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Vermiremoval of heavy metal in sewage sludge by utilising *Lumbricus rubellus*

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ABSTRACT

Experiments were conducted to remove heavy metals (Cr, Cd, Pb, Cu and Zn) from urban sewage sludge (SS) amended with spent mushroom compost (SMC) using worms, *Lumbricus rubellus*, for 105 days, after 21 days of pre-composting. Five combinations of SS/SMC treatments were prepared in triplicate along with a control for each treatment in microcosms. Analysis of the earthworms' multiplication and growth and laboratory analysis were conducted during the tenth and fifteenth week of vermicomposting. Our result showed that the final biomass of earthworms (mg) and final number of earthworms showed significant differences between treatments i.e. $F=554.70$, $P=0.00$ and $F=729.10$, $P=0.00$ respectively. The heavy metals Cr, Cd and Pb contained in vermicompost were lower than initial concentrations, with 90–98.7percent removal on week ten. However, concentrations of Cu and Zn, that are considered as micronutrients, were higher than initial concentrations, but they were 10–200-fold lower than the EU and USA biosolid compost limits and Malaysian Recommended Site Screening Levels for Contaminated Land (SSLs). An increment of heavy metals were recorded in vermicompost for all treatments on week fifteen compared to week ten, while concentration of heavy metals in earthworms' tissue were lower compared to vermicompost. Hence, it is suggested that earthworms begin to discharge heavy metals into their surroundings and it was evident that the earthworms' heavy metals excretion period was within the interval of ten to fifteen weeks.

1. Introduction

The toxicity of heavy metals and the danger of bioaccumulation in the food chain represent a major environmental health problem. Disposal of sewage sludge (SS) by spreading it on land causes environmental hazards to terrestrial ecosystems and humans due to toxic pollutants such as heavy metals, toxic organic compounds (e.g. polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorobenzenes, aromatic and alkyl amine) and pathogenic agents (bacteria, protozoa and viruses) contained in the sludge. Moreover, overwhelming complexity and unpredictable substances enter sewers from hospitals and industrial plants inundated by run-off from non-point sources, and thus SS is classified as very hazardous and highly toxic (Rockefeller, 2002). In a developing country like Malaysia, about five million m^3 of SS are generated per year due to increasing population and urbanisation (Ahmadun and Alam, 2002). The total cost of managing SS has been estimated at RM 1 billion ($\approx$ USD 0.3 billion) annually, and the volume of sludge is expected to rise to about seven million m^3 by the year 2020 (Ahmadun and Alam, 2002). SS is the end product of 5500 sewage plants and approximately 14,400 km of sewers, mostly situated in urban areas, serving a population of more than twelve million people. In fact, the total Malaysian population as reported by the Department of Statistics Malaysia in the 2010 census (updated 5 August 2011—corrigendum) was 28.3 million, and will account for an enormous amount of SS production, disposal problems and impacts on humans in the future. In spite of this, sewage treatment methods do not involve any tertiary treatment, which comprises the removal of toxic substances including heavy metals and further removal of suspended solids and organic matter. Discarding the final SS leads to further concentration of the pollutants in the environment so, it is apparent that alternative methods are necessary in order to achieve sustainable management of wastewater treatment by-products. In this way monitoring of the toxicity can be facilitated and a reduction in the amount of hazardous agents released into the environment can be realised.

Apart from the population increase, which is followed by an increase in the volume of SS, the demand for food industry and food variety also increases correspondingly. Cultivation of mushrooms, a protein-rich food results in an abundance of organic waste being generated since mushroom farmers in Malaysia discard more than 4000 t of spent mushroom compost (SMC) each month (Azizi et al., 2011). Efficient and practical methods of

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managing this valuable organic waste are needed in order to optimise its usage and to ensure the safe disposal. In the management of SMC, it is commonly sent to landfill or openly burnt at mushroom farms and its' potential in recycling has been applied in livestock food consumption. The possibility of using SMC as earthworm feed material in vermicomposting was proposed by Nik Nor Izyan et al. (2009), and the effect on the concentration of heavy metals was tested by Azizi et al. (2011) with SMC contents of 25, 50, 75 and 100 percent. Hence, this work further utilises SMC as an amendment for removing heavy metals in sewage sludge.

Earthworms are a key indicator of ecosystem health and many studies have been performed on the response of earthworms to metals (Nahmani et al., 2007). Vermiremoval is an enhancement of the natural process that integrates earthworms' and microbes' role as an efficient tool in accumulating heavy metal in the earthworms' tissues. Moreover, its ability to safely remove metals in polluted environments is widely known. Earthworms ingest, grind and digest organic waste with the help of aerobic and anaerobic microflora in their gut, converting it into much finer, humified, microbially active material (Maboeta and Van Rensburg, 2003). Vermicompost or vermicast produced from the process is stable and homogenous, has desirable aesthetics, may have reduced levels of contaminants, and furthermore is a valuable, marketable and superior plant growth medium (Aranda et al., 1999). From the bibliographic studies, the utilisation of organic waste to stabilise and treat SS has become a popular trend. Many efforts have been made to recycle SS into a safe and value-added product – i.e. vermicompost – by using epigeic and anecic earthworms (Gupta and Garg, 2008; Khwairakpam and Bhargava, 2009; Suthar, 2010; Azizi et al., 2011). Most researchers use *Eisenia foetida, Perionyx excavatus, Eudrilus eugeniae* and *Lampito mauritii* as their bioremediation agent to inoculate the SS. However, there is a paucity of research on the capacity of *Lumbricus rubellus* or red worms for vermicomposting of SS. Additionally, our study supports the conclusion proposed by Nahmani et al. (2007) who suggested that earthworm species other than *Eisenia foetida* should be evaluated to compare this earthworm to other, soil-dwelling species.

