



## EFFECTS OF NITROGEN RICH ORGANIC WASTE MATERIALS ON THE ORGANIC HUMIFICATION PROCESS DURING BIODEGRADATION

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### ABSTRACT

Nitrogenous waste, poultry droppings is generated in huge amounts and cause serious hazards to the environment. In this experiment, poultry droppings (PD) amended with sugar cane pressmud (PM) and cow dung (CD) were used as substrate for exotic earthworm species *Eudrilus eugeniae* and *Eisenia fetida* to stabilize and standardize the recycling the nutrients on the basis of changes in humification process during experimentation. The humification process during worm unworked composting and vermicomposting was carried out and observed results were suggested that total humification process in the vermicompost produced by both species of earthworms *E. eugeniae* and *E. fetida* were significantly enhanced than initial substrate and worm unworked natural compost. Though, among the different treatments VT1, VT2 and VT3 treatments for *E. eugeniae* and VT6, VT7 and VT8 for *E. fetida* treatments showed significantly ( $p < 0.05$ ) higher level of total humic acid content (HA) and humification index (HI) and reduction of total fulvic acid (FA) and humic carbon content (HC) than other treatments of both worms and treatments without earthworms (WW11 to WW15).

### KEY WORDS

Organic waste, Poultry droppings, Earthworms, Humification, Vermicompost.

### INTRODUCTION

Composting using epigeic earthworms is an appropriate technology to manage different types of organic solid wastes material and produce nutrient rich organic fertilizer from it. However, humification parameter is one of the excellent indicators of degradation process of organic waste material during vermicomposting process [1]. India is one of the largest producers of poultry in the world and the nitrogen rich poultry manure availability is estimated to be 12.1 million tons and, in the poultry, industry produces huge amount of droppings that accumulated in the litter turns it into significance sources odorous gases including amines, amides, mercaptans, and disulphides. These noxious gases can cause several diseases especially respiratory disease in animals and humans [2, 3]. Nevertheless, poultry

droppings along with litter have useful nutrients, and are consequently used as organic fertilizer for agronomic purpose [4]. However, uncontrolled and excess applications of poultry droppings to agricultural field can cause environmental problems due to their tremendously high levels of nitrogen as ammonia, low level of pH, and heat generation [5]. Therefore, successful degradation of poultry droppings to be studied by vermicomposting technology using earthworm's *E. eugeniae* and *E. fetida* has been inevitable. Similarly, sugar industries with 400 sugar mills rank as the second major agro industry in the country. The cane-sugar manufacturing has a number of co-products of immense potential worth and the co-products include pressmud and molasses. Out of which pressmud is produced during clarification of sugarcane

juice and 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 processing [5, 6]. Nevertheless, pressmud is directly practical to soil as fertilizer; the fawn present might deteriorate the physical properties such as permeability, aeration, soil texture and composition and with the passage of time the deterioration might get worsen [5].

In this study, the main focal point is to use one of the sugar industries by products i.e. pressmud (excellent food stuff for earthworms when mixed with cow dung), which is converted in to vermicompost mixed with poultry droppings using exotic epigeic earthworms [7]. Cow dung enhances the microbial populations and activity in the initial decomposition process during vermicomposting and also enhances the growth and reproductions of earthworms [8, 9]. Therefore, the present study emphasizes the enhancement of humification process during vermicomposting of poultry droppings amended with cow dung and sugar cane pressmud in different proportions using the exotic earthworm species *E. eugeniae* and *E. fetida* to produce nutrient rich fertilizer. The exotic earthworm species *E. eugeniae* and *E. fetida* was used in this study in view of the fact that it can tolerate wide range of pH, moderate temperature and moisture level [10]. In addition, the earthworm casts contain calcium that buffers the pH level of the substrate material and facilitates rapid degradation of organic waste materials [11, 12]. Several earthworms can also eliminate the destructive pathogens through devouring them and also with discharge of antibacterial coelomic fluid and heavy metals (by bio-accumulation) [13]. Therefore, the aims of this study were to assess the ability of aforesaid earthworm species used as degrader to efficiently decompose selected organic wastes (poultry droppings with bulking agent cow dung and pressmud) into stabilized nutrient rich product by monitoring the humification parameters (humic acid, fulvic acid, humification index and humic carbon).

