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Evolution of humic material in different organic waste during vermicomposring using *Perionyx ceylanensis*

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Abstract
Humification during vermicomposting and composting of poultry droppings (PD), rice husk (RH) and sugarcane press mud (SPM) was investigated to reveal its correlation with humic composition. The aim of this study was PD was mixed with RH and SPM at various proportions to make different treatments (VCPT1-VCPT7) and kept for natural stabilization for 15 days and subsequently vermicomposted using native earthworm species *Perionyx ceylanensis* for a period of 75 days under laboratory conditions. Controls for the above treatment proportions were also included without the inoculation of worms (CT1-CT7). The humification process during composting and vermicomposting was carried out and the results were suggested that humic acid (HA) content in the vermicompost were significantly altered than initial substrate and natural compost. However, among the different vermicomposting and composting treatments VCPT2, VCPT3, VCPT6 and VCPT7 treatments showed significantly ($p<0.05$) higher level of humic acid (HA) and humification index (HI) and also reduction of fulvic acid (FA) and humic carbon (HC) content than other treatments. Hence, it was concluded that mixing of SPM and RH as mixing agent in appropriate quantity of PD creates suitable medium for humic material rich vermicompost production.

Keywords: vermicomposting, composting, humification, earthworms, organic waste

1. Introduction

Humic acids (HA) and Fulvic acids (FA) are one of most important active fractions of organic matter and they improve the absorption of mineral nutrients by plants and also the most resistant fraction to microbial degradation of the organic matter in soil. HA and FA are heterogeneous mixtures of a variety of organic compounds of functional groups, consisting of aromatic, aliphatic, phenolic and quinolic with varying molecular sizes and properties (Gu *et. al*., 1995) [1]. Further, humic substances are necessary for life on this earth and they influence plant growth through their effect on the physical, chemical and biological properties of soil and improve seed germination and plant growth (Jayam and Manivannan, 2017) [2]. Earthworm can increase the velocity of decomposition of organic residues and also produce several bioactive humic substances (Vinceslas-Akpa and Loquet, 1997) [3]. Vermicomposting is a simple biotechnological process of composting using different organic waste, in which certain species of earthworms are used to improve the process of organic 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) [4]. From the agricultural point of view, earthworm activity on humic acid could be considered as the most important component of the humic substances. Thus, earthworms, microorganisms and humic acid content are closely associated with soil fertility and plant growth.

Biomass generation production has increased in recent years due to increased agricultural and agro-industrial production and its processing is a serious problem for present society (Amir *et al*., 2003) [5]. With the increase in organic wastes, landfill space has become the limiting factor for disposal as a result recycling through available local earthworms species has become an attractive option for the treatment of these residues. Poultry and livestock industries are growing rapidly along with the human population and production of animal waste are potential sources of many major environmental problems. India is one of the main producers of poultry in the world and the poultry manure availability is estimated to be 12.1 million tons (The week end leader, 2014) [6]. In the poultry farm large amount of droppings that accumulated in the litter turns it into importance sources of contamination i.e. odorous gases including amines, amides, mercaptans, sulphides and disulphides. These noxious gases can cause respiratory disease in animals and humans (Schiffamn and Williams, 2005) [7]. However, poultry droppings along with litter has useful nutrients, and is therefore used as organic fertilizer but uncontrolled decomposition and excess applications to soil can cause environmental problems due to their extremely high levels of nitrogen as ammonia, low pH, and heat generation (Moore *et al.*, 1995) [8].

In addition, India is one of the primary growers of sugarcane with an estimated production of approximately 300 million tons in the marketing for every year. Huge amount of solid waste streams generated during sugar manufacturing process including sugarcane trash, bagasse, press mud and bagasse. Mostly sugarcane industry generates enormous amount of residue after the sugarcane juice has been clarified commonly known as press mud. For about 134 million tons of sugarcane crushed, 4.0 million tons of filter mud are produced

(Manivannan *et al.*, 2004) [9]. There is a major disposal problem for the filter mud although it is fairly rich in organic nutrients; it finds little use as bio-fertilizer (Sen and Chandra, 2006) [10]. Similarly, rice husk as by-product of the rice milling and is extensively available in rice producing country like India (Soltani *et al.*, 2015) [11]. Approximately 500 million tons paddy produced by world every year and 120 million tonnes of paddy produced by India, it gives around 24 million tonnes of RH per year (Shwetha *et al.*, 2014) [12]. Hence, the present work is aimed to study the total humic material content produced during vermicomposting by local earthworms in RH, PD and SPM in different ratios in order to agronomic utilization.

