Effect on heavy metals concentration from vermiconversion of agro-waste mixed with landfill leachate

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A B S T R A C T

Spent Pleurotus sajor-caju compost mixed with livestock excreta, i.e. cow dung or goat manure, was contaminated with landfill leachate and vermiremediated in 75 days. Results showed an extreme decrease of heavy metals, i.e. Cd, Cr and Pb up to 99.81% removal as effect of vermiconversion process employing epigeic earthworms i.e. Lumbricus rubellus. In addition, there were increments of Cu and Zn from 15.01% to 85.63%, which was expected as non-accumulative in L. rubellus and secreted out as contained in vermicompost. This phenomenon is due to dual effects of heavy metal excretion period and mineralisation. Nonetheless, the increments were 50-fold below the limit set by EU and USA compost limits and the Malaysian Recommended Site Screening Levels for Contaminated Land (SSLs). Moreover, the vermicompost C:N ratio range is 20.65–22.93 and it can be an advantageous tool to revitalise insalubrious soil by acting as soil stabiliser or conditioner.

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1. Introduction

Malaysia’s high annual rainfall due to the tropical climate coupled with high moisture contents from the incoming waste stream have led to the significant impact of leachate generation from landfills. As reported by Kortegast et al. (2007), the resulting base flow of leachate in the Bukit Tagar landfill (15–20% of the mass of incoming waste) is significantly higher than that experienced even in Hong Kong (5–10% of the waste mass) and is a major contributor to the total flow (approximately 60% annually at Bukit Tagar). In addition, Kortegast et al. (2007) also provided calibrated estimates of infiltration for intermediate cover slopes and confirmed the critical nature of rigorous water exclusion measures in such a wet climate as auxiliary evidence to the total flow of leachate in the Bukit Tagar landfill. Leachate treatment plants in the landfill treat up to 1000 cubic metres of leachate per day and irrigate a 120-acre field (instead of discharging into open-water solution treatments, the adsorbents used were difficult to separate from the wastewater, while the soil treatments were highly priced relative to the large hectare areas.

Proper and profitable management via recycling of agricultural waste generated, including livestock excreta, into a valuable product such as compost is an environmentally sound practice. Integrating leachate with an enormous amount of organic waste for earthworm feed materials in vermiconversion could be an effective technique in the bioremediation of contaminated landfill leachate. Vermiconversion is an efficient eco-biotechnology tool utilising earthworms to decompose organic waste into a valuable product, henceforth called vermicompost, as a final product. This eco-biotechnology tool involves the aerobic, biodiation and stabilisation of non-thermophilic processes of organic waste facilitated by earthworms to fragment, mix and promote microbial activity. Technically, the vermiconversion process involves physical/mechanical (mixing and grinding) and biochemical activities (microbial decomposition in the earthworms' intestine) (Loh et al., 2005). The effect of heavy metals concentration in vermiconversion utilising Lumbricus rubellus has been tested in previous
To ensure only bags (Malaya. Clitellated earthworms (L. rubellus) consumed by earthworms. Cow dung (CD) was procured from a different treatments and design of the work are shown in Fig. 1. CD, in a 2:1 ratio as feed and bedding materials, respectively. Stock culture used organic and agricultural waste, i.e. SMC and selected from stock cultures maintained in the Earthworm Reservoir at room temperature 25 ± 3°C for one day and stored in a digestion block at 170°C for 2 h. The prepared samples were transferred into centrifuge tubes and final volume of 50 mL was made by adding ultra-pure (Mili-Q) water. Digestion of the vermicompost samples was by microwave method. The parameters of microwave digester were IR temperature 260°C, pressure 180 bar and frequency ranging between 50 and 60 Hz (Gupta et al., 2010). The heavy metals were extracted using extraction of the diethylene-triaminepentaacetic acid (DTPA) method (Fernández-Gómez et al., 2012).

