Cow dung and tea waste amendments enhance the biotransformation of textile mill sludge into vermicomposts using *Eisenia fetida*

Vinay Kumar Badhwar (vinaybadhwar.vb@gmail.com)
Guru Nanak Dev Engineering College https://orcid.org/0000-0001-5234-0427
Charanjit Singh
Guru Nanak Dev Engineering College

Research Article

Keywords: Textile Mill Sludge, Tea Waste, Cow Dung, Eisenia fetida, Vermicompost

Posted Date: April 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-438691/v1

Abstract

Vermicomposting of textile mill sludge (TMS) with cow dung (CD) and tea waste (TW) as amendments was done in seven different combinations using *Eisenia fetida* for 90 days. Results revealed that pH decreased from 7.68–8.63 to 7.09–7.59. TOC content and C/N ratio reductions were in range of 15.71–20.08% and 39.33–50.05% respectively ($P < 0.05$). The macronutrients in the form of TN, TP and TK increased 0.38–0.64 folds, 1.07–2.27 folds and 0.56–1.98 folds respectively after the end of bioconversion process ($P < 0.05$), among increases in ash content and EC. The biomass and cocoon production of *E.fetida* increased significantly ($P < 0.05$), while increasing mortality rate of earthworms was observed in treatments containing 50% or more TMS content. The bacterial population beneficial for degradation of organic matter increased significantly over initial substrates ($P < 0.05$). Increased humification index in the end product indicated better maturity of vermicompost as observed in treatments containing higher proportions of amendments. The addition of amendments favored earthworm activity which significantly decreased the heavy metal concentration (Fe, Cu, Pb, Zn) in the end product. The study concluded that sustainable utilization of TMS could be achieved for the cleaner and enriched vermicompost production by addition of amendments CD and TW in proportions of 50% and above.

Highlights

- The addition of cow dung and tea waste as amendments proved successful in vermicompost production.
- Higher proportions of textile mill sludge hindered the growth and reproduction of earthworms.
- Increasing beneficial bacterial population and humification index indicated better vermicompost maturity.
- Amendments favored earthworm activity which reduced heavy metal concentrations in vermicompost.

1. Introduction

Industrial waste management has been a rising concern due to the rapid industrialization and demand driving factors like per capita expenditure and increasing urbanization. The textile industry plays a major role in expanding Indian economy and is the 2nd largest exporter of textiles and clothing in the entire world. The textile exports are expected to reach US$ 82 billion in 2021 from US$ 39 billion in 2019 (https://www.ibef.org/industry/indian-textiles-and-apparel-industry-analysis-presentation). The fast fashion, falling prices and disposable clothing have led to a rise in consumerism which require large quantities of oil, water and non-renewable resources for production of synthetic fibres and toxic chemicals that are applied onto those fibres for dyeing, another water intensive operation. It is estimated that, globally, around 80–100 billion unused garments are sent to landfills every year (https://textilevaluechain.in/2020/04/10/textile-waste-management/). There are 2324 textile industries in total in India as per Central Pollution Control Board (2007) in which the annual artificial dye production is about 700,000 tonnes (Robinson et al., 2001). The dyes in the textile industry have been classified into five different groups on the basis of chemical nature – triphenyl methane, azo, polymeric, heterocyclic and reactive dyes (Banat et al., 1996). 830 million m^3/ year of fresh water is utilized by Indian textile industries for different wet operations like bleaching, desizing, dyeing, etc. (Jegatheesan et al., 2016), which expels out colorful effluent at the end. In order to remove color and traces of cotton and dyes, chemicals like ferric chloride, alum, polyelectrolyte and lime are added in Effluent Treatment Plants (ETPs), where accumulation of sludge occurs in primary and secondary clarifiers (Jahagirdar et al., 2013). The consumption of 50 m^3/hr of water results in 1–10 ton/day of sludge generation on wet basis (Balan and Monteiro, 2001).This textile mill sludge

(TMS) is very troublesome for environment and is composed of complex dyes, perishable organics, surfactants, heavy metals, auxiliaries and polycyclic aromatic hydrocarbons persistent in nature (Liang et al., 2017). Approximately, 4.65 MT of TMS was produced in China in 2016 as per China Environment Statistical Yearbook (Man et al., 2018). Destructive methods such as open burning, land filling practices and incineration is illegally practiced by industries in management of TMS which results in hazardous air pollution. The stringent waste disposal regulations/ restrictions imposed by the controlling authorities has resulted in textile sludge disposal in open dumps, agricultural fields, along the railway tracks and roadsides and in unorganized landfills, thereby causing public health hazards through soil and ground water contamination. Cost effective technologies are required for the safe disposal of textile industry sludge and usage of nutrients present in it, thus, promoting circular economy and sustainability in the total environment. The textile mill sludge has high organic and nutrient contents and could well be utilized as fertilizer/ soil conditioner in soils with low yielding capacities (Rosa et al., 2007a).

Being a popular beverage, the global consumption of tea is about 3.5 million tonnes per year (Mahaly et al., 2018) and it has been estimated that the black tea and green tea production is expected to reach 3.5MT and 4.14MT in 2027 respectively at an annual growth rate of 7.5% and 2.5% respectively (http://www.fao.org/3/BU642en/bu642en.pdf). Tea waste (TW) is disposed of in larger quantities in the environment, just like TMS. Researchers have used TW in bioremediation processes as novel adsorbent, but only few studies have used it in vermicomposting process (Badhwar et al., 2020; Hussain et al., 2018; Kaur et al., 2014; Mahaly et al., 2018). Cow dung has also been used by most of the researchers as bulking agent and amendment in the process of vermicomposting (Badhwar et al., 2020; Negi and Suthar, 2018; Sharma and Garg, 2020; Suthar, 2010). Badhwar et al. (2020) used major proportions of cow dung (60%) in all the treatment units for the bioconversion of paper mill sludge and tea waste using *Eisenia fetida*. The amendments, thus, play a crucial role in bioconversion of biosolids such as TMS. The addition of amendments/ bulking substrates is helpful for survival of earthworms as they may not be able to survive the harsh conditions of different raw wastes. Amendments decrease the harmful effects of substrates that could alter the vermicomposting process in their absence. This study was intended to investigate the effectiveness of both TW and CD as amendments in vermicomposting of TMS.

Vermicomposting is an eco-friendly sustainable method of biotransformation of different organic and non-hazardous agro-industrial wastes. The waste fed to earthworms gets fragmented and aerated after passage through gut of earthworms, and is excreted out in the form of earthworm castings. These castings are rich in macro and micro-nutrients required for plants' growth and conditioning of soil (Lavelle et al., 2006). These castings are formed as a result of biochemical actions like enzymatic digestion, enrichment of nitrogen and transport of organic and inorganic materials, taking place in the earthworm gut in association with microbial action (Edwards and Lofty, 1972). The mutual relationship of earthworms with ingested microflora results in high enzymic activity and effective degradation of organic matter available in substrates. The selection of species of earthworm is crucial before initiating vermiconversion process. *Eisenia fetida* is an epigeic earthworm that has good tolerance to different non-hazardous industrial wastes and has been used by various researchers in vermicomposting of wastes such as bakery industry sludge, tannery sludge, pulp and paper sludge, rice straw, etc. (Sharma and Garg, 2018; Ravindran et al., 2008; Yadav and Garg, 2019; Yuvaraj et al., 2020). Vermiculture technology is odourless, cost-effective, sustainable and much preferable than traditional composting in terms of availability of nutrients (Suthar, 2009a).

The biotransformation of different industrial wastes using a variety of earthworms has been reported by researchers in recent past (Bhat et al. 2015; Sharma and Garg, 2020; Negi and Suthar, 2018; Yuvaraj et al. 2018). Vermistabilization of paper mill sludge was done using earthworm species *Perionyx excavatus* which showed 48.51% reduction of TOC with maximum TP and TK contents of 83.33% and 73.33% respectively in PMS: CD = 1:1, while 100% CD (control mixture) showed maximum TN content of 88.23% (Yuvaraj et al., 2018). Singh et al. (2010a) vermicomposted bio-sludge from beverage industry in a short period of 110 days using *Eisenia fetida* and concluded that it has to be mixed with cattle dung in 50:50 ratio considering the toxicity of sludge. Vermicomposting of textile mill sludge spiked with horse dung and cow dung using *Eisenia fetida* resulted in increase in nitrogen and phosphorus contents and decrease of C/N ratio from 66.1-148.3 to 20.4–26.9 in the final substrate after six-month pilot-scale study done by Garg et al. (2009). Ravindran et al. (2008) reported increase in earthworm *Eisenia fetida* biomass from 12.5 to 50 g and maximum decrease of C/N ratio to 15.5 after biotransformation of animal flesh (ANFL) generated from tannery industry into nutrient-enriched vermicompost using cow dung and agricultural residues in 3:1:1 mixing ratios in feed mixtures. Therefore, considering leaching and production of primary and secondary pollutants generated from conventional disposal methods of open dumping, landfilling and incineration and human-ill effects associated with them, zero-waste technology of vermicomposting is most preferable and feasible method of industrial waste management and regulating recovery of resource from waste.

