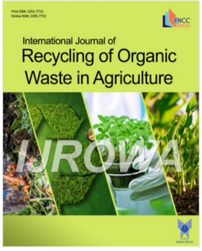

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Vermicomposting of coir pith and cow manure: Influence of initial total phenolic content on earthworms' performance

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Original Research

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Abstract:

Purpose: Vermicomposting of phenolics-rich lignocellulosic materials takes a long time to reach maturity, while worm mortality and weight loss are often encountered. Phenolic compounds have antimicrobial properties which may affect the vermicomposting process. The present study aims at investigating the effect of the initial total phenolic content (TPC) on coir pith vermicomposting.
Method: The earthworm activities and performance during vermicomposting of coir pith and cow manure containing 4.8 (T_0), 6.4 (T_1), 7.2 (T_2), 8.8 (T_3), 10.3 (T_4) and 12.7 (T_5) mg initial TPC g^{-1} substrate using *Eudrilus eugeniae* were investigated.
Results: Total carbon loss increased as initial TPC increased and only T_0 and T_1 reached maturity. Earthworm mortality and weight loss increased as initial TPC of materials increased in a concentration dependent manner. High performance liquid chromatography (HPLC) results showed that the total numbers of water-soluble phenolics of coir pith and cow manure were, respectively, 17 and 16 out of which 5 and 4 were unique to coir pith and cow manure, respectively. By the end of vermicomposting, almost all of phenolics present in cow manure disappeared while those in coir pith remained intact, albeit, reduced in concentrations. 4-hydroxybenzoic acid and *p*-coumaric acid were detected in all treatments. Disappearance of 4.90 and 6.35 minute-peaks was observed with T_0, T_1 and T_2 whilst a new phenolic (5.30 minute-peak) was detected only in T_1 indicating the better degradation of phenolic compounds.
Conclusion: Results indicated a significant role of initial TPC on earthworm activities during coir pith vermicomposting.

Keywords: Coir pith; Earthworm; *Eudrilus eugeniae*; Initial total phenolic content; Mortality

1. Introduction

Vermicomposting process consists primarily of two subprocesses, namely, earthworm gut-associated process and cast-associated process (Domínguez, 2004) whereby organic wastes are biodegraded into stable humic-like substances, vermicompost, by taking advantage of an interaction between earthworms and microorganisms (Saha et al., 2012). As a result of physical and biochemical modification taking place during the process, the resultant vermicompost is of higher qualities in terms of macro/micronutrients, plant growth hormones, enzymes, and vitamins, including diversity and richness of lignocellulose degrading microorganisms (Prabhu et al., 1998; Tognetti et al., 2005) which are thus capable of sustaining better plant growth.

Earthworms have been classified into three ecological groups which are epigeic, endogeic, and anecic based on their feeding and burrowing behaviors. Generally, an epigeic species is more suitable for vermicomposting due to its high consumption rate of organic matter, ability to tolerate a wide range of environmental factors, and high reproduction rate (Domínguez, 2004). *Eudrilus eugeniae*, one of the tropical earthworm species widely used in commercial vermiculture (Giraddi, 2008), consumes various kinds of agricultural, urban, and industrial wastes such as olive oil waste, sugarcane waste, oil palm fiber waste including coir pith (Jeyabal and Kuppuswamy, 2001; Nattudurai et al., 2014). *E. eugeniae* per se could produce several enzymes, e.g., protease, cellulase, dehydrogenase (Ravindran et al., 2015), chitinase, and cellobiose (Kadam and Pathade, 2017). Additionally, the activities of enzymes produced by *E. eugeniae*(gut wall tissues) were significantly lower than those of the whole gut content (with the presence of microorganisms capable of producing enzymes). Earthworms involve primarily in increasing in surface to volume ratio of starting organic materials and, at the same time, secrete mucus, enzymes, and casts that are rich in available nutrients for further microbial degradation (Zhao et al., 2010).

Coir pith, a byproduct from coir fiber production via the mechanical defibering process of either retted or unretted coconut husks (Ravindranath, 1999), contains 23 − 43% cellulose, 3 − 12% hemicellulose, 37 − 54% lignin, 2.5% TPC, and 75 − 156% C/N ratio (Abad et al., 2002; Shashirekha and Rajarathnam, 2007) rendering it recalcitrant to degradation. In Thailand, coir fiber is produced from unretted coconut husk; therefore, the phenolic content of coir pith remains intact. Although a small portion of coir pith finds its application in farming and particle board making, large quantities are discarded inattentively as waste resulting in environmental problems. For efficient management, attempts have been made to transform coir pith into organic fertilizer, employing different composting and vermicomposting techniques. However, due to its lignocellulosic nature, vermicomposting of coir pith took a long time. Jeyabal and Kuppuswamy (2001) reported that vermicomposting of coir pith-based feedstock with cow manure, biodigested slurry or sugarcane pressmud at 50:50 (w/w), using *E. eugeniae*, took 85 days to reach maturity whereas the weed-based materials took only 55 days. Viji and Neelanarayanan (2014) further reported that the vermicomposting of predigested-coir pith (42 days) and cow manure at 50:50 (w/w), by *E. eugeniae*, took 63 days to reach maturity. Patil et al. (2017) showed that vermicomposting of partially decomposed coir waste (80%) with cow manure (20%) took 60 days to maturation. Generally, the long degradation period of vermicomposting of ligno-cellulosic materials was attributed to the high lignin content and the preference of earthworms for high N feed but low C/N, C/P, lignin, polyphenolic/N, and condensed tannins feed (Hendriksen, 1990; Kasurinen et al., 2007; Rief et al., 2012).

In addition, high mortality of earthworms was also encountered during vermicomposting of phenolics-rich feed-stock. Ganesh et al. (2009) reported the high mortality rate and weight loss of earthworms during vermicomposting of pre-composted acacia leaf litter containing polyphenol (12.5%) and lignin (14.7%). Chittavanij (2011) reported that when both *E. eugeniae* and *Pheretima peguana* were cultured in coir pith mixed with cow manure and soil (1:1:2 ratio) mortalities of both worms were observed continually from day 0, day 21, and day 42 of cultivation. Moreover, vermicomposting of coir pith-based feedstocks resulted in a smaller number of cocoons and juveniles of earthworms (Murali and Neelanarayanan, 2011; Viji and Neelanarayanan, 2014). Tahir and Hamid (2012) also found significant weight loss and mortality during vermicomposting of coconut husk with goat manure but attributed to the acidic pH of the mixture. Sabrina et al. (2009), reported that during vermicomposting of oil palm empty fruit bunch (EFB) and oil palm frond together with cow dung, *Pontoscolex corethrurus* died immediately whereas only *Amynthas rodoricensis* survived in frond and, in 2012, found that after applying composted EFB, containing 10% phenolics, to an oil palm plantation, approximately 20% mortality of native earthworms was observed. Further, Sabrina et al. (2009) found that the addition of cow manure and 1-month-old pre-composted EFB could reduce of mortality rate and, at the same time, sustain the growth of earthworms.

During vermicomposting of EFB, it was anticipated that phenolic compounds present in EFB were responsible for earthworms' mortality (Sabrina et al., 2012). Moreover, it is well known that phenolic compounds have antimicrobial activities. Polyphenols-rich pine bark extract showed high antimicrobial activity against several microorganisms such as *Candida* spp. and *Saccharomyces cerevisiae* (Romani et al., 2006). Phenolic compounds, such as gallic acid, were active against *Alternaria* spp., *Botrytis cinerea* and *Penicillium digitatum* (Lattanzio et al., 1996). Several studies have shown that tannin and phenolic compounds released during coir pith degradation (Prabha et al., 2009) and/or extracted from plants exhibited antimicrobial activities against bacteria, such as *Listeria monocytogenes*, *Bacillus coagulans*, *Escherichia coli*, *Shigella flexneri*, *Salmonella Typhi*, *Klebsiella pneumoniae*, *Citrobacter koseri* and *Staphylococcus aureus* (Sung et al., 2012; Manandhar et al., 2019), fungi, such as *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium expansum*, *Fusarium moniliforme*, *Trichophyton mentagrophytes* (Okoi et al., 2013; Kebede et al., 2021) and yeast, such as *Candida albicans*, *Candida zeylanoide*, *Cryptococcus laurentii* and *Filobasidiella neoformans* (Romani et al., 2006; Kebede et al., 2021). Further, phenolic compounds could precipitate proteins via several mechanisms such as covalent bonds, hydro-phobic interactions and hydrogen bonds (Yilmaz et al., 2022). Therefore, the precipitation of extracellular enzymes produced by earthworms and microorganisms may also attribute to the mortality and weight loss of earthworms cultivated in phenolics-rich substrates.

Regardless of the difficulty encountered during vermicomposting of high TPC containing lignocellulosic wastes, information regarding their possible role is limited. It is possible that earthworm activity and vermicomposting performance are hampered by phenolic compounds; hence, insight into the possible role of these TPC present in feed material is crucial. The present study aims to investigate the influence of TPC present in feedstock, e.g., coir pith and cow manure, on vermicomposting performance, physico-chemical change and bio-logical change using *E. eugeniae* and to assess their effects on the survival and growth of earthworms.

