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Vermicomposting of Sewage Sludge by Lumbricus rubellus using Spent Mushroom Compost as Feed Material: Effect on Concentration of Heavy Metals

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Abstract

Vermicomposting of sewage sludge (SS) using spent mushroom compost from Pleurotus sajor-caju as feed material was conducted to determine the effect on the concentration of heavy metals, namely Cr, Cd, Pb, Cu, and Zn. Previous studies have reported the feasibility of brandling worms, Eisenia foetida, for vermicomposting SS, whereas we conducted vermicomposting by employing red worms, Lumbricus rubellus, with a combination of different percentages of SS and spent mushroom compost (SMC) for 70 days subsequent to 21 days of precomposting. The vermicompost produced in treatments with a low percentage of SS were fine in texture, dark in colour and odourless in contrast to the initial physical characteristics. Results indicate that growth in earthworm numbers and biomass gain was maximum at 25 : 75 (TD) of SS : SMC compared to other treatments with 5 and 8 fold increases, respectively. The heavy metals contained in vermicompost were 0.25 ~ 11.57-fold higher than the initial concentration due to mineralization and excretion of non-accumulated heavy metals existent in the earthworms' gut, which were present prior to treatments. Even so, the concentration was below the limits set by EU and US biosolid compost standards and safe to be utilized as a biofertilizer and soil conditioner.

Keywords: mass balance, mineralization, urban sewage sludge, Pleurotus sajor-caju compost, vermiculture

1. Introduction

In Malaysia, about 5 million m^3 of sewage sludge (SS) is generated per year due to an increase in population and urbanization [1]. The total cost of managing this waste is estimated to be 1 billion RM (≈ 0.3 billion US$) annually, and this volume of sludge is expected to rise to about 7 million m^3 by 2020 [1]. This SS is an end product from 5,500 sewage treatment plants with approximately 14,400 km of sewers, mostly situated in urban areas, serving more than 12 million people. According to the Department of Statistics in 2010 (updated July 2, 2010), the Malaysian population of 28.25 million will certainly generate an enormous amount of SS and cause a disposal problem in the future. This problem is not limited to Malaysia, but it is also applicable to any global urban centre or highly populated country, as SS is a universal challenge. Management of SS in Malaysia involves final SS (~ 20 tonnes per STP per day), which is currently disposed of in open land or landfills, because SS recycling has not been commercially applied and has only been tested on a research scale. Nevertheless, the newly conceded act under Regulation 16, which enacted applications for disposal of SS on land, is accompanied by a prescribed fee of RM 500 (≈ 151.51 US$) and an additional sewage-related license fee under Regulation 24. This includes a fee/kg for contaminants discharged onto any soil or other land other than inland waters specified in subparagraphs 5(1) (a), (c), or (e) and refers to limits beyond those set for selected parameters, namely biological oxygen demand at 20°C for RM 0.05/kg (≈ 0.015/kg US$), oil, and grease for RM 250/kg (≈ 75.75/kg US$) and ammoniacal nitrogen for RM 500/kg (≈ 151.51/kg US$) [2]. Regrettably, specific permission permits for limits based on heavy metal content in soil as post-disposal monitoring have not been developed.

The amount of spent mushroom compost (SMC) is expected to increase due to a rise in population and demands from the food sector industry. Mushrooms are largely consumed due to their taste, nutrient content and numerous bioactive compounds, which may promote human wellbeing, including the prevention and treatment of several illnesses [3-5]. Due to their high demand, mushroom cultivation has become an industrial crop in Malaysia. Additionally, mushroom production is one of the largest solid-substrate fermentation industries in the world [6]. Mushroom farmers in Malaysia, discard more than 4,000 tonnes of SMC each month, which is commonly sent to dumping sites or openly burnt on mushroom farms. Efficient and practical methods, in terms of minimal capital investment and operating commitment, to manage this valuable organic waste are needed to optimize its use and to ensure the safe disposal of SMC. For these reasons, and in conjunction with the new regulatory constraints on SS coupled with future growing capacity, managing the abundance of different types of wastes has become very challenging and important to sustain a healthy environment.