Therefore, the main objectives of this study were (1) to investigate the variation in physico-chemical parameters after addition of cow dung and tea waste as amendments for vermicomposting of textile mill sludge using earthworm *Eisenia fetida*, (2) evaluate biological growth patterns of earthworms and bacterial population beneficial for the enhanced mineralization rate of substrate, (3) access the efficacy of amendments and earthworms in terms of heavy metal removal and maturity of vermicompost determined by humification index.

2. Materials And Methods

2.1. Collection of earthworms, CD, TMS and TW

The epigeic earthworms were randomly collected from the Water Treatment Plant of college campus. The cow dung (urine-free) was brought from a local shed. The textile mill sludge was collected from Khurana Textile Mills, Ludhiana. The tea waste was collected from all kitchens of college campus and nearby tea stalls. Both TMS and TW were air-dried for 5 days, grinded and sieved through 2mm mesh for further use.

2.2. Experimental design

In all, seven different treatment combinations of CD, TMS and TW were set-up on dry weight basis and 5 kg of the final mixture was made in each treatment. Plastic containers of size 50.8 cm x 34.3 cm x 15.25 cm were used for the study and triplicates of each treatment were prepared for drawing statistical inferences. The following treatments were prepared:

Treatment I (T1) – 100% CD

Treatment II (T2) – 75% CD + 25% TMS (3:1)

Treatment III (T3) – 50% CD + 25% TMS + 25% TW (2:1:1)

Treatment IV (T4) – 25% CD + 25% TMS + 50% TW (1:1:2)

Treatment V (T5) – 25% CD + 50% TMS + 25% TW (1:2:1)

Treatment VI (T6) – 75% TMS + 25% TW (3:1)

Treatment VII (T7) – 100% TMS

The treatment combinations were so prepared to analyze the suitability of TMS in the process of bioconversion, considering the varying C/N ratio of TMS. All the treatments were pre-composted for 15 days by maintaining 70 ± 5% moisture content and turning the mix at regular intervals for proper aeration and degradation mechanism. After 15 days, 30 earthworms *E.fetida* (average biomass = 287.4 ± 0.57 mg/ worm) were added in each treatment and all the treatments were kept in a dark room at room temperature and 75–85% moisture content. The samples were collected at the 45th day and 90th day of study and were dried for 1 day at 60°C in hot-air oven, before being kept in airtight plastic containers for further testing and analysis of physico-chemical parameters (pH, EC, Ash content, TOC, TN, TP, TK and C/N ratio). The biological properties such as cocoon population, mortality rate and biomass changes of earthworms were also analysed at the end of vermicomposting as suggested by Negi and Suthar (2013).

2.3 Analytical Methods

2.3.1. Physico-chemical analysis

The measurement of pH and EC was done using procedure given by Tandon (2017). For pH analysis, digital pH meter was used. Distilled water aqueous extract of vermicompost (1:10 w/v) was used for pH, while it was filtered using Whatman filter paper No. 42 for EC analysis using digital conductivity meter. Ash content and TOC were determined using Nelson and Sommers method (1982). Macro Kjedahl method by Humphries (1956) was used for TN determination. Tandon (2017) was used to analyze TP (diacid digestion method – 9:4 mixture of HNO_3:$HClO_4$) and TK content (acid digestion method, followed by flame photometry), while C/N ratio was simply obtained by dividing TOC content with TN content of respective samples. The humification index was determined according to Zbytniewski and Buszewski (2005). 1 g sample was shaked with 50 ml of 0.5M NaOH for 2 hours, left overnight and centrifuged at 3000 revolutions/minute for 25 minutes using REMI R-8C Laboratory centrifuge. Finally, Agilent Cary 60 UV – VIS spectrophotometer was used to check the absorbance of supernatants at wavelengths 472 nm (A472) and 664 nm (A664). The humification index is evaluated by the ratio of A472/A664. Heavy metals (Fe, Cu, Pb, Zn) were determined by employing the method as outlined by Pedersen and van Gestel (2001). 1g sample was digested with HNO_3 and $HClO_4$ mixture and diluted with deionised water. After filtering with Whatman no.42 filter paper, the supernatant was estimated using Atomic Absorption Spectrophotometer (Agilent Technologies Spectra 240FS AA).

2.3.2 Beneficial bacterial population analysis

Standard pour plate and serial dilution method were used to evaluate the total number of colony forming units (CFU) of nitrogen fixing, phosphorus and potassium solubilizing bacteria. The procedure was followed as per Dubey and Maheshwari (2005). For preparation of stock solution, 1 g sample was taken in 10 ml deionised water and serial dilutions up to 10^{-6} were made. 1 ml of aliquot was poured onto plates containing agar media, i.e., Jensen's medium (N_2-fixing), Pikovskaya's agar (P-solubilizing) and Aleksandrow agar (K-solubilizing) respectively. The plates were then incubated at 30°C for 48 hours. The bacterial population was obtained by multiplication of number of cells per plate with the dilution factor. The results were expressed as CFU/g.

2.4. Decomposition rate

The rate of decomposition of substrates is equal to the ratio of reduction in the weight of substrate after the end of study (i.e., after 90 days) to the initial weight of substrate just before the addition of earthworms (i.e., on 0th day) (Goswami and Kalita, 2000).

Decomposition rate (%) = $[(W_i - W_f)/ W_i] * 100$

where, W_i = Initial weight of substrates before addition of earthworms and W_f = Final weight of substrates after vermicomposting

2.5. Statistical analysis

SPSS software version 22.0 was used to evaluate the statistical significance between treatment units and their relation to physcio-chemical parameters. Two-way analysis of variance (ANOVA) was done to find the mean value difference at significant level (P □ 0.05), followed by Tukey's honestly significant difference (HSD) multiple comparison test. All the data has been expressed as mean ± standard error of mean (SEM).

3. Results And Discussion

3.1. Physico-chemical characteristics of raw CD, TMS and TW

The physico-chemical parameters of CD, TMS and TW were presented in Table 1. The TMS and CD were slightly alkaline while TW was acidic in nature. Maximum Ash content was to be seen in TW (87.62 ± 0.3 %), while maximum C/N ratio was seen in TMS (111.48 ± 1.25) and clearly indicates the imbalance of organic matter and nitrogen content. Therefore, CD and TW were added to TMS for fulfillment of nutrient content and degradation of organic matter in the process of vermicomposting. Moreover, the compost maturity and biological growth patterns of earthworms were also assessed after addition of amendments. The main characteristics of 'fresh' solid textile mill sludge observed by Kaushik and Garg (2003) were: total solids (192 g/kg); pH (8.4); TOC (138 g/kg); TKN (0.66 g/kg) and C/ N ratio (230). The difference in characteristics in comparison with our study could have been due to textile mill sludge age of both studies.

3.2. Decomposition rate (%)

The decomposition rate of all the treatments after 90 days of vermicomposting with *E. fetida*, was found to have statistically significant difference (P □ 0.05) as shown in Fig. 1. The decreasing order of rate of decomposition was: T1 (61.7%) > T2 (55.8%) > T3 (52.9%) > T4 (48.4%) > T5 (35.7%) > T6 (21.0%) > T7 (7.3%) and clearly depicted unsuitability of higher TMS content treatments (T5, T6 & T7) to earthworms in the degradation process. This could well have been due to toxicity and varying C/N ratio of TMS that earthworms were not able to consume and die. Contrarily, treatments T1, T2, T3 & T4 showed good rate of degradation which can be attributed to higher proportions of amendments in the form of CD and TW. The rate of decomposition is also said to depend on the species of earthworms inoculated in vermicomposting and on the physico-chemicals characteristics of substrates (Prakash and Karmegam, 2010).

3.3. Changes in Physico-chemical characteristics of vermicompost

3.3.1 Changes in pH and Electrical Conductivity (EC)

In this study, pH of substrates decreased after the end of 90 days vermicomposting period. The pH content significantly varied (P □ 0.05) between 7.68 ± 0.05 to 8.63 ± 0.05 at the 0th day (Table 2) and decreased to 7.54 ± 0.04 to 7.96 ± 0.02 (Table 3) and 7.09 ± 0.03 to 7.59 ± 0.05 (Table 4) after 45 days and 90 days of vermicomposting respectively. The production of humic and fulvic acids from microbial activity during organic matter degradation may have resulted in this decrease (Badhwar et al., 2020; Swarnam et al., 2016). The production of CO_2 as a result of metabolic activities may also have resulted in decrease of pH (Suthar, 2009b). Yu et al. (2020) attributed the decrease in pH to the volatilization of ammonia, i.e., nitrification of NH_4^+-N and NH_3 into stable NO_3^-. A decrease in pH to 6.61–7.01 from 7.52–7.80 was reported by Sharma and Garg (2020) after biotransforming *Parthenium hysteropus*, a toxic weed into vermicompost with buffalo dung using *E.fetida*.

