



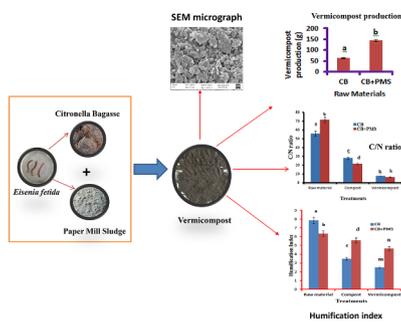
Vermicomposting of citronella bagasse and paper mill sludge mixture employing *Eisenia fetida*

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GRAPHICAL ABSTRACT



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ABSTRACT

The vermicomposting potential of *Eisenia fetida* on citronella bagasse and paper mill sludge mixture was studied. The experiment was carried out in pots by taking a mixture of citronella bagasse and paper mill sludge in 3:2 ratios. The physico-chemical properties such as pH, conductivity, total organic carbon, nitrogen, phosphorus, potassium, calcium, trace elements and heavy metals were studied in the end products. The ash content, humification index, C/N ratio and scanning electron microscopic analysis were done to understand the maturity of the vermicompost. Results revealed that bioconversion of citronella bagasse and paper mill sludge mixture is accompanied with reduction of C/N ratio and humification index; enhancement of nutrients profile, nitrogen fixing, phosphate and potassium solubilizing bacterial population. SEM analysis showed that there was more disintegration in vermicompost samples than the initial raw materials and compost. Further, earthworm population and biomass has significantly increased by the end of the experimental trials.

1. Introduction

Citronella bagasse and paper mill sludge are the by-products of agro based industry. *Cymbopogon winterianus* Jowitt which is commonly known as java citronella is an aromatic, perennial, evergreen, clump-forming grass belonging to family Poaceae and cultivated commercially in several countries of the world for production of citronella oil.

Citronella bagasse released in bulk amount during steam distillation which is the principal process of oil extraction in citronella oil industries. According to an estimate, India produces more than six million tons of aromatic spent biomass annually (Bassi et al., 2006). This waste biomass has entirely no value since animals do not consume it due to their aroma scent. However, it is reported that a small portion of this waste biomass is used for heat generation during the steam distillation process and in

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some cases; it is also used as mulching agent (Hesse et al., 2008). In our previous study, we have reported the problems associated with citronella bagasse and vermicomposting potential of *Eudrilus eugeniae* and *Perionyx excavatus* for bioconversion of this waste biomass to vermicompost (Deka et al., 2011a,b). However, potential of other earthworm species is still needed to explore to end up the lacuna for biomanagement of citronella bagasse, besides, blending of citronella bagasse with other organic substrate is essential in order to obtain good quality end products.

Paper mill sludge as mentioned above is the by-products, released in huge amount from paper and pulp industries. Vermicomposting has already been established as an efficient tool for bioconversion of paper mill sludge into nutrient rich end products (Mohapatra et al., 2019). Even reports are also available regarding mixing of paper waste with other substrates such as rice straw and cowdung for vermicomposting (Sharma and Garg, 2018). Besides, other utility aspects of paper mill sludge such as liming material for fish culture; manufacturing of eco-friendly bricks has also been enumerated (Goel and Kalamdhad, 2017).

Vermitechnology is an eco-friendly technique that has gained more popularity throughout the globe due to the ability to neutralize the bio-waste associated burdens. Vermicomposting is a process that involves joint action of earthworms and microorganisms for stabilization of organic wastes. This technique has been widely used for bioconversion of waste such as household waste, agricultural residues, agro-industrial waste, paper mill sludge, fly ash etc to valuable end products known as vermicompost (Parthasarathi et al., 2016). Nevertheless, low levels of macronutrients still a major problem for wider application of vermicompost in commercial scale. Therefore, enhancement of nutrients level in the vermicompost is still a major challenge and to overcome the issue blending of several wastes has been suggested as an alternative option (Barthod et al., 2018).

The earthworm *Eisenia fetida* is found extensively around the world in various habitats and its vermicomposting potential has already been established. For example, *Eisenia fetida* has been utilized for vermicomposting of agro-industrial waste such as bakery industry sludge (Yadav et al., 2015), fruit and vegetable processing industry waste (Sharma and Garg, 2017), paper and pulp mill sludge (Suthar et al., 2014), biogas plant slurry (Hanc and Dreslova, 2016) etc. However, reports regarding use of *Eisenia fetida* for vermicomposting of citronella bagasse are very scanty. Besides, blending of citronella bagasse with other substrate materials for vermicomposting has not been attempted earlier although type and combination as well as the mixing of substrate materials is very important to obtain enriched product (Vig et al., 2011). Keeping this in mind the present investigation has been designed to study the potential of *Eisenia fetida* for vermicomposting of citronella bagasse and paper mill sludge mixture. The efficiency of vermicomposting process was measured in terms of physico-chemical and nutrients profiles, microbial profiles, earthworm population and biomass, trace element and heavy metal profile, stability parameters such as ash contents, C/N ratio, humification index and scanning electron microscope (SEM) imaging of the end products.