As a consequence of the new regulations, growing interest in SS and organic waste recycling is now a key focus, with a view to reduce disposal costs, decrease waste transportation and abide by regulatory standards. Current scientific research has focused on the use of organic waste, and the intended use of vermicomposting to stabilize and treat SS during recycling has become a popular trend. This is because SS recycling requires an amendment to dilute the concentration of hazardous substances. In this research, the abundance and nutrient content of SMC were determined, making it a possible type of feed material in the vermicomposting process. The types of feed materials (such as heavy metals, nutrients, etc.) are pertinent to the vermicomposting process. Vermicomposting of SS (municipal or urban) from different stages of sewage treatment plants (primary and final) using epigeic (surface dwellers) i.e. Eisenia foetida, Perionyx excavatus, Eudrilus eugeniae, and anecic earthworms (subsoil dwellers and vertically burrowing feeders) i.e. Lampito mauritii have been implemented and well documented [7-10]. Previous work has shown that organic waste acts as a bulking agent during SS vermicomposting and not only supports E. foetida growth but also lowers the risk of earthworm mortality [11]. Cow dung acts as a mixture in primary SS vermicomposting; however, this is not of prime concern in vermiculture (E. foetida production), as adding 30 ~ 40% primary SS to cow dung is acceptable due to the quality of the vermicompost obtained [8]. Nevertheless, data on SS vermicomposting, essentially an effect of the process by epigeic Lumbricus rubellus (red worms), is exceptionally limited as is the utilization of SMC as a feed material. Furthermore, earthworms (L. rubellus and E. foetida) accumulate significant levels of Cd, Cu, Pb, and Zn from soils contaminated with SS [12-15]. However, another study showed that the vermicomposting of SS mixed with raw vegetable fruit mash appears to increase the content of heavy metals in worm (E. foetida) castings slightly [16].

Therefore, the aim of this work was to determine the potential of SMC as feed material for L. rubellus during vermicomposting of the final SS and to assess the effect of this process on the concentration of heavy metals, namely Cr, Cd, Pb, Cu, and Zn in a mixture of substrates.

2. Materials and Methods

2.1. Experimental design

The final SS generated from the activated sludge process was collected from a sewage treatment plant located in Kuala Lumpur. The SS (also termed sludge cake) was black, had a foul odour and was semi-solid with a moisture content of 60%. The SS was collected in large-sized round plastic buckets (45 L). After collection, non-biodegradable materials such as chewing gum, glass, metal, rubber bands and plastic, as well as the sewage treatment plant residue were removed manually before being placed inside microcosms (experimental containers). SMC was procured from a mushroom farm in Tanjung Sepat, Selangor that produces more than a tonne of Pleurotus sajor-caju per day. SMC discarded after 6 months of cultivation consisted of sawdust and P. sajor-caju mycelia in plastic bags (~ 600 g each). Clitellated earthworms (L. rubellus) were randomly selected from stock cultures maintained in our laboratory. The stock culture used organic and agricultural waste, i.e. SMC sawdust and cow dung, in a 2 : 1 ratio respectively, as feed and bedding materials. The initial physicochemical characteristics of the SS and SMC are shown in Table 1.

Table 1. Initial physico-chemical characteristics of SS and SMC

Treatments were performed in 25 microcosms (360 × 280 × 200 each) artificially designed with a net (250 × 100mm) covering the centre of the lid to allow for aeration, to prevent an interruption and to ensure that the microclimate was maintained. Five treatments were prepared with four replicates plus one control, each without earthworms. The composition and ratio of substrate mixture (SS : SMC) and its initial dry weight in these five treatments were as follows: T_A 100% of SS; T_B 75 : 25 (3.75 kg : 1.25 kg); T_C 50 : 50 (2.5 kg : 2.5 kg); T_D 25 : 75 (1.25 kg : 3.75 kg); and T_E 100% of SMC. All microcosms were maintained in an earthworm reservoir (shed area) at ambient conditions (room temperature 25 ± 3°C, 60 ~ 80% relative humidity).