The results obtained for EC were statistically significant (P 0.05) and increased as compared to initial levels of EC of substrates. EC of substrates increased to 1.23 ± 0.03 to 1.99 ± 0.05 mS/cm after 90 days (Table 4) from 0.83 ± 0.03 to 1.78 ± 0.07 mS/cm on 0th day (Table 2). He et al. (2016) described the increase of EC to the release of inorganic ions and soluble salts like nitrates and phosphates.Yuvaraj et al. (2020) also reported increase in values of EC (10.1–24.2 %) after vermistabilizing textile mill wastewater sludge with *Eudrilus eugeniae* and *Perionyx excavates* for 60 days. Negi and Suthar (2018) stated that the main reason of increasing EC is the release of ammonium based compounds during the vermicomposting process.

3.3.2 Changes in Ash Content and Total Organic Carbon (TOC) content

In this study, ash content of substrates increased with increase in duration of vermicomposting and the difference in mean values were statistically significant (P 0.05) as shown by treatments T1 to T7 (Table 2,3,4). Increase in ash content is directly related to the breakdown of organic substrates by earthworms in different units. The microflora present in the gut of earthworm breaks down the complex organic matter and increase the decomposition rate of substrates resulting in increasing ash content (Aira et al., 2006). It was also observed from decreasing rate of ash contents from treatments T2 to T7 that earthworms were not able to stabilize the increasing proportions of TMS to a good effect unlike T1. Khwairakpam and Bhargava (2009a) stated that there is a direct relationship between the ash content and rate of mineralization of the substrates. The results are comparable to Gupta et al. (2007) who vermicomposted water hyacinth and reported 16.5–56.5% increase in ash content.

The biotransformation of TMS using vermicomposting showed a significant decrease in the reduction of TOC content (P 0.05). The order of decrease of TOC content after 90 days of study was: T2 (20.08%) > T1 (19.86%) > T3 (19.47%) > T4 (19.03%) > T5 (17.82%) > T6 (16.55%) > T7 (15.71%); (Table 2, 4). This decrease in TOC can be attributed to increasing ash contents of treatment units and symbiotic relationship between earthworms and microorganisms resulting in degradation of organic matter. The results obtained can be correlated with Arumugam et al. (2017) who used *Eudrillus eugeinea* to degrade paper waste and reported a maximum decrease of 26.52% in the form of TOC. The enzymes of *E.fetida* and microbial community degraded the organic carbon for its growth and reproduction, which is released in the form of CO_2 and results in decline of TOC (Badhwar et al., 2020; Sharma and Garg, 2017). Badhwar et al. (2020) also stated that this decrease in TOC gives clear indication that the vermicompost is getting mature and resulting in higher humic content, beneficial for the growth of plants and fertility of soil. A decline of 28.69–38.49% in TOC was seen in study of Paul et al. (2019) who used biochar as amendment in vermicomposting vegetable waste.

3.3.3 Changes in Total Nitrogen (TN), Total Phosphorus (TP) and Total Potassium (TK) content

The macronutrients (NPK) showed significant increase after 90 days of vermicomposting (P 0.05). The increase in TN content in this process was seen from 0.18–1.06% on 0th day (Table 2) to 0.25–1.65% after 90th day (Table 4). Maximum TN content was seen in T1 and decreased with decreasing CD content and increasing TMS contents in treatments. This can be due to the higher TN of CD (Table 1) and mineralization of organics of TMS and TW along with the efficacy of earthworms in consuming CD as depicted by the maximum increase of 55.23% in their biomass (Table 5). Negi and Suthar (2020) have also reported the significance of adding CD with organic substrates and other suitable amendments in the bioconversion process and increment of TN content. The increase in TN in vermicomposts is also due to inclusion of mucus, body fluid of earthworms, their excretory products and dead earthworm tissues (Bhat et al., 2015). The results of this study are similar to Karwal and Kaushik (2020) who reported maximum increase in TN content in 3:1 combination (Buffalo Dung + Pressmud: Fly ash) after vermicomposting for 90 days.

An increase in TP content of 1.07–2.27 folds was significantly seen after the end of study period ($P \leq 0.05$) and treatment T2 (CD: TMS = 3:1) reported a maximum increase of 1.19 ± 0.03% (Table 4). The metabolism and growth of plants gets augumented with increase in TP content (Singh and Kalamdhad, 2016; Gong et al., 2019). The enzymic (phosphatase) activity occurring in the earthworm gut with microflora partially and the simultaneous release of phosphorus in castings of earthworm contribute in enhancement of TP content (Badhwar et al., 2020). Suthar (2006) used *Perionyx excavatus* for vermicomposting and reported a maximum increase in TP content of 72.8% in combination (guar gum industrial waste + cow dung + saw dust in 3: 1:1) after 150 days. The symbiotic relationship of earthworm's gut microflora and secreted fluids and mucus helps in release of available phosphorus and promotes effective decomposition of ingested substrates (Suthar, 2007).

After 90 days of study period, TK content increased significantly from 0.09–0.89% to 0.14–1.98% and treatment T3 recorded the maximum increase of 1.98 folds, while T7 showed minimum increase of 0.56 folds, as compared to initial values ($P \leq 0.05$); (Table 2, 4). The production of organic acids from earthworm activity results in dissolution of K^+ ions in the mix, which could have increased the TK content in treatment units (Zhi-wei et al., 2019). An increase of 26.3% was also seen in 150 days study of Surendra (2007) who made vermicompost using vegetable waste and leaf litter with *Perionyx sansibaricus*.

The study done by Suthar (2010) in vermicomposting sugar mill industry sludge with three different amendments (biogas plant slurry, cow dung and wheat straw) also reported increase in TN (215.2%), TP (230.4%) and TK (253.3%). The increase in major nutrients in vermicompost is clearly attributed to the mutual activities performed by earthworms and microorganisms in converting complex organics into soluble forms (Badhwar at al., 2020). Figure 2 indicates the increasing nutrient content of treatment T3 (CD: TMS: TW = 2:1:1) and proved to a suitable combination for use in bioconversion using vermicomposting.

3.3.4 Carbon-Nitrogen balance (C/N ratio)

C/N ratio is a crucial indicator in the assessment of quality and maturity of compost and determines the humification rate of organic matter in the process of vermicomposting (Kaushik and Garg, 2003; Sharma and Garg, 2018). C/N ratio showed a significant decrease from initial level in all the treatment units ($P \leq 0.05$) as seen in Tables 2, 3 and 4. Minimum decrease (39.33%) in C/N ratio was seen in T7 due to complete exposure of TMS to earthworms. This clearly indicates the role of amendments and mixing proportions of substrates in balancing the C/N ratio of substrates and higher rate of decomposition. The decrease is generally due to respiration of earthworms and microbes, leading to mineralization of liable organics (Hanc and Chadimova, 2014). C/N ratio in between 25–30 indicates intense stabilization of organic matter; below 20 represents superior quality of compost, while below 15 can be considered for application in agricultural fields (Edwards and Bohlen, 1996; Zhang and Sun, 2014). Balachandar et al. (2019) made use of *Eudrilus eugeniae* in making vermicompost of pressmud by adding cow dung and nitrogenous green manures (*Gliricidia sepium* and *Leucaena leucocephala*) and reported decrease in C/N ratio from 48.09–61.96% to 42.05–52.73% after 50 days of study. A generalized decrease in C/N ratio depicts uniform rate of decomposition of substrates which can be clearly observed from increasing cocoon production, increasing biomass of earthworms along with minimal mortality rate.

3.4 Growth of *E. fetida*, cocoon production and mortality rate

The biological properties of *Eisenia fetida* showed significant improvement over its initial values in terms of biomass change and cocoon production ($P ≤ 0.05$) (Table 5). Increase in biomass of all the treatments were observed after the 90 day vermicomposting period and was in the order: T1 (55.23%) > T2 (53.77%) > T3 (46.89%) > T4 (38.01%) > T5 (29.49%) > T6 (24.25%) > T7 (20.49%). It clearly explains that the earthworms preferred consuming lesser proportions of TMS. The survival rate of earthworms is affected by higher concentrations of industrial wastewater sludge/wastes (Suthar, 2010). The cocoon production showed good progress with the increasing humic content of vermicompost. Maximum cocoons (33.50 ± 2.50) were seen in T2 followed by T3 (31.00 ± 1.50) and T1 (27.00 ± 1.00), while minimum was recorded in T7 (13.50 ± 0.50). The toxicity levels of different treatment units and varying proportions of amendments added to textile mill sludge may have affected rate of cocoon production. Maximum mortality rate of 44% was also seen in T7. Adverse effects in the survival and growth patterns of earthworms *E.fetida* was seen with increasing proportions of rice straw in study accomplished by Sharma and Garg (2018). Garg and Kaushik (2005) reported fluctuations in production of cocoons with time and, i.e., it increased at the the start but declined after 8th week of vermicomposting. Factors such as porosity and water holding capacity of substrate also contribute to biomass change and earthworm population change.

