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Effects of Various C/N Ratios During Vermicomposting of Sewage Sludge Using *Eisenia fetida*

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ABSTRACT

Sewage sludge is an unavoidable by-product of wastewater treatment processes; its disposal is generally costly or easy to contaminate the environment. Being rich in micro-and macronutrients; therefore, the present study is aimed at alteration of sewage sludge into quality compost product using vermicomposting. In addition, nutrients balance plays a crucial role in the composting process which is expressed as, Carbon to Nitrogen (C/N) ratio. Hence, compost materials were prepared by mixing sewage sludge, cattle manure and saw dust in five different proportions (R1, C/N 15; R2, C/N 20; R3, C/N 25; R4, C/N 30 and R5, control) based on C/N ratios employing an epigeic earthworm *Eisenia fetida*. The results showed that carbon content was decreased during the process and nitrogen content was enhanced. The C/N ratio decreased with time in all the reactors indicating a stabilization of the waste and it can be used in agricultural fields as manure. It was observed that the trial R4 of C/N ratio 30 using sewage sludge along with cattle manure and saw dust produced the best compost, showed higher loss in Total Organic Carbon (TOC), soluble Biochemical Oxygen Demand (BOD), soluble Chemical Oxygen Demand (COD) and higher gain in total nitrogen and phosphorus, implying the total amount of biodegradable organic material is stabilized. In addition, higher final concentration of nutrients and limited metal content suited the quality of compost.

Key words: Vermicomposting, *Eisenia fetida,* sewage sludge, cattle manure, C/N ratio

INTRODUCTION

Presently, sewage sludge is a by-product generated at wastewater treatment plants as a result of the treatment of wastes released from a variety of sources including homes, industries, medical facilities, street runoff and businesses (Singh and Agrawal, 2008). The problem of efficient disposal and management of sewage sludge has become more rigorous due to rapidly increasing of population, economic growth and increasing number of treatment plants. Application of sewage sludge in agriculture will be the most convenient methods of disposal compared to several available alternatives. In addition, sewage sludges have high nutritive value for plants and hence; its application as a soil conditioner and/or fertilizer is widely recommended (Bettiol, 2004). The application of sewage sludge in soils not only accelerates the microbial respiration in cultivable lands; also enhances the crop production as they are rich in nutrients and have higher organic content. But the direct use of these wastes may cause new problems derived from the presence of heavy metals, pathogenic microorganisms, bad odors or phytotoxic organic compounds (Diez *et al.*, 2001; Garcia *et al.*, 1991). On the other hand, the long-term application of sewage sludge in cultivable soils may have depressive effects on the metabolism of soil microorganisms (Ayusho *et al.*,1996).

In the regard, composting is a successful approach for the economical and sustainable recycling of sewage sludge (Fermor, 1993; Tuomela *et al.*, 2000). During composting, organic wastes are transformed through the successive activities of different microbes to a more stable and complex organic matter, resulting that can be helpful in agriculture purposes; made composting a promising alternative (He *et al.*, 1992). The rate and extent of these transformations will depend on the nature of the starting materials and the types of composting. On the other hand, the disadvantages are the long duration of the composting process, regular aeration required and loss of nutrients (e.g., gassing off of nitrogen).

In contrast to traditional microbial waste treatment, vermicomposting has been reported to be feasible, commercial and rapid process for the efficient management of the organic wastes (Harris *et al.*, 1990; Logsdon, 1994; Khwairakpam and Bhargava, 2009a). Hence, 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 involves the combined action of earthworms and microorganisms for the stabilization of organic waste. Eventhough microbes are considered to play a major role in organic matter transformation, earthworms are the important drivers in substrate conditioning. Species recognized as potentially useful to degrade the organic wastes were *Eisenia fetida*, *Eisenia andrei*, *Eudrilus eugeniae*, *Perionyx excavatus* and *Perionyx sansibaricus* (Khwairakpam and Bhargava, 2009a; Wong and Griffiths, 1991; Suthar, 2007). *Eisenia fetida* proved best for processing organic wastes (Edwards and Bater, 1992) and is suitable for vermiculture, because it has a wide temperature tolerance (Reinecke *et al.*, 1992). The survival, growth, mortality and reproduction of these species have been studied methodically in the laboratory, using organic wastes of sewage sludge. The cast excreta thus obtained was used as bio-organic fertilizer.

It has been found that vermicomposting of sewage sludge blended with other materials, e.g., garden wastes, food wastes, paper pulp sludge or other carbon-rich wastes can accelerate decomposition by maceration (Maboeta and von Rensburg, 2003). Moreover, these bulking materials help in improving the C/N by supplying carbon and preventing nitrogen loss (Dominguez *et al.*, 1997). While nutrients balance plays a crucial role in the decomposition process which is expressed as, Carbon to Nitrogen (C/N) ratio. Where, the organic carbon and inorganic nitrogen are involved in cell growth, synthesis and all other vital metabolic activities. To achieve proper nutrition, carbon and nitrogen must be present in the substrate at the accurate ratio. The degradation of organic materials can be accelerated by increasing the C/N ratio to the optimum value by mixing with easily biodegradable carbonaceous compounds (Nakasaki *et al.*, 1992). In addition, the organic fraction of cattle manure plays an important role in increasing soil organic matter and tilth; improving soil structure and water infiltration; also having the tendency to bind the substrates together. Many literatures are available on the vermicomposting of animal excreta, sewage sludge and agroindustrial wastes (Bansal and Kapoor, 2000). However, there is little information available on the combination of sewage sludge, cattle manure and saw dust for the production of vermicompost under optimal conditions based on different C/N ratios.

Therefore, the objective of this study was to study a comparative analysis on vermi-stabilization of sewage sludge using *Eisenia fetida* mixed with cattle manure and saw dust in four different roportions based on C/N ratios (i.e. 15 (R1), 20 (R2), 25 (R3) and 30 (R4)) with blank reactor (R5). Control for each proportion was also analyzed (i.e. CR1, CR2, CR3, CR4 and CR5). The feasibility of sewage sludge vermicomposting using *Eisenia fetida* is evaluated in terms of physico-chemical and bio-chemical parameters; the growth performance *E. fetida* in sludge vermicomposting system was also monitored during this study.

MATERIALS AND METHODS

Vermiculture: The recognized earthworm species (*Eisenia fetida*) was brought from Central Plantation Crops Research Institute (CPCRI), Indian Council of Agricultural Research, Guwahati, India. *E. fetida* were cultured in hopper bottom Perspex bins (450×300×450 mm), fabricated in the laboratory. For aeration and drainage purpose 16 holes of 10 mm diameter were drilled along the longer sides and 16 holes at the bottom, respectively. Hopper was used to collect leachate (if any). Partially degraded cow dung was added for culturing the earthworms.

