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Vermistabilization of municipal sewage sludge amended with sugarcane trash using epigeic *Eisenia fetida* (Oligochaeta)

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ABSTRACT

Efforts have been made in this study to stabilize the sewage sludge mixed with sugarcane trash in four different proportions: 20% (T_1); 40% (T_2); 60% (T_3) and 80% (T_4), under laboratory conditions using epigeic earthworm (Oligochaeta) *Eisenia fetida*. The composting potential of worm was also evaluated in 100% sewage sludge treatment (T_5). The changes in chemical properties of substrate was measured at the end. The vermicomposted material showed decrease in organic C (4.8–12.7%) and exchangeable K (3.2–15.3%) content, whereas increase in total N (5.9–25.1%) and available P (1.2–10.9%), exchangeable Ca (2.3–10.9%) and exchangeable Mg (4.5–14.0%) contents. Vermicomposting process caused considerable reduction in concentration of diethylene-triaminepentaacetic acid (DTPA) extractable metals: Cu (4.98–30.5%), Fe (5.08–12.64%), Mn (3.31–18.0%), Zn (2.52–15.90%) and Pb (2.38–20.0%). *E. fetida* showed the better growth performances in first three treatments (T_1–T_3) possibly due to higher content of organic matter (supplied by bulking agent, i.e. sugarcane trash). The earthworm mortality was higher in vermibeds those contained more sludge proportions. Study revealed that vermicomposting might be an efficient technology to convert negligible municipal sewage sludge into value-added products. The feasibility of earthworms to mitigate the metal toxicity and to enhance the nutrient profile might be useful to convert noxious sludge into useful products, at low-input basis.

1. Introduction

The urbanization resulted in generation of large amount of wastewater and sludge. In India about 7300 million cubic meter of wastewater is generated per annum, but most of it remained unutilized and discharged directly in to rivers. High cost and energy required for the conventional treatment technologies are major reasons for poor collection and treatment of wastewater in the developing countries. The sludge generated in enormous quantity creates a problem of their safe disposal. Among the several available alternatives for disposing of sewage sludge, one of the most convenient is using it in agriculture. According to Appelhof [1] appropriate disposal involves both maximum cost-effective recovery of recyclable constituents and transformation of non-recoverable material into forms, which do not present environmental hazards. In general, sewage sludges have high nutritive value for plants and therefore; its application as a soil conditioner and/or fertilizer is widely recommended [2]. In addition to modifying the physico-chemical environment of the soil, sewage sludge drastically modifies the soil biological systems. Therefore, much attention is required during the use of unprocessed sludge materials for crop production.

Earthworms have been used as an alternative tool to convert a great proportion of organic waste resource into a product with relatively higher concentration of plant nutrients, microbial population, soil enzymes and humic acids contents. Vermicomposting is a stabilization of organic waste materials involving the joint action of earthworms and microorganisms. Although, microbes are responsible for biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering the biological activity [3,4]. Benitez et al. [5] concluded that in vermicomposting process, inoculated earthworms maintain aerobic condition in the organic wastes, convert a portion of the organic material into worm biomass and respiration products, and expel the remaining partially stabilized product (vermicompost). Epigeic earthworms could stabilize the sewage sludge potentially and can accelerate the rates of destruction of sludge volatile solids in aerobic sludge greatly, which probably decreases the putrefaction; occurring because of anaerobic conditions. So, the main cause of increased rates of degradation of sludge is probably the increased aeration and turnover of wastes by the earthworms. Nevertheless, much work is still required to design a technology with efficient and cost-effective recovery of wastes into value-added product by applying potential earthworm resources. Many

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aspects of vermicomposting still need to be researched, evaluated, and resolved to ensure the consistent success of such a process for a wide range of wastes.

The presence of certain soil contaminants, such as heavy metals, organic compounds, and human pathogens in sewage sludge causes the alternation in biological property especially the microbial activity [6] in vermicomposting sub-system. Metal availability in sewage sludge can alter the composting potential of earthworms. Since, organic matter represents predominantly substances rich in humic materials, i.e. humic acid, fulvic acid and humin; metal binding by humic substance is a control on metal behaviour and availability in soil/manures [7]. Therefore, the mixing of organic-rich external supplement material, e.g. plant-derived wastes, crop residues, animal excreta, agro-industrial byproducts, paper-pulp sludge, etc. in sludge decomposition system can accelerate the earthworm's composting efficiency. Studies showed that in USA many pilot schemes to recycle sewage sludge have been using thickened sewage sludge layer over sawdust to create a material suitable for vermiculture. It has been found that mixing the sewage sludge with other materials (e.g. garden wastes, paper-pulp sludge, or other lignin-rich wastes) before vermicomposting process could accelerate the decomposition rate in vermireactors. Maboeta and Van Rensburg [8] studied the vermicomposting of sewage sludge spiked with industrially produced wood chips and reported that vermicomposting of sewage sludge with bulking material is more effective than composting of sewage sludge alone. Nevertheless, the attempts at vermicomposting of sludge have suffered the effects of extravagant claims, made often by entrepreneurs or environmental zealots, and lacking in rigorous scientific research [9].

Sugarcane trash is a major by-product of sugarcane industry and produced in millions of tones per year. It has been suggested as a potential soil conditioner due to having efficient plant nutritive values. Moreover, the mixing of sugarcane trash as an amendment material, in vermicomposting of some non-traditional waste materials such as sewage sludge, not only can enhances the nutritive value of end product but, at the same time also suppresses the toxicity by metals through supplying a considerable amount of organic matter.