2. Materials and methods

2.1. Collection of *Eisenia fetida*, citronella bagasse and paper mill sludge

The experimental earthworm species *Eisenia fetida* was collected directly from the vermicomposting research unit of Institute of Advanced Study in Science and Technology, Guwahati, Assam, India. A stock culture of the collected earthworms was maintained in the laboratory before use in the experiment.

The distillate waste material of citronella (i.e. citronella bagasse) was collected from citronella oil industry of Rajapara, Assam, India. The citronella bagasse was air dried and cleaved into small fragments for use in the experiment. The paper mill sludge generated from the final step of paper production process was picked up from Nagaon Paper Mill, Jagirod, Assam, India; processed in the laboratory for experimental use.

2.2. Experimental set up

Two treatments were taken during the experimental trials. In one treatment, only citronella bagasse (CB) was taken as the substrate material whereas in the other the citronella bagasse (CB) and paper mill sludge (PMS) were mixed thoroughly in 3:2 ratio (CB = 150 g, PMS = 100 g). Both the substrate/raw materials were pre-decomposed for 15 days in order to make them palatable for the experimental earthworms (Deka et al., 2011a). The experiment was performed in plastic pots of 2 L capacity (diameter 17 cm, depth 18.5 cm). The vermicomposting beds in the experimental pots were prepared by pieces of small bricks and stones, soil and decomposed cowdung and were placed at the bottom of each experimental pot. Twenty numbers of 20 days old individuals of earthworms with an initial average weight of 0.176 g were introduced into the experimental pots from the stock culture. The experiment was carried out in ambient condition where temperature ranges from 25 to 30 °C during the entire duration of the experiment. Besides, moisture level was maintained at 70 ± 10% by sprinkling distilled water in the compost medium when required (Yadav and Garg, 2009). A similar control setup was also maintained without earthworms. In each case 250 g of substrate material were taken maintaining three replicas for statistical comparison. The pots were covered with pieces of newspaper and small holes were made over it for proper aeration. The duration of the experiment was 45 days. The vermicompost was harvested on the appearance of black coloured granules at the top of the pots. The vermicompost outputs of each treatment were calculated out on a dry weight basis. Earthworm numbers were counted manually whereas earthworm biomass was measured on a fresh weight basis.

2.3. Physico-chemical and FT-IR analysis

The air dried samples of vermicompost, compost and raw material were used for the analysis of physico-chemical parameters. All the samples were analyzed in triplicate and the average results were taken for comparisons. The pH and conductivity values were measured in 1:5 (w/v) water suspension using digital pH (Biochem PM79) and conductivity meter (Systronics 304), respectively. Nelson and Sommers (1982) method was used to measure the ash content of the samples. Total organic carbon of the samples was measured by Walkley and Black titration method (Jackson, 1975). Micro kjeldhal method was used to determine nitrogen content (Jackson, 1975). C/N ratio was calculated from the values of total organic carbon and nitrogen content. The available phosphorus in the samples was determined spectrophotometrically (Shimadzu UV 1601) following the stannous chloride method (APHA, 1998). The total potassium and calcium contents in the samples were determined in flame photometer following acid digestion method (APHA, 1998). The humification index was determined by employing the method as outlined by Zbytniewski and Buszewski (2005). Briefly, one gram sample for each of raw materials, compost and vermicompost were shaken separately with 50 mL of 0.5 M NaOH for two hours, left overnight and centrifuged (REMI R-8C Laboratory centrifuge) at 3000 rpm for 25 min. Finally, the absorbance of supernatants were measured in spectrophotometer (Systronics UV-VIS Spectrophotometer 119) at 472 nm (A_{472}) and 664 nm (A_{664}) and humification index was found out as the ratio of A_{472}/A_{664} . For trace elements and heavy metals profile, the samples (0.5 g of each sample) were digested in a microwave digester (Milestone, Ethos 900) in a solution containing 9 mL nitric acid and 3 mL of chloride acid according to the EPA method 3051 (EPA 1996) and then analyzed by atomic absorption spectrophotometer (Shimadzu AA -7000). The Fourier-transform infrared (FT-IR) spectra of the samples were found out by FTIR spectrophotometer (Nicolet 6700) as per the method outlined by Gupta and Garg (2009). In brief, samples (5 mg of each) were mixed with 400 mg KBr, homogenized in an agate mortar and pressed into a pellet and finally, spectra were recorded in mid infrared area within the wave number 4000–400 cm^{-1} (Deka et al., 2011a,b).

Table 1
Showing the physico-chemical characteristics of the raw materials, compost and vermicompost samples.

Treatment	pH	Conductivity (mS/ds)	Ash content (g/kg)	Organic Carbon (g/kg)
CB Raw material	6.57 ± 0.32a	1.54 ± 0.04a	128 ± 4.3a	652.3 ± 8.4a
CB + PMS Raw material	8.51 ± 0.37b	2.41 ± 0.06b	245 ± 4.6b	693.3 ± 7.8b
CB Compost	6.47 ± 0.15a	1.84 ± 0.02c	131 ± 3.9c	494.5 ± 10.8c
CB + PMS Compost	8.11 ± 0.09a	2.92 ± 0.05d	363 ± 6.2d	209.3 ± 9.4d
CB Vermicompost	6.01 ± 0.01p	1.89 ± 0.05p	145 ± 3.3p	140 ± 3.1p
CB + PMS Vermicompost	6.45 ± 0.03q	3.0 ± 0.02q	437 ± 6.7q	96.9 ± 2.4q

Mean value ± SD, n = 3; Different letters in the same column indicates statistically significant values (Pair *t* test, *P* < 0.05, two tails). CB = Citronella bagasse; PMS = Paper Mill Sludge.