Compost material: Sewage sludge, cattle manure and saw dust was used for preparation of different waste mixtures. Sewage sludge was collected from the sewage treatment plant of the Indian Institute of Technology Guwahati campus. The treatment plant consists of aerated lagoon system with two units; one unit is acting in stand-by mode for maintenance purposes. Though, this treatment activity is considered to be secondary treatment. Therefore, the sludge procured from the treatment plant is called as secondary sludge. Fresh cattle manure was obtained from nearby Amingaon village. Saw dust were purchased from the nearby rice mill and saw mill, respectively. The compost material was prepared by mixing different proportions (i.e., C/N 15 (R1), 20 (R2), 25 (R3) and 30 (R4) with blank reactor (R5) of the collected waste as described in Table 1. Since the nitrogen content is very high in sewage sludge in the range of 1.91% and also with high metals it can be successfully composted along with cattle manure and saw dust. Control for each proportion was also analyzed (i.e. CR1, CR2, CR3, CR4 and CR5). C/N ratios of the compost mixture were calculated as follows Eq. 1 and 2 (Cornell University, 1996):

$$G = \frac{(M_1 \times Q_1 + M_2 \times Q_2 + M_3 \times Q_3)}{Q_1 + Q_2 + Q_3} \tag{1}$$

where, Q_n is mass of material n (wet weight basis); G is moisture goal (%); M_n is moisture content (%) of material n:

$$R = \frac{Q_1(C_1 \times (100 - M_1)) + Q_2(C_2 \times (100 - M_2)) + Q_3(C_3 \times (100 - M_3)) + \ldots\ldots\ldots}{Q_1(N_1 \times (100 - M_1)) + Q_2(N_2 \times (100 - M_2)) + Q_3(N_3 \times (100 - M_3)) + \ldots\ldots\ldots} \tag{2}$$

where, R is C/N ratio of compost mixture; Q_n is mass of material n (wet weight basis); C_n is carbon (%) of material n; N_n is nitrogen (%) of material n; M_n is moisture content (%) of material n.

By simplifying and rearranging the above equation, the mass of the third material required would be Eq. 3:

$$Q_3 = \frac{RQ_1N_1(100 - M_1) + RQ_2N_2(100 - M_2) - Q_1C_1(100 - M_1) - Q_2C_2(100 - M_2)}{C_3(100 - M_3) - RN_3(100 - M_3)} \tag{3}$$

Table 1: Waste composition and initial characteristics of waste materials

Reactors/Parameters	Waste materials (kg) Sewage sludge	Cattle manure	Sawdust
R1	1.30	0.16	0.04
R2	1.04	0.39	0.07
R3	0.98	0.39	0.13
R4	0.87	0.45	0.18
R5	1.50	--	--
Moisture content (%)	34.16±2.03	80.77±0.04	10.25±0.26
pH	6.03±0.01	6.61±0.07	6.16±0.01
Electrical conductivity (dS m^{-1})	2.77±0.01	3.28±0.21	0.39±0.01
Ash content (%)	61.54±0.32	29.88±3.85	2.41±0.05
Total organic carbon (TOC) (%)	21.37±0.18	38.96±2.14	54.22±0.03
Total nitrogen (%)	1.91±0.22	1.47±0.20	0.40±0.02
Nitrate Nitrogen (NO_3^-N) (%)	0.006±0.004	0.045±0.036	ND*
Ammonical Nitrogen (NH_4^-N) (%)	1.47±0.04	0.54±0.02	0.05±0.02
Total phosphorous (%)	4.99±0.29	4.29±0.26	1.69±0.36
Available phosphorus (%)	1.75±0.09	2.76±0.12	0.98±0.16
C/N ratio	11.19±1.21	26.44±2.50	135.88±7.25
Sodium (Na) (g kg^{-1} dry matter)	1.03±0.28	0.94±0.12	0.55±0.09
Potassium (K) (g kg^{-1} dry matter)	4.83±0.35	6.17±0.19	1.95±0.05
Calcium (Ca) (g kg^{-1} dry matter)	2.03±0.34	1.55±0.21	0.80±0.17
Iron (Fe) (g kg^{-1} dry matter)	1.18±0.29	6.61±0.32	2.19±0.08
Nickel (Ni) (mg kg^{-1} dry matter)	278.0±19.3	231.5±11.4	221.5±23.5
Chromium (Cr) (mg kg^{-1} dry matter)	198.5±0.4	89.2±0.2	124.5±0.5
Manganese (Mn) (mg kg^{-1} dry matter)	355.3±23.0	496.1±17.5	148.5±19.5
Cadmium (Cd) (mg kg^{-1} dry matter)	37.0±2.8	51.5±6.3	58.0±4.3
Copper (Cu) (mg kg^{-1} dry matter)	174.5±10.5	45.5±9.0	37.5±6.5
Lead (Pb) (mg kg^{-1} dry matter)	130.1±8.0	80.5±5.8	155.0±4.5
Ziuc (Zn) (mg kg^{-1} dry matter)	967.2±23.3	124.3±11.5	101.9±17.3
Chemical oxygen demand (COD) (mg L^{-1})	692.5±21.8	351.7±5.1	1196.8±10.3
Biochemical oxygen demand (BOD) (mg L^{-1})	278.4±14.6	235.9±11.2	731.4±15.7

*ND: Not detected

Experimental setup: The experiments were conducted in triplicate, in locally made bamboo containers (reactor) of volume 90.47×10^4 mm^3 (radius-120 mm and depth-90 mm). The containers were kept in the laboratory at room temperature. Bedding size was restricted to 10 cm in all the containers using a mixture of hay (155 g), cattle manure (375 g), mix ture of banana leaves and tree leaves (280 g), respectively which were partially degraded for two week. Approximately, 120 earthworms (*E. fetida*), having both clitellated and juvenile, were inoculated in the bedding for acclimatization to the new environment then the substrate was added the next day. Control reactors were carried out in same manner for degradation the substrate without any worms.

Working volume was maintained to 1.5 kg of five different proportions of sewage sludge, cattle manure and saw dust were added to each of the reactors and they are referred to as R1, R2, R3, R4 and R5, respectively. Control for each mixture was also kept (i.e., CR1, CR2, CR3, CR4 and CR5). The quantity of the substrate was decided based on the findings that the earthworms can consume the material half their body weight per day under favorable conditions (Haimi and Huhta, 1986). The moisture level was maintained about 50-60% throughout the study periodically sprinkling of adequate quantity of tap (potable) water. However, the reactors were covered with gunny bags to prevent moisture loss.

Parameters analysis: About 170 g of homogenized wet samples (free from earthworms, hatchlings and cocoons) were taken out at 0, 15, 30 and 45 day of composting period and stored at 4°C immediately for analysis. The sub-samples were air dried immediately, ground to pass through 0.2 mm sieve and stored for physico-chemical analysis. The 0 day refers to the sample taken out before earthworm inoculation. The percentage of moisture content was calculated following Eq. 4 (Schwab *et al.*, 1994):

$$\text{Moisture (\%)} \frac{\text{Weight}_{\text{Wet}} - \text{Weight}_{\text{Dry}}}{\text{Weight}_{\text{Wet}}} \times 100 \quad (4)$$

Volatile solid/organic matter content was determined by loss ignition method (on dry mass basis) at 550°C for 2 h. The total organic carbon content was calculated from volatile solids (Mohee *et al.*, 2008).