2. Materials and method

Materials

Materials employed for vermicomposting in this study were coir pith, cow manure, and *E. eugeniae* earthworms. Coir pith was collected from a coconut processing factory in Bangkok, Thailand whereas the cow manure was purchased from a local worm breeder in Samut Sakhon province, Thailand. Prior to vermicomposting, cow manure was soaked in water for three cycles of 24 hours each to remove excess ammonia which is reportedly toxic to earthworms (Domínguez, 2004). The physical and chemical properties of both coir pith and cow manure are provided in Table 1. *E. eugeniae*, commonly known as African night crawler, was purchased from a local worm breeder in Samut Sakhon province, Thailand. They were maintained by culturing in plastic trays containing cow manure at room temperature. Moisture content was kept at $70-80\%$ using tap water every week. Adult earthworms with a weight of $0.9-1.2$ g were used in this study.

Experimental setup

The experiments were conducted in $30 \times 50 \times 17$ cm (width$\times$length$\times$height) plastic containers using coir pith mixed with cow manure as substrates. Five different treatments of coir pith and cow manure mixtures were prepared in such a way that the initial TPC was 6.4, 7.2, 8.8, 10.3 and 12.7 mg g^{-1} sample, and designated, respectively, as T_1, T_2, T_3, T_4, and T_5. In other words, the coir pith to cow manure ratios employed for T_1, T_2, T_3, T_4, and T_5 were, respectively, 20:80, 30:70, 50:50, 70:30 and 100:0 (w/w). Cow manure only (containing 4.80 mg TPC g^{-1} sample) was also included as control and designated as T_0. Characteristics of initial feed materials are shown in Table 1. Fifty earthworms were placed in each plastic container and supplied with 0.9 kg-feed kg-worm^{-1} day^{-1} of feed materials as earthworms can consume feed materials equivalent to their own weight a day (Riggle and Holmes, 1994). Moisture content was adjusted and maintained daily at $70-80\%$ for the whole period of vermicomposting. All treatments were performed in duplicate. Samples were taken periodically every 7 days for physical and chemical analysis, vermicast collection and microbial population enumeration. All experiments were performed in triplicate.

Analytical method

Earthworm activity

The number and weight of earthworms of all treatments were hand-sorted, weighed, and recorded once a week.

Physical and chemical properties

Changes in the color of materials of all treatments were measured using a Chroma meter (Konica Minolta Chroma meter CR-400, Japan). Based on the Munsell color system, the color dimensions were separated into three dimensions: hue, value (lightness), and chroma (purity). Hue is a color that can be represented by a letter code, such as red (R), green (G), blue (B), yellow-red (YR), green (GY), and so on. Value, which ranges from 0 (representing black) to 10 (representing white), quantifies how light or dark a color is so that a value of 2 will appear darker

Table 1. Physical and chemical characteristics of coir pith, cow manure and treatments employed in this study.

Parameter	T_0	T_1	T_2	T_3	T_4	T_5
pH	8.03 ± 0.15^{a}	7.68 ± 0.03^{b}	7.40 ± 0.08^{c}	6.72 ± 0.01^{d}	6.07 ± 0.13^{e}	5.55 ± 0.08^{f}
EC (dS m^{-1})	0.70 ± 0.19^{a}	0.95 ± 0.07^{a}	1.06 ± 0.21^{ab}	1.13 ± 0.02^{b}	1.20 ± 0.00^{b}	1.31 ± 0.30^{b}
Ammonia (mg g^{-1})	3.26 ± 0.70^{a}	2.85 ± 1.24^{a}	2.65 ± 0.66^{a}	2.24 ± 0.35^{ab}	1.83 ± 0.68^{ab}	1.21 ± 0.47^{b}
TC (% dry wt.)	36.86 ± 0.75^{e}	43.35 ± 0.63^{d}	45.04 ± 0.28^{c}	48.30 ± 1.69^{b}	50.42 ± 0.67^{b}	52.54 ± 0.66^{a}
TKN (% dry wt.)	2.01 ± 0.23^{a}	1.78 ± 0.09^{ab}	1.44 ± 0.19^{b}	1.24 ± 0.20^{bc}	1.10 ± 0.12^{c}	0.93 ± 0.03^{c}
C/N ratio	18.38 ± 2.43^{f}	24.33 ± 0.93^{e}	31.46 ± 2.92^{d}	39.31 ± 3.58^{c}	46.23 ± 4.48^{b}	56.31 ± 2.32^{a}
TPC (mg g^{-1})	4.80 ± 0.13^{f}	6.38 ± 0.23^{e}	7.17 ± 0.17^{d}	8.75 ± 0.30^{c}	10.33 ± 0.35^{b}	12.70 ± 0.42^{a}

Means ($\pm$SD) in the same row with different letters are significantly different from each other ($p < 0.05$). T_0, T_1, T_2, T_3, T_4, and T_5 represent, respectively, treatments containing coir pith and cow manure at 0:100, 20:80, 30:70, 50:50, 70:30 and 100:0 (w/w) ratios.

than a value of 6. Chroma, which ranges from dull (low chroma, typically 2 or lower) to intense (high chroma), is a numerical representation of a color intensity. The sample was diluted with distilled water (1:10 w/v) for determination of pH and EC using a pH meter (pH consort C830, Belgium) and a conductivity meter (Jenway 4310, Bibby Scientific Limited, UK), respectively. Moisture content and organic matter (OM) of the sample were measured by drying at 105° C for 24 hours and incinerated at 550° C for 5 hours, respectively (AOAC, 1995). Total carbon (TC) was calculated according to the equation ($TC = OM/1.8$) as suggested by Jiménez and García (1992). Total Kjeldahl nitrogen (TKN) and ammonia concentration of all treatments were determined using Kjeldahl method (AOAC, 1995). TPC was quantified using Folin-Ciocalteu method (Makkar, 2003). Samples were ground and sieved through a 0.5 mm mesh screen. Ground samples (1 g) were separately added to a round-bottomed flask, containing 100 mL water, fitted with a Soxhlet extractor. The whole setup was digested in a water bath at 100° C for 60 minutes. The supernatants were collected and kept on ice. 50 μL of supernatants were transferred into separate test tubes; then, distilled water was added to make up the volume to 500 μL. Subsequently, 250 μL of 1 N Folin-Ciocalteu reagent and 1.25 mL of 20% sodium carbonate solution were introduced. The absorbance was read at 725 nm wavelength using a UV-Vis spectrophotometer (Thermo scientific, Helios Omega, USA) after incubation for 40 minutes. TPC present in samples was obtained by comparing the measured absorbance against the standard curve previously prepared with tannic acid (Himedia, India).

Microbial properties

To collect vermicast for microbial population analysis, earthworms from each treatment were held on wet filter paper at room temperature for 24 hours in the dark to have the gut contents purged. The fungal, bacterial, and actinomycete populations of vermicast were enumerated by dilution plate count technique (Johnson and Case, 1989). One gram of vermicast (fresh weight) was serially diluted in sterilized water and 100 μL of samples, subsequently, were spread over the surface of agar, namely, nutrient agar, starch casein agar, and potato dextrose agar for enumerating bacteria, actinomycetes, and fungi, respectively. The plates of each sample were incubated at optimal temperature (30° C) for 3 days. The microbial colonies after incubation were counted as Colony Forming Unit (CFU g^{-1} dry weight of sample). Experiments were performed in triplicate.

Qualitative analysis of phenolic compounds of coir pith vermicompost

One hundred mg of samples were extracted using 5 mL of deionized water by homogenization for 30 minutes. The mixtures were then centrifuged at 2500 rpm for 10 minutes at 4° C. The supernatants were collected and analyzed using reverse-phase high performance liquid chromatography (RP-HPLC). These analyses were performed on a Waters e2695 separations module HPLC system equipped with Waters 2998 photodiode array (PDA) detector. The column used was a reversed-phase Symmetry C18 (Waters) (5 μm packing, 250×4.6 mm). The eluents were 1% acetic acid (A) and CH_3CN (B). A multi-step linear solvent gradient was used, starting from 5 to 80% B, over a 35-minute period, at a flow rate of 1 mL min^{-1}. Phenolic compounds were identified according to the retention time of 280 nm wavelength chromatogram against standards, including gallic acid, gallocatechin, epigallocatechin, catechin, resorcinol, epicatechin, 4-hydroxybenzoic acid, catechol, ellagic acid, *p*-coumaric acid, guaiacol, quercetin, and kaempferol.

Statistical analysis

Analysis of variance (ANOVA), Fisher's least significant difference (LSD) test, at the confidence level of 95%, was conducted using Minitab 18 (Minitab Inc., United States).

Table 2. Physical changes during coir pith vermicomposting.

