

Vermicomposting of Vegetable Waste Amended with Different Sources of Agro-Industrial By-Product Using *Lumbricus rubellus*

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Abstract

Vermicomposting of vegetable waste (VW) spiked with multiple sources of agro-industrial waste was conducted in microcosms for 18 days of pre-composting and a subsequent 70 days (10 weeks) of vermicomposting by utilizing epigeic *Lumbricus rubellus*. Nutrient element and heavy metal content in vermicompost produced were evaluated by comparing with different agro-industrial waste as amended materials. Earthworm multiplication and growth showed the highest increment in 100% of spent mushroom compost (SMC) (+323.72% for biomass and +38.10% for number). Significant differences ($P < 0.05$) between earthworm biomass and number was identified in treatment of 100% cow dung (CD), cow dung:vegetable waste I (CD:VW I), 100% of spent mushroom compost (SMC), and spent mushroom compost:vegetable waste I (SMC:VW I). The highest nutrient element i.e. N, P, and K content in vermicompost was paddy straw:vegetable waste II (PS:VW II) 1.37 ± 0.040 , 0.37 ± 0.057 , and 1.29 ± 0.050 respectively and the lowest C:N ratio in cow dung:vegetable waste I (CD:VW I) (19.62 ± 0.11), which indicates an advanced degree of compost maturity. Heavy metal, i.e. Cd, Cr, Pb, Cu, and Zn content in vermicompost from all vermicomposts were lower compared to the compost limits set by the USA, European countries, and the Malaysian Recommended Site Screening Levels for Contaminated Land (SSLs). Thus, *L. rubellus* is feasible in bioconverting VW spiked with agro-industrial waste into vermicompost, and the product possesses agronomic potential as well as environmental soundness in contrast to synthesized chemical fertilizer.

Keywords: biofertilizer, earthworms, heavy metal, nutrient element, recycling, vermitechnology

Introduction

Out of 3,500 tons of waste in a Malaysian landfill, at least 50% is organic waste. Vegetable waste (VW) is categorized as one of the organic wastes from the agro-industrial sector, including livestock farming. Vegetable waste is

easy to putrefy due to its natural characteristics and the putrefaction period occurs in time of harvesting, transportation handling, storage, marketing, and additional processes that generating waste. Moreover, these vegetable processes attract flies, mosquitoes, cockroaches, rats, and other pests that are disease vectors for humans and could potentially cause pollution for the environment. A practical and perspicacious method should be ascertained on VW management so that nutrient recovery can be restored for human benefits

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Table 1. Substrates mixture in ratio for each vermibed.

Vermibed (Substrate mixture)	Ratio (kg)	Description
CD (control)	3.5	Cow dung only
CD:VW I	1.75:1.75	1 part cow dung:1 part vegetable waste
CD:VW II	1.17:2.33	1 part cow dung:2 parts vegetable waste
VW (control)	3.5	Vegetable waste only
PS:VW I	1.75:1.75	1 part paddy straw:1 part vegetable waste
PS:VW II	1.17:2.33	1 part paddy straw:2 parts vegetable waste
SMC (control)	3.5	Spent mushroom compost only
SMC:VW I	1.75:1.75	1 part spent mushroom compost:1 part vegetable waste
SMC:VW II	1.75:2.33	1 part spent mushroom compost:2 parts vegetable waste

as well as for the environment. Since VW and other organic wastes are recyclable, conversion into value-added product by means of composting or thermocomposting into nutritive compost is indeed environmentally feasible due to its cost-effectiveness, and the process involved is chemically free. Even so, thermocomposting has been adopted as a basic tool for on-site waste decomposition. Some disadvantages of traditional thermocomposting methods include the long duration of the process, frequent aeration required, loss of nutrients (e.g., grassing off nitrogen), and a heterogenous end product [1]. In nature, co-operation between microbes, fungi, and other detritivores such as detritus-feeder invertebrates (i.e. earthworms) in decomposition of a variety organic waste termed as vermicomposting is more efficient in converting organic waste into a value added product, i.e. vermicompost.

