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Vermicomposting of Cattle Manure using Mono- and Polycultures of Three Earthworm Species

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ABSTRACT

Vermicomposting can be the most economical and sustainable option for cattle waste management. Therefore, three different earthworm species *Eisenia fetida*, *Eudrilus eugeniae*, *Perionyx excavatus* in individual (monoculture) and combinations (polyculture) were utilized to conserve the potential nutrients from cattle manure. Vermicomposting resulted in lowering of pH, total organic carbon (TOC), C/N ratio, coliforms and increase in electrical conductivity (EC). Macronutrients (Na, K and Ca), total nitrogen (TN) and total phosphorous (TP) was observed to increase with vermicomposting time, clearly indicating the conservation of potential nutrients from cattle manure. Compost stability studies revealed that the final compost became very stable with a final oxygen uptake rate (OUR) of 2.68-3.11 mg g^{-1} volatile solids (VS) day^{-1} and 2.12-2.87 mg g^{-1} VS day^{-1}. Therefore, it is evident that vermicomposting can be carried out in mono as well as polycultures without any mortality.

Keywords: *Eisenia fetida*, *Eudrilus eugeniae*, *Perionyx excavatus*

Abbreviations: ANOVA, analysis of variance; **BCM**, blended cattle manure; **C/N**, carbon to nitrogen ratio; **EC**, electrical conductivity; **FS**, fecal streptococci; **FC**, fecal coliform; **MPN**, most probable number; **NH_4-N**, ammonical nitrogen; **NO_3-N**, nitrate nitrogen; **TOC**, total organic carbon; **TN**, total nitrogen; **TP**, total phosphorous; **TC**, total coliform; **OUR**, oxygen uptake rate; **VS**, volatile solids

INTRODUCTION

As a sequel to the age-old practices and socio-economic dependence of rural populations on livestock, India is endowed with the largest livestock population in the world. It accounts for 57% of the world's buffalo population and 15% of the cattle population. In 2003, it had 185.18 million cattle, 97.92 million buffaloes, 0.75 million horses, 124.35 million goats, 61.47 million sheep, 0.63 million camels and 0.61 million donkeys (Livestock Census Report 2003). According to the National Accounts Statistics (2003), Government of India, only 40-50% of the cattle waste produced annually is used as manure while the remaining is used as domestic fuel. Waste products are often dumped or stored without accounting for the environmental costs. Various methods to store the waste, such as open stockpiles, compost piles, basins, or lagoons are normally adopted which results in considerable loss of nutrients from the waste. Hence, the fertilizer values of animal waste are not being fully utilized. A sustainable approach to handle this will be to treat and reprocess organic waste on-site to produce useful products employing management alternatives, which should be eco-friendly, cheap and fast.

Vermicomposting can be the most economical and sustainable option for cattle waste management. Vermicomposting of different livestock excreta were done by different researchers (Domínguez *et al*. 2001; Gunadi and Edwards 2003). Domínguez *et al*. (2001) studied the growth and reproduction of *Eudrilus eugeniae* in cattle waste solids while Gunadi and Edwards (2003) studied the growth, fecundity, and mortality of the 'epegeic' earthworm *Eisenia fetida*, in a range of different wastes such as fresh cattle manure, fresh pig manure etc. Literature on vermicomposting of cattle manure using monocultures is available but sparse literature is presented on polycultures. Only few studies are available on polycultures of *Aporrectodea caliginosa* and *Lumbricus terrestris* (Nikita *et al*. 2007), *Microscolex dubius* (Fletcher), *Eisenia fetida* (Sav.) and *Allolobophora trapezoides* (Abbott 1980) which documented that *Eisenia* and *Allolobophora* yielded a stable coexistence within 11 weeks but limited literature is available on the polycultures of *E. fetida*, *E. eugeniae* and *P. excavatus*. Suthar and Singh (2008) studied decomposition potential of traditional monoculture and some novel polyculture vermireactors of three earthworm species *E. fetida*, *P. excavatus* and *Lampito mauritii*. Other studies on monoculture and polyculture of *E. fetida*, *E. eugeniae* and *P. excavatus* have been carried out using sewage sludge (Khwairakpam and Bhargava 2009a) and filter mud (Khwairakpam and Bhargava 2009b). Therefore, this paper aims to investigate the physicochemical and biological changes in cattle manure due to vermicomposting and the return of nutrients from the waste to the compost produced by mono- and polycultures of *E. fetida*, *E. eugeniae* and *P. excavatus*.

MATERIALS AND METHODS

Earthworm cultures

Three composting species of earthworms, two exotic (*E. fetida*, *E. eugeniae*) and one indigenous (*P. excavatus*) were chosen for the experiment. *E. fetida*, being the most commonly used worm for vermicomposting was utilized for cattle manure (Macci *et al*. 2010). Domínguez *et al*. (2001) reported that *E. eugeniae* is 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. *P. excavatus* is reported to cause notable excellent changes in organic waste resources and could be used efficiently to combat the problem of waste resource management on a low-input basis (Suthar 2007). In the present study, exotic earthworms, *E. fetida* and *E. eugeniae*, were cultured in the laboratory and were randomly picked for experimentation as described

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in Khwairakpam and Bhargava (2009a, 2009b). The indigenous species, *P. excavatus* was collected from the drainage area in the Indian Institute of Technology, Roorkee campus by hand sorting method. The species were identified at the National Zoological Survey of India, Solan, India, before culturing in the laboratory.

Blended cattle manure

Cattle manure was procured from a dairy and nearby villages in Roorkee, India. The cattle manure was decomposed for 2 weeks before using it for the vermicomposting process. The partially degraded cattle manure (0.95 kg) was then blended with saw dust (0.05 kg) to improve the C/N ratio. The C/N ratio plays an important role in determining the quality of compost; thus, sawdust was added as a bulking agent to increase the C/N ratio to 26 as earthworms can grow better when the C/N ratio of the material is about 25 (Butt 1993; Ndegwa and Thompson 2000). The obtained mixture, blended cattle manure (BCM), was used as the raw material for the vermicomposting process. The initial characteristics of BCM are: pH, 7.9 ± 0.2; electrical conductivity (EC), 0.24 ± 0.05 S m^{-1}; ash content, 11.8 ± 0.3 %; total organic carbon (TOC), 51.1 ± 1 %; total nitrogen (TN), 1.9 ± 0.15 %; total phosphorous (TP), 6.1 ± 0.1 g kg^{-1}; C/N, 25.7 ± 1.5; sodium (Na), 0.6 ± 0.05 %; potassium (K), 1.6 ± 0.5 %; calcium (Ca), 3.0 ± 0.75 %.