## MATERIAL AND METHODS

### Earthworm species and organic waste materials

Ten days old poultry droppings (PD) were collected from Rasi poultry farms, Rasipuram, Namakkal district, Tamil Nadu, India. Fifteen days old sugar industry waste

pressmud (PM) was obtained from effluent treatment plant of E.I.D. Parry sugar factory located at Nellikkuppam, Cuddalore district, Tamil Nadu, India. Ten days old cow dung (CD) was collected from the agricultural dairy farm, Faculty of Agriculture, Annamalai University, Tamil Nadu, India. Exotic epigeic earthworm species *E. eugeniae* and *E. fetida* were cultured and developed outside the laboratory as stock culture on partially degraded cow dung as feed, respectively. For experimental point both earthworm's *E. eugeniae* and *E. fetida* were randomly picked from the stock culture and used for degradation of afore said organic waste in different ratios.

### Experimental design

In the present study, different proportions of selected waste, poultry droppings (PD) with amendment material sugar industry pressmud (PM) and cow dung (CD) were prepared (Table 1). Poultry droppings (PD), sugar industry pressmud (PM) and cow dung (CD) was weighed (dry weight) in the above said treatment description and mixed well with 60 -75% moisture content. The waste mixtures of PD, PM and CD in the above said treatment description were transferred to separate troughs with 40cm diameter × 60cm depth, respectively. Subsequent to transferred in the plastic troughs all the mixture compositions of PD, PM and CD were allowed for ten days of initial natural stabilization. VT1, VT2, VT3, VT4, and VT5 treatments were composed of different proportions of PD, PM and CD with *E. eugeniae* and VT6, VT7, VT8, VT9 and VT10 treatments were composed of different proportions of PD, PM and CD with *E. fetida*. Treatments of WWT11, WWT12, WWT13, WWT14 and WWT15 were composed of different proportions of PD, PM and CD without earthworms (worm unworked – control treatments). The experimental treatments were kept in six replicates in a completely randomized block design and matured aforementioned earthworms were used, with an average weight with a developed clitellum. The troughs were filled with 3kg substrate in above combinations and the experimental troughs were kept under shade and sprinkled with equal quantity of water to ensure that the substrate moisture content was maintained at approximately 60-75%. After the completion of pre-inoculation period of ten days, the mature clitellated earthworm's *E. eugeniae* and *E. fetida* were weighed and introduced in to respective treatment trails [14].

**TABLE 1: The composition of selected waste in different experimental treatments**

Experimental Treatment	Treatment Description	Ratio
<b><i>Eudrilus eugeniae</i></b>		
VT1	Poultry droppings with press mud	1:1
VT2	Poultry droppings with Cow dung	1:1
VT3	Poultry droppings with press mud and Cow dung	1:1:1
VT4	Poultry droppings with press mud	2:1
VT5	Poultry droppings with Cow dung	2:1
<b><i>Eisenia fetida</i></b>		
VT6	Poultry droppings with press mud	1:1
VT7	Poultry droppings with Cow dung	1:1
VT8	Poultry droppings with press mud and Cow dung	1:1:1
VT9	Poultry droppings with press mud	2:1
VT10	Poultry droppings with Cow dung	2:1
<b>Without Worms (Control)</b>		
WWT11	Poultry droppings with press mud	1:1
WWT12	Poultry droppings with Cow dung	1:1
WWT13	Poultry droppings with press mud and Cow dung	1:1:1
WWT14	Poultry droppings with press mud	2:1
WWT15	Poultry droppings with Cow dung	2:1

### Analysis of humus composition

Humus composition was analyzed according to the method described by Kumada [15] with some modifications of Zhang et al., [16]. The humic acid content was extracted by adopting the procedure as described by Schnitzer [17]. Five grams 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 coagulate was separated from 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 HA contents are expressed in mg/5g substrates. All the reported data are the arithmetic means of three 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 DISCUSSION

The humification process (HA, FA, HI, and HC) during worm unworked composting and vermicomposting was carried out and data are given in Tables 2-5. Results evidences that vermicomposting increased humic acid (HA) level while, reduced condensed the fulvic acid (FA) level, which showed the obvious humification development during the vermicomposting of PD, PM

