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Research Article

ENHANCEMENT OF HUMUS COMPOSITION BY EARTHWORMS DURING BIOTRANSFORMATION OF COFFEE PULP AMENDED WITH SUGAR INDUSTRIAL WASTES

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ABSTRACT

The aim of this work was evaluate the enhancement of humus composition by exotic *Eisenia fetida* and an indigenous *Perionyx excavatus* during biotransformation of coffee pulp (CP) amended with sugar industrial wastes press mud (SPM) and bagasse (SBG). In the present study, the CP was amended with SPM and SBG at different proportions, kept for pre-treatment for 7 days and subsequently vermicomposted for a period of 90 days under laboratory conditions. The vermicompost were sampled at 30, 60 and 90 days for the evaluation of sequential changes in humus composition (humic acid, fulvic acid, humification index and humic carbon). The results were revealed that humic acid content in the vermicompost of both species of worms were significantly altered than initial substrate and natural compost. In contrast, fulvic acid and humic carbon content decreased during vermicomposting process. However, among the different treatments TE1, TE2 and TE3 treatments for *E. fetida* and TP7, TP8 and TP9 for *P. excavatus* treatments showed significantly ($p<0.05$) higher level of humic acid (HA) and humification index (HI) and reduction of fulvic acid (FA) and humic carbon (HC) content than other treatments of both worms and treatments without earthworms. Further, the results also suggested that treatments without earthworm did not show any significant effect on the activities of humus composition than vermicomposting.

Keywords: Sugar industry waste, Coffee pulp, Vermicomposting, Humification process.

INTRODUCTION

Coffee is one of the worldwide agricultural products and is the second chief product traded in the world subsequently to oil. Annually, large amount of coffee by-products are generated throughout coffee processing. In recent past, emphasis on use of organic manures has assumed increased significance as it finds a place in organic farming and as well in integrated nutrient system. Coffee processing units those are situated in coffee growing areas pretense threat to the environment because of unsafe discarding of coffee pulp, husk and effluents leading to pollution of water and land around the processing units (Pushpa and Manonmani, 2008). On the other hand, the cane-sugar manufacturing has a number of co-products of immense potential worth (press mud, bagasse and molasses). Press mud and bagasse are directly applied to soil as manure; the buff present might worsen the physical properties such as permeability, aeration, soil structure and composition etc., and with the passage of time the deterioration might get worsen (Mnivannan, 2005). Hence, in this study the main focus is to use the sugar industries by products by - product i.e. press mud and bagasse (tremendous organic amendments for vermicomposting) which is converted in to vermicompost mixed with coffee pulp using *Eisenia fetida* and *Perionyx excavatus*. Due to the high content of organic matter, macro and micro nutrients in sugar industry waste (press mud and bagasse) and coffee pulp, land-application is commonly regarded as the most effective way of recycling this organic waste (Lundin *et al.,* 2004). Some phytotoxicity content in press mud, bagasse and coffee pulp, such as polyphenols, tannins and caffeine, have been

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reported to pose a serious environmental risk to the agricultural ecosystem (Jarup, 2003). Therefore, reducing the phyto-availability of toxic material is critical in recycling sugar industry waste and coffee pulp safely.

The organic matter is known to play vital roles in the interaction and transport of various toxic organic or inorganic chemicals and in nutrient cycling throughout the environment (Tang *et. al*., 2006). Humus composition, the most resistant fraction to microbial degradation of the organic matter in soil, are complex polymeric organic acids with a wide range of molecular weights and they are heterogeneous mixtures of a variety of organic compounds, consisting of aromatic, aliphatic, phenolic, and quinolic functional groups with varying molecular sizes and properties (Elisabete *et al.,* 2013). Further, humic substances are one of most active fractions of organic matter, they improve the absorption of nutrients by plants and soil microorganisms, have a positive effect on the dynamic of N and P in soil, stimulate plant respiration and the photosynthesis process, and favor the formation of soil aggregates, etc. (Hernandez *et. al*., 2007). Humic substances, mostly consisting of humic acids and fulvic acids, are known for their significant influence on the mobility and phyto-availability of metals in soils (Stevenson, 1994).

