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Heavy metal contaminated soil bioremediation via vermicomposting with spent mushroom compost

Sim Cheng-Kim[a], Azizi Abu Bakar[a,b], Noor Zalina Mahmood[a,b,*], Noorlidah Abdullah[a,b]

[a] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Mushroom Research Centre, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

*Corresponding author, e-mail: alin@um.edu.my

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ABSTRACT: The present work focuses on the use of *Lumbricus rubellus* to remediate leachate-contaminated soil containing heavy metals such as Cu, Mn, Pb, Fe, Cr, Ni, Zn, and As. Three types of treatment were carried out consisting of spent mushroom compost and organic soil in a 2:1 ratio (T1), cow dung and organic soil in a 2:1 ratio (T2), and organic soil (T3) with 30 clitelated earthworms in the respective treatments. The vermiremediation took 90 days to complete. At the end of the experiment, T1 provided the greatest reduction (50%) in the concentration of all heavy metal elements. This result reveals that vermiremediation is a suitable technology to remove heavy metals from soil.

KEYWORDS: earthworms, organic soil, vermiremediation

INTRODUCTION

Vermicomposting is a bioconversion process that uses earthworms to convert biodegradable matter into vermicast. This technology is used globally for solid waste management[1]. Solid waste is a long standing issue in many countries due to a variety of wastes often being dumped together. In Malaysia, food waste has increased from 37% in 2004 to 59% in 2009 and occupies more than half of the waste composition[2]. In 2013, 15 000 tonnes of food and kitchen waste are generated daily. Kuala Lumpur alone has claimed to produce 3000 tonnes of this waste alone every day.

Due to the rapid rate of waste generation, inevitably more land will be required for disposal sites, which will cause land scarcity and consequently an increase in land prices, especially urban areas. The most popular and common disposal method practised in Malaysia is the landfill method due to the economic advantages[3]; up to 95% of all solid waste collected is deposited in landfills. There are approximately 300 disposal sites in Malaysia, 111 of which are mere open-dump sites[4]. Open-dump sites, i.e., without proper lining and treatment, will lead to the leakage of leachate into the soil or discharge directly into water courses without any treatment. This poses a risk and threatens not only the aquatic ecosystem but also human health. This is mainly due to bioaccumulation of toxic compounds throughout the food chain. Moreover, the odour emitted by the leachate can cause health problems such as nausea, headaches, drowsiness, and fatigue[5]. In addition, the waste in Malaysia is known to have high moisture content, and the humid weather assists in increasing the rate of leachate generation. Undeniably, considerable effort is needed to find a solution for the adverse impact of landfill leachate.

In this study, the bioremediation of a landfill leachate was conducted by employing earthworms. The introduction of earthworms was intended to facilitate the process of bioremediation, also known as vermiremediation. Vermiremediation is carried out through vermicomposting. The vermicomposting process consists of two different phases, i.e., the active phase and maturation phase. The former phase involves the process of waste by earthworm while the later stage is taken place by microbes to further decompose the waste processed by earthworm[6]. The process of organic waste decomposition includes all modifications to the decaying organic matter and microorganisms during intestinal transit. By breaking down organic waste into useful products called vermicompost, the effects of pollution are minimized. This has been recognized as a potential method for the management of municipal solid waste, which has gained considerable interest in the Philippines, Nigeria, Thailand, Hong Kong, Singapore, China, Italy, the Netherlands, and India[7]. Several studies[8,9] have shown that vermicomposting is able to achieve safe and low pathogen levels, which is facilitated by microbial and enzymatic activity. Vermicomposting also improves nutrient availability, as the use of earthworms increases the nitrogen mineralization rate[10]. Moreover, vermicompost can act as a buffering material by limiting the acid phase and enhancing waste biostabilization as well as a biofilter by removing heavy metals from the leachate by adsorption[11]. In addition, bioremediation is cheap and has few potential harmful effects to the environment. The aim of this work was to identify the most effective treatment for the removal of heavy metals from leachate-polluted soil.

Table 1 Physicochemical characteristics of Ayer Hitam landfill leachate (AH).

