



Dynamics of physicochemical characteristics during vermicomposting of sugarcane bagasse amended with green manure

R Prinitha*, S Senthilmurugan

Department of Zoology, Annamalai University, Chidambaram, Tamil Nadu, India

Abstract

Vermicomposting is the bio-oxidation and stabilization of organic matter involving the action of earthworm and microorganism, turning waste into a valuable soil amendment called vermicompost. Vermicomposting is an ecofriendly process that converts organic substrates into value added vermicompost with the help of earthworms and microflora. In this study, the sugar industrial byproduct, sugarcane bagasse was vermicomposted in combination with cow dung (1:1) in addition to the green manure plant *Gliricidia sepium* as amendment material employing the earthworm, *Eudrilus eugeniae*. Prior to vermicomposting, the substrate combinations were predecomposed for 15 days by inoculating *Pleurotus sajor-caju* spawn. Results showed that pH and electrical conductivity declined from the initial stage, total Kjeldahl nitrogen, sodium, total phosphorus, total potassium, magnesium, calcium, zinc, copper and iron levels were highly increased. Based on the present study findings, it is recommended that the sugarcane bagasse+cow dung combination amended with the green manure plant, *G. sepium* in 2:1:1 ratio is suitable for producing nutrient rich vermicomposting utilizing the epigeic earthworm, *E. eugeniae*.

Keywords: Earthworms, green manure, *Gliricidia sepium*, *Pleurotus sajor-caju*, vermicompost

Introduction

The soil invertebrates of the Lumbricidae family, which include earthworms, are prevalent in both temperate and tropical regions. Although self-fertilization is rare, earthworms are hermaphrodites, meaning they have both male and female reproductive organs. Earthworms grow a unique ring-shaped area of epidermis known as the clitellum when they achieve sexual maturity. This zone is home to specific glands that produce a variety of materials used to form the viscous, girdle-like structures known as cocoons. Although earthworm cocoons appear to be quite little, their real sizes can vary significantly depending on the species. Furthermore, when earthworms transition from their freshly laid stage to their hatching stage, their cocoons undergo periodic color changes (Stephenson, 1930) [47].

The great scientist (Romanes, 1881) [40] completed and releasing his final scientific book, which was titled "The formation of vegetable mould through the action of worms with observations on their habits". The gizzards of earthworms are responsible for the grinding process, which allows them to eat a substantial quantity of organic materials and digestive tract of earthworms can aid in the fragmentation of big particles. However, the function of the calciferous glands related to earthworms is not adequately explained. Recent investigations clearly indicated that these glands (calciferous) could probably play a significant role in maintaining the earthworm's water balance (Morgan *et al.*, 2002) [30]. After a many years, a number of studies -e.g., (Ferrière, 1980; Judas, 1992; Lavelle, 1978; Mariani *et al.*, 2001; Ponomaryova, 1950) [16, 21, 28, 29, 34] have been done on the intestinal content of earthworms. It has been strongly recommended by these investigations that the intake of soil particles is significantly different depending on the feeding habits and biological group of the earthworm species.

Because they transform waste materials into vermicompost, which has the consistency of humus and a black, odorless color, earthworms are crucial to the vermicomposting process. (Samal *et al.*, 2018; Suthar and Singh, 2008) [41, 50].

Furthermore, the vermicomposting process helps to remove a number of gasses that are linked to global warming. Vermicompost eventually has far smaller particles than raw garbage. Vermiculture is the sporadic process of providing earthworms with organic waste to aid in their growth or development (Xie *et al.*, 2016) [53]. The vermicomposting process is being successfully used in the modern day to manage sugarcane wastes, leaf litter, and sewage sludge. When the right circumstances are met in terms of temperature, pH, and moisture content, earthworms may efficiently consume a wide range of organic wastes and produce homogenous material known as cast or vermicast (Samal *et al.*, 2019) [42]. the vermicast has a greater capacity to hold water, a wide range of microbial communities, nutritional components, and a variety of ionic elements (Elvira *et al.*, 1998) [15]. Vermicomposting can be performed on any size, from the intimate setting of a bench to an industrial operation. In addition, there are a variety of physical, chemical, and biological characteristics that can be used to determine that vermicompost has reached its maturity. Earthworms have pores in their skin that allow them to breathe. The earthworm's body is cylindrical in shape, long and bilaterally symmetrical, and has a thin cuticle layer surrounding them. Furthermore, the worm gut is divided into three sections, namely the foregut, midgut, and hindgut, and is linked to various digestive glands (Kaushik and Garg, 2004) [25].

According to previous studies, lysine-rich protein makes up 65% of the dry weight of the earthworms' bodies. The juvenile earthworms have between 100 and 150 distinct segments, and each of these segments is capable of independently contracting and relaxing. In addition, earthworm bodies are devoid of bones, and each segment is composed of bristles known as setae. These setae considerably regulate earthworm mobility in the soil (Hait and Tare, 2012; Reinecke and Venter, 1987) [19, 38]. The natural state of earthworms is that they are hermaphrodites, and the appearance of a clitellum indicates that they have

reached sexual maturity. The two most frequent methods of reproduction are copulation and cross-fertilization. Earthworms lay their eggs in a small spherical-shaped capsule called a cocoon, which incubates for 3-6 weeks (Aira *et al.*, 2002; Brown *et al.*, 2000; Dominguez, 2004) [1, 9, 14]. The earthworm's ability to reproduce is directly proportional to the amount of readily available organic food material in the local environment. Different kinds of earthworms have diverse life spans, habitats, and agroecosystems. Earthworms frequently live a variety of soils, organic compost, and areas close to water sources. In addition, the distribution of earthworm species is determined by the soil's pH level, temperature, level of moisture, and C:N ratio (Dlamini and Haynes, 2004) [13]. The vast majority of earthworms are only found in organically rich soil systems; they are absent from sandy soil, soil with a high clay content, soil with an acidic pH, and coarse-textured soil. The average daily feeding rate of different species of earthworms ranges from 100 to 300 mg/g of worm body weight (Garg and Gupta, 2011) [17]. Though there are several species of earthworms available naturally in soil, only the epigeic category of earthworms is found to be efficient in the bioconversion of organic wastes in the vermicomposting system. The earthworm species like *Eisenia fetida*, *Eudrilus eugeniae*, *Perionyx ceylanensis*, and *Perionyx excavatus* are some of the examples of epigeic

earthworms (Biruntha *et al.*, 2020; Karmegam *et al.*, 2019; Paul *et al.*, 2020) [8, 24, 33]. In the present study, *Eudrilus eugeniae* - a voracious feeder of organic materials have been used for the vermicomposting of sugarcane bagasse and cow dung amended with leafy green manure plant, *Gliricidia sepium*. Hence, the present study has been aimed to amend the green manure plant along with the vermicomposting substrate (sugarcane bagasse + cow dung combinations) using the epigeic earthworm, *E. eugeniae* and to evaluate the dynamics of physico-chemical characteristics during the vermicomposting of sugarcane bagasse amended with cow dung and *G. sepium*.

Materials and methods

Collection of sugarcane bagasse, cow dung and *G. sepium*

Sugarcane bagasse was procured from a private sugar mill near Chidambaram. The cow dung was collected from nearby cattle farm in fresh condition and allowed to stabilize for one week and used for the study. The leaves of the green manure plant, *G. sepium* was collected from the peri-urban region of Chidambaram town, Tamil Nadu, India, dried under shade for a week and used for vermicomposting and worm growth studies.