2. Materials and Methods

Collection of organic wastes and Selection of earthworms

The poultry droppings (PD) 7 days old were collected from Indian feeds farm, Perumal kovilmedu, Namakkal district, Tamil Nadu, India. Sugar cane press mud (SPM) was obtained from Thiru Arooran Sugars Limited, located at Tirumandangudi, Thanjavur district, Taminadu, India. Rice Husk (RH) was collected from a modern rice mill, Vandikkara Street, Thanjavur, Tamil Nadu, India. The poultry droppings were transported to the laboratory using sterile plastic bags. PD and SPM were sundried separately for 15 days to remove the odor and noxious gases. Indigenous, efficient epigeic species *Perionyx ceylanensis* (Mich.) were selected for their survival and 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 earthworms.

Experimental design and vermicomposting conditions

In the present study, different treatments containing different ratios of poultry droppings (PD) with bulking material Sugar industry press mud (SPM) and rice husk (RH) mixtures were prepared (Table 1). Each was weighed (dry weight) in the above said description and treatments of VCPT1, VCPT2, VCPT3, VCPT4, VCPT5, VCPT6 and VCPT7 were composed of different proportions of PD, RH and SPM with *P. ceylanensis*. The waste mixtures were transferred to separate plastic troughs with 40cm diameter x 60cm depth, respectively. The troughs were filled with 5kg substrate per troughs in above combinations. After transferred in the plastic troughs all the mixture compositions of PD, RH and SPM 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. Matured earthworms were used in this experiment, with an average weight of 108 to 112mg of *P. ceylanensis* with a developed clitellum. The above treatments 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* were weighed and inoculated in to respective each treatment (Manivannan *et al.*, 2004) [9]. Controls for the above treatments CT1, CT2, CT3, CT4, CT5, CT6 and CT7 were also included without the inoculation of *P. ceylanensis*. Periodically the vermicomposting and composting 200g samples in each treatment was collected on day 25, 50 and 75 were used to extract the humic substances.

Extraction of humic substances

Humus composition was analyzed according to the method described by Kumada (1987) [13] with some modifications (Xiong *et al.*, 2010) [14]. The humic acid content was extracted by adopting the procedure as described by Schnitzer (1978) [15]. 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 6NHCl 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 humic acid contents are expressed in mg/5g substrates. Changes in humification index (HI) and humic carbon (HC) was calculated using the obtained value of humic acid and fulvic acid.

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.

3. Results and Discussion

These results revealed that vermicomposting significantly increased humic acid (HA) level while, condensed the fulvic acid content (FA), which showed the obvious humification during the vermicomposting of PD mixed with SPM and RH using local earthworm species *P. ceylanensis* (Tables 1- 4). Table 1 evidences that vermicomposting of PD mixed with SPM and RH increased humic acid level while reduced the fulvic acid level, which clearly showed the noticeable humification during the vermicomposting and shows that maximum humic acid content was observed in VCPT2, VCPT3, VCPT6 and VCPT7 treatments than other treatments studied. The results indicated that, rapid break down of organic materials during vermicomposting, produced enhanced humic material 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 earthworms (Manivannan, 2005) [16]. The higher HA content of the treatment during the vermicomposting period may be attributed to the higher content of readily available organic matter used in this study which could be easily decomposed at that time, resulting in higher rate of humification. Additionally, fiber-structure of amendment material RH and 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) [17].

Whereas, reduced the fulvic acid (FA) level during vermicomposting was recorded (Table 2), which showed that obvious humification during the process of vermicomposting than natural composting (without earthworms). The FA content decreased in all the treatments during vermicomposting period than natural composting. 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) [18]. 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 (Fukushima *et al.*, 2009) [19] 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) [20].

Table 3 evidences that vermicomposting of PD mixed with SPM and RH increased humification index (HI) level, which clearly showed the noticeable humification during the vermicomposting and shows that maximum humification index level was observed in VCPT2, VCPT3, VCPT6 and VCPT7 treatments than other treatments studied. The vermicomposting condition turned to be alkalescency 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 HI (Jayam and Manivannan, 2017) [2]. Therefore, earthworms fragment the organic substrates, stimulate microbial activities greatly and increase rates of mineralization, rapidly converting the wastes into humus-like substances (Zhou *et al.*, 2014) [21].

