Chapter 6

Assessment of polyaromatic hydrocarbons (PAHs) removal kinetics and budget during vermicomposting

Introduction

The load of polycyclic aromatic hydrocarbons (PAHs) on terrestrial and aquatic ecosystems has alarmingly increased over the last few decades owing to rapid industrialization and urbanization [1]. The PAHs, well-known persistent organic pollutants, induce several mutagenic, carcinogenic, and teratogenic disorders in human beings [2]. Among numerous PAH compounds, 16 congeners have been designated as priority PAHs by the USEPA, considering their occurrence level and toxic impacts. These organic pollutants mainly emit from incomplete combustion of biomass and fossil fuels (coal and petroleum), readily precipitating on soil and solid waste dumps. The deposition rate of PAHs in the soil is generally high due to their high hydrophobicity and low solubility [3]. Several reports on the detection of PAHs in solid waste residues are available in the literature. Sisinno et al. (2003)[4] reported a significant occurrence of quite a few PAHs (benzo[a]anthracence, benzo[a]fluoranthene, benzo[a]pyrene, fluoranthene, chrysene, etc.) in the solid waste samples of Poland. Recently, Guo et al. (2020)has found all the 16 priority PAHs in the range of 0.38 to 14.0 mg kg-1 in urban yard trash. PAHs are also found in the residues from MSW incineration units in the UK[6]. These studies indicate that PAH remediation pathways through biological techniques need to be assessed from mechanistic viewpoints.

Among different remediation technologies(physical, chemical, and biological), bioremediation approaches are regarded as the most efficient from both ecological and economic aspects[7]. Composting techniques showed good promise in removing vis-à-vis detoxifying PAHs from contaminated materials [8]. Mattei et al. (2016)[9] also reported ~50% removal efficiency of the co-composting technique in sewage-sludge-based feedstocks. Similarly, ~60-80% reduction in PAH concentrations was observed in compost amended oil-contaminated soil [10]. In another interesting study, composting resulted in >80% removal of 16 PAHs in 30 days, while vermicomposting with *Eisenia fetida* resulted in nearly 100% removal of 2-6 ring PAHs in contaminated feedstocks [11]. The PAH reduction efficacy of composting techniques largely depends on the nature and concentration of PAHs in the feedstocks. For instance, the reduction rate of high molecular weight PAHs (e.g., benzo[a]pyrene) exhibited high sorption tendency and

resistance to microbial decay than the low molecular weight compounds (e.g., fluorene, anthracene, phenanthrene, etc.) during composting of sewage sludge and green wastes [12,13]. Rorat et al. (2017)[15] also reported high PAH removal efficiency of vermitechnology in sewage-sludge-based vermibeds using *E. andrei* as a composting agent. As such, some epigeic (*E. fetida* and *E. andrei*) and epi-endogeic (*Lumbricusrubellus*) earthworms showed high tolerance to PAH exposure,and their presence substantially reduced the PAH levels in the substrate [16]. However, similar studies with *Eudrilus eugeniae* are rarely found. This epigeic earthworm (*E. eugeniae*) is a well-known vermiremediation agent and has been widely used for the bioconversion of toxic waste materials [17–19]. Moreover, there is no report on PAH accumulation ability *of E. eugeniae*. Previous researchers have shown that earthworms readily accumulate lipophilic organic pollutants, including PAHs in soil [20,21]. In contrast, Esmaeili et al. (2022)[22] observed that the PAH accumulation pattern in earthworms dramatically fluctuates depending on feeding habits, level of exposure, and ecotypes of earthworm species in contaminated soil. Therefore, it is indeed necessary to study the PAH bioavailability patterns in vermicomposting systems from mechanistic viewpoints.

Earthworms and their gut-associated microorganisms rapidly decompose and mineralize complex waste materials into nutrient-rich products. The presence of earthworms leads to the effective removal of pollutants irrespective of whether the worms accumulate the contaminants or not [23]. Concentration-mass-based calculations could appreciate the removal and apportionment budget of the pollutants; however, no standard equations are available for assessing the budget of the PAH removal process for composting systems. No information about the relationship between nutrient dynamics and PAH removal kinetics during composting and vermicomposting is known. This can be accomplished by deriving the budget of PAH apportionment in different matrixes like earthworm biomass and mineralized feedstock. Therefore, it is quintessential to formulate functional equations for budgeting the PAH removal process in composting systems to understand the differential removal mechanism in the absence or presence of earthworms.

Hence, the key objectives of the present study were – (1) to assess the PAH removal/accumulation potential of *Eudrilus eugeniae* in comparison with *Eisenia fetida*, the most widely studied earthworm species for removal of USEPA recognized toxic PAHs; (2) to comprehend the relationship among PAH bioavailability, mobility of

nutrients (C, N, P, and K), and microbial activity during composting and vermicomposting; and (3) to evolve and apply suitable budget equations for developing a clear understanding on PAH apportionment patterns during biocomposting. Therefore, a yard experiment was formulated by spiking 13 recognized priority PAHs in different concentrations in cow dung-based substrate under aerobic composting and vermicomposting. The vermireactors were incubated with equal representation (fresh weight basis) of *E. fetida* and *E. eugeniae*. Subsequently, periodic changes in PAH profiles, nutrient bioavailability, and microbial activities were enumerated. The earthworm fecundity and PAH accumulation patterns were also assessed. Furthermore, the identified research objectives were addressed with the help of correlation statistics and newly evolved PAH budget equations.

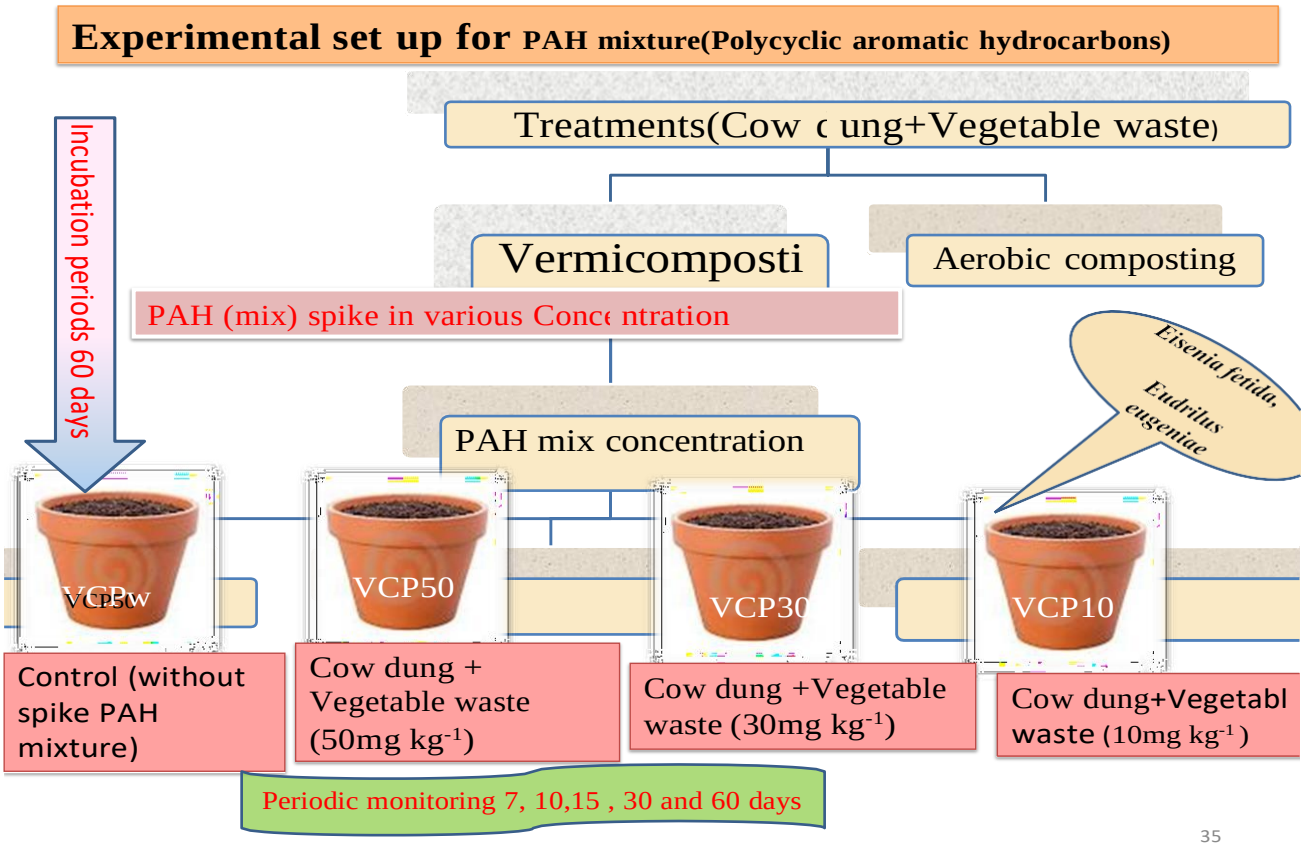
Materials and methods

Experimental setup and PAH spiking

The composting and vermicomposting were conducted in standard earthen reactors of 3 kg capacity, as detailed in our published article [24]. The reactors were kept on the concrete floor of the composting yard of the Department of Environmental Science, Tezpur University, Tezpur, Assam, India. Urine-free cow dung (CD) and vegetable waste (VW)were homogenized at a 1:1 ratio and used as substrates for composting and vermicomposting. However, the concentrations of the 13 target PAH compounds were below the detection limit of the HPLC (Waters Corp., USA) in the prepared feedstocks for composting and vermicomposting. Well-grown adult specimens of the two earthworm species (*Eisenia fetida* and *Eudrilus eugeniae*), each weighing about 2-3 g, were carefully sorted from the vermiculture unit of the department and introduced in the vermireactors in equal proportion (1:1) at 10 worms per kg (fresh weight) of the feedstock (i.e., 15 specimens of each species, constituting 30 specimens, in total 3 kg of feedstock). Subsequently, three different concentrations of the 13 PAH compounds were spiked in such a manner that the concentrations of each PAH were uniform for the designated composting and vermicomposting reactors. The sets of PAH-freecomposting and vermicomposting substrates were also maintained as controls. The final treatment combinations are detailed below.

Vermicomposting		Aerobic Composting	
VCPw –	Without PAH spiking	CPw –	Without PAH spiking
VCP50 –	PAH-spiked @ 50 mg kg-1	CP50 –	PAH-spiked @ 50 mg kg-1
VCP30 –	PAH-spiked @ 30 mg kg-1	CP30 –	PAH-spiked @ 30 mg kg-1
VCP10 –	PAH-spiked @ 10 mg kg-1	CP10 –	PAH-spiked @ 10 mg kg-1

The experiment was performed with three replicates of each treatment, and samples were drawn at 0, 7, 10, 15, 30, and 60 days respectively from each feedstock for various analyses.



Chemical and microbial analyses

The changes in pH [25], total nitrogen (TN) [26], total organic C (TOC) [27], available phosphorous (Av. P) [28], and available potassium (Av. K) in the compost and vermicompost samples were assessed at 0, 30, and 60 days following standard chemical procedures.