Experimental design and vermicomposting

Table 1: Combination of vermibed substrates (1:1)

Treatment	Substrate combination (v/v) 1kg			Ratio
	Sugarcane bagasse (SCB)	Cow dung (CD)	Leaves of <i>Gliricidia sepium</i> (GSL)	
VCT1	50%	50%	-	1:1:0
VCT2	66.67%	16.67%	16.67%	4:1:1
VCT3	60%	20%	20%	3:1:1
VCT4	50%	25%	25%	2:1:1
VCT5	50%	-	50%	1:0:1

The substrate, sugarcane bagasse was collected from the nearby sugar industry and transported to the laboratory. The sugarcane bagasse was chopped into 3-4 cm pieces and shade dried for a week. Fresh cow dung was procured from adjacent cattle shed and shade dried for a week and used for the study. The leafy biomass of the green manure plant, *G. sepium* was collected from nearby village for the amendment to the vermicomposting substrate. The mushroom spawn (*Pleurotus sajor-caju*) was obtained from local market. Different combinations of the substrates were prepared and used for vermicomposting trial to select suitable combination for producing enriched vermicompost (Table 1). For precomposting, each combination of substrates was filled alternatively with the layer (2 g/layer) of mushroom spawn in plastic containers and 50-60% moisture content was maintained for 15 days under dark condition (Balachandar *et al.*, 2021) [4].

The precomposted substrate mixtures were filled in uniform plastic containers and watered to hold 70-80% moisture and allowed for 24 h stabilization. Then, 20 mature clitellate earthworms (*E. eugeniae*) were added to each experimental set-up. For each experimental set-up, triplicates were maintained. The experimental sets were kept in a dark room with a temperature of 27 ± 2 °C. The moisture content in the substrates was maintained by adding water when needed until the end of the vermicomposting, i.e., 50 days. A separate experimental set for each treatment without earthworms was maintained as a control.

Physicochemical analysis of vermibed substrates

The pH of the sample was determined by the method of Jackson (1973) [20] using pH meter with a combined electrode (Elico Model LI-120 pH meter). The electrical conductivity of the sample was determined by the method of Jackson (1973) [20] using the Conductivity Bridge (Type CM-82T). The determination of organic carbon in the samples was carried out as per the procedure of Walkley and Black (1934) [52] method. The total nitrogen content of the sample was estimated by the Micro Kjeldahl method and phosphorous by colorimetric method. Total potassium content of the sample was determined by flame photometric method. Sodium, calcium and magnesium content of the sample was determined as per the procedure of Tandon (1993) [51].

Total sulphur was normally estimated by wet ashing of plant tissue sample (as described under phosphorus – diacid digestion) and the sulphate content in the digest was then determined by barium sulphate turbidimetry method. Zinc and Copper content were estimated using Atomic Absorption Spectrophotometer (AAS) according to the procedure outlined by Tandon (1993) [51]. The iron concentration was estimated using spectrophotometer (Model CL-27) as per the procedure outlined by Jackson (1973) [20]. The Mn concentration was estimated using spectrophotometer (Model CL-27) as per the procedure described by Chopra and Kanwar (1991) [12]. The ratio of

the percentage of carbon to that of nitrogen i.e., C:N ratio was calculated by dividing the percentage of carbon estimated for the sample with the percentage of nitrogen estimated for the same sample. The ratio of the percentage of carbon to that of phosphorous i.e., C/P ratio was calculated by dividing the percentage of carbon estimated for the sample with the percentage of phosphorus estimated for the same sample in the present investigation. The percentage increase/decrease of various physico-chemical parameters over the worm-unworked substrates were calculated as hereunder (Ramalingam and Ranganathan, 2001) [37].

Results and discussion

Initial and final physicochemical characteristics of vermibed substrates, dynamics of physicochemical characteristics during vermicomposting of sugarcane bagasse + cow dung (1:1) amended with leafy green manure plant, *G. sepium* (50 days), percentage increase/decrease of physicochemical characteristics of vermicompost over initial substrates.

Dynamics of pH, electrical conductivity, total Kjeldahl nitrogen, phosphorus and potassium

pH

In most of the treatments, the pH showed reduction in final vermicompost except VCT3 which exhibited slight increase from the initial values (Table 2; Fig. 1A). The overall pH change reflected a reduction from the initial substrates, however, the difference between the initial and the final pH change between treatments was not significant ($P > 0.05$). The dynamics of pH values in most of the treatments followed similar patterns. For instance, the dynamics of pH during vermicomposting of sugarcane bagasse substrates were 7.91, 7.85, 7.66, 7.56, 7.40 and 7.28 in VCT1 treatment while it was 7.32, 7.19, 7.22, 7.21, 7.15 and 7.11 in VCT4 respectively on 0, 10, 20, 30, 40 and 50 days of vermicomposting duration.

The basic requirements during the process of vermicomposting include suitable bedding, food source, adequate moisture and aeration, suitable temperature and pH. Vermicomposting of various organic wastes has been shown to produce a product rich in chelating and phytohormonal substances (Prakash and Karmegam, 2010a; Singh *et al.*, 2020; Yuvaraj *et al.*, 2021) [35, 45, 54]. The range of pH in vermicompost was well within the desired range of 7.0–8.5 for agricultural use (Aulinas Masó and Bonmatí Blasi, 2008) [2]. The reduction of pH during vermicomposting has been reported by various vermicomposting studies with and without biochar addition (Birintha *et al.*, 2020; Paul *et al.*, 2020; Suthar *et al.*, 2012) [8, 33, 49]. Cai *et al.* (2020) [11] evaluated the pH values shows near to neutral (7.1–7.6) at the final vermicomposts. pH value was decreased in soybean meal: sugarcane bagasse mixtures with an increasing sugarcane bagasse percentage. But, there is no significant results of pH were identified between vermicompost and compost with 100% of sugarcane bagasse (Cai *et al.*, 2022) [10].

Bhat *et al.* (2017) [6] reported the pH values showed significant changes ($p < 0.05$) with different concentration of pressmud and bagasse waste mixture. The vermicomposting reveals the increase in pH was reported in all the final results of pressmud and bagasse mixtures in

120 days, which ranged from 8.71 to 9.15 in the final feed mixtures. Pressmud from sugar industry waste combined with cow dung and employed an epigeic earthworm *E. fetida*. The pH value was decreased during vermicomposting, it is due to the transformation of the minerals of nitrogen and phosphorus into nitrites, nitrates, and orthophosphates as well as the bioconversion of the organic material into intermediate forms of organic acids (Sangwan *et al.*, 2010) [43].

The formation of organic and phenolic chemicals as a result of the breakdown of organic materials may have been the cause of the pH decrease. (Gusain and Suthar, 2020; Paul *et al.*, 2020) [18, 33]. The pH value of the noteworthy vermicomposting process utilizing sugarcane bagasse and the earthworm *E. eugeniae* tended toward neutrality (Bhandarkar *et al.*, 2014) [5]. Using *E. eugeniae* and green manure plants, the pressmud vermicompost process was carried out. After 50 days of treatment, the pH values decreased, as reported by (Balachandar *et al.*, 2020) [3].

Electrical conductivity (EC)

Electrical conductivity was used to evaluate the quantity of soluble salts in organic substrates and is an important parameter to assess the quality and maturity of final vermicompost for agricultural application. The electrical conductivity (EC) showed increase from the initial levels with significant variation between treatments ($F = 11.385$; $P < 0.05$) (Table 2; Fig. 2B). The range of initial EC values was $0.81 \pm 0.05 - 1.23 \pm 0.07$ dS/m whereas, it was $1.05 \pm 0.06 - 1.62 \pm 0.10$ dS/m in the final vermicompost harvested on 50th day of vermicomposting. A maximum of 1.62 ± 0.10 dS/m EC was recorded in VCT4 followed by VCT3 (1.38 ± 0.08 dS/m). The EC change in VCT3 vermibed substrate was 1.18, 1.20, 1.22, 1.10, 1.31 and 1.38 dS/m on 0, 10, 20, 30, 40 and 50th day while it was 1.23, 1.27, 1.30, 1.46, 1.55 and 1.62 dS/m with a maximum EC value of 1.62 dS/m in VCT4 vermicompost. Overall, the level of enhancement of EC was higher in earthworm introduced and green manure amended treatments than the treatments without green manure.

The increase of EC could be attributed to the presence of mineral salts in available form. The range of pH and organic carbon varied greatly between different vermicomposts is due to the physicochemical nature of organic substrates. Here, the variation is also accompanied by the amendment materials added along with organic wastes. Earlier reports on the changes in pH and EC are in line with the findings of the present study (Karmegam *et al.*, 2021; Paul *et al.*, 2020) [23, 33]. Bhat *et al.* (2017) [6] evaluated the enhancement of press-mud wastes and sugarcane bagasse wastes by the vermicomposting earthworm *E. fetida*. It indicates the decline in EC of the vermicompost final ratios ($p < 0.05$). Below the standard value of 4 mS cm^{-1} , the final EC value of feed mixtures ranged from 2.13 to 3.76 mS cm^{-1} , confirming the vermicompost's ability to be utilized as excellent manure.