2. Materials and methods

2.1. Landfill leachate, spent mushroom compost and earthworm preparation

Landfill leachate samples were collected from the inlet feed of a leachate treatment facility in Bukit Tigar located in the sparsely populated area of the Hulu Selangor district, approximately 50 km from Kuala Lumpur city. The samples were collected via grab sampling in a large-sized round plastic black bin (45 L). Spent mushroom compost (SMC) was procured from a mushroom farm that produces more than a tonne of Pleurotus sajor-caju per day in Tanjung Sepat, Selangor. SMC discarded after six months of cultivation consisted of sawdust and P. sajor-caju mycelia in plastic bags (~600 g each). SMC with any visible mould was discarded to ensure only P. sajor-caju mycelia (milky white in colour) were consumed by earthworms. Cow dung (CD) was procured from a livestock farm in Putrajaya and goat manure (GM) was acquired from the Mini Farm, Institute of Biological Sciences, University of Malaya. Clitellated earthworms (L. rubellus) were randomly selected from stock cultures maintained in the Earthworm Reservoir at Institute of Biological Sciences, University of Malaya. The stock culture used organic and agricultural waste, i.e. SMC and CD, in a 2:1 ratio as feed and bedding materials, respectively.

2.2. Experimental design

The experiment was conducted using epigeic L. rubellus or red worms in microcosm (360 mm × 280 mm × 200 mm) artificially designed with a net (250 mm × 100 mm) covering the centre of the lid to allow aeration, to prevent any interruption of pests and to imitate climatic conditions (Azizi et al., 2011, 2013). The experiment was prepared in triplicates with 2 L of raw landfill leachate in each replicate. The composition of substrates in four different treatments and design of the work are shown in Fig. 1. During the pre-composting period, pH and temperature were monitored until the optimum level of pH 7 ± 1 and temperature of 27 ± 1°C was achieved and stabilised by manual turning. This period, which is also termed thermo-composting, effectively inactivates pathogen (Nair et al., 2006) and prevents the exposure of earthworms to high temperatures during the initial thermophilic stage of microbial decomposition (Loh et al., 2005). All of the microcosms were kept in the Earthworm Reservoir (shed area) with identical ambient conditions (room temperature 25 ± 3°C, relative humidity 60–80%). Following 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 at day 0 of vermicomversion. The samples were air dried in the reservoir at room temperature 25 ± 3°C for one day and stored in plastic vials (airtight). During the vermicomversion process, the moisture content of feed materials was maintained at 70 ± 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 which are potentially toxic to earthworms. No extra mixtures of feed materials were added during this experimental stage. On day 75, the upper layer of the vermicompost (100 g, 70% moisture content) produced in the microcosms was sampled (similar to the technique used for day 0 sampling) for laboratory analysis before all of the earthworms were removed manually by hand sorting. The upper layer was sampled because it was the first layer converted into vermicompost. The total number and biomass of living earthworms were measured every 15 days (0, 15, 30, 45, 60 and 75) by quantification and weighing scale after hand sorting and removing all of the extraneous material using tissue paper on the earthworms’ bodies. Heavy metal mass balance was calculated according to Azizi et al. (2011, 2013):

\[
\text{Input content} = \text{Output content} = \text{Biomass or Number on day 75} - \text{Biomass or Number on day 0}
\]

2.3. Heavy metal analysis

2.3.1. Analytical procedure for vermicompost samples

All the chemicals and reagents used for estimation of toxic heavy metals were of analytical grade (E-Merck, UK). Stock certified standard solution of Cr, Cd, Pb, Cu and Zn containing 1000 ppm of each metal was used as stock solutions with appropriate dilution. Four samples of vermicompost from each treatment (in triplicates) were powdered in an electrical blender. Homogenised powder (0.25 g) of each was weighed in a quartz vessel and 8 mL aqua regia was added. The samples were digested for 12 h. The prepared samples were transferred into centrifuge tubes and final volume of 50 mL was made by adding ultra-pure (Mili-Q) water. Digestion of the vermicompost samples was by microwave method. The parameters of microwave digester were IR temperature 260°C, pressure 180 bar and frequency ranging between 50 and 60 Hz (Gupta et al., 2010). The heavy metals were extracted using extraction of the diethylene-triaminepentaacetic acid (DTPA) method (Fernández-Gómez et al., 2012).

2.3.2. Instrumentation

Atomic absorption spectrometry was carried out on a PerkinElmer Model Analyst 800 with hollow cathode lamp (HCL) and electrode less discharge lamp (EDL). Electrode discharge lamp was used for volatile and non-volatile toxic heavy metals analysis under optimum operating conditions with an air-acetylene flame and argon gas. The instrument was controlled by a personal computer using Winlab software. Graphite furnace was used to measure (non-volatile) Cu, Zn, Pb, Cr and Cd metals (Gupta et al., 2010). Limit of quantification (LOQ) was determined by dilution until the response level of the sample was 10 times (S/N = 10:1) that of noise. The limits of quantification for Cd, Pb, Cu and Zn were 0.001 mg kg⁻¹ and Cr 0.0005 mg kg⁻¹.