After 21 days of pre-composting all treatments (feed mixtures), 500 g (~ 150 g dry weight) of each feed mixture was randomly collected from each treatment to analyse heavy metals at day 0 of vermicomposting. Samples were air dried in the reservoir at room temperature (25 ± 3°C) for 1 day and stored in plastic airtight vials for heavy metal analysis. Fifty clitelated earthworms per microcosm, of approximately the same size (5078.0 ± 458.36 mg, mean ± SEM), were used in the experiment. The earthworms were introduced into each microcosm, which contained the feed mixtures. During the pre-composting period, pH and temperature were monitored to be certain that the optimal pH of 7 ± 1 and temperature (27 ± 1°C) were achieved and maintained by manual turning. Pathogens are effectively inactivated during this period, also termed thermocomposting [17]. Pre-composting was also performed to avoid exposure of earthworms to high temperatures during the initial thermophilic stage of microbial decomposition [18]. The pH for T_A and T_E remained as the initial pH indicated in Table 1 throughout the process.

The vermicomposting lasted 70 days. During this process, the moisture content of feed materials was maintained at 70 ± 10% by periodic sprinkling of an adequate quantity of distilled water [19] using wash bottles (80 ~ 160 mL per microcosm) and simultaneously turned manually once every 3 ~ 4 days to remove any stagnant water and odour and to eliminate volatile gases that are potentially toxic to earthworms. No other mixtures or feed materials were added during the experimental stage. At the end of the study, earthworms were manually removed, and the total number and biomass were measured to determine their growth.

The upper layer of vermicompost produced in the microcosm was sampled (500 g) to analyse heavy metals before the earthworms were removed [20]. The samples were prepared using the same method at day 0 for heavy metal analysis. The upper layer (2 ~ 3 cm) was sampled, because it is the first layer that is converted into a vermicast. The number of living earthworms was determined after hand sorting and removing all extraneous material. Heavy metal mass balance was developed to determine the flow source of heavy metals analysed. The heavy metal mass balance was calculated as follows:

Input content *(heavy metal in feed material + microbe)* = *Output content* *(heavy metal in vermicast + microbe).*

Total mass of the feed mixture was weighed on days 0 and 70. Mass balance was calculated as follows:

Input mass *(initial mass of feed material)* = *Output mass* *(total mass of product).*

where: Output mass = Product mass (earthworm biomass gain + vermicompost) + Microbial respiration product (microbe biomass + H2O + CO2 + inorganic salts).

2.2. Analysis of physicochemical qualities and heavy metals

Total organic carbon was determined using the partialoxidation method [21]. Total Kjeldahl Nitrogen (TKN) was estimated by Kjeldahl digestion with concentrated H_2SO_4 (1 : 20, w/v) followed by distillation [22]. Total phosphorus was detected using a colorimetric method with ammonium molybdate in HCl [23]. Total potassium and heavy metals, i.e. Cr, Cu, Cd, Pb, and Zn were measured by the ignition method using a Perkin Elmer model 3110 Double Beam Atomic Absorption spectrophotometer after digesting the sample with concentrated HNO_3 : concentrated $HClO_4$ (4 : 1, v/v) [12], and the C : N ratio was calculated. pH and electrical conductivity were determined using a doubledistilled water suspension of each substrate and vermicompost in a 1 : 10 ratio (w/v). Ash content was measured according to Nelson and Sommers [24]. Biological oxygen demand and chemical oxygen demand were analysed using standard methods 405.1 and 5220C, respectively [25].

2.3. Statistical analysis

The statistical analysis was conducted using SPSS 16.0 standard version (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance was performed to analyse significant differences between treatments during vermicomposting. Tukey's t-test was used to assess the effects of heavy metals on earthworm growth. The relationship between the percentage of SMC in each treatment and the percentage biomass gain and loss in earthworms was determined by regression analysis. P-values < 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Earthworm growth during vermicomposting

Significant differences were observed in the final biomass of L. rubellus earthworms (mg) (F = 24.88, P = 0.00) and in the final number of earthworms (F = 15.32, P = 0.00) among different feed mixture treatments. Furthermore, other growth parameters also revealed statistical differences for biomass gain and loss (%) (F = 13.13, P = 0.00), biomass gain and loss rate (mg/day) (F = 24.97, P = 0.00), number gain and loss (%) (F = 15.32, P = 0.00) and mortality rate (%) (F = 9.23, P = 0.008). Earthworms presented maximum and minimum mean biomass on TD (40762.5 ± 4976.67 mg) and TA (1870.0 ± 1195.92 mg), respectively (Table 2).