The percentage of nitrogen content was calculated using Kjeldahl method following Eq. 5 (Codell and Verderame, 1954):

$$\text{Total nitrogen(\%)} = \frac{1.4\,(R - S)\,N}{W} \quad (5)$$

where, R is volume of H_2SO_4 to titrate boric acid (mL); S is volume of H_2SO_4 to titrate blank (mL), W is weight of sample (g) and N is normality of H_2SO_4. The carbon-to-nitrogen ratio was determined by dividing the total organic carbon content to the total nitrogen content. Each sub-samples was analyzed for the following parameters: pH and electrical conductivity (EC) (1:10 w/v waste: Water extract with pH and EC meters); total nitrogen using Kjeldahl method, ammoniacal nitrogen (NH_4^-N) and nitrate nitrogen (NO_3^-N) using KCl extraction; available and total phosphorus by acid digestion using stannous chloride method (APHA, AWWA and WPCF, 1995), potassium, sodium and calcium (acid digest) using flame photometry, trace elements including Cr, Ni, Fe, Cd, Pb, Zn and Cu (acid digest) were analyzed using atomic absorption spectroscopy. The biodegradable organic matter was measured as soluble Biochemical Oxygen Demand (BOD) by the dilution method (APHA, AWWA and WPCF, 1995) and soluble Chemical Oxygen Demand (COD) by the dichromate method by Codell and Verderame (1954) for supernatant of the blended mixture of 10 g sample in 100 mL deionized water. In addition earthworm growth related parameters like earthworm biomass and total mortality were measured at the end of every 15th day of the experiment.

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's HSD test was used as a *post hoc* analysis to compare the means using Statistica software.

RESULTS AND DISCUSSION

pH: pH value was observed to increase for all the reactors during 45 days of vermicomposting period (Table 2). Maximum increase in pH was observed in R4 with pH value 7.42. An increase in the pH of final vermicompost possibly due to excess amount of organic nitrogen not required by microbes is released as ammonia, which gets dissolved in water and increases the pH of the vermicompost (Rynk *et al.*, 1992). Similar pH variation was also observed by (Datar *et al.*, 1997). However, other researchers have reported decrease in pH during vermicomposting (Mitchell, 1997; Ndegwa *et al.*, 2000). The respective controls, however, showed same trend in pH variation during 45 days of vermicomposting but shows lesser values as compared to the reactors. The pH variations were significant ($p<0.0001$) in all the reactors.

Table 2: Variation in pH, EC and ash content during vermicomposting

Reactors	0 day	15 day	30 day	45 day
pH				
R1	6.53 ± 0.04^{a}	7.01 ± 0.04^{ade}	7.06 ± 0.04^{a}	7.21 ± 0.01^{ae}
CR1	6.62 ± 0.01^{b}	6.86 ± 0.02^{b}	6.93 ± 0.02^{b}	7.06 ± 0.01^{bf}
R2	6.88 ± 0.07^{cf}	7.12 ± 0.09^{de}	7.17 ± 0.01^{ce}	7.42 ± 0.06^{c}
CR2	6.75 ± 0.02^{dg}	6.89 ± 0.02^{ab}	7.09±0.073	7.15 ± 0.01^{ab}
R3	6.82 ± 0.02^{df}	7.00 ± 0.01^{abde}	7.14 ± 0.02^{ac}	7.27 ± 0.03^{e}
CR3	6.81 ± 0.01^{cdf}	6.90 ± 0.02^{ab}	7.05 ± 0.03^{a}	7.18 ± 0.02^{ae}
R4	6.69 ± 0.02^{bg}	7.07 ± 0.03^{e}	7.26 ± 0.01^{e}	7.42 ± 0.07^{c}
CR4	6.65 ± 0.03^{b}	6.99 ± 0.06^{abde}	7.09 ± 0.03^{ac}	7.20 ± 0.04^{ae}
R5	6.11 ± 0.03^{e}	6.60 ± 0.08^{c}	6.77 ± 0.04^{d}	7.01 ± 0.03^{f}
CR5	6.06 ± 0.01^{e}	6.49 ± 0.03^{c}	6.75 ± 0.02^{d}	6.90 ± 0.01^{d}
Electrical condnctivity (dS m^{-1})				
R1	3.22 ± 0.48^{a}	3.11 ± 0.06^{ad}	3.24 ± 0.05^{ad}	3.52 ± 0.07^{ad}
CR1	2.76 ± 0.01^{b}	2.91 ± 0.01^{b}	3.06 ± 0.04^{b}	3.18 ± 0.03^{b}
R2	2.92 ± 0.04^{a}	3.11 ± 0.06^{ad}	3.45 ± 0.05^{ce}	3.58 ± 0.07^{a}
CR2	2.83 ± 0.02^{a}	2.99 ± 0.03^{ab}	3.17 ± 0.02^{bd}	3.25 ± 0.02^{b}
R3	3.06 ± 0.09^{a}	3.22 ± 0.10^{dc}	3.52 ± 0.07^{ce}	3.71 ± 0.04^{c}
CR3	3.03 ± 0.02^{a}	3.28 ± 0.02^{cde}	3.37 ± 0.03^{ac}	3.46 ± 0.02^{de}
R4	3.24 ± 0.04^{ab}	3.40 ± 0.09^{e}	3.57 ± 0.04^{e}	3.73 ± 0.04^{c}
CR4	3.17 ± 0.02^{a}	3.31 ± 0.03^{ce}	3.39 ± 0.03^{ac}	3.55 ± 0.03^{ad}
R5	3.01 ± 0.06^{a}	3.17 ± 0.12^{acd}	3.24 ± 0.11^{ad}	3.47 ± 0.03^{ade}
CR5	2.95 ± 0.03^{a}	3.03 ± 0.02^{ab}	3.19 ± 0.02^{bd}	3.37 ± 0.01^{e}
Ash content (%)				
R1	51.67 ± 0.59^{ac}	58.57 ± 0.59^{ade}	63.12 ± 0.67^{ae}	65.80 ± 0.67^{a}
CR1	51.48 ± 0.33^{ab}	56.81 ± 0.18^{abf}	59.48 ± 0.39^{db}	61.67 ± 0.41^{b}
R2	52.73 ± 1.04^{ac}	59.09 ± 0.14^{abd}	63.32 ± 1.17^{ae}	68.19 ± 0.63^{c}
CR2	53.49 ± 0.23^{cdf}	57.12 ± 0.09^{ab}	61.59 ± 0.31^{abde}	64.23 ± 0.30^{e}
R3	49.80 ± 0.89^{b}	56.33 ± 1.21^{f}	60.55 ± 0.58^{db}	65.08 ± 0.28^{c}
CR3	45.48 ± 0.40^{e}	50.54 ± 0.28^{c}	55.80 ± 0.58^{c}	57.63 ± 0.28^{f}
R4	45.99 ± 0.85^{e}	55.48 ± 2.19^{bf}	62.86 ± 1.89^{abe}	69.94 ± 0.45^{g}
CR4	46.08 ± 0.43^{e}	51.95 ± 0.26^{c}	56.82 ± 0.08^{c}	59.77 ± 0.38^{b}
R5	54.63 ± 0.79^{fg}	59.86 ± 0.44^{e}	63.99 ± 0.73^{e}	67.50 ± 0.44^{c}
CR5	55.65 ± 0.33^{g}	59.59 ± 0.16^{ae}	62.34 ± 0.38^{abd}	63.43 ± 0.41^{ae}