Vermicomposting is a simple biotransformation process of organic solid waste, in which certain species of earthworms are used to improve the process of waste conversion and produce enhanced end product. During vermicomposting, earthworms fragment the organic waste, stimulate microbial activity and increase rates of mineralization, rapidly converting the wastes into humus composition having diverse microbial population (Xiong, 2010). Especially epigeic species of earthworms can increase the velocity of decomposition of organic residues and also produce several bioactive humic substances (Tamizhazhagan *et al.,* 2016). From the agricultural point of view, the humic acid could be considered as the most important component of the humus composition. Therefore, earthworms, microorganisms and humus composition are closely associated with soil fertility. Therefore, the present work is aimed to study the changes in humus composition in the different treatments of coffee pulp and sugar industry waste during vermicomposting and natural composting to standardize biotransformation efficiency.

MATERIALS AND METHODS

Collection of sugar industry waste and coffee pulp

The sugar industry by product press mud (SPM) and bagasse (SBG) was procured from E.I.D. Parry's sugar mill located at Nellikuppam, Cuddalore District, Tamil Nadu, India. Fresh SPM was kept under shade for two weeks to remove the foul smell before using for the experimental process. Coffee pulp (CP) was collected from the coffee plantation processing industry (T.T. L. P. Ltd) at Yercaud in Salem district, Tamilnadu, India.

Earthworm cultures

Two composting species of earthworm's exotic *E. fetida* and indigenous *P. excavatus* were chosen for the study. Both worms were obtained from the stock culture which was cultured in cow dung in the laboratory, Department of Zoology, Annamalai University, India. The worms were stocked in cement tank and one month old cow dung was used as substrate to maintain the both earthworms.

Experimental Design

The substrates (treatments) were prepared by using CP amended with sugar industry waste SPM and SBG in different ratio (Table 1). Coffee pulp and sugar industry waste was weighed (dry weight proportion) in the above said description and mixed well with optimum moisture content. The waste mixtures, CP, SPM and SBG of different proportions were transferred to respective plastic troughs with 40 cm diameter x 60 cm depth. After transferred in to the plastic troughs all the mixture compositions in different treatments were allowed for seven days of initial natural decomposition. All the experimental treatments were kept in six replicate for each treatment in a completely randomized block design. The troughs were kept under shade and moisture content was maintained around 65-75% for all treatments by periodic sprinkling of water. The temperature in the laboratory was maintained at around 25 ± 2°C, which is the optimum temperature for earthworms during vermicomposting. After the completion of pre-inoculation period of seven days, the clitellated *E. fetida* and *P. excavatus* were weighed (15gm kg^{-1} of substrate) and inoculated in to respective treatment containing 3 kg of substrate. The vermicompost were sampled at 30, 60 and 90 days for the evaluation of sequential changes in humus composition.

Extraction of humic-like substances

Humus composition was analyzed according to the method described by Kumada (1987) with some modifications of Xiong *et al.,* 2010. The humic acid content was extracted by adopting the procedure as described by Schnitzer (1972). Five gram of fine sieved sample was dissolved in 100 ml of 0.5N NaOH. The liquid was shaken for one hour in a mechanical shaker and allowed to stand at room temperature for 24 hrs. The dark brown liquid was filtered through Whatman No.1 filter paper. The filtrate was collected in a glass jar, acidified with 6N HCl to pH1. After 3hrs the supernatant liquid (fulvic acids) was separated from the coagulate (humic acids) by siphoning off. Then coagulate was dialyzed extensively against distilled water till free of chloride and finally dried in hot air oven at 40°C. The FA fractions were isolated by the procedure recommended by the International Humic Substance

Society and the humification indices and humic carbon were calculated using the procedure of Roletto *et al.*, 1985.