Experimental set up

The experiments were conducted in triplicate, in perforated cylindrical plastic 6 L containers. The containers were kept in a temperature controlled experimentation room at 25 ± 1°C, which is the optimum temperature range for all three species (Reinecke *et al.* 1992; Viljoen and Reinecke 1992). 10 cm bedding of vermicompost was kept in all the containers. Approximately 50 g of earthworms, equivalent to ~100-120 earthworms, both clitellated (adult) and juvenile (young) were used for the experiment. The earthworms were placed on the bedding and kept undisturbed for one day so that they could enter into the bedding material and get acclimatized to the new environment. The substrate BCM (1.2 kg) was placed on the surface of the bedding the next day so that the earthworms could come up and start consuming the substrate. The earthworms used for experimentation were mono- and polycultures of *E. fetida*, *E. eugeniae* and *P. excavatus*: i) *E. fetida* (R_1), ii) *E. eugeniae* (R_2), iii) *P. excavatus* (R_3), iv) *E. fetida* + *E. eugeniae* (R_4), v) *E. fetida* + *E. eugeniae* + *P. excavatus* (R_5), vi) *E. eugeniae* + *P. excavatus* (R_6), vii) *E. fetida* + *P. excavatus* (R_7), viii) control (R_8). The polycultures were prepared using the earthworm species in equal proportions (w/w) and one control (without any worms) was also kept for comparison.

The moisture level was maintained at about 50-60% throughout the study period by periodic sprinkling of tap (potable) water. To prevent moisture loss, the experimental containers were covered with jute gunny bags (which include apertures for aeration).

Compost analysis

About 110 g of homogenized wet samples (free from earthworms, hatchlings and cocoons) were taken out on day zero and on the 15^{th}, 30^{th} and 45^{th} day of the composting period. Day zero refers to the sample taken out before earthworm inoculation. Triplicate samples were collected and stored at 4°C for assessment of stability parameters i.e. oxygen uptake rate (OUR) and CO_2 evolution as described in Kalamdhad *et al.* (2008). Bacterial population (1: 10, w/v, waste: water extract) including total coliforms (TC), fecal streptococci (FS) and fecal coliforms (FC) was measured by the multiple fermentation method using lactose broth (APHA 1998). Sub-samples were air dried, ground to pass through a 0.2 mm sieve for further analysis. Each sub-sample was analyzed for the following parameters: pH and EC (1: 10, w/v, waste: water extract), ash content (550°C for 2 h) (loss on ignition), TN using the Kjeldahl method, ammonical nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) using KCL extraction (Tiquia and Tam 2000), TOC determined by a Shimadzu (TOC-V_{CSN}) Solid Sample Module (SSM-5000A), TP by acid digestion using the stannous chloride method (APHA 1998), potassium (K), calcium (Ca) and sodium (Na) by acid digestion using a flame photometer (TMF-45, Toshniwal, India), trace element Fe (acid digest) was analyzed using atomic absorption spectroscopy (GBC Avanta 1.31) (APHA 1998). In addition, earthworm growth-related parameters like earthworm biomass and total mortality were measured at the end of the vermicomposting process. The earthworms were separated from the compost by light separation method in which unsorted finished compost was placed under a light for few minutes. The earthworms tend to move downwards away from the light towards the bottom. When all the earthworms move away at the bottom; the upper compost, free of adult earthworms was collected as the finished vermicompost. Cocoons and hatchlings were sorted from the finished vermicompost by hand sorting method using magnifying glass if required. Hatchlings were very small and delicate so they were measured without washing and weighing. After this earthworms at the bottom were washed in tap water to remove the adhering material from their bodies and subsequently weighed on live weight basis.

Statistical analysis

All results reported are the means of three replicates. The results were statistically analyzed at $P = 0.05$ using one-way analysis of variance (ANOVA) and Tukey's HSD test was used as a post-*hoc* analysis to compare the means (SPSS Package, v. 16).

RESULTS AND DISCUSSION

pH

There were only slight changes in the pH value of the BCM as shown in **Table 1**. pH decreased from an alkaline range (7.9) to 7.3, 7.8, 7.5 in monoculture reactors, 7.4, 7.1, 7.1, 7.3 in polyculture reactors except for the control (8.1). Other workers (Atiyeh *et al.* 2000; Gunadi and Edwards 2003) have also reported similar observations. pH was also observed to be reduced for both mono and polyculture reactors when substrates like sewage sludge and filter mud were utilized (Khwairakpam and Bhargava 2009a, 2009b). The lower pH in the final products may be due to CO_2 and organic acids produced during microbial metabolism (Elvira *et al.* 1998). On analyzing the results by ANOVA (**Table 1**), the decrease in pH values was insignificant ($P < 0.05$) between the reactors.

Electrical conductivity (EC)

The level of soluble salts was lower in the presence of earthworms. This indicates that vermicompost may be a suitable material for both soil amendments and plant growth, without producing toxic effects due to high salt concentrations. There were very little reductions in EC for all the reactors (**Table 1**), which may be attributed to loss of soluble salts by leaching and/or microbial immobilization, and/or to formation of insoluble salts. The reductions in the monocultures were almost similar to the reduction in the polycultures, no significant differences were observed. However a gradual increase in EC was observed for other substrates (Khwairakpam and Bhargava 2009a, 2009b). The maximum reduction in the monocultures was observed in R_1 (0.3 S m^{-1}) and R_5 (0.32 S m^{-1}) in the polycultures. On analyzing the results by ANOVA (**Table 1**), the decrease in EC values was insignificant ($P < 0.05$) between the reactors.

Ash content

The ash content is an important indicative parameter for decomposition and mineralization of the substrate. The ash content was observed to be increasing with the increase in vermicomposting time. Faster rate of increase in ash content indicated the higher rate of volatilization, which is a good measure of degradation of the organic waste. In the monocultures there were increments of 1-2 folds, while the polycultures showed the fastest rate of volatilization and simultaneous 1.5-3.5 folds (approximately) increase in ash content (**Table 1**). Similar observations have also been reported

Table 1 Variation in pH, EC and ash content during vermicomposting.

