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Effect of earthworms activity on humus composition during biological stabilization of coffee pulp amended with pressmud

M Sri Jayam, * S Manivannan
Department of Zoology, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Abstract
Coffee pulp is generated in huge amounts and cause serious hazards to the environment. In this experiment, coffee pulp (CP) with organic amendment sugare cane press mud (SPM) were used as substrate for native earthworm species *P. ceylanensis* and *L. mauritii* to stabilize and standardize the recycling technique on the basis of changes in humic composition during vermicomposting. The humification process during composting and vermicomposting was carried out and the results were suggested that humic acid content in the vermicompost of both species of worms were significantly altered than initial substrate and natural compost. However, among the different treatments PT4, PT5 and PT6 treatments for *P. ceylanensis* and LT10, LT11 and LT12 for *L. mauritii* 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 the same treatment without earthworms.

Keywords: vermicomposting, earthworms, humic material, coffee pulp

Introduction

Vermicomposting is a simple biotechnological process of composting, 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-like substances having diverse microbial population (Elvira *et al.,* 1998). 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) [2]. Large potentialities exist for recycling of both pulp and husk of coffee that can be composted and used as manure for several crops.

Coffee pulp could be useful because of its high content of carbohydrates and proteins. However, the presence of caffeine, tannins and polyphenols limits its utilization. However, composting and/or vermicomposting of these wastes over attractive recycling alternatives (Pandey *et al.,* 2000) [3]. The cane-sugar manufacturing has a number of co-products of immense potential worth. The co-products include pressmud and molasses. Out of which pressmud is produced during clarification of sugarcane juice. About 3.5 – 4.3% of sugarcane packed down end up as pressmud i.e. 36 - 40 kg of pressmud is obtained after one ton of cane crushing. However, pressmud is 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) [4]. In this research, the main focus is to use one of the sugar industries by products by - product i.e. pressmud (excellent organic amendments for vermicomposting) which is converted in to vermicompost mixed with coffee pulp using local earthworms (Mnivannan, 2005) [4].

Humic substances are essential for life on this earth and they influence plant growth and equilibrium in ecosystems through their effect on the physical, chemical and biological properties of soil and improve seed germination and plant growth and enhance plant nutrient absorption capacity. Earthworm can increase the velocity of decomposition of organic residues and also produce several bioactive humic substances (Vinceslas-Akpa and Loquet, 1997; Tamizhazhagan *et al.*, 2016) [5, 10]. Humic acids (HA) comprise one of the major fractions of humic substances. From the agricultural point of view, the humic acid could be considered as the most important component of the humic substances. So, microorganisms and humic acid content are closely associated with soil fertility. Hence the present work is aimed to study the total humic material content in the mixtures of CP and SPM during vermicomposting to standardize composing efficiency.

Material and Methods

Collection of coffee pulp (CP) and sugar industry press mud (SPM)

The coffee pulp (CP) waste of was collected from the JSP plantation coffee seed processing industry at Yercaud in Salem district, Tamilnadu, India. Sugar industry by product press mud (SPM), also called filter mud 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 2-3 weeks to remove the foul smell before using for the experimental process.

Selection of local earthworms

Indigenous, efficient epigeic species *Perionyx ceylanensis* (Mich.) were compared with another local earthworm species *Lampito mauritii* (Kinberg) for their survival and degradation efficiency of selected waste materials. Indigenous earthworm's *P. ceylanensis* and *L. mauritii* were obtained from the stock culture which was cultivated 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

In the present study, different proportions of Coffee pulp (CP) with bulking material Sugar industry press mud (SPM) mixtures were prepared (Table 1). Coffee pulp (CP) and Sugar industry press mud (SPM) was weighed (dry weight) in the above said description and mixed well with 65-75% moisture content. The waste mixtures, CP and SPM were transferred to separate plastic troughs with 40cm diameter x 60cm depth, respectively. After transferred in the plastic troughs all the mixture compositions of CP and SPM were allowed for seven days of initial natural decomposition. PT1, PT2, PT3, PT4, PT5 and PT6 treatments were composed of different proportions of CP and SPM with *P. ceylanensis*. LT6, LT7, LT8, LT9, LT10 and LT12 treatments were composed of different proportions of CP and SPM with *L. mauritii*. Treatments of CT13, CT14, CT15, CT16, CT17 and CT18 were composed of different proportions of CP and SPM without earthworms (Table 1). 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 irrigated with equal quantity of water to ensure that the substrate moisture content was maintained at approximately 65-75%. After the completion of pre-inoculation period of 7days, the clitellated *P. ceylanensis and L. mauritii* were weighed and inoculated in to respective each treatment (Manivannan *et al.*, 2005) [4].