2.4. Analysis of beneficial bacterial population

The total number of nitrogen fixing, phosphate solubilizing and potassium solubilizing bacteria were calculated using standard pour plate and serial dilution method outlined by Dubey and Maheshwari (2005). The number of colony forming units (CFU) was expressed as CFU g⁻¹. The stock solution was prepared by taking 1 g of sample in 10 mL sterile water. Serial dilutions up to 10⁻⁶ were made and 1 mL of aliquot was poured onto plates containing agar media i.e. Jensen's medium for nitrogen fixing, Pikovskaya's agar for phosphate solubilizing and Aleksandrov agar for potassium solubilizing bacteria, respectively.

2.5. Scanning electron microscopy

The raw materials, compost and vermicompost samples were dried at 70 ± 2 °C until constant weight is obtained so that all the moisture content were removed properly. By using double-sided adhesive carbon tape the samples were fixed on a metallic sample holder and coated with gold through sputter coater for clear visibility of picture (Bhat et al., 2015). Micrographs of surface morphology of the samples were recorded at different magnifications of scanning electron microscopy (Gemini, Sigma-300 series).

2.6. Statistical analysis

Independent sample *t* test (two tailed, *P* < 0.05) was used to compare the vermicompost production from the initial substrate materials. Pair *t* test was used to compare the physico chemical properties and nutrient analysis of raw materials, compost and vermicompost. ANOVA, LSD test (*P* < 0.01) was used to compare the results of earthworm population and biomass, microbial analysis, humification index, trace element and heavy metal profiles.

3. Results and discussion

3.1. Vermicompost production

The amount of vermicompost generated from citronella bagasse (CB) and citronella bagasse plus paper mill sludge mixture (CB + PMS) after 45 days of the experimental trial has been evaluated. The amount of substrate/raw materials was taken as 250 g (on a dry weight basis) for vermicomposting trials in each treatment. The results revealed that *Eisenia fetida* can produce 63 ± 2 g and 145 ± 4 g of vermicompost from CB and CB + PMS, respectively. Thus the experimental earthworm produces 25.2% and 58% of vermicompost from the substrate materials i.e. CB and CB + PMS, respectively. Different factors such as nature, composition and nutrients profile of the substrate materials influence the rate of vermicompost production (Malińska et al., 2017). The present investigation reveals that blending of paper mill sludge with citronella bagasse positively influences the rate of vermicompost production. Moreover, the high rate of production of vermicompost can be linked with the active reproductive and other metabolic activities of the earthworms during the time of the experiment. It has been reported that 59.32% and 54.86%

vermicompost production is possible during summer and winter periods, respectively by employing *Perionyx excavatus* after 90 days of experimental trial on citronella bagasse (Deka et al., 2011a). Pattnaik and Reddy (2010) observed 63% production of vermicompost from urban green waste employing the earthworm species *Eisenia fetida*.

3.2. Physico-chemical analysis, macronutrient composition and C/N ratio

The physico-chemical compositions that include pH, electrical conductivity, total organic carbon and ash contents of raw material, compost and vermicompost have been presented in Table 1. The results showed that there were changes in the studied parameters in the end product as compared to the initial level of the raw materials and control (i.e. compost).

3.2.1. Changes in pH level

The results revealed that there was a significant reduction in pH levels in the vermicompost samples as against the initial values of the substrate materials. The pH value of raw material (CB) was 6.57 ± 0.32 whereas after blending with paper mill sludge pH level of CB + PMS mixture was 8.51 ± 0.37. The pH value in vermicompost samples obtained from CB and CB + PMS mixture was found to be 6.01 ± 0.01 and 6.45 ± 0.03, respectively after the 45 days of experimental trials. There was a marginal shift in pH level in control (i.e. compost) samples and it was found to be 6.47 ± 0.15 and 8.11 ± 0.09 in the compost samples obtained from CB and CB + PMS mixture, respectively. The decrease level of pH in the vermicompost samples has been already reported by earlier workers. For example, Bhat et al., (2015) reported about the reduction in pH values during vermicomposting of sugarcane bagasse and cattle dung which was attributed to the production of organic acids such as humic and fulvic acids during the process. Earthworms have the capacity to neutralize the substrate material by the end of the vermicomposting process. Even the pH level in the end products may vary with the degradation pattern of various substrate materials as the production of different types of organic intermediates are possible during the vermicomposting process. Moreover, production of CO₂, NO₃, mineralization of the phosphorus and nitrogen is also a primary reason for decrease in pH level in the vermicompost products (Suthar 2009; Ananthavalli et al., 2019).