Reactors	pH			EC (S m^{-1})			Ash Content (%)		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
R_1	7.9 ± 0.15 acd	7.6 ± 0.10 a	7.3 ± 0.1 ac	0.29 ± 0.09 a	0.28 ± 0.09 a	0.3 ± 0.08 a	15.6 ± 0.4 a	16.0 ± 0.4 a	21.2 ± 0.4 ad
R_2	7.8 ± 0.10 ad	7.6 ± 0.10 a	7.8 ± 0.1 b	0.29 ± 0.09 a	0.27 ± 0.06 a	0.31 ± 0.08 a	16.8 ± 0.5 a	18.6 ± 0.4 bg	23.4 ± 0.5 c
R_3	8.1 ± 0.20 acb	8.1 ± 0.20 bd	7.5 ± 0.1 c	0.34 ± 0.10 a	0.27 ± 0.07 a	0.37 ± 0.08 a	12.6 ± 0.4 b	14.1 ± 0.4 c	22.2 ± 0.4 ac
R_4	8 ± 0.20 abcd	7.9 ± 0.15 abc	7.4 ± 0.1 ac	0.34 ± 0.10 a	0.26 ± 0.05 a	0.36 ± 0.09 a	11.9 ± 0.3 b	18.4 ± 0.5 g	25.8 ± 0.5 e
R_5	7.6 ± 0.10 d	7.7 ± 0.10 ac	7.1 ± 0.1 a	0.32 ± 0.1 0a	0.23 ± 0.04 a	0.31 ± 0.08 a	22.0 ± 0.6 c	37.5 ± 0.9 d	40.7 ± 1.0 f
R_6	8.4 ± 0.20 cb	7.7 ± 0.10 ac	7.2 ± 0.1 a	0.37 ± 0.10 a	0.26 ± 0.05 a	0.34 ± 0.08 a	12.5 ± 0.3 b	30.5 ± 0.8 e	37.8 ± 0.8 b
R_{17}	8.4 ± 0.20 b	8.1 ± 0.20 c	7.3 ± 0.1 ac	0.35 ± 0.10 a	0.24 ± 0.05 a	0.35 ± 0.08 a	14.8 ± 0.3 d	25.5 ± 0.6 f	37.1 ± 0.6 b
R_8	7.8 ± 0.10 a	7.9 ± 0.10 abc	8.1 ± 0.2 b	0.34 ± 0.10 a	0.31 ± 0.09 a	0.38 ± 0.10 a	12.4 ± 0.3 b	15.1 ± 0.3 ac	19.6 ± 0.4 d

All data represent average of triplicates. Values followed by the same letter within each column are not significantly different.

Table 2 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	48.9 ± 0.8 ac	48.6 ± 0.8 acd	45.7 ± 0.5 a	2.1 ± 0.20 a	2.2 ± 0.21 a	4.3 ± 0.32 a	0.28 ± 0.03 acde	0.25 ± 0.03 a	0.32 ± 0.02 a
R_2	48.2 ± 0.8 c	47.1 ± 0.7 c	44.4 ± 0.4 a	2.2 ± 0.20 a	3.1 ± 0.25 bd	4.1 ± 0.31 a	0.21 ± 0.02 b	0.24 ± 0.02 ac	0.31 ± 0.03 a
R_3	50.6 ± 0.9 ac	49.8 ± 0.9 d	45.1 ± 0.5 a	2.2 ± 0.18 a	2.7 ± 0.23 ab	3.9 ± 0.25 a	0.23 ± 0.02 ab	0.25 ± 0.03 a	0.31 ± 0.02 a
R_4	51.0 ± 1.0 a	47.2 ± 0.8 ac	43.0 ± 0.3 a	2.0 ± 0.16 a	3.5 ± 0.30 dc	3.8 ± 0.25 a	0.24 ± 0.02 ab	0.22 ± 0.02 ac	0.29 ± 0.02 a
R_5	45.2 ± 0.5 b	36.2 ± 0.2 b	34.3 ± 0.3 b	2.1 ± 0.17 a	3.4 ± 0.30 dc	4.1 ± 0.31 a	0.34 ± 0.03 c	0.46 ± 0.04 b	0.55 ± 0.04 b
R_6	50.7 ± 1.0 a	40.2 ± 0.6 e	36.1 ± 0.5 b	2.3 ± 0.20 a	3.9 ± 0.30 c	4.5 ± 0.35 a	0.31 ± 0.03 dc	0.17 ± 0.01 c	0.59 ± 0.05 b
R_{17}	49.4 ± 0.9 ac	43.1 ± 0.7 f	36.5 ± 0.4 b	2.1 ± 0.16 a	2.8 ± 0.26 abd	4.1 ± 0.31 a	0.32 ± 0.03 ce	0.45 ± 0.04 b	0.59 ± 0.06 b
R_8	50.8 ± 1.0a	49.2 ± 0.8 ad	46.6 ± 0.7 a	2.0 ± 0.15 a	2.3 ± 0.22 a	2.6 ± 0.18 b	0.19 ± 0.01 b	0.16 ± 0.02 a	0.29 ± 0.07 a

All data represent average of triplicates. Values followed by the same letter within each column are not significantly different.

by Atiyeh *et al*. (2000) and Singh *et al*. (2003). 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. On analyzing the results by ANOVA, the increase in ash content varied significantly between the reactors ($P < 0.05$).

Total organic carbon

TOC decreased as the vermicomposting time increased as shown in **Table 2**. Percentage decrease in monocultures as well as polycultures was very fluctuating 4-11% and 10-32%. The highest reduction was observed in R_5 of the polycultures (32.7%). The observed results are supported by those of other authors (Elvira *et al*. 1998; Kaviraj and Sharma 2003) who have reported 20-45% loss of carbon as CO_2 during vermicomposting of municipal or industrial wastes. On analyzing the results by ANOVA, TOC varied significantly between the reactors ($P < 0.05$).

Total nitrogen, ammonical nitrogen, nitrate nitrogen

TN content increased as a result of loss of dry mass with significant differences between the reactors (**Table 2**). A 2.0-2.1–fold increase was observed in the reactors R_1, R_2, R_3 (monocultures) while a 1.93-2.30–fold increase was observed in the reactors R_4, R_5, R_6, R_7 (polycultures). The maximum increase was observed in R_6 (2.3–fold) of the polycultures and the minimum increase was observed in R_8 (control) with 1.32–fold only. Increase in total nitrogen has also been observed by other authors (Suthar and Singh 2008; Garg and Yadav 2009). The loss of dry mass (organic carbon) in terms of CO_2 as well as water loss by evaporation during mineralization of organic matter (Viel *et al*. 1987) might have determined the relative increase in nitrogen. However, in general, the final content of nitrogen in vermicomposting is dependent on initial nitrogen present in the waste and the extent of decomposition (Crawford 1983). 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 this element is lost as volatile ammonia at high pH values (Hartenstein and Hartenstein 1981). On analyzing the results by ANOVA, TN varied significantly between the reactors ($P < 0.05$).