Vermicomposting using sugarcane bagasse with the earthworm *E. eugeniae*, shows the increase in EC was significantly different ($p < 0.01$). EC was increased in all the concentrations of bagasse. The maximum increase in EC was in B100 (102.02%) reported by (Bhat *et al.*, 2015) [7].

EC was decreased 1.02 to 0.87 % it is because of loss of weight in organic manure and the release of various mineral salts through nitrogen fixation (Pandit and Maheshwari, 2012) [31]. Effective vermicompost was prepared using the earthworm *E. eugeniae* showed the increase of EC and it was 1.86–2.90 dS m⁻¹ (Balachandar *et al.*, 2020; Cai *et al.*, 2022) [3, 10].

Total Kjeldahl nitrogen (TKN)

The TKN contents in initial vermibed substrates of all treatment exhibited increase towards the progression of vermicomposting days (Table 2; Fig. 1C). The initial TKN content in the treatments, VCT1, VCT2, VCT3, VCT4 and VCT5 was 1.14 ± 0.07, 1.44 ± 0.09, 1.50 ± 0.10, 1.55 ± 0.10 and 1.61 ± 0.11% respectively which rose to 1.32 ± 0.08, 1.72 ± 0.10, 1.95 ± 0.12, 2.42 ± 0.15 and 1.79 ± 0.11% in respective treatments after 50 days of vermicomposting. A higher TKN content of 2.42% was observed in VCT4 (2:1:1 ratio) followed by VCT3- (3:1:1 ratio) and VCT5 (1:0:1 ratio). The change of TKN content in the vermicomposting treatments from initial levels to final elevated levels was significantly different (F = 22.135; P<0.01). However, the dynamics of TKN during vermicomposting sugarcane bagasse+cow dung amended with the green manure plant, *G. sepium* exhibited variation between treatments. The dynamics of TKN during vermicomposting in VCT4 was 1.53, 1.79, 1.86, 1.92, 2.21 and 2.42% on day 0, 10, 20, 30, 40 and 50 respectively. The change of TKN in VCT 5 substrates without cow dung was 1.61, 1.80, 1.75, 1.79, 1.80 and 1.79% on day 0, 10, 20, 30, 40 and 50 respectively. Similar trend of TKN dynamics was observed for the rest of the treatments, VCT1, VCT2 and VCT3 with a significant variation in the final TKN.

The increase in total N content was higher in vermicomposts than composts, where CD increment resulted in increased nutrient contents. Many authors reported that losses in organic carbon might be responsible for N upgrading. Also, the addition of N in the form of mucous, excretory substances which were not initially present in feed substrates has been reported (Karmegam and Daniel, 2009; Sangwan *et al.*, 2008) [22, 44]. The higher percent increase of EC and NPK in vermicompost produced by *P. ceylanensis* than in the compost in this study may be attributed to the mineralization process caused by earthworm action along with microorganisms on organic materials.

The addition of N in the form of mucous, excretory substances which were not initially present in feed substrates has been reported (Balachandar *et al.*, 2021; Rini *et al.*, 2020; Singh *et al.*, 2020) [4, 39, 45]. The higher percent increase of NPK in vermicompost produced by *E. eugeniae* than in the control in this study might be attributed to the mineralization process caused by earthworm action, along with micro-organisms on organic materials due to the presence of cow dung and nutrient conservation in presence of biochar (Paul *et al.*, 2020; Yuvaraj *et al.*, 2021) [33, 54].

Nitrogen is one of the most important nutrients for growth and development of plants and its enrichment during the bioconversion process specifies the beneficial role of earthworms in nitrogen enhancement of waste mixtures. Kumar *et al.* (2010) [27] evaluated the nitrogen content using the sugarcane bagasse through treatment with microorganisms and employed with *Drawida willsi*

earthworm, it reveals during initial pre-decomposition nitrogen content increased in all treatments after that there is no significant increase of TKN. During vermicomposting process, nitrogen content was declined due to ammonification, NH₃ volatilization and denitrification.

Bhat *et al.* (2017) [6] reported that TKN highly increased in the final products of ratios and it ranges from 0.55 to 1.72%. The maximum increase in percentage of TKN was in (1:1) ratio of press-mud and bagasse wastes and it showed 48.57%. TKN was highly increased in the bagasse wastes significantly (p <0.01). The maximum increase in TKN was in 100% bagasse waste which exhibited 230.76%. TKN increased from initial levels in almost all the mixtures of bagasse wastes. Here, earthworms are responsible for the enhancement of the nitrogen content of vermicomposts (Bhat *et al.*, 2015) [7]. The final total nitrogen increase is attributed to the earthworms which enrich the nitrogen content of vermicompost with the help of microorganisms through nitrogen fixation (Pandit and Maheshwari, 2012) [31].

Total phosphorus (TP)

The TP contents were elevated in final vermicompost in comparison with that of the initial values (Table 2; Fig. 1D). The overall TP change reflects enhancement from the initial substrates and the difference between the initial and the final TP contents was statistically significant (F = 8.2006; P<0.05). The dynamics of TP contents were in most of the treatments followed similar pattern of increment. For instance, the dynamics of TP during vermicomposting of sugarcane bagasse substrates in VCT2 treatment were 0.94, 0.96, 0.99, 1.19, 1.24 and 1.26% while it was 1.19, 1.22, 1.30, 1.45, 1.48 and 1.54% in VCT4 treatment respectively on 0, 10, 20, 30, 40 and 50 days of vermicomposting intervals.

Krishnamoorthy (1990) [26] reported that the rise in the level of P content during vermicomposting is probably due to mineralization and mobilization of P due to bacterial and faecal phosphatase activity of earthworms. The elevated level of Zn and Fe in vermicompost indicates accelerated mineralization with selective feeding by earthworms on materials containing these metals. The increased level of macro and micronutrients in the vermicomposts were in conformity with the results of earlier works (Parthasarathi and Ranganathan, 1999; Suthar, 2007) [32, 48].

Total potassium (TK)

The TK contents were also elevated in the final vermicompost of most of all the treatments in comparison with that of the initial values. The overall TK change reflects enhancement from the initial substrates and the difference between the initial and the final TK contents was statistically highly significant (F = 46.151; P<0.001) (Table 2; Fig. 1E). The dynamics of TK contents were in most of the treatments followed similar pattern of increment. For instance, the dynamics of TK during vermicomposting of sugarcane bagasse substrates in VCT3 treatment were 0.94, 0.92, 0.99, 1.17, 1.29 and 1.35% while it was 0.91, 0.91, 1.01, 1.20, 1.32 and 1.40% in VCT4 treatment respectively on 0, 10, 20, 30, 40 and 50 days of vermicomposting intervals.