3.2.2. Changes in electrical conductivity

There was a significant increase in electrical conductivity values in both vermicompost and compost samples as against the initial values of the raw materials. The electrical conductivity values of CB and CB + PMS mixture were 1.54 ± 0.04 mS/ds and 2.41 ± 0.06 mS/ds which was found within the range of 1.89 ± 0.05–3.00 ± 0.02 mS/ds in the vermicompost and 1.84 ± 0.02–2.92 ± 0.05 mS/ds in the compost samples. Thus both compost and vermicompost samples showed 1.2 fold increases in electrical conductivity values over the initial substrate materials. Increase in EC values could be attributed to the loss of weight of organic matter during the vermicomposting process besides release of several salts such as ammonium, potassium and phosphate in the end products (Yadav and Garg, 2011).

Table 2
Showing the macronutrient composition of the raw materials, compost and vermicompost samples.

Treatment	Total N (g/kg)	Available P (mg/kg)	Total K (mg/kg)	Total Ca (g/kg)
CB Raw material	11.7 ± 0.15a	171.86 ± 0.82a	620.16 ± 1.62a	0.006 ± 0.001a
CB + PMS Raw material	9.67 ± 0.19b	83.86 ± 0.44b	5004.5 ± 0.59b	7.12 ± 0.54b
CB Compost	17.7 ± 0.21c	488 ± 2.65c	654.93 ± 1.21c	0.008 ± 0.011a
CB + PMS Compost	9.76 ± 0.31d	125.70 ± 0.65d	6016 ± 2.65d	7.34 ± 1.02c
CB Vermicompost	18.4 ± 0.22k	284.4 ± 0.62k	242.77 ± 0.57k	0.034 ± 0.001d
CB + PMS Vermicompost	15.2 ± 0.25z	185.38 ± 2.10z	2825.3 ± 3.14z	8.8 ± 0.89k

Mean value ± SD, n = 3; Different letters in the same column indicates statistically different values (Pair t test P < 0.05, two tails). CB = Citronella bagasse; PMS = Paper Mill Sludge.

3.2.3. Changes in ash content

The ash content of both compost and vermicompost samples were found to be much higher than the initial raw materials. The ash content of raw materials i.e. CB and CB + PMS were 0.13 ± 0.003 g and 0.25 ± 0.004 g per gram dry weight, respectively. The ash content in vermicompost and compost samples obtained from CB raw material was found to be 0.145 ± 0.003 g and 0.131 ± 0.002 g per gram dry weight showing 13.3% and 2.3% increase, respectively over the initial value found in the raw materials. Whereas, the ash content in vermicompost and compost samples obtained from CB + PMS were found to be 0.437 ± 0.006 g and 0.363 ± 0.002 g per gram dry weight, respectively. Thus vermicompost showed 78.4% increase in ash content value while the compost showed 48.2% increase as against the initial raw material (i.e. CB + PMS). The ash content is an important indicator for decomposition and mineralization of vermicomposting materials and higher the ash values more the mineralization/decomposition in vermicompost materials. Thus the higher ash level in the vermicompost samples as against the compost and raw materials samples may be attributed to enhanced mineralization due to the activity of *Eisenia fetida*. The “community conditioning” resulting from homogenization of the substrate material by earthworms led to accumulation of microorganisms that are specialized in breaking down compounds released by earthworms, improving the rate of decomposition and mineralization of the substrate (Aira et al., 2006; Griffiths et al., 2001). Further, the results also suggest that blending of PMS with CB enhances the ash values of both compost and vermicompost samples. This could be attributed to the import of nutrients, palatability of the substrate material for enhanced earthworms’ and microbial activity after mixing of PMS with CB which in turn results in more mineralization of mixed substrate. The present findings can be corroborated with the previous findings who have reported a higher level of ash values in the vermicompost materials (Karmegam et al., 2019).

3.2.4. Changes in total organic carbon

The total organic carbon (TOC) content was found to be lower in compost and vermicompost irrespective of the initial raw materials used for the experiments. In the treatment where CB was used as raw material the reduction of TOC was 78.5% in vermicompost and 24.2% in compost as compared to the initial raw material. Whereas, in the treatment where CB + PMS was utilized as raw material the reduction of TOC was found to be 86% and 69.8% in vermicompost and compost, respectively. The reduction in total organic carbon in the vermicomposting experiment has been already reported by earlier researchers. Sharma and Garg (2017) have reported about 39.9–48.2% reduction in TOC during the vermicomposting of food and vegetable processing industry waste. The decrease in TOC content in the vermicompost could be attributed to the increasing earthworm population during vermicomposting process. The utilization of organic carbon by earthworms and microorganisms, release of carbon in the form of CO₂ due to microbial respiration mainly cause the loss of TOC during vermicomposting (Yadav and Garg, 2016a; Sharma and Garg, 2017). Here, the greater loss of carbon in the vermicompost samples in comparison to compost may be due to differences in carbon mineralization. Further mixing of PMS with CB could improve the substrate condition which

accelerate both microbial and earthworm’s activity and cause more loss of CO₂ thereby resulting more decrease in TOC loss.

