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Vermitechnology for sewage sludge recycling

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ABSTRACT

The present paper is aimed at safe reuse and recycling of sewage sludge (SS) and production of good quality compost using vermicomposting. Three different earthworm species *Eisenia fetida (E. fetida)*, *Eudrilus eugeniae (E. eugeniae)*, *Perionyx excavatus (P. excavatus)* in individual and combinations were utilized to compare the suitability of worm species for composting of sewage sludge as well as the quality of the end product. The sewage sludge without blending can be directly converted into good quality fertilizer (vermicompost). Vermicomposting resulted in reduction in C/N ratio 25.6 to 6–9, TOC (25%) but increase in electrical conductivity (EC) (47–51%), total nitrogen (TN) (2.4–2.8 times), potassium (45–71%), calcium (49–62%), sodium (62–82%) and total phosphorous (TP) (1.5–1.8 times), which indicated that sewage sludge can be recycled as a good quality fertilizer. The present study also inferred that the application of sewage sludge in the agricultural fields after vermicomposting would not have any adverse effect as the heavy metals (Cu, Mn, Pb and Zn) are now within the permissible limits.

1. Introduction

Large scale urbanization, a consequence of economic development is leading to production of huge quantities of waste water in India and posing serious environmental problems for their disposal. The treatment and disposal of sludge produced during waste treatment is one of the most critical environmental issues of today. Sludge produced is large in volume and hazardous. Hence, studies related to its safe handling, disposal and recycling techniques are important. Another issue of concern is that the sewage sludge (SS) and effluents are frequently disposed off on agricultural lands as fertilizer and irrigation purpose, respectively, due to their nutrient contents, especially N and P without any treatments, but they may induce plant and soil toxicity and may have depressive effects on the metabolism of soil microorganisms [1]. But it is advisable for them to undergo an additional stabilization treatment [2]. Therefore, there is a need for ecologically sound technologies which are not only cost-effective, but also sustainable in terms of possible recovery of recyclable constituents from sewage sludges as they are rich in nutrients and have higher organic content.

A sustainable approach to handle this will be to convert it to a useful recyclable product at site by an eco-friendly and economical method. Vermicomposting is a decomposition process involving interactions between earthworms and microorganisms and it is an economical, viable and sustainable option for sewage sludge management. It is easy to operate and can be conducted in contained space to produce a good quality product (fertilizer). Earthworms have been successfully used in the vermicomposting of urban, industrial and agricultural wastes in order to produce organic fertilizers and obtain protein for animal feed. Several epigeics (*Eisenia fetida*, *Eisenia andrei*, *Eudrilus eugeniae*, *Perionyx excavatus* and *Perionyx sansibaricus*) have been identified as potential candidates to decompose organic waste materials [3,4]. Research into the potential use of earthworms to break down and manage sewage sludge began in the late 1970s and the use of earthworms in sludge management has been termed vermicomposting or vermistabilization [5]. In its basic form, this is a low-cost technology system that primarily uses earthworms in the processing or treatment of organic wastes [6]. In nature, several different earthworm species may exist in the same acre of soil, each filling a different niche and using different substrates for food. Therefore, it is possible that a combination of worm species (mixed culture) in a vermicomposting process could accomplish greater stabilization than a single species (pure culture). Literatures on vermicomposting using pure cultures are available but sparse literatures are available on mixed cultures. Other authors [7,8] have reported that polyculture reactor can decompose organic matter more efficiently by accelerating its key microbial properties. But some authors [9] have reported that polyculture did not show any advantage over monoculture in the vermicomposting process. Some authors [10] have also documented that *E. eugeniae* and *P. excavatus* do not coexist comfortably in mixed cultures probably due to competition for food among the earthworms species. *E. fetida* is being used widespread in exist-

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ing vermicomposting systems and also proven of its potential for processing of relatively moist organic materials such as municipal biosolids and animal manure slurries [11]. *E. eugeniae* also reported as a fast-growing and productive earthworm in animal waste that is ideally suited as a source of animal feed protein as well as for rapid organic waste conversion [12]. Literature is available reporting that when tried *P. excavatus* gave excellent changes in organic waste resources and could be used efficiently to combat the problem of waste resources management at low-input basis [13].

Therefore, the objective of the present paper is to try out different earthworm combinations (pure culture or mixed culture) for vermicomposting of sewage sludge. In addition, the paper aims to verify whether different earthworm species can coexist in the same environment and also the safe reuse and recycle of sewage sludge producing a good quality end product.

2. Materials and methods

2.1. Earthworm cultures

Three composting species of earthworms two exotic (*E. fetida* and *E. eugeniae*) and one indigenous (*P. excavatus*) were chosen for the experiment. In the present study exotic earthworms *E. fetida* and *E. eugeniae* were cultured in the laboratory and were randomly picked for experimentation. The indigenous species, *P. excavatus* was collected from the drainage area in Indian Institute of Technology Roorkee campus by hand sorting method. The species were identified at National Zoological Survey of India, Solan, India, before culturing in the field laboratory.