The objective of this work was to study the efficient composting of sewage sludge spiked with organic supplement, i.e. sugarcane trash using an epigeic earthworm species: *Eisenia fetida* (Savigny), under laboratory conditions. The influence of bulking material, i.e. sugarcane trash on composting and growth performance of *E. fetida* in sludge vermicomposting system was also monitored during this study.

2. Materials and methods

2.1. Earthworm

Originally the composting earthworm, i.e. *E. fetida* (Savigny) of different age groups were obtained from vermicomposting unit of a cowshed: Shree Gaushala, Sri Ganganagar, India. Stock earthworms were cultured, in the laboratory, on partially decomposed cow dung mixed with leaf litter of *Mangifera indica*. Fresh cocoons of stock earthworm were incubated at 25 °C [10] in laboratory and hatchlings were collected. The young earthworms were used for further vermicomposting experiments.

2.2. Sewage sludge and sugarcane trash

Municipal sewage sludge (contained 35% dry matter) was obtained after filtering the municipal black wastewater sludge. Sewage sludge was collected in large-sized plastic buckets. Collected material was brought to the laboratory and filtered through a sand filter unit (made from stones and pebbles layer (15 cm) at base and sand layer at the top (45 cm), filled in a large-sized plastic pot container). The sludge remained at the top of the filter unit was further used for vermicomposting experiments. The washed-water obtained from filtration unit was not discarded because it also contains some soluble forms of nutrients. It was used to moisten the vermibeds during vermicomposting process. The characteristics of sludge are reported in Table 1. Sugarcane trash was obtained locally from local sugar mill unit, Sri Ganganagar, India. The cane trash was allowed to dry slightly in sunlight and was chopped into small bits (>1 cm). The chemical characteristics of sugarcane trash are also reported in Table 1.

Table 1
Chemical structure of sewage sludge and sugarcane trash used for experimentation

Parameter	Sewage sludge	Sugarcane trash
pH	7.4 ± 0.02	-
C_{ogr} (g kg^{-1})	267.13 ± 2.67	391.2 ± 2.67
N_{tot} (g kg^{-1})	26.65 ± 1.11	25.2 ± 0.58
P_{avail} (g kg^{-1})	42.98 ± 2.62	15.7 ± 0.97
K_{exch} (g kg^{-1})	4.96 ± 0.04	6.8 ± 0.07
Ca_{exch} (g kg^{-1})	44.97 ± 1.66	60.2 ± 1.29
Mg_{exch} (g kg^{-1})	23.36 ± 0.74	10.3 ± 0.48
C:N ratio	11.4 ± 0.5	15.49 ± 0.7
Fe (mg kg^{-1})	362.5 ± 0.9	389.4 ± 0.7
Zn (mg kg^{-1})	321.7 ± 1.1	359.4 ± 1.4
Cu (mg kg^{-1})	45.5 ± 0.9	19.45 ± 0.5
Mn (mg kg^{-1})	304.5 ± 2.4	189.4 ± 0.9
Pb (mg kg^{-1})	19.9 ± 0.4	8.45 ± 0.2

2.3. Treatment design

At the beginning, sewage sludge containing 70–85% moisture was mixed with chopped sugarcane trash in different ratios. Four different combinations of sewage sludge and sugarcane trash were prepared (Table 2). One treatment (fifth) was composed of pure sewage sludge without any dilutions. Sugarcane trash acted as bulking or amendment material in sewage sludge vermicomposting trial container. Experimental pot containers were pretreated (manually aerated every day for 15 days) to reduce the characteristics smell of putrescible substances and biotoxic compounds, formed under anaerobiosis [11]. Although, most of the sludges are anaerobic and, when fresh, can be toxic to *E. fetida* and when they are dewatered after becoming aerobic they are readily acceptable to earthworms [12]. Plastic circular pot containers of appropriate size (28 cm diameter and 30 cm in depth) with pierced lid for aeration were used for laboratory screening of different treatment combinations of sewage sludge. Experimental beddings were kept in triplicate for each treatment, and the control treatment had the same setup without earthworm. The organic wastes material

Table 2
Composition of treatments used for experimentation

Treatment nos.	Treatment description	Sewage sludge (g)[a]	Sugarcane trash (g)[a]
T_1	SS[b] (20%)[c] + SCT[d] (80%)	150	600
T_2	SS (40%) + SCT (60%)	300	450
T_3	SS (60%) + SCT (40%)	450	300
T_4	SS (80%) + SCT (20%)	600	150
T_5	SS (100%) + SCT (0%)	750	0

[a] Dry weight basis.
[b] Sewage sludge.
[c] The figures in parentheses indicates the percent content in the initial substrate material.
[d] Sugarcane trash.

in container served as bedding as well as food for composting earthworms. About 3-week old 20 clitellated, *E. fetida* (live weight ~289–295 mg) were collected from stock culture and released in to different plastic pot containers containing 750 g of (dry weight basis) substrate material. Although, in initial, moisture level in substrates was appropriate (≈70%), but after 4 weeks it was maintained around 65–70%, throughout the study period by periodic sprinkling of adequate quantity of tap water, if required. To prevent moisture loss, the experimental pots were covered with paddy straw. Containers were placed in a humid and dark room with a temperature of 28.4 ± 0.3 °C. In all experimental containers 50 g (dry weight basis) fresh bedding material (chopped, <1 cm sugarcane trash) was added after each 30 days interval (i.e. after 30 and 60 days of starting of experiment), so that food limit could not influence the worm's growth performance. To determine the mineralization/decomposition rate in worm-processed material, homogenized samples of substrate material (10 g dry weight basis) were drawn at day 0 (initial) and at 90 days (vermicomposted substrate, at end) from each experimental container for chemical analysis.