The contents of humic carbon (HC) declined in all the treatments of *P. ceylanensis* (VCPT2, VCPT3, VCPT6 and VCPT7) and decreased probably due to the dramatic decrease of fulvic acid (FA) in all the treatments during degradation of PD with SPM and RH (Table 4). Whereas, in the vermicomposting treatments with higher levels of SPM and RH with PD significantly reduced levels of FA than treatments with lower SPM and RH mixed with PD and the differences among VCPT2, VCPT3, VCPT6 and VCPT7 treatments were statistically significant. Value less than 1-2% fulvic acid in the last material implied that simply available carbon (C) in the substrate was reduced and constancy of the end product was increased level. In the present study, contents of humic carbon (HC) decreased in all the treatments during vermicomposting period. Interestingly, in all the vermicomposting treatments the HC recorded was greater than one percent (except for VCPT1, VCPT4 and VCPT5 treatment) after vermicomposting, at the end, vermicompost obtained after 50 day could be considered mature. The HC 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) [22]. 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 (Fukushima *et al.*, 2009) [23]. 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.

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

Treatments	Humic acid (%)		
	Days		
	25	50	75
Perionyx ceylanensis			
VCPT1	1.64 ± 0.7^{b}	3.88 ± 0.4^{de}	3.06 ± 0.6^{c}
VCPT2	2.00 ± 0.6^{cd}	3.93 ± 0.4^{e}	3.29 ± 0.4^{cd}
VCPT3	2.02 ± 0.2^{cd}	3.95 ± 0.2^{e}	3.35 ± 0.2^{d}
VCPT4	1.76 ± 0.4^{b}	3.51 ± 0.7^{d}	3.25 ± 0.5^{cd}
VCPT5	1.71 ± 0.5^{b}	3.60 ± 0.5^{d}	3.06 ± 0.3^{c}
VCPT6	1.94 ± 0.7^{c}	3.88 ± 0.2^{de}	3.25 ± 0.5^{cd}
VCPT7	1.96 ± 0.5^{c}	3.91 ± 0.4^{e}	3.27 ± 0.4^{cd}
Composting without worms			
CT1	1.21 ± 0.5^{a}	2.90 ± 0.4^{b}	2.50 ± 0.5^{ab}
CT2	1.36 ± 0.5^{ab}	3.12 ± 0.3^{c}	2.62 ± 0.4^{b}
CT3	1.30 ± 0.4^{ab}	3.17 ± 0.2^{c}	2.58 ± 0.4^{ab}
CT4	1.15 ± 0.4^{a}	2.55 ± 0.4^{a}	2.47 ± 0.2^{a}
CT5	1.20 ± 0.2^{a}	2.95 ± 0.5^{b}	2.37 ± 0.5^{a}
CT6	1.30 ± 0.3^{ab}	3.05 ± 0.1^{bc}	2.50 ± 0.3^{ab}
CT7	1.29 ± 0.2^{ab}	3.08 ± 0.5^{bc}	2.51 ± 0.4^{ab}

VCPT1 to VCPT7 – Treatments with *Perionyx ceylanensis*; CT1 to CT7 - Composting without worms. 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 content of the produced vermicompost and composts

Treatments	Fulvic acid (%)		
	Days		
	25	50	75
Perionyx ceylanensis			
VCPT1	5.44 ± 0.4^{d}	1.24 ± 0.3^{a}	1.17 ± 0.2^{a}
VCPT2	5.50 ± 0.3^{d}	1.21 ± 0.3^{a}	1.19 ± 0.6^{a}
VCPT3	5.50 ± 0.5^{d}	1.21 ± 0.4^{a}	1.19 ± 0.9^{a}
VCPT4	4.52 ± 0.3^{bc}	2.18 ± 0.5^{b}	2.05 ± 0.3^{bc}
VCPT5	5.18 ± 0.7^{c}	1.20 ± 0.6^{a}	1.18 ± 0.4^{a}
VCPT6	5.36 ± 0.2^{cd}	2.13 ± 0.5^{b}	1.91 ± 0.4^{b}
VCPT7	5.36 ± 0.6^{cd}	2.13 ± 0.5^{b}	1.91 ± 0.5^{b}
Composting without worms			
CT1	3.42 ± 0.3^{a}	3.04 ± 0.3^{c}	2.78 ± 0.2^{c}
CT2	5.21 ± 0.5^{c}	1.20 ± 0.7^{a}	1.16 ± 0.3^{a}
CT3	4.27 ± 0.3^{b}	2.15 ± 0.2^{b}	2.07 ± 0.4^{a}
CT4	4.31 ± 0.2^{b}	2.15 ± 0.2^{b}	1.89 ± 0.4^{b}
CT5	4.30 ± 0.2^{b}	2.20 ± 0.4^{b}	1.94 ± 0.3^{b}
CT6	4.20 ± 0.4^{b}	2.10 ± 0.2^{b}	2.03 ± 0.2^{bc}
CT7	4.25 ± 0.2^{b}	2.13 ± 0.3^{b}	2.08 ± 0.5^{bc}

VCPT1 to VCPT7 – Treatments with *Perionyx ceylanensis*; CT1 to CT7 - Composting without worms. 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 of vermicompost and composts using different organic waste