Treatment	Time	T_0	T_1	T_2	T_3	T_4	T_5
Texture	Day 0	coarse/ compact	coarse/ compact	coarse/ compact	coarse/ compact	coarse/ compact	coir pith (coarse/ porous)
	Day 35	soft/loose	soft/loose	soft/loose	soft/loose	soft/loose	coir pith (coarse/ porous)
Texture	Day 0	8.6YR, 2.9/0.9	5.4YR, 2.9/0.6	4.2YR, 2.9/0.8	3.2YR, 3.1/1.3	2.5YR, 3.1/1.6	2.2YR, 3.2/1.9
	Day 35	8.0YR, 2.9/0.8	5.8YR, 2.7/0.7	4.5YR, 2.8/0.7	3.2YR, 3.0/1.1	2.7YR, 2.7/1.0	2.1YR, 2.9/1.4
Weight reduction (% DW)	Day 35	42.5 ± 2.6	41.6 ± 2.8	43.5 ± 4.8	24.3 ± 2.6	16.9 ± 2.5	3.02 ± 0.8

Munsell color system: hue; value/chroma

3. Results and discussion

Physicochemical changes during vermicomposting process

Texture, color and weight loss

In the present study, physical changes in terms of texture, color, and weight loss were observed during the vermicomposting process (Table 2). On day 0, T_5 containing 12.7 mg g^{-1} of initial TPC was of very dark reddish-grey color (2.2YR, 3.2/1.9) which is characteristic of coir pith, whereas the rest (T_0, T_1, T_2, T_3 and T_4) were of yellow to light reddish color (2.5YR, 3.1/1.6 - 8.6YR, 2.9/0.9). At the end of vermicomposting (day 35), major changes in color (hue, value and chroma) and texture were observed in T_0, T_1 and T_2 (containing low initial TPC at 4.80, 6.38 and 7.17 mg g^{-1}, respectively) providing that color of T_0, T_1 and T_2 became brownish black (4.5YR, 2.8/0.7 - 8YR, 2.9/0.8) with a crumbly soil-like texture indicating a greater extent of biodegradation. Similar observation was reported by Karmegam et al. (2021) who vermicomposted coir pith together with *Sesbania sesban* leaves and cow manure. However, for treatment containing high initial TPC (12.7 mg g^{-1}), T_5, it appeared that coir pith simply underwent a diminution process by earthworms as the texture of cast was still that of coir pith (Supplementary Fig. 1) suggesting a lower degree of biodegradation. The weight of T_0, T_1 and T_2 reduced considerably by $41-43\%$ of the initial weight while the lowest weight loss was observed with T_5, approximately 3%. The weight loss was primarily due to microbial respiration and mineralization of organic materials in the form of CO_2, NH_3, and H_2O (Hanc and Chadimova, 2014) as well as earthworm catabolism, which primarily causes CO_2 loss from respiration (Jayakumar et al., 2011). Changes in texture, color, and weight of T_5 between day 35 and day 0 were marginal. It is likely that the activities of both earthworms and microbes were very low due to the presence of high phenolics as well as lignin ($30-50\%$) which is recalcitrant to natural decomposition (Abad et al., 2002). Initial TPC of coir pith appeared to play a significant role in the vermicomposting of coir pith and cow manure in this study.

pH and EC

At the onset of experiment, the initial pH of all treatments decreased with an increase in the initial TPC of substrates, in other words, the amount of coir pith addition. The initial pH of all treatments (Table 3), T_0, T_1, T_2, T_3, T_4, and T_5, were 8.0, 7.7, 7.4, 6.7, 6.1, and 5.6, respectively, which are suitable for growth and reproduction (pH $5-9$) of epigeic earthworms (Domínguez, 2004). During vermicomposting, pH of T_0, T_1, and T_2 decreased continuously to the final values of 7.5, 7.2, and 7.1, respectively, on day 35, while pH of treatments containing moderate and high initial TPC (T_3, T_4, and T_5) increased slightly and reached the values of 6.7, 6.3, and 5.8, respectively. The decline in pH of T_0, T_1, and T_2 could be due to the volatilization of NH_3 (Rupani et al., 2019), CO_2, and organic acids produced as a result of activities of microflora and fauna present in raw materials and earthworms themselves (Biruntha et al., 2020). Consequently, it is apparent that treatments with low initial TPC and neutral pH (T_0, T_1, and T_2) were more susceptible to microbial degradation than treatments with acidic pH and high initial TPC (T_4 and T_5).

Initial EC values of all treatments ranged from $0.70-1.31$ dS m^{-1} at the beginning of vermicomposting (Table 3). During the vermicomposting process, the EC values of T_0, T_1, T_2, and T_3 (0.70, 0.95, 1.06, and 1.13 dS m^{-1}, respectively) significantly increased ($p < 0.05$), reaching the final values of 1.05, 1.16, 1.19, and 1.39 dS m^{-1}, respectively. In contrast, the changes in EC values of T_4 and T_5 were marginal. The shift in EC values during vermicomposting could be explained by the presence of soluble salts released from coir pith by earthworms (Mago et al., 2021) and also microbial degradation of organic substrates (Majlessi et al., 2012; Biruntha et al., 2020; Jayakumar et al., 2022). Results obtained, therefore, also suggested that the decomposition of treatments containing moderate and high initial TPC (T_3, T_4, and T_5) were less than those of T_0, T_1, and T_2 which contained low initial TPC.

Total carbon (TC), Total Kjeldahl Nitrogen (TKN), Ammonia, and C/N ratio

The reduction in TC during vermicomposting indicates the rate of organic matter degradation. Initially, the TC of all treatments varied within the range of $36.9-52.5\%$. It is evident from Table 3 that the extent of TC reduction declined as the initial TPC present in vermicomposting substrates increased. TC of T_0, T_1, T_2, T_3, T_4, and T_5 reduced to 31.2, 40.9, 44.7, 46.7, 49.7, and 52.3%, respectively, after 35 days of vermicomposting. T_0 had the highest TC reduction and TC loss, 15.3 and 35.5%, respectively, following by T_1 (5.5 and 23.0%) and T_2 (5.9 and 24.9%). Slight TC reduction and TC loss were observed when the initial TPC of starting substrate was high, particularly, T_5, approximately 0.5 and 8.4%, respectively, indicating virtually no decomposition as the reduction in TC was attributed to the carbon loss in the form of CO_2 by the combined action of microorganisms and earthworms (Boruah et al., 2019). Therefore, it is apparent that the biodegradation was higher in T_0, T_1, and T_2 which may be due to the lower initial TPC and lignin content of substrates (Table 1). In addition to high initial TPC, T_3, T_4, and T_5 contained also high lignin content which may contribute to low biodegradation. Results corroborated well with that reported by Patil et al. who suggested that the high proportion of lignin in raw materials retarded the decomposition of coir waste during vermicomposting (Patil et al., 2017).

Initially, the TKN of all treatments ranged from $0.93-2.01\%$ (Table 3) and then increased slightly which is in accordance with results reported in the literature (Viji and Neelanarayanan, 2014; Jayakumar et al., 2022) probably because of organic matter mineralization during

Table 3. Changes in chemical parameters during vermicomposting of coir pith.

