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Degradation of Polynuclear Aromatic Hydrocarbons under Bench-Scale Compost Conditions

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The relationship between biomass growth and degradation of polynuclear aromatic hydrocarbons (PAHs) in soil, and subsequent toxicity reduction, was evaluated in 10 in-vessel, bench-scale compost units. Field soil was acquired from the Reilly Tar and Chemical Company Superfund site in St. Louis Park, MN (Reilly soil) and brought to the U.S. Environmental Protection Agency Test and Evaluation Facility in Cincinnati, OH for a 12-week composting study. Five separate amendment conditions were applied in duplicate to Reilly soil to stimulate varying degrees of biomass growth. Amendments included standard nutrients (SN) adjusted to C:N:P = 100:5:1, based on total organic carbon, plus 1% cow manure, modified OECD nutrients adjusted to C:N:P = 100:5:1 plus 1% cow manure, SN plus 1% activated sludge, SN plus 5% activated sludge, and SN plus 5% autoclaved sludge. All reactors contained 30% (w/w) corn cobs. All amendment conditions resulted in decreased concentrations of PAHs with two to four rings in their molecular structure. No reduction in concentrations of five- or six-ring PAHs occurred during the 12-week study. No significant differences resulted between the final concentrations achieved through any of the amendment conditions. Starting concentrations of total PAHs ranged from 1606 to 4445 mg/kg, and final concentrations ranged from 888 to 1556 mg/kg in the reactors. Contaminant concentration plateaus appeared in all treatment curves by the eighth week. Once a concentration plateau was attained, little further PAH removal occurred during the remaining treatment, and all treatments moved closer to a similar concentration plateau value. Therefore, percent removal of PAHs from Reilly soil correlated with starting PAH concentrations but not with final concentrations. Rates of removal of PAHs during the first 4 weeks of compost treatment correlated strongly with starting PAH concentration but did not correlate with reactor biomass concentration. Several toxicity bioassays in earthworms and plants were used to evaluate the efficacy of compost biomass to reduce toxicity of PAH-contaminated soil. Earthworms (*Lumbricus terrestris* and *Eisenia fetida andrei*) were exposed to contaminated soil mixed with artificial soil in 6% to 100% dilutions (w/w), and survival was assessed after 14 days. Seed germination and root elongation tests were evaluated in lettuce and oats, and genotoxicity (mitotic aberrations) testing was performed on *Allium cepa* (onion). Composting of PAH contaminated soil decreased toxicity to earthworms and oat roots but had no significant effect on lettuce root toxicity. Untreated soil evoked genotoxicity in the *Allium* assay. After composting, no significant genotoxicity was observed in Reilly soil. Two challenges for future research on compost treatment of soils contaminated with PAHs involve increasing the removal of five- and six-ring compounds and achieving total removal that plateaus at a lower level. Whether this can be achieved by optimizing compost biomass development is uncertain. Continued evaluation of the amount and physiological status of compost biomass may provide information on the long-term ability of composting to destroy large PAHs.

Introduction

Composting has been used to degrade solid waste materials such as yard waste, sewage sludge, and food wastes. More recently, composting has been investigated as a remediation technology for hazardous waste (*1*−*4*). Laboratory and field-scale work has been conducted to determine the treatability of polynuclear aromatic hydrocarbons (PAHs) and explosives in the composting environment. PAHs are a concern at many sites, including former wood-treating facilities and manufactured gas plants.

Composting conditions differ from other ex-situ soil treatment systems in that bulking agents are added to the compost mixture to increase porosity and serve as sources of easily assimilated carbon for biomass growth. Aerobic metabolism generates heat resulting in significant temperature increases that bring about changes in the microbial population and physiology in the compost mixture. The conventional aerobic compost process passes through four major microbiological phases identified by temperature: mesophilic (30−45 °C); thermophilic (45−75 °C); cooling; and maturation. The greatest microbial diversity has been observed in the mesophilic stage. The thermophilic stage is characterized by spore-forming bacteria (Bacillus spp.) (*5*) and thermophilic fungi (*6*). Microbial recolonization during the cooling phase brings the appearance of mesophilic fungi whose spores withstood the high temperatures of the thermophilic stage. In the final compost stage (maturation), most digestible organic matter has been consumed by the microbial population and the composted material is considered stable.

Previous research has suggested that the degrading fraction of microorganisms in soil increases with an increase in overall biomass content (*7*). Unpublished research conducted in our laboratory showed that the number of phenanthrene degrading microorganisms in a compost system changed according to the total amount of compost biomass. This research sought to evaluate the relationship between aerobic biomass development and removal of 19 individual PAHs and toxicity from field soil during the composting process in in-vessel reactors located at the U.S. Environmental Protection Agency (EPA) Test & Evaluation (T&E) Facility in Cincinnati, OH. Five compost amendment conditions were formulated from different nutrients or amendments to the reactor mixtures. Operating parameters

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TABLE 1. Experimental Design for 12-Week Compost Treatment of Reilly Soil[a]

experimental condition	reactor number									
	1	2	3	4	5	6	7	8	9	10
soil + std* nutr + cobs + 1% cow manure	X					X				
soil + OECD + cobs + 1% cow manure		X					X			
soil + std nutr + cobs +1% activated sludge			X					X		
soil + std nutr + cobs + 5% activated sludge				X					X	
soil + std nutr + cobs + 5% autoclaved sludge					X					X

[a] Each treatment included duplicate reactors. All reactors contained corn cobs (70/30 soil/cobs, w/w). Standard and modified OECD nutrients were adjusted to carbon:nitrogen:phosphorus (C:N:P) = 100:5:1

of interest included aeration, moisture dynamics, and heat production. Toxicity tests were conducted to evaluate the effect of composting on soil toxicity.

Materials and Methods

Five experimental conditions were tested in duplicate using 10 bench-scale compost reactors. Experimental conditions are shown in Table 1. Each of the test conditions utilized a 70% soil and 30% corn cob mixture on a dry weight basis. Carbon:nitrogen:phosphorus (C:N:P) was adjusted to a ratio of 100:5:1 based on total organic content of each compost mixture. The standard compost mixture consisted of 1% (w/w) cow manure added to soil and corn cobs. Other conditions included addition of modified OECD fertilizer, 1% (w/w) activated sludge, 5% activated sludge, and 5% previously autoclaved sludge. A portion of the activated sludge was autoclaved in an attempt to alter biomass composition at the start of the composting process. OECD fertilizer (*8*) has been shown to improve biodegradation of soil contaminants (*9*). Therefore, we chose it as a test condition to evaluate its effects on biomass development. The OECD fertilizer was modified by adjusting C:N:P to 100:5:1.