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p<0.05$)

Electrical conductivity (EC): A gradual increase in EC value was observed during 45 days for all the reactors including respective controls (Table 2). The EC was increased in the range of 8.5-18.4% for vermireactors and 10.7-13.2% for control, respectively. The increase in EC may be due to the loss of weight of organic matter and release of different mineral salts in available forms (such as phosphate, ammonium and potassium) as reported by other researchers (Garg *et al.*, 2006; Suthar, 2007). The differences in EC between reactors and respective controls were found significant ($p<0.0001$).

Ash content: The ash content is an important indicative parameter for decomposition and mineralization of the vermicomposting material. The ash content increased with the composting time by about 21.4% for R1; 22.6% for R2; 26.9% for R3; 34.2% for R4 and 19.1% for R5 and 16.5% for CR1; 16.7% for CR2; 23.7% for CR3; 24.9% for CR4 and 13.6% for CR5, respectively, due to the loss of organic matter through microbial degradation (Table 2). Faster rate of increase in ash content indicated the higher rate of volatilization, which is a good measure of degradation of the organic waste (Khwairakpam and Bhargava, 2009b). The maximum increase in the ash content was observed in R4 indicates that more degradation takes place during vermicomposting. The increase in the ash content illustrates that earthworms are consuming the wastes at a faster rate and the microbial assimilation is also performing the degradation process rapidly (Gupta and Garg, 2008). The ash contents in vermicomposting reactors were significantly different from control samples ($p<0.0001$).

Nitrogen dynamics: Total nitrogen consists of the inorganic forms of nitrogen; ammonium (NH_4-N) and nitrate (NO_3-N). Total nitrogen as shown in Fig. 1 was higher in final products as

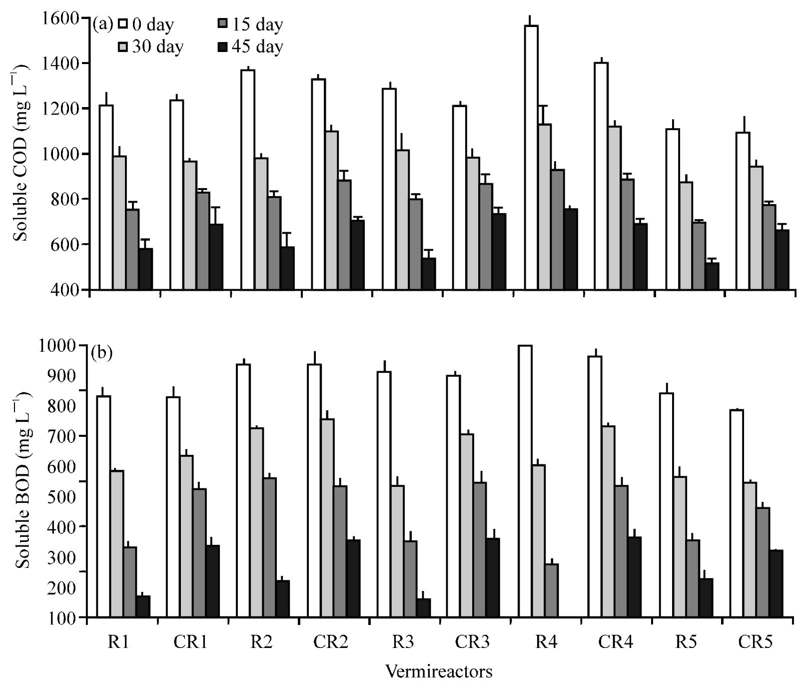

Fig. 1(a-b): Nitrogen dynamincs during vermicomposting compared to initial substrates with 1.62-2.01, 1.22-1.49, 1.10-1.45, 0.94-1.62, 1.86-2.10% increase in R1, R2, R3, R4 and R5, respectively. Initial total nitrogen content was higher in R5 followed by R1 due to the presence of huge amount of sewage sludge, which contains higher total nitrogen content. However, the maximum increase was observed in R4 followed by a similar increment in the rest of the reactors; in addition, the control had the minimum increase. Total nitrogen increased during the vermicomposting process due to the net loss of dry mass in terms of CO_2 during oxidization of organic matter (Fang *et al.*, 1999; Huang *et al.*, 2004).

The changes in concentration of NH_4-N and NO_3-N in all reactors followed the similar trend during the vermicomposting. During the process, NH_4-N concentration decreased from 0.74-0.14% in R1, 0.80-0.11% in R2, 0.71-0.15% in R3, 0.77-0.04% in R4 and 0.81-0.18% in R5, respectively (Fig. 1). However, the maximum decrease was observed in R4 provides better compost as compared to other reactors; in addition, the control had the minimum decrease. Higher initial NH_4-N concentration could be due to the conversion of organic nitrogen to NH_4-N through volatilization and immobilization (Huang *et al.*, 2004; Hirari *et al.*, 1983). It has been noted that the absence or decrease in NH_4-N is an indicator of both high-quality compost (Hirari *et al.*, 1983).

Initial nitrate is almost absent in the cattle manure and saw dust but higher concentration is prevalent in sewage sludge. The concentration of NO_3-N was almost nil at 0 day, due to inhibition by excessive amount of ammonia. A gradual increase was observed in NO_3-N concentration in all reactors including respective controls (Fig. 1). However, a decrease in NH_4-N occurred which correspond to an increase in NO_3-N at the end of the vermicomposting process. 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 nitrogen would be due to immobihzation/denitrification or both. Variation in total nitrogen content, NH_4-N and NO_3-N was observed to be significant in all the reactors ($p<0.0001$).