Vermicomposting is a simple biotechnological process in which earthworms are employed to convert the organic waste material into vermicompost or excellent organic compost [2]. Moreover, vermicomposting emerges as one of the most feasible alternative techniques compared to conventional aerobic composting. This process is not only rapid, easily controllable, cost effective, energy saving, and zero waste, but also accomplishes the most efficient recycling of organic waste and nutrients [3]. Various physical and biochemical processes are affected by earthworms. The physical processes include substrate aeration, mixing, and grinding. The biochemical processes are affected by microbial decomposition of substrates in the intestine of the earthworm [4]. During the process, the important plant nutrients in the materials are released and converted through microbial action into forms that are much more soluble and available to plants than those in the parent compounds [5]. The vermicompost produced is an excellent soil conditioner that has higher nutrient availability for plant growth, since it is homogenous, has desirable aesthetics, reducing levels of contamination, and tends to hold more nutrients over a longer period [6]. However, previous work on vermicomposting of organic waste by Azizi et al. [7], 60-70% moisture content of feed materials was maintained during the process, but it is problematic to implement such

moisture content with VW nature characteristics (i.e. easily putrescible) unless substrate amendment is added in the vermicomposting process.

Therefore, the objective of this work is to convert VW into a bioproduct spiked with agro-industrial waste as amendment, viz., paddy straw, cow dung, and spent mushroom compost by employing *Lumbricus rubellus* or red worms. In the meantime, to compare nutrient elements and heavy metal content in the produced product (i.e. vermicompost) from different agro-industrial waste as amended materials in the vermicomposting process.

Materials and Methods

Substrate, Microcosm, and Earthworm Preparation

Approximately 47 kg of vegetable waste (VW), mainly leafy vegetables such as cabbage, lettuce, and cauliflower, were collected in gunny sacks from a daily-fresh market in Putrajaya. Other types of VW, i.e. onion, garlic, peppers, tomato, potato, peas, chilies, and citrus, were not collected since they would have hindered the earthworms' palatability. The collected VW were dried for one day under direct sunlight on newspapers. Foreign residues such as rubber bands, plastic, and wrapping paper were discarded prior to sun bathing. Cow dung (CD) was collected from a country cow farm in Serdang, Selangor, and was dried for 11 days before stabilization in the pre-composting period. Meanwhile, paddy straw (PS) and sawdust-based spent mushroom compost (SMC) were obtained from an agricultural farm in Jenderam Hulu, Selangor, and the Mushroom House in the Institute of Biological Science, University of Malaya, respectively. Earthworms utilized in this study were *L. rubellus*, which was cultured in a mixture of a variety of organic waste sources. Experiments were conducted in microcosm (360 mm×280 mm×200 mm) artificially designed with a net (250 mm×100 mm) covering the center of the lid to allow aeration, to prevent any interruption by pests, and to provide microclimatic conditions [7-9]. All of

the microcosms were kept in an earthworm reservoir (shed area) in the Institute of Biological Science, University of Malaya, with identical ambient conditions (room temperature $25\pm 3^\circ\text{C}$, relative humidity 60-80%). Each microcosm contained a total of 3.5 kg of substrates mixture (Table 1).

Pre-Composting Period

Pre-composting is the process of stabilizing the substrates in terms of pH, temperature, and moisture before vermicomposting. The pre-composting period is to avoid exposure of earthworms to high temperature during the initial thermophilic stage of microbial decomposition [10] and provide appropriate conditions for earthworms in mass reduction, moisture management, and pathogen reduction [11]. In this study, the mixtures of substrates were stabilized in a pre-composting period for 18 days, starting from the first day the substrates were mixed. During the pre-composting period, pH and temperature were monitored to ensure optimum level of pH 7 ± 1 , and a temperature of $27\pm 1^\circ\text{C}$ was achieved and stabilized by manual turning.