Contaminated Soil. Soil contaminated with PAHs was obtained from the Reilly Tar and Chemical Superfund site in St. Louis Park, MN (Reilly soil) for use in this study. Test soil was contaminated with creosote and contained 19 PAHs that were measured during the study. The Reilly site was home to creosote manufacturing and wood preserving operations for about 80 years. Industrial operations at the site were halted in 1972. Since that time, the Reilly site has undergone remedial action by covering all contaminated soil with clean soil. Twenty-two 55-gallon drums of contaminated soil were excavated from the site and shipped to the EPA T&E Facility. Soil was screened to $^1/_2$ in. particle size by passing it through a two-stage vibrating 1-in. screen to remove large rocks and debris. The undersized material was placed in a cement mixer and blended for 30 min to obtain a homogeneous soil for testing. Several drums of the blended soil were passed through a $^1/_2$-in. screen for use in the composting study.

Reactor Design. Ten 208-L, insulated, stainless steel compost reactors were fabricated to provide closely monitored and controlled conditions required for treatability studies (Figure 1). These fully enclosed, computer monitored, bench-scale reactors each held about 100 kg total compost mixture. The reactor units stood upright with air flowing vertically up through the compost mixture for 23.5 h/day. Air flow to the composter was provided by the T&E Facility high-pressure air system. Air exited the reactor from the top and passed through a high efficiency particulate absolute (HEPA) filter before leaving the T&E Facility through the facility exhaust system. Slipstreams of inlet and exhaust gas passed through a series of monitoring instruments (relative humidity, oxygen, carbon dioxide, and methane) to determine gas composition.

Compost moisture content was measured twice a week in each reactor unit. Moisture was adjusted to 30−35% in the compost mixture. Moisture condensers inside the compost units promoted retention of moisture within the reactor to avoid excessive water evaporation during aeration. If moisture fell below 30%, a water distribution system inside the reactor was used to add water to the reaction mixture without opening the reactor. Cylindrical reactor design permitted mixing of reactor contents, by rolling each unit for approximately 30 min daily Monday through Friday, to break up anaerobic pockets and avoid packing of the compost mixture. All reactors were mixed simultaneously by placing them on rollers over a modified conveyor belt that forced the reactors to turn in unison. Baffles inside reactors promoted mixing during rolling. Two 2-in. sample ports at the top of each unit permitted sampling of the compost mixture for chemical and physical analysis.

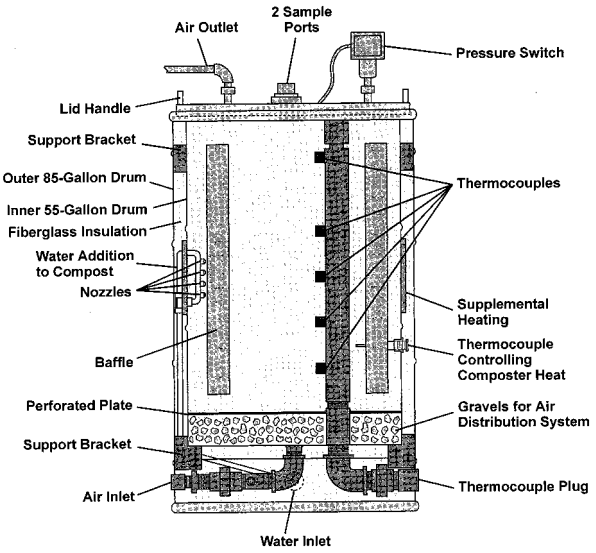

FIGURE 1. Schematic of compost reactor.

Insulation between the 208-L stainless steel reactor core and an 300-L outer shell reduced heat loss from the reactor during thermogenic aerobic activity. Each composter housed five thermocouples connected to a central computer for on-line temperature measurements. Thermocouples resided at four equally spaced vertical locations within the compost mixture, and a fifth thermocouple tracked temperature in the headspace of the reactor. Air flow was set at 10 L/min throughout the experiment. The composters were monitored by an automated control system composed of a control panel and computer.

Sample Analysis. Duplicate samples were collected from the reactors for PAH, biomass, and nutrient (ammonia nitrogen, nitrate nitrogen, and orthophosphate) analysis immediately after loading reactors (T0) and after 1, 2, 4, 8, and 12 weeks of treatment (T1, T2, T4, T8, and T12, respectively). Samples were collected through a port in the top of the reactor using a core sampler. An aliquot of compost was analyzed for moisture content by weighing before and

after drying for 12 h in an oven at 103 °C. Since soil was bulked with corn cobs, we report PAH concentrations based on the inorganic ash content of the compost mixture. This removes potential bias due to dilution by organic bulking agents. Ash content was determined using a loss-on-ignition (LOI) procedure. Duplicate 10-g samples were dried for 12 h at 103 °C and then transferred to a muffle furnace held at 550 °C for 12 h to burn the organic matter. Ash content was calculated from the ratio of pre- and postignition sample weights. Ash content results were averaged, and the averaged values were used to calculate PAH concentrations in the soil as mg PAH/kg ash.

PAH Analysis. PAH compounds in compost samples were extracted using an EPA operating procedure. Briefly, a 4-g sample of compost was mixed with 10 g of sodium sulfate and 20 mL of methylene chloride/acetone (1/1 v/v, optima grade, Fisher Scientific) in a 40 mL extraction vial. The vial was shaken horizontally on a reciprocating shaker (Eberbach Corp., Model 6000) for 18 h at 165 oscillations per min and then centrifuged at 220 g for 30 min to separate the soil and solvent. The solvent layer was then taken for analysis. A surrogate compound (2-fluorobiphenyl, 1 mg/soil sample, purity > 97.5%, Aldrich Chemical Co.) was added to all samples, including blanks and quality control samples, prior to extraction to monitor extraction efficiency. Usually, surrogate recovery was well within specifications. If surrogate recovery for a sample fell outside acceptable limits, the sample was voided unless a concrete explanation was available.