Growth and cocoon production in each experimental container was measured at 15, 30, 45, 60, 75 and 90 day. Earthworms and cocoons, produced during experiment, were separated from the substrate material by hand sorting, after which worms were washed in tap water to remove adhering material from their body, and subsequently weighed on a live weight basis. Than all measured earthworms were returned to the concerned container. Separated cocoon were counted and introduced in separate bedding containing the same material in which their parents were reared. On the basis of obtained data of biomass and cocoon numbers, other growth parameters of earthworm, i.e. growth rate (mg day^{-1}), maximum weight achieved and reproduction rate (cocoon $worm^{-1}$ day^{-1}) were produced with the help of recorded data, for different studied treatments.

2.4. Chemical analysis

The pH was measured using digital pH meter (Systronic made) in 1/10 (w/v) aqueous solution. Organic carbon was determined by the partial-oxidation method [13]. Total nitrogen was measured by micro Kjeldahl method [14]. C:N ratio was calculated from the measured value of C and N. Extractable phosphorous was determined by following Olson's sodium bicarbonate extraction method [15]. Exchangeable elements (K, Ca and Mg) were determined after extracting the sample using ammonium acetate extractable method [16]; analyzed by PerkinElmer model 3110 double beam atomic absorption spectrophotometer (AAS). The concentration of heavy metals, i.e. Cu, Fe, Zn, Mn, and Pb was determined by following diethylene-triaminepentaacetic acid (DTPA) extraction method; analyzed by AAS.

2.5. Statistical analysis

One-way ANOVA was used to analyze the significant difference between treatments. Duncan multiple-ranged test was also performed to identify the homogeneous type of the bedding material in respect to earthworm's growth parameters (earthworm weight gain, individual growth rate, total cocoons numbers, cocoon production rate, and total population mortality, etc.) and chemical parameters of initial substrates. A paired-sample *t*-test was performed between control (compost without worms) and experiment (vermicompost with earthworms) for different chemical parameters.

3. Results and discussion

3.1. Physico-chemical changes during vermicomposting

Statistically vermicomposted material showed significant difference in organic C ($F = 985.2$, $P < 0.001$), total N ($F = 26.9$, $P < 0.001$), available P ($F = 753.2$, $P < 0.001$), exchangeable K ($F = 299.3$, $P < 0.001$), exchangeable Ca ($F = 115.9$, $P < 0.001$) and exchangeable Mg ($F = 853.1$, $P < 0.001$) content than their initial (at starting of experiment) levels. The pH of the substrate material was lower in all treatments than their initial values (Table 3). The reduction in pH was between the ranges of 3.5–9.5% for different treatments studied (Table 4). The shift in pH during the study could be due to microbial decomposition during the process of vermicomposting. Elvira et al. [17] concluded that production of CO_2 and organic acids by microbial decomposition during vermicomposting lowers the pH of substrate. Similarly, Ndegwa et al. [18] pointed out that a shift in pH might be related to the mineralization of the nitrogen and phosphorus into nitrites/nitrates and orthophosphates and bioconversion of the organic material into intermediate species of the organic acids. Organic C was lower in final product, i.e. vermicompost, when compared to the initial level in the substrate (Table 4). The organic C loss was in the ranges of 17.5 (T_5)% to 67.0% (T_3 treatment) (Table 4). However, statistically the C loss was not different between T_1 and T_2 treatments (ANOVA; Duncan multiple-ranged test: $P = 0.397$). Comparatively, substrate with earthworm (vermicompost) showed the greater carbon loss as compared to control containers (all significant: *t*-test), which suggests earthworm-mediated rapid organic matter mineralization rate in vermibeds. According to Dominguez [12] vermicomposting is a combined operation of earthworm and microorganisms in which earthworm fragments and homogenizes the ingested material through muscular action of their foregut and also adds mucus and enzymes to ingested material and thereby increases the surface area for microbial action while, microorganisms perform the biochemical degradation of waste material providing some extracellular enzymes required for organic waste decomposition within the worm's gut. Moreover, this biological mutuality caused C loss in the form of CO_2 from the substrates during the decomposition and mineralization of organic waste [4,19]. The conversion of some part of organic fractions of waste into worm biomass can also reduce the C loss form the substrate. Present result is consistent with previous reports that earthworm inoculation in wastes accelerates the rate of carbon mineralization. Nevertheless, in present study the difference among treatments, for C loss patterns, was directly related to the proportion of amendment material in treatment substrates. As data indicates (Table 4), earthworm showed more C loss in beddings than those containing a great proportion of sugarcane trash. It indicates that the amount of organic matter in substrate affects the rate of microbial enzymes production and their activities. Benitez et al. [5] concluded that β-glucosidase and BBA-hydrolysing protease, which are hydrolytic enzymes involved in the C and N cycles, respectively, showed sharp decrease as a consequence of the decrease in available organic substances. Present result extends and confirms above hypothesis.