Vermicomposting Process

On day 18 of pre-composting, 100 g (~30 g dry weight) sample was taken from each treatment for 0 week nutrient element analysis. At the same time as the substrates' pH, moisture content and temperature were stable and 35 clitellated *L. rubellus* were introduced into each microcosm after the biomass of the earthworms was weighed. During the vermicomposting process, the moisture content of feed materials was maintained at $70\pm 10\%$ by periodic sprinkling of an adequate quantity of distilled water using wash bottles (80-160 ml per microcosm), together with manual turning once every few days to remove any stagnant water and odour and to eliminate volatile gases that are potentially toxic to earthworms and indirectly removing ammonia, odour, and excess water. No extra mixtures of feed materials were added during this experimental stage. On day 70 (week 10), the numbers of individual *L. rubellus* in each microcosm were calculated by hand sorting, and its biomass weighed. 100 g (~30 g dry weight) sample of vermicompost from each microcosm (total 27 microcosms) was

harvested in plastic vials (air tight) for chemical analysis to determine the nutrient element and heavy metal content in the vermicompost produced. The multiplication and growth of earthworms was calculated as:

$$\frac{(\text{Biomass or Number on day 70} - \text{Biomass or Number on day 0})}{\text{Biomass or Number on day 0}} \times 100$$

Laboratory Analysis

Total organic carbon (TOC) was determined by a partial oxidation method [12]. Total Kjeldahl nitrogen (TKN) was estimated by Kjeldahl digestion with concentrated H_2SO_4 (1:20, w/v) followed by distillation [13]. Total phosphorus (TP) was detected by with the colorimetric method using ammonium molybdate in HCl [14]. Total potassium (TK) and heavy metals viz., Cr, Cu, Cd, Pb, and Zn were measured by the ignition method using a Perkin Elmer model 3110 double beam atomic absorption spectrophotometer, AAS after digestion of the sample with concentrated HNO_3 :concentrated HClO_4 (4:1, v/v). [10]. C:N ratio analyzed through calculation.

Statistical Analysis

Statistical analysis was carried out using SPSS for Windows version 16.0 (SPSS, Inc., Chichago, IL, USA). One-way analysis of variance (ANOVA) was carried out to analyze the significant difference of the data obtained between treatments during vermicomposting at 0.05% level of significance. Paired samples t-test was used to identify the significant differences for each microcosm between percentage of number and biomass of earthworms during the vermicomposting process.

Results and Discussion

Earthworm Multiplication and Growth

Earthworms' multiplication and growth during 70 days (10 weeks) of vermicomposting are illustrated in Fig. 1.

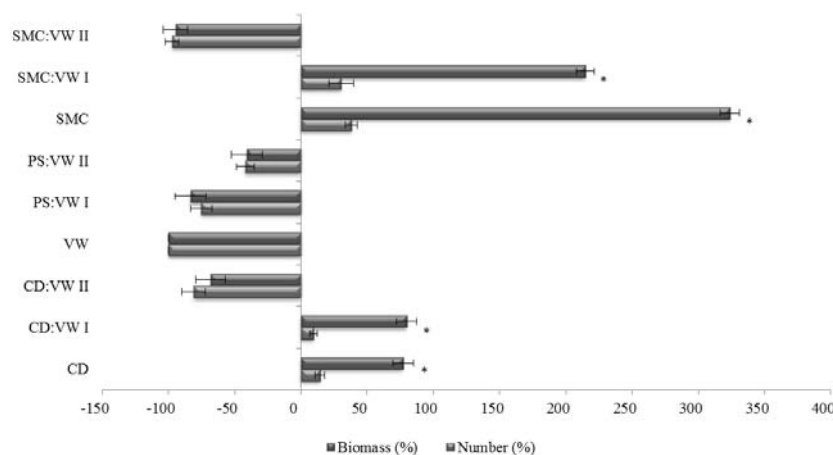


Fig. 1. Earthworm multiplication and growth during vermicomposting.

Table 2 . Nutrient elements i.e. N, P, and K content (mg·kg⁻¹) and C:N ratio in vermibeds in week 0 and vermicompost in week 10.