Concentrations of 19 PAH compounds were determined in the extracts by gas chromatography with flame ionization detection (GC-FID) according to EPA Method 8100 (10). Analysis was performed on a Hewlett-Packard 5890 Plus GC (Palo Alto, CA) equipped with a 30-m Supelco SPB-5 column (0.53 mm inside diameter, 0.50 μm film thickness). Flow and temperature were maintained constant throughout the study. Data were analyzed by one-way analysis of variance (ANOVA) to test for significant differences between mean PAH concentrations. The Pearson product moment correlation coefficient, r, was determined to reveal any correlation between percent PAH removal and starting and final PAH concentrations.

Inorganic Nutrients. Total Kjeldahl nitrogen (TKN) analysis was performed by Galbraith Laboratories, Knoxville, TN on T0 and T4 samples. Ammonia nitrate and orthophosphate analyses were performed by Data Chem Laboratories, Cincinnati, OH on T0, T4, and T8 samples.

Biomass. Biomass was estimated as total phospholipid-associated phosphate in the samples. Five grams of soil was suspended in 7.5 mL of dichloromethane (DCM) and 15 mL of methanol to dissolve microbial membranes and extract lipid components. After standing overnight in the dark at 4 °C, liquid phases were separated by adding 7.5 mL of DCM and 7.5 mL of 50 mM phosphate buffer (pH 7.4), shaking, and storing overnight at 4 °C. Samples were centrifuged at 250 g for 2 min, and the upper water−methanol fraction was aspirated leaving lipids dissolved in DCM. Fourteen milliliters of the DCM-lipid fraction was filtered through a Watman 2V filter. The filter was rinsed three times with 0.75 mL of DCM. The DCM−lipid solution was dried under nitrogen in a 37 °C water bath. The dry material was considered to be the total extractable lipid from the sample. Dried samples were reconstituted with 1.0 mL of chloroform, and 0.1 mL was withdrawn for biomass determination. Phospholipids were digested by adding 0.45 mL of sulfuric acid saturated with potassium persulfate and incubating overnight at 100 °C in sealed ampules. After digestion, the released lipid phosphate was complexed with 0.1 mL of ammonium molybdate and 0.45 mL of malachite green. After sitting at room temperature for 30 min, samples were analyzed colorimetrically at 610 nm for determination of total lipid phosphate. Total biomass in a sample was proportional to total lipid phosphate.

Significant differences between treatment regimens was evaluated by one-way ANOVA. If differences were found to exist at $p \leq 0.05$, differences between individual means ($p \leq 0.05$) were evaluated using the least-significant-difference (LSD) test for multiple comparisons of means (*11*).

Toxicity Testing. A 25-kg sample of untreated soil was collected prior to the compost treatability study and was stored at 4 °C until toxicity testing. A 25-kg sample of composted soil that received modified OECD nutrients was collected at the end of the treatability study for testing. Due to resource limitations, only one treatment regimen of composted soil was tested for toxicity. Earthworm acute toxicity and plant seed germination/root elongation tests were performed on the soil directly as previously described (*12*).

Earthworm Acute Toxicity. The 14-day standard earthworm artificial soil toxicity test (*13*−*15*) was used to assess the potential soil toxicity before and after remediation. Two species of earthworm were chosen as test organisms in the study: *Lumbricus terrestris* (nightcrawler) and *Eisenia fetida andrei* (red worm). *Lumbricus* worms were obtained from the North Carolina Biological Supply Company (Burlington, NC) and *Eisenia* were originally obtained from the EPA Environmental Research Laboratory in Corvallis, OR and maintained as cultures in the National Environmental Research Laboratory in Cincinnati, OH. Composted and untreated soil was sieved through a 2.5-mm screen to remove corn cobs that were added to the compost mixture at T_o as well as small stones and other debris present during the composting process. Sieved soil from each sample was mixed with artificial soil (10% sphagnum peat, 20% kaolinite clay, and 70% fine sand by weight) at varying dilutions (0−100%). Ten adult earthworms, in triplicate, were added to either 400 or 200 g of the test soil mixture for *Lumbricus* and *Eisenia*, respectively, in covered glass containers. 2-Chloroacetamide (30 mg/kg in artificial soil) was used as positive control. The containers were incubated at 15 or 23 °C for *Lumbricus* or *Eisenia*, respectively, for a total of 14 days. To assess mortality, worms were considered dead when they did not respond to a gentle mechanical stimulus.

Seed Germination/Root Elongation Test. Seed germination and root elongation toxicity testing was performed according to EPA protocols (*16*), as modified by Chang et al. (*12*). Triplicate soil samples were mixed with silica sand (20 mesh) at varying dilutions (0−100%) and placed in 100-mm Petri dishes. One species of dicotyledon (lettuce, *Lactuca sativa*) and one species of monocotyledon (oats, *Avena sativa*) were used in this study. For each dish, either 20 lettuce or 10 oat seeds were placed on top of the test soil mixture. 2-Chloroacetamide (22 mg/kg for lettuce and 35 mg/kg for oats in silica sand) was used as positive control. Phytotoxicity results are based on the successful germination of the test seed (LC_{50}) analyzed by a Trimmed Spearman-Karber method (*17*), and the effective concentration (EC_{50}) that reduced root growth by 50% was interpolated from a dose−response curve by regression analysis using SigmaPlot graphic software. Statistical analysis was performed using Dunnett's Test for multiple mean comparison (*18*). Differences were considered significant if $p \leq 0.05$.