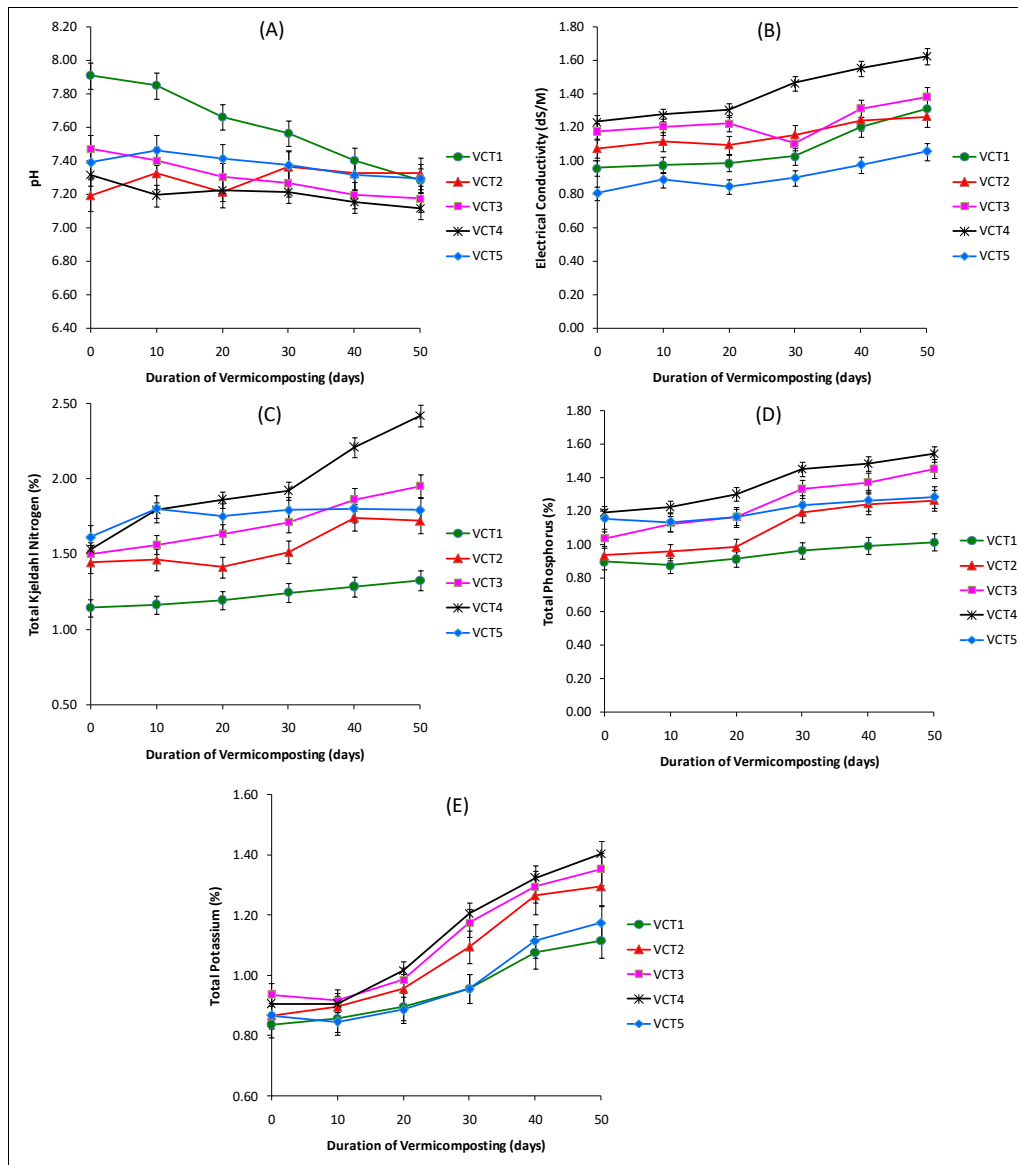


Fig. 1: Dynamics of (A) pH, (B) electrical conductivity, (C) total Kjeldahl nitrogen, (D) total phosphorus, and (E) total potassium during vermicomposting of sugarcane bagasse + cow dung (1:1) amended with leafy green manure plant, *Gliricidia sepium* (50 days). [Vermibed combination (Sugarcane bagasse:Cowdung:*Gliricidia*): VCT1 - 1:1:0; VCT2 - 4:1:1; VCT3 - 3:1:1; VCT4 - 2:1:1; VCT5 - 1:0:1].

Dynamics of total calcium, sodium, magnesium, and sulphur during the vermicomposting of sugarcane bagasse vermibed substrates

Calcium (Ca)

In most of the treatments, the Ca contents showed increment in the final vermicompost from the initial values. The overall Ca content change reflected increase from the initial substrates, with a difference between initial and final Ca change between treatments (F = 8.272; P<0.05). The dynamics of total Ca contents in most of the treatments followed similar pattern. For instance, the dynamics of calcium during vermicomposting of sugarcane bagasse substrates were 0.70, 0.72, 0.76, 0.72, 0.79 and 0.73% in VCT1 treatment while it was 0.88, 0.91, 0.98, 1.01, 1.13 and 1.16% in VCT4 respectively on 0, 10, 20, 30, 40 and 50 days of vermicomposting treatments (Table 3; Fig. 2A).

Ca is a dynamic plant nutrient required for growth and development of plants. Total calcium content was significantly increased in different ratios of waste materials. The initial total calcium content in the different ratios ranges from 2.41 to 3.23%, while the final total calcium content

was increased between 2.99 and 6.83%. Here, earthworm *E. fetida* enhanced the calcium content in the final mixtures through enriching the mineralization process and decomposition during vermicomposting. The enrichment of nutrient content in the final product is mainly due to the presence of earthworms and microorganisms which reduces the waste (Bhat *et al.*, 2017)^[6].

The Ca content is higher in the final compost than initial substrates. Ca content was increased from 1.56 to 2.27% during the vermicomposting period (Sangwan *et al.*, 2010)^[43]. It explains that gut process associated with Ca metabolism are primarily responsible for improvement of inorganic calcium content in *E. fetida* (Pandit and Maheshwari, 2012)^[31].

Sodium (Na)

The Na contents showed increase from the initial levels with a highly significant variation between treatments (F = 31.129; P<0.001). The initial Na levels was 0.41 ± 0.02, 0.29 ± 0.01, 0.31 ± 0.02, 0.34 ± 0.03 and 0.40 ± 0.04% respectively in VCT1, VCT2, VCT3, VCT4 and VCT5,

while it was 0.46 ± 0.02 , 0.50 ± 0.04 , 0.54 ± 0.03 , 0.57 ± 0.05 , and $0.53 \pm 0.03\%$ in the final vermicompost harvested on 50th day of vermicomposting. A maximum of 0.57% of Na was recorded in VCT4 followed by VCT3 (0.54%). The Na contents change in VCT3 vermibed substrate was 0.31, 0.33, 0.39, 0.43, 0.51 and 0.54% on 0, 10, 20, 30, 40 and 50th day while it was 0.34, 0.35, 0.41, 0.47, 0.54 and 0.57% with a maximum Na content of 0.57% in VCT4 vermicompost (Table 3; Fig. 2B).

Magnesium (Mg)

The Mg contents in initial vermibed substrates of all treatment exhibited increase towards the progression of vermicomposting days. However, the dynamics of Mg during vermicomposting sugarcane bagasse + cow dung amended with the green manure plant, *G. sepium* exhibited variation between treatments. The initial Mg content in the treatments, VCT1, VCT2, VCT3, VCT4 and VCT5 was 0.42 ± 0.01 , 0.35 ± 0.03 , 0.39 ± 0.02 , 0.41 ± 0.04 and $0.40 \pm 0.01\%$ respectively which rose to 0.47 ± 0.02 , 0.54 ± 0.03 , 0.56 ± 0.04 , 0.58 ± 0.03 and $0.50 \pm 0.02\%$ in respective treatments after 50 days of vermicomposting. A higher Mg

content of 0.58% was observed in VCT4 (2:1:1 ratio) followed by VCT3 (3:1:1 ratio) and VCT2 (4:1:1 ratio). The change of Mg content in the vermicomposting treatments from initial levels to final elevated levels was highly significantly different ($F = 33.875$; $P < 0.001$) (Table 2; Fig. 2C).

Sulphur

The sulphur contents were elevated in final vermicompost in comparison with that of the initial values. The overall sulphur content change reflected enhancement from the initial substrates and the difference between the initial and the final sulphur contents was statistically significant ($F = 37.818$; $P < 0.001$). The dynamics of sulphur contents in most of the treatments followed similar pattern of increment. For instance, the dynamics of sulphur during vermicomposting of sugarcane bagasse in VCT2 treatment were 0.21, 0.21, 0.24, 0.26, 0.31 and 0.30% while it was 0.22, 0.24, 0.29, 0.32, 0.36 and 0.36% in VCT4 treatment respectively on 0, 10, 20, 30, 40 and 50 days of vermicomposting intervals (Fig. 2).