The changes in microbial attributes in the feedstocks were analyzed at 0 and 60 days, respectively. The microbial biomass C (MBC) and compost respiration in the freshly collected samples were measured following standard procedures [29,30]. In addition, the colony-forming units (CFU) of bacterial, actinomycetes, and fungi populations were enumerated in the compost and vermicompost samples after Parmer and Schimdt(1964).

pH

The methodology for pH determination of sample has been described in the previous chapter 4 (see section *4.3.1*)

Total Kjeldahl N (TKN)

The methodology for Total Kjeldahl N (TKN) determination of sample has been described in the previous chapter 4(see section *4.3.3*)

Total organic C (TOC)

The methodology for Total organic C (TOC) determination of sample has been described in the previous chapter 4(see section *4.3.2*)

Available P

The methodology for Available P determination of sample has been described in the previous chapter 4(see section *4.3.4*)

Available K

The methodology for Available K determination of sample has been described in the previous chapter 5(see section *5.4.6*)

Microbial biomass C (MBC)

The methodology for Microbial biomass C (MBC) determination of sample has been described in the previous chapter 4(see section *4.5.2*)

Microbial growth

The methodology for *Microbial growth* determination of sample has been described in the previous chapter 4(see section *4.5.1*)

Working standards, calibration,extraction, and estimation of PAHs in compost, vermicompost, and earthworms

The detection of PAHs in different compost and vermicompost samples was performed in HPLC (Waters Corp., USA) using an ultraviolet (UV) detector at 230 nm over a runtime of 30 minutes through a C-18 column (Symmetry, Waters Corp., USA) with a flow rate of 1 mL min-1 and acetonitrile in the mobile phase. The LC grade mix liquid standards of the 13 PAHs (EPA 525 PAH MixB) and other organic solvents (n-hexane and acetonitrile) were procured from Merck. A 500-ppm stock standard solution of the PAHs was prepared in acetonitrile. Subsequently, six different concentrations of the 13 PAHs were prepared through step-wise dilution with acetonitrile. The six liquid-phase working standards were then used to derive the calibration curves for each compound in HPLC (Waters).

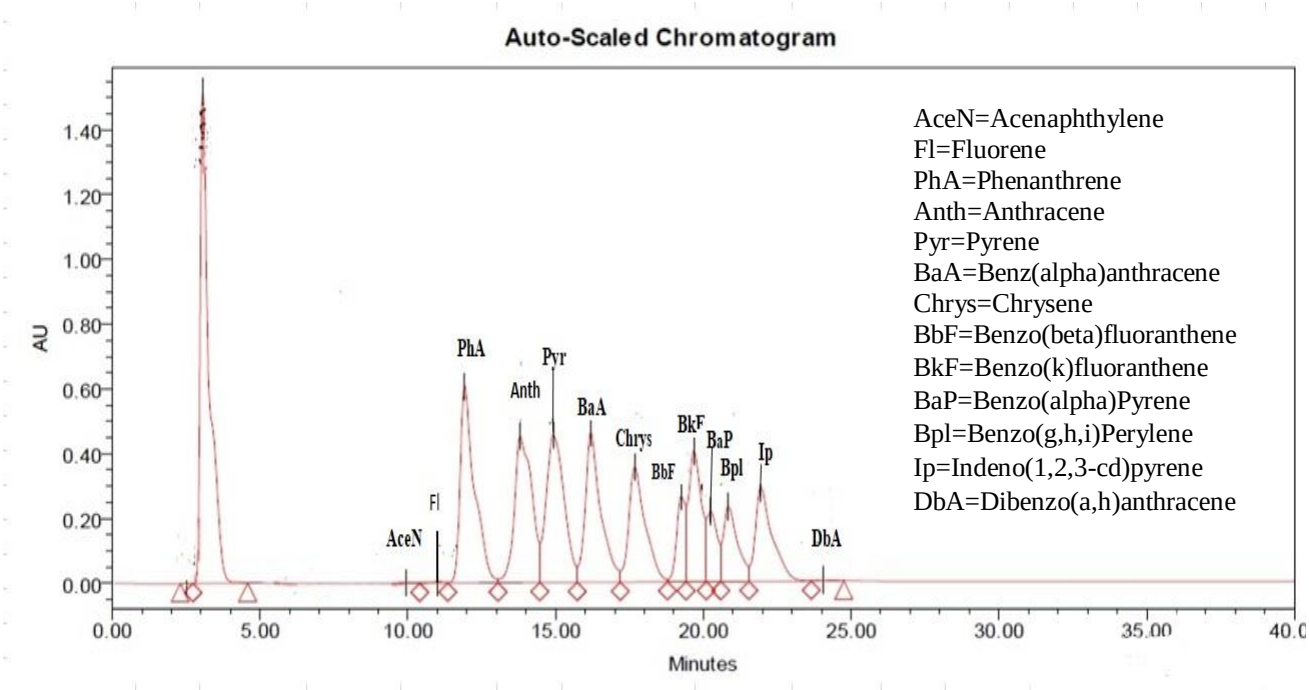


Fig 6.4: HPLC Chromatogram of 13 PAH mixture(Polycyclic aromatic hydrocarbons)

The QA-QC attributes like method detection limit (MDL) and relative standard errors (RSE %) were computed by taking seven replicates of the lowest working standard (~0.025 µg mL-1). The absolute mass (µg) of each compound in 1 mL of the standard was used to calculate the MDL values. The details of all 13 compounds, along with their MDLs and RSEs, are presented in table 1.

Table 6.20: Basic chemical details, abbreviated names, and QA-QC information of the studied PAHs

Target compound name Full chemical name	Abbreviated names	No. of fused rings	Mol. Wt. (g mol-1)	Density (g mL-1)	Molecular formula	CAS number	MDL* (µg g-1)	RSE (Relative standard error)
Acenaphthylene	AceN	3	152.19	0.899	C12H8	208-96-8	0.0063	0.43
Fluorene	Fl	3	166.223	1.2	C13H10	86-73-7	0.0114	10.16
Phenanthrene	PhA	3	178.23	1.18	C14H10	85-01-8	0.0960	1.60
Anthracene	Anth	3	178.234	1.28	C14H10	120-12-7	0.0047	0.81
Pyrene	Pyr	4	202.256	1.271	C16H10	129-00-0	0.0042	2.36
Benz(alpha)anthracene	BaA	4	228.294	1.19	C18H12	56-55-3	0.0054	8.53
Chrysene	Chrys	4	228.294	1.274	C18H12	218-01-9	0.0053	12.75
Benzo(beta)fluoranthene	BbF	5	252.316	1.286	C20H12	205-99-2	0.0127	6.03
Benzo(k)fluoranthene	BkF	5	252.316	1.286	C20H12	207-08-9	0.0088	5.07
Benzo(alpha)pyrene	BaP	5	252.316	1.24	C20H12	50-32-8	0.0042	16.52
Benzo(g,h,i) perylene	Bpl	6	276.3307	1.378	C22H12	191-24-2	0.0076	0.01
Indeno (1,2,3-cd) pyrene	Ip	6	276.3	1.18	C22H12	193-39-5	0.0118	0.37
Dibenzo(a,h)anthracene	DbA	6	278.3466	1.232	C22H14	53-70-3	0.0036	40.43

*MDL – Mean detection limi

The PAH extraction from compost and vermicompost samples was conducted following the USEPA method (EPA 3550C). In short, a 10 g sample was collected from the composting and vermicomposting beds at different time points (0 d, 7 d, 10 d, 15 d, 30 d, and 60 d) and extracted in a hexane /acetone (4:3) solvent system and then sonicated for 3 minutes. This procedure was repeated thrice, and the accumulated volume of the three extractions was centrifuged (4000×g for 5 minutes), concentrated, and then exchanged to acetonitrile in a rotary evaporator (Eyela, Japan) to 5 ml and stored at 4oC till they were analyzed in HPLC. On the other hand, a previously standardized method reported by Contreras-Ramos et al. (2009)[33] was followed for extracting the PAHs accumulated in two earthworm species (*Eisenia fetida* and *Eudrilus eugeniae*). Three actively growingmature earthworm specimenswere collected from each vermibed after 30 d of incubation. Then, the earthworm specimens were cleaned with double distilled water and kept in a moist filter paper overnight for gut evacuation. Eventually, the earthworms were freeze-killed in liquid N2,ground in a mortar pestle, and reconstituted in plastic tubes with a sodium sulfate[34]. The fats were removed by sonication with KOH and hexane 1:1 (v/v) solutions for 10 minutes at 45 oC. Then the PAHs were separated from the worm body tissues by centrifuging for 15 minutes at 13700×g, followed by freezing with dry ice, and the supernatants were collected. The decanted supernatants were mixed with 5 ml hexane, and the process of sonication followed by centrifugation was repeated three times. Afterward, the whole extract was cleaned through a deactivated alumina column (10%). The cleaned sections were re-activated with hexane, eluted in acetone, filtered using a 0.45-micron syringe filter, and used for HPLC analysis.

PAH removal efficiency and budget equations

The removal and budget equations were computed considering the changes in PAH levels after 30 days of incubation. The magnitude of variations in all 13 PAH compounds was most prominent within this period. The removal efficiency (RE) of the composting and vermicomposting systems for the 13 PAHs was calculated with the formula no. 1, given below:

$$Removal\ efficiency\ (RE)\% = \frac{PAH\ concentration\ \ at\ 0\ d - PAH\ concentration\ \ at\ 30\ d}{PAH\ concentration\ \ at\ 0\ d} \times 100 \ldots\ldots(1)$$

Formulano. 1 was evolved by modifying the formula for computing metal removal efficiency of composting systems reported by Sahariah et al. (2015)[36].

The formula (no. 2 to 8) for the apportionment budget of the 13 PAHs, as furnished below,wasevolvedbased on the principles of grade and separation potential for the pollutants during the composting processes [37,38]:

$$Red_m = M_{if} - M_{ff}$$

……(2)

Here, Redm – Mass (in mg) PAH reduction after vermicomposting and composting

Mif – Initial mass (in mg) in the feedstock (i.e., at 0 d)

Mff – Final mass (in mg) in the feedstock (i.e., at 30 d)

The concentrations of different PAHs at a given time point in mg kg-1were multiplied by the weight of feedstock to derive the mass of the compounds.

$$MEf_{acc}\ (mg) = \frac{Ef_{con.}}{1000} \times W_{avb} \times W_n$$

……..(3)

Here, MEfacc – Mass of PAH accumulated by *Eisenia fetida* in mg

Efcon. – HPLC-derived concentrations of PAHs in µg g-1

Wavb – Average biomass of earthworms (*E. fetida*)

Wn - Number of earthworms at 30 d in feedstock

$$MEu_{acc}\ (mg) = \frac{Eu_{con.}}{1000} \times W_{avb} \times W_n$$

…….....(4)

Here, MEuacc – Mass of PAH accumulated by *Eudrilus eugeniae* in mg, and other notations follow the same norm used for *Eisenia fetida*.

$$TME_{acc} = MEf_{acc} + MEu_{acc}$$

……..…(5)

Here, TMEacc – Gross total of PAHs (mg) accumulated by the earthworms at 30 d

$$the\ TME_{acc}\ \% = \frac{TME_{acc}}{Red_m} \times 100$$

……..(6)

$$M_{imb} = Red_m - TME_{acc}$$

……(7)

Here, Mimb – Mass of PAH immobilized in feedstock biomass in mg

$$M_{mb}\ \% = \frac{M_{imb}}{Red_m} \times 100$$

…….(

Statistical analyses

The temporal dynamics of various parameters, including the 13 PAH compounds in composting and vermicomposting samples, were evaluated by two-way ANOVA with three observations per cell in SPSS software. The time and treatment were considered two influencing factors for all the studied attributes. Then, the least significant difference (LSD) posthoc test was performed to compare the significance of variations among treatments because the p values for all the attributes were less than 0.05. Moreover, Pearson's correlation statistics were performed to assess the impacts of various chemical and microbial attributes on the bioavailability of the 13 PAH compounds during the biocomposting processes.