Total organic carbon (TOC): TOC was lower in the final products as compared to the initial values (Fig. 2). The maximum carbon loss was observed in R4 (44.3%) followed by R3 (36.5%), R2 (32.6%), R1 (29.2%) and R5 (28.3%), respectively. All the respective control reactors showed a similar pattern of change in TOC, with a reduction in the range of 19.8-28.4% at the end of the process. During vermicomposting a large fraction of TOC is lost as CO_2 due to the consumption of the available carbon as a source of energy by the earthworms and microbes that

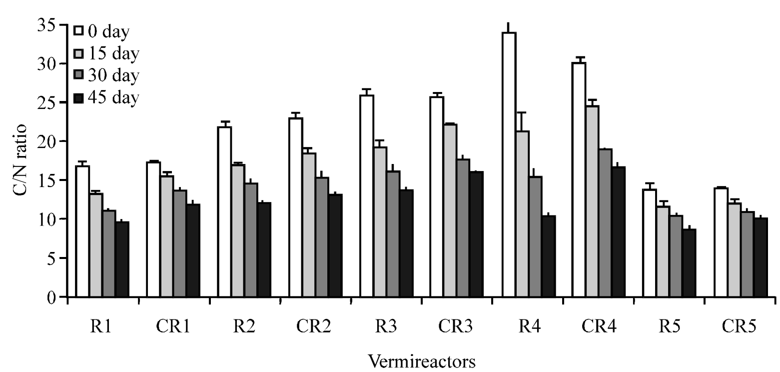

Fig. 2: Total organic carbon content during vermicomposting

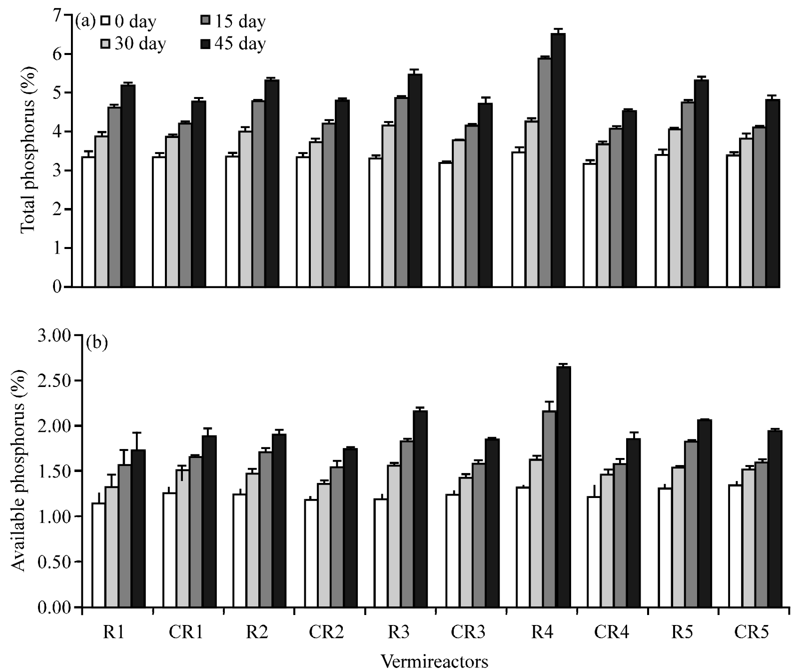

Fig. 3(a-b): Variation in total and available phosphorus during vermicomposting (a) Total phosphorus (%) and (b) Available phosphorus (%)

cause reduction of TOC value in vermicompost samples (Khwairakpam and Bhargava, 2009b). TOC value in the different vermicomposting reactors were statistically significant ($p<0.0001$).

Phosphorus: Phosphorous content gradually increased during the vermicomposting process because of the mineralization of the organic matter. The changes of total and available phosphorus with a gradual increase were observed throughout the vermicomposting period (Fig. 3). The maximum increase in total phosphorus was observed in R4 (46.9%) followed by R3 (39.6%), R2 (35.8%), R5 (35.5%) and R1 (34.9%) and in control reactors, the maximum increase in total phosphorus was observed in CR3 (31.9%) followed by CR2 (29.5%), CR5 (29.4%), CR1 (29.3%) and CR4 (29.1%), respectively. Increase in total phosphorus during vermicomposting maybe due to mineralization and mobilization of phosphorus by bacterial and faecal phosphatase activity of earthworms (Edwards and Lofty, 1972). Increase in total phosphorus was attributed to direct action of worm gut enzymes and indirectly by stimulation of the micro flora. Similar observations were found for available phosphorus. On analyzing the results by ANOVA, the variation in total phosphorus and available phosphorus content on all the sampling days obtained from different reactors was significant ($p<0.0001$).

Nutrients (Na, K, Ca and Fe): Table 3 shows the concentration of the macronutrients; namely total K, Na, Ca and Fe in all reactors throughout the vermicomposting process. These nutrients are used as mineral fertilizers in the compost. All reactors showed a similar pattern of changes in macronutrients. Macronutrients K, Na, Ca and Fe were gradually increase till the end of the composting due to due to the net loss of dry mass. R4 showed greater amounts of the three macronutrients except calcium throughout the composting process as compared to other reactors could be due to higher amount of cattle manure coupled with sewage sludge which represents