Analysis of humus composition

Humus composition was analyzed according to the method described by Kumada (1987) with some modifications (Xiong *et al.*, 2010). The humic acid content was extracted by adopting the procedure as described by Schnitzer (1978) [7]. 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 24hrs. 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 dialysed extensively against distilled water till free of chloride and finally dried in hot air oven at 40°C. The humic acid contents are expressed in mg/5g substrates.

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

The humification process during composting and vermicomposting (HA, FA, HI, and HC) was carried out and data are given in Tables 1to 4. Results evidences that vermicomposting increased humic acid (HA) level while, condensed the fulvic acid (FA) level, which showed the obvious humification development during the vermicomposting of CP and SPM by indigenous earthworm species *P. ceylanensis* and *L. mauritti*. Table 1 evidences that vermicomposting by both worms increased humic acid level while reduced the fulvic acid level, which clearly showed the noticeable humification process during the vermicomposting and shows that maximum humic acid content was observed in PT4, PT5 and PT6 for *P. ceylanensis* and LT10, LT11 and LT12 for *L. mauritti* and minimum was recorded in PT1, PT2 and PT3 for *P. ceylanensis* and LT7, LT8 and LT9 for *L. mauritti* and all the composting treatments (CT13 – CT18). While, reduced the fulvic acid (FA) level during vermicomposting period respectively, which showed that obvious humification process during the process of vermicomposting than composting without earthworms (Table 2).

The contents of humic carbon (HC) declined in all the treatments for both species of worms (*P. ceylanensis* and *L. mauritti)*. In the present experiment, contents of humic carbon (HC) decreased probably due to the dramatic decrease of fulvic acid (FA) in all the treatments (PT1 – PT6; LT1 – LT6) during degradation of CP with amendment SPM by *P. ceylanensis* and *L. mauritti*. Whereas, in the presence of *P. ceylanensis* and *L. mauritti*, treatments with higher levels of CP (PT1 and PT3 for *P. ceylanensis*; LT7 and LT9 for *L. mauritti*) maintained significantly reduced levels of humic acid than treatments with lower CP mixed with SPM (Table 4). In the present observation, the fulvic acid (FA) content reduced in all the treatments (PT1 – PT6; LT1 – LT6) during vermicomposting. Value less than 1-2% fulvicacid in the last material implied that simply available carbon (C) in the substrate was reduced and constancy of the end product was increased level. Humicacid and fulvicacid (HI) index increased in all treatments with highest change in the PT4, PT5 and PT6 for *P. ceylanensis* and LT10, LT11 and LT12 for *L. mauritti,* followed by other treatment and composting without worms at the end of experiment and the differences among treatments for both worms were statistically significant (Table 3). Interestingly, in all the treatments of *P. ceylanensis* and *L. mauritti* the HC ratio (HA/FA) recorded was greater than one percent (except for CP alone treatment for both worms) after vermicomposting, at the end, vermicompost obtained after 50 day could be considered mature (stabilized material). In the present study, contents of HC (HA+FA) decreased in all the treatments (PT1 – PT6; LT1 – LT6) during vermicomposting period. The treatments of PT4, PT5 and PT6 for *P. ceylanensis* and LT10, LT11 and LT12 for *L. mauritti* achieved the maximum reduction of HC during vermicomposting, followed by the other treatments studied.