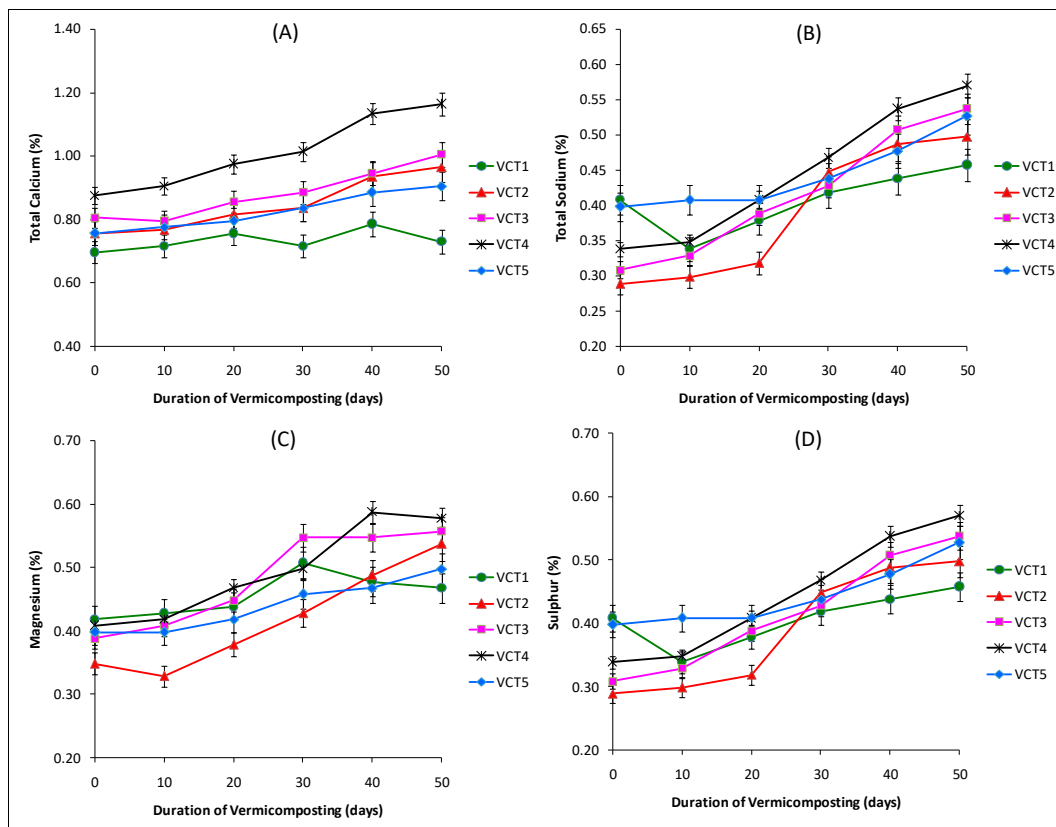


Fig. 2: Dynamics of (A) total calcium, (B) sodium, (C) magnesium, and (D) sulphur during vermicomposting of sugarcane bagasse + cow dung (1:1) amended with leafy green manure plant, *Gliricidia sepium* (50 days).

[Vermibed combination (Sugarcane bagasse: Cowdung: *Gliricidia*): VCT1 - 1:1:0; VCT2 - 4:1:1; VCT3 - 3:1:1; VCT4 - 2:1:1; VCT5 - 1:0:1].

Dynamics of manganese, copper, iron and zinc during the vermicomposting of sugarcane bagasse vermibed substrates

Manganese (Mn)

The contents of Mn showed increment in the final vermicompost from the initial values. The overall Mn content change reflected increase from the initial substrates, with a difference between initial and final Mn change ($F =$

14.480 ; $P < 0.01$). For instance, the dynamics of Mn during vermicomposting of sugarcane bagasse substrates were 16.92, 17.41, 18.01, 18.31, 18.51 and 18.61 ppm in VCT1 treatment while it was 16.92, 17.01, 17.11, 17.71, 17.91 and 18.71 ppm in VCT4 respectively on 0, 10, 20, 30, 40 and 50 days of vermicomposting treatments (Table 4; Fig. 3A).

Copper (Cu)

The Cu contents in the vermicompost showed increase from the initial levels with a highly significant variation ($F = 21.910$; $P < 0.01$) (Table 4; Fig. 3B). The initial Cu levels was 10.95 ± 0.66 , 11.94 ± 0.72 , 12.24 ± 0.73 , 12.64 ± 0.76 and 13.03 ± 0.78 ppm respectively in VCT1, VCT2, VCT3, VCT4 and VCT5, while it was 14.43 ± 0.87 , 13.83 ± 0.83 , 14.29 ± 0.86 , 14.77 ± 0.89 , and 13.43 ± 0.81 ppm in the final vermicompost harvested on 50th day of vermicomposting. A maximum of 14.77 ppm of copper was recorded in VCT4 followed by VCT1 (14.43 ppm). The Cu contents change in VCT3 vermibed substrate was 12.24, 12.74, 13.13, 13.83, 14.03, and 14.29 ppm on 0, 10, 20, 30, 40 and 50th day while it 12.64, 12.94, 14.43, 13.73, 14.33 and 14.77 ppm with a maximum Cu content of 14.77 ppm in VCT4 vermicompost.

Iron (Fe)

The change of Fe content in the vermicomposting treatments from initial levels to final elevated levels was not significantly different ($F = 3.7208$; $P > 0.05$). However, the dynamics of Fe during vermicomposting sugarcane bagasse+cow dung amended with the green manure plant, *G. sepium* exhibited variation between treatments (Table 4;

Fig. 3C). The dynamics of Fe during vermicomposting in VCT3 was 126.96, 127.36, 132.34, 134.82, 137.71 and 138.31 ppm on day 0, 10, 20, 30, 40 and 50 respectively. The change of Fe in VCT 5 substrates without cow dung was 130.64, 123.88, 125.97, 126.46, 129.36 and 130.44 ppm on day 0, 10, 20, 30, 40 and 50 respectively. Similar trend of Fe dynamics was observed for the rest of the treatments, VCT1, VCT2 and VCT4 with a non-significant variation in the final Fe content.

Zinc (Zn)

The contents of Zn were elevated in final vermicompost in comparison with that of the initial values. The overall Zn content change reflected enhancement from the initial substrates and the difference between the initial and the final Zn contents was not statistically significant ($F = 0.7194$; $P > 0.05$) (Table 4; Fig. 3D). The dynamics of Zn contents in most of the treatments followed similar pattern of increment. For instance, the dynamics of Zn during vermicomposting of sugarcane bagasse in VCT2 treatment were 37.81, 39.20, 40.99, 41.91, 42.79 and 42.69 ppm while it was 38.81, 39.20, 38.51, 40.50, 40.59 and 40.80 ppm in VCT4 treatment respectively on 0, 10, 20, 30, 40 and 50 days of vermicomposting intervals.