3.2.5. Macronutrients composition (N, P, K, Ca)

The results of N, P, K and Ca are presented in Table 2. The results revealed that there were significant enhancements in N and P content in the vermicompost samples as compared to the compost and initial values of the raw materials. There was 1.6 fold increase in total kjeldhal nitrogen (TKN) content in the vermicompost samples irrespective of the raw materials used in the experiment. In the case of compost samples, it was found in the range of 1.01–1.2 fold by the end of the experimental trials. Our previous study reported about 3.4 to 4.6 fold increase in TKN in vermicompost samples obtained from citronella bagasse and cowdung mixture by employing *Perionyx excavatus* and *Eudrilus eugeniae* (Deka et al., 2011a). Higher TKN value during vermicomposting of food and vegetable processing industry waste by employing *Eisenia fetida* was also reported recently by Sharma and Garg (2017). Several factors such as nitrogen level in the raw materials, earthworms’ body fluid, mucus, excretory products and decaying tissues of dead earthworms are associated with increase in nitrogen content in the end products (Suthar, 2009; Bhat et al., 2015). In conclusion, less increase in nitrogen level in the present study as against our previous reports may be associated with type of earthworm species and nature of the substrate material used in the experiment.

The concentration of available phosphorus was also found higher in both vermicompost and compost as against the raw materials used in the experimental trials (Table 3). Again, phosphorus level was found significantly higher in vermicompost than compost samples. The treatment where CB + PMS was used as raw material showed 121.1% increase in available Phosphorus in vermicompost and 49.9% increase in compost. Again, the increase in available phosphorus was found to be 183.9% in vermicompost and 65.5% in compost in the treatment involving CB only. Moreover, phosphorus content of CB vermicompost was found to be significantly higher than the CB + PMS vermicompost (Table 3). Yadav and Garg (2016a, b) have reported up to 29.5–75% increase in available phosphorus in the vermicompost samples than the initial waste material. Furthermore, 97.9% increase in available phosphorus was recorded in the vermicomposting of vegetable greenhouse waste (Fernández-Gómez et al., 2010). Depending upon the difference in the rate of mineralization the available phosphorus in the end products may vary. Release of phosphorus into the vermicompost by phosphate solubilizing microbes

Table 3
Showing the changes in earthworm population and biomass during vermicomposting by *Eisenia fetida*.

Substrate	Population		Live biomass (g)		Biomass change (%)
	Initial	Final	Initial	Final	
CB	20	41 ± 1.3b	0.176 ± 0.01a	0.390 ± 0.07d	121.59
CB + PMS	20	49 ± 1.5a	0.172 ± 0.03a	0.359 ± 0.02c	108.72

Mean value ± SD, n = 3; Different letters shows statistically different values (ANOVA, LSD test, P < 0.01). CB = Citronella bagasse; PMS = Paper Mill Sludge.

and phosphatase enzyme present in earthworm gut can contribute to the available phosphorus (Suthar, 2009)

In CB treatment, the potassium content was found to be increased by 5.6% in compost and decreased by 60.9% in vermicompost. On the other hand, in CB + PMS treatment the value of potassium increased in the compost by 20.2% and decreased in the vermicompost by 43.5% as compared to the initial raw materials. The decrease in potassium level during vermicomposting has been also reported by earlier workers (Elvira et al., 1996). From the experiment it can be interpreted that earthworms may utilize the potassium present in the vermicompost for their growth and reproduction resulting in the decrease of potassium content in the final products. Nevertheless another planned experiment is needed to understand the proper reason for decrease in K value during the vermicomposting process.

Total calcium content of CB vermicompost has increased from 0.006 g/kg to 0.034 g/kg resulting 466.7% enhancement over the initial value. Whereas, the calcium content of CB + PMS vermicompost was found to be increased from 7.1 g/kg to 8.8 g/kg which counted 23.6% increase against the initial value of the waste mixture. Sharma and Garg (2019) also reported up to 76.1% increase in the total calcium content of vermicompost produced from lignocellulosic waste utilizing the earthworm species *Eisenia fetida*. Production of calcium carbonate due to the catalytic activity of carbonic anhydrase present in earthworm gut can be a potential contributor to the high level of calcium in the vermicompost (Sharma and Garg 2019). Here it can be concluded that the increase in total calcium value in the final product can be attributed to the mineralization of calcium due to the increased physiological and metabolic activity due to the conducive environment of the earthworms during the vermicomposting period.

3.2.6. Changes in C/N ratio

The results showed that there was a sharp decline in C/N ratio both in vermicompost and compost samples as compared to the initial level of raw materials. The decrease was found to be higher in vermicompost than the compost samples irrespective of the treatment used during the study. As presented in Fig. 1, the treatment where CB was used as substrate material reduction in C/N values was found to be 86.4% in vermicompost and 49.9% in compost samples. Whereas, the treatment containing CB + PMS as raw material showed 91.1% decrease in vermicompost and 70.1% in compost. The decrease in C/N values may be attributed to the loss of carbon as CO₂ due to microbial respiration (Alidadi et al., 2016) and the simultaneous increase in nitrogen by worms in the form of mucus and nitrogenous excretory products (Lv et al., 2018) during vermicomposting. The higher decrease in C/N ratio values in the vermicompost samples of CB + PMS may be attributed to the suitability of the substrate materials which promote more microbial and earthworm's activity. The present findings showed similarity with the previous worker (Gupta and Garg, 2008) that reports up to 85.2% reduction in C/N value in the vermicompost and also with that of Deka et al., 2011b who reported 83.5% reduction in C/N level in the vermicompost. The quality of vermicompost is regarded as superior if the C/N ratio is less than 20 and a ratio below 15 indicates the suitability for agronomic use (Edwards and Bohlen, 1996).