NH_4-N and NO_3-N increased for all worm worked reactors (mono- and polycultures) with the increase in vermicomposting time (**Tables 2, 3**). On analyzing the results by ANOVA, NH_4-N and NO_3-N varied significantly between the reactors ($P < 0.05$) on 15^{th}, 30^{th} and 45^{th} sampling days.

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 nitrogen unless the ratio is in the order of 20 or less (Edwards and Bohlen 1996). The final C/N ratios for the monocultures were in the range of 9-10 while that of polycultures and control were 9-11 and 16.8, 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 (Senesi 1989). The decrease in C/N ratio over time might also be attributed to increase in the earthworm population (Ndegwa and Thompson 2000), 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 nitrogen excrements, increases levels of nitrogen and lowers the C/N ratios (Senapati *et al*. 1980). On analyzing the results by ANOVA, C/N varied significantly between the reactors ($P < 0.05$).

Total phosphorous

TP increased by the end of the vermicomposting process probably because of the mineralization of the organic matter. The increase in TP from the initial (6.1 g kg^{-1}) in monocultures R_1 (7 g kg^{-1}), R_2 (6.8 g kg^{-1}) and R_3 (6.3 g kg^{-1}) and control (6.2 g kg^{-1}) were much lesser when compared to the polycultures R_4 (6.5 g kg^{-1}), R_5 (12.6 g kg^{-1}), R_6 (8.2 g kg^{-1}) and R_7 (12. g kg^{-1}) as shown in **Table 3**. Increase in TP during vermicomposting is probably through mineralization and mobilization of phosphorus by bacterial and phosphatase activity of earthworms (Edwards and Lofty 1972). Increase in TP was attributed to direct action of worm gut enzymes and indirectly by stimulation of the microflora. On analyzing the results by ANOVA, TP varied significantly between the reactors ($P < 0.05$).

Table 3 Variation in NO_3-N, K and TP during vermicomposting.

Reactors	NO_3-N (%)			K (%)			TP (g kg^{-1})		
	15 days	**30 days**	**45 days**	**15 days**	**30 days**	**45 days**	**15 days**	**30 days**	**45 days**
R_1	0.15 ± 0.01 a	0.21 ± 0.03 ac	0.25 ± 0.03 a	1.7 ± 0.6 a	2.4 ± 0.60 a	2.6 ± 0.70 a	6.1 ±0.1 a	6.5 ± 0.21 ace	7 ± 0.20 a
R_2	ND	0.19 ± 0.02 ab	0.23 ± 0.03 a	1.6 ± 0.5 a	1.8 ± 0.60 a	2.1 ± 0.60 a	6.2 ± 0.15 a	6.6 ± 0.30 c	6.8 ± 0.18 ab
R_3	ND	0.16 ± 0.01 ab	0.2 ± 0.02 ac	1.6 ± 0.5 a	1.9 ± 0.60 a	2.1 ± 0.55 a	6.1 ± 0.15 b	6.1 ± 0.15 b	6.3 ± 0.19 b
R_4	ND	0.12 ± 0.01 b	0.18 ± 0.02 ac	1.7 ± 0.5 a	2.3 ± 0.60a	2.4 ± 0.70 a	6.1 ± 0.10 a	6.3 ± 0.1 0acd	6.5 ± 0.21 ab
R_5	0.18 ± 0.01 cd	0.27 ± 0.03 c	0.45 ± 0.05 be	1.7 ± 0.5 a	2 ± 0.55 a	2.7 ± 0.75 a	6.2 ± 0.10 a	6.5 ± 0.1 0acd	12.6 ± 0.30 c
R_6	0.17 ± 0.01 ac	0.38 ± 0.04 de	0.58 ± 0.07 de	1.7 ± 0.5 a	2.1 ± 0.55 a	2.6 ± 0.65 a	6.4 ± 0.20 a	6.7 ± 0.10 ac	8.2 ± 0.28 d
R_{17}	0.2 ± 0.02 d	0.41 ± 0.05 e	0.54 ± 0.06 e	1.7 ± 0.5 a	2.3 ± 0.65 a	2.7 ± 0.75 a	6.1 ± 0.10 a	6.1 ± 0.1 0d	12.3 ± 0.3 ce
R_8	ND	ND	0.11 ± 0.01 c	1.6 ± 0.5 a	1.7 ± 0.50 a	1.8 ± 0.50 a	6.1 ± 0.10 a	6.2 ± 0.10 ed	6.2 ± 0.10 b

All data represent average of triplicates. Values followed by the same letter within each column are not significantly different.
ND: Non-detectable