Table 3: Variations in macronutrients during vermicomposting

Days	R1	CR1	R2	CR2	R3	CR3	R4	CR4	R5	CR5
Sodium (g kg^{-1})										
0	1.84±0.13^{a}	1.71±0.04^{ac}	1.49±0.08^{beg}	1.65±0.06^{ab}	1.54±0.08^{cbfe}	1.71±0.03^{af}	1.29±0.03^{dgh}	1.37±0.03^{eh}	1.15±0.09^{d}	1.24±0.06^{dh}
15	2.23±0.20^{a}	2.03±0.08^{ab}	1.88±0.04^{b}	1.84±0.09^{b}	2.04±0.14^{ab}	2.01±0.08^{ab}	1.83±0.09^{bc}	1.74±0.07^{be}	1.31±0.17^{df}	1.52±0.08^{cef}
30	2.80±0.12^{a}	2.21±0.02^{b}	2.09±0.06^{b}	2.12±0.03^{b}	2.61±0.04^{a}	2.23±0.09^{b}	2.76±0.12^{a}	2.30±0.06^{b}	1.59±0.07^{c}	1.69±0.06^{c}
45	3.37±0.27^{a}	2.71±0.03^{b}	2.53±0.10b^{c}	2.25±0.06^{cd}	3.26±0.12^{a}	2.42±0.07^{bc}	3.43±0.14^{a}	2.68±0.06^{b}	2.54±0.03^{bc}	2.07±0.04^{d}
Potassium (g kg^{-1})										
0	6.53±0.08^{a}	6.24±0.11^{b}	7.43±0.04^{c}	7.53±0.02^{cd}	7.67±0.13^{cd}	7.71±0.04^{d}	8.23±0.04^{e}	8.13±0.08^{e}	5.11±0.10f	4.68±0.11^{g}
15	6.81±0.09^{a}	6.63±0.05^{a}	7.80±0.12^{b}	7.82±0.07^{b}	7.89±0.02^{b}	7.92±0.03^{b}	8.79±0.09^{c}	8.58±0.04^{d}	5.40±0.09^{e}	4.87±0.04^{f}
30	7.10±0.03^{a}	6.97±0.05^{b}	8.18±0.06^{c}	8.07±0.04^{cd}	8.09±0.03^{cd}	8.05±0.03^{d}	9.30±0.03^{e}	8.76±0.03^{f}	5.63±0.06^{g}	5.08±0.06^{h}
45	7.61±0.07^{a}	7.25±0.13^{b}	8.60±0.09^{c}	8.37±0.03^{df}	8.53±0.04^{cd}	8.25±0.04^{f}	9.83±0.09^{e}	9.09±0.04^{g}	5.91±0.06^{h}	5.31±0.04^{i}
Calcium (g kg^{-1})										
0	3.35±0.06^{a}	3.31±0.03^{a}	4.46±0.11^{b}	4.41±0.11^{b}	3.92±0.07^{c}	3.83±0.07^{c}	4.33±0.06^{b}	4.35±0.08^{b}	2.44±0.10^{e}	2.48±0.03^{c}
15	3.94±0.07^{a}	3.77±0.07^{a}	5.82±0.02^{b}	4.79±0.06^{c}	4.74±0.13^{c}	4.27±0.08^{d}	5.32±0.09^{e}	4.81±0.02^{c}	3.07±0.10^{f}	2.81±0.03^{g}
30	4.52±0.11^{a}	4.30±0.09^{a}	6.26±0.11^{b}	5.21±0.01^{ce}	5.37±0.13^{c}	4.81±0.07^{d}	5.91±0.04^{f}	5.07±0.05^{e}	3.90±0.06^{g}	3.25±0.04^{h}
45	5.11±0.06^{a}	4.84±0.03^{b}	6.77±0.07^{c}	5.90±0.07^{d}	5.88±0.04^{d}	5.21±0.10^{ae}	6.40±0.05^{f}	5.37±0.09^{e}	4.31±0.07^{g}	3.88±0.07^{h}
Iron (g kg^{-1})										
0	5.71±0.13^{a}	5.54±0.16^{a}	6.42±0.04^{b}	6.22±0.04^{b}	7.33±0.08^{c}	7.37±0.04^{c}	7.70±0.14^{d}	7.75±0.02^{d}	4.55±0.06^{e}	4.26±0.06^{f}
15	6.83±0.12^{a}	5.96±0.06^{b}	8.35±0.10^{c}	7.07±0.06^{a}	9.00±0.08^{d}	8.28±0.04^{c}	9.69±0.09^{e}	8.30±0.04^{c}	5.52±0.18^{f}	4.91±0.03^{g}
30	7.45±0.07^{a}	6.58±0.11^{b}	9.18±0.09^{c}	7.92±0.03^{d}	9.80±0.09^{e}	9.73±0.04^{e}	11.77±0.12^{f}	8.85±0.03^{g}	6.19±0.06^{h}	5.74±0.06^{i}
45	8.99±0.12^{a}	7.29±0.06^{b}	10.56±0.09^{c}	8.52±0.11^{d}	11.12±0.20^{e}	10.84±0.03^{ce}	12.24±0.22^{f}	9.44±0.06^{g}	7.08±0.03^{b}	6.32±0.07^{h}

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, p<0.05)

Table 4:. Variations in trace elements (Nickel, Cadmium, Copper and Zinc) during vermicomposting

Days	R1	CR1	R2	CR2	R3	CR3	R4	CR4	R5	CR5
Nickel (mg kg^{-1})										
0	250.3±0.8^{a}	247.0±0.5^{a}	239.8±0.8^{bd}	241.8±0.8^{d}	236.0±1.5^{b}	235.8±0.8^{b}	224.8±3.3^{c}	220.8±1.3^{c}	262.0±2.5^{e}	265.8±1.3^{e}
15	262.8±0.8^{a}	258.8±0.8^{ab}	261.0±1.5^{ab}	256.8±0.8^{bc}	252.8±1.8^{cd}	253.3±0.8^{cd}	249.8±2.8^{d}	240.5±1.0^{e}	281.8±2.8^{f}	277.0±1.5^{g}
30	275.0±1.5^{a}	265.5±1.0^{b}	274.3±1.3^{a}	270.3±1.3^{c}	269.3±0.8^{c}	262.3±1.3^{b}	264.5±1.0^{b}	258.5±1.0^{d}	295.5±1.5^{e}	285.5±2.0^{f}
45	291.5±3.0^{a}	272.3±0.8^{be}	292.0±0.5^{a}	288.5±2.0^{a}	289.3±1.8^{a}	276.3±0.8^{bc}	278.5±4.0^{c}	267.8±1.3^{e}	309.3±1.3^{d}	292.3±1.8^{a}
Cadmium (mg kg^{-1})										
0	48.3±0.8^{a}	46.8±0.3^{a}	52.3±1.3^{bd}	51.0±0.5^{b}	54.3±0.8^{c}	53.3±0.3^{cdf}	51.8±0.3^{bf}	52.3±0.3^{bf}	39.3±0.8^{e}	38.0±0.5^{e}
15	54.0±0.5^{a}	51.0±0.5^{b}	58.3±0.3^{c}	54.3±0.3^{a}	58.8±0.3^{c}	55.3±0.8^{ad}	58.8±0.8^{c}	55.8±0.8^{d}	47.0±0.5^{e}	40.8±0.3^{f}
30	57.8±0.3^{a}	54.3±1.3^{b}	64.8±0.8^{c}	58.8±0.3^{a}	63.8±0.3^{c}	57.8±0.3^{a}	65.5±1.0^{c}	58.3±0.3^{a}	53.3±1.3^{b}	43.5±0.5^{d}
45	62.3±0.3^{a}	58.0±0.5^{b}	71.5±1.0^{c}	61.8±0.3^{a}	70.0±0.5^{c}	61.8±0.3^{a}	70.3±0.8^{c}	61.8±0.8^{a}	58.5±1.0^{b}	47.0±0.5^{d}
Copper (mg kg^{-1})										
0	189.0±1.5^{a}	185.8±0.8^{b}	175.3±1.3^{c}	175.0±0.5^{c}	173.0±0.5^{c}	173.0±0.5^{c}	162.5±1.0^{d}	161.8±0.8^{d}	165.8±0.8^{e}	166.0±0.5^{e}
15	195.8±0.8^{a}	189.3±0.3^{b}	182.8±0.8^{c}	178.8±0.3^{d}	180.5±1.0^{e}	175.8±0.3^{f}	170.3±0.8^{g}	166.0±0.5^{h}	170.0±0.5^{g}	167.8±0.3^{i}
30	202.3±1.3^{a}	193.8±0.3^{b}	189.0±0.5^{c}	184.5±1.0^{d}	188.8±0.8^{c}	179.3±0.3^{e}	176.8±0.8^{f}	168.8±0.3^{g}	176.5±1.0^{f}	170.8±0.3^{g}
45	208.0±0.5^{a}	197.5±0.5^{b}	193.8±0.8^{c}	188.3±0.3^{d}	194.8±0.8^{c}	183.8±0.8^{e}	182.0±0.5^{f}	173.0±0.5^{g}	182.0±0.5^{f}	174.5±0.5^{g}
Zinc (mg kg^{-1})										
0	1141.5±4.0^{a}	1131.0±1.5^{a}	1064.0±5.5^{b}	1052.8±1.3^{b}	985.8±2.3^{c}	977.0±1.5^{c}	941.0±6.5^{d}	929.3±0.8^{d}	1007.5±9.0^{e}	1024.3±0.8^{f}
15	1183.5±2.5^{a}	1149.8±1.3^{b}	1080.8±1.3^{c}	1068.8±0.8^{d}	1017.0±2.5^{e}	990.8±1.3^{f}	984.8±2.8^{f}	956.8±1.8^{g}	1056.8±5.8^{h}	1040.5±2.0^{i}
30	1208.3±3.3^{a}	1164.3±0.8^{b}	1106.0±2.5^{c}	1078.3±1.3^{d}	1062.3±3.3^{e}	1011.5±2.0^{f}	1023.0±3.5^{g}	972.3±0.8^{h}	1082.5±2.0^{d}	1063.8±1.3^{e}
45	1251.0±2.5^{a}	1186.3±1.3^{b}	1138.5±3.5^{c}	1094.3±0.8^{d}	1090.0±2.5^{d}	1030.0±1.5^{e}	1078.3±4.3^{f}	996.0±1.5^{g}	1111.3±2.3^{h}	1088.5±4.0^{d}