Table 1: Humic acid parameters of the produced vermicompost and composts

Treatments	Humic acid (%)		
	Days		
	25	50	75
Perionyx ceylanensis			
PT1	1.36 ± 0.5^{a}	3.12 ± 0.3^{a}	2.62 ± 0.4
PT2	1.77 ± 0.3^{b}	3.53 ± 0.4^{c}	2.27 ± 0.3^{c}
PT3	1.70 ± 0.2^{b}	3.60 ± 0.2^{b}	3.11 ± 0.3^{c}
PT4	1.64 ± 0.5^{b}	3.88 ± 0.2^{b}	3.06 ± 0.4^{b}
PT5	2.02 ± 0.3^{c}	3.95 ± 0.2^{c}	3.35± 0.2^{b}
PT6	1.96 ± 0.4^{c}	3.91 ± 0.3^{c}	3.27 ± 0.4^{c}
Lampito mauritii			
LT7	1.37 ± 0.3^{a}	3.09 ± 0.2^{a}	2.60 ± 0.3^{a}
LT8	1.76 ± 0.5^{b}	3.51 ± 0.2^{b}	3.25 ± 0.5^{c}
LT9	1.71 ± 0.4^{b}	3.60 ± 0.3^{b}	3.06 ± 0.3^{b}
LT10	1.65 ± 0.2^{b}	3.85 ± 0.3^{c}	3.28 ± 0.3^{c}
LT11	2.00 ± 0.4^{c}	3.93 ± 0.4^{c}	3.29 ± 0.2^{b}
LT12	1.94 ± 0.3^{c}	3.88 ± 0.2^{c}	3.25 ± 0.4^{c}
Composting (without worms)			
CT13	1.15 ± 0.4^{a}	2.55 ± 0.4^{a}	2.47 ± 0.2^{a}
CT14	1.21 ± 0.3^{b}	2.90 ± 0.3^{c}	2.50± 0.3^{c}
CT15	1.20 ± 0.2^{b}	2.95 ± 0.5^{b}	2.37 ± 0.5^{c}
CT16	1.30 ± 0.3^{b}	3.05 ± 0.1^{b}	2.50 ± 0.3^{b}
CT17	1.30 ± 0.4^{c}	3.17 ± 0.2^{c}	2.58 ± 0.4^{b}
CT18	1.29 ± 0.3^{c}	3.08 ± 0.3^{c}	2.51 ± 0.3^{c}

Above values are reported as mean ± standard deviation among six replicates; Different letters in a row are significant at *P<0.05* (ANOVA; Tukey's test).

Table 2: Fulvic acid parameters of the produced vermicompost and composts

Treatments	Fulvic acid (%)		
	Days		
	25	50	75
Perionyx ceylanensis			
PT1	5.21 ± 0.5^{b}	1.20 ± 0.7^{a}	1.16 ± 0.3^{a}
PT2	4.90 ± 0.1^{a}	2.11 ± 0.4^{a}	1.98 ± 0.4^{a}
PT3	5.18 ± 0.8^{b}	1.20 ± 0.5^{a}	1.18 ± 0.5^{a}
PT4	5.44 ± 0.4^{bc}	1.24 ± 0.3^{a}	1.17 ± 0.2^{a}
PT5	5.50 ± 0.5^{bc}	1.21 ± 0.4^{a}	1.19 ± 0.9^{a}
PT6	5.36 ± 0.6^{bc}	2.13 ± 0.5^{b}	1.91 ± 0.5^{b}
Lampito mauritii			
LT7	5.21 ± 0.5^{a}	1.21 ± 0.3^{a}	1.15 ± 0.5^{a}
LT8	4.52 ± 0.3^{a}	2.18 ± 0.5^{a}	2.05 ± 0.3^{a}
LT9	5.18 ± 0.7^{a}	1.20 ± 0.6^{a}	1.18 ± 0.4^{a}
LT10	5.44 ± 0.6^{b}	1.24 ± 0.4^{a}	1.17 ± 0.5^{a}
LT11	5.50 ± 0.3^{b}	1.21 ± 0.3^{a}	1.19 ± 0.6^{a}
LT12	5.36 ± 0.2^{b}	2.13 ± 0.5^{b}	1.91 ± 0.4^{b}
Composting (without worms)			
CT13	4.31 ± 0.2^{a}	2.15 ± 0.2^{a}	1.89 ± 0.4^{a}
CT14	3.42 ± 0.3^{a}	3.04 ± 0.3^{a}	2.78 ± 0.2^{a}
CT15	4.30 ± 0.2^{a}	2.20 ± 0.4^{a}	1.94 ± 0.3^{a}
CT16	4.20 ± 0.4^{a}	2.10 ± 0.2^{a}	2.03 ± 0.2^{a}
CT17	4.27 ± 0.3^{a}	2.15 ± 0.2^{a}	2.07 ± 0.4^{a}
CT18	4.25 ± 0.2^{a}	2.13 ± 0.3^{a}	2.08 ± 0.5^{a}

Above values are reported as mean ± standard deviation among six replicates; Different letters in a row are significant at *P<0.05* (ANOVA; Tukey's test).