Treatments	Humification index (HI)		
	Days		
	25	50	75
Perionyx ceylanensis			
VCPT1	0.28 ± 0.5[a]	2.68 ± 0.2[b]	2.57 ± 0.4[b]
VCPT2	0.23 ± 0.5[a]	1.37 ± 0.3[ab]	1.34 ± 0.5[ab]
VCPT3	0.23 ± 0.3[a]	1.38 ± 0.5[ab]	2.31 ± 0.3[b]
VCPT4	0.36 ± 0.6[a]	3.16 ± 0.3[c]	2.85 ± 0.3[c]
VCPT5	0.31 ± 0.5[a]	2.83 ± 0.5[b]	2.79 ± 0.2[bc]
VCPT6	0.28 ± 0.4[a]	3.04 ± 0.3[c]	2.60 ± 0.4[b]
VCPT7	0.29 ± 0.4[a]	2.63 ± 0.4[b]	2.50 ± 0.5[b]
Composting without worms			
CT1	1.04 ± 0.2[bc]	1.09 ± 0.3[a]	1.02 ± 0.2[a]
CT2	0.29 ± 0.5[a]	2.98 ± 0.5[bc]	2.65 ± 0.4[b]
CT3	0.94 ± 0.4[b]	1.30 ± 0.3[ab]	1.21 ± 0.5[ab]
CT4	1.05 ± 0.3[bc]	1.18 ± 0.2[a]	1.15 ± 0.3[a]
CT5	1.02 ± 0.4[bc]	1.18 ± 0.4[a]	1.13 ± 0.3[a]
CT6	0.96 ± 0.5[b]	1.29 ± 0.5[ab]	1.20 ± 0.4[ab]
CT7	0.94 ± 0.3[b]	1.28 ± 0.4[ab]	1.20 ± 0.3[ab]

VCPT1 to VCPT7 – Treatments with *Perionyx ceylanensis*; CT1 to CT7 - Composting without worms. 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 humic carbon (HC) contents of vermicompost and composts

Treatments	HC (%)		
	Days		
	25	50	75
Perionyx ceylanensis			
VCPT1	7.08 ± 0.7[d]	5.12 ± 0.3[b]	4.23 ± 0.6[b]
VCPT2	7.50 ± 0.3[e]	5.14 ± 0.4[b]	4.48 ± 0.4[bc]
VCPT3	7.52 ± 0.6[e]	5.16 ± 0.6[b]	4.54 ± 0.4[c]
VCPT4	6.28 ± 0.4[c]	5.69 ± 0.3[c]	5.30 ± 0.4[de]
VCPT5	6.89 ± 0.6[cd]	4.80 ± 0.4[ab]	4.24 ± 0.3[b]
VCPT6	7.05 ±0.3[d]	6.01 ± 0.3[d]	5.16 ± 0.4[d]
VCPT7	7.32 ± 0.9[de]	6.04 ± 0.5[d]	5.18 ± 0.9[d]
Composting without worms			
CT1	4.73 ± 0.6[a]	4.94 ± 0.5[ab]	4.28 ± 0.3[b]
CT2	6.57 ± 0.5[c]	4.32 ± 0.2[a]	3.78 ± 0.4[a]
CT3	5.57 ± 0.6[b]	5.32 ± 0.6[bc]	4.65 ± 0.6[c]
CT4	5.46 ± 0.4[b]	4.70 ± 0.3[a]	4.36 ± 0.8[bc]
CT5	5.55 ± 0.3[b]	4.35 ± 0.7[a]	4.31 ± 0.6[bc]
CT6	5.50 ± 0.6[b]	5.15 ± 0.3[b]	4.53 ± 0.6[c]
CT7	5.54 ±0.2[b]	5.21 ± 0.5[b]	4.59 ± 0.5[c]

VCPT1 to VCPT7 – Treatments with *Perionyx ceylanensis*; CT1 to CT7 - Composting without worms. 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).

4. Conclusion
In the present work, humification process was found to have significantly alerted in the final vermicompost obtained from all the vermicomposting treatments over natural compost. The increased level of humic material i.e. humic acid (HA) and humification index (HI) and reduction of fulvic acid (FA) and humic carbon (HC) content was observed in VCPT2, VCPT3, VCPT6 and VCPT7 treatments than other treatments and the similar treatment without *P. ceylanensis* could be due to the higher nutrient and microbial concentration in the initial substrate and multiplication of microbes while passing through the gut of *P. ceylanensis*. Hence it was concluded that mixing of RH and SPM as bulking agent in right quantity of PD creates suitable medium for better humification process during vermicomposting.

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