2.2. Sewage sludge (SS)

Sewage sludge was procured from sewage treatment plant at Haridwar, India. The sewage sludge was dried in direct sunlight for 2 weeks with periodic turning to bring its moisture content to 50%. The physico-chemical characteristics of SS are given in Table 1.

Table 1
Initial physico-chemical characteristics of SS before composting

S. no.	Parameter	Sewage sludge (SS)
1	pH	6.88 ± 0.1
2	EC (S/m)	0.28 ± 0.08
3	Ash content (%)	42.16 ± 0.5
4	TOC (%)	33.54 ± 0.44
5	TN (%)	1.31 ± 0.1
6	TP (g/kg)	7.97 ± 0.1
7	C/N	25.6 ± 1.5
8	COD (mg/L)	1500 ± 75
9	BOD (mg/L)	580 ± 15
10	Fe (%)	0.63 ± 0.03
11	Cu (mg/kg)	158.2 ± 20
12	Mn (mg/kg)	290.6 ± 30
13	Zn (mg/kg)	612 ± 45
14	Pb (mg/kg)	49.4 ± 6
15	Na (%)	0.5 ± 0.05
16	K (%)	0.86 ± 0.56
17	Ca (%)	5.39 ± 0.68

All data represent average of triplicates.

2.3. Experimental set up

The experiments were conducted in triplicate, in perforated cylindrical plastic containers of capacity 6 L. The temperature in the experimentation room was maintained at 25 ± 1 °C which is the optimum temperature range for all the three species [14,15]. 10 cm bedding was kept in all the containers using old vermicompost. Approximately 50 g (~100–120 in numbers) of earthworms, having both clitellated and juvenile, were inoculated in the bedding for acclimatization of the earthworms to the new environment then SS was added the next day. The mixed cultures were prepared using the earthworm species in equal proportions and one control was also kept for degradation without any worms. The inoculated earthworms were pure cultures as well as mixed cultures of *E. fetida*, *E. eugeniae*, and *P. excavatus* which are shown in Table 2.

1.2 kg of SS was added to each of the reactors. The quantity of the SS was decided based on the data reported in the literature, that the earthworms can consume the material half their body weight per day under favorable conditions [16]. C/N ratio plays an important role in determining the quality of compost hence, saw dust was added as a bulking agent to increase the C/N ratio to 25.6 as earthworm can grow better when C/N ratio of material is about 25 [17]. The moisture level was maintained about 50–60% through out the study period by periodic sprinkling of adequate quantity of tap (potable) water. To prevent moisture loss, the experimental containers were covered with gunny bags. The measurements for total organic carbon (TOC), total nitrogen (TN), ammonical nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorous (TP), exchangeable potassium (K), sodium (Na), calcium (Ca), C/N ratio, electrical conductivity (EC), pH, biological oxygen demand (BOD), chemical oxygen demand (COD) and coliforms were carried out before the introduction of earthworms that is 0 day and on 15th, 30th and 45th day of composting. In addition earthworm growth related parameters like earthworm biomass; and total mortality were measured at the end of the vermicomposting process. These analyses were either carried out on samples immediately after sampling (bacteriological) or within 2 days (the samples were stored at 4 °C until analyzed) for physico-chemical parameters. The values reported are the mean of the triplicates.

2.4. Compost analysis

110 g of homogenized wet samples (free from earthworms, hatchlings and cocoons) were taken out at 0 day and 15 days interval of composting period. The 0 day refers to the substrate taken out before earthworm inoculation. Temperature and moisture content

Table 2
Details of earthworm inoculation in reactors

S. no.	Reactor names	Earthworm combinations (EWs)	Weight of EWs (g)	No. of earthworms
1	R_1	*E. fetida*	50	120
2	R_2	*E. eugeniae*	50	100
3	R_3	*P. excavatus*	50	120
4	R_4	*E. fetida + E. eugeniae*	50	110
5	R_5	*E. fetida + E. eugeniae + P. excavatus*	50	105
6	R_6	*E. eugeniae + P. excavatus*	50	115
7	R_7	*E. fetida + P. excavatus*	50	120
8	R_8	Control	Nil	Nil

All data represent average of triplicates.