***Allium* Mitotic Aberration Assay**. The *Allium* test was conducted according to the method of Rank and Nielson (*19*) as modified by Meier et al. (*20*). Aqueous extracts of soil were diluted to 0, 25%, 50%, and 100% (undiluted) with synthetic freshwater (SFW) for testing. 4-Nitroquinoline-n-oxide at a concentration of 0.125 μg/mL was used as positive control. Abnormalities scored included fragments, lagging chromosomes, bridges, and multipolar, polyploid, and c-mitotic type cells. Mitotic index (MI) and percent aberrant

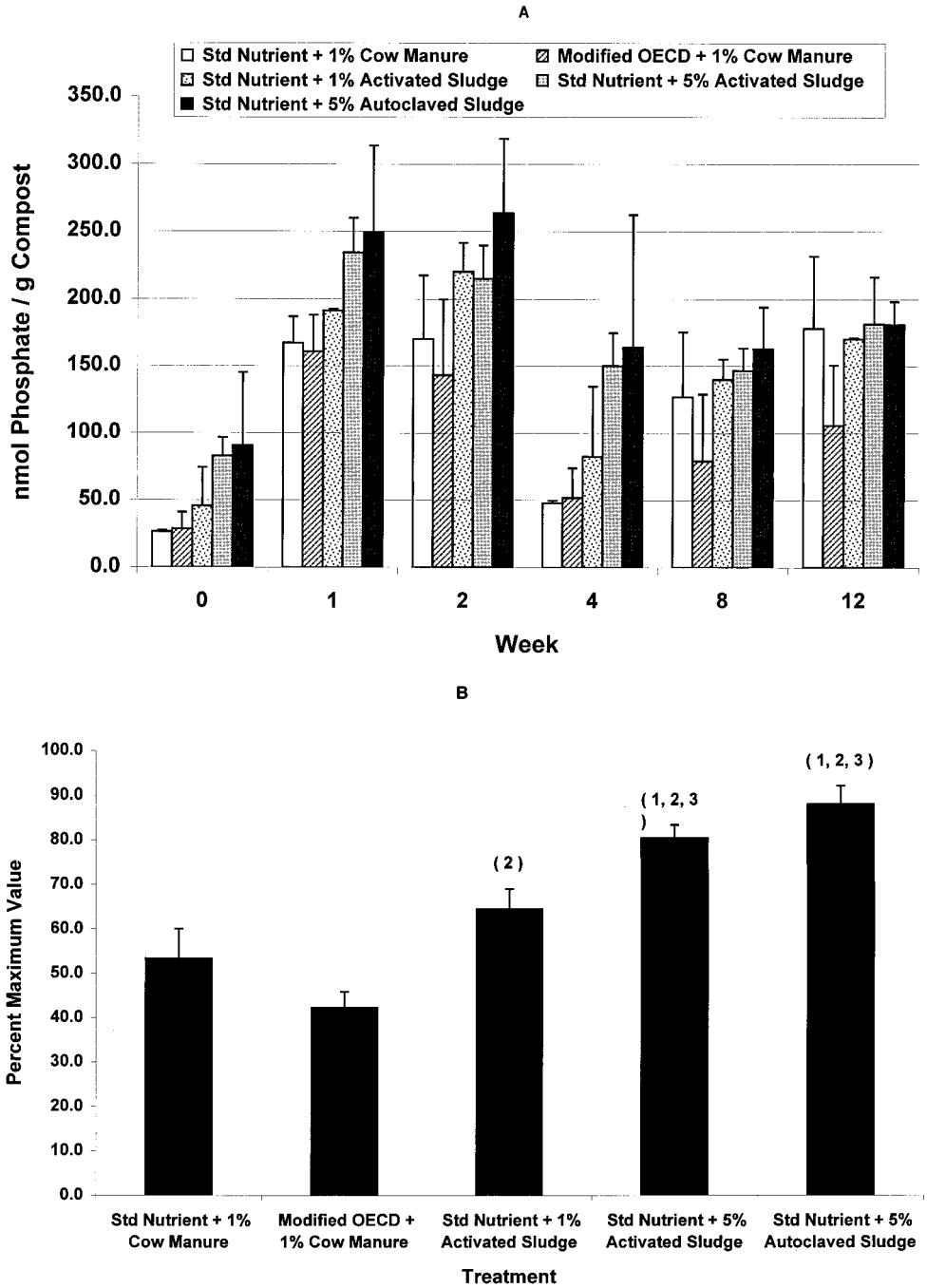

FIGURE 2. (A) Biomass concentration in compost reactors. Compost biomass concentration was estimated by lipid-associated phosphate concentration. Values (nmol phosphate/g compost) represent means ± standard deviation. (B) Relative biomass in compost reactors. Relative biomass was calculated by normalizing the biomass value for each reactor as percent of the highest biomass value among the reactors at each time point. Values represent means ± standard deviation for relative biomass for each treatment regimen (duplicate reactors) over all time points. (1) Significantly different from std nutrient + 1% cow manure ($p < 0.001$). (2) Significantly different from modified OECD nutrient ($p < 0.01$). (3) Significantly different from std nutrient + 1% activated sludge ($p < 0.05$).

cells (AB) were analyzed statistically by nonparametric ANOVA following arcsine square root transformation of the data. Analysis was performed using SAS software (version 6.12) using PROC RANK to rank data and PROC GLM for two-way ANOVA. The two factors in ANOVA were soil treatment and dose. Dunnett's multiple mean comparison test was used to test for differences between the negative control and each dose group. Significant differences between groups were determined using Duncan's test. The reason two-way ANOVA was employed in this study was that other treated soils besides compost treated soil were included in the toxicity tests.

Results

Reactor Temperature. Temperatures in all reactors climbed to the upper mesophilic and lower thermophilic ranges (41–53 °C) during the first 15 days of composting and subsequently dropped to ambient conditions. This temperature profile indicated that all reactors contained active aerobic compost mixtures. Reactors containing 5% sludge, activated or autoclaved, attained higher temperatures than reactors containing less or no sludge. On day 3 of composting, compost reactor number 4 (5% activated sludge) was observed to have lower temperature than other composters and a plugged air

TABLE 2. Small (Two + Three Rings), Four-Ring, and Large (Five + Six Rings) PAH Concentrations at the Beginning and Week 12 of Compost Treatment[a]

experimental condition	reactor no.	total PAH (mg/kg) week 0	total PAH (mg/kg) week 12	percent removal (%)	mean ± SE week 0	mean ± SE week 12	mean percent removal (%)
std nutr + 1% cow manure	1	3367	1396	58.5	3080.0 ± 405.9	1310.5 ± 120.9	57.5 ± 1.2
	6	2793	1225	56.1			
modified OECD + 1% cow manure	2	4445	1211	72.8	4315.0 ± 183.8	1141.5 ± 98.3	73.5 ± 0.8
	7	4185	1072	74.4			
std nutr + 1% activated sludge	3	4058	1556	61.7	3144.0 ± 1292.6	1190.5 ± 516.9	62.1 ± 0.7
	8	2230	825	63.0			
std nutr + 5% activated sludge	4	1606	888	44.7	2235.0 ± 889.5	899.0 ± 15.6	59.8 ± 11.8
	9	2864	910	68.2			
std nutr + 5% autoclaved sludge	5	3607	935	74.1	3141.5 ± 658.3	1051.0 ± 164.0	66.5 ± 8.8
	10	2676	1167	56.4			

[a] Values on the left show concentrations for individual reactors. Values on the right show the mean ± standard error for duplicate reactors.

line was discovered. After the air line obstruction was removed and air was readjusted to 10 L/min, the reactor temperature rose to above 50 °C.