Combining these anthropogenic wastes in order to revitalise the health of polluted environments following the accumulation of heavy metals by using earthworms is a significant green technology. In the meantime, the trend of research in earthworms' bioaccumulation has limited to some extend of experiment period but at longer period has rarely been studied and documented. This study on the duration of heavy metal removal from the wastes also investigates the change of heavy metal content in vermicompost after its removal period. Therefore, this work aimed to study the removal of heavy metal in SS using SMC as an amendment or feed material for *L. rubellus* via vermicomposting and to investigate the effect of heavy metals content until 105 days (15 weeks) of the vermicomposting process.

2. Methods

2.1. Experimental design

The final SS was collected from a sewage treatment plant (STP) situated in Kota Damansara, Kuala Lumpur. The SS was black in colour with a foul odour, semi-solid, had a moisture content of 60 ± 10 percent, and was collected in large-sized round plastic buckets (45 L). Subsequent to collection, non-biodegradable materials such as chewing gum, glass pieces, metal pieces, rubber bands, plastics etc. were removed manually. Before being placed in microcosms, SS was dried under direct sunlight for a week in open plastic containers (720 mm × 400 mm × 200 mm) with periodic manual turning to ensure complete drying. SS was mixed with SMC procured from a mushroom farm producing more than a tonne of *Pleurotus sajor-caju* per day in Tanjung Sepat, Selangor. No pesticide was used to grow the mushrooms because of the concern that food mushrooms grown for human consumption would absorb the toxic pesticide, leading to further accumulation, hence the SMC is chemical free. SMC discarded after six months of cultivation consists of sawdust and *P. sajor-caju* mycelia in plastic bags of ~600 g each. In this study, SMC with any visible mould was discarded to ensure that only *P. sajor-caju* mycelia (milky white in colour) were consumed by earthworms. The plastics bags were ripped off and the sawdust-based SMC was then weighed. Earthworms (*L. rubellus*) were randomly selected from stock cultures maintained in the Earthworm Reservoir, Institute of Biological Sciences (ISB), University of Malaya. The stock culture used organic and agricultural waste, i.e. sawdust-based SMC and cow dung, as feed and bedding materials. The initial physico–chemical characteristics of the SS and SMC are tabulated in Table 1.

The treatments were performed in 25 microcosms or experimental containers (360 mm × 280 mm × 200 mm) artificially designed with a net (250 mm × 100 mm) covering at the centre of the lid to allow for aeration, to prevent any disturbance by pests, and to provide microclimatic conditions (Azizi et al., 2011). The experiment was prepared in triplicates with a control for each treatment; one microcosm without earthworms acted as the control. The compositions of SS and SMC in five different treatments were twenty percent:80 percent (T_A); 40 percent:60 percent (T_B); 60 percent:40 percent (T_C); 80 percent:twenty percent (T_D) and 100 percent:0 percent (T_E). Total dry-weight (SS+SMC) of each treatment was 3.50 kg per microcosm. All the microcosms were kept in an earthworm reservoir (shed area) under identical ambient conditions (room temperature 25 ± 3 °C, relative humidity 60–80 percent).

After 21 days (three weeks) of pre-composting, 100 g (~30 g dry weight) of the feed mixtures were randomly collected from each treatment for laboratory analysis on day 0 of vermicomposting. The samples were air dried in the reservoir at room temperature, 25 ± 3 °C, for one day and stored in airtight plastic vials. Thirty-five mature or clitellate *L. rubellus* earthworms, weighing $84.9 \times 10^2 \pm$ 116 mg (mean ± SEM) and of approximately the same size, were introduced into each microcosm. During the pre-composting period, pH and temperature were monitored to make sure that the optimum pH of 7 ± 1 and temperature of 27 ± 1 °C were achieved and stabilised by manual turning. Artificial perforated straw was spiked inside the mixture to enhance aeration of the inner part of the mixture in between manual turning. This period, which is also termed thermo-composting, effectively inactivates pathogens (Nair et al., 2006) and avoids exposure of the earthworms to high temperature during the initial thermophilic stage of microbial decomposition (Loh et al., 2005). However, the pH range for T_E could not be maintained at the optimal level since homogenous substrates were used without mixtures or amendment and the initial pH remained as shown in Table 1.

The vermicomposting process lasted for 105 days (Gupta and Garg, 2008), during which two laboratory analyses were carried out on day 70 and 105 for heavy metal analysis of the vermicompost. If an increment of heavy metal was recorded in the vermicompost, analysis of the earthworms' tissue was conducted, and if a decrease in heavy metal was reported, nutrient element analysis of the vermicompost was performed. During the vermicomposting process, the moisture content of feed materials was maintained at 70 ± 10 percent by periodic sprinkling of an adequate quantity of distilled water (Sangwan et al., 2008) 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 which are potentially toxic to earthworms. No extra mixtures of feed materials were added during this experimental stage.