Total N was significantly higher in the end product (Table 4) than initial substrate material (Table 3). Nitrogen enhancement ranged from 5.9% (T_4)% to 25.1% (T_3). Dramatically, no clear pattern was evidenced for nitrogen increase among T_1–T_3 treatments with earthworms (ANOVA; Duncan multiple-ranged test: $P = 0.062$) (Table 4). Comparatively the vermicomposted material showed nearly 4.87–17.11% more nitrogen content than control treatments (all, $P < 0.05$: *t*-test) (Table 4). It is suggested that in addition to releasing N from compost material, earthworms also enhance nitrogen levels by adding their excretory products, mucus, body fluid,

Table 3
Chemical compositions (g kg^{-1}) of substrates at starts used for experimentation (mean ± S.D.; n = 3)

Treatment[a]	pH	C_{org}	N_{tot}	C:N ratio	P_{avail}	K_{exch}	Ca_{exch}	Mg_{exch}
T_1	7.85 ± 0.03 d	390.7 ± 0.8 e	24.3 ± 0.5 c	16.1 ± 0.3 d	10.8 ± 0.5 a	7.1 ± 0.07 e	57.6 ± 0.03 e	12.6 ± 0.1 a
T_2	7.71 ± 0.03 c	343.7 ± 1.4 d	23.4 ± 0.4 b	14.7 ± 0.2 c	17.5 ± 0.5 b	6.8 ± 0.04 d	53.7 ± 0.04 d	15.7 ± 0.1 b
T_3	7.66 ± 0.02 c	320.4 ± 0.6 c	23.5 ± 0.4 b	13.6 ± 0.3 b	25.4 ± 0.4 c	6.3 ± 0.1 c	50.3 ± 0.03 c	16.5 ± 0.2 c
T_4	7.52 ± 0.03 b	289.5 ± 0.6 b	21.3 ± 0.4 a	13.9 ± 0.4 b	38.7 ± 0.3 d	5.4 ± 0.1 b	41.7 ± 1.0 a	20.7 ± 0.2 d
T_5	7.40 ± 0.03 a	267.6 ± 1.9 a	26.8 ± 0.2 c	10.0 ± 2.1 a	43.7 ± 0.2 e	5.1 ± 1.6 a	46.6 ± 0.12 b	23.5 ± 0.1 e

Mean values followed by different letters are statistically different (ANOVA, Duncan multiple-ranged test; $P < 0.05$.
[a] Refer to Table 2, for explanation of treatments.

enzymes, etc. to the substrate. Decaying tissues of dead worms also add a significant amount of N to vermicomposting sub-system. The N mineralization was more rapid in bedding those contained a great proportion of amendment material, i.e. sugarcane trash, which suggested that the nitrogen enrichment process might be related to the amount of organic matter supplied by bulking agents in vermicomposting system. According to Benitez et al. [5] the hydrolytic enzyme production, which plays an important role in C and N cycle in waste decomposition system, is drastically influenced by the availability of easily degradable organic compounds in the substrates. However, the greater concentration of total N in T_5 treatment was not related to decomposition or nitrification activity. It was related more likely to the higher earthworm mortality that enriched the bedding with N content due to decaying earthworm tissue [20]. In general, earthworm contains about 60–70% (of dry mass) proteins in their body tissue, and this pool of N returned to the soil upon mineralization.

After vermicomposting, all treatments showed higher concentrations of available P in vermicomposted material than initial material. The increase in P was in the order: T_3 (24.4%) > T_2 (20.6%) > T_1 (19.8%) > T_4 (11.6%) > T_5 (6.9%). As compared to control bedding vermicomposted material showed the great concentration of available P, after 90 days of experimentation (Table 4). The difference between control and experimental container was statistically significant for different treatments (Table 4), except in T_2 (P = 0.134) and T_5 (P = 0.512) treatment. When organic matter passes through the gut of earthworm, results in some amount of phosphorus is converted to more available form. The release of phosphorus in available form is performed partly by earthworm gut phosphatases, and further release of P might be attributed to the P-solubilizing microorganisms present in worm casts. Recently Suthar [21] and Gupta and Garg [22] claimed a higher concentration of available P in earthworm processed organic waste. In present study, however, there was a consistent trend of decreasing P mineralization rate with increasing proportion of sewage sludge. This difference could be attributed to the increased level of heavy metals and other toxic compounds in substrates.

Exchangeable K content in ready vermicompost was lower (Table 4) than initial substrate material. The loss in exchangeable K was in the order: T_3 (15.3%) > T_2 (12.0%) > T_4 (11.1%) > T_1 (9.2%) > T_5 (3.2%). Dramatically, the substrate material of control containers showed the higher concentration (4.1–18.5% more) of exchangeable K than vermicomposted material (all significant, t-test: $P < 0.05$). No remarkable differences among T_1–T_3 vermibeds (P = 0.107) was observed for K content. The loss of available K in the presence of earthworm is evidenced in some previous studies [21,23]. Benitez et al. [2] correlated the K loss with leaching process, during vermicomposting. Garg and Kaushik [24] reported a similar trend of potassium loss during vermicomposting process. Dramatically the control treatments showed the higher content of exchangeable K in end products. Therefore, in this study the loss of exchangeable K in vermibeds was not the result of leaching process. There might be possibility of potassium absorption or assimilation by inhabiting earthworms as potassium is important physiological supplement

Table 4
Chemical compositions (g kg^{-1}) of substrates after 90 days of experimentation (mean ± S.D.; n = 3)