3.3. Earthworm population and biomass

The production of earthworm is an important and integrated aspect of any vermicomposting experiment (Ananthavalli et al., 2019). The results of earthworm population and biomass are presented in the Table 3. In the treatment where CB + PMS were used as raw material, initially 20 individuals of *Eisenia fetida* were introduced for vermicomposting of the raw materials. The results showed 2.5 fold increases in *Eisenia fetida* population in CB + PMS treatment whereas it was 2.1 fold in case of CB treatment by end of the experimental trials. Initial average biomass of the experimental earthworm was 0.172 ± 0.03 g and it was found to be 0.359 ± 0.02 g at the end of the experiment. The biomass of each earthworm increased by 2.1 fold or 108.7% from the initial value. Again, in treatment containing CB as raw material, 20 earthworms were

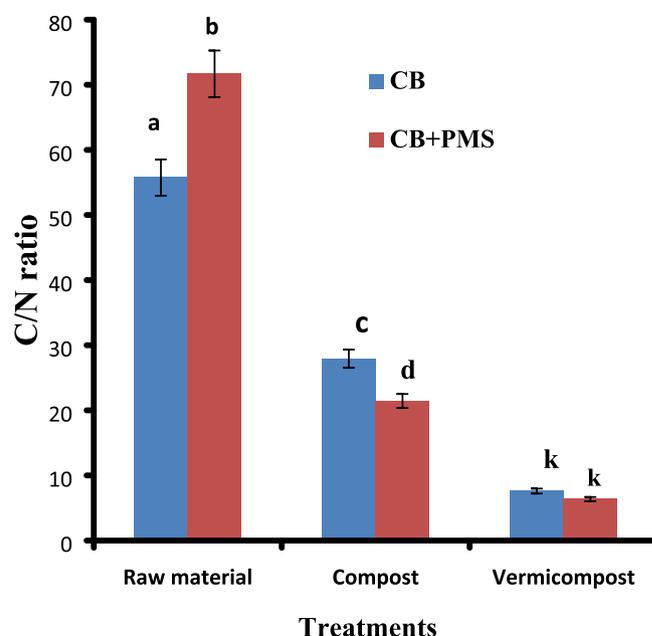


Fig. 1. C/N values of the raw materials, compost and vermicompost. Values are mean, n = 3, error bars indicate SD. Significant differences indicated by different letters.

introduced initially and by the end of the experiment 41 earthworms were recovered. There were 2.1 fold increases in the population of earthworms. The initial average biomass was 0.176 ± 0.03 g, which enhanced to 0.390 ± 0.07 g during the experiment. The earthworm biomass was increased by 2.2 fold or 121.6% from the initial weight. The reproductive activity and survivability of the earthworm are directly correlated with the environmental variability (Suthar, 2009). This finding is understandable since earthworms were actively participating in its physiological activities during the time of the experiment.

3.4. Beneficial bacterial populations

The total population of N₂ fixer, P and K solubilizing bacteria were determined in vermicompost and the results were compared with compost and raw materials. The results were expressed in CFU g⁻¹ and presented in Table 4. The results showed that there was a significant enhancement in the population of N₂ fixer, P and K solubilizer bacteria in both compost and vermicompost samples as against the initial raw material (Table 5). Further, the population of N₂ fixing, P and K solubilizing bacteria were found to be higher in the vermicompost and compost samples obtained from CB + PMS than the CB treatment. Irrespective of the treatments, the maximum number of all the studied bacterial population was found in the vermicompost samples. The present findings are conformity with the previous works (Ravindran et al., 2015) who have reported about the increase in the beneficial microbial population in vermicompost materials. The nature of organic waste, conducive environment during vermicomposting trials and high nutrient status of the end products (vermicompost) may provide a suitable habitat for proliferation and interaction of microbes which ultimately enhance the total beneficial bacterial population. Besides, metabolic and physiological activities of earthworms may also responsible for the significant increase in beneficial microbial population (Malińska et al., 2017).

3.5. FT-IR spectroscopy analysis of vermicomposting process

The FT-IR analysis reveals the presence or absence of peaks for different metabolites based on which the degradation or stabilization of organic waste can be confirmed. The major absorbance bands of the FT-

Table 4
Showing the nitrogen fixing, phosphate and potassium solubilizing bacterial population in raw materials, compost and vermicompost samples.