2.4. Total organic carbon, total Kjeldahl nitrogen analysis and C:N ratio

As regards method used for the total organic carbon, we followed Saint-Laurent et al. (2014), which was developed by Yeomans and Bremner (1988). The samples were placed in a digestion tube and 5 mL of acidified dichromate solution (K₂Cr₂O₇–H₂SO₄) was added for 30 min. The tube was placed in a preheated digestion block at 170°C for 30 min. The sample was cooled and 0.3 mL of the N-phenylanthranilic acid-based indicator and sodium carbonate were added. After these steps, titration was performed with ammonium ferrous sulphate solution at 0.05 mol l⁻¹. Total
nitrogen was determined by the Kjeldahl method (Quikchem Method, 1996). The samples were placed in glass tubes, and concentrated sulphuric acid (H₂SO₄) was used for the reagent after the tubes were placed on a block digester, which was preheated and heated to 390 ± 5 °C and digested for 2 h. The solution was filtered with no. 2 filter paper, and the ammonium content was analysed using a Flow Injection Analyzer based on Lachat Method No. 13-107-06-2-D. This method was repeated from Saint-Laurent et al. (2014). C:N ratio was analysed through calculation.

2.5. Statistical analysis

Statistical analysis was carried out using SPSS 16.0 (Standard Version). One-way analysis of variance (ANOVA) was carried out to analyse the significant difference of the data obtained between the treatments during vermicomposting at a 0.05% level of significance. LSD test was used to identify the homogeneous type of the microcosms for their heavy metals and nutrient elements.

3. Results and discussion

3.1. Multiplication and growth of L. rubellus

The highest earthworm number was observed at day 75 in the CD:SMC II group with a 15.24% increase, followed by CD:SMC I (11.43%) and GM:SMC I and II with 9.52% each. The biomass growth of earthworms (g) in different treatments is illustrated in Fig. 2, with the highest growth in GM:SMC II and the lowest growth in CD:SMC I. The increase in biomass growth rate was significantly different (\(F = 3.788; \ p < 0.05\)) showing the following trend: GM:SMC II > GM:SMC I > CD:SMC II > CD:SMC I with values of 14.32 g day\(^{-1}\), 12.49 g day\(^{-1}\), 8.57 g day\(^{-1}\) and 7.16 g day\(^{-1}\), respectively. It can be postulated that combination of CD and SMC is an effective bedding for earthworms breeding whilst GM presented as earthworms’ growth supplement when it combined with SMC due to its effect on earthworms biomass.

3.2. Heavy metal concentration

The heavy metal concentration in vermicompost resulting from the vermiconversion process are shown in Table 1. The treatments resulted in a radical decrease in the heavy metal concentration. Cd and Pb reflected 90% and 80% removal in all the treatments, respectively. In contrast, Cr depicted the highest removal in GM:SMC II (99.81%) followed by CD:SMC II (99.71%) and both CD:SMC I and GM:SMC I (95%). Nevertheless, an increase of heavy metal was recorded for Cu with 65.01% in CD:SMC II and 85.63% in GM:SMC I. Zn also showed an increase but only in GM:SMC I (95%). The highest removal of Cu was in GM:SMC II (87.57%) and the lowest rate of removal was in CD:SMC I (58.96%). Similar results were recorded for Zn, with the highest in GM:SMC II (79.08%) followed by CD:SMC II (42.93%) and CD:SMC I (17.32%). The results suggest that soil chemical properties, especially organic matter contents (organic waste and livestock excreta), ingested by earthworms,
earthworm tissue after passing through the alimentary canal following ingestion by the earthworms. Moreover, there were no holes beneath the microcosms that could cause leaching and drainage of heavy metals or cations. Increments of Cu and Zn can be explained by Lukkari et al. (2006), who stated that the binding of metals to organic matter (more tightly bound fractions) partly reduced the availability of metals for earthworms. In addition, the increments might also be due to the 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 (Azizi et al., 2013). Previous work suggested that earthworms, i.e. *L. rubellus*, begin to discharge heavy metals into their surroundings and it was evident that the earthworms’ heavy metals excretion period was within the interval of 10–15 weeks (Azizi et al., 2013). Since this vermiconversion process lasted for 75 days, that is more than 10 weeks, the concentration of heavy metal in vermicompost was higher compared with the initial concentrations because of the heavy metal excretion period. Heavy metal mass balance (Table 1) showed that 4.46 mg kg\(^{-1}\) of Cu is expected as non-accumulative in the earthworm’s gut or tissues for CD:SMC II treatment and 4.23 mg kg\(^{-1}\) for GM:SMC I treatment. A similar situation concerns Zn with 7.31 mg kg\(^{-1}\) for GM:SMC I treatment even though this requires further analysis in the earthworm’s alimentary canal and its tissue burden. Besides, all of the concentrations of heavy metals produced in vermicompost were lower compared with EU and USA compost limits (Brinton, 2000) and