Results and discussion

Changes in PAH concentration and removal efficiency (RE)

The data on changes in the concentrations of 13 PAHs and composting and removal efficiency of the system are presented in Fig. 6.1a-6.1f and tables 6.1-6.13.

Table 6.1: Temporal variation in the concentration (mg kg-1) of 3-ring Acenaphthylene (AceN) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	31.39±11.5	24.81±8.03	10.95±2.3	4.03±0.4	0.0001±0.00001
VCP30	33.00±3.4	15.05±0.5	4.64±0.6	0.10±0.01	0.0003±0.0002
VCP10	23.96±2.4	12.02±1.2	1.22±0.1	0.0004±0.00002	0.0002±0.0001
CP50	38.05±11.1	27.81±8.5	15.03±3.8	10.05±1.14	2.01±0.2

CP30	22.80±8.3	16.04±1.6	5.6±0.1	0.20±0.02	0.669±0.1
CP10	9.48±2.7	9.08±1.4	9.09±0.9	0.117±0.02	0.03±0.002
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	1.39				

Table 6.2: Temporal variation in the concentration (mg kg-1) of 3-ring Fluorene (Fl) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	39.76±6.3	21.10±4.1	10.51±2.8	11.37±0.6	0.02±0.002
VCP30	21.0±4.04	12.99±2.1	6.53±0.7	0.02±0.007	0.004±0.005
VCP10	5.37±1.6	1.41±0.15	0.12±0.013	0.0001±0.00006	0.00002±0.000001
CP50	39.76±6.3	27.98±4.8	15.90±3.8	12.56±0.7	0.12±0.012
CP30	27.32±5.0	25.90±3.8	13.30±1.3	0.13±0.007	0.001±0.002
CP10	6.45±1.7	2.45±0.3	0.23±0.024	0.01±0.001	0.0002±0.00001
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	1.59				

Table 6.3: Temporal variation in the concentration (mg kg-1) of 3-ring Phenanthrene (Phn) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	34.65±12.7	24.55±5.6	5.62±2.6	3.98±0.7	0.10±0.01
VCP30	20.97±5.1	10.32±12.5	6.72±1.04	0.04±0.004	0.0002±0.0001
VCP10	8.43±0.9	2.51±0.3	0.67±0.04	0.0027±0.0001	0.0001±0.0001
CP50	38.88±13.9	26.49±6.9	10.17±2.9	7.22±0.8	0.33±0.02
CP30	24.81±5.7	14.81±2.5	5.39±1.6	0.24±0.03	0.0002±0.0002

CP10	8.09±0.9	5.68±0.3	1.23±0.07	0.003±0.0003	0.0003±0.0004
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	1.33				

Table 6.4: Temporal variation in the concentration (mg kg-1) of 3-ring Anthracene (Anth) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	36.20±11.6	30.56±6.2	5.97±3.6	10.03±1.3	0.02±0.002
VCP30	27.33±8.7	12.84±6.7	2.37±0.7	5.67±0.6	3±0.3
VCP10	6.89±2.4	1.21±1.2	0.99±0.4	0.13±0.1	0.03±0.06
CP50	40.56±11.4	27.78±8.2	10.06±4.1	14.88±1.2	6.98±0.7
CP30	27.24±9.2	19.11±6.3	5.59±1.6	5.95±0.6	4.98±0.5
CP10	9.79±2.4	5.45±2.7	1.12±1.1	0.99±0.2	0.09±0.01
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	1.04				

Table 6.5:Temporal variation in the concentration (mg kg-1) of 4-ring Pyrene (Pyr) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	38.85±4.9	21.95±2.2	11.96±1.7	5.07±0.5	0.05±0.005
VCP30	26.88±4.1	11.98±1.2	3.01±0.4	0.02±0.002	0.00012±0.00002
VCP10	9.94±2.2	8.20±0.8	0.05±0.003	0.003±0.0002	0.003±0.006
CP50	38.33±3.7	28.06±0.9	12.96±1.9	11.84±5.5	1.50±0.2
CP30	24.88±4.4	17.92±4.00	9.39±1.5	0.27±0.03	0.0007±0.001
CP10	9.06±2.3	7.92±0.9	0.35±0.13	0.11±0.01	0.10±0.01
p treatment	<0.001				

p day	<0.001
p t*d	<0.001
LSD for treatment	0.75

Table 6.6: Temporal variation in the concentration (mg kg-1) of 4-ring Chrysene (Chrys) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	42.30±6.6	17.31±2.2	10.07±7.70	0.17±0.02	0.01±0.001
VCP30	22.10±2.9	12.08±1.1	5.99±0.6	0.50±0.03	0.001±0.002
VCP10	8.24±1.8	5.20±1.05	4.02±0.4	0.53±0.03	0.0004±0.00049
CP50	40.17±7.1	20.28±3.2	16.26±1.7	1.73±0.2	0.15±0.015
CP30	21.72±3.3	13.62±1.4	10.08±1.1	0.94±0.2	0.02±0.002
CP10	8.06±1.9	5.93±2.7	2.67±0.3	0.73±0.04	0.02±0.002
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	0.61				

Table6.7:Temporal variation in the concentration (mg kg-1) of 4-ring Benz(alpha)anthracene (BaA) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	41.88±14.8	37.40±3.9	17.79±1.8	5.82±0.8	0.11±0.01
VCP30	23.77±2.5	18.27±1.9	10.22±1.05	0.07±0.12	0.003±0.002
VCP10	8.66±1.4	4.51±0.4	1.12±0.09	0.0002±0.0001	0.0001±0.00002
CP50	41.90±14.5	30.87±7.2	25.92±5.7	8.13±1.0	0.36±0.04
CP30	23.85±2.4	21.11±2.4	13.52±3.2	0.53±0.05	0.0002±0.00002
CP10	9.97±2.2	6.22±1.07	3.91±0.9	1.12±0.12	0.0005±0.00004
p treatment	<0.001				
p day	<0.001				

p t*d	<0.001
LSD for treatment	1.42

Table 6.8: Temporal variation in the concentration (mg kg-1) of 5-ring Benzo(alpha)pyrene (BaP) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	35.23±7.8	21.25±3.3	10.35±1.1	0.41±0.04	0.09±0.01
VCP30	27.00±5.7	12.64±2.1	9.12±1.1	0.11±0.01	0.00013±0.0001
VCP10	9.06±1.9	3.02±0.9	0.0016±0.0009	0.0002±0.0002	0.0001±0.0001
CP50	32.78±7.8	32.19±5.2	25.53±2.4	4.78±0.51	0.63±0.06
CP30	28.80±5.3	20.46±2.4	12.05±1.1	0.38±0.04	0.02±0.001
CP10	9.10±2.1	3.97±1.3	0.01±0.0005	0.004±0.00037	0.001±0.00012
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	1.03				

Table 6.9: Temporal variation in the concentration (mg kg-1) of 5-ring Benzo(k)fluoranthene (BKF) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	35.88±9.2	20.88±6.4	11.93±2.9	11.7±1.2	0.38±0.038
VCP30	21.67±6.0	18.13±2.2	3.02±1.7	0.03±0.003	0.0001±0.00006
VCP10	6.93±2.8	2.96±1.9	0.01±0.001	0.00004±0.000004	0.0001±0.00006
CP50	33.89±9.7	26.28±7.9	15.77±3.05	3.98±2.5	0.74±0.08
CP30	22.03±6.3	13.86±4.5	6.00±2.7	0.58±0.0083	0.003±0.0022
CP10	6.94±2.5	3.95±2.1	0.31±0.02	0.16±0.016	0.0001±0.00001
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	0.85				

Table 10: Temporal variation in the concentration (mg kg-1) of 5-ring Benzo(beta)fluoranthene (BbF) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	37.96±7.7	23.68±3.5	8.48±0.9	1.70±0.2	0.17±0.1
VCP30	24.40±6.5	13.34±1.2	1.68±0.6	0.11±0.01	0.04±0.03
VCP10	9.70±5.5	8.00±0.8	2.99±0.3	0.001±0.0001	0.0001±0.000003
CP50	37.96±7.8	23.68±3.6	16.53±1.6	2.34±0.3	0.26±0.03
CP30	27.50±4.9	20.68±2.1	4.39±0.4	0.33±0.03	0.05±0.0051
CP10	9.11±1.2	8.69±0.9	4.99±0.5	0.004±0.0004	0.001±0.0001
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	0.65				

Table 11: Temporal variation in the concentration (mg kg-1) of 6-ring Benzo(g,h,i) perylene (Bpl) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	37.77±7.8	20.95±2.09	9.98±1.6	5.51±0.6	4.03±0.4
VCP30	23.94±3.4	10.51±0.6	1.87±0.2	0.07±0.006	0.0002±0.00006
VCP10	7.38±2.04	3.04±0.6	0.10±0.006	0.0001±0.00006	0.0001±0.00006
CP50	37.80±8.08	23.93±3.5	11.48±2.2	7.56±1.7	8.06±0.8
CP30	25.93±3.7	12.54±2.1	5.58±0.6	0.51±0.051	0.02±0.001
CP10	6.94±2.6	4.03±1.05	0.87±0.051	0.005±0.0001	0.0056±0.0003
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	0.49				

Table 12: Temporal variation in the concentration (mg kg-1) of 6-ringIndeno (1,2,3-cd) pyrene (Ip) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	35.49±6.6	21.42±3.2	11.48±1.2	0.75±0.1	0.01±0.001
VCP30	22.56±6.7	17.33±1.7	2.08±0.2	0.07±0.007	0.0033±0.003
VCP10	4.8±0.3	0.55±0.4	0.04±0.034	0.002±0.0002	0.0002±0.00006
CP50	35.14±6.5	19.63±1.1	14.50±1.5	2.29±0.2	0.17±0.016
CP30	24.81±3.7	20.25±36.3	2.14±0.7	1.04±0.6	0.34±0.3
CP10	7.8±0.3	3.55±0.4	0.54±0.04	0.002±0.0019	0.003±0.0027
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	0.93				

Table 13: Temporal variation in the concentration (mg kg-1) of 6-ring Dibenzo(a,h)anthracene (DbA) under various treatments. Values are presented as mean ± standard deviation