Parameter	Time (days)	T_0	T_1	T_2	T_3	T_4	T_5
pH	0	$8.00 \pm 0.12^{a,C}$	$7.68 \pm 0.03^{b,A}$	$7.38 \pm 0.08^{c,A}$	$6.72 \pm 0.01^{d,BC}$	$6.05 \pm 0.13^{e,C}$	$5.55 \pm 0.08^{f,D}$
	7	$8.37 \pm 0.02^{a,A}$	$7.58 \pm 0.02^{b,B}$	$7.27 \pm 0.03^{c,B}$	$6.78 \pm 0.10^{d,ABC}$	$6.33 \pm 0.02^{e,B}$	$5.66 \pm 0.01^{f,C}$
	14	$8.12 \pm 0.08^{a,B}$	$7.40 \pm 0.01^{b,C}$	$7.15 \pm 0.01^{c,C}$	$6.84 \pm 0.01^{d,A}$	$6.40 \pm 0.05^{e,AB}$	$5.78 \pm 0.04^{f,B}$
	21	$7.87 \pm 0.08^{a,D}$	$7.36 \pm 0.03^{b,D}$	$7.07 \pm 0.01^{c,D}$	$6.80 \pm 0.06^{d,AB}$	$6.50 \pm 0.06^{e,A}$	$5.76 \pm 0.02^{f,B}$
	28	$7.81 \pm 0.01^{a,D}$	$7.28 \pm 0.03^{b,E}$	$7.08 \pm 0.01^{c,D}$	$6.76 \pm 0.04^{d,ABC}$	$6.42 \pm 0.02^{e,AB}$	$5.89 \pm 0.05^{f,A}$
	35	$7.54 \pm 0.01^{a,E}$	$7.24 \pm 0.01^{b,E}$	$7.09 \pm 0.02^{c,D}$	$6.69 \pm 0.05^{d,C}$	$6.34 \pm 0.04^{e,B}$	$5.77 \pm 0.02^{f,B}$
EC (dS m^{-1})	0	$0.70 \pm 0.19^{d,CD}$	$0.95 \pm 0.07^{c,B}$	$1.06 \pm 0.21^{bc,A}$	$1.13 \pm 0.02^{abc,C}$	$1.20 \pm 0.00^{ab,AB}$	$1.31 \pm 0.30^{a,AB}$
	7	$0.58 \pm 0.09^{c,D}$	$1.06 \pm 0.06^{b,AB}$	$1.10 \pm 0.23^{ab,A}$	$1.21 \pm 0.22^{ab,BC}$	$1.06 \pm 0.10^{b,C}$	$1.25 \pm 0.02^{a,AB}$
	14	$0.76 \pm 0.19^{b,BC}$	$1.10 \pm 0.07^{a,A}$	$1.14 \pm 0.19^{a,A}$	$1.16 \pm 0.11^{a,C}$	$1.22 \pm 0.16^{a,AB}$	$1.21 \pm 0.04^{a,AB}$
	21	$0.90 \pm 0.02^{d,AB}$	$1.13 \pm 0.06^{c,A}$	$1.23 \pm 0.02^{b,A}$	$1.23 \pm 0.05^{b,BC}$	$1.16 \pm 0.02^{c,BC}$	$1.34 \pm 0.02^{a,A}$
	28	$0.85 \pm 0.03^{c,BC}$	$1.10 \pm 0.19^{b,A}$	$1.15 \pm 0.03^{ab,A}$	$1.30 \pm 0.06^{a,AB}$	$1.20 \pm 0.05^{ab,AB}$	$1.17 \pm 0.22^{ab,AB}$
	35	$1.05 \pm 0.18^{d,A}$	$1.16 \pm 0.12^{bcd,A}$	$1.19 \pm 0.14^{bc,A}$	$1.39 \pm 0.06^{a,A}$	$1.27 \pm 0.06^{ab,A}$	$1.12 \pm 0.01^{cd,B}$
TC (%g g^{-1} dry wt.)	0	$36.86 \pm 0.16^{e,A}$	$43.57 \pm 0.16^{d,A}$	$46.33 \pm 2.24^{c,A}$	$48.70 \pm 1.47^{b,A}$	$50.85 \pm 0.95^{a,A}$	$52.54 \pm 0.21^{a,A}$
	7	$35.41 \pm 0.24^{f,B}$	$42.99 \pm 0.05^{e,AB}$	$45.69 \pm 0.68^{d,AB}$	$48.54 \pm 0.34^{c,A}$	$50.33 \pm 0.22^{b,A}$	$51.31 \pm 0.03^{a,CD}$
	14	$34.62 \pm 0.28^{f,C}$	$42.33 \pm 0.22^{e,BC}$	$45.23 \pm 0.34^{d,AB}$	$47.78 \pm 0.23^{c,AB}$	$50.05 \pm 0.55^{b,AB}$	$51.52 \pm 0.21^{a,BCD}$
	21	$32.96 \pm 0.39^{f,D}$	$41.88 \pm 0.43^{e,CD}$	$43.44 \pm 0.42^{d,C}$	$47.83 \pm 1.97^{c,B}$	$49.39 \pm 0.23^{b,B}$	$51.16 \pm 0.31^{a,D}$
	28	$32.86 \pm 0.32^{f,D}$	$41.11 \pm 0.91^{e,DE}$	$43.71 \pm 0.26^{d,C}$	$47.72 \pm 1.50^{c,AB}$	$49.35 \pm 0.25^{b,B}$	$51.87 \pm 0.33^{a,AB}$
	35	$31.23 \pm 0.32^{f,E}$	$40.94 \pm 0.40^{e,E}$	$44.79 \pm 1.09^{d,BC}$	$46.74 \pm 0.13^{c,AB}$	$49.79 \pm 0.49^{b,B}$	$52.30 \pm 0.27^{a,AB}$
TKN (%g g^{-1} dry wt.)	0	$2.01 \pm 0.23^{a,B}$	$1.78 \pm 0.09^{b,B}$	$1.44 \pm 0.19^{c,A}$	$1.24 \pm 0.20^{d,A}$	$1.10 \pm 0.12^{de,A}$	$0.93 \pm 0.03^{e,B}$
	7	$2.15 \pm 0.23^{a,B}$	$2.07 \pm 0.11^{a,A}$	$1.58 \pm 0.25^{b,A}$	$1.23 \pm 0.36^{c,A}$	$1.15 \pm 0.35^{c,A}$	$1.02 \pm 0.04^{c,AB}$
	14	$2.20 \pm 0.38^{a,AB}$	$2.02 \pm 0.27^{ab,A}$	$1.61 \pm 0.49^{ab,A}$	$1.36 \pm 0.62^{cd,A}$	$1.15 \pm 0.06^{cd,A}$	$1.01 \pm 0.19^{d,AB}$
	21	$2.29 \pm 0.32^{a,AB}$	$2.00 \pm 0.18^{ab,A}$	$1.70 \pm 0.51^{bc,A}$	$1.37 \pm 0.55^{cd,A}$	$1.17 \pm 0.11^{d,A}$	$1.07 \pm 0.04^{d,AB}$
	28	$2.59 \pm 0.24^{a,A}$	$2.00 \pm 0.13^{b,A}$	$1.70 \pm 0.34^{b,A}$	$1.52 \pm 0.04^{bc,A}$	$1.18 \pm 0.13^{c,A}$	$1.10 \pm 0.20^{c,A}$
	35	$2.55 \pm 0.42^{a,A}$	$2.01 \pm 0.14^{b,A}$	$1.78 \pm 0.16^{bc,A}$	$1.60 \pm 0.17^{c,A}$	$1.19 \pm 0.01^{d,A}$	$1.13 \pm 0.03^{d,A}$
Ammonia (mg g^{-1})	0	$3.26 \pm 0.70^{a,A}$	$3.32 \pm 1.24^{a,A}$	$3.30 \pm 0.66^{a,A}$	$2.65 \pm 0.35^{ab,A}$	$1.81 \pm 0.68^{bc,A}$	$1.21 \pm 0.47^{c,A}$
	7	$3.17 \pm 0.01^{a,A}$	$2.54 \pm 0.22^{b,B}$	$2.34 \pm 0.62^{bc,B}$	$2.05 \pm 0.29^{c,B}$	$1.51 \pm 0.57^{d,A}$	$1.28 \pm 0.16^{d,A}$
	14	$3.34 \pm 0.24^{a,A}$	$1.91 \pm 0.09^{b,BCD}$	$1.86 \pm 0.66^{b,BC}$	$1.45 \pm 0.81^{b,C}$	$1.50 \pm 0.08^{b,A}$	$0.79 \pm 0.11^{c,B}$
	21	$2.98 \pm 0.17^{a,A}$	$2.25 \pm 0.19^{b,BC}$	$1.59 \pm 0.12^{cd,C}$	$1.42 \pm 0.12^{cd,C}$	$1.65 \pm 0.36^{c,A}$	$1.31 \pm 0.39^{d,A}$
	28	$2.55 \pm 0.24^{a,B}$	$1.79 \pm 0.14^{b,CD}$	$1.53 \pm 0.67^{bc,C}$	$1.78 \pm 0.29^{b,BC}$	$1.59 \pm 0.45^{bc,A}$	$1.19 \pm 0.13^{c,A}$
	35	$2.17 \pm 0.08^{a,B}$	$1.55 \pm 0.10^{bc,D}$	$1.41 \pm 0.52^{cd,C}$	$1.78 \pm 0.15^{b,BC}$	$1.26 \pm 0.17^{cd,A}$	$1.17 \pm 0.05^{d,A}$
C/N ratio	0	$18.38 \pm 2.43^{f,A}$	$24.33 \pm 0.93^{e,A}$	$32.75 \pm 2.45^{d,A}$	$39.31 \pm 3.58^{c,A}$	$46.23 \pm 4.48^{b,A}$	$56.31 \pm 2.32^{a,A}$
	7	$16.49 \pm 1.74^{c,AB}$	$20.67 \pm 0.94^{bc,B}$	$28.70 \pm 3.59^{b,AB}$	$41.19 \pm 12.04^{a,A}$	$46.05 \pm 14.37^{a,A}$	$50.42 \pm 2.17^{a,AB}$
	14	$15.77 \pm 2.18^{e,B}$	$21.40 \pm 3.13^{de,B}$	$29.93 \pm 9.66^{cd,AB}$	$38.53 \pm 16.59^{bc,A}$	$42.97 \pm 3.63^{ab,A}$	$52.20 \pm 10.16^{a,AB}$
	21	$14.34 \pm 2.29^{d,BC}$	$20.71 \pm 1.38^{cd,B}$	$26.23 \pm 7.46^{c,AB}$	$36.70 \pm 16.48^{b,A}$	$42.10 \pm 3.19^{ab,A}$	$47.69 \pm 1.41^{a,B}$
	28	$12.41 \pm 0.72^{e,C}$	$21.09 \pm 1.33^{d,B}$	$26.73 \pm 4.34^{cd,AB}$	$31.27 \pm 0.24^{c,A}$	$42.42 \pm 5.06^{b,A}$	$48.62 \pm 9.86^{a,B}$
	35	$12.38 \pm 1.91^{f,C}$	$20.45 \pm 5.91^{e,B}$	$25.19 \pm 1.50^{d,B}$	$29.31 \pm 1.66^{c,A}$	$41.88 \pm 1.88^{b,A}$	$46.13 \pm 0.56^{a,B}$

Means (±SD) in the same row with different lowercase letters are significantly different from each treatment and means in the same column with different uppercase letters are significantly different from each time ($p < 0.05$).

Table 4. Regression equation of normalized worm number and individual worm weight.