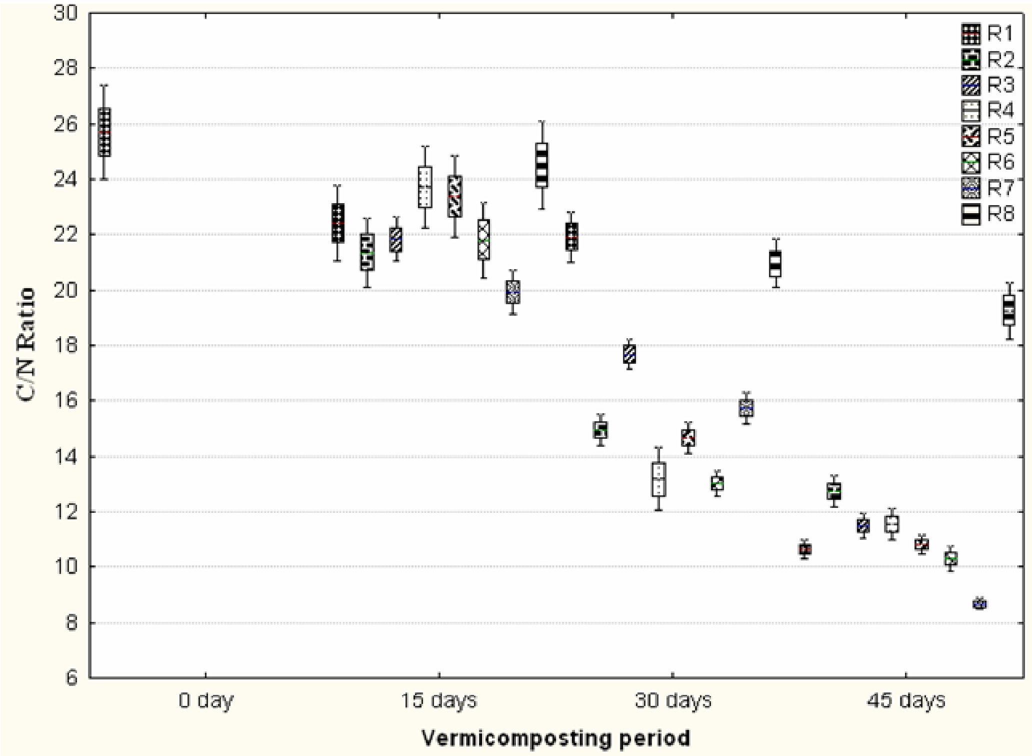

Fig. 1 Variation in C/N ratio during the vermicomposting of cattle manure (n=3).

Macronutrients (K, Na, Ca and Fe)

Potassium was observed to be increasing in all the reactors; it increased by 5-40% in monocultures and in the polycultures the increment was 10-40% and the control increased by 20-28% (**Table 3**). The microflora also influences the level of available potassium. Acid production by the microorganisms is the major mechanism for solubilizing of insoluble potassium. The important acids in phosphorus solubilisation are carbonic, nitric, and sulfuric (Kaviraj and Sharma 2003). On analyzing the results by ANOVA, K value was insignificant ($P < 0.05$) between the reactors.

Sodium reduced by up to 20-50% for the monocultures, 30-70% for polycultures and 20-30% for the control (**Table 4**). There was slight increase in sodium concentration for all the reactors except for R_5 and the control. Similar observations have been reported by Elvira *et al.* (1998) during the vermicomposting of paper-pulp mill sludge by *E. andrei*. On analyzing the results by ANOVA, Na varied significantly between the reactors ($P < 0.05$).

There was increase in calcium concentration for all the reactors; the increments are in the range of 20-27, 13-25 and 2-4% for the monocultures, polycultures and control respectively (**Table 4**). The highest increment was observed in the reactor with monoculture R_2. In contrast, a decrease in potassium (Elvira *et al.* 1998) and no significant increase in calcium (Elvira *et al.* 1996) have been reported for the vermicomposting of paper-pulp mill sludge. Kaushik and Garg (2003) also reported slightly lower calcium content than in the final cast after vermicomposting of cow dung mixed with solid textile mill sludge. They have attributed this decrease to leaching of the soluble elements by excess water that drained through mass. As water was sprinkled in less quantities in this study that there was no excess water which avoided the leaching of minerals with leachate. On analyzing the results by ANOVA, Ca value was insignificant ($P < 0.05$) between the reactors.

The Fe concentration increased up to 7-8–fold, 3-10–fold and 5-7–fold in the reactors with the monocultures, polycultures and the control (**Table 5**), respectively of which the maximum increase was observed in R_6. On analyzing the results by ANOVA, the Fe value was insignificant ($P < 0.05$) between the reactors on 45th day sample.

Coliforms

Coliforms are the indicators of the presence of pathogens. Use of such an indicator, as opposed to the actual disease-causing organisms, is advantageous as the indicators generally occur at higher frequencies than the pathogens and are simpler and safe to detect. The average number of fecal coliforms was 4.3×10^6 at the beginning of the composting process and decreased considerably to 2.3×10^4-1.5×10^4 MPN g^{-1} for monocultures, 2.1×10^4-2.3×10^3 MPN g^{-1} for polycultures and 2.3×10^5 MPN g^{-1} for the control (**Table 5**). The reduction was presumably because of the elimination of the coliforms as they enter the food chain of the earthworm. Fecal Streptococci are commonly considered to be

Table 4 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	0.58 ± 0.04 a	0.5 ± 0.03 a	0.3 ± 0.02 ac	3.2 ± 0.8 a	3.3 ± 0.85 a	3.6 ± 0.85 a	0.35 ± 0.01 a	0.51 ± 0.04 a	4.79 ± 1.4 a
R_2	0.5 ± 0.03 a	0.28 ± 0.02 c	0.24 ± 0.02 a	3.2 ± 0.8 a	3.6 ± 0.9 a	3.8 ± 0.95 a	0.46 ± 0.03 a	0.57 ± 0.04 a	5.39 ± 1.6 a
R_3	0.34 ± 0.03 b	0.36 ± 0.03 bc	0.28 ± 0.02 a	3.2 ± 0.8 a	3.3 ± 0.9 a	3.6 ± 0.80 a	0.57 ± 0.04 a	0.71 ± 0.05 a	4.88 ± 1.5 a
R_4	0.54 ± 0.04 a	0.42 ± 0.04 ab	0.14 ± 0.01 d	3 ± 0.75 a	3.1 ± 0.85 a	3.4 ± 0.80a	0.58 ± 0.04 a	0.38 ± 0.02 a	2.36 ± 0.9 a
R_5	0.54 ± 0.04 a	0.46 ± 0.04 a	0.3 ± 0.03 ac	3.2 ± 0.85 a	3.6 ± 0.9 a	3.9 ± 1.00 a	4.88 ± 1.20 b	4.94 ± 1.30 b	5.7 ± 1.54 a
R_6	0.5 ± 0.04 a	0.42 ± 0.03 ab	0.36 ± 0.03 cb	3.2 ± 0.8 a	3.3 ± 0.95 a	3.4 ± 0.85 a	5.11 ± 1.40 b	6.09 ± 1.70 b	6.41 ± 1.7 a
R_{17}	0.56 ± 0.04 a	0.36 ± 0.03 bc	0.26 ± 0.02 a	3.1 ± 0.75 a	3.6 ± 0.95 a	3.8 ± 1.00 a	4.91 ± 1.10 b	6.09 ± 1.60 b	6.39 ± 1.6 a
R_8	0.53 ± 0.04 a	0.44 ± 0.04 ab	0.42 ± 0.03 b	3 ± 0.75 a	3.1 ± 0.85 a	3.1 ± 0.85 a	0.49 ± 0.04 a	5.84 ± 1.50 b	4.52 ± 1.4 a

All data represent average of triplicates. Values followed by the same letter within each column are not significantly different.