Table 3: Changes in the Humification index properties of vermicompost and composts

Treatments	Humification index (HI)		
	Days		
	25	50	75
Perionyx ceylanensis			
PT1	0.29 ± 0.5^{a}	2.98 ± 0.5^{b}	2.65 ± 0.4^{b}
PT2	0.37 ± 0.4^{a}	3.13 ± 0.4^{c}	2.59 ± 0.5^{b}
PT3	0.31 ± 0.2^{a}	2.83 ± 0.3^{b}	2.79 ± 0.2^{c}
PT4	0.28 ± 0.5^{a}	2.68 ± 0.2^{b}	2.57 ± 0.4^{b}
PT5	0.23 ± 0.3^{a}	1.37 ± 0.5^{a}	2.34 ± 0.3^{a}
PT6	0.29 ± 0.4^{a}	2.63 ± 0.4^{b}	2.50 ± 0.5^{b}
Lampito mauritii			
LT7	0.29 ± 0.4^{a}	2.92 ± 0.5^{b}	2.68 ± 0.4^{b}
LT8	0.36 ± 0.6^{a}	3.16 ± 0.3^{c}	2.85 ± 0.3^{bc}
LT9	0.31 ± 0.5^{a}	2.83 ± 0.5^{b}	2.79 ± 0.2^{bc}
LT10	0.28 ± 0.3^{a}	2.67 ± 0.4^{b}	2.57 ± 0.3^{b}
LT11	0.23 ± 0.5^{a}	1.37 ± 0.3^{a}	1.34 ± 0.5^{a}
LT12	0.28 ± 0.4^{a}	3.04 ± 0.3^{c}	2.60 ± 0.4^{b}
Composting(without worms)			
CT13	1.05 ± 0.3^{a}	1.18 ± 0.2^{a}	1.15 ± 0.3^{a}
CT14	1.04 ± 0.2^{a}	1.09 ± 0.3^{a}	1.02 ± 0.2^{a}
CT15	1.02 ± 0.4^{a}	1.18 ± 0.4^{a}	1.13 ± 0.3^{a}
CT16	0.96 ± 0.5^{a}	1.29 ± 0.5^{ab}	1.20 ± 0.4^{ab}
CT17	0.94 ± 0.4^{a}	1.30 ± 0.3^{ab}	1.21 ± 0.5^{ab}
CT18	0.94 ± 0.3^{a}	1.28 ± 0.4^{ab}	1.20 ± 0.3^{ab}

Above values are reported as mean ± standard deviation among six replicates; Different letters in a row are significant at *P<0.05* (ANOVA; Tukey's test).

Table 4: Changes in the Humic carbon (HC) properties of vermicompost and composts

Treatments	Humic carbon (%) HA+FA		
	Days		
	25	50	75
Perionyx ceylanensis			
PT1	6.57 ± 0.5^{a}	4.32 ± 0.2^{a}	3.78 ± 0.4^{a}
PT2	6.67 ± 0.4^{b}	5.64 ± 0.6^{b}	6.39 ± 0.5^{c}
PT3	6.88 ± 0.3^{ab}	4.80 ± 0.7^{a}	4.29 ± 0.8^{ab}
PT4	7.08 ± 0.7^{b}	5.12 ± 0.3^{b}	4.23 ± 0.6^{ab}
PT5	7.52 ± 0.6^{b}	5.16 ± 0.6^{b}	4.54 ± 0.4^{ab}
PT6	7.32 ± 0.9^{b}	6.04 ± 0.5^{c}	5.18 ± 0.9^{b}
Lampito mauritii			
LT7	6.58 ± 0.4^{a}	4.30 ± 0.4^{a}	3.75 ± 0.4^{a}
LT8	6.28 ± 0.4^{a}	5.69 ± 0.3^{b}	5.30 ± 0.4^{c}
LT9	6.89 ± 0.6^{ab}	4.80 ± 0.4^{a}	4.24 ± 0.3^{b}
LT10	7.09 ± 0.4^{ab}	5.09 ± 0.4^{ab}	4.45 ± 0.4^{b}
LT11	7.50 ± 0.3^{b}	5.14 ± 0.4^{ab}	4.48 ± 0.4^{b}
LT12	7.05 ±0.3^{ab}	6.01 ± 0.3^{c}	5.16 ± 0.4^{c}
Composting (without worms)			
CT13	5.46 ± 0.4^{b}	4.70 ± 0.3^{a}	4.36 ± 0.8^{a}
CT14	4.73 ± 0.6^{a}	4.94 ± 0.5^{ab}	4.28 ± 0.3^{a}
CT15	5.55 ± 0.3^{b}	4.35 ± 0.7^{a}	4.31 ± 0.6^{a}
CT16	5.50 ± 0.6^{b}	5.15 ± 0.3^{b}	4.53 ± 0.6^{ab}
CT17	5.57 ± 0.6^{b}	5.32 ± 0.6^{bc}	4.65 ± 0.6^{ab}
CT18	5.54 ±0.2^{b}	5.21 ± 0.5^{b}	4.59 ± 0.5^{ab}