Treatment	7 Day	10 Days	15 Days	30 Days	60 Days
VCP50	32.59±7.3	19.87±2.6	9.76±1.05	0.14±0.01	0.0001±0.00001
VCP30	19.56±1.7	5.07±0.4	0.26±0.02	0.18±0.01	0.0001±0.00006
VCP10	8.15±0.9	0.32±0.03	0.001±0.0001	0.0001±0.00001	0.0001±0.00006
CP50	32.50±9.0	22.04±3.4	17.72±2.3	8.11±0.9	0.01±0.001
CP30	26.61±2.7	10.32±1.04	5.26±0.5	1.17±0.2	0.01±0.0005
CP10	8.05±1.1	3.57±0.8	0.10±0.011	0.005±0.001	0.0310±0.002
p treatment	<0.001				
p day	<0.001				
p t*d	<0.001				
LSD for treatment	0.76				

Table 6.14: Removal efficiency (%) of the 13 PAHs as observed under the different feedstocks of both composting and vermicomposting pathways

Treatments	**Bpl (6-ring)**	**Ip (6-ring)**	**DbA (6-ring)**	**BaP (5-ring)**	**BkF (5-ring)**	**BbF (5-ring)**	**Pyr (4-ring)**	**Chrys (4-ring)**	**BaA (4-ring)**	**Phn (3-ring)**	**AceN (3-ring)**	**Anth (3-ring)**	**Fl (3-ring)**
CP50	84.88±7.82	95.42±6.54	83.78±7.33	90.44±6.20	92.04±5.12	95.32±5.32	76.32±3.21	96.54±6.23	83.74±7.22	85.56±5.45	79.90±3.98	70.24±5.04	74.88±4.36
CP30	98.3±8.11	96.53±6.33	96.10±6.43	98.73±7.04	98.07±5.03	98.90±7.31	99.10±4.21	96.87±5.81	98.23±5.22	99.20±7.22	99.33±7.34	80.17±6.13	99.57±5.74
CP10	99.95±6.55	99.98±6.87	99.95±7.01	99.96±5.15	98.40±5.97	99.96±6.51	98.90±4.54	92.70±7.21	88.80±4.05	99.97±5.05	98.83±5.75	90.10±8.21	99.90±8.05
VCP50	88.98±5.22	98.50±5.45	99.72±6.02	99.18±4.88	76.60±5.04	96.60±6.07	89.86±4.63	99.66±4.32	88.36±6.31	80.10±6.15	91.94±7.13	79.94±3.28	77.26±3.76
VCP30	99.76±6.21	99.77±7.12	99.40±5.22	99.63±5.22	99.90±4.77	99.63±6.22	99.93±4.82	98.33±6.51	99.77±5.01	99.87±5.86	99.67±6.73	81.10±4.33	99.93±5.46
VCP10	100.00±6.32	99.98±5.33	100.00±6.03	100.00±7.09	100.00±6.22	99.99±6.11	99.97±4.73	94.70±4.78	100.00±6.21	99.97±6.43	100.00±6.32	98.70±4.78	100.00±5.45
p (Tr)	<0.01												
p (Comp)*	<0.001												
p (Tr×Comp)	<0.05												
LSD (Tr)	1.895												

All 13 PAHs sharply reduced within seven days of spiking in composting and vermicomposting reactors, while the extent of reduction was remarkably greater under vermicomposting compared to composting. The PAH compounds degrade rapidly under aerobic conditions[39]. Therefore, the degradation of all 13 PAH compounds was highly rapid under vermicomposting and composting, as both processes are aerobic in nature [35]. The low molecular weight (3 ring structure) PAHs [phenanthrene (Phn), anthracene (Anth), acenaphthylene (AceN), and fluorene (Fl)] reduced by 20-40% to 2-12% within a week under vermicomposting and composting respectively **(Fig. 6.1a and table6.1-6.4).** In contrast, the high molecular weight PAHs (4-6 ring structure) [e.g., benzo(a)anthracene (BaA), pyrene (Pyr), benzo(b)fluoranthene (BbF), chrysene (Chrys), and others] decreased by about 15-52% and 7-15% under vermicomposting and composting respectively within seven days **(Fig. 6.1b-6.1d and tables 6.5-6.13).** At 60 d, levels of the 4-6 ring PAHs were less than 1 mg kg-1 in all the feedstocks, except for Anth; the occurrence of other 3-ring (AceN, Phn, and Fl) compounds was also negligible in the end **(Fig. 6.1a)**. Such rapid reduction of PAHs under aerobic conditions in the composting systems, including vermicomposting, has been reported in earlier studies [11,40].

However, the efficiency of both the systems regarding removal of different PAHs was significantly greater in low dose spiked reactors (VCP10 and CP10) as compared to high dose spiked feedstocks (VCP50 and CP50) **(Fig. 6.1a-6.1d)**; which implies that degradation efficiency of the earthworms and microorganisms was largely concentration-dependent. This could be better appreciated from the removal efficiency (RE) data **(Fig. 6.1e & 6.1f; table 6.14**). The RE ranged between 93.05 – 99.57% for vermicomposting and 88.72 – 97.87% for composting for the 4-6 ring PAHs after 30-day incubation. For example, the RE for benzo(a)pyrene (BaP), a 5-ring compound, was 99 – 100% under vermicomposting at 30 days **(Fig. 6.1f& table 6.14);** while the RE in the 30 and 10 mg kg-1 spiked vermibeds was equally efficient for dibenzo(a,h)anthracene (DaA) **(Fig. 6.1f& table 6.14)**, Pyrene (Pyr) **(Fig. 6.1e& table 6.14)**, benzo(k)fluoranthene (BkF) (**Fig. 6.1f; table 6.14)**, benzo(g,h,i)perylene (Bpl) **(Fig. 6.1f& table 6.14)**, and BaA **(Fig. 6.1e & table 6.14)** (pfor treatment<0.01; LSD = 1.89). The prolific PAH removal potential of vermicomposting system was mainly due to the presence of earthworms. Although high concentration (VCP50) exposure resulted in slight retardation of PAH removal efficacy of vermicomposting, the RE in VCP50was significantly greater than in

composting (CP50). Such initial concentration-dependent removal under composting and vermicomposting have been reported in earlier studies [11,14,40]. The REs of the vermicomposting system (82.31 – 99.67%) were comparatively greater than composting (77.65 – 97.20%) for the 3-ring PAHs (Phn, Anth, AceN, and Fl) at the 30 d **(Fig. 6.1e& table 6.14)**. For example, the RE for AceN and Phn was 92 – 100% under vermicomposting systems (pfor treatment<0.01; LSD = 1.89). On the other hand, RE for AceN and Phnin composting reactors ranged between 79.9 – 99.3% and 85.6 – 99.9%, respectively. Nevertheless, the magnitude of removal potency of both the systems was lower for the 3-ring (i.e., low molecular weight) as compared to that of the 4-6 ring PAHs (i.e., high molecular weight) ($p<0.001$). These results suggest that the PAH removal efficiency in earthworm-modifiedsystemsvaries considerably depending on the PAHs' molecular mass; Esmaeili et al. (2022)[22] have also recently reported a similar observation. The sequestration of PAHs in organic substrates increases with the greater number of fused aromatic rings vis-à-vis a greater hydrophobicity [41]. The biomass decomposition process differentiates vermicomposting from composting regarding the formation of humic substances that efficiently work as a pollutant-capturing matrix [38]. Due to their lipophilic nature, the PAHs exhibit a high affinity toward binding to such matrices, and the sequestration intensifies with the increase in thenumber of aromatic rings [13,42]. Therefore, the availability of PAHs is limited for microbe-mediated degradation under such conditions [8]. This could be appreciated from the results of PAH budget equations in the following section.

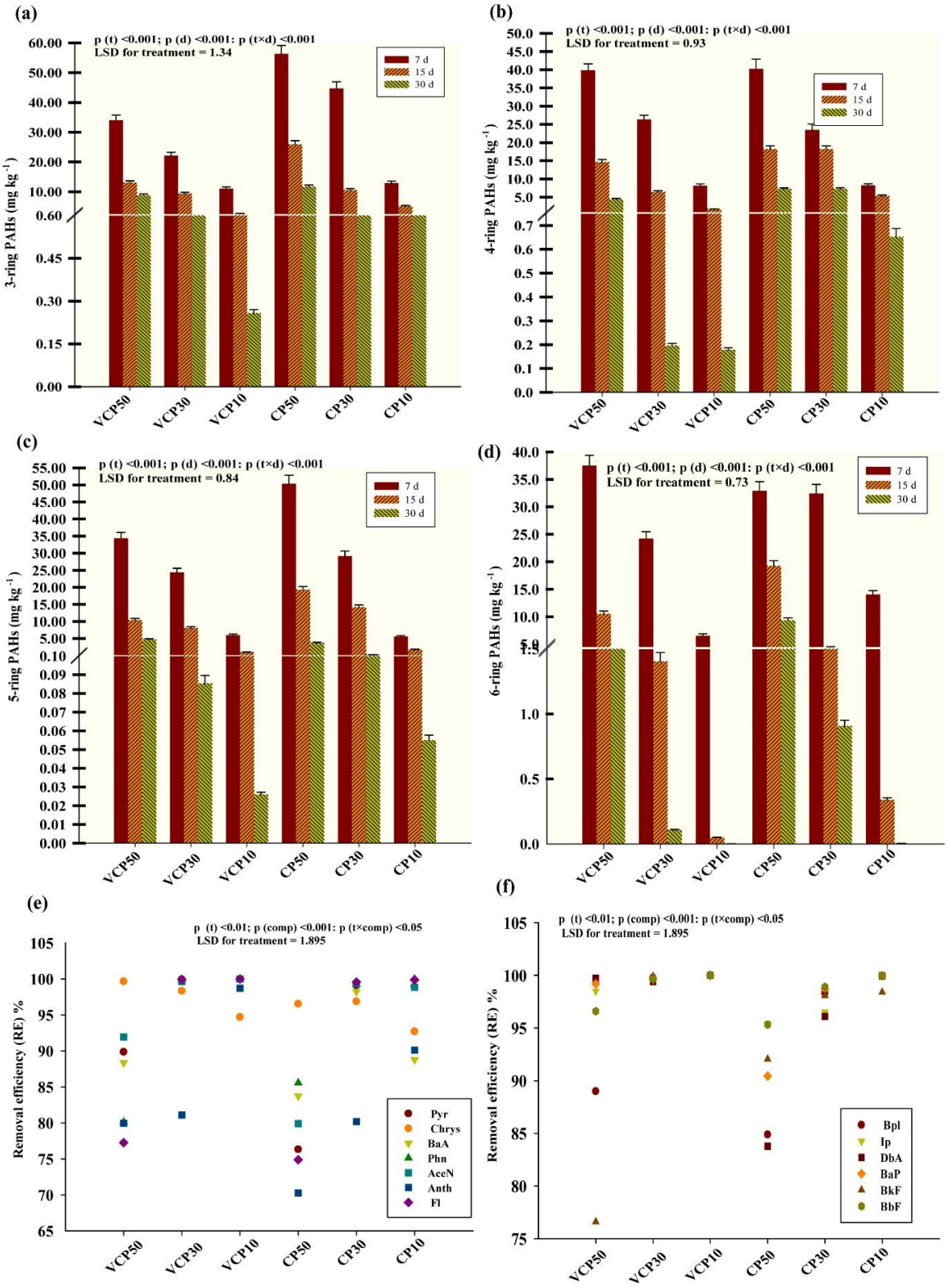


Fig. 6.1: Changes in the concentration of PAHs during composting and vermicomposting and their corresponding removal efficiency at 30 days after incubation. (a), (b), (c), and (d)representconcentration of 3, 4, 5, and 6 ring PAH compounds, respectively; (e)depicts the removal efficiency for 3 & 4 ring PAHs; and (f)depicts the removal efficiency for 5 & 6 ring PAHs. Values are presented as mean ± standard deviation.