and CD by exotic earthworm species *E. eugeniae* and *E. fetida*. Table 2 and 3 evidences that vermicomposting by *E. eugeniae* and *E. fetida* increased humic acid level while reduced the fulvic acid level, which clearly showed the noticeable enhancement humification process during the vermicomposting. Further, tables 2 and 3 shows that maximum humic acid content was observed in VT1, VT2 and VT3 for *E. eugeniae* and VT6, VT7 and VT8 for *E. fetida* and minimum was recorded in VT4 and VT5 for *E. eugeniae* and VT9 and VT10 for *E. fetida* and all the worm unworked composting treatments (WWT11 – WWT5). Whereas, reduced the fulvic acid level during vermicomposting period respectively, which showed that obvious humification process during the process of vermicomposting than composting without earthworms (WWT11 – WWT5). The higher HA content of the treatment during the vermicomposting period may be attributed to the higher content of readily available organic matter from PM which could be easily decomposed at that time, resulting in higher rate of HA formation. Additionally, fiber-structure of amendment material PM components such as lignin, which are known to provide more stable phenolic compounds required as starting material for humification processes [18, 19, 20]. HA were generated from other forms of humic substances, such as FA. The quantities of HA contents observed in this study were similar to the levels reported by Xiong *et al.* [21]. 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 [22]. Further, 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. The bio-oxidation of these compounds resulted in the production of substances with more stable structures in mature vermicompost [23, 24]. Interestingly, in all the treatments of *E. eugeniae* and *E. fetida* the humification index recorded was greater than one percent after vermicomposting, at the end, vermicompost obtained after 50 day could be considered mature (stabilized material). The contents of humiccarbon (HC) declined in all the treatments for both species of worms (*E. eugeniae* and *E. fetida*) (Table 4 and 5). In the present experiment, contents of humiccarbon (HC) decreased probably due to the dramatic decrease of fulvic acid (FA) in all the

treatments during degradation of PD with amendment PM and CD by *E. eugeniae* and *E. fetida*. During the humification process, 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 [25]. The FA also condensed to HA during mineralization of waste material, resulting in a sharp increase in HA [23]. Therefore, earthworms fragment the organic substrates, stimulate microbial activities greatly and increase rates of mineralization, rapidly converting the wastes into humus-like substances [18]. In view of the fact that 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 may be varied depending on the earthworm species inoculated to the organic substrates. Results suggested that inoculation of *E. eugeniae* and *E. fetida* in the initial organic substrates during vermicomposting was most effective in terms of increasing the humic acids content of final vermicompost.

**TABLE 2: Humic acid (HA) parameters of vermicompost and composts produced from different treatments**

Treatments	HA (%)		
	Days		
	25	50	75
<b><i>E. eugeniae</i></b>			
VT1	1.64 ± 0.5 <sup>ab</sup>	3.88 ± 0.2 <sup>b</sup>	3.26 ± 0.4 <sup>bc</sup>
VT2	1.96 ± 0.4 <sup>b</sup>	3.91 ± 0.3 <sup>bc</sup>	3.27 ± 0.4 <sup>b</sup>
VT3	2.02 ± 0.3 <sup>b</sup>	3.95 ± 0.2 <sup>bc</sup>	3.35 ± 0.2 <sup>b</sup>
VT4	1.77 ± 0.3 <sup>ab</sup>	3.53 ± 0.4 <sup>ab</sup>	2.27 ± 0.3 <sup>a</sup>
VT5	1.36 ± 0.5 <sup>a</sup>	3.12 ± 0.3 <sup>a</sup>	2.62 ± 0.4 <sup>a</sup>
<b><i>E. fetida</i></b>			
VT6	1.65 ± 0.2 <sup>b</sup>	3.85 ± 0.3 <sup>c</sup>	3.28 ± 0.3 <sup>b</sup>
VT7	1.94 ± 0.3 <sup>c</sup>	3.88 ± 0.2 <sup>c</sup>	3.25 ± 0.4 <sup>b</sup>
VT8	2.00 ± 0.4 <sup>c</sup>	3.93 ± 0.4 <sup>c</sup>	3.29 ± 0.2 <sup>b</sup>
VT9	1.37 ± 0.3 <sup>a</sup>	3.09 ± 0.2 <sup>a</sup>	2.60 ± 0.3 <sup>a</sup>
VT10	1.76 ± 0.5 <sup>b</sup>	3.51 ± 0.2 <sup>b</sup>	3.25 ± 0.5 <sup>b</sup>
<b>Without Worms</b>			
WWT11	1.30 ± 0.3 <sup>b</sup>	3.05 ± 0.1 <sup>bc</sup>	2.50 ± 0.3 <sup>a</sup>
WWT12	1.29 ± 0.3 <sup>b</sup>	3.08 ± 0.3 <sup>c</sup>	2.51 ± 0.3 <sup>a</sup>
WWT13	1.30 ± 0.4 <sup>b</sup>	3.17 ± 0.2 <sup>c</sup>	2.58 ± 0.4 <sup>a</sup>
WWT14	1.15 ± 0.4 <sup>a</sup>	2.55 ± 0.4 <sup>a</sup>	2.47 ± 0.2 <sup>a</sup>
WWT15	1.21 ± 0.3 <sup>ab</sup>	2.90 ± 0.3 <sup>ab</sup>	2.50 ± 0.3 <sup>a</sup>