**Table 1**

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>CD: SMC I</th>
<th>CD: SMC II</th>
<th>GM: SMC I</th>
<th>GM: SMC II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy metal concentration in each treatment (day 0)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>1.00 (\times) 10(^{-2}) ± 0.00b</td>
<td>1.00 (\times) 10(^{-2}) ± 0.00a</td>
<td>1.00 (\times) 10(^{-2}) ± 0.00d</td>
<td>1.00 (\times) 10(^{-2}) ± 0.00c</td>
</tr>
<tr>
<td>Cr</td>
<td>1.00 (\times) 10(^{-2}) ± 0.00d</td>
<td>1.00 (\times) 10(^{-2}) ± 0.00e</td>
<td>26.0 (\times) 10(^{-2}) ± 0.00f</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>1.00 (\times) 10(^{-2}) ± 0.000a</td>
<td>1.00 (\times) 10(^{-2}) ± 0.000c</td>
<td>1.00 (\times) 10(^{-2}) ± 0.000d</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>3.46 ± 0.00e</td>
<td>6.86 ± 0.000d + 4.46(^{c})</td>
<td>4.94 ± 0.000c + 4.23</td>
<td>10.3 ± 0.00a</td>
</tr>
<tr>
<td>Zn</td>
<td>25.8 ± 0.00c</td>
<td>73.0 ± 0.000d</td>
<td>48.7 ± 0.000c + 7.31</td>
<td>10.3 ± 0.00a</td>
</tr>
<tr>
<td><strong>Heavy metals concentration in vermicompost (day 75)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>1.00 (\times) 10(^{-3}) ± 0.00d + 9.00 (\times) 10(^{-3})</td>
<td>1.00 (\times) 10(^{-3}) ± 0.00d + 9.00 (\times) 10(^{-3})</td>
<td>1.00 (\times) 10(^{-3}) ± 0.00d + 9.00 (\times) 10(^{-3})</td>
<td>1.00 (\times) 10(^{-3}) ± 0.00d + 9.00 (\times) 10(^{-3})</td>
</tr>
<tr>
<td>Cr</td>
<td>5.00 (\times) 10(^{-3}) ± 0.000d + 95.0 (\times) 10(^{-4})</td>
<td>5.00 (\times) 10(^{-3}) ± 0.000d + 17.0 (\times) 10(^{-2})</td>
<td>5.00 (\times) 10(^{-3}) ± 0.000d + 95.0 (\times) 10(^{-2})</td>
<td>5.00 (\times) 10(^{-3}) ± 0.000d + 26.0 (\times) 10(^{-2})</td>
</tr>
<tr>
<td>Pb</td>
<td>2.00 (\times) 10(^{-3}) ± 0.000d + 0.008</td>
<td>2.00 (\times) 10(^{-3}) ± 0.000d + 8.00 (\times) 10(^{-3})</td>
<td>2.00 (\times) 10(^{-3}) ± 0.000d + 8.00 (\times) 10(^{-3})</td>
<td>2.00 (\times) 10(^{-3}) ± 0.000d + 8.00 (\times) 10(^{-3})</td>
</tr>
<tr>
<td>Cu</td>
<td>1.42 ± 7.20 (\times) 10(^{-3}) b + 2.04</td>
<td>11.3 ± 7.22c</td>
<td>9.17 ± 5.43a</td>
<td>1.28 ± 1.22d + 9.02</td>
</tr>
<tr>
<td>Zn</td>
<td>21.3 ± 1.64bc + 4.47</td>
<td>41.7 ± 3.12ab + 31.3</td>
<td>56.0 ± 3.52 cd</td>
<td>19.1 ± 2.84bc + 72.2</td>
</tr>
</tbody>
</table>

CD: cow dung; GM: goat manure; SMC: spent mushroom compost.
Values are mean and standard error (mean ± S.E.M.; n = 3) followed by different letters is statistically different (ANOVA; LSD, P < 0.05).

ab Heavy metal concentration expected as non-accumulative in the earthworms introduced for each treatment (day 0).

b Heavy metal concentration expected as accumulated in the earthworms introduced for each treatment (day 75).