Treatment[a]	pH			C_{org}			N_{tot}		
	Control[b]	Experiment[c]	t-test[d]	Control	Experiment	t-test	Control	Experiment	t-test
T_1	7.7 ± 0.05	7.1 ± 0.03	P = 0.002	288.3 ± 8.02	159.2 ± 3.5	P = 0.003	25.4 ± 0.54	28.5 ± 0.90	P = 0.009
T_2	7.6 ± 0.03	6.7 ± 0.03	P = 0.001	262.3 ± 3.06	138.7 ± 1.6	P = 0.000	24.2 ± 0.41	27.3 ± 1.1	P = 0.069
T_3	7.6 ± 0.02	7.1 ± 0.02	P = 0.000	272.2 ± 2.65	105.6 ± 2.8	P = 0.000	25.2 ± 0.55	29.5 ± 1.0	P = 0.007
T_4	7.5 ± 0.04	7.2 ± 0.03	P = 0.000	256.7 ± 2.50	148.6 ± 1.7	P = 0.000	21.6 ± 0.38	22.6 ± 0.7	P = 0.040
T_5	7.2 ± 0.03	7.0 ± 0.04	P = 0.019	242.0 ± 2.81	220.8 ± 1.6	P = 0.005	27.4 ± 0.29	28.8 ± 2.7	P = 0.070
	C:N ratio			K_{exch}			P_{avail}		
T_1	11.4 ± 0.55	5.6 ± 0.1	P = 0.003	6.8 ± 0.04	6.4 ± 0.04 [e]	P = 0.037	11.7 ± 0.2	12.9 ± 0.04	P = 0.009
T_2	10.9 ± 0.29	5.1 ± 0.2	P = 0.002	6.8 ± 0.04	5.9 ± 0.04 [d]	P = 0.000	18.1 ± 0.1	21.1 ± 0.78	P = 0.134
T_3	10.8 ± 0.30	3.6 ± 0.2	P = 0.000	6.4 ± 0.11	5.4 ± 0.05 [c]	P = 0.009	27.2 ± 0.1	31.6 ± 0.59	P = 0.010
T_4	12.0 ± 0.10	6.4 ± 0.1	P = 0.000	5.4 ± 0.10	4.8 ± 0.04 [b]	P = 0.016	39.6 ± 0.1	43.2 ± 0.59	P = 0.512
T_5	8.8 ± 0.13	7.7 ± 0.3	P = 0.016	5.1 ± 0.05	4.9 ± 0.05 [a]	P = 0.028	44.2 ± 0.1	46.7 ± 0.24	P = 0.041

	Ca_{exch}			Mg_{exch}		
T_1	58.6 ± 0.21	58.7 ± 1.1	P = 0.902	12.9 ± 0.11	13.8 ± 0.1	P = 0.009
T_2	54.2 ± 0.62	56.5 ± 1.6	P = 0.389	16.0 ± 0.03	17.4 ± 0.2	P = 0.004
T_3	52.0 ± 0.59	55.8 ± 0.7	P = 0.024	18.1 ± 0.54	18.8 ± 0.2	P = 0.116
T_4	43.2 ± 0.59	42.8 ± 0.4	P = 0.670	21.0 ± 0.05	21.8 ± 0.2	P = 0.023
T_5	46.7 ± 0.24	47.6 ± 0.5	P = 0.077	23.7 ± 0.16	24.5 ± 0.5	P = 0.091

[a] Refer to Table 2, for explanation of treatments.
[b] Control—compost proceed without worms.
[c] Experiment—vermicompost proceed with worms.
[d] Paired sample t-test.

for animals. A detailed study of potassium metabolism in earthworm during vermicomposting process is still required to support the above hypothesis.

The concentration of exchangeable Ca and Mg was higher in vermicomposted material in different treatments. The enhancement in exchangeable Ca and Mg recorded between the ranges of 1.9% (T_5) to 10.9% (T_3) and 4.3% (T_5)–13.9% (T_3), respectively (Table 4). The vermicomposted container showed more concentration of available forms of Ca and Mg than experimental control containers. However, there was no significant difference (*t*-test) between control and experimental container in respect to contents of Ca and Mg, except in T_3 and T_5 treatments. In general, earthworm plays an important role in transformation of plant metabolites into more available forms, which can be further metabolized by microbial communities associated with their casts [12]. This study is accordance with previous workers who reported a significant increase in the level of Ca, after the completion of vermicomposting process [20,25]. Earthworm activity changes a proportion of Ca and Mg from bound forms to free forms, which can be further, utilized or absorbed by earthworms themselves as physiological supplement or could be lost through leaching process, although further detailed study is required to support this hypothesis.

The C:N ratios of substrate materials showed the drastic changes after 90 days of experimentation (F = 197.5, P < 0.001). As compared to initial values, the decrease in C:N ratios was in the order: $T_3 > T_1 > T_2 > T_4 > T_5$ (Table 4). However, C:N ratio, which has been studied extensively as an indicator of compost maturity, decreases drastically during vermicomposting processes [26,27]. In vermicomposting sub-system, the loss of carbon as carbon dioxide due to respiratory activities of earthworms and associated microflora, and simultaneously adding of nitrogen in substrate material by inoculated earthworms (through. production of mucus, enzymes and nitrogenous excrements) lowers the C:N ratio of the substrate [20,26].