On days 70 and 105, the upper layer of vermicompost produced in the microcosm was sampled (100 g, 70 percent moisture content) for laboratory analysis before all of the earthworms were removed (Nik Nor Izyan et al., 2009). The upper layer was sampled because it is the first layer being converted into vermicast. The total number and biomass of living earthworms were measured after hand sorting and removal of all extraneous material to determine their multiplication and growth. Two days before the measurements were taken, none

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

Parameter	SS[a]	SMC[b]
pH	6.89 at 32.0 °C	5.37 at 29.6 °C
EC (mS/cm)	1.13	1.56
Ash content (%)	15.8	27.5
TOC (%)	66.3	56.3
TKN (%)	5.35	2.35
TP (%)	1.10	1.23
TK (%)	0.490	0.980
C:N ratio	12.4	24.0
BOD (ppm)	49	1080
COD (ppm)	185	4120

[a] Sewage sludge.
[b] Spent mushroom compost; TOC: total organic carbon; TKN: total Kjeldahl nitrogen; TP: total phosphorous; TK: total potassium; BOD: biochemical oxygen demand; COD: chemical oxygen demand.

of the treatments were watered, to make the sifting of vermicompost easier (Adi and Noor, 2009). Living earthworms (~5 g of biomass) were sampled on day 105 from each treatment by hand sorting and left for a depuration period of 12 h on filter paper in 500 ml glass beakers in a laboratory before the analysis of heavy metal in the earthworms' tissue. The depuration period is the time allowed for earthworms to egest any contaminants from their gut so that subsequent analysis records earthworms' body burden and is not biased by residual contaminants (Nahmani et al., 2007). Heavy metal mass balance was developed to determine the flow source of heavy metals analysed. The heavy metal mass balance was calculated according to Azizi et al. (2011):

$$\text{Input content}_{(\text{heavy metal in feed material}+\text{microbe})} = \text{Output content}_{(\text{heavy metal in vermicast}+\text{microbe})}.$$

The heavy metal content increment and removal, biomass and earthworms gain and loss and mortality were calculated as follows:

$$\frac{(\text{Analysis on day 70 or 105} - \text{Analysis on day 0})}{\text{Analysis on day 0}} \times 100$$

The biomass gain and loss rate was calculated as follows:

$$\frac{\text{Biomass (week 10 or week 15)} - \text{Biomass (initial)}}{\text{70 days or 105 days}}$$

2.2. Physico–chemical qualities and heavy metal analysis

Total organic carbon (TOC) was determined by the partial-oxidation method (Walkley and Black, 1934). Total Kjeldahl nitrogen (TKN) was estimated by Kjeldahl digestion with concentrated H_2SO_4 (1:20, w/v) followed by distillation (Bremner and Mulvaney, 1982). Total phosphorus (TP) was detected by using the colourimetric method using ammonium molybdate in HCl (John, 1970). Total potassium (TK) and the heavy metals 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 a concentrated HNO_3 to concentrated $HClO_4$ ratio of 4:1 (v/v) (Loh et al., 2005). The C:N ratio was analysed through calculation. The pH and electrical conductivity (EC) were determined using a double-distilled water suspension of each substrate and vermicompost in the ratio of 1:10 (w/v). Ash content was measured by Nelson and Sommers (1982). BOD and COD were analysed using the standard methods APHA 5220 C and APHA 5210 B respectively.

2.3. Statistical analysis

Statistical analysis was carried out using the computer software package SPSS 16.0 (Standard Version). One-way analysis of variance (ANOVA) was performed to analyse the significant differences between treatments during vermicomposting at 0.05 percent level of significance. Tukey's *t*-test was used to identify the homogeneous types of the microcosms for their heavy metals, nutrient elements, and earthworms' multiplication and growth parameters. The relationship between the percentage of SMC in each treatment and the earthworms' percentage biomass gain and loss were analysed using regression analysis.

3. Results and discussion

3.1. Earthworm multiplication and growth during vermicomposting

Lumbricus rubellus showed significant differences among treatments (feed mixtures) with regard to biomass(mg) of earthworms ($F=500.50$, $P=0.00$; Table 2) and number of earthworms ($F=438.65$, $P=0.00$; Table 2) on week ten as well as biomass (mg) of earthworms ($F=554.70$, $P=0.00$) and final number of earthworms ($F=729.10$, $P=0.00$) on week fifteen. Furthermore, other growth parameters also revealed statistical differences in biomass gain and loss (percent; $F=466.58$, $P=0.00$), rate of biomass gain and loss (mg day^{-1}; $F=418.50$, $P=0.00$), and number gain and loss (percent; $F=438.64$, $P=0.00$) on week ten. Week fifteen also yielded significant differences in biomass gain and loss (percent; $F=554.70$, $P=0.00$), rate of biomass gain and loss (mg day^{-1}; $F=358.08$, $P=0.00$), and number gain and loss (percent; $F=729.11$, $P=0.00$). The earthworms' rate of change in biomass (mg day^{-1}) observed on weeks ten and fifteen followed the order: $T_A > T_B > T_C > T_D > T_E$, whereas biomass gain and loss (percent) on weeks ten and fifteen5 followed the order: $T_A > T_B > T_C > T_D$ and T_E. The earthworms' number gain and loss (percent) on weeks ten and fifteen was in the order $T_A > T_C > T_B > T_D$ and T_E, and only T_D and T_E recorded a mortality rate of 100 percent.The increasing percentage of SS in the microcosms promoted a decrease in biomass and number of *L. rubellus* and this also agreed with previous work of municipal sewage sludge vermistabilisation amended with sugarcane trash using *Eisenia foetida* (Suthar, 2009). In addition, the highest mortality rate was recorded when the amount of SS was 80 percent or above. This finding is in agreement with Gupta and Garg (2008), who utilised primary SS in vermicomposting by using *Eisenia foetida* and experienced a decrease of biomass gain in higher primary SS composition. In addition, previous work also recorded that an increasing percentage of SS in microcosms promoted a decrease in the biomass and number of *L. rubellus* (Azizi et al., 2011). Hence, 80–100 percent of SS in T_D and T_E as feed materials might be due to the difference in chemical composition of waste mixtures used for vermicomposting, although this needs further studies. Yadav and Garg (2011) concluded that the survival rate of earthworms also depends upon the rate of food consumption during acclimatisation of worms in the waste mixtures. Changes in the chemical composition of feed, changes in the pH of the substrate, a higher C:N ratio of the initial substrate, and production of toxic or foul smelling gases (ammonia carbon dioxide, nitrogen oxides, etc.) may be some of the factors responsible for earthworm mortality (Flegel and Schreder, 2000). High consumption and an ample amount of food in the vermibeds may have led to the increases in earthworm multiplication and growth (biomass gain) recorded in T_A–T_C, while scarcity of food (organic waste) caused earthworms to compete, but led to mortality in T_D and T_E. This also further suggests that the palatability and quality of food (in terms of their chemistry) directly affected the survival, growth rate and reproduction potential of earthworms (Gajalakshmi et al., 2005; Ndegwa and Thompson, 2000). On the other hand, there is a dramatic decrease in the biomass growth rate of earthworms during the later phase (week fifteen) as summarised in Table 2. The decreased growth rate in the last phase of vermicomposting may be due to exhaustion of the feed substrate and this situation has also been reported by other researchers (Sangwan et al., 2008; Yadav and Garg, 2011).