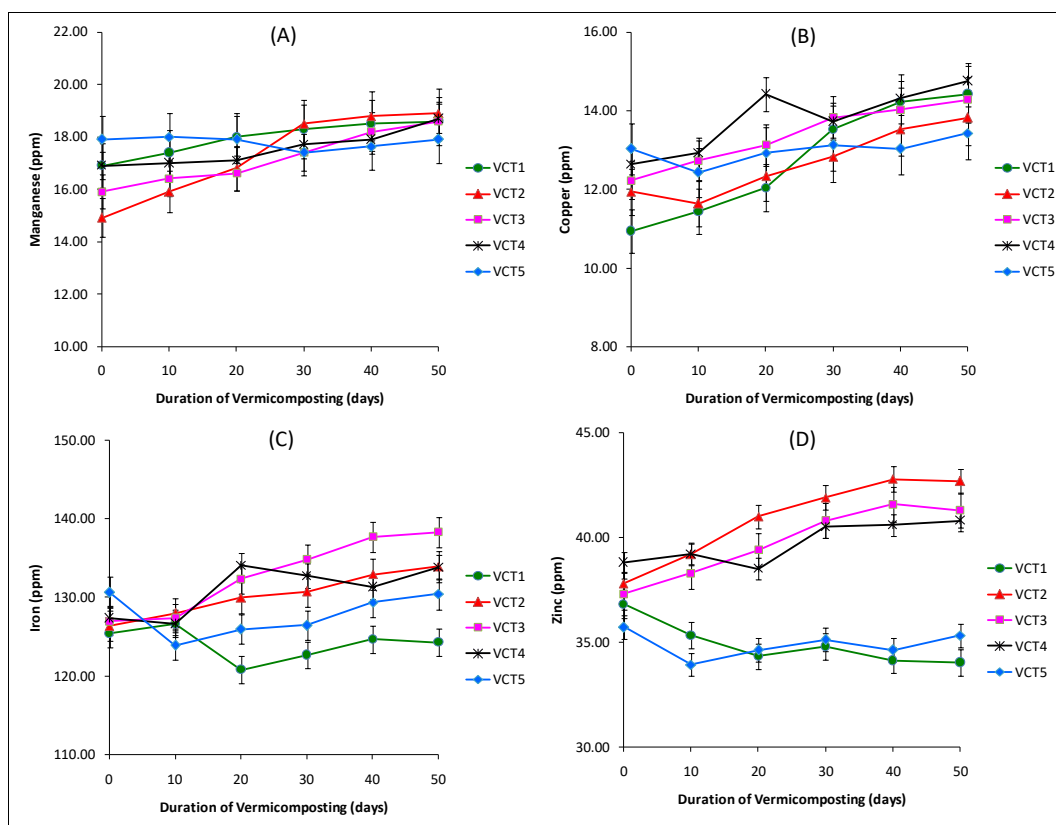


Fig 3: Dynamics of (A) manganese, (B) copper, (C) iron, and (D) zinc during vermicomposting of sugarcane bagasse + cow dung (1:1) amended with leafy green manure plant, *Gliricidia sepium* (50 days).
 [Vermibed combination (Sugarcane bagasse: Cowdung: *Gliricidia*): VCT1 - 1:1:0; VCT2 - 4:1:1; VCT3 - 3:1:1; VCT4 - 2:1:1; VCT5 - 1:0:1].

C/N ratio

The TOC contents, C/N ratio and C/P ratio showed decline from the initial levels in all the treatments (Table 5; Fig. 4). The C/N ratio of the vermicompost declined from the initial levels with a highly significant variation ($F = 19.7885$; $P < 0.01$). The initial C/N ratio was 35.65 ± 1.23 , 31.03 ± 1.40 , 30.75 ± 1.38 , 27.93 ± 1.26 and 28.21 ± 1.41

respectively in VCT1, VCT2, VCT3, VCT4 and VCT5, while it was 25.11 ± 1.13 , 21.10 ± 0.74 , 19.23 ± 0.75 , 15.06 ± 0.68 and 23.44 ± 1.06 in the final vermicompost recovered from vermibeds on 50th day of vermicomposting. A narrowed C/N ratio of 15.06 was recorded in VCT4 followed by VCT3 (19.23). The C/N ratio change in VCT3 vermibed substrate was 30.75, 29.94, 27.11, 24.07, 20.96

and 19.23 on 0, 10, 20, 30, 40 and 50th day while it was 27.93, 25.00, 22.26, 20.10, 16.85 and 15.06 with a lower C/N ratio of 15.06 ± 0.68 in VCT4 vermicompost. Due to the steady decline in TOC, and TP increment, the C/P ratio also showed similar results.

The C:N ratio is used for the determination of the maturity of final product, i.e., vermicompost for its agricultural utility. C:N ratio of <20 indicates maturity and stability of wastes and is beneficial for agricultural use. Pressmud and bagasse wastes have been used in the vermiremediation process. Cattle dung was added to the wastes in different ratios with the earthworm *E. fetida* which is used to evaluate the C:N ratio from initial in all the final ratios was decreased significantly which initially ranges from 36.6 to 58.8 and it is reduced in finally from 22.5 to 31.4. the maximum reduction of C:N ratio is seen in pressmud and bagasse wastes with (1:1) ratio (Bhat *et al.*, 2017)^[6].

Sangwan *et al.* (2010)^[43] reported that press mud combined with cow dung and employed with *E. fetida* shows significant decrease of C:N ratio. Initial treatment of vermicomposting shows C:N ratios were ranges from 22–42 and finally C:N ratios were reduced, ranges from 14–16. This reduction is due to the loss of C as CO₂ in the

respiration process and level of nitrogen is enhanced through nitrogen fixation. Bhat *et al.* (2015)^[7] evaluated the maximum decrease in C: N ratio was found in (1:1) ratio mixture is 73.35%. Decline of C:N ratio may be due to loss of C through respiration in the form of CO₂ and also with an increase of nitrogen and stabilization of press mud waste by the action of *E. fetida*.

Vermicomposting of press-mud with *E. fetida*, *E. eugeniae*, *Lampito mauritii*, and *P. ceylanensis* showed significant decrease of C:N ratio reveals the decline of organic carbon due to the respiratory activity of different types of earthworm and microorganisms, and mineralization process (Prakash and Karmegam, 2010b)^[36]. C:N ratio was initially 715.71% and after 45 days highly reduced to 54.44% using the sugarcane bagasse waste with *E. eugeniae* earthworm in the vermicomposting (Bhandarkar *et al.*, 2014)^[5]. Balachandar *et al.* (2020)^[3] reported Sugarcane press-mud with cow dung and nitrogenous green manures was used to prepare composting and it is employed with the earthworm *E. eugeniae* which reveals initial C:N ratio ranged between 22.25 and 28.76%, reduced finally 12 and 16.66 for vermicomposts (Balachandar *et al.*, 2020)^[3].

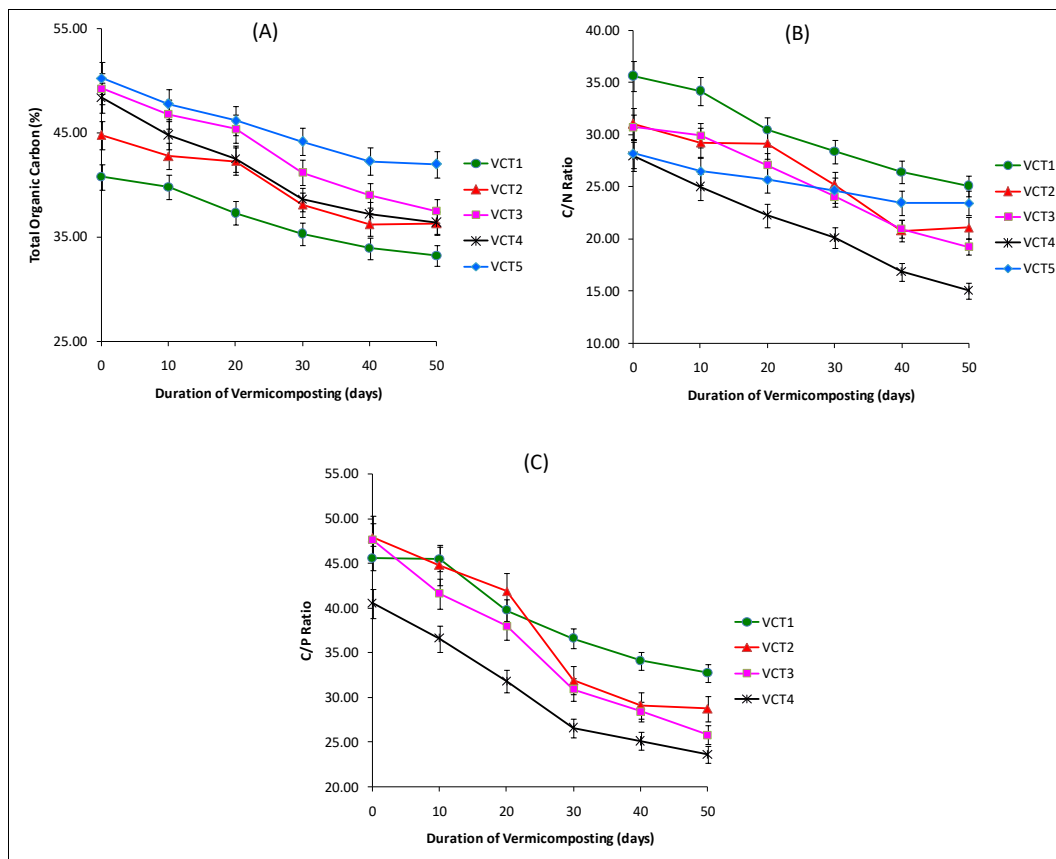


Fig 4: Dynamics of (A) total organic carbon, (B) C/N ratio, and (C) C/P ratio during vermicomposting of sugarcane bagasse + cow dung (1:1) amended with leafy green manure plant, *Gliricidia sepium* (50 days).
 [Vermibed combination (Sugarcane bagasse:Cowdung:*Gliricidia*): VCT1 - 1:1:0; VCT2 - 4:1:1; VCT3 - 3:1:1; VCT4 - 2:1:1; VCT5 - 1:0:1].