3.2. DTPA extractable metal concentration in end products

As compared to initial levels (Table 5) the concentrations of DTPA extractable metals decreased markedly in vermicomposted material. The reduction (% of their initial values) was in the ranges: 4.98–30.5 for Cu, 5.08–12.64 for Fe, 3.31–18.0 for Mn, 2.52–15.90 for Zn, and 2.38–20.0 for Pb (Fig. 1a–e). However, metal loss was comparatively higher in experimental vermibeds those showed the maximum earthworm activities, e.g. T_2 and T_3. On the other hand, T_5 treatment, which exhibited the minimum mineralization rate and even earthworm biomass production, showed the least metal loss during the vermicomposting process. As presented in Table 6, it is clear that presence of earthworms caused a significant metal removal from the substrate material. Comparatively, the vermicomposted substrate showed a lower level of Cu (5.0–37.4% lower), Fe (4.6–11.8%), Mn (3.1–21.3%), Zn (8.6–18.3%), and Pb (2.4–19.3%), than control treatments (Fig. 1a–e). However, the data did not exhibit the direct relationship between earthworm's activities and metal loss from the substrates, but data of experimental control containers support the hypothesis that metal loss from the substrate was due to presence of earthworms in substrates. Suthar and Singh [20] reported the considerable amount of metals in tissues of earthworms inoculated in distillery sludge for long periods. They correlated the metal loss form substrate with earthworm tissues metal level. In this study, the difference in the metal loss patterns among different treatments was possibly related to the availability of metals in substrates. Earthworms could accumulate some amount of metals although; further analysis of tissues of inoculated earthworms is needed to support the proposed hypothesis. Organic matter ingested by earthworms undergoes chemical and microbial changes; when it passes through the gut some part of the organic matter is digested, and pH and microbial activity of the gut increases. As a result, the possibilities of binding of metals to ions and carbonates (i.e. more soluble fractions) increase in ingested material [28]. So, the rate of bioaccumulation of such soluble forms of metals could be increased when it passes through worm's gut. Moreover, cutaneous absorption of metals is also evidenced in earthworms. Metal contents in end product is also strongly codetermined by physico-chemical edaphic interactions, including factors such as pH, Ca concentration, organic matter content, cation-exchange capacity, etc. Suthar et al. [29] suggested that soil chemical properties especially organic matter content plays an important role in metal accumulation level in tissues of earthworms.

Table 5
DTPA extractable metal contents (mg kg^{-1}) in initial substrate material (mean ± S.D., n = 3)

Treatment[a]	Cu	Fe	Mn	Zn	Pb
T_1	9.7 ± 0.1	76.7 ± 0.3	63.6 ± 1.9	73.0 ± 0.8	1.8 ± 0.1
T_2	18.8 ± 0.1	147.5 ± 0.5	130.3 ± 0.7	129.5 ± 0.3	3.8 ± 0.1
T_3	24.3 ± 0.3	215.3 ± 2.0	150.5 ± 1.8	148.3 ± 1.3	6.0 ± 0.1
T_4	31.1 ± 0.1	293.6 ± 1.9	206.0 ± 2.3	207.2 ± 2.3	9.9 ± 0.2
T_5	48.1 ± 0.1	369.3 ± 0.9	325.9 ± 1.1	325.6 ± 3.0	16.8 ± 0.3

[a] Refer to Table 2, for explanation of treatments.

3.3. Earthworm growth and reproduction performance in different treatments

Earthworm showed significant difference in growth and reproduction parameters, i.e. mean individual live weight at end (F = 2056.4, P = 0.001), individual biomass gain (F = 2257.6, P < 0.001), maximum individual growth rate (mg day^{-1}) (F = 2233.3, P < 0.001), total cocoon numbers (F = 205.6, P < 0.001) and mean reproduction rate (cocoon $worm^{-1}$ day^{-1}) (F = 61.13, P < 0.001), among different treatments. *E. fetida* showed significantly higher individual live weight in T_2 (790. 2 ± 4.5 mg) followed by T_2 (651.1 ± 4.4 mg), T_3 (635.3 ± 4.9 mg), T_4 (573.2 ± 2.9 mg) and T_5 treatment (394.0 ± 8.9 mg) (P = 0.005, for all), during the experimentation (Fig. 2a). The maximum and minimum growth rate (of individual worm (mg day^{-1})) was also recorded in T_2 and T_5 treatment, respectively. However, individual growth rate (mg day^{-1}) of *E. fetida* in T_2 treatment was ≈38–388% higher than other treatments studied (P < 0.001, for all). The order of growth rate among treatments was: $T_2 > T_1 > T_3 > T_4 > T_5$ (Table 6). There was a consistent pattern in worm growth rate tented to be decreased with increasing sludge concentration in treatments, expect in T_2 vermibed. The decreasing composting efficiency in treatments with higher contents of sewage sludge was due to changed chemical environment in the substrate, which possibly affected the earthworm's potential and survival rate. Since organic matter content plays an important role in decomposition process due to its direct relation with microbial population and their mineralization activities. Therefore, higher growth rate in first three treatments (T_1–T_3) could be claimed to higher organic matter contents in these treatments, supplied by the sugarcane trash. A significant relationship (R^2 = 0.65, P < 0.05) between earthworm growth (mg day^{-1}) and sugarcane trash proportion in vermibed (Fig. 3) supports the proposed hypothesis. Moreover, the metal concentration, which tends to increase with increasing sludge proportion, also influences the worm's biological activities in waste decomposing system. In general, organic matter represents the predominantly substances rich in humic materials (humic acid, fulvic acid and humin) and thereby metal binding by humic substance is a control on metal behaviour