Table 3
Variation in pH, EC and ash content during vermicomposting

Reactors	pH			EC (S/m)			Ash content (%)		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
R_1	5.4 ± 0.1ac	6.4 ± 0.1a	6.7 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.06a	0.5 ± 0.11a	46.3 ± 0.67a	47.3 ± 0.7ac	47.9 ± 0.5af
R_2	5.4 ± 0.2ac	6.6 ± 0.1a	6.8 ± 0.1a	0.2 ± 0.06a	0.3 ± 0.07a	0.5 ± 0.09a	47.2 ± 0.78a	47.76 ± 0.83abc	51.3 ± 0.7b
R_3	6.0 ± 0.1b	6.0 ± 0.1b	6.9 ± 0.2ab	0.3 ± 0.05a	0.4 ± 0.06a	0.5 ± 0.12a	49.3 ± 0.5b	49.4 ± 0.65b	56.8 ± 0.6c
R_4	5.7 ± 0.1abd	6.5 ± 0.2a	6.6 ± 0.1a	0.3 ± 0.09a	0.3 ± 0.05a	0.5 ± 0.09a	46.9 ± 0.45a	47.7 ± 0.56abc	49.0 ± 0.46ad
R_5	5.9 ± 0.1bd	6.4 ± 0.2a	6.9 ± 0.2ab	0.3 ± 0.06a	0.3 ± 0.03a	0.4 ± 0.1a	46.7 ± 0.4a	48.7 ± 0.8ab	49.6 ± 0.56bd
R_6	5.7 ± 0.2abd	6.5 ± 0.1a	7.2 ± 0.1b	0.3 ± 0.07a	0.3 ± 0.07a	0.4 ± 0.06a	47.2 ± 0.5a	49.2 ± 0.85b	52.1 ± 0.78e
R_{17}	5.3 ± 0.1c	6.4 ± 0.1a	6.9 ± 0.1ab	0.3 ± 0.08a	0.4 ± 0.1a	0.5 ± 0.09a	45.9 ± 0.4a	47.82 ± 0.45abc	48.3 ± 0.5ad
R_8	5.5 ± 0.1cd	6.7 ± 0.1a	7.2 ± 0.1b	0.3 ± 0.04a	0.3 ± 0.08a	0.4 ± 0.07a	44.1 ± 0.38a	45.8 ± 0.4c	46.1 ± 0.6f

Values followed by the same letter within each column are not significantly different.

was maintained throughout the composting period. The experiments were replicated thrice for each earthworm combinations. 10 g of the wet sample was used for the biological analysis viz. BOD, COD and coliform analysis and the rest was oven dried at 110 °C, ground in stainless steel blender, passed through 0.2 mm sieve and stored in plastic vials for further chemical analysis. Each dried sample was analyzed for the following parameters: ash (550 °C for 2 h) (loss on ignition) and pH (1:10 w/v waste: water extract), total nitrogen using Kjeldahl method, NH_4^+-N and NO_3^--N using KCL extraction [18], TOC determined by Shimadzu (TOC-V_{CSN}) Solid Sample Module (SSM-5000A), total phosphorus (acid digest) using stannous chloride method [19], potassium, calcium and sodium (acid digest) using flame photometer. The presence of bacterial population including total coliforms, fecal streptococci and fecal coliforms were analyzed by multiple fermentation method using Lactose broth. Biodegradable organic matter measured as BOD by the dilution method and COD by the dichromate method (APHA Standard Methods).

2.5. *Statistical analysis*

All results reported are the means of three replicate. The results were statistically analyzed at 0.05 levels using one way analysis of variance (ANOVA) and Tukey HSD test was used as a post hoc analysis to compare the means (SPSS Package, Version 16).

3. Results and discussion

3.1. *pH*

There were only slight changes in the pH value of the sewage sludge as shown in Table 3. Initially pH was observed to decrease for all the reactors in 15 days but later increased to almost neutral after 30 and 45 days, respectively. Maximum pH was observed to be 7.26 ± 0.1 for R_6 while it was 7.24 ± 0.1 for the respective control, R_8. Other researchers have reported decrease in pH during vermicomposting [20,17]. Increase in pH was also reported by some authors during vermicomposting [21]. The lower pH in the final products may be due to CO_2 and organic acids produced during microbial metabolism [16]. pH value varied significantly ($p < 0.05$) for 15th, 30th and 45th days, respectively as per the ANOVA analysis of variance.

3.2. *Electrical conductivity (EC)*

A gradual increase in EC was observed with time in all the reactors (Table 3). The electrical conductivity was increased in the range of 47–51% for pure cultures, 34–51% for mixed cultures and 30% for control respectively. The increase in EC might have been due to the loss of weight of organic matter and release of different mineral salts in available forms (such as phosphate, ammonium, potassium) as reported by other researchers [22]. The variation in EC was non-significant ($p > 0.05$) on all the sampling days as per the ANOVA analysis of variance.

3.3. *Ash content*

The ash content is an important indicative parameter for decomposition and mineralization of the substrate. The content of ash increased with composting time about 12.13%, 17.84%, 25.87% for pure cultures, 14.08%, 15%, 19.14%, 12.71% for mixed cultures and 8.54% for control respectively, owing to the loss of organic matter through microbial degradation (Table 3). Faster rate of increase in ash content indicated the higher rate of volatilization, which is a good measure of degradation of the organic waste. The maximum increase in the ash content was observed in the reactor R_3 (25.87%) with the pure culture which indicated that more decomposition took place during vermicomposting. The increase in the ash content shows that earthworms are consuming the wastes in a faster rate and the microbial assimilation is also performing the decomposition process in a good pace. The variation in ash content on all the sampling days varied significantly ($p < 0.005$).