Table 2. Earthworms multiplication in biomass and number for five different treatments (mean ± S.E.M.; n = 4)

Earthworm biomass rate (mg/day) and biomass gain and loss (%) were observed in the following sequence: TD > TC > TB > TE > TA. In contrast, the number of earthworms at the end of the experiment (day 70) and earthworm number gain and loss (%) were observed in the following order: TD > TC > TE > TB > TA. An increasing percentage of SS in the microcosms promoted a decrease in the biomass and number of L. rubellus. In addition, the highest mortality rate and biomass loss were recorded with a TA of 100% SS (63.6 ± 4.86%). This finding is in agreement with that of Gupta and Garg [8], who vermicomposted primary SS with E. foetida, which resulted in a decrease in earthworm biomass. The gain or loss in the number of earthworms was proportional to the gain or loss in earthworm biomass, except for TB and TE (Table 3). This may have been caused by the chemical nature of the feed materials (organic waste), which influences earthworm palatability directly or indirectly and consequently affects the efficiency of earthworms in a decomposition system [26]. Hence, 100% of SMC in TE as feed material without bedding directly affected the palatability of earthworms and created an unfavourable environment for earthworms. After 70 days of treatment, 100% SMC resulted in a lower increase and biomass of earthworms compared to SMC and SS mixtures. Earthworm mortality rate (%) is shown as TA, TB, and TE (Table 3), although the number of earthworms increased in TC and TD on the final day of vermicomposting.

Table 3. Earthworms multiplication in biomass, number and mortality rate for five different treatments (mean ± S.E.M.; n = 4)

This difference may have been caused by the quality and chemical profile of the organic waste, but this needs further experimental confirmation. This result further suggested that the palatability and quality of food (in terms of chemistry) directly affected the survival rate, growth and reproductive potential of earthworms [27,28].

3.2. Heavy metal concentrations in the final vermicompost

In contrast with organic compounds, heavy metals cannot be degraded and their cleanup requires immobilization, reduction or removal of toxicity. Some heavy metals at low doses are essential micronutrients for plants, but at higher doses, they may cause metabolic disorders and inhibit growth [25]. Our findings showed that heavy metal concentrations in the different vermicompost treatments were significantly ($P < 0.05$) higher than those of initial levels except for Cr ($F = 0.92$, $P = 0.48$) (Table 4). These results are supported by those of Gupta and Garg [8], who observed an increase in heavy metals, i.e. Cr, Pb, Fe, Cu, and Zn following vermicomposting of primary SS and cow dung, and those of Elvira et al. [29] who reported an increase in heavy metal (Fe, Mn, Cu, Zn, Pb, and Ni) concentrations during vermicomposting of sludge from the paper mill and dairy industries. Additionally, Deolalikar et al. [30] claimed that a reduction in feed material weight and volume due to breakdown of organic matter during vermicomposting may be a reason for the increase in heavy metal concentrations in vermicompost; this was illustrated by the decreased output mass as product mass in the mass balance (Table 4).