Treatments	N ₂ fixing (CFU g ⁻¹)	P solubilizing (CFU g ⁻¹)	K solubilizing (CFU g ⁻¹)
CB Raw material	6.52 × 10 ⁶ ± 0.22d	4.25 × 10 ⁶ ± 0.52d	7.33 × 10 ⁶ ± 1.53d
CB + PMS Raw materials	35 × 10 ⁶ ± 1.52a	1.3 × 10 ⁶ ± 0.578a	35.67 × 10 ⁶ ± 8.0a
CB Compost	12 × 10 ⁶ ± 8.50e	12 × 10 ⁶ ± 4.04e	13.3 × 10 ⁶ ± 1.53e
CB + PMS Compost	53 × 10 ⁶ ± 5b	61 × 10 ⁶ ± 3.51b	84.33 × 10 ⁶ ± 5.5b
CB Vermicompost	60 × 10 ⁶ ± 3.65f	48 × 10 ⁶ ± 7.12f	28.67 × 10 ⁶ ± 3.1f
CB + PMS Vermicompost	64 × 10 ⁶ ± 2.65c	11 × 10 ⁶ ± 2c	133.7 × 10 ⁶ ± 5.5c

Mean values ± SD, n = 3; Different letters in the same column indicates statistically different values (ANOVA, LSD test, P < 0.01). CB = Citronella bagasse; PMS = Paper Mill Sludge.

IR spectra of raw materials, compost and vermicompost samples were presented in Table 5. The bands of FT-IR spectra of the studied samples have been interpreted based on the references of Pavia et al. (2001), Wang et al. (2004), Gupta and Garg (2009).

The bands which were common to all the samples were found at 3100–3000 cm⁻¹ (C–H stretching for Alkenes) and 2900–2800 cm⁻¹ (C–H stretching for alkynes). Distinct peak in the range of 1660–1600 cm⁻¹ (Aromatic and olefinic C=C, C=O in carboxyl; amide (I), ketone and quinone groups) were observed in raw materials and compost of both the treatments but absent in vermicompost samples suggesting the decrease in the carboxylic group and aromatic structure by end of the vermicomposting process. Deformation of bands in and around 1100 cm⁻¹ and 2925 cm⁻¹ were observed in vermicompost samples which confirms the degradation of raw material for cellulose, hemicelluloses, fats and lipids (Ravindran and Sekaran, 2010). Further, there was a decrease in peak intensities at 3600–3100 cm⁻¹ which can be attributed to the decomposition of carbohydrates as a result of decrease of atomic groups and structure of OH and CH₂ (Wang et al., 2004). Moreover, the peak intensity at 1045 cm⁻¹ (C–O stretch of polysaccharide) has decreased which can be due to the enhanced mineralization of the vermicomposting samples (Smidt and Meissl 2007).

3.6. Humification index and SEM analysis

The results of humification index have been presented graphically in Fig. 2. The results showed that humification index values have decreased significantly in the vermicompost samples irrespective of the initial raw materials used in the experiment. The humification index of CB raw material was 7.9 ± 0.2 which changes to 3.5 ± 0.08 and 2.5 ± 0.2 in compost and vermicompost samples, respectively. The percentage decrease of humification index of CB compost and CB vermicompost from initial raw material was recorded as 55.7% and 68.4%, respectively. Whereas, the humification index of CB + PMS mixture was 6.4 ± 0.1 and it changes to 5.6 ± 0.3 and 4.7 ± 0.04, respectively in compost and vermicompost samples. The humification index of CB + PMS compost and vermicompost were decreased by 12.5% and 26.6%, respectively. The higher level of organic material humification and a high degree of aromatic condensation is indicated by a smaller value of A₄₇₂/A₆₆₄ in the vermicompost which suggests the suitability of the end product over initial raw material (Zbytyniewski and Buszewski, 2005). The

Table 5
Showing bands and peaks obtained from FTIR spectroscopy in raw materials, compost and vermicompost samples.

Bands and peaks (cm ⁻¹)	Assignments	Remarks
3650–3100	H bonded OH groups of alcohols, phenols and aldehydes	Present in CB compost.
3100–3000	C–H stretching for alkenes	Present in all the samples
2900–2800	C–H stretching for alkynes	Present in all the samples
2550	S–H group for mercaptans	Present in CB + PMS compost sample
1810–1760	C=O group for anhydride	Present in CB + PMS vermicompost, CB + PMS compost samples
1750–1730	C=O group for este	Present in CB + PMS raw material
1660–1600	Aromatic and olefinic C=C, C=O in carboxyl amide (I), Ketone and quinone groups	Present in all the raw materials and compost samples

CB = Citronella bagasse; PMS = Paper Mill Sludge.