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, p<0.05)

comparatively higher concentration of nutrients. On analyzing the results by ANOVA, significant differences in macronutrients were observed between all the reactors ($p<0.0001$).

Trace elements: The total concentrations of regulated trace elements (Ni, Cd, Cu, Zn, Mn, Cr and Pb) in vermicompost are shown in Table 4 and 5. Most of the elements are actually needed by plants for normal growth, although in limited quantities. However, in higher concentrations they are likely to have detrimental effects upon plant growth. Certain trace elements are not

Table 5: Variations in trace elements (Manganese, Chromium and Lead) during vermicomposting

Reactors	0 day	15 day	30 day	45 day
Manganese (mg kg^{-1})				
R1	475.0±10.0ac	507.5±2.5a	565.0±5.0a	622.5±7.5a
CR1	457.5±2.5c	487.5±7.5b	510.0±5.0be	537.5±2.5b
R2	487.5±7.5a	520.0±5.0a	565.0±5.0a	602.5±7.5c
CR2	480.0±10.0a	507.5±2.5a	537.5±7.5c	577.5±2.5d
R3	432.5±7.5b	477.5±7.5b	517.5±7.5b	562.5±2.5e
CR3	437.5±2.5b	457.5±2.5ce	477.5±7.5d	500.0±5.0f
R4	430.0±5.0b	472.5±12.5bc	497.5±2.5e	527.5±2.5b
CR4	422.5±2.5b	450.0±5.0e	467.5±2.5d	502.5±7.5f
R5	315.0±5.0d	385.0±10.0d	412.5±2.5f	442.5±2.5g
CR5	317.5±2.5d	347.5±2.5f	377.5±2.5g	400.0±5.0h
Chromium (mg kg^{-1})				
R1	205.8±1.8a	237.8±0.3a	267.5±2.0a	295.8±1.8a
CR1	205.5±1.0a	218.3±1.3b	235.8±1.8b	248.8±0.3b
R2	196.8±1.8b	222.8±1.8ce	248.3±1.3c	281.0±2.5c
CR2	196.8±0.3b	204.8±0.8d	214.0±0.5d	222.3±1.3d
R3	188.3±0.8c	221.0±1.5be	258.5±1.5a	295.0±2.5e
CR3	189.3±0.8c	196.3±1.3f	203.3±0.8e	213.8±0.3f
R4	175.3±0.8d	209.5±1.0g	238.3±0.8f	268.8±0.8b
CR4	173.8±0.8d	185.3±0.8h	189.8±0.3g	194.8±0.3g
R5	154.3±2.8e	167.3±1.8i	177.8±0.8g	196.8±0.3h
CR5	153.5±1.0e	157.8±0.8j	164.3±0.8h	167.8±0.8i
Lead (mg kg^{-1})				
R1	227.0±1.5a	250.3±0.8a	282.3±0.8a	319.3±0.8a
CR1	226.8±0.8a	234.8±0.8b	242.3±0.8b	248.3±0.8b
R2	249.0±0.5b	285.8±1.8c	307.0±1.5c	336.3±1.3c
CR2	246.8±0.3b	253.3±0.8a	257.8±0.3d	264.8±0.8d
R3	288.0±2.5c	308.0±0.5d	348.5±1.0e	370.8±0.8e
CR3	287.8±0.3c	293.5±1.0e	297.8±0.8f	304.8±0.3f
R4	271.3±1.3d	300.5±1.0f	331.5±1.0g	360.3±1.3g
CR4	275.0±0.5e	281.3±0.8g	285.8±0.3h	291.8±0.8h
R5	152.8±1.8f	186.0±3.5h	214.3±0.8i	247.3±1.8b
CR5	152.3±0.8f	159.5±1.0i	167.8±0.8j	175.3±0.8i

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p<0.05$)

biodegradable and become toxic at limited concentration; therefore, measuring the concentration of these elements can provide fertilizer requirements of plants. The increase of total metal content was due to weight loss in the course of composting following organic matter decomposition, release of CO_2 and mineralization processes (Amir *et al.*, 2005). Similar variation in total metal concentration during vermicomposting was observed by (Hartenstein and Hartenstein, 1981; Elvira *et al.*, 1985; Deolalikar *et al.*, 2005). The total metal concentrations in final compost were low in all reactors and are considered as soil fertilizer/conditioner with good quality according to the standards to ensure safe application of compost laid down in Municipal Waste Management and Handling Rules notified by the Ministry of Environment and Forest, Government of India CPHEEO (2000) and Canadian Council of Ministers of the Environment (CCME, 1995). Significant differences in trace elements were observed between all the reactors ($p<0.0001$).

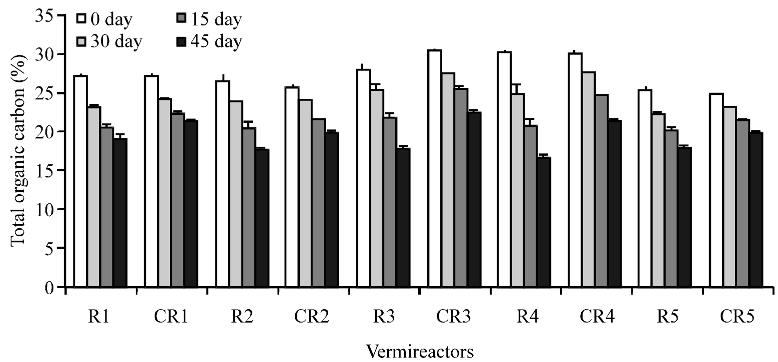

Fig. 4: Variation in Carbon/Nitrogen ratio during vermicomposting

Table 6: Variation in earthworm biomass during vermicomposting

Reactors	0 day	15 day	30 day	45 day
Total earthworm biomass				
R1	120	139±12a	198±8a	323±15a
R2	120	153±2ab	199±2a	318±4a
R3	120	156±4b	226±14b	342±17a
R4	120	174±5c	262±8c	402±29b
R5	120	115±5d	172±8d	315±2a
Coocons				
R1	ND*	8±1a	26±7ab	55±16a
R2	ND	14±5a	25±2ab	47±7a
R3	ND	13±3a	34±1a	86±3a
R4	ND	12±3a	37±2a	89±4a
R5	ND	11±4a	18±10b	58±35a
Jnveniles+New Hatchlings				
R1	-	131±12a	172±14ac	268±1ab
R2	-	139±7a	174±0ac	272±11ab
R3	-	144±7ab	192±15ab	256±20a
R4	-	162±2b	225±10b	314±26b
R5	-	104±9c	154±18c	257±33ab