Table 4
Variation in TOC, TN and NH_4-N during vermicomposting

Reactors	TOC (%)			TN (%)			NH_4^+-N (%)		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
R_1	31 ± 0.3acd	30.5 ± 0.3ac	30.1 ± 0.2a	2.7 ± 0.20a	2.9 ± 0.2a	3.2 ± 0.23acd	0.58 ± 0.02a	0.53 ± 0.01a	0.43 ± 0.01ace
R_2	30.6 ± 0.2a	30.2 ± 0.2a	28.2 ± 0.13a	2.3 ± 0.2ab	2.6 ± 0.18ab	3.7 ± 0.24ad	0.61 ± 0.03a	0.57 ± 0.02ab	0.45 ± 0.01ae
R_3	29.4 ± 0.15b	29.3 ± 0.18b	25 ± 0.15a	2.7 ± 0.2a	2.7 ± 0.18a	3.6 ± 0.25ad	0.61 ± 0.02a	0.60 ± 0.03b	0.47 ± 0.01ce
R_4	30.7 ± 0.2ac	29.5 ± 0.2b	28.7 ± 0.2a	2.4 ± 0.18a	2.6 ± 0.13ab	3.1 ± 0.21acd	0.53 ± 0.01a	0.53 ± 0.01a	0.32 ± 0.01b
R_5	29.7 ± 0.1b	29.1 ± 0.3b	22.5 ± 0.21a	2.4 ± 0.18a	2.5 ± 0.11ab	3.1 ± 0.2ac	0.53 ± 0.02a	0.47 ± 0.01c	0.36 ± 0.01dcb
R_6	30.5 ± 0.2a	29.4 ± 0.3b	27.7 ± 0.15a	2.4 ± 0.17a	2.6 ± 0.12ab	2.9 ± 0.19c	0.58 ± 0.01a	0.57 ± 0.02ab	0.34 ± 0.02ae
R_7	31.3 ± 0.2cd	30.2 ± 0.2a	29.9 ± 0.2a	2.9 ± 0.21a	3.1 ± 0.23a	3.7 ± 0.28d	0.54 ± 0.01a	0.53 ± 0.01a	0.38 ± 0.02ae
R_8	32.4 ± 0.3d	31.0 ± 0.1c	30.6 ± 0.1a	1.8 ± 0.1b	2.1 ± 0.16b	2.2 ± 0.21b	0.57 ± 0.01a	0.53 ± 0.02a	0.51 ± 0.02ce

Values followed by the same letter within each column are not significantly different.

3.4. TOC

A large fraction of TOC was lost as CO_2 as well as due to the consumption of the available carbon as a source of energy by the earthworms and the microorganisms in all the reactors. All the reactors showed a similar pattern of change in TOC, which reduced from the initial value in the range of 10–25% in case of pure cultures, 10–17% in case of mixed cultures and 20% in control after the whole period of decomposition (Table 4). The maximum reduction was observed in R_3 with the pure culture (25.46%). The observed results are supported by those of other authors [23] who have reported 20–45% loss of carbon as CO_2 during vermicomposting of municipal or industrial wastes. TOC varied significantly ($p < 0.05$) on 15th and 30th sampling days but showed non-significant ($p > 0.05$) variation on 45th day of sampling.

3.5. Total nitrogen (TN), ammonical nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N)

The total nitrogen consists of the inorganic forms of nitrogen (ammonium (NH_4^+-N) and nitrate (NO_3^--N)) and organic nitrogen (N_{org}). Total nitrogen as shown in Table 4 was higher in final products than the initial substrates with 2.4–2.8, 2.2–2.9 and 1.7 times increase in the pure cultures, mixed cultures and control, respectively. The maximum increase was observed in R_7 of the mixed cultures followed by a similar increment in the rest of the reactors; however, the control had the minimum increase. The reduction in dry mass (organic carbon in terms of CO_2) due to substrate utilization by microbes and worms and their metabolic activities as well as water loss by evaporation during mineralization of organic matter might have led to relative increase in nitrogen [24]. However, in general the final content of nitrogen in vermicomposting is dependent on initial nitrogen present in the waste and the extent of decomposition. Earthworm activity enriches the nitrogen profile of vermicompost through microbial mediated nitrogen transformation, through addition of mucus and nitrogenous wastes secreted by earthworms. Decrease in pH may be an important factor in nitrogen retention as N_2 is lost as volatile ammonia at high pH values. The difference in TN content in the end products from different reactors was significant ($p < 0.05$) on all the sampling days.

The changes in total N concentration in all the reactors were more or less equal to those of the N_{org}. This increase in the N_{org} in the reactors can be attributed as a consequence of strong degradation of organic carbon compounds [18]. The exchangeable NH_4^+-N (Table 4) in the vermicompost was always greater than the NO_3^--N (Table 5) during the experimentation period. A decrease in NH_4^+-N occurred which corresponded with an increase in NO_3^--N at the end of the vermicomposting process. However, the rapid decrease in NH_4^+-N during composting did not coincide with a rapid increase in NO_3^--N. The difference between various forms of N would be due to immobilization/denitrification or both. Significant variation ($p < 0.05$) was observed in NO_3^--N, organic-N and NH_4^+-N variation on all the sampling days except for 15th day sampling for NH_4^+-N as per the ANOVA analysis of variance.