HC (Humic carbon) =HA+FA; CP- Coffee Pulp; SPM- Sugar industry Press mud; Above values are reported as mean ± standard deviation among six replicates; Different letters in a row are significant at *P<0.05* (ANOVA; Tukey's test).

Discussion

Vrmicomposting results indicated rapid break down of organic materials, producing humic material (HM) during the stabilization process than natural composting. In the present study, HA FA, HI and HC extracted from the final vermicompost studied having higher value, suggest aggregated humic macromolecule and complete humification level enhanced by earhworms. HM derived from vermicompost are higher than those for HM extracted from initial materials, indicating that HM obtained after the vermicomposting process, are supramolecules association of a larger proportion of lesser molecules and have more humification process (Manivannan, 2005) [4]. The higher HA content of the treatment during the vermicomposting period may be attributed to the higher content of readily available organic matter from SPM which could be easily decomposed at that time, resulting in higher rate of HA formation. Additionally, fiber-structure of amendment material SPM components such as lignin, which are known to provide more stable phenolic compounds required as starting material for humification processes (Campitelli and Ceppi, 2008) [1, 8].

The FA content decreased in all the treatments during vermicomposting period. A value less than 1% FA in the final product obscure that easily available carbon in the vermicompost was reduced and stability of the vermicompost increased. On the contrary, FA content was reduced after vermicomposting. 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 (Manivannan, 2005; Fukushima *et al.*, 2009) [4, 11, 14] due to relatively high mobility and availability of compounds; 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. It is reported that to some extent the FA are precursors for the formation of HA (Doane *et al.*, 2003) [12]. The degradation of the readily available organic substances, including the FA, provided energy to the microorganism. The bio-oxidation of these compounds resulted in the production of substances with more stable structures in mature vermicompost.

The humic acids-to-fulvic acid (HA/FA) ratio 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; Doane *et al.*, 2003) [13, 12]. 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. The complicated ring structures in HA had positive correlation with vermicompost maturity and degree of humification (Fukushima *et al.*, 2009) [11, 14]. Additionally, the vermicomposting condition turned to be alkalescence and substrates had higher molecular weights as the vermicompost were aged; this situation catalyzed the degradation of FA due to acidic functional groups and lower molecular weight. The FA also condensed to HA during mineralization of waste material, resulting in a sharp increase in HA (Zhou *et al.*, 2014) [15]. Therefore, earthworms fragment the organic substrates, stimulate microbial activities greatly and increase rates of mineralization, rapidly converting the wastes into humus-like substances. Since narrower C/N ratio of organic matter facilitates higher rate of decomposition, organic wastes with lesser C/N ratios show higher HA content in vermicompost using earthworms. Therefore, it was concluded that inoculation of earthworms in initial organic substrates significantly ($P<0.05$) increased the humic acids content of resulted vermicompost, but their effect on humification varied depending on the earthworm species inoculated to the organic substrates. Results suggested that inoculation of *L. mauritii* and *P. excavates* in the initial organic substrates during vermicomposting was most effective in terms of increasing the humic acids content of final vermicompost, while the values of PT4, PT5. PT6, LT10, LT11 and LT12 treatments were statistically not significant.

Conclusion

In the present study, humic material were found to have significantly alerted in the vermicompost of *P. ceylanensis* and *L. mauritii* obtained from all the treatments over initial substrate and natural compost. The significantly increased 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 the same treatment without earthworms could be due to the higher nutrient concentration in the substrate and cast, 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. Finally it was concluded that mixing of SPM as bulking agent in appropriate quantity of CP creates suitable medium for earthworms for stabilization.

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