Inorganic Nutrients. In all composters, ammonia nitrogen, nitrate nitrogen, and orthophosphate concentrations decreased over the study period. In most reactors, inorganic nutrients were depleted in approximately 4 weeks indicating that they were mostly consumed during the active (thermogenic) stage of composting. Nutrients were not replenished during the study period. TKN concentrations remained relatively constant over the course of the study. Moisture content in the reactors ranged between 22% and 35% after the compost reactions were underway.

Biomass. Compost reactors containing sludge sustained higher biomass concentrations than did reactors containing cow manure during the first 4 weeks (Figure 2A,B). The greatest amount of biomass appeared between the first and second weeks of composting. This period corresponded to the highest reactor temperatures and, by inference, the greatest aerobic activity in the compost mixture. After 8 weeks of composting, all composters contained similar amounts of biomass. High variance about the mean precluded definitive statements about statistical differences in non-normalized data (Figure 2A). Therefore, weekly biomass data were normalized as percent of the highest biomass value at each time point so that relative biomass values could be computed for each reactor over the entire course of the study. Figure 2B shows the relative proportions of biomass for each treatment averaged over 12 weeks. Reactors containing modified OECD nutrients had significantly lower biomass than any reactors containing sludge. Reactors containing 5% sludge, either activated or autoclaved, sustained the highest average biomass concentrations during the study. A portion of the sludge was autoclaved in an attempt to differentiate between effects of nutrients and microorganisms in the added sludge. It was hoped that autoclaving would sterilize the sludge. It became evident, however, that the autoclaved sludge was not sterile since reactors containing autoclaved sludge showed biomass concentrations comparable to 5% activated sludge condition. Autoclaved sludge may not have been completely sterilized due to the large volume required for composting and its high water content.

PAH Concentrations. Total PAH concentrations at the beginning and end of the 12-week compost treatment study, and percent decline for each treatment regimen are shown in Table 2. Despite extensive blending, PAHs remained unevenly distributed in the soil. Starting total PAH concentrations ranged from 1606 mg/kg in reactor 4 to 4445 mg/kg in reactor 2. Final PAH concentrations ranged from 888 mg/kg (reactor 4) to 1556 mg/kg (reactor 3) and were statistically similar across all treatment regimens as determined by one-way ANOVA.

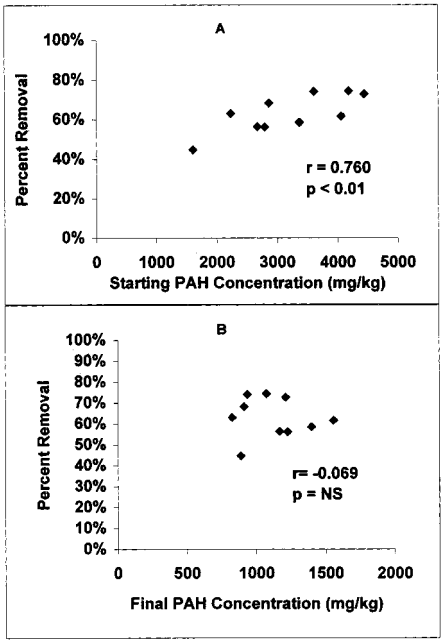

FIGURE 3. (A) Scatter diagram showing correlation between percent total PAH removal and starting concentrations of total PAHs in each reactor. (B) Scatter diagram showing lack of correlation between percent total PAH removal and final concentration of total PAHs in each reactor.

Percent total PAH removal during composting correlated positively to starting concentration, yielding a Pearson correlation coefficient of $r = 0.760$ ($p < 0.01$) (Figure 3A). Percent PAH removal did not correlate with final PAH concentrations ($r = -0.069$, $p > 0.40$) (Figure 3B). These results can be expected if treatment removes PAHs until a plateau concentration is reached and all treatments tend toward a common plateau value.

Rates of small PAH (two and three rings) removal during the first 4 weeks ranged from 84 mg/kg-week (reactor 4) to 567 mg/kg-week (reactor 2) as estimated by linear regression of small PAH concentration over T_0 to T_4. Rates for four-ring PAH ranged from 25 mg/kg-week (reactor 5) to 232 mg/kg-week (reactor 1). Figure 4A,B shows scatter diagrams of small PAH removal rates against small PAH starting concentrations and average biomass in respective reactors during the first 4 weeks of composting. Rates of small and four-ring PAH removal correlated strongly to starting small PAH concentration ($r = 0.9840$, $p < 0.001$, Figure 4A) and starting four-ring concentrations ($r = 0.7094$, $p < 0.05$, scatter diagram not shown), respectively, with no correlation to treatment regimen or reactor biomass (Figure 4B). During the first 2

TABLE 3. Total PAH Concentrations at the Beginning and Week 12 of Compost Treatment[a]

	week 0 (mg PAH/kg ash) number of rings			week 12 (mg PAH/kg ash) number of rings		
experimental condition	2 + 3	4	5 + 6	2 + 3	4	5 + 6
std nutr + 1% cow manure	1604	1093	385	290	487	534
modified OECD + 1% cow manure	2628	1245	444	217	468	457
std nutrient + 1% activated sludge	1633	1110	402	303	406	483
std nutr + 5% activated sludge	962	945	328	121	332	448
std nutr + 5% autoclaved sludge	1524	1122	497	178	442	432
average percent removal				86.7%	61.3%	−14.5%

[a] Values represent the average of duplicate reactors rounded to the nearest whole number.