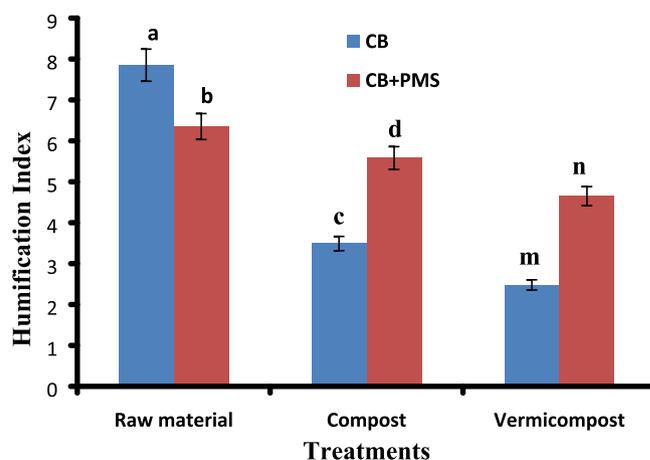


Fig. 2. “Humification index” of raw materials, compost and vermicompost. Values are mean, n = 3, error bars indicate SD. Significant differences indicated by different letters.

humification index below 5 is indicative of a high level of humification of organic matter in the waste/raw material (Zbytyniewski and Buszewski, 2005). Here it is assumed that the combined association of earthworms and microbes accelerate the decomposition and stabilization of organic waste thereby lowering in humification index.

The micrographs obtained from Scanning Electron microscope reveal significant disintegration in the vermicompost samples relative to a more contiguous and robust structure present in the initial raw material (micrographs are attached as electronic annex). A similar disaggregation with less intensity was also observed in the compost samples. The results of the SEM analysis shows similarities with earlier studies of Sharma and Garg (2018) who reported about the changing pattern of surface morphology in the vermicompost samples. Both microbes and earthworms were responsible for the progressive break down of the initial raw material which was reflected in the scanning electron micrograph.

3.7. Trace element and heavy metal

Trace elements present in the samples (Zn, Cu, Ni, Fe) are an

Table 6
Showing trace elements and heavy metals composition in the raw materials, compost and vermicompost samples.

Substrate	Zn (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Co (mg/kg)	Cr (mg/kg)	Fe (mg/kg)
*Raw materials	9.167 ± 0.54a	0.333 ± 0.02a	3.916 ± 0.11a	3 ± 0.09a	1.5 ± 0.12a	1.83 ± 0.33a	8.333 ± 0.54a
Compost	8.25 ± 0.32b	0.167 ± 0.04b	2.083 ± 0.21b	0.083 ± 0.05b	0.75 ± 0.04b	0.083 ± 0.03b	1.833 ± 0.05b
CB + PMS Vermicompost	8.571 ± 0.89c	0.429 ± 0.09c	4.285 ± 0.98c	4.285 ± 0.82c	1.857 ± 0.33c	3.571 ± 0.21c	15 ± 2.26c
CB Vermicompost	14.28 ± 1.45d	0.286 ± 0.32d	3.928 ± 0.85d	2.143 ± 0.32d	1 ± 0.05d	2.571 ± 0.09d	16.428 ± 2.11d

Mean value ± SD, n = 3; Different letters in the same column indicates statistically different values (ANOVA, LSD test, P < 0.01). CB = Citronella bagasse; PMS = Paper Mill Sludge.

*Represent the CB and CB + PMS mixture.

essential part of the plant growth and nutrition, although the exposure periods and concentrations level of heavy metals (Cd, Co, Cr) can be toxic to both plants and rhizosphere microorganisms which can alter their diversity, abundance and distribution pattern (Malley et al., 2006). The results of trace elements and heavy metal analysis are presented in Table 6. The concentration of Cu, Cr and Fe was found to be increased in the vermicompost sample of CB by 1.003, 1.4, and 1.9 fold whereas it was found to be 1.09, 1.9, 1.8 fold, respectively in the vermicompost samples obtained from CB + PMS mixture. The concentration of Zn increased by 1.6 fold in CB vermicompost but decreased by 0.9 fold in CB + PMS vermicompost. The concentration of Cd, Ni and Co was increased in vermicompost sample of CB + PMS mixture by 1.3, 1.4 and 1.2 fold although it was found to be decreased by 0.86, 0.71 and 0.67 fold, respectively in the vermicompost samples of CB.

Both increase and decrease in heavy metal and trace element concentrations in various vermicompost samples were already justified by previous workers in this field. For example, Li et al., 2010 reported about decrease in the concentration of heavy metals and Yadav and Garg, 2011 reported about increase in the concentration of heavy metals in vermicompost samples. The decrease in the concentration of heavy metals can be associated with accumulation of heavy metals in the body of *Eisenia fetida* in association with different environmental factors (Liu et al., 2012). The concentration effect caused by volume and weight loss resulting from the mineralization of organic matter during vermicomposting can be a possible reason for the increase in heavy metal concentration (Garg and Gupta 2011). Though some of the metal concentration was found to be increased in the vermicompost as compared to the initial raw material, which was still very much under the international permissible levels (Mohee and Soobhany, 2014) for compost that indicates its suitability for their use in agricultural field and as plotting media in horticulture.

4. Conclusion

The bioconversion of citronella bagasse is possible by employing *Eisenia fetida*. The blending of citronella bagasse with paper mill sludge significantly improves the quality and quantity of the end products. The reduction in humification index and C/N ratio; enhancement of nutrients profile, increase in beneficial bacterial population indicates the superiority of vermicompost over traditional compost. The FT-IR spectroscopy of the vermicompost showed a reduction in the carboxylic group and aliphatic structures. Although the concentrations of a few heavy metals was found higher in the vermicompost samples but it was found below the limits as established by international regulations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2019.122147>.

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