3.2. Heavy metal content in vermicompost (week ten)

In contrast with organic compounds, heavy metals cannot be degraded and their clean-up requires immobilisation, toxicity reduction or removal. Some heavy metals are essential micronutrients for plants at low doses, but in higher doses they may cause metabolic disorders and growth inhibition in most plant species (Sinha et al., 2005). Our findings showed that vermicomposting of material by different treatments had a significant ($P < 0.05$) effect on some heavy metals, i.e. Cu ($F=54.14$, $P=0.00$) and Zn ($F=17.18$, $P=0.00$), while it was not significant for others ($P > 0.05$). Concentrations of heavy metals, namely Cr, Cd and Pb, on week ten of vermicomposting (T_A–T_E) were lower than initial concentrations in the raw materials except for Pb in T_D and T_E. Cu and Zn were found to have increased on week ten of vermicomposting compared to initial concentrations (Table 3). Our results are supported by Suthar et al. (2008), who showed that soil chemical properties, especially organic matter contents, ingested by earthworms play an important role in the accumulation of metals in earthworms' tissues. In addition, heavy metals content decreased in vermicompost and accumulated in the earthworms' tissue as it passed through the alimentary canal of the earthworms, and there were no holes beneath the microcosms that could be the cause of leaching and drainage of heavy metals or cations. Increments of Cu and Zn on week ten of vermicomposting (except Cu in T_A and T_B) might be caused by

Table 2
Earthworms growth in biomass and multiplication in number for five different treatments (mean ± S.E.M.; $n=3$)

Treatment	Biomass of earthworm (mg)			Biomass gain/loss (%)		Biomass gain/loss rate (mg day^{-1})	
	Initial	Week 10	Week 15	Week 10	Week 15	Week 10	Week 15
T_A	8790 ± 204	18.5×10^3 ± 538a	18.9×10^3 ± 693ab	110. ± 6.20de	120. ± 5.50ab	138 ± 7.10de	98.1 ± 5.60ab
T_B	8780 ± 39.7	17.1×10^3 ± 545a	10.5×10^3 ± 340.ab	94.9 ± 5.60ab	19.0 ± 4.20cd	119 ± 7.40ab	15.9 ± 3.40cf
T_C	8860 ± 158	15.7×10^3 ± 545ab	800. ± 218cd	76.9 ± 7.30ae	−90.8 ± 2.60ab	97.1 ± 8.40ae	−75.7 ± 3.10ab
T_D	80.0×10^2 ± 108	0.00cd	0.00de	−100.bc	−100.ae	−114 ± 1.60bc	−114 ± 1.60cg
T_E	8040 ± 307	0.00cd	0.00de	−100.bc	−100.ae	−115 ± 4.40bc	−115 ± 4.40cg
	Number of earthworms			Earthworm number gain/loss (%)		Mortality (%)	
	Initial	Week 10	Week 15	Week 10	Week 15	Week 10	Week 15
T_A	35.0	40.3 ± 1.70ab	248 ± 8.11ab	14.9 ± 4.70ab	610. ± 23.2ab	–	–
T_B	35.0	37.0 ± 1.10ab	68.0 ± 3.21ab	5.70 ± 3.10ab	94.3 ± 9.18ab	–	–
T_C	35.0	38.0 ± 1.10ab	5.33 ± 1.45ac	8.57 ± 3.10ab	−84.8 ± 4.15ac	–	84.8 ± 4.15ac
T_D	35.0	0.00bc	0.00bd	−100.bc	−100.bd	100.bc	100.bd
T_E	35.0	0.00bc	0.00bd	−100.bc	−100.bd	100.bc	100.bd

– Nil, no mortality calculated in the treatment.
Mean value followed by different letters is statistically different (ANOVA; Tukey's test, $P < 0.05$). The experiment lasted until the 105th day.

Table 3
Heavy metal content and heavy metal mass balance from five different treatments in week 10.