Earthworm fecundity and PAH accumulation pattern in two earthworm species

The data on earthworm count, body weight, and PAH-bioaccumulation are presented in **Fig. 6.2a-6.2d and table 6.15**. Overall, the results indicate that the population growth of both species was initially affected due to PAH exposure **(Fig. 6.2a & 6.2b)**. There was a sharp decrease in the count of *E. eugenia*e (13 – 23%) after 30 d depending on PAH exposure level **(Fig 6.2a)**. This indicates that the earthworms fled from the reactors due to PAH exposure. However, the 60d data suggests that the earthworm population noticeably revived its growth after 30 days **(Fig. 6.2a)**. In the end, the *E. fetida* population was highest in VCP30, followed by VCP50, while the counts of *E.eugeniae* were significantly high in VCP10 and VCP30 (pfor treatment<0.001; LSD = 0.07). The body weights of both species substantially increased over time in PAH-spiked reactors and were more significant in such reactors as compared to the control pots (VCPwEf and VCPwEu) **(Fig. 6.2b)**. For example, the specimens of *E. fetida* in VCP50 at 30d and 60d were heaviest, followed by VCP30 and VCP10 (pfor treatment<0.001; LSD = 0.62). On the other hand, the body weights of E. eugeniae in all the PAH spiked vermireactors were significantly greater than those of the untreated ones after 60 days (VCPwEu) **(Fig. 6.2b)**. Exposure to toxic conditions enhances body weight, thereby inducing bioaccumulation and detoxification activities in the earthworms [19,43].

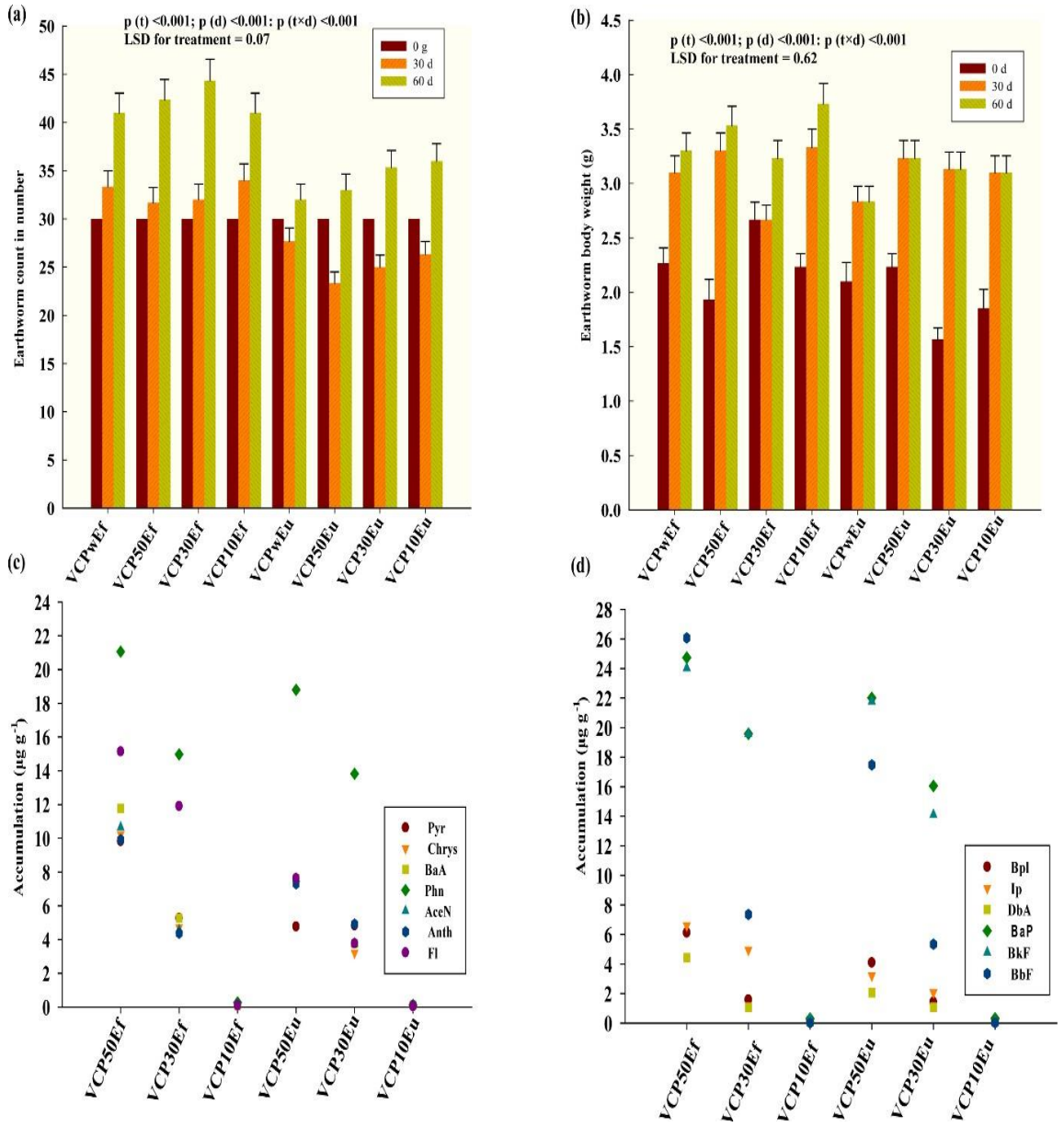


Fig. 6.2 : Health, proliferation, and PAH-bioaccumulation potential of *Eisenia fetida* and *Eudrilus eugeniae*: (a) earthworm count; (b) body weight; (c)bioaccumulation pattern of 3 & 4 ring PAHs; and (d)bioaccumulation pattern of 5 & 6 ring PAHs. Values are presented as mean ± standard deviation

Concordantly, a significant accumulation of the 13 PAHs depending on the exposure levels by the earthworm was observed in the present experiment (**Fig. 6.2c &6.2d; table6.15)**. In general, accumulation potential by *E. fetida* for all 13 PAHs was significantly greater than *E. eugeniae*. However, the preference of accumulation in both the earthworms was in the order: 5-ring PAHs (BaP, BkF, and BbF) **(Fig. 6.2d; table 6.15)** > 3-ring PAHs (Phn, AceN, Anth, and Fl) = 4-ring PAHs (BaA, Chrys, and Pyr) **(Fig. 6.2c; table 6.15)** > 6-ring PAHs (Ip, Bpl, and Dba) **(Fig. 6.2d; table 6.15)**. In

VCP50 feedstock, the preference for accumulation of *E. fetida*was highest for BbF, followed by BaP, BkF, and Phn. In contrast,*E. eugeniae*preferred the PAHs for collection in the order: BaP≥BkF>Phn>BbF in VCP50, while both the species preferred BaP the most in VCP 30 **(Fig. 6.2c&6.2d; table 6.15)**. Therefore, a clear trend of compound-specific preferential accumulation of potential in earthworms was evident in the present study. These results strongly indicate that the initial shock of PAH exposure greatly arrested the population growth; however, the earthworms probably revived their development through their unique defense mechanism. The body weight gain upon PAH exposure was a strong indication of an efficient defense mechanism of the earthworms. Although results are in good agreement with previous findings[32], some studies have found 25 – 70% weight loss in PAH exposed earthworms [44,45]. Factors like ambient conditions, feedstock compatibility, and nutrient contents can influence earthworm defense mechanisms at cellular levels [19]. Almost nothing is known about the PAH accumulation potential of *E. eugeniae*. Therefore, this research is probably the first report on a comparative assessment of the PAH accumulation ability of *E. eugeniae* with that of *E. fetida.*

Table 6.15: Accumulation pattern of the 13 PAHs by *E. fetida* and *E. eugeniae* in the spiked feedstocks

	Phn µg g-1		**AceN µg g-1**		**Anth µg g-1**		**Fl µg g-1**	
	EW*Ef*	**EW*Eu***	**EW*Ef***	**EW*Eu***	**EW*Ef***	**EW*Eu***	**EW*Ef***	**EW*Eu***
VCP50	21.06±0.03	18.80±3.3	10.65±0.01	7.62±0.01	9.92±1.0	7.30±3.4	15.14±1.6	7.62±0.01
VCP30	14.97±0.8	13.82±0.1	4.57±0.04	3.76±0.7	4.37±0.5	4.92±1.0	11.90±1.6	3.76±0.7
VCP10	0.28±0.05	0.11±0.1	0.12±0.1	0.11±0.1	0.13±0.04	0.13±0.05	0.08±0.1	0.03±0.002
p value	<0.001		<0.001		<0.001		<0.001	
p value (treatment)	<0.001		<0.001		<0.001		<0.001	
LSD (Treatment)	1.127		1.897		1.23		0.343	

	Pyr µg g-1		**Chrys µg g-1**		**BaA µg g-1**	
	EW*Ef*	**EW*Eu***	**EW*Ef***	**EW*Eu***	**EW*Ef***	**EW*Eu***
VCP50	9.82±1.01	4.77±1.3	10.22±0.01	7.46±0.2	11.78±1.2	7.62±0.01
VCP30	5.27±1.01	4.83±1.6	4.72±0.83	3.21±0.1	5.28±1.0	3.76±0.7
VCP10	0.13±0.11	0.11±0.1	0.13±0.08	0.08±0.1	0.08±0.1	0.11±0.1
p value	<0.001		<0.001		<0.001	
p value (treatment)	<0.001		<0.001		<0.001	
LSD (Treatment)	41.897		0.289		1.128	

	BaP µg g-1		**BkF µg g-1**		**BbF µg g-1**	
	EW*Ef*	**EW*Eu***	**EW*Ef***	**EW*Eu***	**EW*Ef***	**EW*Eu***
VCP50	24.73±3.7	22.01±2.9	24.03±3.7	21.77±9.4	26.07±0.03	17.48±1.8
VCP30	19.58±3.7	16.05±2.3	19.58±3.7	14.10±5.7	7.36±3.68	5.34±4.9
VCP10	0.31±0.14	.32±2.9	0.31±0.14	0.12±0.1	0.02±0.01	0.02±0.01
p value	<0.001		<0.001		<0.001	
p value (treatment)	<0.001		<0.001		<0.001	