Parameter (mg/l)	Standard	AH
BOD_5	20	2497 ± 221
COD_5	400	4000 ± 313
TSS	50	800 ± 15
NH_3-N	5	3200 ± 185
TOC	NA	$45\,070 \pm 1044$
TN	NA	1700 ± 150
pH	6.0–9.0	8.19 ± 0.17

MATERIALS AND METHODS

Experimental design

Spent mushroom compost (SMC), previously used to grow mushrooms, was obtained from the mushroom house at the University of Malaya; it consisted of sawdust and *Pleurotus sajor-caju* mycelia. Cow dung was collected from a farm in Serdang, Selangor, while organic soil was purchased at a nursery site. Landfill leachate samples were collected from the inlet feed of a leachate treatment facility at Ayer Hitam Landfill located in Puchong, Selangor. This is a closed sanitary landfill with six million tonnes of capacity to receive domestic wastes. It has been operated for 11 years (1995–2006). The samples were collected by the grab sampling method and stored in a black bin. Thirty *Lumbricus rubellus* (clitelated) were randomly chosen from the stock cultures in our earthworm reservoir.

Eighteen microcosms with a size of 360 mm × 280 mm × 200 mm (length × width × height) were prepared for vermicomposting. Each microcosm was designed with a net (250 × 100 mm) at the centre of the lid to allow ventilation or aeration and to prevent the intervention of pest such as mice and flies[12]. Three treatments were conducted with three replicates and one control (without earthworms) for each respective treatment. Each replicate of the treatment and control contained 3 kg of substrate mixture such as spent mushroom compost: organic soil in a 2:1 ratio (T1), cow dung: organic soil in a 2:1 ratio (T2) and organic soil (T3). Then 300 ml of leachate collected from a landfill was added to each of the treatment and control microcosms. The characteristics of the leachate are listed in Table 1.

The mixture then underwent 21 days of pre-composting. The purpose of pre-composting was to stabilize the substrate (feed source), which had been added with the leachate. Pre-composting allows for the process of vermicomposting to be accelerated due to partially break down of the particles. Additionally, it also assists in stabilizing the conditions optimal for the survival of earthworm in terms of pH, moisture, and mass reduction[9]. With this, it reduces potential toxic effects to earthworms. Pre-composting is essential to prevent worm death[13]. During the pre-composting stage, the mixture was periodically stirred for aeration purposes and the moisture content was maintained at 60–70% by spraying with distilled water. Distilled water was selected to avoid the chlorine in tap water, which might affect the chemical composition of the mixture. By the end of the pre-composting period, samples were taken from each of the treatments for initial nutrient and heavy metal analysis. Thirty weighed clitelated earthworms of approximately the same size were then introduced into each treatment. At this stage, 90 days of vermicomposting was carried out. During this period, the moisture content was maintained at 60–70% and the samples were stirred manually for aeration once in every three days. Additionally, pH and temperature were measured every week to ensure that the treatments possessed the optimum conditions (pH 7 ± 1, temperature 27 ± 1 °C) for earthworm development. After 90 days, the earthworms were removed by hand to determine the total number and biomass. The earthworms were cleaned with tissue paper to remove the substrate on the body, which would affect the biomass readings. The number of cocoons in each treatment was counted and approximately 500 g of the substrate mixture was taken for final nutrient and heavy metal analysis. The heavy metal mass balance was previously reported[12]: Input content (heavy metal in feed material + microbe) = Output content (heavy metal in vermicast + microbe). Heavy metal content increases and removal, biomass and earthworm gain/loss and mortality were calculated as $(A_{90} - A_0)/A_0$, where A_0 and A_{90} are the parameters measured at day 0 and day 90, respectively. The biomass gain/loss rate was calculated from $(B_{90} - B_0)/90$, where B_0 and B_{90} are the parameters measured at day 0 and day 90, respectively.