Treatment	0 day (mg kg^{-1}) (Initial[a])	70 days (mg kg^{-1}) (Final[b])	Control[c] (mg kg^{-1})	Increment/removal (%)
	Cr			
T_A	7.50×10^{-2}	$< 1.00 \times 10^{-3}$[d] ± 0.00a + $< 7.40 \times 10^{-2}$[e]	5.00×10^{-4}	−98.7
T_B	$< 1.00 \times 10^{-2}$	$< 1.00 \times 10^{-3}$ ± 0.00a + $< 9.00 \times 10^{-3}$	5.00×10^{-4}	−90.0
T_C	$< 1.00 \times 10^{-2}$	$< 1.00 \times 10^{-3}$ ± 0.00a + $< 9.00 \times 10^{-3}$	5.00×10^{-4}	−90.0
T_D	$< 1.00 \times 10^{-2}$	$< 1.00 \times 10^{-3}$ ± 0.00a + $< 9.00 \times 10^{-3}$	5.00×10^{-4}	−90.0
T_E	$< 1.00 \times 10^{-2}$	$< 1.00 \times 10^{-3}$ ± 0.00a + $< 9.00 \times 10^{-3}$	5.00×10^{-4}	−90.0
	Cd			
T_A	$< 1.00 \times 10^{-2}$	$< 1.00 \times 10^{-3}$ ± 0.00a + $< 9.00 \times 10^{-3}$	1.00×10^{-3}	−90.0
T_B	$< 1.00 \times 10^{-2}$	$< 1.00 \times 10^{-3}$ ± 0.00a + $< 9.00 \times 10^{-3}$	1.00×10^{-3}	−90.0
T_C	$< 1.00 \times 10^{-2}$	$< 1.00 \times 10^{-3}$ ± 0.00a + $< 9.00 \times 10^{-3}$	1.00×10^{-3}	−90.0
T_D	$< 1.00 \times 10^{-2}$	$< 1.00 \times 10^{-3}$ ± 0.00a + $< 9.00 \times 10^{-3}$	1.00×10^{-3}	−90.0
T_E	$< 1.00 \times 10^{-2}$	$< 1.00 \times 10^{-3}$ ± 0.00a + $< 9.00 \times 10^{-3}$	1.00×10^{-3}	−90.0
	Pb			
T_A	$< 1.00 \times 10^{-2}$	$< 2.00 \times 10^{-3}$ ± 0.00b + $< 8.00 \times 10^{-3}$	2.00×10^{-3}	−80.0
T_B	$< 1.00 \times 10^{-2}$	$< 2.00 \times 10^{-3}$ ± 0.00b + $< 8.00 \times 10^{-3}$	2.00×10^{-3}	−80.0
T_C	$< 1.00 \times 10^{-2}$	$< 2.00 \times 10^{-3}$ ± 0.00b + $< 8.00 \times 10^{-3}$	2.00×10^{-3}	−80.0
T_D	$< 1.00 \times 10^{-2} + < 0.586$[f]	0.596 ± 3.00×10^{-1}b	0.671	$+9.26 \times 10^3$
T_E	$< 1.00 \times 10^{-2} + < 0.875$	0.885 ± 7.00×10^{-1}b	0.605	$+15.4 \times 10^3$
	Cu			
T_A	7.89	0.206 ± 2.10×10^{-1}de + 7.68	1.00×10^{-3}	−94.7
T_B	6.67	3.63 ± 3.60×10^{-1}ab + 3.04	8.11	−40.2
T_C	7.22 + 3.04	10.3 ± 1.16ae	7.55	+58.2
T_D	3.85 + 28.7	32.6 ± 2.63bc	27.2	+814
T_E	3.97 + 25.4	29.3 ± 3.46bc	34.1	+726
	Zn			
T_A	30.3 + 11.4	41.7 ± 3.80ab	29.7	+50.4
T_B	28.0 + 39.0	67.0 ± 2.20ab	89.8	+147
T_C	32.4 + 89.2	122 ± 6.20ab	106	+294
T_D	12.1 + 249	261 ± 40.5bc	270	$+2.39 \times 10^3$
T_E	27.1 + 268	295 ± 45.9bc	263	$+1.16 \times 10^3$

[a] Initial heavy metals content of the feed materials in the microcosms. In heavy metal mass balance this is: input content (heavy metal in feed material + microbe).
[b] Final vermicompost; values are mean and standard error (mean ± S.E.M.; $n=3$) followed by different letters is statistically different (ANOVA; Tukey's test, $P < 0.05$). In heavy metal mass balance this is: output content (heavy metal in vermicast + microbe).
[c] Experimental control-no earthworms introduced.
[d] Heavy metal content in vermicompost [70 days (Final) column].
[e] Heavy metal content expected accumulated in earthworms introduced for each treatment [70 days (Final) column].
[f] Heavy metal content expected as non-accumulative in earthworms introduced for each treatment [0 day (Initial) column].

earthworms' selection and large consumption of organic waste to achieve appropriate nutrition. This is because earthworms promote microclimatic conditions in microcosms that increase the loss of organic carbon through the earthworms' feeding mechanism and microbial degradation and thus further concentrate the heavy metals in vermibeds. In the meantime, the bound metals in ingested feed materials were incorporated with the earthworms' gut enzymes directly and indirectly by stimulation of the microflora. Hence metals are liberated in free forms due to the enzymatic action in the earthworms' gut (Suthar, 2007). In some other cases, Lukkari et al. (2006) stated that binding of metals to organic matter (more tightly bound fractions) partly reduced the availability of metals for earthworms. Therefore, the concentration of heavy metal in vermicompost was higher compared to initial concentrations. Generally, the concentrations of heavy metals were lower in treatments compared to control (Table 3), except: T_E for Cr; T_D and T_E for Pb; T_B and T_E for Cu; and T_B and T_D for Zn. Hartenstein and Hartenstein (1981) found that heavy metals in castings showed a greater increase than those in sludge without earthworms (control) due to the mineralisation process, which is accelerated by earthworms during sludge decomposition and stabilisation. High levels of heavy metals in vermicompost compared to compost (control) in a study by Benitez et al. (1999) indicated that the decrease in dehydrogenase (DH-ase) and hydrolysis (β-glucosidase, urease, BAA-hydrolysing protease and phosphatase) activities as available organic compounds decreased on week seven of SS vermicomposting. Therefore it can be inferred that the mechanism of breakdown by enzymes had stopped by week eight. This is followed by another study (Malley et al., 2006) that tested the impact of heavy metals on enzymatic activities and concluded that high concentrations of Cu and Zn in a vermicomposting system inhibited the dehydrogenase and microbial activity. These facts indirectly support our findings of high Cu and Zn concentrations, which decrease during enzyme and microbial activity, and the removal of heavy metals is slowed. As a consequence, it is clear that the concentrations of heavy metals in the final vermicompost obtained from mixtures of SS and SMC with up to100 percent SS were smaller than the limits set for compost in European countries, USA and the Malaysian Recommended Site Screening Levels for Contaminated Land (SSLs) (Brinton, 2000 and DOE, 2009).