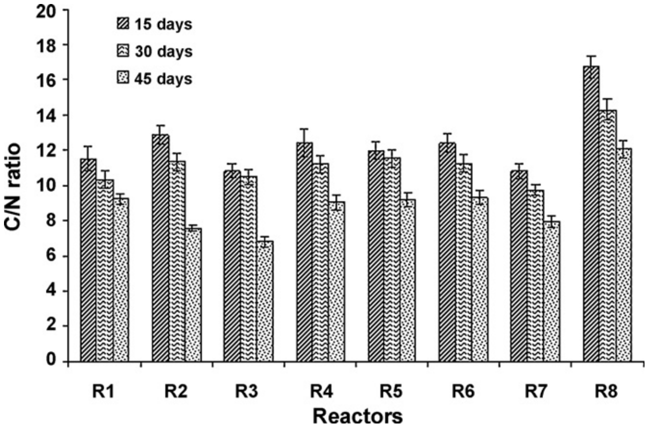

Fig. 1. Variations in C/N ratio during vermicomposting.

3.6. C/N ratio

The C/N ratio is used as an index for maturity of organic wastes as well as a very important parameter because plants cannot assimilate N unless the ratio is in the order of 20 or less [25]. The C/N ratios of the product for the pure cultures were in the range of 6–9 while that of mixed cultures and control were 7–9 and 12, which were less than 20 (Fig. 1). And a decline in C/N ratio to less than 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes [26]. So, in the present study, a high degree of organic matter stabilization was achieved in all the reactors. The decrease in C/N ratio over time might also be attributed to increase in the earthworm population [27], which led to rapid decrease in organic carbon due to enhanced oxidation of the organic matter. The release of part of the carbon as carbon dioxide (CO_2) in the process of respiration, production of mucus and N excrements, increases levels of N and lowers the C/N ratios [28]. A high significance ($p < 0.05$) was observed in C/N ratio for all the reactors on all the sampling days.

3.7. Total phosphorous (TP)

Total phosphorous increased by the end of the vermicomposting process probably because of the mineralization of the organic matter. TP was 1.5–1.8 and 1.2–1.9 times higher in the reactors with pure cultures (R_1, R_2 and R_3) and with mixed cultures (R_4, R_5 and R_6) (Table 5). The reactor R_7 showed no change in the TP value from the initial while R_8 showed a decrease of 0.98 times from the initial. The maximum increase was observed in R_5 with 1.92 times higher

Table 5
Variation in NO_3^--N, K and TP during vermicomposting

Reactors	NO_3^--N (%)			K (%)			TP (g/kg)		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
R_1	0.15 ± 0.01ad	0.23 ± 0.02ae	0.35 ± 0.02a	1.2 ± 0.87a	1.5 ± 0.98a	1.5 ± 0.24ab	10.2 ± 0.2a	14.0 ± 0.2a	14.6 ± 0.2a
R_2	ND	0.12 ± 0.01b	0.27 ± 0.01b	1.2 ± 0.86a	1.5 ± 0.89a	1.6 ± 0.9ab	8.1 ± 0.1b	9.2 ± 0.1bg	12.6 ± 0.2b
R_3	ND	0.05 ± 0.01c	0.48 ± 0.02c	1.2 ± 0.89a	1.3 ± 0.87a	2.9 ± 0.2a	4.8 ± 0.08c	5.6 ± 0.08c	11.9 ± 0.1c
R_4	0.19 ± 0.01b	0.21 ± 0.02ae	0.52 ± 0.02c	1.1 ± 0.76a	1.3 ± 0.77a	1.4 ± 0.27b	7.9 ± 0.1b	9.3 ± 0.1bg	9.4 ± 0.1d
R_5	0.23 ± 0.02c	0.45 ± 0.03d	0.51 ± 0.01c	1.1 ± 0.57a	1.5 ± 0.91a	1.5 ± 0.7ab	8.8 ± 0.15d	9.1 ± 0.09g	15.3 ± 0.2e
R_6	0.17 ± 0.01ab	0.25 ± 0.02a	0.32 ± 0.01a	1.0 ± 0.67a	1.3 ± 0.77a	1.5 ± 0.19ab	7.0 ± 0.1e	9.5 ± 0.1db	9.8 ± 0.07fd
R_7	0.14 ± 0.01d	0.18 ± 0.01e	0.27 ± 0.01d	1.0 ± 0.78a	1.3 ± 0.87a	1.7 ± 0.5ab	5.0 ± 0.08fc	5.1 ± 0.05e	7.9 ± 0.04g
R_8	0.16 ± 0.01ad	0.18 ± 0.02a	0.19 ± 0.01de	0.93 ± 0.6a	0.82 ± 0.67a	0.96 ± 0.67b	6.1 ± 0.1g	6.4 ± 0.06f	6.8 ± 0.05hg

ND, not detected. Values followed by the same letter within each column are not significantly different.