3.5 Beneficial bacterial population

The total beneficial bacterial population of nitrogen fixing, phosphorus and potassium solubilizing bacteria showed a significant increase in final substrates (90th day) over initial substrates (0th day) as seen in Table 6 and is indicative of faster mineralization of organic matter (Sharma and Garg, 2018) ($P ≤ 0.05$). The results can be correlated with the findings of Balachandar (2019) who reported a maximum bacterial population of 1.67 CFU x 10^8 g^{-1} after vermicomposting pressmud with cow dung and nitrogenous green manures (*Gliricidia sepium* and *Leucaena leucocephala*) using *Eudrilus eugeniae.* The increase in bacterial population, however, decreased with the increasing TMS content in treatment units as seen in T5, T6 and T7. This clearly indicates the importance of both the amendments CD and TW in this bioconversion process as depicted by treatments T1, T2, T3 and T4 and the enzymic activities performed by earthworms in conjunction with microbes. The change in bacterial population is mostly dependent up on the nature of organic substrate and the symbiotic relationship between earthworms and microbes in the process of vermitechnology.

3.6 Humification index

The humification index of various treatment units for initial and final substrates is presented graphically in Fig. 3. The significantly increasing values of humification index are indicative of mineralization of substrate and enhancement of humic acid ($P < 0.05$). Treatments with high CD content showed higher rate of humification which suggests its suitability in the process of decomposition by earthworms and microbes. The results are in correlation with Zhang and Sun (2017) who made use cow dung and spent coffee grounds for the enhancement of two-stage cocomposting of green waste. Conversely, Boruah et al. (2019) employed *E.fetida* for the vermiconversion of citronella bagasse (CB) and paper mill sludge mixture and reported 68.4% and 26.6% decrease in humification index of CB and CB + PMS (3:2) vermicompost, respectively over initial mixtures. The difference in results can be attributed to the nature of organic substrate to be degraded and the nature of amendments that are added in the vermicomposting process.

3.7 Heavy Metals (Fe, Cu, Pb, Zn)

The changes in concentrations of heavy metals (Fe, Cu, Pb, Zn) was also evaluated and showed a significant decrease over initial substrates as seen in Table 7 (P □ 0.05). This reduction could have been due to the heavy metal accumulation within the gut of earthworms. Samal et al., 2019 stated that heavy metals have strong affinity to metal binding protein (metallothionein) available within the earthworms, which limits heavy metal effect in excreted casts. Suleiman et al., 2017 justified this decrease to the prolific defence mechanism of *E.fetida* who has higher metal accumulation capacity among other earthworm species that helps in effective bioremediation of industrial wastes. The addition of amendments CD and TMS also favored the decrease in heavy metals in this study as depicted by treatments T2, T3, T4 and T5. Some studies have also reported increase in heavy metal concentrations in the final substrates (Yadav and Garg, 2011; Gong et al., 2019). Gong et al. (2019) used spent mushroom substrate and cattle manure as amendments to enhance biotransform garden waste into vermicompost employing *Eisenia fetida* and reported increase in heavy metals: Cu (35.6–62.4%), Zn (27.4–91.1%), Cr (34.2–87.1%), Pb (37.2–60.7%), Cd (32.7–87.7%) and Ni (22.0–59.0%) for Ni. This could have been a consequence of loss of carbon in the form of CO_2 and increasing decomposition rate of substrates (Yadav and Garg, 2011).

4. Conclusion

The addition of amendments CD and TW (50% or more) in bioconversion of TMS using *E.fetida* accelerated degradation rate of substrates and enhanced growth patterns of earthworms. The increased beneficial bacterial population and higher humification index indicates high rate of organic matter degradation and better vermicompost maturity of the end product. The study observed that higher proportions of TMS (50% and above) hindered the performance of earthworms and increased their mortality rate. The addition of amendments favored the earthworm activity resulted in decrease of heavy metal concentrations in the final vermicompost. The physico-chemical characteristics indicated increased nutrient content and effective stabilization of organic matter of substrate. Therefore, vermicomposting of textile mill sludge can be done by addition of cow dung and tea waste as amending materials for eradication of disposal issues of industrial wastes, enriched vermicompost production and cleaner environment.

Declarations

Acknowledgement

The authors would like to thank Dr. Puneet Pal Singh Cheema, Prof. Sukhwinderpal Singh and Prof. Balihar Singh for providing research facilities in the Environment Engineering Laboratory and mentoring at every stage of this work.

Funding Source

No specific grants were received from funding agencies in the commercial, public, or not-for-profit sectors.

Conflict of interest statement

The authors declare on mutual understanding that there is no conflict of interest, whatsoever, in this work.

References

Aira, M., Monroy, F., Domínguez, J., 2006. *Eisenia fetida* (*Oligochaeta*, *Lumbricidae*) activates fungal growth, triggering cellulose decomposition during vermicomposting. Microb. Ecol. 52, 738–747. https://doi.org/10.1007/s00248-006-9109-x.

Arumugam, K., Renganathan, S., Babalola, O.O., Muthunarayanan, V., 2017. Investigation on paper cup waste degradation by bacterial consortium and Eudrillus eugeinea through vermicomposting, Waste Manag. 74, 185–193.

Badhwar, V.K., Singh, S., Singh, B., 2020. Biotransformation of paper mill sludge and tea waste with cow dung using vermicomposting. Bioresour Technol., 318, 124097. https://doi.org/10.1016/j.biortech.2020.124097.

Balachandar, R., Baskaran, L., Yuvaraj, A., Thangaraj, R., Subbaiya, R., Ravindran, B., Chang, S.W., Karmegam, N., 2019. Enriched pressmud vermicompost production with green manure plants using *Eudrilus eugeniae*, Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.122578.

Balan, D.S.L., Monteiro, R.T.R., 2001. Decolonization of textile indigo dye by ligninolytic fungi. J. Biotechnol. 89,141-145. https://doi.org/10.1016/S0168-1656(01)00304-2.

Banat, I.M., Nigam, P., Singh, D., Marchant, R., 1996. Microbial decolorization of textile-dye containing effluents: a review. Bioresour. Technol. 58, 217–227. https://doi. org/10.1016/S0960-8524(96)00113-7.

Bhat, S.A., Singh, J., Vig, A.P., 2015. Potential utilization of bagasse as feed material for earthworm Eisenia fetida and production of vermicompost. Springerplus 4. https://doi.org/10.1186/s40064-014-0780-y.

CPCB (Central Pollution Control Board), 2007. Advance Methods for Treatment of Textile Industry Effluents. Publication No.RERES/7/2007, New Delhi.

Dubey, R., Maheshwari, D., 2005. Practical Microbiology. S. Chand Comp. LtdS. Chand Comp. Ltd, New Delhi.

Edwards, C.A., Lofty, J.R., 1972. Biology of earthworms. Chapman and Hall, London.

Edwards, C., and Bohlen, P., 1996. Biology and ecology of earthworms. Springer Science & Business Media Volume 3.

Garg, V.K., Kaushik, P., 2005. Vermistabilization of textile mill sludge spiked with poultry droppings by an epigeic earthworm *Eisenia foetida*. Bioresour Technol. 96, 1063-1071.

Garg, V.K., Gupta, R., Kaushik, P., 2009. Vermicomposting of solid textile mill sludge spiked with cow dung and horse dung: a pilot-scale study. Int J Environ Pollun. 38(4), 385–396.

Gong, X., Li, S., Carson, M. A., Chang, S. X., Wu, Q., Wang, L., Sun, X., 2019. Spent mushroom substrate and cattle manure amendments enhance the transformation of garden waste into vermicomposts using the earthworm *Eisenia fetida*. J. Environ. Manag. 248, 109263. https://doi.org/10.1016/j.jenvman.2019.109263.

Goswami, M.C., Kalita, B., 2000. Efficiency of some indigenous earthworm species of Assam and its characterization through vermitechnology. Indian J. Environ. Ecoplann. 3, 351–354.