Table 5 Variation in FS, FC during vermicomposting.

Reactors	FS (MPN g^{-1})			FC (MPN g^{-1})		
	15 days	**30 days**	**45 days**	**15 days**	**30 days**	**45 days**
R_1	$7.5\times10^4 \pm 1000$ a	$4.3\times10^3 \pm 600$ a	$2.4\times10^2 \pm 70$ af	$1.5\times10^5 \pm 980$ a	$4.3\times10^4 \pm 800$ a	$2.1\times10^4 \pm 700$ ag
R_2	$4.3\times10^4 \pm 900$ b	$3.9\times10^3 \pm 500$ b	$2.4\times10^2 \pm 70$ b	$9.3\times10^5 \pm 990$ b	$2.3\times10^5 \pm 900$ b	$2.3\times10^4 \pm 760$ b
R_3	$1.5\times10^4 \pm 900$ c	$7.5\times10^3 \pm 700$ a	$1.2\times10^2 \pm 50$ c	$9.3\times10^5 \pm 1000$ b	$4.3\times10^4 \pm 700$ a	$1.5\times10^4 \pm 600$ c
R_4	$9.3\times10^4 \pm 1000$ e	$3.9\times10^3 \pm 550$ c	$4.3\times10^2 \pm 90$ d	$9.3\times10^5 \pm 1900$ ef	$3.9\times10^4 \pm 680$ c	$9.3\times10^3 \pm 400$ d
R_5	$9.3\times10^3 \pm 600$ c	$4.3\times10^2 \pm 100$ d	40 ± 5 e	$3.9\times10^5 \pm 890$ c	$2.1\times10^4 \pm 650$ d	$2.3\times10^3 \pm 300$ e
R_6	$2.1\times10^4 \pm 1000$ c	$9.3\times10^2 \pm 100$ e	60 ± 3 b	$7.5\times10^5 \pm 1000$ f	$9.3\times10^4 \pm 700$ e	$2.3\times10^4 \pm 800$ b
R_{17}	$2.1\times10^3 \pm 300$ c	$2.3\times10^2 \pm 70$ f	$1.5\times10^2 \pm 20$ f	$3.9\times10^5 \pm 1000$ d	$7.5\times10^4 \pm 700$ f	$2.1\times10^4 \pm 800$ g
R_8	$3.9\times10^5 \pm 1000$ d	$7.5\times10^4 \pm 900$ g	$4.3\times10^4 \pm 300$ g	$4.3\times10^6 \pm 1000$ b	$9.3\times10^5 \pm 900$ g	$2.3\times10^5 \pm 900$ f

All data represent average of triplicates. Values followed by the same letter within each column are not significantly different.

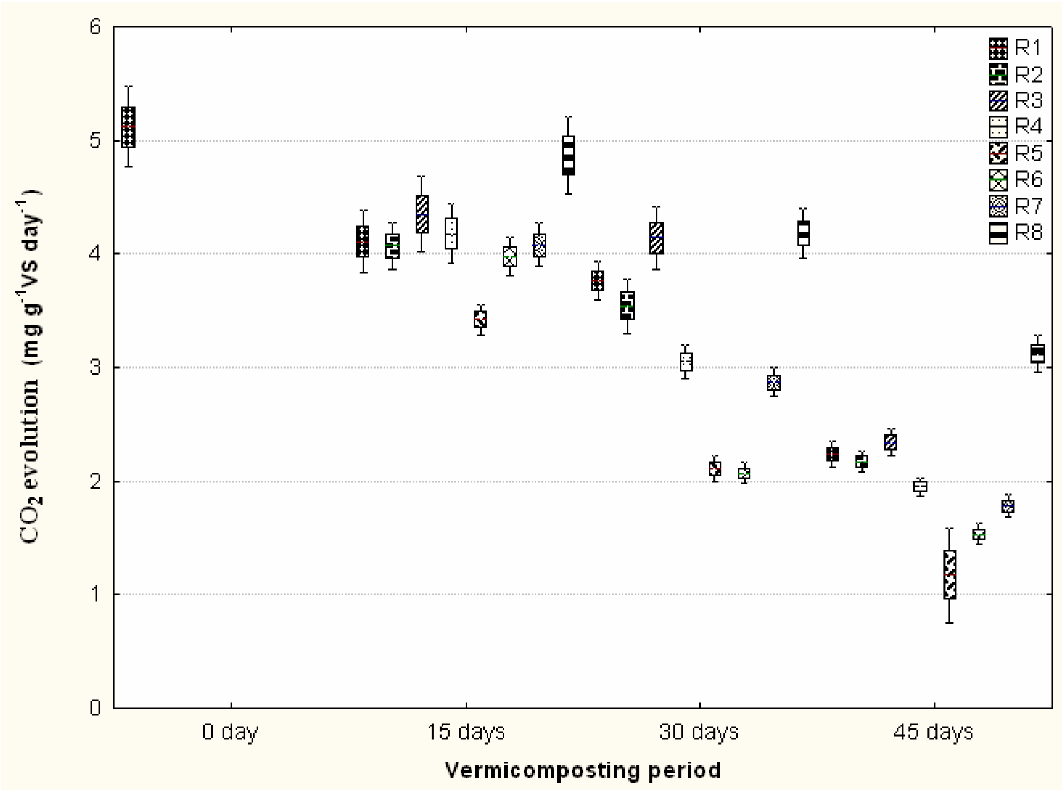

Fig. 2 CO_2 evolution during the vermicomposting of cattle manure (n=3).

the best indicator of fecal contamination. They are more resistant to different environmental factors than the coliforms. The number of fecal Streptococci showed a distinct reduction from 3.9×10^5 MPN g^{-1} to 2.4×10^2-1.2×10^2 MPN g^{-1} for monocultures, 4.3×10^2 -4.2×10^1 MPN g^{-1} for polycultures and 4.3×10^4 MPN g^{-1} for the control. On analyzing the results by ANOVA, TC, FC, FS values were significant ($P < 0.05$) between the reactors.