Percentage increase/decrease of physicochemical parameters in initial substrates over final vermicompost

The overall trend of decrease of pH, TOC, C/N ratio and C/P ratio was invariably observed in most of the treatments while the other parameters showed increase over initial vermicombed substrates. The pH in the vermicomposts recorded a decrease of 7.91, 3.97, 2.77 and 1.30% from the initial

levels in VCT1, VCT3, VCT4, and VCT5 respectively while VCT2 showed slight increase (1.81%). The increase percentage of EC was 36.91, 17.96, 17.52, 31.54 and 30.94% respectively in the vermicomposts of the treatments, VCT1, VCT2, VCT3, VCT4, and VCT5 with a maximum increment in VCT1 followed by VCT5. The TKN content recorded a maximum increase of 58.03% in the

vermicompost of VCT4 over the initial substrate while it was 40.38% for TP in VCT3 and 54.95% for TK in VCT4. The increase of Ca was 32.95% in VCT4 vermicompost over initial substrate which was followed by VCT2 and VCT3. The Na change percentage in vermicompost was from initial values 74.19% in VCT3 treatment followed by VCT2 and VCT4, whereas it was 52.49% for Mg in VCT2. The range of sulphur increase percentage was from 40.00 – 63.64% with a higher increase in VCT4. The maximum percentage of Mn, Cu, iron and Zn was 26.67, 31.82, 8.39 and 12.89% respectively in VCT2, VCT1, VCT3 and VCT2 vermicomposts over initial contents. The decline percentage of TOC, C/N ratio and C/P ratio in vermicompost over initial values were 24.69% (VCT4), 46.08% (VCT4), and 45.75% (VCT3). The decrease of C/N ratio was 29.56, 32.02, 37.44, 46.08 and 16.90% in the vermicomposts of VCT1, VCT2, VCT3, VCT4 and VCT5 respectively over the initial levels.

Conclusions

The present research has been aimed to bioconvert the sugar industrial waste, sugarcane bagasse with cow dung through the amendment of leafy green manure plant, *G. sepium* employing the epigeic earthworm, *E. eugeniae* to produce enriched vermicompost. For this, different combination of vermibed materials were prepared and predecomposed using mushroom spawn (*Pleurotus sajor-caju*) for 15 days and then the earthworms were inoculated. The vermicomposting setup was maintained for 50 days. The vermibed combinations used were sugarcane bagasse + cow dung + *G. sepium* in the order of following treatment combinations: VCT1 - 1:1:0; VCT2 - 4:1:1; VCT3 - 3:1:1; VCT4 - 2:1:1; VCT5 - 1:0:1. During vermicomposting, the vermibed samples were taken on 0 (initial), 10, 20, 30, 40 and 50 days intervals and subjected to the analyses of physico-chemical characteristics. Overall findings revealed that the vermibed combination, VCT4 - 2:1:1 is found to be superior in nutrient contents.

References

- Aira M, Monroy F, Domínguez J, Mato S. How earthworm density affects microbial biomass and activity in pig manure. *Eur J Soil Biol*,2002;38:7-10.
- Aulinas Masó M, Bonmatí Blasi A. Evaluation of composting as a strategy for managing organic wastes from a municipal market in Nicaragua. *Bioresour Technol*,2008;99:5120–5124. <https://doi.org/10.1016/J.BIORTECH.2007.09.083>
- Balachandar R, Baskaran L, Yuvaraj A, Thangaraj R, Subbaiya R, Ravindran B, *et al.* Enriched pressmud vermicompost production with green manure plants using *Eudrilus eugeniae*. *Bioresour Technol*,2020;299:122578. <https://doi.org/10.1016/j.biortech.2019.122578>
- Balachandar R, Biruntha M, Yuvaraj A, Thangaraj R, Subbaiya R, Govarathanan M, *et al.* Earthworm intervened nutrient recovery and greener production of vermicompost from *Ipomoea staphylina* – An invasive weed with emerging environmental challenges. *Chemosphere*,2021;263:128080. <https://doi.org/10.1016/j.chemosphere.2020.128080>
- Bhandarkar BA, Kakde SA, Sonar SK, Sayyed AS. Vermicomposting from bagasse by using *Eudrilus eugeniae*. *Int J Res Eng Technol*, 2014, 3.
- Bhat SA, Singh J, Vig AP. Amelioration and degradation of pressmud and bagasse wastes using vermitechnology. *Bioresour Technol*,2017;243:1097–1104. <https://doi.org/10.1016/j.biortech.2017.07.093>
- Bhat SA, Singh J, Vig AP. Potential utilization of bagasse as feed material for earthworm *Eisenia fetida* and production of vermicompost. *Springerplus*,2015;4:11. <https://doi.org/10.1186/s40064-014-0780-y>
- Biruntha M, Karmegam N, Archana J, Karunai Selvi B, John Paul JA, Balamuralikrishnan B, *et al.* Vermiconversion of biowastes with low-to-high C/N ratio into value added vermicompost. *Bioresour Technol*,2020;297:122398. <https://doi.org/10.1016/j.biortech.2019.122398>
- Brown GG, Barois I, Lavelle P. Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. *Eur J Soil Biol*,2000;36:177–198.
- Cai L, Gong X, Ding H, Li S, Hao D, Yu K, *et al.* Vermicomposting with food processing waste mixtures of soybean meal and sugarcane bagasse. *Environ Technol Innov*,2022;28:102699. <https://doi.org/10.1016/j.eti.2022.102699>
- Cai L, Sun X, Hao D, Li S, Gong X, Ding H, *et al.* Sugarcane bagasse amendment improves the quality of green waste vermicompost and the growth of *Eisenia fetida*. *Front Environ Sci Eng*,2020;14:61. <https://doi.org/10.1007/s11783-020-1240-2>
- Chopra SL, Kanwar JS. Analytical agricultural chemistry. New Delhi: Kalyani Publ, 1991.
- Dlamini TC, Haynes RJ. Influence of agricultural land use on the size and composition of earthworm communities in northern KwaZulu-Natal, South Africa. *Appl Soil Ecol*,2004;27:77–88.
- Dominguez J. State-of-the-art and new perspectives on vermicomposting research. In: Edwards CA, editor. *Earthworm Ecology*,2nd ed. Boca Raton, FL: CRC Press LLC, 2004, 401–24.
- Elvira C, Sampedro L, Benitez E, Nogales R. Vermicomposting of sludges from paper mill and dairy industries with *Eisenia andrei*: A pilot-scale study. *Bioresour Technol*,1998;63:205-11.
- Ferrière G. Fonctions des lombriciens. VII. Une méthode d'analyse la matière Org. *Pedobiologia*,1980;20:263–273.
- Garg VK, Gupta R. Optimization of cow dung spiked pre-consumer processing vegetable waste for vermicomposting using *Eisenia fetida*. *Ecotoxicol Environ Saf*,2011;74:19–24.
- Gusain R, Suthar S. Vermicomposting of duckweed (*Spirodela polyrhiza*) by employing *Eisenia fetida*: Changes in nutrient contents, microbial enzyme activities and earthworm biodynamics. *Bioresour Technol*, 2020, 123585. <https://doi.org/10.1016/j.biortech.2020.123585>
- Hait S, Tare V. Transformation and availability of nutrients and heavy metals during integrated composting-vermicomposting of sewage sludges. *Ecotoxicol Environ Saf*,2012;79:214–24.
- Jackson ML. Soil chemical analysis. New Delhi, India: Prentice Hall India Pvt. Ltd., 1973.