Statistical Analysis

All the reported data are the arithmetic means of six replicates. Two way analysis of variance (ANOVA) was done to determine any significant difference among the treatments at 0.05% level of significance.

RESULTS AND DISCUSSIONS

The composition of humus content (HA, FA, HI, and HC) during vermicomposting using *E. fetida* and *P. excavatus* and composting was carried out and results are given in Tables 2 to 5. Tables 2 and 3 evidences that vermicomposting increased humic acid (HA) level while reduced the fulvic acid (FA) level, which showed the significant humification development during the vermicomposting of CP amended with SPM and SBG by *E. fetida* and *P. excavatus*. Further, vermicomposting by both worms increased humic acid level and shows that maximum humic acid content was in TE1, TE2 and TE3 treatments for *E. fetida* and TP7, TP8 and TP9 for *P. excavatus* and minimum was in TE4, TE5 and TE6 treatments for *E. fetida*, TP10, TP11 and TP12 for *P. excavatus* and all the composting treatments (TW13 – TW18) (Table 3). While, *E. fetida* and *P. excavatus* inoculated treatments with higher ratios of CP and/or CP alone maintained significantly reduced levels of humic acid than treatments with lower CP ratio with amendment material (SPM and SBG). The higher HA content of the treatments during the vermicomposting period may be attributed to the higher content of readily available organic matter from CP, SPM and SBG which could be easily decomposed at that time, resulting in higher rate of HA formation (Manivannan, 2005). Additionally, fiber structure of amendment material (SBG) components such as lignin, which are known to provide more stable phenolic compounds required as starting material for humification processes (Campitelli and Ceppi, 2008).

On the contrary, the fulvic acid (FA) content reduced in all the treatments during vermicomposting. the maximum reductions of FA was observed, respectively, in TE1, TE2 and TE3 treatments for *E. fetida* and TP7, TP8 and TP9 for *P. excavatus* and minimum reduction of FA was recorded in TE4, TE5 and TE6 treatments for *E. fetida*, TP10, TP11 and TP12 for *E. fetida* and all the composting treatments (TW13 – TW18) (Table 3). Similar fluctuations were also found in a previous study when kitchen waste was used, due to initial instability of HA formation and transformation under the influence of microbial reaction and thermophilic temperature (Smidt *et al.*, 2008). Due to the presence of more acid functional groups and lower molecular weight, the water solubility of FA is higher than HA; thus FA content was relatively higher at the initial vermicomposting phase as reported before due to relatively high mobility and availability of compounds (Manivannan, 2005) and the immature condition of vermicompost. During vermicomposting, the gut microbes utilized FA for their metabolism and involved in the organic matter transformation towards HA (Fukushima *et al.*, 2009). Further, the degradation of the readily available organic substances, including the FA, provided energy to the microorganism and the bio-oxidation of these compounds resulted in the production of substances with more stable structures in mature vermicompost.

