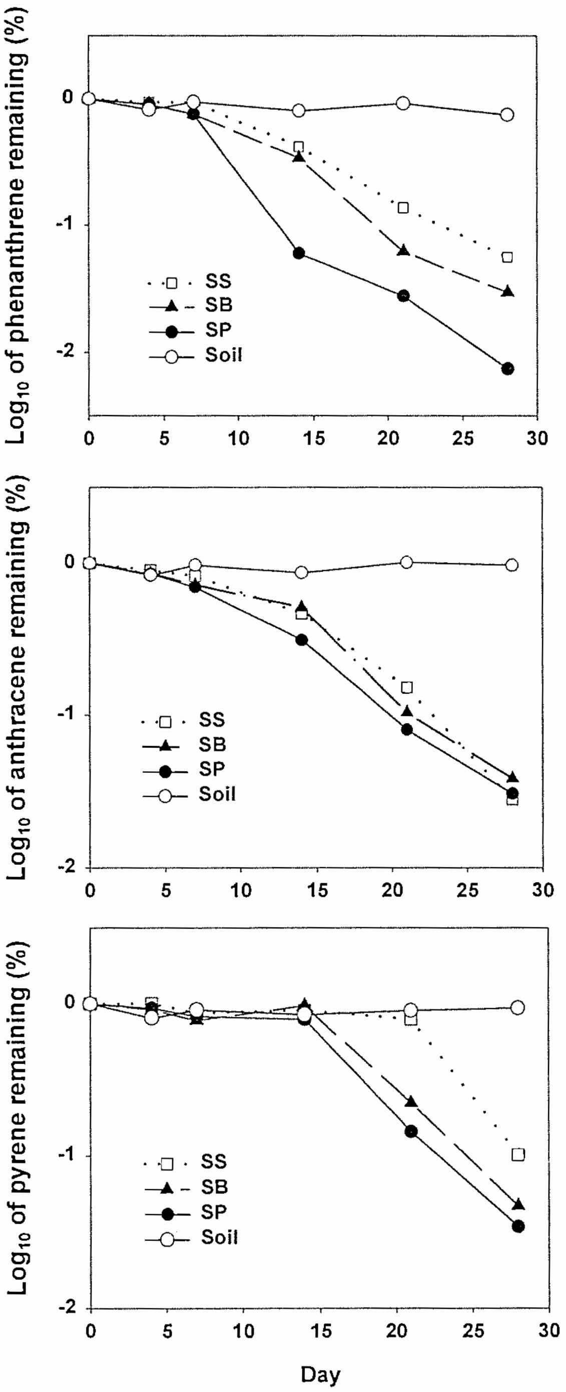
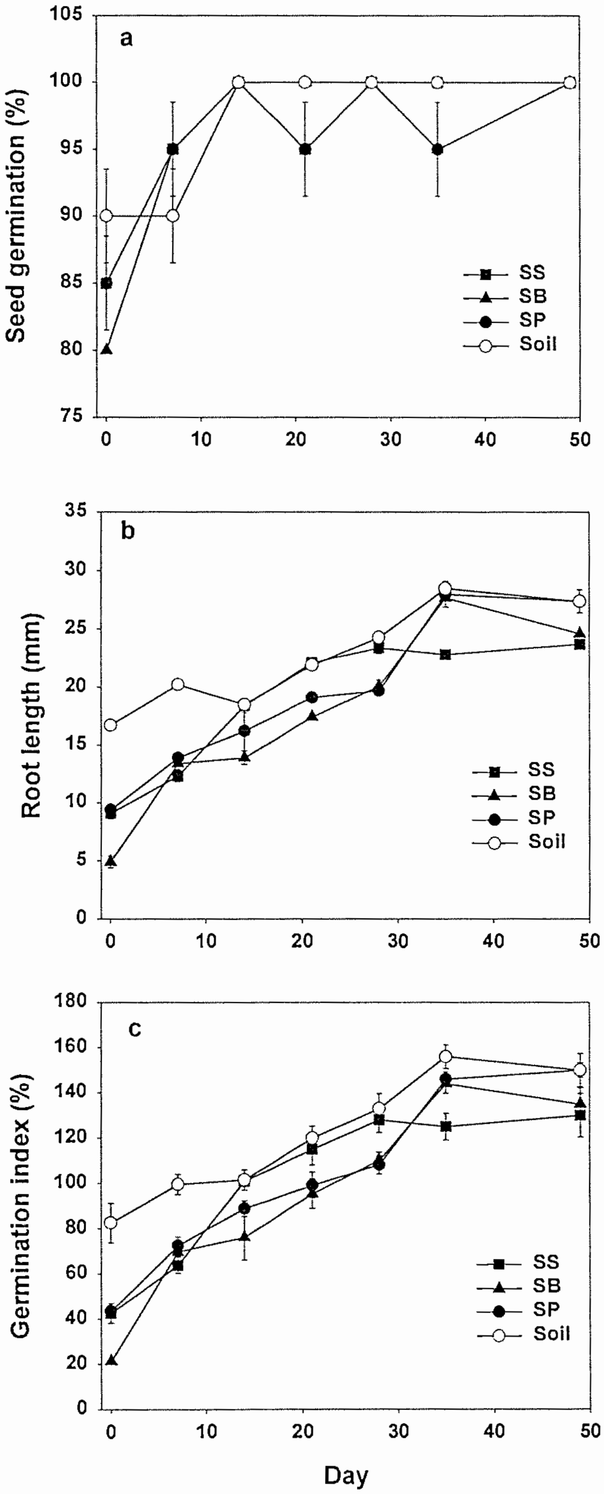

Log₁₀ of phenanthrene remaining (%)
0
-1
-2
0
5
10
15
20
25
30
SS
SB
SP
Soil
Log₁₀ of anthracene remaining (%)
0
-1
-2
0
5
10
15
20
25
30
SS
SB
SP
Soil
Log₁₀ of pyrene remaining (%)
0
-1
-2
0
5
10
15
20
25
30
SS
SB
SP
Soil
Day

Figure 5



a
Seed germination (%)
105
100
95
90
85
80
75
0
10
20
30
40
50
SS
SB
SP
Soil
b
Root length (mm)
35
30
25
20
15
10
5
0
0
10
20
30
40
50
SS
SB
SP
Soil
c
Germination index (%)
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
SS
SB
SP
Soil
Day