Vermibed	Week 0			Week 10		
	NPK content					
	N	P	K	N	P	K
^a CD (control)	1.45	0.57	1.98	1.22±0.053	0.23±0.030	1.18±0.056
CD:VW I	1.22	0.43	1.65	1.25±0.040	0.24±0.015	1.27±0.082
CD:VW II	1.10	0.35	1.14	1.20±0.045	0.29±0.025	1.27±0.076
VW (control)	1.38	0.66	1.75	1.38±0.045	0.11±0.032	1.18±0.035
PS:VW I	1.18	0.67	1.24	1.30±0.080	0.37±0.050	1.29±0.075
PS:VW II	1.18	0.44	1.07	1.37±0.040	0.37±0.057	1.29±0.050
SMC (control)	1.19	0.67	1.22	1.18±0.032	0.29±0.040	1.08±0.047
SMC:VW I	1.15	0.48	1.22	1.18±0.020	0.27±0.020	1.31±0.031
SMC:VW II	1.23	0.37	1.13	1.31±0.031	0.30±0.070	1.23±0.056
C:N ratio						
CD (control)	30.24			26.87±2.79		
CD:VW I	28.59			19.62±0.11		
CD:VW II	15.68			27.17±0.95		
VW (control)	27.14			24.66±2.67		
PS:VW I	26.47			23.39±1.88		
PS:VW II	22.05			21.26±1.25		
SMC (control)	21.20			21.65±1.67		
SMC:VW I	28.75			25.76±1.13		
SMC:VW II	29.59			22.30±0.10		

Values are mean and standard deviation (mean±SD; n = 3).

^aRefer to Table 1 for vermibed description.

The highest biomass and number increment was in vermibed of 100% SMC with +323.72% and +38.10%, respectively, whereas the lowest in biomass and number was in vermibed of 100% VW with -100% in both biomass and number. In the paired samples t-test conducted for all vermibeds between both biomass and number, the result showed that a significant difference ($P < 0.05$) in vermibeds 100% of CD, CD:VW I, 100% SMC and SMC:VW I. In fact, those significantly different vermibeds were only vermibeds yielding an increment percentage in both biomass and number. It was evidence that the earthworms in 100% of VW recorded 100% mortality starting from the first two weeks of the vermicomposting process and gradual circumstances of mortality occurred for SMC:VW II, PS:VW I, CD:VW II, and PS:VW II (in descending trend).

Multiplication and growth of earthworm biomass and number are conditional to earthworm palatability on the feedstock mixture or vermibed quality. Multiple increments in SMC vermibed (i.e. SMC:VW I and 100% of SMC, Fig. 1) might be due to the presence of fungi in the forms of mycelia, which provided a nutritious food source for earthworms' physiological development. Michael Bonkowski et

al. [15] analyzed the possible explanation for fungal preferences by earthworms to focus on the nutritional value of the fungus, or the presence of antibiotics or other deterrent metabolites in or around mycelia. Hence, it can be inferred that fungi primarily function as indicators of food quality to earthworms or other microbes/detritivores in soil. Apart from that, the SMC sawdust base used in this study acted as an absorbent to VW releasing moisture content, thus reducing the amount of distilled water utilized to moisten the feedstock mixture during the vermicomposting process. Mortality of earthworms recorded possibly derived from the high moisture content of VW, which was released during the intermediate period of the monitoring schedule. During that period, the earthworms experienced asphyxiate conditions, which led to a lethal phase. A similar situation occurred on PS vermibeds, i.e. PS:VW I and PS:VW II, where the results reflect that PS was not able to absorb excess moisture content released by VW and resulted in quick water stagnation in the microcosms followed by earthworm mortality. Moreover earthworm mortality also was reported in vermibeds of more than 50% of VW i.e. SMC:VW II, PS:VW II, CD:VW II and 100% of VW, and

Table 5. Comparison of heavy metals ($\text{mg}\cdot\text{kg}^{-1}$) contained in vermicompost with EU, USA compost limits and Malaysian site screening levels (SSLs).

Heavy metal	EU limit range ^a	USA biosolids limit ^b	Malaysian Site Screening Levels (SSLs) ^c		Vermicompost ^d
			Residential soil	Industrial soil	
Cr	70-200	1,200	70	810	0.0005
Cd	0.7-10	39	280	14,000	0.001
Pb	70-1,000	300	400	800	0.002
Cu	70-600	1,500	3,100	41,000	0.001-7.50
Zn	210-4,000	2,800	23,000	310,000	8.03-73.24

^{ab} Limits set for compost applied in European countries and United States [34].

^c Recommended levels for Malaysian contaminated site screening based on Contaminated Land Management and Control Guidelines No. 1 [35].