Initial phenolic content (mg g^{-1})	Normalized worm number		Normalized individual worm weight	
	Regression equation	R^2	Regression equation	R^2
4.8	$y = -0.0115x + 0.98$	0.96	$y = -0.0047x + 1.34$	0.06
6.4	$y = -0.0053x + 0.98$	0.95	$y = -0.0070x + 0.99$	0.92
7.2	$y = -0.0098x + 1.03$	0.94	$y = -0.0094x + 1.02$	0.89
8.8	$y = -0.0127x + 1.05$	0.90	$y = -0.0124x + 0.97$	0.90
10.3	$y = -0.0124x + 1.06$	0.92	$y = -0.0139x + 0.99$	0.97
12.7	$y = -0.0185x + 1.07$	0.96	$y = -0.0157x + 0.96$	0.95

Note: y is mortality (%) or individual weight loss (%) and x is vermicomposting time (days).

vermicomposting (Kaushik and Garg, 2004). The final TKN of T_0, T_1 and T_2 (low initial TPC treatments) were higher than those of treatments with moderate and high initial TPC (T_3, T_4, and T_5) which may be due possibly to the higher proportion of cow manure in T_1 and T_2. Additionally, the excretion of earthworms, i.e., mucus, enzymes, and casts and decomposition of dead earthworm tissues also led to an increase in the nitrogen content of the vermicompost (Juárez et al., 2011; Biruntha et al., 2020).
Table 3 shows the initial ammonia concentration of all treatments ranging from $1.2 - 3.3$ mg g^{-1}. It appeared that the concentration of ammonia increased as the proportion of added cow manure increased (Table 1). It was reported that ammonia is highly toxic to earthworms (Domínguez, 2004) particularly when the concentration present in organic wastes is higher than 1 mg g^{-1}; however, the toxicity of ammonia on earthworms varied according to ammonium forms. Hughes et al. investigated the toxicity of NH_4Cl and $(NH_4)_2SO_4$ on the survival of *Eisenia fetida* and found that NH_4Cl was of relatively low toxicity with LC_{50} of 1.49 g kg^{-1} while $(NH_4)_2SO_4$ posed no adverse effect on survival at 2 g kg^{-1} concentration (Hughes et al., 2008). After 35 days of vermicomposting, the decrease in NH_3 concentration was observed in all treatments regardless of the initial TPC. The highest reduction of NH_3 concentration was observed in T_1 and T_2 ($53 - 57\%$), while the lowest reduction was found in T_5 (3%). The shift in NH_3 content during vermicomposting could be due to nitrification by microbial activities and ammonia volatilization (Wu et al., 2010).
The changes in C/N ratio were of a similar trend to those of total carbon. The initial C/N ratios of all treatments were $18 - 56$ (Table 3). An increase in coir pith proportion of the starting materials led to a significant increase in the initial C/N ratio as well as initial TPC. On day 35 of vermicomposting, the C/N ratio of all treatments decreased inversely proportional to initial TPC and the amount of coir pith added. Further, the lowest final C/N ratio was resulted in T_0, following by T_1, T_2, T_3, T_4, and T_5. The decline in C/N ratio was probably due to the loss of carbon as CO_2 via respiration and mineralization of labile organic compounds leading to a reduction in the weight and volume of vermicompost piles (Tahir and Hamid, 2012). Results further showed that T_1 reached maturity within 35 days which is faster than that reported by Patil et al. who found that vermicomposting of partially decomposed coir pith with cow manure at 20:80 (% (w/w)) reached maturity within 60 days (Patil et al., 2017). However, information on initial C/N ratio and initial TPC was not provided rendering comparison difficult. These results indicated that vermicomposting of non-pretreated coir pith could be accomplished when initial TPC was adjusted to an appropriate concentration.

Microbial population changes

Organic substrates are stabilized by mutual interaction between earthworms and microorganisms during vermicomposting (Hussain et al., 2018). Organic wastes are crushed and ground by earthworm gizzard and passed on to the intestine for enzymatic actions (Kumar et al., 2020). The gut microbial populations play an important role in the earthworm diet by releasing enzymes in the intestines, thus, aiding the breakdown of organic matter (Ghosh et al., 2022). Earthworms ingest feedstock and then excrete vermicasts containing organic matter, minerals, and beneficial microorganisms. Since changes in microbial community play a major role during vermicomposting (Devi et al., 2023), the microbial populations (bacteria, fungi, and actinomycetes) present in vermicasts taken from all treatments (T_0, T_1, T_2, T_3, T_4, and T_5) during 35 days of vermicomposting were enumerated by dilution plate method.
The bacterial, fungal, and actinomycete populations in vermicasts of all treatments regardless of initial TPC were not significantly different ($p > 0.05$) during the first week of vermicomposting providing that the highest bacterial and fungal counts of 14.4 and 9.3 log CFU g^{-1} were observed on day 0 of vermicomposting. Significant declines in bacterial and fungal populations of all treatments were, respectively, observed on day 14 and day 21 while the actinomycete population remained virtually unchanged within the range of $7.4 - 9.9$ log CFU g^{-1} throughout the vermicomposting process (Fig. 1A – C) given that

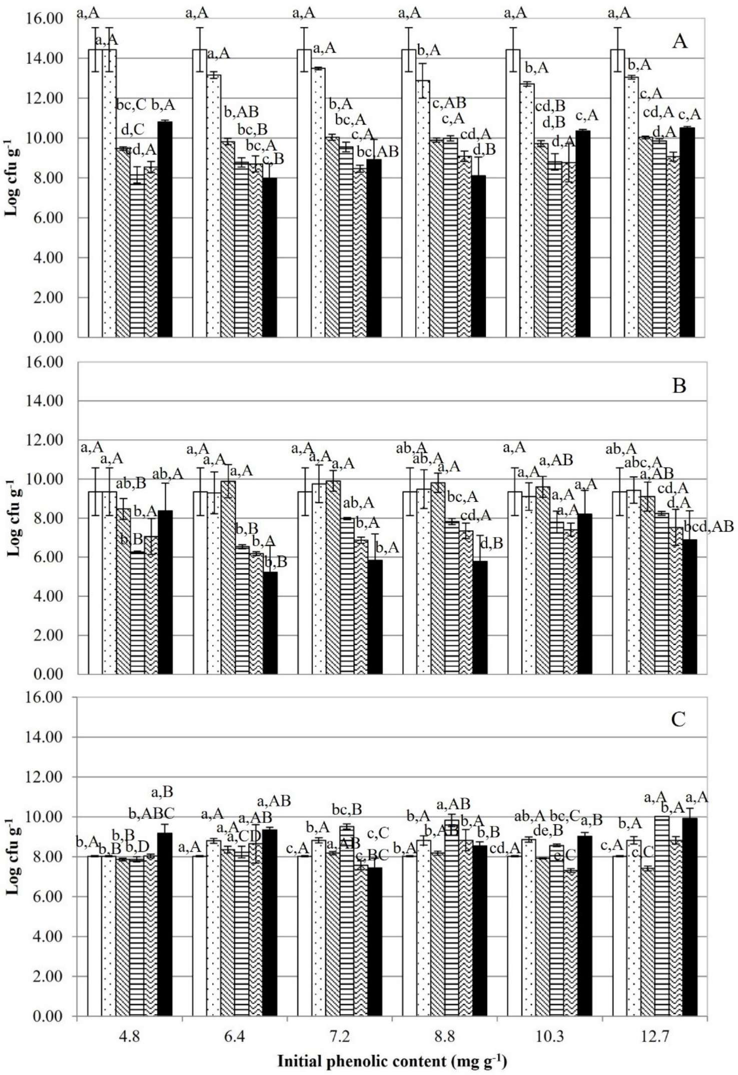

Figure 1. Changes in microbial population (A) total bacteria, (B) fungi and (C) actinomycetes in the casts of *E. eugeniae* during vermicomposting.
Means (±SD) in the same treatment with different lowercase letters are significantly different from each other and means in the same vermicomposting time with different uppercase letters are significantly different from each other ($p < 0.05$).
Symbol: □ (day 0), ▩ (day 7), ▧ (day 14), ▤ (day 21), ▨ (day 28), ■ (day 35).

by the end of vermicomposting, the bacterial, fungal and actinomycete populations were $8.1 - 10.8$, $5.2 - 8.4$ and $7.8 - 10.0$ log CFU g^{-1}, respectively. It is worth noting that the high microbial population found in vermicasts on day 0 may be due to the fact that earthworms employed in this study were reared on the nutrient-rich cow manure which is capable of sustaining good microbial growth. The decrease in bacterial and fungal populations after day 7 and day 14 may be due to (i) the change of nutrient environment, i.e., the transition from cow manure-only feed to cow manure-coir pith mixture, thus, containing less nutrients, (ii) selective feeding behaviors of earthworms on both bacteria (Gómez-Brandón et al., 2011) and fungi (Chen et al., 2023), (iii) precipitation and deactivation of enzymes originating from both gut microbes and earthworms by phenolic compounds (Liebeke et al., 2015) and (iv) susceptibility of gut microbes to phenolic compound toxicity present in coir pith (Aziz et al., 1998; Alamri and Moustafa, 2012).