Table 4. Heavy metal (mg/kg) and mass balance for five different treatments

In contrast, this phenomenon may have been caused by selecting earthworms for their large organic waste consumption to achieve the appropriate nutritional requirement; thus, the mineralization process was considered a result of cooperation with microbes. In relation to the mineralization process, earthworms promote microclimatic conditions in microcosms which increase the loss of feed mixture mass (Table 4) and further concentrate heavy metals in vermibeds. Bound metals in ingested feed materials are incorporated by gut enzymes in the earthworm directly and indirectly by microfloral stimulation; thus, heavy metals are liberated to free forms due to enzymatic action in the earthworm gut [31]. Lukkari et al. [32] stated that heavy metals bound to organic matter (more tightly bound fractions) partly reduce the availability of metals to earthworms. Therefore, from this study, it can be concluded that the concentration of a heavy metals in vermicompost was higher than the initial concentration due to the excretion of worm castes coupled with heavy metals, which primarily reduced the ability of the heavy metal to accumulate in earthworm tissue. The expected amount of non-accumulated heavy metal content in earthworms derived from the heavy metal mass balance calculation is shown in Table 4. Generally, the concentrations of heavy metals were lower in trials compared to the control (Table 4), except for TE of Cr, Cu, and Zn and TA of Cd, Pb, and Zn. Harstenstein and Harstenstein [33] noted that heavy metals in castings increase more than those in activated sludge without earthworms due to the accelerated mineralization process during sludge decomposition and stabilization. Moreover, a study by Benitez et al. [34] indicated that a decrease in dehydrogenase (DH-ase) and hydrolyse (β-glucosidase, urease, BAA-hydrolysing protease and phosphatase) activities as available organic compounds decreased on week 7 of SS vermicomposting. Therefore, it can be deduced that the enzyme metabolic mechanism stops by week 8. Another study [35], which tested the impact of heavy metals on enzymatic activities, concluded that a high concentration of Cu and Zn in a vermicomposting system inhibits DH-ase and microbial activity. These observations indirectly support our finding that the removal of selected heavy metals (in our study, Cu and Zn) was retarded when enzyme and microbial activity decreased. Despite these consequences, it is clear that the heavy metal concentrations in the final vermicompost obtained from mixtures of SS and SMC (up to 100% of SS) were lower than the limits set for compost in EU countries and the USA [36] (Table 5), and that the vermicompost produced had a C : N ratio of 27.27 ~ 28.11.

Table 5. Comparison of heavy metal (mg/kg) contained in vermicompost with EU and USA compost limits

As stated by Bishop and Godfrey [37], adequate stabilization during sewage composting is achieved when the C : N ratio is 25 ~ 35. because microorganisms require 30 parts of C per unit of N. However, the nutrient content of the product (vermicompost) resulted in 1.14 ~ 1.31% TKN, 0.23 ~ 0.30% P, and 1.11 ~ 1.36% K. Table 6 summarizes the heavy metal results using epigeic E. foetida and several other species.

Table 6. Previous selected work on vermicomposting of sewage sludge

According to these studies, L. rubellus also caused an increase in heavy metal content in SS vermicompost using different types of feed materials (Table 6). Only E. foetida in SS amended with sugarcane trash resulted in a decrease in heavy metal content. Therefore, apart from earthworm species, feed material can also be a factor influencing the heavy metal concentration of vermicompost.

3.3. Suitability of SMC composition during vermicomposting

SMC is decomposed through vermicomposting using L. rubellus, and a previous study by Nik Nor Izyan et al. [20] showed that the highest percentage of nutrient elements in vermicompost is obtained when 80 : 20 of cow dung : SMC is used. The relationship between the percentage of SMC and earthworm growth rate (mg/day), examined by a regression analysis (results not shown), resulted in a weak correlation between these two parameters (R2 = 0.17). Consequently, the percentage of SMC used in the treatments was not related to L. rubellus growth rate, so other proportions of SMC and SS are recommended. The composition of organic waste with SS caused a reduction in heavy metal content when Suthar [38] recorded losses of heavy metals in experimental vermibeds of 80, 60, 40, and 20% sugarcane trash amended with SS. Sawdust-based SMC [39] is physically wet and putrefies quickly; therefore, it needs to be processed and its physical capacity, i.e. storage period and nutritional value, need to be improved. This SMC usually serves as animal feed for ruminants. Because of the nature of sawdust-based SMC, the highest population of earthworms was encountered in TC and TD; the combination of SMC and SS yielded an odourless vermicompost with a fine texture, although for higher ratios of SS, the substrates were clumped, as observed by Kaushik and Garg [40] with vermicomposted material of mixed soil textile mill sludge and cow dung. Removing heavy metals using earthworms is feasible and reduces the concentration of heavy metals considerably, although a longer period of vermicomposting is required based on earlier studies; Yadav and Garg [41] indicated 84 days, whereas Suthar [42] indicated 90 days.

4. Conclusion

Vermicomposting is a realistic and practical solution for solving the problem of SS and SMC disposal. The present findings showed that increases in earthworm numbers and growth were maximum in a 25 : 75 ratio of SS : SMC, suggesting that adding an appropriate amount of SS (25 ~ 50%) to SMC can be used as feed material for vermicomposting. A lower percentage of SS in vermibeds is recommended if the main objective is vermiculture (i.e. earthworm production). Notably, applying SS vermicompost as a soil stabilizer or fertilizer would not have an adverse impact on heavy metal content.

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