^d Vermicompost from vermicomposting in week 10.

als and N-fixing bacteria. Moreover, the earthworm itself has its role in generating N content derived from mucus, nitrogenous excretory substances, growing stimulating hormones, and enzymes [18]. Apart from that, decaying tissues of dead earthworms might contribute to the N content in the vermicompost produced, and similar analysis discussed by Suthar [19] approved this suggestion. Decrease in N content is irregularly reported in vermicomposting work, and the occurrence from this study showed that palatability of earthworms on the mixture of the vermibeds hindered the capability of earthworms to facilitate microclimatic condition for the N-fixing bacteria in enriching nutrient content. Moreover, the mortality of earthworms also prevented the secretion of earthworm mucus (polysaccharide) to moisten its body surface for supplementing vermibeds with N-fixer bacteria [20], and consequently the microbial propagation was retarded. Pramanik et al. [21] have reported that acid production during organic matter decomposition by the microorganisms is the major mechanism for solubilization of insoluble P and K, which subsequently results in an increase in P and K contents in vermicompost.

On common circumstances of the vermicomposting process, the earthworms gut enzymes, i.e. acid phosphatases and alkaline phosphatases converted some P into more available forms in gut intestine [22] and the possible action from P-solubilizing microorganisms present in vermicompost is the reason for the increase of P in vermicompost [23], although this study recorded a decrease of P content. The decrease of P is a similar scenario as experienced by Orozco et al. [24], which recorded lower P content in coffee pulp waste after vermicomposting. These differences in the P content after vermicomposting can be attributed to the differences in the chemical nature of the initial raw materials [25], even though there is no possible leaching of P by the excess water that drained through the microcosms. Further study is needed on determining the chemical nature of the vegetable waste. Besides, the situation can possibly be due to part of the N, P, K, and micronutrients being removed from the vermicompost before analysis and assimilated by the earthworms [26]. The decrease in nutrient element content is also caused by the mortality of earth-

worms that was supposedly performing the vermicomposting process. Indeed, a modification on vermibeds amendment to correct earthworm palatability is required in future study. The C:N ratio reflects the spectra of changing C and N concentrations of the biowaste during the composting/vermicomposting process [27]. According to Senesi [28], a C:N ratio < 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of organic waste maturity. Conversely, a high C:N ratio reflects a decrease in biological activity and consequently slow degradation due to the mortality of earthworms. In this study, mortality of earthworms and earthworm palatability has suspended the spectra of C:N ratio to achieve as low as <20, except for CD:VW I treatment. Nevertheless, all treatments recorded a C:N ratio below 30, which measured as adequate C:N ratio because it is considered that the microorganisms require 30 parts of C per unit of N [29].

Heavy Metal Content

Heavy metals content in vermicompost was analyzed on day 70 (week 10) to investigate safe utilization of the vermicompost in agronomic applications. Heavy metals content in all vermibeds is shown in Table 4. Cd, Cr, and Pb content in all vermibeds recorded similar content, i.e. $0.001 \text{ mg}\cdot\text{kg}^{-1}$, $0.0005 \text{ mg}\cdot\text{kg}^{-1}$, and $0.002 \text{ mg}\cdot\text{kg}^{-1}$, respectively. The highest contents for Cu were CD:VW I ($7.50\pm 3.62 \text{ mg}\cdot\text{kg}^{-1}$) and CD:VW II ($73.24\pm 9.92 \text{ mg}\cdot\text{kg}^{-1}$) for Zn. The lowest Cu recorded for PS:VW I, PS:VW II and 100% of SMC ($0.001\pm 0.00 \text{ mg}\cdot\text{kg}^{-1}$), whereas Zn was the lowest content recorded in 100% of SMC ($8.03\pm 4.34 \text{ mg}\cdot\text{kg}^{-1}$).

In small amounts, many of these elements (heavy metals) may be essential for plant growth; however, in higher concentrations they are likely to have detrimental effects on plant growth [30]. All concentrations of heavy metals tabulated in the vermibeds were lower compared to the permissible limits of heavy metals content in compost set by the USA, European countries, and the Malaysian Recommended Site Screening Levels for Contaminated Land (SSLs) (Table 5). Hence, the vermicompost produced

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