Some studies have demonstrated that phenolic compounds released during coir pith degradation by cyanobacteria, *Oscillatoria annae* (Prabha et al., 2009), and basidiomycetes fungus, *Pleurotus sajor-caju*, were resorcinol, guaiacol, catechol and other unidentified compounds (Reghuvaran and Ravindranath, 2012). Further, extracts obtained from several parts of coconut exhibited antimicrobial, antifungal, and antiviral activities. Oliveira et al. reported that ethyl acetate extract of crude aqueous extract of coconut husk fiber was of higher antimicrobial activity than crude extract, 78 and 156 μg ml^{-1}, respectively (Oliveira et al., 2013), against *Staphylococcus aureus*, both methicillin sensitive and resistant strains. Singla et al. found that the ethanolic extract of coconut shell was active against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *S. aureus*, and *Micrococcus* *luteus* (Singla et al., 2011). Venkataraman et al. reported that, at 100 μg ml^{-1} concentration, alcoholic extract of dried coconut shell actively inhibited *Microsporum canis*, *Microsporum gypseum*, *Microsporum audouinii*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Trichophyton violaceum* (Venkataraman et al., 1980). Additionally, the antimicrobial activities of phenolics extracted from plants have also been shown to possess inhibitory activities against bacteria, such as *Listeria monocytogenes*, *B. coagulans*, *Escherichia coli* and *Shigella flexneri* (Sung et al., 2012), fungi, such as *Aspergillus niger*, *Penicillium expansum*, and *Fusarium moniliforme* (Okoi et al., 2013) and yeast, such as *Candida albicans*, *Cryptococcus laurentii*, and *Filobasidiella neoformans* (Romani et al., 2006). Hence, the decrease in bacterial and fungal populations during vermicomposting may be attributed to the negative impact of phenolic compounds present in coir pith. A detailed study is required to understand how these phenolic compounds affect the microbial diversity in earthworm gut and vermicasts.

Influence of initial TPC on Earthworm activity: Biomass and population

The number and individual weight of earthworms tended to decline as the initial TPC present in substrates increased during the whole process of vermicomposting. Initially, all 50 adult earthworms, with average weight of 1.1 g worm^{-1}, were added to each treatment. Results showed that weight losses were observed for all treatments from the start until the end of vermicomposting except for T_0 in that an increase in earthworm weight was observed, 53.23%, in the first week and then gradually and continuously lost weight, approximately 3.6%, by the end of vermicomposting. On day 35, earthworms in T_1 and T_2 containing initial TPC of 6.4 and 7.2 mg g^{-1} suffered 25.5 and 30.7% weight loss, respectively, while weight losses of 42.1, 49.6 and 57.6%, respectively, were noted for T_3, T_4, and T_5. Even though earthworms used in this study were reared in cow manure, high mortality was still observed in T_0 and T_1, e.g., 10 and 38% on day 7, respectively, while rather low worm mortalities, i.e., 3, 3, 2 and 1% were observed with T_2, T_3, T_4, and T_5, respectively. It is worthy to note that as the vermicomposting process progressed, a significant increase in worm mortalities of approximately 31, 47, 43, and 63% for T_2, T_3, T_4, and T_5, respectively, on day 35, were observed. Initially, high mortality observed in T_0 and T_1 may be due probably to high initial ammonia concentration, approximately 3.3 and 2.9 folds higher than the toxic threshold, $>$ 1 mg g^{-1}, reported in literature (Domínguez, 2004). However, regardless of the low initial mortality of T_2, T_3, T_4, and T_5, it is possible that apparently high weight losses may be due to the selective feeding behavior of earthworms as well as the presence of high initial TPC. Results found are in good agreement with that reported by Ganesh et al. who vermicomposted pre-composted acacia leaf litter containing 12.5% soluble polyphenols using *Lampito mauritii* and *E. eugeniae* and found that regardless of reactor geometry employed and nitrogen source addition, earthworm mortality was persistently high and earthworms lost weight continuously even though earthworms consumed a large amount of feed substrate (Ganesh et al., 2009). The authors suggested that some constituents present in acacia leaves that are not generally toxic to plants may be responsible for such high mortality and weight losses and that even though earthworms preferred feeds of low C/N ratio to those of low nitrogen content, the presence of polyphenolics suppressed such preferences which can also be observed in this study whose initial TPC and cow manure proportion were, respectively, of the following order: $T_0 < T_1 < T_2 < T_3 < T_4 < T_5$ and $T_0 > T_1 > T_2 > T_3 > T_4 > T_5$. De Silva and van Gestel investigated the feasibility of utilizing paddy husk, sawdust, non-composted coir pith, and composted coir pith for the preparation of artificial soil and found that only the mixture containing 10% non-composted coir pith led to 40% mortality of *Eisenia andrei* (Silva and Gestel, 2009). The authors found further that *E. andrei* aggregated to feed on cow manure added daily as feed to artificial soil containing sawdust and non-composted coir pith. Additionally, Karmegam et al. reported that cow manure addition was necessary for good growth and reproduction of earthworms, e.g., *E. fetida* and *E. eugeniae* (Karmegam et al., 2021). Sanchez-Hernandez et al. tested the ecotoxicity of olive mill waste (OMW) sediment containing 873 mg kg^{-1} total soluble phenolics on *Lumbricus terrestris* and reported also that mortality was dose dependent providing that 40 and 80% OMW amended soil led to the high mortality as well as weight losses (Silva and Gestel, 2009). Sabrina et al. showed further that EFB was highly toxic to *P. corethrurus* and *A. rodoricensis* and to a lesser extent to *E. fetida* (Sabrina et al., 2009). The authors reported further that, during EFB vermicomposting using *E. fetida* at several stocking densities, e.g., 1:15, 1:10, 1:7.5, 1:6, and 1:5 (earthworm to media ratio), even though 5 mg of cow dung was fed for each worm daily, worm weight losses were apparent by the end of vermicomposting and that mortality was evident by week 4 post vermicomposting in that the higher the stocking density the higher the mortality.

Results found in this study (Table 4) indicated that initial TPC negatively affected both worm number (survival) and weight. For each initial TPC, as vermicomposting progressed, worm weight and worm number decreased continuously ($R^2 > 0.89$ and 0.90, respectively). It should be noted that since *E. eugeniae* gained weight initially, no relationship ($R^2 = 0.06$) was, therefore, found for T_0, cow manure only, containing 4.8 mg g^{-1} initial TPC and that high initial ammonia concentration present in T_0 may possibly be responsible for high mortality ($R^2 = 0.96$).

Furthermore, the results found above indicated that the initial TPC of vermicomposting materials negatively affected the survival and weight gain of *E. eugeniae*. However, HPLC results (see below) indicated that almost all of phenolic compounds present in cow manure, namely, T_0, disappeared by the end of vermicomposting. As earthworms employed in this study were reared in cow manure of the same lot, then, TPC present in cow manure should play no role in both mortality and weight loss of

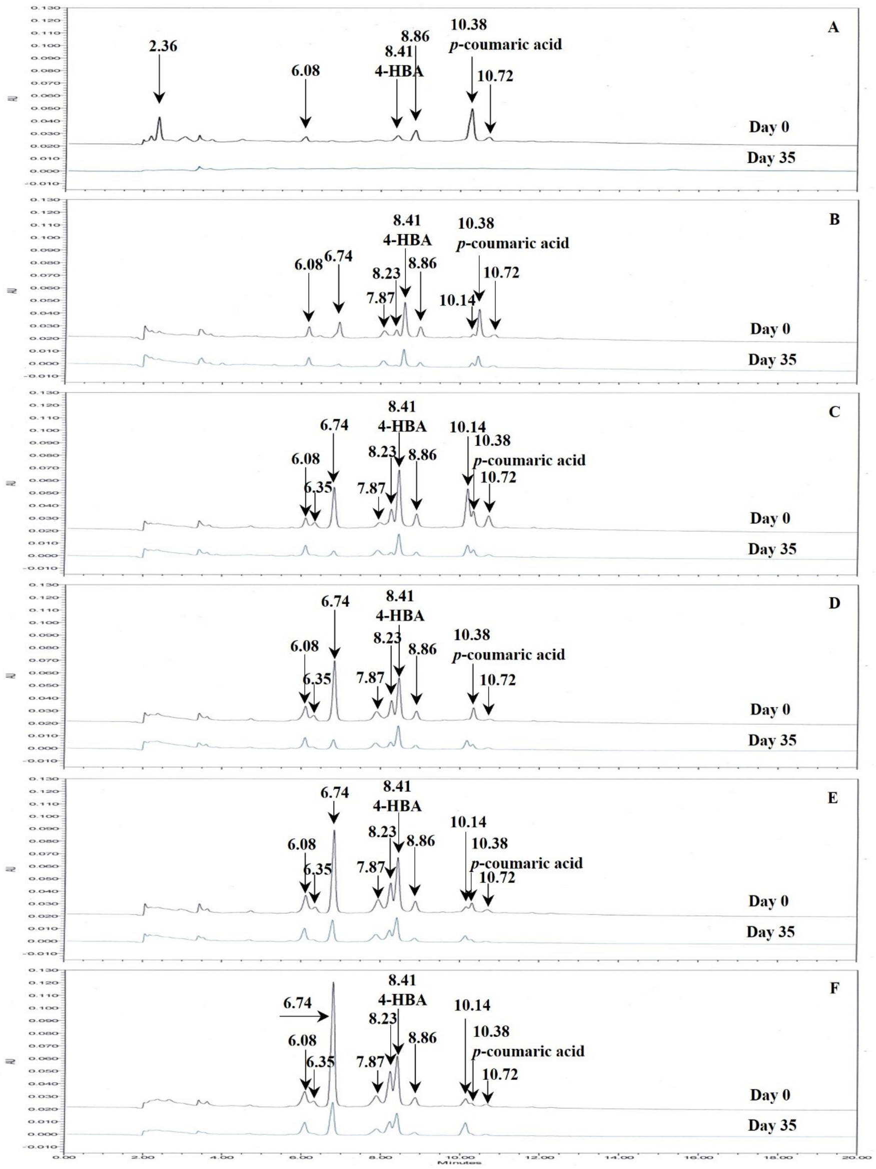

Figure 2. Reverse-phase HPLC-UV trace (280 nm) of coir pith vermicompost extract: (A) T_0, (B) T_1, (C) T_2, (D) T_3, (E) T_4 and (F) T_5.