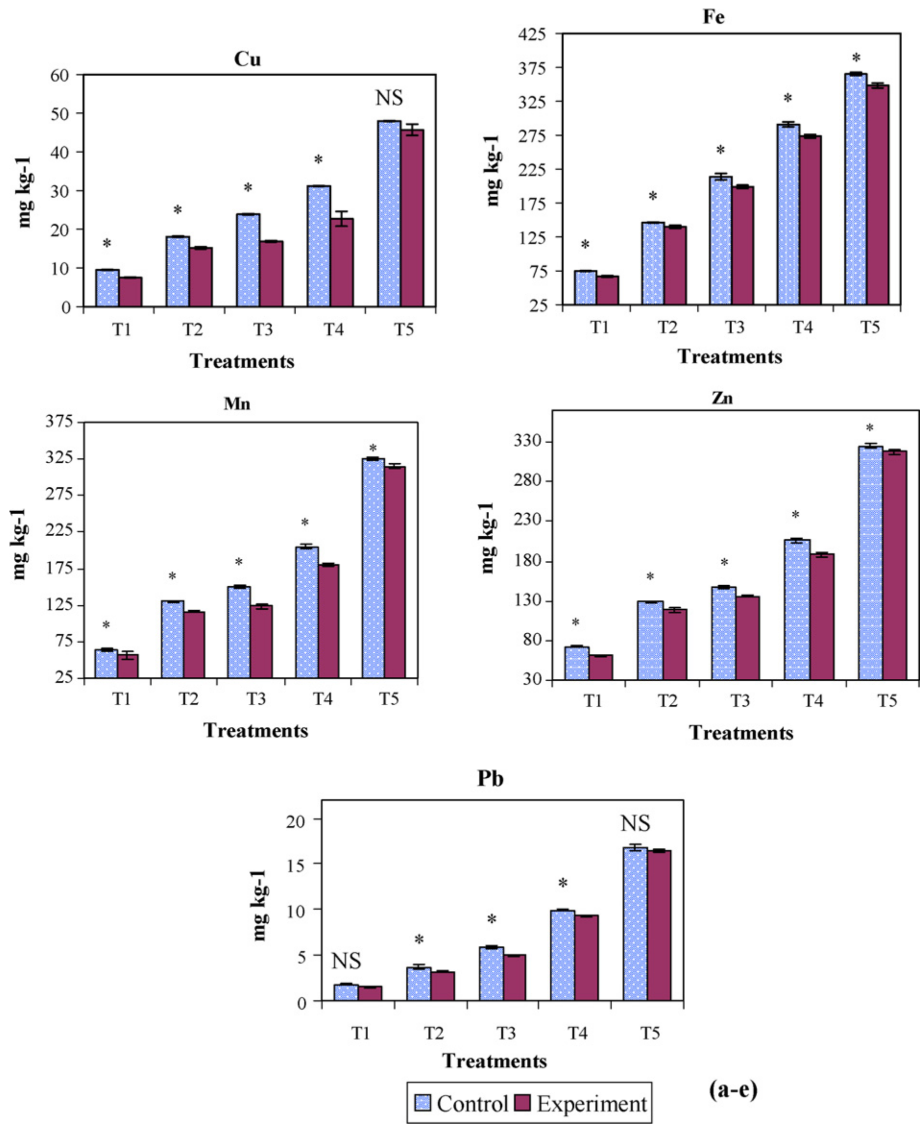

Fig. 1. DTPA extractable metal contents (mg kg^{-1}) in end material (mean ± S.D., $n = 3$). The symbol star (*) indicates the significant difference between control and experiment vermibed for metal content.

and availability in soil/manures [7]. The better performance of earthworms in first three treatments could be a related to the marked supply of organic material in vermibeds.

The amount of sewage sludge in vermibed affected the cocoon production rate in *E. fetida* (Fig. 2b). *E. fetida* showed the maximum cocoon production in T_2 (171.3 ± 7.1) followed by T_2 (114.4 ± 9.1), T_3 (82.3 ± 5.9), T_4 (39.3 ± 7.0) and T_5 treatment (13.3 ± 4.2) ($P = 0.005$, for all). Although, the reproduction rate (cocoon $worm^{-1}$ day^{-1}) ranged between 0.017 ± 0.009 (T_5) and 0.120 ± 0.008 (T_2), among different treatments (Table 6), but it did not show a significant difference between T_4 and T_5 treatments (ANOVA; Duncan multiple-ranged test: $P = 0.098$). Cocoon production rate among different treatments could be attributed to the quality of the substrate used for worm feeding. Suthar [19] concluded that the important difference between the rates of cocoon production in the two organic wastes must be related to the quality of the waste material, which is one of the most important factors in determining onset of cocoon production. It is suggested that the chemical nature

Table 6
Growth and reproduction performance of *E. fetida* in different treatments (mean ± S.D., $n = 3$)

Treatment[a]	Initial individual live weight (mg)	Biomass gain (mg)	Individual growth rate (mg day^{-1})	Total earthworm mortality during experimentation (%)	Reproduction rate (cocoons $worm^{-1}$ day^{-1})
T_1	292.8 ± 0.6 b	358.3 ± 4.9 d	3.98 ± 0.05 d	5.0 ± 7.1 a	0.067 ± 0.009 c
T_2	294.5 ± 0.6 c	495.7 ± 5.0 e	5.51 ± 0.04 e	8.4 ± 7.6 ab	0.120 ± 0.008 d
T_3	295.7 ± 0.7 c	339.6 ± 4.4 c	3.73 ± 0.04 c	21.7 ± 5.7 bc	0.047 ± 0.005 b
T_4	289.4 ± 0.7 a	283.8 ± 3.7 b	3.15 ± 0.03 b	30.0 ± 5.0 c	0.030 ± 0.001 a
T_5	292.5 ± 1.7 b	101.0 ± 7.3 a	1.13 ± 0.07 a	55.0 ± 13.2 d	0.017 ± 0.009 a

Mean value followed by different letters is statistically different (ANOVA; Duncan multiple-ranged test, $P < 0.05$).
[a] For treatment composition see text.