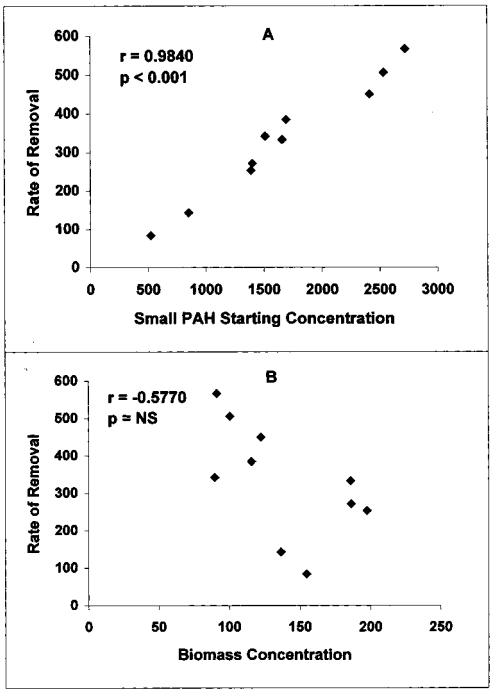

FIGURE 4. (A) Scatter diagram showing correlation between rates of small PAH removal and starting concentrations of small PAHs in each reactor over 4 weeks. (B) Scatter diagram showing lack of correlation between rates of small PAH removal and average biomass concentrations in each reactor over 4 weeks.

weeks of composting, reactors containing 5% activated sludge had the lowest removal rates of total PAH, while reactors containing OECD fertilizer had the highest (68, 345, 597, 755, and 939 mg/kg/week for 5% activated sludge, 1% activated sludge, 5% autoclaved sludge, SN-corn cobs, and OECD-corn cobs, respectively).

Small PAHs were reduced by an average of 87% in all composters after 12 weeks, and four-ring PAHs were reduced by an average of 61% (Table 3). None of the amendment conditions appeared effective in degrading large PAHs (five and six rings) in this study.

Concentrations of individual PAHs in compost samples listed in order of increasing GC retention time, corresponding roughly to increasing molecular weight, are shown in Table 4. Smaller PAHs were removed more effectively than large ones. 2-Methylnaphthalene was reduced to nondetectable levels (2 mg/kg) by all treatments except 1% activated sludge (7.5 mg/kg). Naphthalene was less effectively removed with final concentrations ranging from 19.0 mg/kg (5% autoclaved sludge) to 36.9 mg/kg (1% activated sludge). Chrysene was the largest PAH to be reduced during the compost process. Plots for each composting condition show PAH concentrations for different sizes of PAHs based on the number of rings contained in the molecule (Figure 5). Small, four-ring, and large PAH concentrations declined at different rates with smaller PAHs disappearing before larger ones. Concentrations of five- and six-ring PAHs did not decline during 12 weeks of composttreatment. Decline in total concentrations of PAHs was accounted for mostly by disappearance of small PAHs. Most of the concentration reduction occurred within the first 4 weeks of treatment with a plateau forming by 8 weeks. This resulted in curves with a biphasic, "hockey-stick" shape indicating relatively rapid removal of PAHs during the initial phase of treatment followed by slow removal during the later phase.

Toxicity. Toxicity testing of Reilly soil was performed before and after compost treatment. The LC_{50} of untreated soil ranged from 13% (*Eisenia*) to 33% (*Lumbricus*) contaminated soil mixed with artificial soil (Table 5). None of the earthworms tested survived 14 days of exposure to 100% untreated Reilly soil. Composting decreased Reilly soil toxicity to earthworms compared with untreated soil. The LC_{50} for composted soil exceeded 100% for both *Eisenia* and *Lumbricus*. This observation was consistent with decreased concentrations of soil PAHs.

In phytotoxicity studies, Reilly soil had little effect on seed germination either before or after compost treatment (Table 6). However, untreated Reilly soil exhibited significant inhibition of root elongation in both lettuce and oats (EC_{50} = 95% and 77% untreated soil, respectively). Composted soil also inhibited root elongation in lettuce (EC_{50} = 90% composted soil) but showed a decreased effect against oat roots (EC_{50} > 100% composted soil). Neither untreated Reilly soil nor composted soil showed any effect on *Allium* mitotic index results (Table 7). However, untreated soil fostered significant anaphase aberrations in *Allium*. After compost treatment of Reilly soil, no significant *Allium* anaphase aberrations appeared compared to negative control.

Discussion

Removal of PAHs. Temperatures remained below 55 °C throughout the composting process. Unpublished research in our laboratory indicated that phenanthrene-degrading microorganisms disappeared at temperatures exceeding 60 °C. This suggested that thermophilic temperatures might destroy some PAH degraders. Therefore, the strategy was to establish active, thermogenic compost conditions for maximal metabolic activity without destroying PAH-degrading capabilities. PAH-degrading microorganisms were not monitored during this study, but since reactor temperatures remained below 55 °C, no thermal inhibitory effects on the microorganisms were anticipated. Reactor temperatures remained in the thermophilic range through the first 2 weeks of composting. Compost systems often exhibit thermophilic conditions for much longer than 2 weeks. The shorter thermophilic time in this study was probably due to the high soil/bulking agent ratio (70/30). A higher fraction of soil has the advantage of greater soil throughput in a space-limited

TABLE 4. Individual PAH Concentrations at the Beginning and Week 12 of Compost Treatment[a]