The humic carbon (HC) content declined in all the treatments during vermicomposting for both species of worms (*E. fetida* and *P. excavatus)* (Table 4). In the present study, humic carbon (HC) content decreased probably due to the dramatic decrease of fulvic acid (FA) in all the treatments during degradation of CP with amendment of SPM and SBG by *E. fetida* and *P. excavatus*. While, humification index (HI) increased in all treatments with highest change was observed in TE1, TE2 and TE3 treatments for *E. fetida* and TP7, TP8 and TP9 for *P. excavatus*, followed by other treatments and composting without worms at the end of experiment and the differences among treatments for both worms were statistically significant (Table 5). Interestingly, in all the treatments of *E. fetida* and *P. excavatus* the HI recorded was greater than one percent (except for TE6 and TP12 and all the composting treatments) at the end of experiment. Therefore, vermicompost obtained after 90 day could be considered mature (stabilized material). The ratio of humic acids-to-fulvic acid is widely used to describe the relative speed of HA and FA transformation as well as the maturity of the final vermicompost (Dev and Antil, 2011). During the humification process, the lignin in the treatment provided rich substrates for aromatization and oxidation. As a result, the cores of humic substances were constructed and oxygen-containing HA functional groups increased and the complicated ring structures in HA had positive correlation with vermicompost maturity and degree of humification (Fukushima *et al.*, 2009; Zhou *et al.*, 2014). Therefore, Vermicomposting results indicated rapid break down of organic materials, producing humus composition during stabilization process than natural composting. In the present study, HA, FA, HC and HI values of the final vermicompost having optimal value suggest aggregated humic macromolecule and complete humification level enhanced by earthworms. In addition, earthworms fragment the organic substrates, stimulate microbial activities greatly and increase rates of mineralization, rapidly converting the wastes into humus-like substances. Results of this study suggested that inoculation of *E. fetida* and *P. excavatus* in the suitable organic substrates during vermicomposting was most effective in terms of increasing the humic acids content of final product.

Table 1. Experimental design of vermicomposting of coffee pulp in different combination with sugar industry waste.

Treatment No.	Treatment description	Proportion
	Esenia fetida	
TE1	Coffee pulp + Bagasse	1:1
TE2	Coffee pulp + Press mud	1:1
TE3	Coffee pulp + Bagasse + Press mud	1:1:1
TE4	Coffee pulp + Bagasse	2:1
TE5	Coffee pulp + Press mud	2:1
TE6	Coffee pulp	100%
	Perionyx excavates	
TP7	Coffee pulp + Bagasse	1:1
TP8	Coffee pulp + Press mud	1:1
TP9	Coffee pulp + Bagasse + Press mud	1:1:1
TP10	Coffee pulp + Bagasse	2:1
TP11	Coffee pulp + Press mud	2:1
TP12	Coffee pulp	100%
	Without earthworms	
TW13	Coffee pulp + Bagasse	1:1
TW14	Coffee pulp + Press mud	1:1
TW15	Coffee pulp + Bagasse + Press mud	1:1:1
TW16	Coffee pulp + Bagasse	2:1
TW17	Coffee pulp + Press mud	2:1
TW18	Coffee pulp	100%

TE1 – TE6: treatments with *E. fetida*: TP7 – TP12: treatments with *P. excavatus*; TW13 – TW18: treatments without earthworm.

Table 2. Changes in humic acid parameters during vermicomposting of sugar industry waste amended with coffee pulp in different treatments.

Treatments	Humic acid (%)		
	Days		
	30	60	90
	Eisenia fetida		
TE1	1.80 ± 0.2	3.47 ± 0.7	3.55 ± 0.2*
TE2	1.85 ± 0.4	3.92 ± 0.2	4.05 ± 0.5*
TE3	1.98 ± 0.5	4.15 ± 0.5	4.27 ± 0.1*
TE4	1.59 ± 0.4	3.07 ± 0.0	3.10 ± 0.5
TE5	1.61 ± 0.3	3.16 ± 0.1	3.27 ± 0.4
TE6	1.27 ± 0.4	1.59 ± 0.3	1.61± 0.2
	Perionyx excavates		
TP7	1.75 ± 0.5	3.43 ± 0.5	3.51 ± 0.6*
TP8	1.82 ± 0.4	3.90 ± 0.7	3.97 ± 0.5*
TP9	1.90 ± 0.4	4.12 ± 0.5	4.18 ± 0.3*
TP10	1.52 ± 0.3	3.02 ± 0.3	3.07 ± 0.8
TP11	1.55 ± 0.5	3.09 ± 0.5	3.13 ± 0.4
TP12	1.25 ± 0.5	1.55 ± 0.4	1.57 ± 0.5
	Without earthworms		
TW13	1.48 ± 0.5	1.60 ± 0.3	1.64 ± 0.5
TW14	1.40 ± 0.2	1.70 ± 0.4	1.75 ± 0.4
TW15	1.51 ± 0.6	1.82 ± 0.6	1.86 ± 0.5
TW16	1.28 ± 0.4	1.47 ± 0.2	1.47 ± 0.7
TW17	1.29 ± 0.3	1.45 ± 0.4	1.48 ± 0.4
TW18	1.21 ± 0.2	1.41 ± 0.2	1.41 ± 0.5