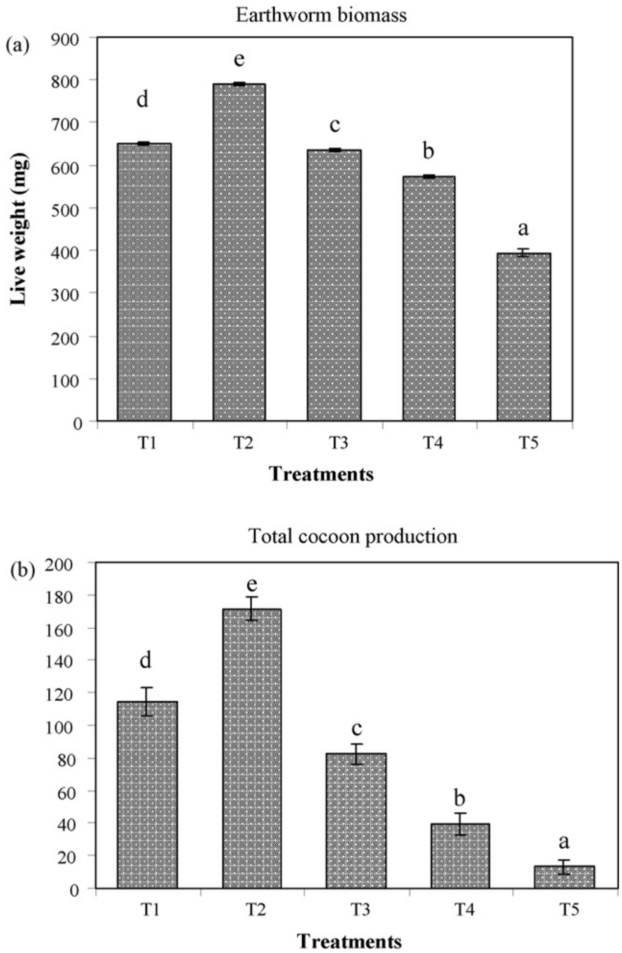

Fig. 2. The biomass production (a) and cocoon production rate (b) in *E. fetida* collected from different treatments. The significant difference ($P < 0.05$) is indicated by different letters.

of feeding stock may be of a primary importance for rearing of earthworms on organic waste resources. The difference in cocoon production rates among different treatments could be due to variation in quality of the substrates. A trend of decreasing cocoon production rate with increasing proportion of sewage sludge was recorded in this study. The availability of food plays an important role in regulating growth parameters of composting earthworms. However, microbes, which play an important role in earthworm diet, are influenced directly by the quality and quantity of organic matter; supplied by the bulking material in vermibed [20]. It is concluded that the increasing proportion of sewage sludge suppresses the growth performance of inoculated earthworms; possibly due to more availability of growth retarding substances (i.e. metals, detergents, grease, etc.) in substrates. This growth-retarding substance directly affects the food availability as well as microbial biomass in decomposing substrate.

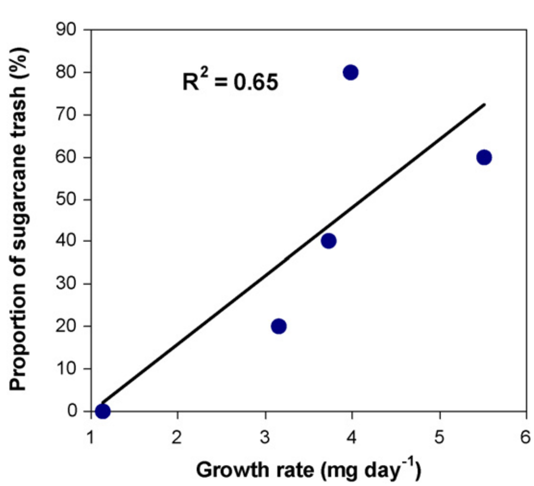

Fig. 3. Relationship between earthworm growth and proportions of sugarcane trash in vermibeds.

Earthworm mortality was between the ranges of 5.0 ± 7.1 (T_1) and 55.0 ± 13.2 (T_5) (Table 5), among treatments. Nevertheless, earthworm mortality did not show significant difference (ANOVA; Duncan multiple-ranged test) between T_1 and T_3 ($P = 0.019$); T_1 and T_2 ($P = 0.067$) and T_2 and T_4 ($P = 0.229$) treatments. The earthworm mortality was higher in treatments, which contained more proportion of sewage sludge in experimental vermibeds. It suggests that at higher concentration sludge affects the survival rate of earthworms in waste decomposing system. Garg and Kaushik [24] found that *E. fetida* showed more mortality in beddings, which contained lower amounts of organic supplements in textile mill sludge vermibeds. The data suggests using appropriate organic supplement (bulking agent) to checks the earthworm mortality, during vermicomposting of such kind of wastes. It has been found that mixing of some other materials (e.g. garden wastes, paper-pulp sludge, or other lignin-rich wastes) in sludge before vermicomposting not only accelerated the rates of decomposition but at the same time also reduced the rate of worm mortality [21] during decomposition process.

4. Conclusions

The use of sludge as raw material in the vermicomposting systems can potentially help to convert this waste into value-added products, i.e. vermicompost. The data clearly suggest that mixing of some bulking agent, e.g. sugarcane trash in sewage sludge not only supports earthworm growth, but at the same time also lowers the risk of earthworm mortality during the process of vermicomposting. The vermicomposts obtained in this study were rich in nitrogen and phosphorus, and had low levels of metals. Moreover, *E. fetida* grew and reproduced favorably in sludge that contained sufficient amount of sugarcane trash. During vermicomposting process some degree of metal toxicity was reduced possibly due to bioaccumulation of some fractions of metals by inhabiting earthworms. The study provides a sound basis that vermicomposting can be a potential technology to convert the noxious community wastes into value-added materials, i.e. vermicompost and earthworm biomass.

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