Oxygen uptake rate

OUR is the most accepted method for the determination of biological activity of a material (Gomez *et al*. 2006). It measures compost stability by evaluating the amount of readily biodegradable organic matter still present in the sample through its carbonaceous oxygen demand. The OUR variation for all the reactors are shown in **Fig. 2**. The microbial activity decreased with composting time as evidenced by the decrease in the values of OURs in all the reactors. Above 50% reduction was observed in the reactors with monocultures and a reduction of above 60-70% was observed in the reactors with polycultures. Maximum reduction was observed in R_5 with 67.02% however, least reduction was observed in control reactor (32.81%) as compared to other reactors, indicating poor decomposition. On an overall the reactors with polycultures showed better reduction than the reactors with monocultures. On analyzing the results by ANOVA, OUR value was significant ($P < 0.05$) between the reactors.

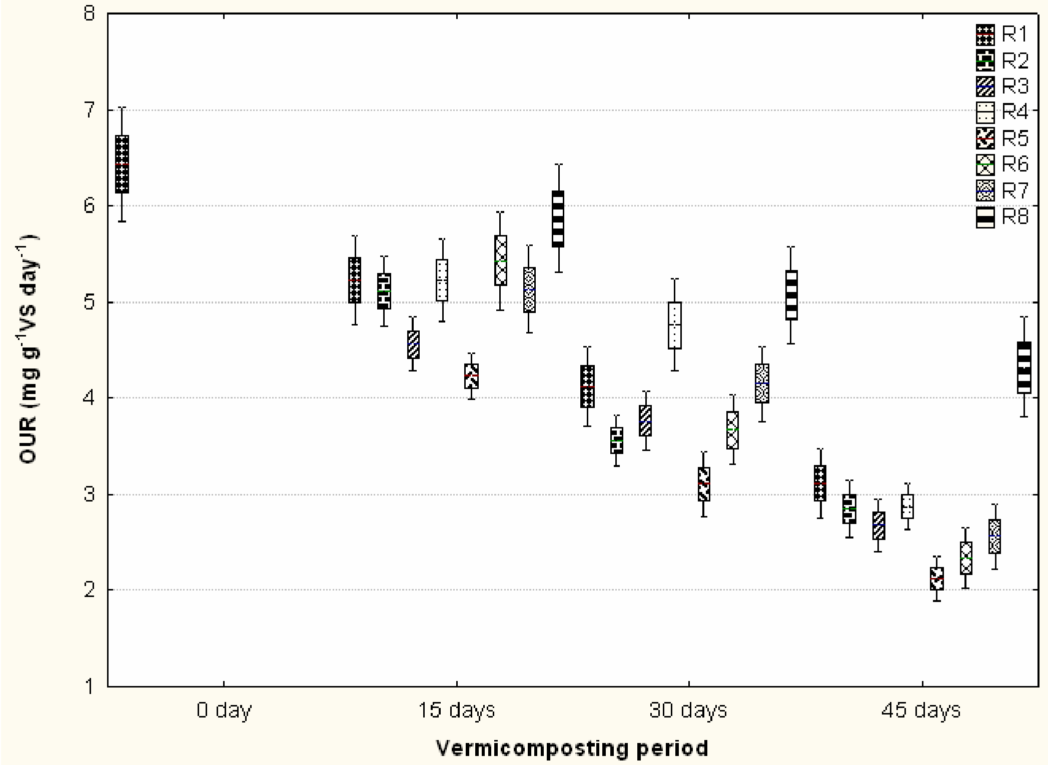

Fig. 3 OUR variation during the vermicomposting of cattle manure (n=3).

Table 6 Live biomass production (earthworms) in different reactors.

Reactors	Earthworms/Combinations	Mean weight of the earthworms in g		Live biomass % change	No. of Cocoons $worm^{-1}day^{-1}$	No. of juveniles hatched/100g of vermicompost
		Initial	Final			
R_1	*E. fetida*	50	55	10	0.37	5
R_2	*E. eugeniae*	50	60	20	0.13	6
R_3	*P. excavatus*	50	50	--	0.22	12
R_4	*E. fetida+ E. eugeniae*	50	55	10	0.10	5
R_5	*E.fetida+ E. eugeniae+ P. excavatus*	50	60	20	0.12	2
R_6	*E. eugeniae+ P. excavatus*	50	60	20	0.03	4
R_{17}	*E. fetida + P. excavatus*	50	64	28	0.05	4
R_8	*E. fetida*	50	55	10	0.37	5

All data represent average of triplicates.

CO_2 evolution

Carbon dioxide evolution is the most direct technique of compost stability because it measures carbon derived directly from the compost being tested. Thus CO_2 evolution directly correlates to aerobic respiration, the truest measure of respiration and hence aerobic biological activity. The CO_2 evolution rates decreased from initial value of 5.1 to 2.4, 2.1, 2.3 mg g^{-1} VS day^{-1} for reactors R_1, R_2 and R_3 while the values were 1.9, 1.3, 1.5 and 1.7 mg g^{-1}VS day^{-1} for reactors R_4, R_5, R_6, and R_7, respectively (**Fig. 3**). On analyzing the results by ANOVA, CO_2 value was significant ($P < 0.05$) between the reactors. The highest decrease in CO_2 evolution (67%) was observed in R_5. The decrease in CO_2 evolution was very low after 45 days of composting in all reactors, indicating the stability of finished compost.

Growth and reproduction of earthworms

The changes in worm biomass for all mono- as well as polycultures over the experimentation period are illustrated in **Table 6**. The earthworm growth and cocoon production for all the combinations of earthworm were more or less similar. The increase in weight of earthworm biomass during the composting period varied between 5-15 g. In general, the greatest increase was recorded in the case of R_7 (28%) and lowest in R_4 (10%). The maximum mean number of cocoons $worm^{-1}$ day^{-1} observed for R_1 (0.37) was the highest of all the cultures while R_6 (0.03) and R_7 (0.05) showed the least value. The mean cocoon production of the polyculture reactors R_4, R_5, R_6, R_7 were very less when compared to monoculture reactors which clearly indicates that monocultures were better off in context of cocoon production. However, in context of the number of juveniles hatched the polyculture reactors showed similar performance as compared to the monocultures reactors.

CONCLUSION

The work reports the potential use of vermicomposting technology in cattle manure management and the return of nutrients. From the present study, it is evident that vermicomposting can be carried out in mono as well as polycultures without any mortality. Reactors with monoculture and polyculture produced good quality compost after 45 days of vermicomposting. However, compost stability studies revealed that compost from polyculture reactors was more matured and stable than the monoculture reactors as confirmed by the OUR results. A considerable amount of worm biomass and cocoons were produced in monoculture reactors, in addition the numbers of juveniles hatched were also the highest among all the reactors. The percentage earthworm biomass change ranged from the lowest of 10 to highest of 28 in the polycultures reactors. On an overall the mono as well as polyculture reactors produced high quality stable compost free from pathogens and no specific differentiation could be inferred between the reactors.