Gupta, R., Mutiyar, P.K., Rawat, N.K., Saini, M.S., Garg, V.K., 2007. Development of a water hyacinth based vermireactor using an epigeic earthworm *Eisenia foetida.* Bioresource Technology, 98, 2605–2610.

Hanc, A., Chadimova, Z., 2014. Nutrient recovery from apple pomace waste by vermicomposting technology. Bioresour. Technol.https://doi.org/10.1016/j.biortech.2014.02.031.

http://www.fao.org/3/BU642en/bu642en.pdf

https://textilevaluechain.in/2020/04/10/textile-waste-management/

https://www.ibef.org/industry/indian-textiles-and-apparel-industry-analysis-presentation

Humphries EC., 1956. Mineral components and ash analysis. Modern Methods of Plant Analysis. Springer, 468-502.

Hussain, S., Anjali, K.P., Hassan, S.T., 2018. Waste tea as a novel adsorbent: a review. *Appl Water Sci* 8, 165. https://doi.org/10.1007/s13201-018-0824-5.

Jahagirdar, S.S., Shrihari, S., Manu, B., 2013. Utilization of Textile Mill Sludge in Burnt Clay Bricks. International Journal of Environmental Protection. 3, 6-13. https://www.researchgate.net/publication/277961152.

Jegatheesan, V., Pramanik, B.K., Chen, J., Navaratna, D., Chang, C.-Y., Shu, L., 2016. Treatment of textile wastewater with membrane bioreactor: a critical review. Bioresour. Technol. 204, 202–212. https://doi.org/10.1016/j.biortech.2016.01.006.

Karwal, K., Kaushik, A., 2020. Co-composting and vermicomposting of coal fly-ash with press mud: Changes in nutrients, micro-nutrients and enzyme activities. Environmental Technology & Innovation 18, 100708. https://doi.org/10.1016/j.eti.2020.100708.

Kaur, S., Kour, G., and Singh, J., 2014. Vermicomposting of tea leaves waste mixed with cow dung with the help of exotic earthworm *Eisenia fetida*. Int. J. Adv. Res. Biol.Sci., 1(9), 229–234.

Kaushik, P., Garg, V.K., 2003. Vermicomposting of mixed solid textile mill sludge and cow dung with the epigeic earthworm *Eisenia foetida*. Bioresour. Technol. 90, 311–316. https://doi.org/10.1016/S0960-8524(03)00146-9.

Khwairakpam, M., Bhargava, R., 2009a. Bioconversion of filter mud using vermicomposting employing two exotic and one local earthworm species. Bioresour Technol., 100 (23), 5846–5852.

Lavelle, P., Decaens, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., 2006. Soil invertebrates and ecosystem services. Eur. J. Soil Biol. 42, 3–15.

Liang, J., Ning, X., Lai, X., Zou, H., Sun, J., Lu, X., 2017. Influence mechanisms of textiledyeing sludge characteristics on degradation of anilines by integrated ultrasoundpermanganate treatment. J Clean Prod. 151, 172–8.

Mahaly, M., Senthilkumar, A.K., Arumugam, S., Kaliyaperumal, C., Karupannan, N., 2018. Vermicomposting of distillery sludge waste with tea leaf residues. Sustainable Environment Research. 28, 223-227.

Man, X., Ning, X.A., Zou, H., Liang, J., Sun, J., Lu, X., Sun, J., 2018. Removal of polycyclic aromatic hydrocarbons (PAHs) from textile dyeing sludge by ultrasound combined zero-valent iron/EDTA/Air system. Chemosphere 191, 839–847. https://doi.org/10.1016/j.ecoenv.2018.10.022.

Negi R, Suthar S., 2013. Vermistabilization of paper mill wastewater sludge using Eisenia fetida. Bioresour Technol., 128,193–198.

Negi, R., Suthar, S., 2018. Degradation of paper mill wastewater sludge and cow dung by brown-rot fungi *Oligoporus placenta* and earthworm (*Eisenia fetida*) during vermicomposting. J. Clean. Prod. 201, 842–852. https://doi.org/10.1016/j.jclepro.2018.08.068.

Nelson, D.W., Sommers, L., 1982. Total carbon, organic carbon, and organic matter. Methods Soil Anal. 539–579.

Pedersen, M.B., van Gestel, C.A.M., 2001. Toxicity of copper to collembolan *Folsomia fimeteria* in relation to the age of soil contamination.Ecotoxicol.Environ.Saf. 49, 54–59.

Prakash, M., Karmegam, N., 2010. Vermistabilization of pressmud using *Perionyx ceylanensis* Mich. Bioresour Technol., 101, 8464–8468. doi:10.1016/j.biortech.2010.06.002.

Ravindran, B., Dinesh, S.L., John Kennedy, L., Sekaran, G., 2008. Vermicomposting of solid waste generated from leather industries using epigeic earthworm Eisenia foetida. Appl. Biochem. Biotechnol., 151, 480–488.

Robinson, T., McMullan, G., Marchant, R., Nigam, P., 2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresour. Technol. 77, 247–255. https://doi.org/10.1016/S0960-8524 (00)00080-8.

Rosa, E.V.C., Giuradelli, T.M., Corrêa, A.X.R., Rörig, L.R., Sshwingel, P.R., Resgalla-Jr, C., Radetski, C.M., 2007a. Ecotoxicological evaluation of the short term effects of fresh and stabilized textile sludges before application in forest soil restoration. Environ. Pollut. 146, 463- 469. https://doi.org/10.1016/j.envpol.2006.07.005.

Samal, K., Mohan, A.R., Chaudhary, N., Moulick, S., 2019. Application of vermitechnology in waste management: A review on mechanism and performance. J. Environ. Chem. Eng. 7, 103392. https://doi.org/10.1016/j.jece.2019.103392.

Sharma, K., Garg, V.K., 2017. Management of food and vegetable processing waste spiked with buffalo waste using earthworms (*Eisenia fetida*). Environ. Sci. Pollut. Res. 24, 7829–7836. https://doi.org/10.1007/s11356-017-8438-2.

Sharma, K., Garg, V., 2018. Comparative analysis of vermicompost quality produced from rice straw and paper waste employing earthworm *Eisenia fetida* (Sav.). Bioresour. Technol. 250, 708–715.

Sharma, K., Garg, V.K., 2020. Conversion of a toxic weed into vermicompost by Eisenia fetida: Nutrient content and earthworm fecundity. *Bioresource Technology Reports.* https://doi.org/10.1016/j.biteb.2020.100530.

Singh, J., Kaur, A., Vig, A.P., Rup, P.J., 2010a. Role of *Eisenia fetida* in rapid recycling of nutrients from bio sludge of beverage industry. Ecotoxicol Environ Saf. 73, 430–435.

Singh, W.R., Kalamdhad, A.S., 2016. Transformation of nutrients and heavy metals during vermicomposting of the invasive green weed Salvinia natans using Eisenia fetida. Int. J. Recycl. Org. Waste Agric. 5, 205–220.

Soobhany, N., Mohee, R., Garg, V.K., 2015. Comparative assessment of heavy metals content during the comparing and vermicomposting of municipal solid waste employing *Eudrilus eugeniae*. Waste Manag., 39, 130-145.

Suleiman, H., Rorat, A., Grobelak, A., Grosser, A., Milczarek M., Płytycz B., Kacprzak M., Vandenbulcke F., 2017. Determination of the performance of vermicomposting process applied to sewage sludge by monitoring of the compost quality and immune responses in three earthworm species: *Eisenia fetida*, *Eisenia andrei* and *Dendrobaena veneta.* Bioresour. Technol. 241, 103–112.

Suthar, S., 2006. Potential utilization of guar gum industrial waste in vermicompost production. Bioresour Technol., 97, 2474-2477.

Suthar, S., 2007. Nutrient changes and biodynamics of epigeic earthworm *Perionyx excavatus*

(Perrier) during recycling of some agriculture wastes. Bioresour. Technol. 98, 1608–1614.

Suthar, S., 2009a. Vermicomposting of vegetable-market solid waste using Eisenia fetida: impact of bulking material on earthworm growth and decomposition rate. Ecol. Eng. 35, 914–920.

Suthar, S., 2009b. Vermistabilization of municipal sewage sludge amended with sugarcane

trash using epigeic *Eisenia fetida* (Oligochaeta). Journal of Hazardous Materials, 163: 199–206.

Suthar, S., 2010. Recycling of agro-industrial sludge through vermitechnology. Ecol. Eng., 36, 1028–1036.

Swarnam, T.P., Velmurugan, A., Pandey, S.K., Roy, S.D., 2016. Enhancing nutrient recovery and compost maturity of coconut husk by vermicomposting technology. Bioresour. Technol. 207, 76–84.

Tandon, H.L.S., (Ed.), 2017. Methods of Analysis of Soils, Plants, Water, Fertilisers and Organic Manures. Fertiliser Development and Consultation Organization, New Delhi India. pp. 204 + xii.