		std nutr + 1% cow manure		modified OECD nutrs + 1% cow manure		std nutr + 1% activated sludge		std nutr + 5% activated sludge		std nutr + 5% autoclaved sludge	
PAH analyte (mg/kg ash)	no. of rings	T0	T12	T0	T12	T0	T12	T0	T12	T0	T12
naphthalene	2	67.6	36.5	288.1	34.1	129.0	36.9	33.4	23.6	49.4	19.0
2-methylnaphthalene	2	49.0	ND[b]	142.4	ND[b]	78.8	7.5	19.6	ND[b]	37.7	ND[b]
acenaphthylene	3	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
acenaphthene	3	206.8	6.4	316.2	10.4	196.9	5.8	124.1	3.2	113.8	ND[b]
dibenzofuran	3	123.6	17.2	211.4	15.6	134.8	9.6	77.0	3.6	85.2	ND[b]
fluorene	3	200.7	15.7	310.6	11.2	195.7	23.0	116.5	3.6	148.3	9.9
phenanthrene	3	538.6	73.7	815.5	72.1	530.7	69.8	321.2	34.0	408.3	54.5
anthracene	3	417.0	140.7	543.2	73.8	366.9	150.1	270.3	52.2	680.8	93.7
fluoranthene	4	493.0	147.6	566.5	150.7	488.7	102.0	416.8	87.9	460.9	134.7
pyrene	4	349.5	115.9	393.0	126.9	363.7	85.5	305.7	71.0	365.7	102.8
benzanthracene	4	113.6	89.7	127.5	79.5	114.1	89.3	96.7	63.4	126.3	79.9
chrysene	4	136.8	133.6	157.3	110.5	142.8	128.6	125.1	110.3	168.7	124.0
benzo[*b*]-pyrene + benzo[*k*]-pyrene	5 5	150.9	193.9	171.4	165.3	159.2	167.7	131.9	156.7	183.4	154.4
benzo[*e*]pyrene	5	63.1	88.6	72.6	74.6	65.7	76.0	52.9	70.7	78.8	69.3
benzo[*a*]pyrene	5	84.3	109.1	96.4	94.8	87.4	94.1	73.2	85.3	108.3	85.3
dibenzoanthracene + indenopyrene	5 6	40.1	63.4	48.4	55.9	42.9	62.6	33.8	60.3	62.7	55.6
benzo(*g*,*h*,*i*)perylene	6	45.5	78.7	54.4	66.1	47.2	81.9	36.5	73.2	62.9	67.8

[a] Values represent the average of duplicate reactors. [b] ND = nondetectable: Detection limit = 2 mg/kg ash.

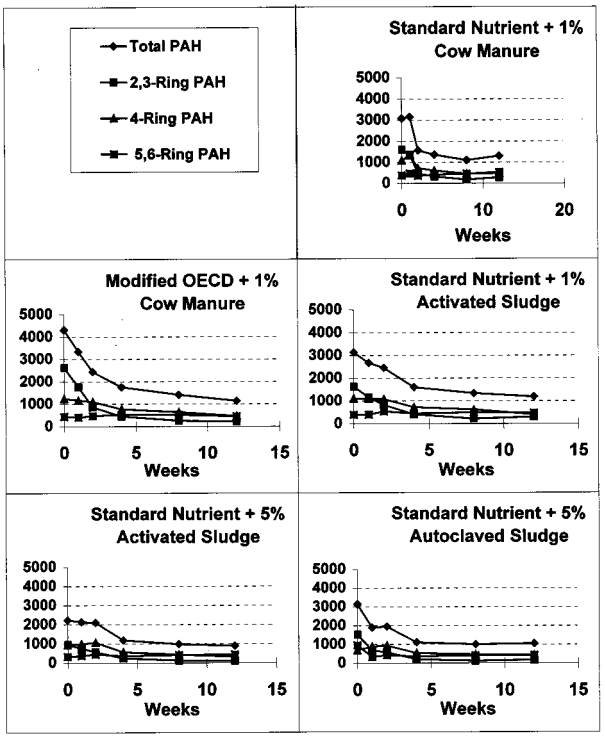

FIGURE 5. Plots of total, small, four-ring, and large PAH concentrations in each treatment mixture over time. Plots show average values for duplicate reactors.

system, and it also tends to keep the temperature below 60 °C where degrading microorganisms begin to expire.

Despite a range of final values, no statistical differences appeared between the final PAH concentrations in any of the five compost groups. PAH concentrations showed little reduction during the last 4 weeks of composting, implying the existence of a plateau (Figure 5). A plateau pattern yields a disappearance curve for PAHs that resembles a "hockey stick" in form. The curve's plateau may indicate a fraction of PAHs in soil that remains inaccessible to microorganisms for biological degradation. Mass transfer may be the rate-limiting step in PAH biodegradation (*21*−*23*). If so, then removal of PAHs below the plateau will require mobilization of soil-bound PAH compounds. Some studies have indicated that before biodegradation can occur, PAHs must be released from soil sequestration into the aqueous media (*24, 25*). Release of hydrophobic chemicals into aqueous media has been shown to be biphasic in nature with rapid release followed by slow, long-term release (*22, 26*).

TABLE 5. Earthworm Survival after 14-Day Acute Toxicity Test on Compost-Treated and Untreated Reilly Soil[a]

	survival (LC_{50})	
soil	*Eisenia fetida*	*Lumbricus terrestris*
untreated	32.9	12.8
compost	>100%	>100%

[a] The treated soil was from the modified OECD treatment regimen. Results are from triplicate treatments, each containing 10 worms.

TABLE 6. Effect of Compost Treatment on Seed Germination and Root Elongation in Oats and Lettuce

	lettuce		oats	
soil	germination (LC 50)	root length (mm)[b]	germination (LC 50)	root length (mm)[b]
untreated	>100%	95.3%	no Effect	77.0%
compost	>100%	89.9%	no effect	>100%

[a] The treated soil was from the modified OECD treatment regimen. [b] Percent of negative control.

TABLE 7. Effect of Compost Treatment on Allium Toxicity[a]

	mitotic index, soil dilution				anaphase aberrations, soil dilution			
soil	25%	50%	100%	overall[a]	25%	50%	100%	overall[a]
untreated	NS[c]	NS[c]	NS[c]	0.7490	<0.05[b]	<0.05	<0.05	0.0054
compost	NS[c]	NS[c]	NS[c]	0.8204	NS[c]	NS[c]	NS[c]	0.0401

[a] *p*-Value from one-way ANOVA. [b] Significantly different from control using Dennett's test. [c] NS = not significant. [d] The treated soil was from the modified OECD treatment regimen.

In addition to soil sequestration of PAHs, a contributing factor might involve changes in biomass characteristics after easily degradable organic material has been consumed by the microorganisms. For example, further analysis of biomass data from this study, using signature phospholipid fatty acid methyl ester (SP-FAME) analysis, revealed onset of long-term stress in the microorganisms after 4 weeks of composting (*27*). Long-term microbial stress, as indicated by increased ratio of the fatty acids cyclo19:0/18:1w7cis (*28*), can appear under conditions of extreme competition for limited nutrients and microorganisms shift their cell membranes to a less permeable state. During long-term stress, degradable chemicals may be less capable of crossing bacterial membranes to become metabolized.