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REFERENCES

Abbott I (1980) Do earthworms compete for food? *Soil Biology and Biochemistry* **12 (6)**, 523-530

Atiyeh RM, Domínguez J, Subler S, Edwards CA (2000) Changes in biochemical properties of cow manure during processing by earthworms (*Eisenia andrei* Bouche) and the effects on seedling growth. *Pedobiologia* **44**, 709-724

APHA (American Public Health Association) (1998) *Standard Methods for the Examination of Water and Wastewater* (17^{th} Edn), APHA, Washington, D.C., pp 992-998

Butt KR (1993) Utilization of solid paper-mill sludge and spent brewery yeast as a feed for soil-dwelling earthworms. *Bioresource Technology* **44**, 105-107

Crawford JH (1983) Review of composting. *Process Biochemistry* **18**, 14-18

Dominguez J, Edwards CA, Ashby J (2001) The biology and population dynamics of *Eudrilus eugeniae* (Kinberg) (Oligochaeta) in cattle waste solids. *Pedobiologia* **45**, 341-353

Edwards CA, Lofty JR (1972) *Biology of Earthworms*, Chapman and Hall, London, UK, 283 pp

Edwards CA, Bohlen PJ (1996) *Biology and Ecology of Earthworms*, Chapman and Hall, London, UK, pp 426

Elvira C, Goicoechea M, Sampedro L, Mato S, Nogales R (1996) Bioconversion of solid paper-pulp mill sludge by earthworms. *Bioresource Technology* **57**, 173-177

Elvira C, Sampedro L, Benitez E, Nogales R (1998) Vermicomposting of sludges from paper mill and dairy industries with *Eisenia andrei*: a pilot scale study. *Bioresource Technology* **63**, 205-211

Gomez RB, Lima FV, Ferrer AS (2006) The use of respiration indices in the composting process: a review. *Waste Management and Research* **24**, 37-47

Gunadi B, Edwards CA (2003) The effect of multiple application of different organic wastes on the growth, fecundity and survival of *Eisenia fetida*. *Pedobiologia* **47**, 321-330

Hartenstein R, Hartenstein F (1981) Physicochemical changes affected in activated sludge by the earthworm *Eisenia fetida*. *Journal of Environmental Quality* **10**, 337-338

Haimi J, Huhta V (1986) Capacity of various organic residues to support adequate earthworm biomass for vermicomposting. *Biology and Fertility of Soils* **2**, 23-27

Kalamdhad AS, Pasha M, Kazmi AA (2008) Stability evaluation of compost by respiration techniques in a rotary drum composter. *Resource Conservation and Recycling* **52**, 829-834

Kaushik P, Garg VK (2003) Vermicomposting of mixed solid textile mill sludge and cow dung with the epigeic earthworm *Eisenia foetida*. *Bioresource Technology* **90**, 311-316

Kaviraj, Sharma S (2003) Municipal solid waste management through vermicomposting employing exotic and local species of earthworms. *Bioresource Technology* **90**, 169-173

Khwairakpam M, Bhargava R (2009a) Vermitechnology for sewage sludge recycling. *Journal of Hazardous Materials* **161 (2/3)**, 948-954

Khwairakpam M, Bhargava R (2009b) Bioconversion of filter mud using vermicomposting employing two exotic and one local earthworm species. *Bioresource Technology* **100 (23)**, 5846-5852

Livestock Census Report (2003) *Directorate of Economics and Statistics*, *Ministry of Agriculture*, Govt. of India, 217 pp

Macci C, Masciandaro G, Ceccanti B (2010) Vermicomposting of olive oil mill wastewaters. *Waste Management and Research* **28**, 738-747

National Accounts Statistics (2003) *Central Statistical Organization*, *Ministry of Programme Planning and Implementation*, Goverment of India, 270 pp

Nikita SEH, Joann KW (2007) Competitive interactions after the growth of *Aporrectodea caliginosa* and *Lumbricus terrestris* (Oligochaeta: Lumbricidae) in single and mixed species laboratory cultures. *European Journal of Soil Biology* **43 (3)**, 142-150

Ndegwa PM, Thompson SA (2000) Effect of C-to-N ratio on vermicomposting of biosolids. *Bioresource Technology* **75 (1)**, 7-12

Reinecke AJ, Viljoen SA, Saayaman RJ (1992) The suitability of *Eudrilus eugeniae*, *Perionyx excavatus* and *Eisenia fetida* (Oligochaeta) for vermicomposting in southern Africa in terms of their temperature requirements. *Soil Biology and Biochemistry* **24**, 1295-1307

Senesi N (1989) Composted materials as organic fertilizers. *Science of the Total Environment* **81/82**, 521-524

Senapati BK, Dash MC, Rane AK, Panda BK (1980) Observation on the effect of earthworms in the decomposition process in soil under laboratory conditions. *Compost Physiology and Ecology* **5**, 140-142

Singh NB, Khare AK, Bhargava DS, Agrawal S (2003) Vermicomposting of tomato skin and seed waste. *Journal Institute of Engineers (India)* **84**, 30-34

Suthar S (2007) Vermicomposting potential of *Perionyx sansibaricus* (Perrier) in different waste materials. *Bioresource Technology* **98**, 1231-1237

Suthar S, Singh S (2008) Feasibility of vermicomposting in biostabilization of sludge from a distillery industry. *Science of the Total Environment* **394**, 237-243

Tiquia SM, Tam NFY (2000) Fate of nitrogen during composting of chicken litter. *Environmental Pollution* **110**, 535-541

Viljoen SA, Reinecke AR (1992) The temperature requirements of the epigeic earthworm species *Eudrilus eugeniae* (Oligochaeta) A laboratory study. *Soil Biology and Biochemistry* **24**, 1345-1350

Viel M, Sayag D, Andre L (1987) Optimisation of agricultural, industrial waste management through in-vessel composting. In: de Bertoldi M (Ed) *Compost: Production, Quality and Use*, Elsevier Applied Science, Essex, pp 230-237

Yadav A, Garg VK (2009) Feasibility of nutrient recovery from industrial sludge by vermicomposting technology. *Journal of Hazardous Materials* **168**, 262-268