3.3. Nutrient element in vermicompost (week ten)

As a decrease in heavy metals was recorded during week ten, nutrient elements were analysed to determine the stabilisation and quality (in terms of nutrient content) of the vermicompost to ensure the safe disposal of SS into the environment, and the results are shown in Table 4. A considerable amount of nutrient elements were found in the vermicompost with regard to Total Kjeldahl N (1.14–1.31 percent), Total P (0.230–0.300 percent) and Total K (1.11–1.36 percent). During the vermicomposting, N content was derived from earthworms' mucus, nitrogenous excretory substances, growth-stimulating hormones, and enzymes (Tripathi and Bhardwaj, 2004). P and K in the vermicompost probably resulted from the direct action of the earthworm gut enzymes and indirectly from stimulation of the microflora (Satchell and Martin, 1984) and total organic C (27.5–31.7 mg kg^{-1}), which signals a reasonable decrease relative to the initial content in each prime substrate. The basis for the decrease of C is the loss of C in the form of CO_2 from the substrates during the decomposition and mineralisation of organic waste. Dominguez and Edwards (2004) reported that earthworms fragment and homogenise the ingested material through muscular action of their foregut and also add mucus and enzymes to the ingested material, thereby increasing the surface area for microbial action, while microorganisms perform the biochemical degradation of waste material, providing some extracellular enzymes within the worm's gut. Thus, the combined action of earthworms and microorganisms brings about the loss of C from the substrates in the form of CO_2.The C:N ratio was within the range of 23.4–26.1,as given in Table 4, and this range was found to be adequate because it is considered that the microorganisms require 30 parts of C per unit of N (Bishop and Godfrey, 1983).

3.4. Heavy metal content in vermicompost and earthworms' tissue (week fifteen)

At this stage, the heavy metals content in earthworms' tissue were lower compared to the heavy metals content in vermicompost (day 105) except for Cr in T_B (Table 5). According to Nahmani et al. (2007), accumulation and excretion rate may differ between metals as reported by Peijnenburg et al. (1999), rapid uptake and equilibration demonstrated for Cr, Ni, Cu and Zn but little uptake for As, Cd and Pb. In fact, no earthworm population was identified in T_D and T_E due to the high proportion of SS, which was unfavourable in terms of palatability by earthworms and consequently hindered their survival. The highest content of heavy metals in earthworms' tissue was Zn (22.1–23.1 mg kg^{-1}) and the lowest was Cd (< 0.001 $mgkg^{-1}$ in all treatments). The high contents of Zn and Pb in earthworms' tissue can be explained by Morgan and Morris (1982), who studied the accumulation and intracellular compartmentalisation of metals in *L. rubellus* and *D. rubida* from highly contaminated soil. From electron microprobe analysis, they identified the major chemical constituents of the chloragogenous tissue, especially in chloragosome granules, as being P, Ca, Zn and Pb, and further confirmed in preliminary analyses of cryosections the presence of Pb and Zn (with Ca and P) together with the absence of Cd in the chloragosomes was high in *L. rubellus* compared to *D. rubida*. The contents of heavy metal in both compost and tissue in this study are basically in accordance with the total content in the feeding mixtures and the contrasting trends in the results from week ten validate the excretion of heavy metals from the earthworms' body burden into its environment. It proves that, at a certain period, earthworms do not ecologically pose a risk of higher food chain contamination, as extend to previous work of Suthar and Singh (2009) on *Eisenia foetida* in municipal sewage sludge vermicomposting. This work suggests that *L. rubellus* inoculation reduced the heavy metals availability in substrate at ten weeks of vermicomposting but ecologically at longer period of fifteen weeks should be considered as to eliminate roles of the earthworms as mediator of heavy

Table 4
Nutrient elements content in vermicompost in week 10.

Nutrient elements (%)	T_A	T_B	T_C	T_D	T_E
TOC	$30.5 \pm 4.70 \times 10^{-1}$a	$31.7 \pm 8.70 \times 10^{-1}$b	30.6 ± 1.42c	$27.5 \pm 9.70 \times 10^{-1}$d	30.7 ± 1.37e
TKN	$1.19 \pm 4.00 \times 10^{-2}$e	$1.22 \pm 4.00 \times 10^{-2}$d	$1.30 \pm 6.00 \times 10^{-2}$c	$1.14 \pm 2.00 \times 10^{-2}$b	$1.31 \pm 2.00 \times 10^{-2}$a
TP	$2.80 \times 10^{-1} \pm 4.00 \times 10^{-2}$c	$3.00 \times 10^{-1} \pm 2.00 \times 10^{-2}$d	$3.00 \times 10^{-1} \pm 5.00 \times 10^{-2}$b	$2.30 \times 10^{-1} \pm 2.00 \times 10^{-2}$a	$3.00 \times 10^{-1} \pm 2.00 \times 10^{-2}$b
TK	$1.16 \pm 3.00 \times 10^{-2}$ac	$1.11 \pm 2.00 \times 10^{-2}$cd	$1.32 \pm 3.00 \times 10^{-2}$ab	$1.36 \pm 3.00 \times 10^{-2}$ab	$1.28 \pm 4.00 \times 10^{-2}$be
C:N ratio	25.7 ± 1.25d	26.1 ± 1.25c	23.6 ± 2.10e	$24.0 \pm 3.60 \times 10^{-1}$a	23.4 ± 1.30b

TOC: Total Organic Carbon; TKN: Total Kjeldahl Nitrogen; TP: Total Phosphorous; TK: Total Potassium.
Mean value followed by different letters is statistically different (ANOVA; Tukey's test, $P < 0.05$).