All values are reported as mean ± standard deviation between six replicates; * Statistical significance value $p < 0.05$.

Table 3. Changes in fulvic acid content during vermicomposting of sugar industry waste amended with coffee pulp in different treatments.

Treatments	Fulvic acid (%)		
	Days		
	30	60	90
	Eisenia fetida		
TE1	5.20 ± 0.5	1.94 ± 0.5	1.82 ± 0.3*
TE2	5.20 ± 0.4	1.85 ± 0.3	1.81 ± 0.4*
TE3	5.11 ± 0.4	1.42 ± 0.3	1.31 ± 0.5*
TE4	5.42 ± 0.2	2.24 ± 0.5	2.17 ± 0.2
TE5	5.28 ± 0.4	2.11 ± 0.4	2.01 ± 0.3
TE6	5.58 ± 0.4	3.28 ± 0.2	3.11 ± 0.3
	Perionyx excavates		
TP7	5.27 ± 0.4	1.95 ± 0.3	1.90 ± 0.2*
TP8	5.25 ± 0.3	1.99 ± 0.5	1.91 ± 0.4*
TP9	5.13 ± 0.3	1.44 ± 0.4	1.33 ± 0.4*
TP10	5.40 ± 0.2	2.20 ± 0.2	2.15 ± 0.3
TP11	5.39 ± 0.2	2.15 ± 0.4	2.06 ± 0.4
TP12	5.62 ± 0.4	3.25 ± 0.2	3.14 ± 0.3
	Without earthworms		
TW13	5.54 ± 0.7	4.32 ± 0.4	3.48 ± 0.4
TW14	5.61 ± 0.5	4.29 ± 0.3	3.12 ± 0.4
TW15	5.75 ± 0.3	4.18 ± 0.5	2.98 ± 0.6
TW16	5.77 ± 0.4	4.55 ± 0.3	2.27 ± 0.5
TW17	5.68 ± 0.6	4.58 ± 0.4	2.25 ± 0.3
TW18	5.82 ± 0.4	4.67 ± 0.3	2.46 ± 0.4

All values are reported as mean ± standard deviation between six replicates; * Statistical significance value $p < 0.05$.

Table 4. Humic carbon content during vermicomposting of sugar industry waste amended with coffee pulp in different treatments.

Treatments	Humic carbon (%) (HC)		
	Days		
	30	60	90
	Eisenia fetida		
TE1	7.01 ± 0.7	5.41 ± 0.2	5.37 ± 0.5*
TE2	7.05 ± 0.8	5.77 ± 0.5	5.86 ± 0.9*
TE3	7.09 ± 0.7	5.57 ± 0.8	5.57 ± 0.6*
TE4	7.01 ± 0.6	5.31 ± 0.5	5.27 ± 0.6
TE5	6.89 ± 0.7	5.27 ± 0.5	5.28 ± 0.7
TE6	6.85 ± 0.8	4.87 ± 0.6	4.72 ± 0.8
	Perionyx excavates		
TP7	7.02 ± 0.9	5.38 ± 0.8	5.41 ± 0.8*
TP8	7.07 ± 0.8	5.89 ± 0.2	5.88 ± 0.9*
TP9	7.03 ± 0.7	5.56 ± 0.9	5.51 ± 0.7*
TP10	6.92 ± 0.6	5.22 ± 0.5	5.22 ± 0.9
TP11	6.94 ± 0.7	5.24 ± 0.9	5.19 ± 0.5
TP12	6.87 ± 0.8	4.80 ± 0.6	4.71 ± 0.8
	Without earthworms		
TW13	7.02 ± 0.2	5.92 ± 0.7	5.12 ± 0.9
TW14	7.01 ± 0.7	5.99 ± 0.7	5.17 ± 0.6
TW15	7.26 ± 0.9	6.05 ± 0.9	5.04 ± 0.9
TW16	7.05 ± 0.8	6.02 ± 0.5	5.04 ± 0.8
TW17	6.97 ± 0.5	6.03 ± 0.8	5.03 ± 0.9
TW18	7.03 ± 0.6	6.08 ± 0.5	5.17 ± 0.9