E. eugeniae. It is, therefore, presumed that the adverse effects on survival and worm weight observed in this study stemmed mainly from TPC present solely in coir pith. The correlation between both worm mortality and weight loss and initial TPC (of only coir pith) was evaluated and found to be (Supplementary Fig. 2):

$$\text{Mortality}(\%) = 3.84\text{TPC}_\text{i} + 14.28 \tag{1}$$

$$\text{Individual Weight Loss}(\%) = 3.19\text{TPC}_\text{i} + 19.18 \tag{2}$$

where TPC_i is the initial TPC with R^2 of 0.88 and 0.97 for Eqs. 1 and 2, respectively.

Sabrina et al., in a field trial experiment, investigated the effects of both raw and composted EFB application on earthworms and found that even though worm mortalities were observed in all experiments (Sabrina et al., 2012), no significant relationship between mortality and total extractable phenolic content was found which is in contrast to this study. Tahir and Hamid reported weight loss (as high as 33%) and mortality (as high as 50%) during vermicomposting of coconut husk together with goat manure (Tahir and Hamid, 2012). The authors presumed that worm mortality was due to changes in pH to acidic one which *E. eugeniae* is highly susceptible. However, no information on the phenolic content of materials is available rendering comparison impossible. It appeared that initial TPC of coir pith played a significant role in both morality and weight loss of *E. eugenia* during vermicomposting. Further, the correlation provided above may be employed to optimize and/or empirically predict the highest amount of starting material containing TPC for vermicomposting leading to acceptable weight loss and mortality of earth-worms, subsequently, efficient vermicomposting and utilization of high TPC raw materials.

Characterization of Phenolic Compounds using HPLC

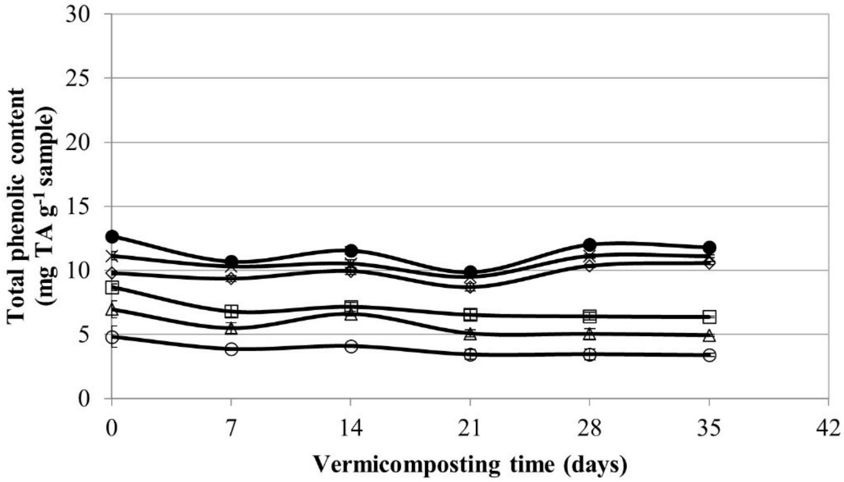

Figure 3. Changes in TPC during vermicomposting of feed materials containing different initial TPC: ◯ (4.8 mg g^{-1}), △ (6.4 mg g^{-1}), □ (7.2 mg g^{-1}), ◊ (8.8 mg g^{-1}), × (10.3 mg g^{-1}) and • (12.7 mg g^{-1}).

Since high mortality of earthworms was noticeable for treatments containing high initial TPC, T_3, T_4, and T_5, in particular, it is of significant interest to semi-quantitatively estimate the presence of TPC in both raw materials and treatments. Chromatograms of water-soluble TPC of raw materials, e.g., cow manure and coir pith, and treatments are given in Fig. 2A – F. Results (Supplementary Table 1) showed that 16 and 17 peaks were found for cow manure (T_0) and coir pith (T_5), respectively, on day 0. It was found further that cow manure and coir pith contained similar phenolic compounds given that 5 (3.61, 4.90, 5.73, 8.23, and 10.14 minutes) and 4 (1.80, 3.02, 3.70, and 4.47 minutes) compounds, respectively, were unique to coir pith and cow manure. Among thirteen standard phenolic compounds tested, only 4-HBA (8.41 minutes) and *p*-coumaric acid (10.38 minutes) were detected in both cow manure and coir pith. Additionally, 20 and 18 peaks were found for T_1 and T_2 (Supplementary Table 1) which were the combination of phenolic compounds present in cow manure and coir pith, whereas for T_3 and T_4, 17 peaks were detected. On day 35 post vermicomposting, phenolic compounds detected in cow manure (T_0) reduced significantly from 16 to 5 peaks while those detected in coir pith (T_5) remained intact albeit declined in concentrations. It is interesting to note that for treatments containing low initial TPC, T_0, T_1 and T_2, disappearance of phenolic compounds, 4.90 and 6.35 minute-peaks, was evident given further that new compound (5.30 minute-peak) was detected only for T_1 indicating degradation of phenolic compounds present in the mixture whereas the number of peaks detected in both T_3 and T_4 remained the same. HPLC results given above agreed well with results obtained for TPC in that TPC of all treatments reduced from 4.8 – 12.7 mg g^{-1} initially to 3.4 – 11.8 mg g^{-1} by the end of vermicomposting, indicating a significant decrease in TPC ($p < 0.05$) of 29.6, 23.0, and 11.5% in T_0, T_1, and T_2, respectively (Fig. 3). The reduction of TPC could be due to degradation by both earthworms and microorganisms which was in good agreement with HPLC results (see below). Earthworm actions, mucus, and enzyme secretion including vermicast production, may promote microbial growth and thereby trigger phenolic compound decomposition (Zhao et al., 2010; Luz et al., 2012; Chen et al., 2015).

Several studies reported that *p*-coumaric acid was found to be present in coconut water and meat (Mahayothee et al., 2016), coconut oil (Seneviratne and Dissanayake, 2008), and coconut testa (Arivalagan et al., 2018) whereas 4-HBA was reported to be present in both coconut husk (Dey et al., 2003; Dey et al., 2005) and coconut testa (Arivalagan et al., 2018). Furthermore, several phenolics such as catechol, catechin, epicatechin, gallic acid, ferulic acid, and chlorogenic acid have also been reported (Esquenazi et al., 2002; Ravindranath and Sarma, 1998; Mendonça-Filho et al., 2004) (Supplementary Table 2). It is rather surprising that most of phenolic compounds (peaks' height) decreased for all treatments which may be attributable to the action of microorganisms innately present in coir pith, cow manure, and earthworms since the population of bacteria, fungi and actinomycetes remained rather high ($\geq$ 8 log) throughout the whole period of vermicomposting (Fig. 1). Limaye et al. (2017) reported that actinomycetes are capable of producing laccase enzyme which is able to degrade phenolic compounds such as lignin. When coir pith was composted by *Pleurotus sajor-caju*, resorcinol, catechol, and guaiacol were identified (Reghuvaran and Ravindranath, 2012) which, however, were not found in coir pith vermicompost prepared in this study. Yahaya et al. (2017) reported that *Pleurotus sajor-caju* could degrade phenolics, e.g., phenol, pyro-catechol, vanillic acid, and 4-hydroxybenzoic acid, present in raw EFB with the aid of laccase, phenolic oxidase, and manganese oxidase (Jeon et al., 2012). Our findings indicated that some of phenolic compounds in starting materials were broken down during the vermicomposting. However, the reduction of phenolic compounds depended on the initial TPC present in substrates and biodegradation conditions (Baddi et al., 2009). It is evident that earthworms could efficiently digest phenolic compounds in treatments containing low TPC (T_1 and T_2) better than those in treatments containing high TPC (T_3, T_4, and T_5).