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

Treatments	FA (%)		
	Days		

	25	50	75
<b><i>E. eugeniae</i></b>			
VT1	5.44 ± 0.4 <sup>bc</sup>	1.24 ± 0.3 <sup>a</sup>	1.17 ± 0.2 <sup>a</sup>
VT2	5.36 ± 0.6 <sup>bc</sup>	2.13 ± 0.5 <sup>b</sup>	1.91 ± 0.5 <sup>b</sup>
VT3	5.50 ± 0.5 <sup>bc</sup>	1.21 ± 0.4 <sup>a</sup>	1.19 ± 0.9 <sup>a</sup>
VT4	4.90 ± 0.1 <sup>a</sup>	2.11 ± 0.4 <sup>a</sup>	1.98 ± 0.4 <sup>b</sup>
VT5	5.21 ± 0.5 <sup>b</sup>	1.25 ± 0.7 <sup>a</sup>	1.16 ± 0.3 <sup>a</sup>
<b><i>E. fetida</i></b>			
VT6	5.44 ± 0.6 <sup>b</sup>	1.20 ± 0.4 <sup>a</sup>	1.17 ± 0.5 <sup>a</sup>
VT7	5.36 ± 0.2 <sup>b</sup>	2.19 ± 0.5 <sup>b</sup>	1.91 ± 0.4 <sup>b</sup>
VT8	5.50 ± 0.3 <sup>b</sup>	1.21 ± 0.3 <sup>a</sup>	1.19 ± 0.6 <sup>a</sup>
VT9	5.21 ± 0.5 <sup>b</sup>	1.27 ± 0.3 <sup>a</sup>	1.15 ± 0.5 <sup>a</sup>
VT10	4.52 ± 0.3 <sup>a</sup>	2.18 ± 0.5 <sup>b</sup>	2.05 ± 0.3 <sup>b</sup>
<b>Without Worms</b>			
WWT11	4.27 ± 0.3 <sup>b</sup>	2.15 ± 0.2 <sup>a</sup>	2.07 ± 0.4 <sup>a</sup>
WWT12	4.25 ± 0.2 <sup>a</sup>	2.13 ± 0.3 <sup>a</sup>	2.08 ± 0.5 <sup>a</sup>
WWT13	4.20 ± 0.4 <sup>b</sup>	2.10 ± 0.2 <sup>a</sup>	2.03 ± 0.2 <sup>a</sup>
WWT14	3.42 ± 0.3 <sup>a</sup>	3.04 ± 0.3 <sup>b</sup>	2.78 ± 0.2 <sup>a</sup>
WWT15	4.31 ± 0.2 <sup>b</sup>	2.15 ± 0.2 <sup>a</sup>	1.89 ± 0.4 <sup>a</sup>

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

**TABLE 4: Changes in the Humification index (HI) of vermicompost and composts produced from different treatments**

Treatments	HI		
	Days		
	25	50	75
<b><i>E. eugeniae</i></b>			
VT1	0.28 ± 0.5 <sup>a</sup>	2.68 ± 0.2 <sup>b</sup>	2.57 ± 0.4 <sup>b</sup>
VT2	0.29 ± 0.4 <sup>a</sup>	2.63 ± 0.4 <sup>b</sup>	2.50 ± 0.5 <sup>b</sup>
VT3	0.23 ± 0.3 <sup>a</sup>	1.37 ± 0.5 <sup>a</sup>	2.34 ± 0.3 <sup>a</sup>
VT4	0.37 ± 0.4 <sup>a</sup>	3.13 ± 0.4 <sup>c</sup>	2.59 ± 0.5 <sup>ab</sup>
VT5	0.29 ± 0.5 <sup>a</sup>	2.98 ± 0.5 <sup>b</sup>	2.65 ± 0.4 <sup>ab</sup>
<b><i>E. fetida</i></b>			
VT6	0.28 ± 0.3 <sup>a</sup>	2.67 ± 0.4 <sup>b</sup>	2.57 ± 0.3 <sup>b</sup>
VT7	0.28 ± 0.4 <sup>a</sup>	3.04 ± 0.3 <sup>c</sup>	2.60 ± 0.4 <sup>b</sup>
VT8	0.23 ± 0.5 <sup>a</sup>	1.37 ± 0.3 <sup>a</sup>	1.34 ± 0.5 <sup>a</sup>
VT9	0.29 ± 0.4 <sup>a</sup>	2.92 ± 0.5 <sup>b</sup>	2.68 ± 0.4 <sup>b</sup>
VT10	0.36 ± 0.6 <sup>a</sup>	3.16 ± 0.3 <sup>c</sup>	2.85 ± 0.3 <sup>bc</sup>
<b>Without Worms</b>			
WWT11	0.94 ± 0.4 <sup>a</sup>	1.30 ± 0.3 <sup>ab</sup>	1.21 ± 0.5 <sup>ab</sup>
WWT12	0.94 ± 0.3 <sup>a</sup>	1.28 ± 0.4 <sup>ab</sup>	1.20 ± 0.3 <sup>ab</sup>
WWT13	0.96 ± 0.5 <sup>a</sup>	1.29 ± 0.5 <sup>ab</sup>	1.20 ± 0.4 <sup>ab</sup>
WWT14	1.04 ± 0.2 <sup>a</sup>	1.09 ± 0.3 <sup>a</sup>	1.02 ± 0.2 <sup>a</sup>
WWT15	1.05 ± 0.3 <sup>a</sup>	1.18 ± 0.2 <sup>a</sup>	1.15 ± 0.3 <sup>a</sup>