LSD (Treatment)	2.112		4.101		2.769	
	Bpl µg g-1		**Ip µg g-1**		**DbA µg g-1**	
	EW*Ef*	**EW*Eu***	**EW*Ef***	**EW*Eu***	**EW*Ef***	**EW*Eu***
VCP50	6.12±0.01	4.09±0.9	6.61±1.7	3.21±0.2	4.43±0.3	2.07±0.9
VCP30	1.58±0.3	1.42±0.3	4.96±1.0	2.08±0.6	1.07±0.5	1.07±0.2
VCP10	0.16±0.2	0.11±0.1	0.03±0.01	0.21±0.2	0.11±0.1	0.07±0.1
p value	<0.001		<0.001		<0.001	
p value (treatment)	<0.001		<0.001		<0.001	
LSD (Treatment)	1.725		0.329		0.353	

Temporal budget of 13 PAHs: Insights on reduction mechanism

The primary data on changes in 13 PAHs in the substrate under composting and vermicomposting suggested that all the compounds sharply reduced over time. Therefore, budget equations were evolved to appreciate the removal kinetics and the underlying reduction mechanism in PAH bioavailability during composting process **(tables 6.16-6.18).** The budget equation for mass reduction (Redm) revealed that the initial concentration of the PAHs and the duration of exposure played a significant role in PAH remediation because according to the two-way ANOVA treatment × day interaction effect was effective for all the 13 PAHs (p for day& p for treatment × day<0.001). Overall, the mass reduction (Redm) of 3-ring PAHs (Phn, AceN, Anth, and Fl) was lower as compared to the HMW (4-6-ring) PAHs. Moreover, the Redm values strongly indicated that the efficacy of vermitechnology in reducing PAH bioavailability for some of the 6-ring and 5-ring PAHs [indeno(1,2,3-cd)pyrene (Ip), dibenzo(a,h)anthracene (DbA), BaP, benzo(beta)fluoranthene (BbF), BkF, and Chrys] were significantly higher than composting (p for treatment <0.01) **(table 6.16)**. The accumulation of the PAHs in earthworm biomass (TMEacc) probably played a complementary role in the PAH removal process, along with the immobilization of the PAHs in humified feedstocks during vermicomposting. In contrast, immobilization/desorption in partially decomposed feedstocks is expected to be the primary mechanism of PAH detoxification in composting [10,11]. Furthermore, the Redm values indicated that the higher the initial exposure concentration of the PAHs, the greater the mass removal. For instance, Redm values at 30d were significantly greater in VCP50 followed by VCP30 as compared to VCP10, CP50, and CP10 (p<0.01). Such trends agreed with a recent report on PAH degradation in composting systems [40].

The budget equations signify that the accumulation of PAHs (TMEacc) by earthworms on a mass basis significantly varied depending on the type of compounds and their initial exposure concentrations in the substrates **(tables 6.16-6.18).** For instance, the TMEacc values ranged between 0.4 – 4.3% and 0.2 – 3.1% in VCP50 and VCP30 feedstocks, respectively, while the values were negligible for the VCP10 feedstocks. Moreover, the mass accumulation ability of *E. fetida* (MEfacc) was significantly more significant as compared to *E. eugeniae* (MEeuacc) for all the PAHs in VCP50 and VCP30 feedstocks (p<0.05; LSD = 0.23) (Tables 2-4). Various studies have reported on the PAH bioaccumulation potential [21,46,47]. However, such potential has rarely been studied for *E. eugeniae*. In the previous section, the

PAH bioaccumulation potential of both the species (*E. fetida* and *E. eugeniae*) has been discussed.

However, the mass accumulation for some compounds like BaP (TMEacc = 4.25 mg), BbF (TMEacc = 4.04 mg), Phn(TMEacc = 3.6 mg), and BkF (TMEacc = 4.2 mg) was considerably high; and TMEaccvalues for some compounds (IP = 1.56 mg, Fl = 2.2 mg, AceN = 1.7 mg, Anth = 1.59 mg, and BaA = 1.33 mg) was fairly pronounced; while the values were < 1 mg for DbA and Bpl in VCP50. These data imply that the accumulation potential of the earthworms was not only dependent on initial concentrations of the PAHs but also highly preferential regarding compound types. Roratet al.(2017) also observed compound-specific preferential accumulation in *E. fetida*. Correspondingly, the Mimb% values were more than 99% for all the 13 compounds in VCP10, where earthworms' accumulation on a mass basis was negligible. While the Mimb% values for DbA and Bpl were greater than 99% in all the vermireactors **(table 6.2)**. Contrastingly, for compounds like BkF **(table 6.16)** and Phn **(table 6.4)**, the Mimb% ranged between 96.53 to 97.42 in VCP50 and VCP30. As such, the Mimb and Mimb% values revealed that the overall contribution of the PAH bioaccumulation ability of earthworms was inferior to that of the immobilization vis-à-vis desorption of the PAHs in humified organic matter during vermicomposting. This may be due to substantial retardation in earthworm fecundity. Eventually, the budget equation exhibits that immobilization by the humified feedstocks was the major pathway of PAH detoxification under vermicomposting. However, the true benefit of earthworm incorporation in the feedstocks lies elsewhere. The occurrence of earthworms in vermireactors enhances the detoxification process in various ways. Earthworms ingest the toxicants with their food and passive adsorption via their skin [32]. In addition, the burrowing and feeding habits of earthworms may facilitate the increase in PAHs adsorption sites in the humified substances through accelerated humification. A similar mechanism has recently been postulated for *E. eugeniae*regarding toxic element removal during vermicomposting [38].

Moreover, the occurrence of earthworms in the vermireactors also augments microbial activity. They act as a bridge between microbes and the PAHs, facilitating the contact between the two fields[16,48]. The results of the present experiment have demonstrated that the microbial population significantly increased due to the presence of earthworms.

Table 6.16: Budget and detailed apportionment of the 6-ring and 5-ring PAHs under composting and vermicomposting systems. Values are represented as mean ± standard deviation

	Ip (6-ring)								**Bpl (6-ring)**							
Treatments	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30d (mg)	TMEacc %	Mimb 30 d (mg)	Mimb %	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc 30d(mg)	TMEacc %	Mimb 30 d (mg)	Mimb %
	15 d	30 d							15 d	30 d						
CP50	109.40±7.5	144.50±8.3							117.86±9.2	131.86±10.5						
CP30	84.01±4.6	87.50±5.2							74.38±4.4	88.78±2.7						
CP10	28.49±2.1	30.00±1.9							27.56±1.1	29.99±1.3						
VCP50	117.86±9.9	148.20±9.5	1.32±0.06	0.24±0.013	1.56±0.09	1.05±0.05	146.64±8.4	98.95±3.1	122.06±6.6	136.78±7.4	0.64±0.03	0.31±0.02	0.95±0.04	0.69±0.04	135.83±3.7	99.31±1.09
VCP30	84.18±5.8	89.83±4.7	1.12±0.10	0.16±0.011	1.28±0.10	1.43±0.07	88.55±3.9	98.57±3.8	84.76±4.8	89.83±5.1	0.14±0.01	0.11±0.01	0.25±0.01	0.27±0.01	89.58±2.5	99.72±4.5
VCP10	29.89±2.1	30.00±2.4	0.003±0.0002	0.02±0.001	0.02±0.001	0.068±0.003	29.97±1.6	99.93±3.3	29.72±1.1	30.00±1.2	0.018±0.001	0.01±0.001	0.03±0.01	0.09±0.005	29.97±1.1	99.91±2.8
p (Tr)	<0.01		<0.05		<0.05	<0.01	<0.01	NS	<0.01		<0.05		<0.05	<0.01	<0.01	NS
p (D)/(E)	<0.01								<0.01							
p (T×D)/T×E	<0.01								<0.01							
LSD (Tr)	6.51		0.63		0.38	0.16	16.33		6.18		0.12		0.36	0.11	20.33	

	DbA (6-ring)							
Treatments	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30d(mg)	TMEacc %	Mimb 30 d (mg)	Mimb %
	15 d	30 d						
CP50	100.38±3.6	130.54±8.1						
CP30	75.27±1.2	87.19±3.5						
CP10	29.72±1.5	29.99±2.2						
VCP50	122.67±2.7	149.66±3.8	0.46±0.03	0.16±0.01	0.62±4.1	0.41±0.03	149.04±1.05	99.59±4.5
VCP30	89.27±2.9	89.57±3.1	0.09±0.004	0.09±0.004	0.08±0.002	0.18±0.01	89.40±2.9	99.80±3.2
VCP10	30.00±1.4	30.00±1.6	0.01±0.001	0.01±0.001	0.006±0.0003	0.02±0.001	29.98±1.4	99.94±2.6
p (Tr)	<0.01		<0.05		<0.05	<0.01	<0.01	NS
p (D)/(E)	<0.01							
p (T×D)/T×E	<0.01							
LSD (Tr)	6.18		0.21		0.35	0.2	22.11	

Continue…

	BaP (5-ring)								**BkF (5-ring)**							
Treatments	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30d(mg)	TMEacc %	Mimb 30 d (mg)	Mimb %	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc 30d (mg)	TMEacc %	Mimb 30 d (mg)	Mimb %
	15 d	30 d							15 d	30 d						

CP50	78.52±3.1	138.53±9.2							116.60±4.3	121.92±2.6						
CP30	56.26±4.2	89.09±4.5							81.54±2.9	89.93±3.1						
CP10	29.98±2.1	29.99±1.8							29.97±2.4	30.00±1.7						
VCP50	121.02±5.1	149.02±9.9	2.59±0.11	1.66±0.11	4.25±0.31	1.09±0.06	147.96±9.2	98.91±6.4	105.84±1.8	140.45±3.5	2.59±0.11	1.64±0.11	4.22±0.26	3.46±0.19	117.69±9.5	96.53±5.6
VCP30	64.46±1.9	89.74±3.9	1.70±0.09	1.26±0.09	2.94±0.16	0.75±0.02	88.14±5.5	99.26±3.9	73.20±3.9	88.61±2.8	1.70±0.08	1.10±0.08	2.79±0.17	3.11±0.25	87.13±3.8	96.89±5.5
VCP10	30.00±2.2	30.00±2.4	2.20±0.12	2.61±0.17	4.81±0.22	0.08±0.004	28.71±1.1	99.93±5.2	29.13±2.1	29.62±1.5	0.03±0.002	0.010±0.001	0.044±0.002	0.15±0.01	29.95±1.1	99.85±6.2
p (Tr)	<0.01		<0.05		<0.05	<0.01	<0.01	NS	<0.01		<0.05		<0.05	<0.01	<0.01	NS
p (D)/(E)	<0.01								<0.01							
p (T×D)/T×E	<0.01								<0.01							
LSD (Tr)	6.51		0.23		0.42	0.19	17.93		6.18		0.21		0.35	0.2	7.33	

Continue

	BbF (5-ring)							
Treatments	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30d(mg)	TMEacc %	Mimb 30 d (mg)	Mimb %
	15 d	30 d						
CP50	103.72±5.4	144.38±9.1						
CP30	77.71±2.9	89.21±4.6						
CP10	16.03	29.99						