Table 5
Heavy metal content and mass balance in vermicompost and earthworms tissue in week 15.

Heavy metal	[a]V (day 70) (mg kg^{-1})	[b]V (day 105) (mg kg^{-1})	[c]E (day 105) (mg kg^{-1})
	T_A		
Cr	$<1.00\times10^{-3}\pm0.00a+4.80$[d]	3.32 ± 1.40d	$1.48\pm1.00\times10^{-1}$b
Cd	$<1.00\times10^{-3}\pm0.00a+1.00\times10^{-3}$	$1.00\times10^{-3}\pm0.00$d	$<1.00\times10^{-3}\pm0.00$e
Pb	$<2.00\times10^{-3}\pm0.00b+5.56$	$3.35\pm9.00\times10^{-1}$a	2.21 ± 0.00c
Cu	$0.206\pm2.10\times10^{-1}de+6.04$	$5.67\pm4.00\times10^{-1}$ab	$5.80\times10^{-1}\pm6.00\times10^{-1}$a
Zn	41.7 ± 3.80ab$+35.7$	54.5 ± 3.80ad	$22.9\pm8.00\times10^{-1}$d
	T_B		
Cr	$<1.00\times10^{-3}\pm0.00a+2.38$	$6.20\times10^{-1}\pm6.00\times10^{-3}$a	$1.76\pm4.00\times10^{-2}$c
Cd	$<1.00\times10^{-3}\pm0.00a+1.00\times10^{-3}$	$1.00\times10^{-3}\pm0.00$c	$<1.00\times10^{-3}\pm0.00$d
Pb	$<2.00\times10^{-3}\pm0.00b+4.79$	$2.49\pm6.00\times10^{-1}$d	$2.30\pm7.00\times10^{-2}$b
Cu	$3.63\pm3.60\times10^{-1}$ab$+9.74\times10^{-1}$	3.34 ± 3.30ab	$1.26\pm6.30\times10^{-1}$e
Zn	67.0 ± 2.20ab	29.3 ± 29.3bd	23.1 ± 2.70c$+14.7$[e]
	T_C		
Cr	$<1.00\times10^{-3}\pm0.00a+4.43$	$2.99\pm6.00\times10^{-1}$e	$1.44\pm2.00\times10^{-1}$a
Cd	$<1.00\times10^{-3}\pm0.00a+1.00\times10^{-3}$	$1.00\times10^{-3}\pm0.00$a	$<1.00\times10^{-3}\pm0.00$f
Pb	$<2.00\times10^{-3}\pm0.00b+8.16$	5.97 ± 1.80ce	$2.19\pm3.00\times10^{-2}$a
Cu	10.3 ± 1.16ae$+6.71$	$16.4\pm6.00\times10^{-1}$ab	$5.50\times10^{-1}\pm5.50\times10^{-1}$d
Zn	122 ± 6.20ab	$140.\pm4.10$ce	22.1 ± 3.21e$+40.4$
	T_D		
Cr	$<1.00\times10^{-3}\pm0.00a+3.89$	$3.89\pm3.30\times10^{-1}$de	–
Cd	$<1.00\times10^{-3}\pm0.00a+1.00\times10^{-3}$	$1.00\times10^{-3}\pm0.00$b	–
Pb	$0.596\pm3.00\times10^{-1}b+4.88$	$5.48\pm3.90\times10^{-1}$b	–
Cu	32.6 ± 2.63bc	30.3 ± 4.73de$+2.30$[f]	–
Zn	261 ± 40.5bc	250 ± 42.6da$+10.8$	–
	T_E		
Cr	$<1.00\times10^{-3}\pm0.00a+1.00\times10^{-3}$	$1.00\times10^{-3}\pm0.00$ed	–
Cd	$<1.00\times10^{-3}\pm0.00a+1.00\times10^{-3}$	$1.00\times10^{-3}\pm0.00$e	–
Pb	$0.885\pm7.00\times10^{-1}b+1.02$	$1.90\pm3.00\times10^{-2}$ec	–
Cu	29.3 ± 3.46bc	$1.00\times10^{-3}\pm0.00$ae$+29.3$	–
Zn	295 ± 45.9bc	$1.00\times10^{-3}\pm0.00$ed$+295$	–

– Nil, no earthworms detected in the treatment.
Mean value followed by different letters is statistically different (ANOVA; Tukey's test, $P<0.05$).
[a] Heavy metal content in vermicompost (day 70).
[b] Heavy metal content in vermicompost (day 105).
[c] Heavy metal content in earthworms' tissue.
[d] Heavy metal content expected as non-accumulative in the earthworms introduced for each treatment.
[e] Heavy metal content expected to be detoxified by storage or metabolised in the earthworms introduced for each treatment.
[f] Heavy metal content expected to be detoxified by storage or metabolised in the earthworms introduced for each treatment before mortality.