Yadav, A., Garg, V.K., 2011. Vermicomposting–an effective tool for the management of invasive weed Parthenium hysterophorus. Bioresour. Technol. 102, 5891–5895.

Yadav, A., Garg, V.K., 2019. Biotransformation of bakery industry sludge into valuable product using vermicomposting. Bioresour Technol., 274, 512–517.

Yu, K., Li, S., Sun, X., Kang, Y., 2020. Maintaining the ratio of hydrosoluble carbon and hydrosoluble nitrogen within the optimal range to accelerate green waste composting. Waste Manage. 105, 405–413.

Yuvaraj, A., Karmegam, N., Thangaraj, R., 2018a. Vermistabilization of paper mill sludge by an epigeic earthworm *Perionyx excavatus*: Mitigation strategies for sustainable environmental management. Ecol. Eng., 120: 187–197. https://doi.org/10.1016/j.ecoleng.2018.06.008.

Yuvaraj A, Karmegam N, Tripathi S, Kannan S, Thangaraj R., 2020. Environment-friendly management of textile mill wastewater sludge using epigeic earthworms: Bioaccumulation of heavy metals and metallothionein production. J Environ Manage., 254 (2020) 109813. https://doi.org/10.1016/j.jenvman.2019.109813.

Zbytniewski, R., Buszewski, B., 2005. Characterization of natural organic matter (NOM) derived from sewage sludge compost. Part 2: Multivariate techniques in the study of compost maturation. Bioresour. Technol. 96, 479–484.

Zhang, L., Sun, X.Y., 2014. Changes in physical, chemical, and microbiological properties during the two-stage co-composting of green waste with spent mushroom compost and biochar. Bioresour Technol., 171, 274–284.

Zhi-wei, S., Tao, S., Wen-jing, D., Jing, W., 2019. Investigation of rice straw and kitchen waste degradation through vermicomposting. Journal of Environmental Management. 243, 269-272.

Tables

Table 1

Physico-chemical characteristics of raw CD, TMS and TW.

Parameters	pH	EC (mS/cm)	Ash Content (%)	TOC (%)	TN (%)	TP (%)	TK (%)	C/N ratio	P-value
Cow Dung (CD)	7.89 ± 0.07	1.62 ± 0.22	19.15 ± 0.12	46.71 ± 1.60	1.35 ± 0.12	0.38 ± 0.05	0.51 ± 0.03	34.47 ± 1.33	0.0001*
Textile Mill Sludge (TMS)	8.26 ± 0.13	2.69 ± 0.08	67.28 ± 0.7	18.98 ± 0.12	0.17 ± 0.03	0.11 ± 0.01	0.08 ± 0.02	111.48 ± 1.25	0.0001*
Tea Waste (TW)	5.23 ± 0.09	2.48 ± 0.06	87.62 ± 0.3	7.18 ± 0.4	0.09 ± 0.02	1.54 ± 0.04	0.83 ± 0.05	78.77 ± 1.02	0.0001*

*All values are statistically significant (P ▯ 0.05).

Table 2

Physico-chemical characteristics of initial substrates (0^{th} day) in different treatments (Values are mean ± SEM of three replicates).

* [SEM= Standard error of mean and all the above mean values indicate that difference between treatments is statistically different] (P ▯ 0.05).

Table 3

Physico-chemical characteristics of substrates after 45 days of vermicomposting with *E.fetida* in different treatments (Values are mean ± SEM of three replicates).

Treatments	pH	EC (mS/cm)	Ash Content (%)	TOC (%)	TN (%)	TP (%)	TK (%)	C/N ratio
T1 [CD (100%)]	8.63 ± 0.05	0.83 ± 0.03	21.48 ± 0.22	45.55 ± 0.65	1.06 ± 0.01	0.42 ± 0.02	0.89 ± 0.03	42.97 ± 0.25
T2 [CD:TMS(3:1)]	8.52 ± 0.08	0.91 ± 0.04	44.38 ± 0.65	32.26 ± 0.40	0.96 ± 0.02	0.38 ± 0.03	0.72 ± 0.02	33.60 ± 0.40
T3 [CD:TMS:TW(2:1:1)]	8.39 ± 0.04	1.17 ± 0.05	49.91 ± 0.10	29.05 ± 0.70	0.84 ± 0.01	0.31 ± 0.05	0.59 ± 0.03	34.58 ± 0.15
T4 [CD:TMS:TW(1:1:2)]	8.24 ± 0.05	1.28 ± 0.08	54.27 ± 0.50	26.53 ± 0.10	0.63 ± 0.03	0.26 ± 0.01	0.45 ± 0.01	42.11 ± 0.10
T5 [CD:TMS:TW(1:2:1)]	8.09 ± 0.07	1.35 ± 0.05	59.35 ± 0.45	23.58 ± 0.60	0.45 ± 0.03	0.22 ± 0.02	0.34 ± 0.02	52.40 ± 0.05
T6 [TMS:TW(3:1)]	7.99 ± 0.04	1.52 ± 0.03	63.16 ± 1.05	21.37 ± 0.25	0.31 ± 0.01	0.19 ± 0.02	0.21 ± 0.04	68.93 ± 0.45
T7 [TMS (100%)]	7.68 ± 0.05	1.78 ± 0.07	69.20 ± 0.30	17.87 ± 0.50	0.18 ± 0.02	0.13 ± 0.01	0.09 ± 0.02	99.28 ± 0.20
P-value	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0056*	0.0001*

Treatments	pH	EC (mS/cm)	Ash Content (%)	TOC (%)	TN (%)	TP (%)	TK (%)	C/N ratio
T1 [CD (100%)]	7.96 ± 0.02	1.14 ± 0.05	31.04 ± 0.34	40.00 ± 0.25	1.36 ± 0.02	0.75 ± 0.01	1.36 ± 0.03	29.42 ± 0.20
T2 [CD:TMS(3:1)]	7.87 ± 0.03	1.36 ± 0.04	51.69 ± 0.26	28.02 ± 0.70	1.21 ± 0.03	0.79 ± 0.04	1.49 ± 0.02	23.15 ± 0.45
T3 [CD:TMS:TW(2:1:1)]	7.83 ± 0.05	1.50 ± 0.03	56.05 ± 0.31	25.49 ± 0.45	1.03 ± 0.04	0.64 ± 0.03	1.07 ± 0.04	24.74 ± 0.35
T4 [CD:TMS:TW(1:1:2)]	7.79 ± 0.06	1.67 ± 0.05	60.17 ± 0.43	23.06 ± 0.15	0.81 ± 0.01	0.56 ± 0.01	0.74 ± 0.02	28.47 ± 0.20
T5 [CD:TMS:TW(1:2:1)]	7.74 ± 0.03	1.69 ± 0.05	64.39 ± 0.65	20.65 ± 0.40	0.63 ± 0.01	0.49 ± 0.03	0.62 ± 0.02	32.78 ± 0.20
T6 [TMS:TW(3:1)]	7.65 ± 0.02	1.76 ± 0.03	67.70 ± 0.81	18.73 ± 0.55	0.42 ± 0.03	0.35 ± 0.01	0.33 ± 0.03	44.60 ± 0.30
T7 [TMS (100%)]	7.54 ± 0.04	1.83 ± 0.04	71.98 ± 0.21	16.25 ± 0.30	0.23 ± 0.01	0.29 ± 0.01	0.11 ± 0.01	70.65 ± 0.50
P-value	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*

* [SEM= Standard error of mean and all the above mean values indicate that difference between treatments is statistically different] (P ▯ 0.05).

Table 4

Physico-chemical characteristics of final substrates after 90 days of vermicomposting with *E.fetida* in different treatments (Values are mean ± SEM of three replicates).

* [SEM= Standard error of mean and all the above mean values indicate that difference between treatments is statistically different] (P ▯ 0.05).