	±1.3	±1.9						
VCP50	126.26±1.2	145.92±6.1	2.73±0.21	1.31±0.09	4.04±0.12	2.77±0.12	141.88±9.2	97.23±2.3
VCP30	85.30±1.6	89.74±4.7	0.63±0.32	0.42±0.31	1.05±0.09	1.17±0.07	88.68±3.9	98.82±2.8
VCP10	21.63±1.9	30.00±1.3	0.002±0.001	0.002±0.0001	0.004±0.0003	0.013±0.001	29.99±1.5	99.99±3.1
p (Tr)	<0.01		<0.05		<0.05	<0.01	<0.01	NS
p (D)/(E)*	<0.01							
p (T×D)/T×E*	<0.01							
LSD (Tr)	6.51		0.22		0.45	0.33	13.43	

**T = Treatment; *D/E = Day or treatment as applicable; Ip = Indeno (1,2,3-cd) pyrene; Bpl= Benzo (g,h,i) peylene; Dba= Bibenzo (a,h) anthracene; BaP=Benzo (alpha) pyrene; BkF= Benzo (k) fluoranthene; BbF= Benzo (beta) fluoranthene*

Table 6.17: Budget and detailed apportionment of the 4-ring PAHs under composting and vermicomposting systems.Values are represented as mean ± standard deviation

	Pyr (4-ring)								**Chrys (4-ring)**							
Treatments	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30d(mg)	TMEacc %	Mimb 30 d (mg)	Mimb %	Redm(mg)	MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30 d(mg)	TMEacc %	Mimb 30 d (mg)	Mimb %	
	15 d	30 d							15 d	30 d						
CP50	113.71±8.5	121.58±10.5							104.47±2.7	145.85±9.8						
CP30	63.71±4.1	89.35±3.3							61.77±2.2	87.74±2.3						
CP10	29.02±1.9	29.74±1.6							22.52±2.9	28.25±1.1						
VCP50	116.51±5.4	137.83±9.2	1.03±0.09	0.36±0.02	1.39±0.06	1.01±0.06	136.44±9.9	98.99±4.3	121.80±8.5	149.60±6.5	1.07±0.06	0.56±0.02	1.63±0.07	1.09±0.04	147.96±8.2	98.91±5.9
VCP30	81.57±3.1	89.95±4.3	0.45±0.02	0.38±0.01	0.93±0.08	0.93±0.07	89.19±4.1	99.07±2.1	73.23±2.1	88.80±2.1	0.41±0.02	0.25±0.01	0.66±0.02	0.74±0.02	88.14±3.6	99.26±2.7
VCP10	29.86±1.5	29.99±1.2	0.01±0.001	0.009±0.0004	0.023±0.001	0.08±0.005	29.97±1.5	99.92±6.9	18.74±1.1	28.73±1.6	0.014±0.001	0.006±0.0002	0.021±0.0001	0.073±0.004	28.71±1.4	99.93±5.3
p (Tr)	<0.01		<0.05		<0.05	<0.01	<0.01	NS	<0.01		<0.05		<0.05	<0.01	<0.01	NS
p (D)/(E)*	<0.01								<0.01							
p (T×D)/T×E*	<0.01								<0.01							
LSD (Tr)	6.51		0.11		0.55	0.17	18.33		6.18		0.21		0.35	0.2	17.03	

Continue…

BaA (4-ring)

Treatments	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30d(mg)	TMEacc %	Mimb 30 d (mg)	Mimb %
	15 d	30 d						
CP50	77.42±1.7	130.49±8.3						
CP30	52.14±2.5	88.73±4.1						
CP10	19.05±1.7	27.31±1.6						
VCP50	100.19±5.4	136.03±9.9	1.23±0.10	0.57±0.04	1.80±0.12	1.33±0.06	134.23±2.9	98.67±5.1
VCP30	61.38±2.8	89.83±3.05	0.46±0.02	0.30±0.02	0.75±0.04	0.83±0.05	89.08±2.1	99.16±4.4
VCP10	26.86±1.3	30.00±1.8	0.009±0.0003	0.009±0.0002	0.02±0.001	0.06±0.003	29.98±2.6	99.94±2.8
p (Tr)	<0.01		<0.05		<0.05	<0.01	<0.01	NS
p (D)/(E)*	<0.01							
p (T×D)/T×E*	<0.01							
LSD (Tr)	6.51		0.31		0.53	0.18	14.66	

Continue

**T = Treatment; *D/E = Day or treatment as applicable;Pyr= Pyrene; Chrys= Chrysene; BaA= Benz (alpha) anthracene*

Continue…

Table 6.18: Budget and detailed apportionment of the 3-ring PAHs under composting and vermicomposting systems.Values are represented as mean ± standard deviation

	Phn (3-ring)								**AceN (3-ring)**							
Treatments	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30d(mg)	TMEacc %	Mimb 30 d (mg)	Mimb %	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30 d (mg)	TMEacc %	Mimb 30 d (mg)	Mimb %
	15 d	30 d							15 d	30 d						
CP50	121.52±4.5	132.67±7.1							107.97±4.3	125.88±3.3						
CP30	74.91±3.3	89.42±6.9							74.32±2.1	89.52±3.1						
CP10	26.56±1.9	30.00±2.1							4.55±4.05	29.72±3.9						
VCP50	134.26±8.1	140.45±6.3	2.20±0.08	1.41±0.05	3.62±0.23	2.58±0.14	136.83±6.4	97.42±6.2	119.34±2.7	140.33±2.5	1.11±0.09	0.57±0.02	0.1	1.20±0.06	138.64±8.1	98.90±3.6
VCP30	71.18±3.9	89.90±4.7	1.29±0.01	1.08±0.04	2.37±0.16	2.64±0.16	87.53±3.7	97.36±4.8	77.01±3.1	89.76±3.1	0.39±0.02	0.29±0.01	0.69±0.04	0.77±0.03	89.07±2.8	99.23±3.1
VCP10	28.12±1.5	29.99±1.3	0.031±0.001	0.009±0.0005	0.047±0.003	0.13±0.01	29.95±2.4	99.86±5.7	26.58±2.9	30.00±3.7	0.013±0.001	0.009±0.0003	0.02±0.001	0.07±0.0009	29.98±2.05	99.92±2.2
p (Tr)	<0.01		<0.05		<0.05	<0.01	<0.01	NS	<0.01		<0.05		<0.05	<0.01	<0.01	NS
p (D)/(E)*	<0.01								<0.01							
p (T×D)/T×E*	<0.01								<0.01							
LSD (Tr)	6.51		0.21		0.35	0.2	12.33		6.18		0.23		0.65	0.28		
	Anth (3-ring)								**Fl (3-ring)**							
Treatments	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d	TMEacc30d (mg)	TMEacc %	Mimb 30 d (mg)	Mimb %	Redm(mg)		MEfacc30 d (mg)	MEuacc 30 d (mg)	TMEacc30	TMEacc %	Mimb 30 d (mg)	Mimb %

Continue

	(mg)								d(mg)					
	15 d	30 d							15 d	30 d				
CP50	121.83±0.9	114.29±2.1							105.48±1.9	119.86±3.4				
CP30	74.35±3.4	75.72±4.1							52.76±3.4	89.69±5.1				
CP10	26.86±1.5	27.62±2.3							29.36±1.1	29.98±1.6				
VCP50	133.28±4.5	125.93±3.1	1.08±0.06	0.55±0.03	1.59±0.11	1.26±0.05	124.34±4.4	98.74±3.1	120.57±1.7	122.71±3.9	1.58±0.05	0.57±0.01	2.16±0.11	1.76±0.07	120.55±5.1	98.24±3.6
VCP30	83.36±1.3	76.39±2.5	0.38±0.02	0.39±0.01	0.76±0.04	0.99±0.06	75.63±2.6	99.002±6.9	71.72±2.9	89.95±3.6	1.03±0.01	0.29±0.01	1.32±0.06	1.47±0.04	88.63±4.8	98.53±2.4
VCP10	27.23±1.07	29.69±1.1	0.014±0.002	0.011±0.0006	0.025±0.001	0.085±0.003	29.66±4.08	99.91±1.6	29.66±2.1	30.00±2.1	0.009±0.0004	0.002±0.0001	0.011±0.001	0.04±0.003	29.99±4.3	99.96±2.2
p (Tr)	<0.01		<0.05		<0.05	<0.01	<0.01	NS	<0.01		<0.05		<0.05	<0.01	<0.01	NS
p (D)/(E)*	<0.01								<0.01							
p (T×D)/T×E*	<0.01								<0.01							
LSD (Tr)	6.51		0.18		0.34	0.19	6.94		6.18		0.33		0.14	0.17	9.89	

**T=Treatment; *D/E= Day or treatment as applicable; Phn=Phenanthrene; AceN=Acenapthylene; Anth=Anthracene; Fl=Fiuorene*

Temporal dynamics in chemical and microbial attributes: Finding the linkages to PAH remediation through correlation statistics

Fig. 3a-h represents the changes in different chemical and microbial attributes during composting and vermicomposting with PAH-contaminated feedstocks. The data on actinomycetes and fungal counts in various feedstocks are presented in supplementary **fig. 6.1 & 6.2**. The organic matter decomposition dynamics in PAH spiked composting and vermicomposting reactors were evaluated against untreated (i.e., VCPw not spiked with PAHs) feedstocks. The pH and TOC gradually reduced from the initial status in all the feedstocks. However, the extent of the pH **(Fig. 6.3a)** and TOC **(Fig. 6.3b)** reduction was more significant in the vermibeds than in the composting beds. In the end, both pH and TOC were lowest in VCP30, and VCP10, followed by others ($p<0.001$; LSD=0.18). The organic matter decomposition process results in nutrient (C, N, P, etc.) mineralization, producing several aliphatic and aromatic acids during decomposition processes [49]. Therefore, a significant reduction in pH and TOC was a strong indication of efficient organic matter mineralizationin the presence of earthworms [35]. Correspondingly, the total N increment was more prolific under vermicomposting (1.06-1.92 folds) as compared to composting (1.02-1.23 folds) ($p<0.001$) **(Fig. 6.3c)**. Earthworm activity promotes N enrichment in the feedstocks by augmenting microbial growth and polysaccharide contents while deriving energy from biomass [50]. However, the results of the current experiment suggest that the N enrichment ability of the earthworms was somewhat inhibited in PAH spiked feedstocks as the N level was significantly greater in the untreated substrate (VCPw) as compared to VCP50 followed by VCP30 and VCP10 (LSD = 0.03). However, the inhibitory effect of PAH contamination was less spectacular regarding P and K availability in the vermibeds. Both P and K significantly increased over time (pfor day<0.001 and in the end, P availability was highest in VCP50, followed by CP30, VCP30, and the other feedstocks (LSD = 6.43) **(Fig. 6.3d)**. While K availability at 60 d in different substrates was in the order: VCP50=VCP10=VCP30=VCPw> CPW=CP10> CP30> CP50 ($p<0.001$; LSD=2.1; **Fig. 6.3e)**. The presence of earthworms enhances the P availability primarily through stabilizing the substrate pH and improving microbial activity and enzyme secretion. In contrast, K availability is augmented through the release of exogenous and endogenous microbial enzymes contributed by the worm intestinal flora [51]. The data on microbial proliferation in PAH contaminated feedstocks strongly justify the reason behind more

remarkable nutrient (N, P, and K) enrichment under vermicomposting compared to composting.