*ND: Not detected, Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p<0.05$)

Carbon-to-nitrogen ratio (C/N ratio): C/N ratio is used as an index for maturity of organic wastes as well as a very important parameter for fertilizer because plants cannot assimilate nitrogen unless the ratio is in the order of 20 or less (Senesi, 1989). The nitrogen increases due to nitrogenous excreta generated by earthworms and loss of carbon as CO_2 through microbial activity lowered the C/N ratio of the end products. The decreased in C/N ratio over time might also be attributed to increase in the earthworm population. Higher reduction in C/N ratio was observed in R4 with 69.4% followed by R3 (47.4%), CR4 (44.8%), R2 (44.7%), CR2 (43.5%), R1 (42.9%), CR3 (38.3%), R5 (36.5%), CR1 (31.0%) and CR5 (27.4%), respectively. Therefore, continuous decrease was observed during all the reactors (Fig. 4). C/N variations were observed to be significant in all the reactors ($p<0.0001$).

Soluble BOD and COD: The percentage of readily biodegradable organic matter is supposed to be an important aspect of compost quality (Bernal *et al.*, 1998). The composting process

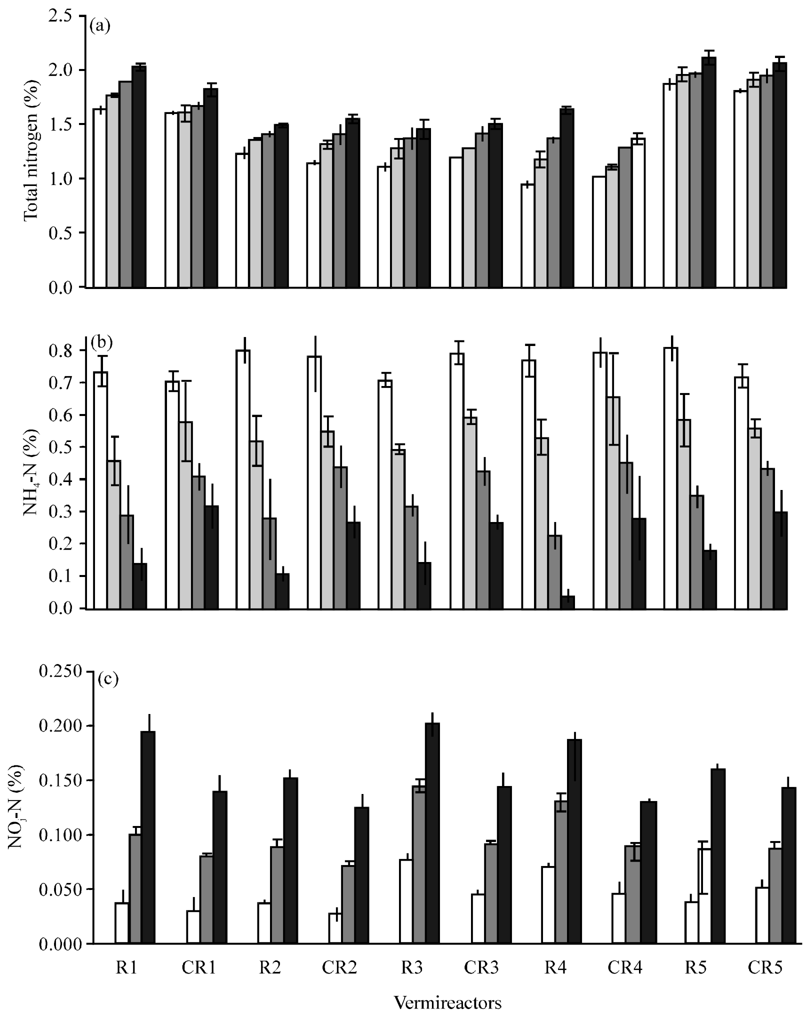

Fig. 5(a-c): Variation in Biochemical oxygen demand and chemical oxygen demand during vermicomposting (a) Total nitrogen, (b) NH_4-N (%) and (c) NO_3-N (%)

occurs until the total amount of biodegradable organic material is stabilized, which is odor free and also a poor breeding substrate for flies and other insects. Even if the compost is stable, care should be taken in its application to soil for crops since the biological processes continue, which can strip the soil of its nutrients (Wang *et al*., 2004). The reduction in BOD values was observed in R1 (835-176 mg L^{-1}), R2 (942-224 mg L^{-1}), R3 (925-165 mg L^{-1}), R4 (1063-91 mg L^{-1}) and R5 (844-231 mg L^{-1}); while, COD values decreased from 1219-586 mg L^{-1} in R1, 1380-589 mg L^{-1} in R2, 1294-542 mg L^{-1} in R3, 1423-477 mg L^{-1} in R4 and 1119-520 mg L^{-1} in R5 at the end of the composting process. Similar observations were found for respective control reactors (Fig. 5). Significant differences in BOD and COD values were observed for all the reactors ($p<0.0001$). As the biological organic content is diminished, BOD and COD are decreased, resulting in decreased emission of CO_2, ultimately indicating stabilization of the compost.

CONCLUSION

Sewage treatment plants exhibits the challenge to societies for disposing the huge amount of sludge but at the same time gives us the prospect for beneficial use by recycling of nutrients.

Sewage sludge, which contains higher concentration of nutrients, could be re-used in agriculture as fertilizer and soil conditioner through vermicomposting technique. The study reveals that R4 (C/N 30) produced more stable compost at the end of 45 days as compared to others; implying that rigorous decomposition was occurred. The decomposition decreased with increasing the sewage sludge content in the reactors due to less availability of readily biodegradable organic. Higher reductions in C/N ratio, soluble BOD and COD in R4 demonstrated the stability, resulting the total biodegradable ingredients are stabilized; in addition, higher final concentration of nutrients and limited metal content suited the quality of compost prepared from sewage sludge in combination with cattle manure and saw dust. On analyzing the results by ANOVA, the physico-chemical and bio-chemical parameters varied significantly ($p<0.001$) during 45 days of the vermicomposting period. Hence, it reveals that the optimal nutrient balance has been occurred in R4, which not only enhanced the affinity of microbes towards substrate but also improved its degradation process; resulting R4 proved to be best combination for the growth and hatchlings of the earthworms. Finally, it can be concluded that R4 (C/N 30) verified to be the best feed combination for vermicomposting technique followed by R3 (C/N 25), R2 (C/N 20), R5 (control) and R1 (C/N 15), respectively.

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