Table 6
Variation in Na, Ca and Fe during vermicomposting

Reactors	Na (%)			Ca (%)			Fe (%)		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
R_1	0.9 ± 0.03aef	0.7 ± 0.03ad	0.9 ± 0.07a	9.6 ± 0.87a	9.8 ± 0.86a	10.7 ± 0.89a	0.67 ± 0.04ab	0.6 ± 0.02ab	0.97 ± 0.06a
R_2	0.8 ± 0.04b	0.9 ± 0.05b	0.9 ± 0.08ab	7.6 ± 0.57b	9.4 ± 0.84ab	10.3 ± 0.98a	0.6 ± 0.02a	0.6 ± 0.02ab	0.98 ± 0.06a
R_3	0.6 ± 0.02acdf	0.7 ± 0.04ad	0.8 ± 0.05ab	8.1 ± 0.6ab	8.6 ± 0.73ab	14.5 ± 1.07b	0.7 ± 0.03ab	0.59 ± 0.02ab	0.95 ± 0.05a
R_4	0.7 ± 0.03ef	0.7 ± 0.03ad	0.7 ± 0.03b	8.4 ± 0.68ab	8.4 ± 0.74ab	9.7 ± 0.78a	0.7 ± 0.04b	0.57 ± 0.02a	0.92 ± 0.04a
R_5	0.5 ± 0.02c	0.8 ± 0.05a	0.9 ± 0.07a	7.9 ± 0.59ab	10.1 ± 0.98a	10.2 ± 0.89a	0.71 ± 0.03b	0.62 ± 0.02ab	0.1 ± 0.01ab
R_6	0.6 ± 0.03ef	0.7 ± 0.03d	0.8 ± 0.04ab	7.6 ± 0.58b	8.5 ± 0.86ab	10.0 ± 0.91a	0.66 ± 0.02ab	0.62 ± 0.01ab	0.92 ± 0.06ab
R_7	0.6 ± 0.02aef	0.7 ± 0.04ad	0.9 ± 0.06a	7.3 ± 0.49b	9.0 ± 0.89ab	9.4 ± .85a	0.7 ± 0.03b	0.64 ± 0.03b	0.96 ± 0.07b
R_8	0.57 ± 0.01dc	0.46 ± 0.01c	0.5 ± 0.02c	7.6 ± 0.57b	7.7 ± 0.67b	8.0 ± 0.65a	0.63 ± 0.02a	0.58 ± 0.01a	0.98 ± 0.07a

Values followed by the same letter within each column are not significantly different.

than the initial value. Increase in TP during vermicomposting is probably through mineralization and mobilization of phosphorus by bacterial and faecal phosphatase activity of earthworms [29]. An increase of 25% in TP of paper waste sludge, after worm activity was found by some authors [30]. Increase in TP was attributed to direct action of worm gut enzymes and indirectly by stimulation of the micro flora. The difference in TP content on all the sampling days obtained from different reactors was significant ($p < 0.05$).

3.8. Macro-nutrients (potassium, sodium, calcium, and iron)

Potassium was observed to be increasing in all the reactors by 45–71%, 38.57%, 38.57% in pure cultures, mixed cultures and the control, respectively (Table 5). A similar increase in potassium was reported by some researchers. Acid production by the microorganisms is the major mechanism for solubilizing of insoluble potassium. The enhanced number of micro flora present in the gut of earthworms in the case of vermicomposting might have played an important role in this process and increased potassium over the control [23]. A decrease in potassium and non-significant increase in calcium have been reported in the vermicomposting process where excess water was used that drained through mass [31]. They have attributed this decrease to leaching of the soluble elements by excess water that drained through mass. It has been reported by some researchers that the leachate collected during vermicomposting process had higher potassium concentrations [32].

Sodium increased by up to 62–88% and 50–88% for the pure cultures and mixed cultures but, there was no change in the sodium concentration for the control. The maximum increase was observed in R_1, R_5 and R_7. Reduction in sodium concentration helps in the reduction of SAR (sodium adsorption ratio—a measure of soil sodicity hazard) [20]. There was increase in calcium concentration for all the reactors. The increments are in the range 49–62%, 43–47% and 37% for the pure cultures, mixed cultures and control, respectively (Table 6). The highest increment was observed in the reactor with pure culture R_3. The increase in Fe (Table 6) concentration was observed as 33–35%, 31–37% and 35% in the reactors with the pure cultures, mixed reactors and the control, respectively, of which the maximum increase was observed in R_5. Significant variation was observed on all the sampling days for Na, Ca and Fe however, K showed a non-significant variation on 15th, 30th sampling day but later on showed a significant variation on 45th day sample.