Treatments	pH	EC (mS/cm)	Ash Content (%)	TOC (%)	TN (%)	TP (%)	TK (%)	C/N ratio
T1 [CD (100%)]	7.09 ± 0.03	1.23 ± 0.03	37.15 ± 0.31	36.46 ± 0.45	1.65 ± 0.02	1.07 ± 0.03	1.83 ± 0.03	22.09 ± 0.35
T2 [CD:TMS(3:1)]	7.21 ± 0.04	1.37 ± 0.02	55.56 ± 0.56	25.78 ± 0.30	1.48 ± 0.03	1.19 ± 0.03	1.98 ± 0.01	17.41 ± 0.20
T3 [CD:TMS:TW(2:1:1)]	7.30 ± 0.06	1.55 ± 0.08	58.72 ± 0.34	23.39 ± 0.10	1.22 ± 0.02	0.97 ± 0.01	1.76 ± 0.02	19.17 ± 0.55
T4 [CD:TMS:TW(1:1:2)]	7.38 ± 0.07	1.72 ± 0.04	62.96 ± 0.50	21.48 ± 0.20	0.96 ± 0.05	0.84 ± 0.01	1.29 ± 0.01	22.37 ± 0.20
T5 [CD:TMS:TW(1:2:1)]	7.47 ± 0.02	1.99 ± 0.05	65.98 ± 0.54	19.37 ± 0.40	0.74 ± 0.03	0.72 ± 0.01	0.95 ± 0.01	26.17 ± 0.45
T6 [TMS:TW(3:1)]	7.52 ± 0.03	1.87 ± 0.03	70.26 ± 0.75	17.83 ± 0.35	0.46 ± 0.02	0.53 ± 0.03	0.43 ± 0.03	38.76 ± 0.65
T7 [TMS (100%)]	7.59 ± 0.05	1.81 ± 0.04	71.84 ± 0.40	15.06 ± 0.60	0.25 ± 0.01	0.27 ± 0.01	0.14 ± 0.04	60.24 ± 0.10
P-value	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0078*	0.0001*	0.0001*

Table 5

Biological properties of earthworms *E.fetida* in different treatments during vermicomposting (Values are mean ± SEM of three replicates).

Treatments	Individual biomass of earthworms (g)		Biomass Change (%)	Total no. of cocoons (n)	Mortality rate (%)
	Initial	Final			
T1 [CD (100%)]	8.79 ± 0.14*	19.63 ± 0.75*	+55.23*	27.00 ± 1.00*	8*
T2 [CD:TMS(3:1)]	8.34 ± 0.47*	18.04 ± 0.63*	+53.77*	33.50 ± 2.50*	8*
T3 [CD:TMS:TW(2:1:1)]	8.23 ± 0.53*	15.48 ± 0.48*	+46.89*	30.50 ± 1.50*	11*
T4 [CD:TMS:TW(1:1:2)]	8.51 ± 0.26*	13.73 ± 0.25*	+38.01*	23.00 ± 2.00*	14*
T5 [CD:TMS:TW(1:2:1)]	8.82 ± 0.18*	12.51 ± 0.14*	+29.49*	21.50 ± 0.50*	23*
T6 [TMS:TW(3:1)]	8.93 ± 0.11*	11.79 ± 0.43*	+24.25*	16.00 ± 1.00*	31*
T7 [TMS (100%)]	8.73 ± 0.38*	10.98 ± 0.24*	+20.49*	13.50 ± 0.50*	44*

* [SEM= Standard error of mean and all the above mean values indicate that difference between treatments is statistically different] (P 0.05).

Table 6

Beneficial nitrogen fixing, phosphate and potassium solubilizing bacterial population in different treatments on 0th day and after 90 days of vermicomposting (Values are mean ± SEM of three replicates).

Treatments	N2-fixing (CFU.g^{-1})	P-solubilizing (CFU.g^{-1})	K-solubilizing (CFU.g^{-1})
Initial Substrates (0th day)			
T1 [CD (100%)]	9.63 x 10^6 ± 1.12*	8.91 x 10^6 ± 0.863*	13.46 x 10^6 ± 1.15*
T2 [CD:TMS(3:1)]	9.15 x 10^6 ± 2.23*	7.23 x 10^6 ± 1.342*	12.81 x 10^6 ± 2.40*
T3 [CD:TMS:TW(2:1:1)]	8.45 x 10^6 ±0.78*	6.08 x 10^6 ±0.754*	10.74 x 10^6 ± 0.54*
T4 [CD:TMS:TW(1:1:2)]	7.64 x 10^6 ± 3.06*	5.45 x 10^6 ± 1.365*	9.23 x 10^6 ± 0.98*
T5 [CD:TMS:TW(1:2:1)]	6.48 x 10^6 ± 1.73*	3.81 x 10^6 ± 2.755*	8.91 x 10^6 ± 3.34*
T6 [TMS:TW(3:1)]	5.93 x 10^6 ± 1.45*	2.37 x 10^6 ± 0.601*	6.15 x 10^6 ± 0.76*
T7 [TMS (100%)]	4.56 x 10^6 ± 0.92*	1.14 x 10^6 ± 0.385*	5.96 x 10^6 ± 1.64*
Final Substrates (90th day)			
T1 [CD (100%)]	76.32 x 10^6 ± 3.56*	43.83 x 10^6 ± 2.45*	92.65 x 10^6 ± 4.91*
T2 [CD:TMS(3:1)]	69.10 x 10^6 ± 5.02*	47.91 x 10^6 ± 4.26*	85.81 x 10^6 ± 3.32*
T3 [CD:TMS:TW(2:1:1)]	55.97 x 10^6 ± 7.95*	51.73 x 10^6 ± 3.34*	79.72 x 10^6 ± 5.53*
T4 [CD:TMS:TW(1:1:2)]	48.32 x 10^6 ± 4.84*	43.85 x 10^6 ± 4.93*	62.63 x 10^6 ± 7.85*
T5 [CD:TMS:TW(1:2:1)]	43.24 x 10^6 ± 4.12*	36.91 x 10^6 ± 4.76*	54.22 x 10^6 ± 5.97*
T6 [TMS:TW(3:1)]	35.75 x 10^6 ± 3.75*	29.01 x 10^6 ± 2.95*	43.01 x 10^6 ± 3.72*
T7 [TMS (100%)]	31.83 x 10^6 ± 5.92*	22.54 x 10^6 ± 1.25*	33.40 x 10^6 ± 2.26*

* [SEM= Standard error of mean and all the above mean values indicate that difference between treatments is statistically different] ($P \le 0.05$).

Table 7

Heavy metals (Fe, Cu, Pb and Zn) in different treatments at 0th day and after 90 days of vermicomposting (Values are mean ± SEM of three replicates).

Treatments	Fe (mg.kg^{-1})	Cu (mg.kg^{-1})	Pb (mg.kg^{-1})	Zn (mg.kg^{-1})
Initial Substrates (0th day)				
T1 [CD (100%)]	18.7 ± 0.38	117.8 ± 0.2	1.09 ± 0.01	71.92 ± 1.2
T2 [CD:TMS(3:1)]	35.3 ± 0.5	105.6 ±0.4	0.95 ± 0.03	76.56 ± 1.4
T3 [CD:TMS:TW(2:1:1)]	46.8 ± 0.1	100.2 ±1.5	0.82 ± 0.02	78.83 ± 0.9
T4 [CD:TMS:TW(1:1:2)]	54.5 ± 0.3	104.6 ± 0.3	0.69 ± 0.06	82.67 ± 1.2
T5 [CD:TMS:TW(1:2:1)]	61.5 ± 0.4	101.2 ± 0.5	0.73 ± 0.04	85.53 ± 1.6
T6 [TMS:TW(3:1)]	79.8 ± 0.5	98.6 ±1.2	0.67 ± 0.04	89.43 ± 1.5
T7 [TMS (100%)]	86.8 ± 0.3	94.6 ±0.4	0.63 ± 0.02	95.43 ± 3.1
Final Substrates (90th day)				
T1 [CD (100%)]	13.5 ± 0.2	69.53 ± 0.4	0.72 ±0.02	32.28 ± 1.3
T2 [CD:TMS(3:1)]	26.8 ± 0.3	73.13 ± 0.2	0.68 ± 0.03	41.85 ± 0.8
T3 [CD:TMS:TW(2:1:1)]	27.4 ± 0.5	74.68 ± 0.3	0.66 ± 0.04	44.60 ± 1.4
T4 [CD:TMS:TW(1:1:2)]	35.3 ± 0.1	76.49 ± 1.1	0.53 ± 0.03	57.54 ± 1.3
T5 [CD:TMS:TW(1:2:1)]	48.4 ± 0.2	78.45 ± 0.2	0.51 ± 0.04	63.45 ± 1.7
T6 [TMS:TW(3:1)]	71.5 ± 0.4	89.7 ± 1.3	0.59 ± 0.01	75.54 ± 1.4
T7 [TMS (100%)]	83.9 ± 0.6	92.1 ± 0.5	0.56 ± 0.04	87.54 ± 2.9