Disappearance of measured nutrients during the compost process raised the question of whether nutrient depletion played a role in the plateau of PAH removal. However, a subsequent experiment in our laboratory that employed nutrient readdition during the composting process indicated that nutrient supply did not affect PAH removal during the plateau stage of compost treatment.

PAH bioremediation results are often reported as percent removal of PAHs. Using percent removal as an index of PAH degradation in this study may be misleading due to the apparent existence of a concentration plateau. Higher starting concentrations will result in higher percent removal as all treatments plateau at similar final concentrations. In this study, percent removal was a function of the starting concentration but not final concentration. Significant correlation was shown between starting concentrations and percent removal ($p < 0.01$), while no correlation was shown between final concentrations and percent removal (Figure 3A,B). When comparing treatment effectiveness for PAH removal where a concentration plateau is encountered, an appropriate estimate of treatment effectiveness is final concentration attained rather than percent removal.

Biomass. One strategy of bioremediation is to create conditions that facilitate increased biomass concentration in an attempt to maximize biodegrative activity (*7*). Other research in our laboratory has shown that counts of culturable phenanthrene degrading microorganisms increased and decreased with corresponding changes in total compost biomass concentrations. Surprisingly, in this study PAH removal rates did not correlate with reactor biomass concentration (Figure 4B) but correlated strongly with starting PAH concentrations (Figure 4A). These results indicate that inferring treatment effectiveness from removal rates in this study would yield misleading conclusions. Correlation of removal rate with starting PAH concentration indicates that PAH removal in this study was strictly a first-order kinetic phenomenon with PAH concentration determining the rate of removal.

Figure 5 shows that total PAH removal rate (68 mg/kg/wk) in reactors containing 5% activated sludge was less than that in reactors containing 5% autoclaved sludge (472 mg/kg/week) during the first 2 weeks of composting. Removal rates in other amendment conditions ranged from 345 to 939 mg/kg/week during the first 2 weeks of composting. It remains uncertain whether low removal rate in reactors containing 5% activated sludge is real or a result of heterogeneity in PAH concentrations in the Reilly soil. However, SP-FAME patterns from microorganisms in reactors containing 5% activated sludge indicated higher long-term stress than those from other reactors during the first 2 weeks of composting (*27*) as signaled by an increased cyclo19:0/18:1w7cis ratio (*28*). This indicates that microorganisms in compost containing 5% activated sludge had shifted their outer membranes to a less permeable state, suggesting they were less capable of consuming PAHs from their surroundings.

Toxicity Testing. The possibility that the appearance of a plateau region in PAH disappearance curves results from inaccessibility of PAHs to microorganisms also raises the possibility that tightly bound PAHs might not be available to evoke toxicity. Therefore, toxicity tests were performed on earthworms and plants to evaluate the effect of compost-treatment on Reilly soil toxicity.

Studies on bioremediation of chemicals in soil have shown that chemicals remaining in soil after biological treatment exhibit significantly reduced leachability into the aqueous phase and decreased bioaccessibility to microorganisms and perhaps reduced bioavailability to macroorganism receptors (*23, 29*−*31*). Therefore, bioavailability of a chemical in soil may not be a function of the measured concentration but a function of physical/chemical properties of the chemical and soil and the amount of time the chemical has been in the soil (*29*).

PAH-associated risk to human health and the environment is commonly estimated based on soil PAH concentration. However, previous studies have indicated that toxicity of PAH-contaminated soils does not correlate well with PAH concentrations in the soil (*23*). Lack of correlation between soil PAH concentration and toxicity after treatment is of interest because it indicates that factors besides PAH concentration are involved in producing toxicity. Such factors may include altered bioavailability of PAHs to test organisms due to binding of PAHs as they age in soil (*32*). Measurement of soil concentration of PAHs alone cannot yield such information. True assessment of health and environmental risk associated with PAH contamination in soil that has undergone treatment requires information about PAH release and transport (availability) and potential toxicity of the treated soil.

Compost treatment of Reilly soil reduced, but did not eliminate, toxicity to earthworms and plants exposed to composted vs untreated soil (Tables 5−7). Compost treatment appeared to eliminate genotoxicity in *Allium* tests that was present after exposure to untreated Reilly soil (Table 7). Absence of effects on seed germination and *Allium* mitotic index in this study illustrates the need for applying a variety of toxicity tests.

Toxicity bioassays assess toxicity of the entire soil sample and toxicity cannot necessarily be associated with PAH concentrations. Studies have shown that toxicity can vary with soil type and organism tested (*20, 33*). Since noncontaminated soil with characteristics similar to Reilly soil was not available for concurrent bioassays, it remains unknown how much toxicity was due to contaminants vs soil characteristics.

Besides the toxicity tests presented here, samples of untreated and composted Reilly soil from this study were evaluated for mutagenicity using the Ames mutagenicity assay by Hughes et al. (*34*) at the U.S. EPA National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC. Both untreated and composted Reilly soil were found to be moderately mutagenic to Salmonella strains YG1041 and YG1042 with S9 addition for metabolic activation of mutagens. There was no statistical difference in mutagenic activity between untreated and composted Reilly soil, indicating persistence of low level mutagenicity throughout 12 weeks of compost treatment of the soil. This is not surprising since the most mutagenic PAHs are five and six-ring compounds, e.g., benzo[*a*]pyrene, which is well-known to produce positive results in Ames mutagenicity assays with S9 addition (*35*).

Conclusion. This short-term, closely controlled study in in-vessel reactors indicated that composting of PAH contaminated field soil can reduce PAH concentrations and toxicity of the soil. General similarity of final PAH concentrations across all treatments reflects the robustness of the

compost process whereby different types of amendments did not significantly alter the final results. Two challenges for research on biodegradation of PAHs in contaminated soils involve increasing the removal of five- and six-ring PAHs and achieving total removal that plateaus at a lower level. Whether this can be achieved by optimizing the compost process remains to be seen. Considerably longer studies are needed to estimate the rate of removal of large PAHs since no apparent decrease in concentrations was observed in five- and six-ring PAHs during the first 12 weeks of composting. A post-composting polishing step such as phytoremediation or land farming might help to achieve lower PAH concentrations after compost treatment. Continued evaluation of biomass characteristics in different compost mixtures may provide more information on the long-term ability of composting to destroy large PAHs.

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