All values are reported as mean ± standard deviation between six replicates; * Statistical significance value $p < 0.05$.

Table 5. Humification index during vermicomposting of sugar industry waste amended with coffee pulp in different treatments.

Treatments	Humification index (HI) (%)		
	Days		
	30	60	90
	Eisenia fetida		
TE1	0.35 ± 0.02	1.81 ± 0.62	1.95± 0.81*
TE2	0.36 ± 0.05	2.11 ± 0.42	2.24 ± 0.48*
TE3	0.39 ± 0.03	3.04 ± 0.93	3.26 ± 0.61*
TE4	0.30 ± 0.02	1.37 ± 0.55	1.43 ± 0.83
TE5	0.30 ± 0.03	1.49 ± 0.42	1.62 ± 0.52
TE6	0.22 ± 0.02	0.48 ± 0.05	0.52 ± 0.05
	Perionyx excavates		
TP7	0.33 ± 0.06	1.76 ± 0.21	1.85 ± 0.23
TP8	0.35 ± 0.02	1.96 ± 0.42	2.08 ± 0.47*
TP9	0.37 ± 0.05	2.86 ± 0.30	3.14 ± 0.52*
TP10	0.28 ± 0.04	1.37 ±0.64	1.43 ± 0.61
TP11	0.29 ± 0.03	0.44 ±0.40	1.52 ± 0.27
TP12	0.22 ± 0.02	0.48 ± 0.02	0.50 ± 0.03
	Without earthworms		
TW13	0.27 ± 0.04	0.37 ± 0.21	0.47 ± 0.17
TW14	0.25 ± 0.05	0.39 ± 0.18	0.51 ± 0.12
TW15	0.26 ± 0.03	0.44 ± 0.22	0.58 ± 0.38
TW16	0.22 ± 0.04	0.32 ± 0.51	0.41 ± 0.51
TW17	0.22 ± 0.04	0.31 ± 0.11	0.41 ± 0.18
TW18	0.21 ± 0.02	0.30 ± 0.03	0.38 ± 0.04

All values are reported as mean ± standard deviation between six replicates;

* Statistical significance value $p < 0.05$.

CONCLUSION

In the present work, humus compositions were found to have significantly alerted in the vermicompost of *E. fetida* and *P. excavatus* obtained from all the treatments over initial substrate material and natural compost (without earthworms). The significant increased level of humic acid (HA) and humification index (HI) and reduction of fulvic acid (FA) and humic carbon (HC) content was observed in TE1, TE2 and TE3 treatments for *E. fetida* and TP7, TP8 and TP9 for *P. excavatus* could be due to the suitable concentration of the substrate and multiplication of microbes while passing through the gut of both worms, optimal moisture and large surface area of casts ideally suited for better feeding, multiplication and activity of humic material. Therefore, amendment material especially sugar industry wastes could improve the complexation ability of HA, but had little influence on that of FA. Finally it was concluded that mixing of SPM and SBG as amendment material in appropriate quantity of CP creates suitable medium for humification process during vermicomposting and this observation was also indicated that *E. fetida* may be a more efficient humification processor than *P. excavatus.*

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