metal transfer to possible higher food chain contamination due to the earthworms' heavy metal excretion period. Even so, different species showed different excretion periods, metabolic physiology and palatability, and so further studies should be undertaken. Regression analysis was performed to determine the relationship between heavy metal content in earthworms' tissue and vermicompost on week fifteen (results not shown) and a weak relationship ($R^2<0.5$) was found. Similar result was supported by Beyer and Cromartie (1987) who reported a weak relationship between soil and earthworms' metal (Pb, Zn, Cu, Cd, Cr, As and Se) body burden. Other parameters may be relevant in terms of biochemical aspects such as enzymatic action, mechanism for mobility and availability of heavy metals in relation to the content of pore water (moisture content), and microbial colonisation to ascertain the heavy metal concentration incorporated in the process, however, this needs further experimental confirmation. As found by Nahmani et al. (2007) in a study relating metal accumulation to soil properties, increases in pH, organic matter content and cation exchange capacity will either increase the sorption of metal ions into the soil (substrate) particles, thereby reducing soil solution metal concentration and increase competition between metal cations at uptake sites, or reduce the concentration of free metal ions in solution by complexation, thereby reducing the availability of metals to earthworms. Nonetheless, the relative difference in the content of heavy metal in earthworms' tissue and vermicompost on week fifteen reflects the fact that there is an increment in heavy metals in vermicompost – from the earthworms – after the decrease in content in vermicompost recorded on week ten. Thus, it is concluded that during the mentioned period, the earthworms had reached the excretion period when the accumulated heavy metals were egested from the earthworms' bodies. Apart from that, there was the possibility of heavy metals, i.e. Cu and Zn (essential elements), being detoxified by storage or metabolised in the earthworms as explained from the analysis of heavy metal mass balance in Table 5 and supported by previous works by Cancio et al. (1995) and Morgan et al. (1995) although further histological analysis is needed to support the above hypothesis.

3.5. Suitability of SMC composition in vermicomposting and heavy metal removal

SMC can be decomposed through vermicomposting by using *L. rubellus*. A previous study by Nik Nor Izyan et al. (2009) showed

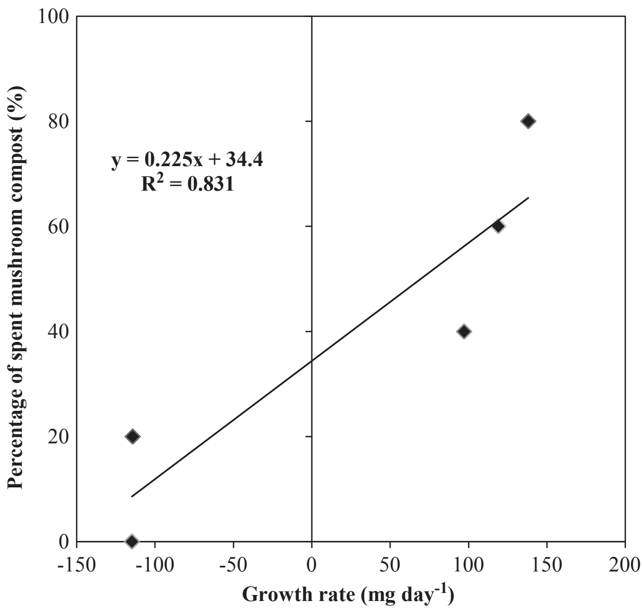

Fig. 1. Relationship between earthworm growth and percentage of spent mushroom compost in vermibeds.

that the highest percentage of nutrient elements in vermicompost was obtained from an 80:20 ratio of cow dung and SMC. In fact, SMC is a nutrient-rich organic by-product of the mushroom industry and many studies have been conducted and have reported its successful use as animal feed worldwide (Kwak et al., 2008). The relationship between percentages of SMC and earthworms' growth rate (mg day^{-1}) in this study was examined by regression coefficient (Fig. 1). The figure shows a close relationship between the two axes ($R^2=0.831$). It is inferred that the percentages of SMC in treatments used was related to the growth rate of *L. rubellus*. In T_A–T_C the combination of SMC and SS yielded a material that was fine in texture, dark in colour, moist and odourless, whereas with higher contents of SS, that is T_D and T_E, the substrates were clumpy and brittle, as also observed by Kaushik and Garg (2003) in vermicomposted material made from a mixture of soil textile mill sludge and cow dung. Importantly, the use of SMC can alter the pH of SS in a mixture, so the mixture results in a desirable pH for earthworms to carry out vermicomposting of hazardous SS. Moreover, mycelia found in the SMC area nutritious food source as earthworms have a substantial dietary intake of edible fungi. An analysis by Bonkowski et al. (2000) found a possible explanation for fungal preferences by earthworms by focusing on the nutritional value of the fungus or the presence of antibiotics or other deterrent metabolites in or around mycelia and concluded that fungi primarily function as indicators of food quality to earthworms or other microbes/detritivores in soil, so much so that without SMC the earthworms would have suffered from difficulty living in an opaque environment where there is poor nutrition and mineral and organic matter in their surroundings as occurs in SS alone.

4. Conclusions

The increasing volume of urban SS disposed in the environment poses a serious threat to the entire ecosystem, especially with regard to the bioaccumulation of heavy metals in crops that are later consumed by humans. Nonetheless, large-scale production of SMC also encounters the same phenomenon and the use of animal feeds should be diversified. As proposed in this study, the combination of SS and SMC led to 90 percent removal of heavy metals, especially Cr, Cd and Pb, through the process of vermicomposting accompanied by maximal earthworm multiplication and growth at an SS to SMC ratio of 20 percent:80 percent(T_A) on week ten. The present findings show that earthworms should be removed from the process before week fifteen because during this stage the excretion of heavy metals from earthworms occurred. Adequate stabilisation of the C:N ratio was achieved, and considerable nutrient content and a desirable physical state were obtained in the vermicompost on week ten. Essentially, application of the week ten vermicompost as soil stabiliser or fertiliser will not pose any antithetical impacts related to the heavy metal contents in the vermicompost.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2012.12.006.

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