21. Judas M. Gut content analysis of earthworms (Lumbricidae) in a beechwood. *Soil Biol Biochem*,1992;24:1413–1417.
22. Karmegam N, Daniel T. Growth, reproductive biology and life cycle of the vermicomposting earthworm, *Perionyx ceylanensis* Mich. (Oligochaeta: Megascolecidae). *Bioresour Technol*,2009;100:4790–6. <https://doi.org/10.1016/j.biortech.2009.05.004>
23. Karmegam N, Jayakumar M, Govarthanam M, Kumar P, Ravindran B, Biruntha M. Precomposting and green manure amendment for effective vermitransformation of hazardous coir industrial waste into enriched vermicompost. *Bioresour Technol*,2021;319:124136. <https://doi.org/10.1016/j.biortech.2020.124136>
24. Karmegam N, Vijayan P, Prakash M, John Paul JA. Vermicomposting of paper industry sludge with cowdung and green manure plants using *Eisenia fetida*: A viable option for cleaner and enriched vermicompost production. *J Clean Prod*,2019;228:718–728. <https://doi.org/10.1016/j.jclepro.2019.04.313>
25. Kaushik P, Garg VK. Dynamics of biological and chemical parameters during vermicomposting of solid textile mill sludge mixed with cow dung and agricultural residues. *Bioresour Technol*,2004;94:203–209.
26. Krishnamoorthy RV. Mineralization of phosphorus by faecal phosphatases of some earthworms of Indian tropics. *Proc Indian Acad Sci (Animal Sci)*, 1990, 509–518.
27. Kumar R, Verma D, Singh BL, Kumar U, Shweta. Composting of sugar-cane waste by-products through treatment with microorganisms and subsequent vermicomposting. *Bioresour Technol*,2010;101:6707–6711. <https://doi.org/10.1016/j.biortech.2010.03.111>
28. Lavelle P. Les vers de terre de la savane de Lamto (Côte d’Ivoire): Peuplements, populations et fonctions dans l’écosystème. *Publ Lab Zool École Norm Univ Paris VI*, 1978.
29. Mariani L, Bernier N, Jiménez JJ, Decaens T. Diet of an anecic earthworm from the Colombian savannas: a question about ecological categories. *CR Acad Sci Paris, Sci la vie*, 2001, 733–742.
30. Morgan AJ, Winters C, Williams AF, Turner M. The transformation of calcitic spherites to excretory concretions in the earthworm calciferous glands: did Darwin miss an ecophysiological function? In: *Proceedings of the Seventh International Symposium on Earthworm Ecology*. Cardiff, Wales, 2002, 1–6.
31. Pandit NP, Maheshwari SK. Optimization of vermicomposting technique for sugarcane waste management by using *Eisenia fetida*. *Int J Biosci*,2012;2:143–155.
32. Parthasarathi K, Ranganathan LS. Longevity of microbial and enzyme activity and their influence on NPK contents in pressmud vermicasts. *Eur J Soil Biol*,1999;35:107–113.
33. Paul S, Kauser H, Jain MS, Khwairakpam M, Kalamdhad AS. Biogenic stabilization and heavy metal immobilization during vermicomposting of vegetable waste with biochar amendment. *J Hazard Mater*, 2020, 121366. <https://doi.org/10.1016/j.jhazmat.2019.121366>
34. Ponomaryova SI. The role of earthworms in forming of stable aggregates under grassland crop rotation. *Pochvovedeniye*, 1950, 476–486.
35. Prakash M, Karmegam N. Dynamics of nutrients and microflora during vermicomposting of mango leaf litter (*Mangifera indica*) using *Perionyx ceylanensis*. *Int J Glob Environ Issues*,2010a;10:339. <https://doi.org/10.1504/IJGENVI.2010.037275>
36. Prakash M, Karmegam N. Vermistabilization of pressmud using *Perionyx ceylanensis* Mich. *Bioresour Technol*,2010b;101:8464–8468. <https://doi.org/10.1016/j.biortech.2010.06.002>
37. Ramalingam R, Ranganathan LS. Vermicomposting of pressmud boosts nutrient quality of compost. *Ecol Environ Conserv*,2001;7:297–299.
38. Reinecke AJ, Venter JM. The influence of moisture on the growth and reproduction of the compost worm *Eisenia fetida* (Oligochaeta). *Biol Fertil Soil*,1987;3:135–141.
39. Rini J, Deepthi MP, Saminathan K, Narendhirakannan RT, Karmegam N, Kathireswari P. Nutrient recovery and vermicompost production from livestock solid wastes with epigeic earthworms. *Bioresour Technol*,2020;313:123690. <https://doi.org/10.1016/j.biortech.2020.123690>
40. Romanes GJ. The formation of vegetable mould through the action of worms, with observations on their habits. *Nature*,1881;24:553–556. <https://doi.org/10.1038/024553a0>
41. Samal K, Dash RR, Bhunia P. A comparative study of macrophytes influence on performance of hybrid vermifilter for wastewater treatment. *J Environ Chem Eng*,2018;6:4714–4726.
42. Samal K, Mohan AR, Chaudhary N, Moulick S. Application of vermitechnology in waste management: A review on mechanism and performance. *J Environ Chem Eng*, 2019, 103392.
43. Sangwan P, Kaushik CP, Garg VK. Vermicomposting of sugar industry waste (press mud) mixed with cow dung employing an epigeic earthworm *Eisenia fetida*. *Waste Manag Res J a Sustain Circ Econ*,2010;28:71–75. <https://doi.org/10.1177/0734242X09336315>
44. Sangwan P, Kaushik CP, Garg VK. Feasibility of utilization of horse dung spiked filter cake in vermicomposters using exotic earthworm *Eisenia fetida*. *Bioresour Technol*,2008;99:2442–2448.
45. Singh A, Karmegam N, Singh GS, Bhadauria T, Chang SW, Awasthi MK, *et al*. Earthworms and vermicompost: an eco-friendly approach for repaying nature’s debt. *Environ Geochem Health*,2020;42:1617–1642. <https://doi.org/10.1007/s10653-019-00510-4>
46. Singh NK, Shahi K, Kumar K, Suthar S. Enhanced vermicomposting of leaf litter by white-rot fungi pretreatment and subsequent feeding by *Eisenia fetida* under a two-stage process. *Bioresour Technol Reports*,2021;13:100609. <https://doi.org/10.1016/J.BITEB.2020.100609>
47. Stephenson J. *The Oligochaeta*. Oxford University Press, London, 1930.
48. Suthar S. Vermicomposting potential of *Perionyx sansibaricus* (Perrier) in different waste materials. *Bioresour Technol*,2007;98:1231–1237.
49. Suthar S, Mutiyar PK, Singh S. Vermicomposting of milk processing industry sludge spiked with plant wastes. *Bioresour Technol*,2012;116:214–219. <https://doi.org/10.1016/j.biortech.2012.03.101>

50. Suthar S, Singh S. Feasibility of vermicomposting in biostabilization of sludge from a distillery industry. *Sci Total Environ*,2008;394:237–243.
51. Tandon HZ. Methods of analysis of soils, plant, water and fertilizers. Fertil Dev Consult Organ, New Delhi, 1993, 1-148.
52. Walkley A, Black IA. An examination of the Degtjareff method for determining soil organic matter and prepared modification of the chronic acid titration method. *Soil Sci*,1934;34:29–38.
53. Xie D, Wu W, Hao X, Jiang D, Li X, Bai L. Vermicomposting of sludge from animal wastewater treatment plant mixed with cow dung or swine manure using *Eisenia fetida*. *Environ Sci Pollut Res*,2016;23:7767–7775.
54. Yuvaraj A, Thangaraj R, Ravindran B, Chang SW, Karmegam N. Centrality of cattle solid wastes in vermicomposting technology – A cleaner resource recovery and biowaste recycling option for agricultural and environmental sustainability. *Environ Pollut*,2021;268:115688.
<https://doi.org/10.1016/j.envpol.2020.115688>