3.9. Heavy metals (Mn, Zn Pb, Cd and Cu)

Heavy metals in small amounts may be essential for plant growth; however, in higher concentrations they are likely to have detrimental effects upon plant growth. So, prior to vermicompost application to the soils, there is a need to determine the heavy metal concentrations in the final vermicomposts. In the experiments heavy metal concentrations (Mn, Zn, Pb and Cu) were slightly lower than in the initial feed mixtures (Table 7). The Cu concentration reduced in all the reactors except for a 2% increase in R_6. The decrease in the metal concentration may be due to the accumulation of metals in the earthworm body as there is no leaching of the cations by extra water drainage. The total metal content of final compost in all the reactors was very low and is considered as soil fertilizer with good quality according to the standards to ensure safe application of compost laid down in Municipal Waste (Management & Handling rules) notified by the Ministry of Environment and Forest, Government of India [33]. Pb showed significant variation on 15th and 30th day sample but no significance was shown for 45th day sample. No significant variation was observed for Mn and Zn while Pb showed significance on all the sampling days.

3.10. BOD and COD

It is generally recognized that the percentage of readily biodegradable organic matter is an important aspect of compost quality. Composting process occurs until all biodegradable organic material is stabilized which is odorless and pathogen free and a poor breeding substrate for breeding of flies and other insects. Even if the compost is stable, care should be taken while applying to soil for crop use because the biological processes will continue and can strip the nutrients of soil [34] hence BOD is also an important parameter to monitor. In all the reactors BOD and COD were reduced (Table 8). The reduction of BOD for the pure cultures R_1, R_2, R_3 and mixed cultures R_4, R_5, R_6, R_7 were of the range 72–91% and 65–85% while that of COD were in the range 46–73% and 29–70%. The maximum reduction in BOD and COD were observed in R_3 with the pure culture. A high significance was observed in the values of BOD and COD for all the reactors ($p < 0.05$).

3.11. Coliforms

Coliforms are the indicators of the presence of pathogens. Use of such an indicator, as opposed to the actual disease-causing organ-

Table 7
Final values (45 days) of heavy metals in reactors

Reactors	Heavy metal content (mg/kg)			
	Mn	Zn	Pb	Cu
R_1	108.6 ± 16a	513 ± 42a	30.6 ± 3.8a	158.2 ± 27a
R_2	99.4 ± 7a	498.26 ± 35a	36.6 ± 3.2a	157.2 ± 25a
R_3	93.8 ± 6a	473.2 ± 31a	35.8 ± 2.5a	136.4 ± 31a
R_4	94.2 ± 5a	441.92 ± 28a	30.8 ± 2.8a	132.2 ± 30a
R_5	110 ± 13a	517.2 ± 37a	31.4 ± 2.7a	131.2 ± 28a
R_6	101.8 ± 11a	523.76 ± 39a	38 ± 3a	161.4 ± 18a
R_7	107.6 ± 12a	494.8 ± 28a	37.4 ± 3.1a	142.8 ± 12a
R_8	109 ± 12a	515.48 ± 38a	35.4 ± 3.6a	158.2 ± 16a

Values followed by the same letter within each column are not significantly different.

Table 8
Variation in BOD, COD during vermicomposting

Reactors	BOD (mg/L)			COD (mg/L)		
	15 days	30 days	45 days	15 days	30 days	45 days
R_1	460 ± 12a	280 ± 5a	158.5 ± 5a	1285.7 ± 55a	1071.8 ± 43a	804.0 ± 37a
R_2	230 ± 8b	150 ± 3c	95 ± 1b	987.5 ± 47b	899.3 ± 32b	634.9 ± 28b
R_3	170 ± 5c	90 ± 1d	50 ± 0.5c	638.5 ± 36c	508.8 ± 25c	398.6 ± 15c
R_4	357.8 ± 10deg	190 ± 3e	85 ± 1b	1043.4 ± 48b	901.5 ± 38b	448.7 ± 23dc
R_5	480 ± 13a	290 ± 8a	150 ± 4a	1306.5 ± 64a	1055.5 ± 42a	1041.9 ± 57ei
R_6	386.7 ± 9deg	310 ± 11f	160 ± 6a	1084.8 ± 51b	1071.2 ± 40a	1062.9 ± 52fe
R_7	316 ± 8f	260 ± 8b	156 ± 3a	1064.5 ± 45b	1037.2 ± 43a	945.5 ± 47gi
R_8	367 ± 9deg	278 ± 9a	230 ± 7d	1207.5 ± 52ad	1087.5 ± 45a	985.6 ± 48hei

Values followed by the same letter within each column are not significantly different.