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

**TABLE 5: Changes in the Humic carbon (HC) properties of vermicompost and composts produced from different treatments**

Treatments	HC (%)		
	Days		
	25	50	75
<b><i>E. eugeniae</i></b>			
VT1	7.08 ± 0.7 <sup>ab</sup>	5.42 ± 0.3 <sup>b</sup>	5.20 ± 0.6 <sup>ab</sup>
VT2	7.32 ± 0.9 <sup>b</sup>	6.04 ± 0.5 <sup>c</sup>	5.18 ± 0.9 <sup>b</sup>
VT3	7.52 ± 0.6 <sup>b</sup>	5.16 ± 0.6 <sup>b</sup>	4.54 ± 0.4 <sup>ab</sup>
VT4	6.67 ± 0.4 <sup>a</sup>	5.12 ± 0.6 <sup>b</sup>	4.29 ± 0.5 <sup>c</sup>
VT5	6.57 ± 0.5 <sup>a</sup>	4.32 ± 0.2 <sup>a</sup>	3.78 ± 0.4 <sup>a</sup>
<b><i>E. fetida</i></b>			
VT6	7.09 ± 0.4 <sup>ab</sup>	5.09 ± 0.4 <sup>ab</sup>	4.45 ± 0.4 <sup>b</sup>
VT7	7.05 ± 0.3 <sup>ab</sup>	5.60 ± 0.3 <sup>b</sup>	5.16 ± 0.4 <sup>c</sup>
VT8	7.50 ± 0.3 <sup>b</sup>	6.01 ± 0.4 <sup>c</sup>	4.48 ± 0.4 <sup>b</sup>
VT9	6.58 ± 0.4 <sup>a</sup>	4.30 ± 0.4 <sup>a</sup>	3.75 ± 0.4 <sup>a</sup>
VT10	6.28 ± 0.4 <sup>a</sup>	5.69 ± 0.3 <sup>b</sup>	5.30 ± 0.4 <sup>a</sup>
<b>Without Worms</b>			
WWT11	5.57 ± 0.6 <sup>b</sup>	5.32 ± 0.6 <sup>bc</sup>	4.65 ± 0.6 <sup>ab</sup>
WWT12	5.54 ± 0.2 <sup>b</sup>	5.21 ± 0.5 <sup>b</sup>	4.59 ± 0.5 <sup>ab</sup>
WWT13	5.50 ± 0.6 <sup>b</sup>	5.15 ± 0.3 <sup>b</sup>	4.53 ± 0.6 <sup>ab</sup>
WWT14	4.73 ± 0.6 <sup>a</sup>	4.94 ± 0.5 <sup>ab</sup>	4.28 ± 0.3 <sup>a</sup>
WWT15	5.46 ± 0.4 <sup>b</sup>	4.70 ± 0.3 <sup>a</sup>	4.36 ± 0.8 <sup>a</sup>

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

## CONCLUSION

Therefore, it was concluded that the possibility of PD amended with bulking agent PM and CD waste decomposition by *E. eugeniae* and *E. fetida* has been evaluated in order to rapid degradation and to produce quality vermicompost with higher agronomic value. The degradation of the waste materials was enhanced, as indicated by reduction in humic carbon in the presence of earthworms than natural worm unworked composting. Our results established that after the adding of PM and CD in appropriate quantities to the PD, it can be used as a raw material in the vermicomposting using *E. eugeniae* and *E. fetida* for nutrients recovery for organic forming.

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