Figures

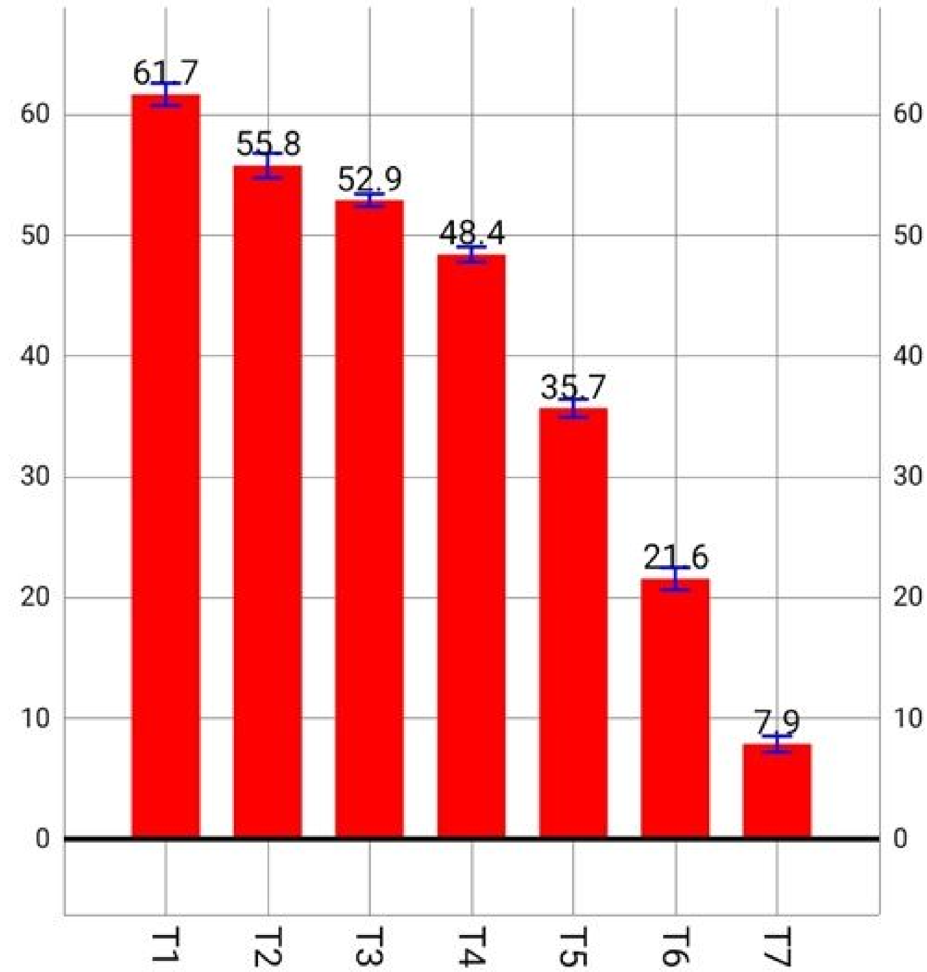

Figure 1

Decomposition rate (vertical axis) of substrates in different treatments (horizontal axis) with E. fetida (90 days). The obtained values are mean of three replicates and error bars indicate ± Standard Error of Mean (SEM). The mean value difference between the treatments are significant at 5% level ($P<0.05$) by Tukey’s honestly significant difference (HSD) multiple comparison test

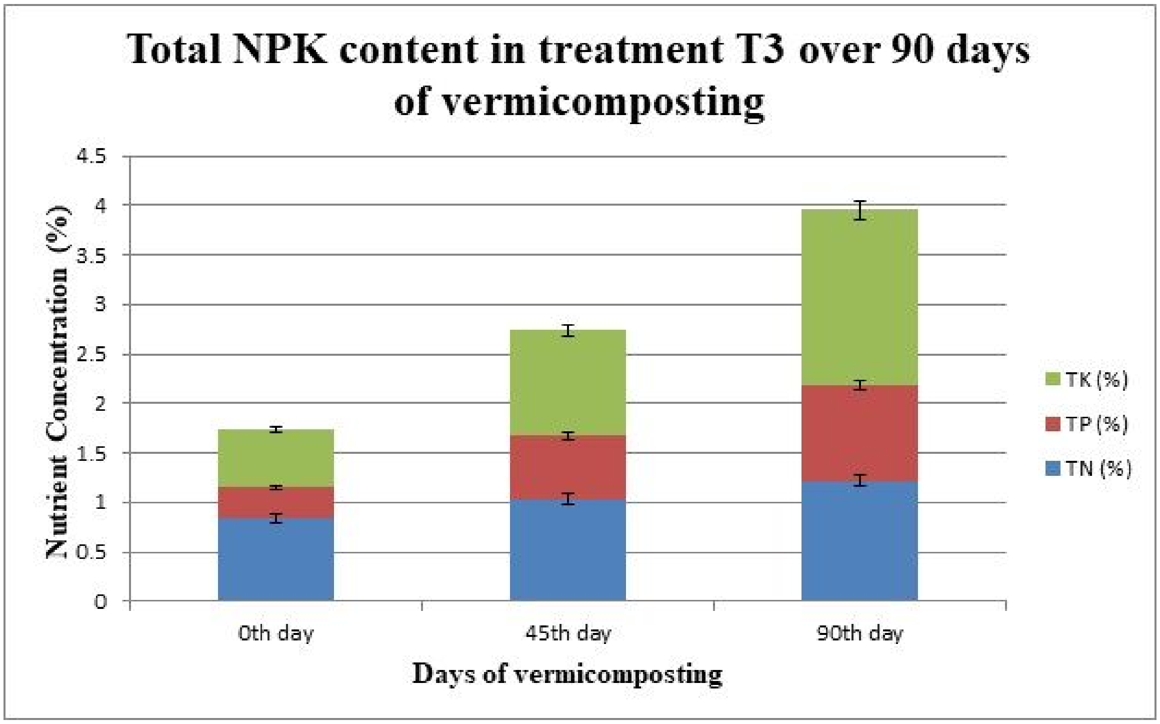

Figure 2

Chart showing the Total N, P and K contents observed in treatment T3 over 90 days of vermicomposting period. The obtained values are mean of three replicates and error bars indicate ± Standard Error of Mean (SEM). The mean value difference between the treatments are significant at 5% level (P<0.05) by Tukey's honestly significant difference (HSD) multiple comparison test.

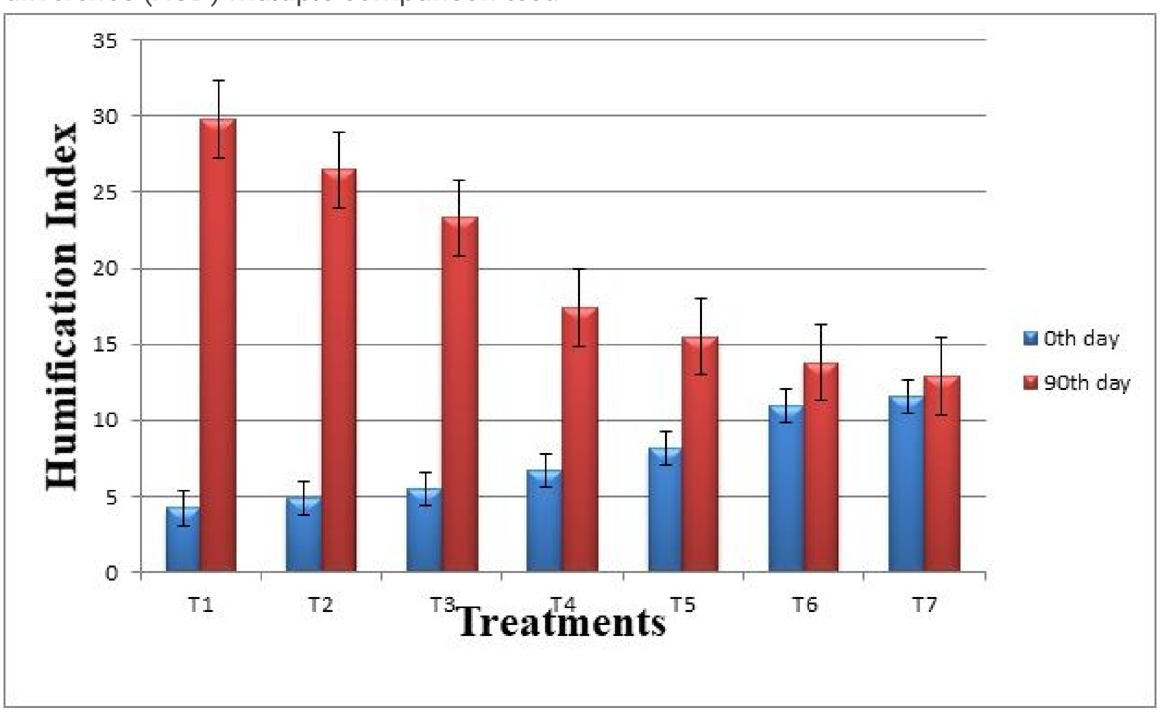

Figure 3

Humification index of initial (0th day) and final (90th day) substrates. Values are mean of three replicates and error bars indicate ± Standard Error of Mean (SEM).). The mean value difference between the treatments are significant at 5% level ($P<0.05$) by Tukey's honestly significant difference (HSD) multiple comparison test.