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



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ABSTRACT

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 courses) following the biological treatments (Sequential Batch Reactors (SBRs), Dissolved Air Floatation (DAF) plant and Reed Beds Polishing by using Phragmites plant). However, the treatments do not specify uptake for certain non-degraded pollutants, namely heavy metals, which potentially migrate into soil and further bioaccumulate in the ecosystem food chain. Heavy metal removal from aqueous solutions and soil has been the focus of research in recent years. The removal of heavy metals is achieved through various techniques, such as electrokinetic treatment, chemical oxidation or reduction, leaching, solidification, vitrification, excavation and off-site treatment (Aboulroos et al., 2006). In the aqueous 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, bioxidation 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

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works on spent mushroom compost and sewage sludge vermicomposting (Azizi et al., 2011, 2013). Hence, vermiconversion of spent mushroom compost supplemented with landfill leachate is explored in the present study. This work examines the effect of vermiconversion activity on heavy metal concentration in the vermicompost produced as well as *L. rubellus* multiplication and growth during the process.

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 Tagar 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 \times 280 mm \times 200 mm) artificially designed with a net (250 mm \times 100 mm) covering the centre of the lid to allow aeration, to prevent any interruption of pests and to imitate microclimatic 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 thermocomposting, effectively inactivates pathogens (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, $100 \text{ g} (\sim 30 \text{ g dry weight})$ of the feed mixtures were randomly collected from each treatment for laboratory analysis at day 0 of vermiconversion. 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 vermiconversion 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 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):

Input content_(heavy metal in feed material + microbe)

 $= Output \ content_{(heavy \ metal \ in \ vermicast \ + \ microbe)}$

The biomass gain and number of earthworms was calculated as:

(Biomass or Number on day 75 – Biomass or Number on day 0) × 100 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 Perkin Elmer 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 ($K_2Cr_2O_7-H_2SO_4$) 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

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