Table 9
Variation in FS, FC during vermicomposting

Reactors	FS (bacteria/g dry weight)			FC (bacteria/g dry weight)		
	15 days	30 days	45 days	15 days	30 days	45 days
R_1	$5 \times 10^3 \pm 300$a	$3 \times 10^3 \pm 240$a	$2.3 \times 10^2 \pm 21$a	$5 \times 10^4 \pm 800$a	$1.3 \times 10^4 \pm 356$a	$2.3 \times 10^3 \pm 178$a
R_2	$1.1 \times 10^4 \pm 600$b	$3 \times 10^3 \pm 230$a	$2.3 \times 10^2 \pm 34$b	$1.4 \times 10^4 \pm 500$bd	$1.3 \times 10^4 \pm 398$a	$2.3 \times 10^3 \pm 157$a
R_3	$1.1 \times 10^4 \pm 650$b	$2.3 \times 10^3 \pm 197$a	$1.3 \times 10^2 \pm 15$c	$5 \times 10^4 \pm 879$a	$2.3 \times 10^3 \pm 167$b	$1.5 \times 10^3 \pm 135$b
R_4	$2.3 \times 10^3 \pm 238$c	$2.3 \times 10^3 \pm 200$a	$1.4 \times 10^2 \pm 16$dc	$2.3 \times 10^4 \pm 700$c	$5 \times 10^3 \pm 189$c	$1.4 \times 10^3 \pm 145$b
R_5	$3 \times 10^4 \pm 700$d	$2.3 \times 10^3 \pm 189$a	$2.3 \times 10^2 \pm 20$a	$1.3 \times 10^4 \pm 356$b	$5 \times 10^3 \pm 200$dc	$1.3 \times 10^3 \pm 188$a
R_6	$8 \times 10^4 \pm 900$e	$2.3 \times 10^4 \pm 545$b	$2.3 \times 10^2 \pm 18$a	$1.5 \times 10^4 \pm 455$d	$1.1 \times 10^4 \pm 123$e	$1.5 \times 10^3 \pm 187$a
R_7	$2.3 \times 10^4 \pm 600$f	$2.3 \times 10^3 \pm 198$a	$1.3 \times 10^2 \pm 14$ec	$5 \times 10^4 \pm 769$a	$2.3 \times 10^3 \pm 167$b	$1.3 \times 10^3 \pm 129$b
R_8	$5 \times 10^3 \pm 290$g	$2.3 \times 10^3 \pm 213$a	$2.3 \times 10^3 \pm 195$a	$1.5 \times 10^4 \pm 345$ed	$1.3 \times 10^4 \pm 300$a	$4.3 \times 10^3 \pm 170$a

Values followed by the same letter within each column are not significantly different.

Table 10
Live biomass production (earthworms) in different reactors

Reactors	Earthworm/combinations	Mean weight of EWs in g		Live biomass% change
		Initial	Final	
R_1	*E. fetida*	50	70	+28.57
R_2	*E. eugeniae*	50	60	+16.66
R_3	*P. excavatus*	50	55	+9.09
R_4	*E. fetida* + *E. eugeniae*	50	70	+28.57
R_5	*E. fetida* + *E. eugeniae* + *P. excavatus*	50	70	+28.57
R_6	*E. eugeniae* + *P. excavatus*	50	60	+16.66
R_7	*E. fetida* + *P. excavatus*	50	55	+9.09

All data represent average of triplicates.

isms, is advantageous as the indicators generally occur at higher frequencies than the pathogens and are simpler and safe to detect. There was reduction in all the reactors in the number of coliforms (Table 9). It was observed as 99–99.9%, 99.7–99.9% and 99.94% reduction in total coliform in the reactors with the pure cultures, mixed cultures and the control, respectively. Fecal streptococci are commonly considered to be the best indicator of fecal population. They are more resistant to different environmental factors than the coliforms. The number of fecal streptococci showed a distinct reduction of 99.5–99.7% for pure cultures, 99.5–99.7% for mixed cultures and 99.5% for the control. The number of fecal coliform was observed to have a reduction of 99.9% for all the reactors. The reduction was presumably because of the elimination of the coliforms as they enter the food chain of the earthworm. The coliforms varied significantly for all the reactors ($p < 0.05$).

3.12. *Earthworm biomass*

The changes in worm biomass for all pure as well as mixed cultures over the experimentation period are illustrated in Table 10. No mortality was observed in any reactor during the whole vermicomposting period. The vermicompost was dark brown (towards blackish) in color and homogeneous after 45 days of earthworm's activity. At the end of the 45 days, the earthworm biomass had increased slowly in all the reactors. The increase in weight of earthworm biomass during the composting period varied between 5 and 20 g which amounts to 10–28% for both the reactors with the pure cultures and mixed cultures. The maximum increase was observed in R_1, R_4 and R_5 with 28% increase.

4. Conclusions

Sewage sludge is a major contributor of toxic heavy metals such as Cd, Pb, etc. to soils due to its direct application as manure. These metals may enter the human and animal body through consumption of the crops. The present study inferred that the application of sewage sludge in the agricultural fields after vermicomposting would not have any adverse effect as these heavy metals are present within the permissible limits. Thus this method converts the hazardous sludge into non-hazardous useful nutritious resource.

The sewage sludge without blending can be directly converted into good quality fertilizer by using *E. fetida*, *E. eugeniae* and *P. excavatus* individually or in combinations. Overall, the pure cultures and mixed cultures worked efficiently almost equally. The mixed cultures showed better performance in terms of TOC, NH_4^+-N, NO_3^--N and bacteriological (BOD, COD and coliforms) while the pure cultures were ahead in terms of TP, K, Na, Ca, C/N and ash content. It was not obvious that mixed culture had any advantage over pure culture in the vermicomposting process but different earthworm species coexisted well and produced good end products.

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