**Table 2**

Comparison of heavy metals (mg kg\(^{-1}\)) contained in vermicompost (day 75) with EU, USA compost limits and Malaysia recommended levels for contaminated sites (SSLs).

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>EU limit range*</th>
<th>USA biosolids limit*</th>
<th>Malaysian Site Screening Levels (SSLs)c</th>
<th>Vermicompostd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residential soil</td>
<td>Industrial soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.7–10</td>
<td>39</td>
<td>70</td>
<td>810</td>
</tr>
<tr>
<td>Cr</td>
<td>70–200</td>
<td>1200</td>
<td>280</td>
<td>14,000</td>
</tr>
<tr>
<td>Pb</td>
<td>70–1000</td>
<td>300</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>Cu</td>
<td>70–600</td>
<td>1500</td>
<td>3100</td>
<td>41,000</td>
</tr>
<tr>
<td>Zn</td>
<td>210–4000</td>
<td>2800</td>
<td>23,000</td>
<td>310,000</td>
</tr>
</tbody>
</table>

ab Limits set for compost applied in European countries and United States (Brinton, 2000).

b Recommended levels for Malaysian contaminated site screening based on Contaminated Land Management and Control Guidelines No. 1 (DOE, 2009).

d Vermicompost produced from the experiment in day 75.
the Malaysian Recommended Site Screening Levels for Contaminated Land (SSLs) (DOE, 2009) (Table 2).

3.3. C:N ratio

C:N ratio is used as an index for the maturity of organic wastes or composted materials. The C:N ratio calculated as shown in Table 3 with 20.65–22.93 range was within the adequate C:N ratio category which is <30. This is because it is considered that the earthworms and microorganisms in the microcosm require 30 parts of C per unit of N (Bishop and Godfrey, 1983). Previous studies found higher C:N ratio compared with this study in vermicompost from saw dust based-SMC with 83.30:1 (Sailila et al., 2010), but lower in vermicompost from reused SMC of pea sprouts cultivation with 11.79:1 (Azizi et al., 2012). This might be due to the effect of landfill leachate contamination that inhibited the activities of the earthworms and microorganisms for further stabilising the feed materials present in the microcosms. Nonetheless, longer period of vermicomposting is suggested in future study to identify greater reduction of C:N ratio which is beyond 75 days. In addition, physical properties of the vermicompost produced were dark in colour, homogenous, odourless and moist, reflecting the bioconversion of the raw substrates into more stable forms.

4. Conclusions

This study demonstrates the removal of heavy metals found in landfill leachate mixed with agricultural waste in the presence of epigeic L. rubellus. Even though increases of Cu and Zn were recorded, this may not hamper the utilisation of the product as soil stabiliser and biofertiliser due to the fact that its contents were recorded, this may not harm the utilisation of the product as soil well as the fate of the heavy metals.

Acknowledgements

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Table 3

<table>
<thead>
<tr>
<th>C:N ratio</th>
<th>CD: SMC I</th>
<th>CD: SMC II</th>
<th>GM: SMC I</th>
<th>GM: SMC II</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)</td>
<td>27.4 ± 6.60 x 10^{-1}a</td>
<td>29.1 ± 8.00 x 10^{-1}c</td>
<td>28.8 ± 1.88b</td>
<td>27.9 ± 1.74d</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>1.23 ± 3.00 x 10^{-4}ac</td>
<td>1.27 ± 3.00 x 10^{-4}ac</td>
<td>1.40 ± 3.00 x 10^{-2}ab</td>
<td>1.24 ± 1.00 x 10^{-2}ad</td>
</tr>
<tr>
<td>N (%)</td>
<td>22.3 ± 6.60 x 10^{-4}d</td>
<td>22.9 ± 19.0 x 10^{-2}b</td>
<td>20.7 ± 1.63c</td>
<td>22.4 ± 1.39a</td>
</tr>
</tbody>
</table>

CD: cow dung; GM: goat manure; SMC: spent mushroom compost. Values are mean and standard error (mean ± S.E.M.; n = 3) followed by different letters is statistically different (ANOVA; LSD, P < 0.05).

References