4. Conclusion

The number of phenolic compounds found in coir pith and cow manure was 17 and 16, respectively, out of which 5 and 4, respectively, were unique to coir pith and cow manure. Biodegradation of phenolic compounds was evident for all treatments regardless of initial TPC except that a new compound was detected in T_1, containing 6.4 mg g^{-1} initial TPC. Vermicomposting performance, especially earthworm activities – mortality and weight loss, was adversely affected in dose dependent manner by initial TPC present in feedstock, coir pith particularly, in this study, in that earthworms' mortality and weight loss increased as the initial TPC of starting materials increased. Better biodegradation of phenolic compounds was achieved when low initial TPC of starting materials for vermicomposting was provided. Therefore, in order to facilitate efficient vermicomposting process, empirical relationship between initial TPC and earthworm activities could be used to optimize the amount of high TPC containing starting materials for vermicomposting.

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Ethical approval
This manuscript does not report on or involve the use of any animal or human data or tissue. So the ethical approval is not applicable.

Author contribution
The authors confirm the study conception and design: S. Tripetchkul, S. Akeprathumchai and K. Pundee; data collection: K. Pundee; analysis and interpretation of results: S. Tripetchkul, S. Akeprathumchai and K. Pundee; draft manuscript preparation: S. Tripetchkul, S. Akeprathumchai and K. Pundee. The results were evaluated by all authors, and the final version of the manuscript was approved.

Availability of data and materials
Data presented in the manuscript are available via request.

Conflict of interest statement
The authors declare that they are no conflict of interest associated with this study.

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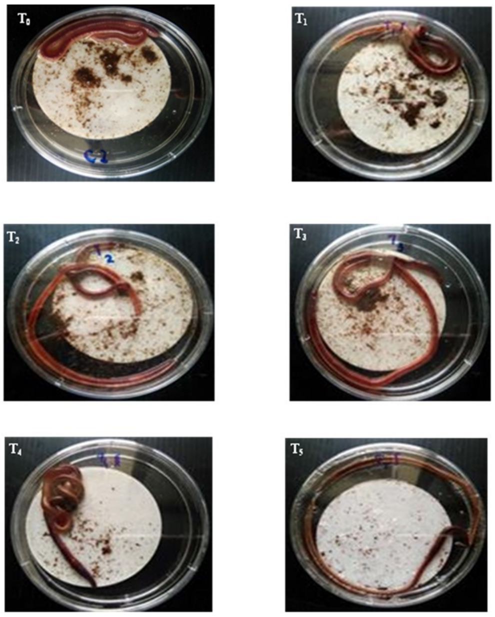
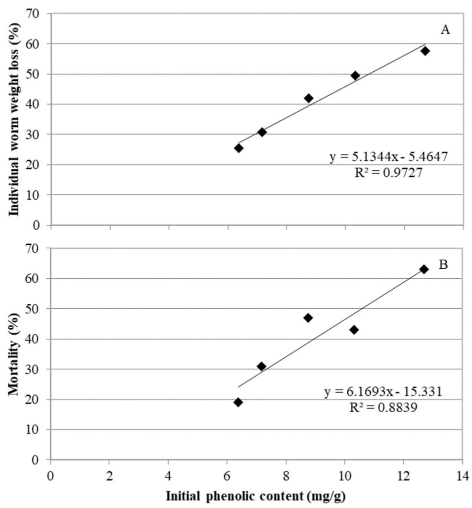
SUPPLEMENTARY FIGURES AND TABLES



Supplementary figure 1: Earthworm cast production on 14th day of the vermicomposting process.



Supplementary figure 2: Correlation between individual weight loss (A), mortality (B) and initial phenolic content* present in the feed materials.

*As HPLC results suggested that phenolic compounds present in the vermicomposting mixture were not toxic to earthworms, it is presumed that phenolic compounds present in coir pith alone were responsible for mortality and weight loss of earthworms found in this study. Therefore, a correlation was re-evaluated by subtracting the (calculated) concentrations of phenolic compounds present in cow manure in accordance to their portion added to the mixture and then the correlation was constructed.

Supplementary Table 1: Phenolics detected in cow manure, coir pith and coir pith vermicompost using E. eugeniae on day 0 and day 35

Retention time	0% (cow manure)		20% coir pith		30% coir pith		50% coir pith		70% coir pith		100% coir pith	
	Day 0	Day 35	Day 0	Day 35	Day 0	Day 35	Day 0	Day 35	Day 0	Day 35	Day 0	Day 35
1.80	√	=	√	=								
1.97	√	×	√	=	√	=	√	=	√	=	√	=
2.16	√	×	√	=	√	=	√	=	√	=	√	=
2.36	√	×	√	=	√	=	√	=	√	=	√	=
3.02	√	×										
3.39	√	—	√	=	√	=	√	=	√	=	√	=
3.61			√	—	√	—	√	—	√	—	√	—
3.70	√	—	√	+								
4.47	√	×	√	—	√	—						
4.90			√	×	√	—	√	—	√	—	√	—
5.30				new								
5.73			√	=	√	=	√	=	√	=	√	=
6.08	√	—	√	=	√	=	√	=	√	=	√	=
6.35	√	×	√	×	√	×	√	—	√	—	√	—
6.74	√	×	√	—	√	—	√	—	√	—	√	—
7.87	√	×	√	—	√	—	√	—	√	—	√	—
8.23			√	—	√	—	√	—	√	—	√	—
8.41	√	×	√	—	√	—	√	—	√	—	√	—
8.86	√	—	√	—	√	—	√	—	√	—	√	—
10.14			√	—	√	+	√	—	√	—	√	—
10.38	√	×	√	—	√	—	√	—	√	—	√	—
10.72	√	×	√	—	√	—	√	—	√	—	√	—

Symbol; √indicate detected peak, × indicate disappear peak, - indicate decrease peak, + indicate increase peak and, = indicate stable peak

Supplementary Table 2: Phenolic compounds found in various parts of coconut.

Phenolics	(Arivalagan et al., 2018)[1,a]	(Reghuvaran and Ravindranath, 2012)[2,b]	(Esquenazi et al., 2002)[3,c]	(Mendonça-Filho et al., 2004)[3,d]	(Dey et al., 2003)[4,b]	(Dey et al., 2005)[4,d]	(Oliveira et al., 2013)[3,e]	(Mahayothee et al., 2016)[5,f]	(Seneviratne and Dissanayake, 2008)[6,g]	(Ravindranath and Sarma, 1998)[4,d]	This study[7,d]
catechol	-	+	-	-	-	-	-	-	-	-	-
resorcinol	-	+	-	-	-	-	-	-	-	+	-
guaiacol	-	+	-	-	-	-	-	-	-	-	-
catechin	+	-	+	+	-	-	+	+	+	-	-
epicatechin	+	-	+	+	-	-	+	-	-	-	-
polymeric procyanidins	-	-	+	-	-	-	+	-	-	-	-
4-hydroxybenzoic acid	+	-	-	-	+	+	-	+	-	-	+
ferulic acid	+	-	-	-	+	+	-	-	+	-	-
gallic acid	+	-	-	-	-	-	+	+	-	-	-
ellagic acid	-	-	-	-	-	-	+	-	-	-	-
procyanidin dimer	-	-	-	-	-	-	+	-	-	-	-
salicylic acid	+	-	-	-	-	-	-	+	-	-	-
syringic acid	+	-	-	-	-	-	-	+	-	-	-
p-coumaric acid	+	-	-	-	-	+	-	+	+	-	+
o-coumaric acid	+	-	-	-	-	-	-	-	-	-	-
m-coumaric acid	-	-	-	-	-	-	-	+	-	-	-
caffeic acid	+	-	-	-	-	-	-	+	+	-	-
pyrogallic acid	-	-	-	-	-	-	-	-	-	+	-
chlorogenic acid	+	-	-	-	-	-	-	-	-	-	-
gentisic acid	+	-	-	-	-	-	-	-	-	-	-
protocatechuic acid	+	-	-	-	-	-	-	-	-	-	-
trans-cinnamic acid	+	-	-	-	-	-	-	-	-	-	-
vanillic acid	+	-	-	-	-	+	-	-	-	-	-
sinapic acid	+	-	-	-	-	-	-	-	-	-	-
2,4-dihydroxybenzoic acid	+	-	-	-	-	-	-	-	-	-	-
3-hydroxybenzoic acid	+	-	-	-	-	-	-	-	-	-	-
apigenin	+	-	-	-	-	-	-	-	-	-	-
hesperetin	+	-	-	-	-	-	-	-	-	-	-
kaempferol	+	-	-	-	-	-	-	-	-	-	-
leutoline	+	-	-	-	-	-	-	-	-	-	-
myricetin	+	-	-	-	-	-	-	-	-	-	-
naringenin	+	-	-	-	-	-	-	-	-	-	-
quercetin	+	-	-	-	-	-	-	-	-	-	-
rutin	+	-	-	-	-	-	-	-	-	-	-
umbelliferone	+	-	-	-	-	-	-	-	-	-	-
epigallocatechin	+	-	-	-	-	-	-	-	-	-	-

The first superscripts indicate sources employed in quantifying phenolics: 1. coconut testa, 2. coir pith compost by *Pleurotus sajor-caju*, 3. coconut husk fiber, 4. coconut husk, 5. coconut water and meat, 6. coconut oil and 7. coir pith/vermicomposted coir pith while the second designate the methods used to quantified the phenolics: a. UPLC coupled with TQD-MS/MS, b. TLC, c. HPLC/DAD, d. HPLC, e. HPLC-ESI/MS f. GC-MS, g. LC-MS.