The MBC increased by 1.21-2.02 folds and 1.22-1.47 under vermicomposting and composting, respectively. In the end (at 60 d), MBC was highest in VCP10, followed by VCP30 and VCP50 (pfor treatment<0.001; LSD = 9.17; **Fig. 6.3f**). The earthworms contribute rich microflora in the vermibeds through the excretion process, thereby greatly enhancingthe microbial biomass [52]. The compost respiration (CR) at 60d was in the order: VCP50 > VCP10 >VCP30 >VCPw>CPw> CP30 >CP10 ($p<0.001$; LSD= 8.83; **Fig. 6.3g**). Interestingly, the variations in CR levels in different feedstocks clearly show the concentration-dependent impact of PAH-induced toxicity on microbial growth. The elevated compost respiration (i.e., basal respiration) in 50 and 30 ppm (mg kg-1) spiked vermibeds (VCP50 and VCP30) indicates that earthworms augmented autochthonous microorganisms in such feedstocks [53]. As such, the growth of autochthonous microbes is one defense mechanism that helps the organisms suppress the toxic impacts of pollutants [54].

Pearson's correlation analysis showed strong correlations between almost all the 13 PAHs and nutrient mobilizing (chemical and microbial) attributes in vermicompost samples **(table 6.19)**. In contrast, correlations among the PAHs and most of the chemical and microbial parameters were weak in the compost **(table 6.19)**. However, the patterns of such relationships were similar in both systems. The TOC in the vermibeds was strongly and positively correlated with all the PAHs except Anth. In contrast, a strong positive correlation was only evidenced between a few compounds and the TOC [BaP ($p<0.05$), BkF ($p<0.05$), and Chrys ($p<0.01$)] under composting. A similar strong and positive correlation was also found among 12 PAHs (except Anth) ($p<0.01$) and pH in the vermibeds **(table 6.19)**. Both the pH and TOC have sharply reduced in the vermibeds. Therefore, the correlation statistics explain that PAH removal could be regulated by the rapidity and efficiency of the organic matter decomposition potential of composting systems. The decomposition mechanism under composting is microbe mediated, while earthworms and microorganisms synergistically conduct the feedstock decomposition in vermicomposting systems [23]. As a result, the balance between humification and mineralization is more effectively maintained in vermicomposting than in composting, which greatly facilitates the pollutant degradation/detoxification in vermicomposting [38,55]. The present study showed a strong negative correlation

between CR and 12 PAHs (except Bpl) **(table 6.19)**. Similarly, MBC was also negatively correlated with BaP ($r = -0.806$; $p<0.05$), BkF ($r = -0.801$; $p<0.05$), Chrys ($r = -0.811$; $p<0.05$), BaA ($r = -0.830$; $p<0.05$), Fl ($r = -0.825$; $p<0.05$), and BbF ($r = -0.878$; $p<0.05$) under vermicomposting. In contrast, the strength of correlations between PAHs and microbial attributes (MBC and CR) was weak under composting. For instance, only Fl and BbF showed significant negative correlations with both the attributes under composting. These results imply that profuse microbial growth in the vermibeds was largely responsible for PAH removal. Earthworms efficiently stimulate the growth of appropriate microbial communities that degrade the PAHs eventually [48]. Due to their burrowing and feeding habits, Earthworms act as a bridge between the PAHs and microorganisms [21].

Table 6.19: Pearson's correlation matrix among the PAHs and biochemical attributes of the vermicomposted and composted feedstock samples

	Vermicomposting					Composting				
PAH compounds	*TOC*	*TN*	*MBC*	*CR*	*pH*	*TOC*	*TN*	*MBC*	*CR*	*pH*
Benzo(alpha)pyrene (BaP)	**0.986****	-0.751	-0.806*	-0.847*	**0.996****	0.949*	-0.714	-0.814	-0.832*	0.803
Benzo(k)fluoranthene (BkF)	**0.971****	-0.705	-0.801*	-0.804*	**0.986****	0.940*	-0.675	-0.814	-0.795	0.830
Chrysene (Chrys)	**0.988****	-0.833*	-0.811*	**-0.909****	**0.986****	**0.990****	-0.851*	-0.791	-0.909	**0.988****
Pyrene (Pyr)	**0.991****	-0.793	-0.766	-0.858*	**0.986****	0.885	-0.616	-0.816*	-0.760	0.914*
Anthracene (Anth)	0.758	-0.796	-0.638	-0.877*	0.747	0.808	-0.635	-0.708	-0.777	0.839
Phenanthrene (Phn)	**0.976****	-0.770	-0.777	-0.857*	**0.988****	0.676	-0.749	-0.753	-0.832*	0.813
Acenaphthylene (AceN)	0.947*	-0.889*	-0.765	**-0.929****	**0.930***	0.726	-0.734	-0.782	-0.854	0.625
Benz(alpha)anthracene (BaA)	0.955*	-0.762	-0.830*	-0.835*	**0.971***	0.740	-0.749	-0.798	-0.800	0.857
Fluorene (Fl)	0.939*	-0.744	-0.825*	-0.862*	**0.952***	0.784	-0.747	-0.813*	-0.856*	**0.990****
Indeno (1,2,3-cd) pyrene (Ip)	0.934*	-0.875*	-0.573	**-0.945***	**0.927***	0.766	-0.738	-0.507	-0.878*	0.786
Benzo(g,h,i) perylene (Bpl)	0.920*	-0.613	-0.780	-0.712	**0.946***	0.631	-0.622	-0.788	-0.719	0.944
Benzo(beta)fluoranthene (BbF)	**0.896****	-0.697	-0.878*	-0.815*	**0.918***	0.691	-0.738	-0.821*	-0.851*	0.953
Dibenzo(a,h)anthracene (DbA)	**0.960****	**-0.895****	-0.764	**-0.933****	**0.945***	0.741	-0.927	-0.698	-0.941	0.921

TOC = Total organic carbon, TN= Total Nitrogen, MBC= Microbial biomass Carbon , CR= Compost respiration

** p<0.01 * p<0.05

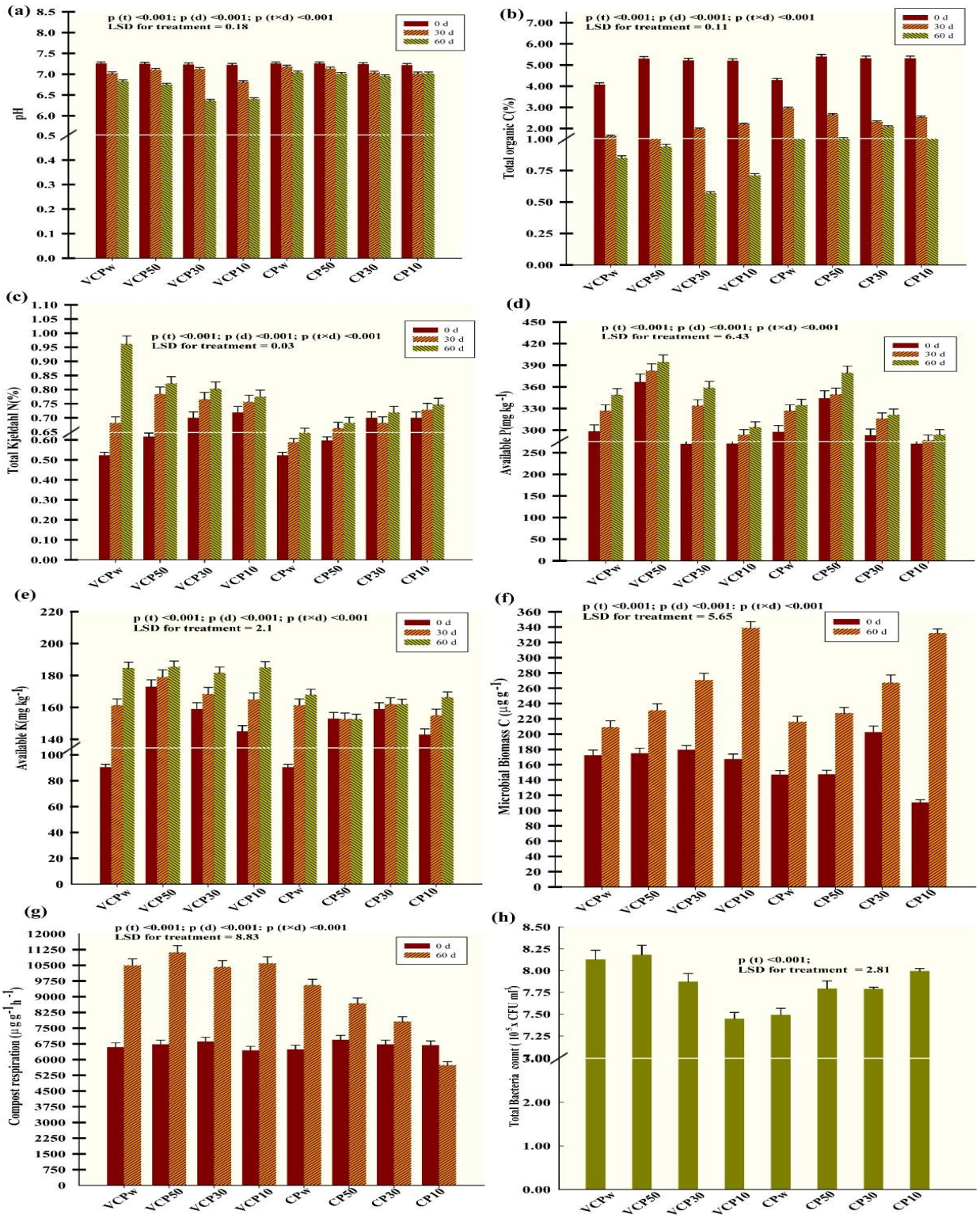


Fig. 6.3: Temporal variations in the physicochemical and microbial attributes of the PAH spiked feedstocks under composting and vermicomposting. (a) pH; (b)total organic C; (c)total N; (d)available P; (e)available K; (f)microbial biomass C; (g)compost respiration; and (h)bacterial count

Conclusions

This investigation exhibits that the PAH accumulation efficacy of *Eudrilus eugeniae* was quite comparable to that of *E. fetida* during vermicomposting of PAH-contaminated biomass. Both species showed a high accumulation preference for BaP, Phn, and BkF. However, the initial concentrations of the PAHs strongly influenced the bioaccumulation capacity of earthworms. Overall, the results indicate that the PAH remediation efficiency of composting and vermicomposting systems wereprimarily regulated by the initial concentrations of the 13 PAHs and their sizes (i.e., number of aromatic rings). The removal efficiency (RE) of vermicomposting was ~100% for the 4-6 ring compounds.On the other hand, the removal of the 3-ring PAHs was dependent on their initial concentrations. According to the mass-basis budget estimation of PAH partitioning during composting and vermicomposting, the bioaccumulation route was not the prime remediation mode. Instead, immobilizing the PAHs in humified organic matter was probably the dominant remediation pathway. The correlation-based assessment vividly clarified that the true benefit of earthworms in vermicomposting systems lies in balancing mineralization-humification kinetics and augmentation of microbial diversity, which in turn probably catalyzes the microbe-mediated detoxification of the PAHs. In-depth future research in this direction might unveil more fascinating PAH remediation mechanisms.

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