Chemical analysis

A sample was taken from each treatment at the initial and final stages of the experiment and dried. The dried sample was then ground using a mortar and pestle. The powder sample was then sieved using a 0.63 µm sieve to obtain the finest particles. This was performed since the cation exchange capacity is higher with fine particles, which enables heavy metals to be held. Next, 0.25–0.30 g of the finest sample was poured into the fluoropolymer liner and inserted into the ceramic vessel jacket with a protective casing outside. HNO_3 (9 ml), H_2O_2 (2 ml), and HF (1 ml) were added into the fluoropolymer and mixed with the sample. The amount and type of acid being added depended on the composition of the sample. This was partly due to the characteristics of the sample that influenced the chemical reaction with the acid being used. The fluoropolymer was sealed and closed with a protective cap. All the pressure vessels were placed on a rotary tray and capped with a rotor lid before put into a microwave for digestion. After one hour, all the pressure vessels were removed and 6 ml of boric acid was added in order to neutralize the solution. The pressure vessels were placed back in the microwave for about half an hour for compensation. The solution was poured out of the fluoropolymer into a centrifuge tube and topped up to 50 ml using distilled water. The mixture of the solution and distilled water was then transferred to a 15 ml reagent tube with the minimum volume of 6 ml. All reagent tubes were labelled and arranged for heavy metal detection using inductively coupled plasma-optical emission spectrometry (Optima 5300 DV). Nutrient analysis such as nitrogen, carbon, phosphorus, and potassium was carried out using standard method of ASTM E778, ASTM E949, ASTM D5198, and ASTMe E926, respectively.

Statistical analysis

The statistical analysis was carried out using one-way ANOVA with SPSS 16.0 to determine significant differences among the parameters analysed during vermiremediation at the 0.05% level of significance.

RESULTS AND DISCUSSION

Earthworm growth and reproduction

Throughout the 90 days of vermiremediation, the development of earthworms was studied (Table 2). Significant differences were shown in both the final biomass ($F = 8.107$, $p = 0.00$) and the final number of earthworms ($F = 41.081$, $p = 0.00$). The highest biomass and gain in number of earthworms were found in T2, i.e., 76% and 757%, respectively. This was partly due to the nutrients available in cow dung. Based on a previous study[14], the growth pattern in earthworms depends on the microbial population and nutrient availability in the feed. It was found that cow dung contains a greater population of decomposing communities, e.g., bacteria, protozoa, nematodes, fungi, and actinomycetes, which the earthworms were able to mineralize at a faster rate[14]. The growth rate could be related to the feed quality and preferences by individual species of earthworm[15], as all feeds provided the earthworms with a sufficient amount of organic matter easy to metabolize with non-assimilated carbohydrates to favour the growth and reproduction of the earthworms[13].

Table 2 Earthworm growth in biomass and increase in number in T1, T2, and T3 (means ± SD, $n = 3$).

Parameter	T1	T2	T3
Biomass (g)			
Initial	9.1 ± 1.4	8.8 ± 0.7	9.43 ± 0.04
90 days	11.6 ± 1.0	15.7 ± 3.4	13.2 ± 1.2
G/L (%)†	28 ± 15	76 ± 27	40 ± 13
G/L rate (g/day)	0.03 ± 0.01	0.08 ± 0.03	0.04 ± 0.01
No. of cocoons	0 ± 0	36 ± 9	11 ± 4
Number G/L (%)	720 ± 157	757 ± 186	522 ± 47
No. of earthworms			
Initial	30	30	30
90 days	246 ± 47	257 ± 56	187 ± 14

† G/L = Gain/Loss.

The number of cocoons produced at the end of the study was determined. The total number of cocoons showed a significant difference ($F = 34.019$, $p = 0.00$). There were no cocoons observed in T1; however, T2 and T3 had 36 and 11 cocoons, respectively. The high production of cocoons in T2 was mainly due to the high nutrient content, especially regarding nitrogen, compared to T1 and T2. It has been shown that greater N fractions enhance cocoon production rates in epigeic earthworms[14], which occurred in T2. Additionally, cocoon production is highly dependent on food availability[16]. SMC is the residual compost waste generated by the mushroom production industry. The composting process and mushroom growing have therefore consumed much of its nitrogen content. This could be the reason why no cocoon was produced in T1. A relationship has been shown between the cocoon production

Table 3 Heavy metal content in T1, T2, and T3 (means ± SD, $n = 3$).

Treatment	Element	Concentration (mg/kg)			Increase/Removal (%)
		0 days	90 days	Control	
T1	Cu	0.082 ± 0.007	0.067 ± 0.004	0.074 ± 0.008	−18 ± 11
	Mn	1.117 ± 0.085	0.949 ± 0.030	1.116 ± 0.096	−14.7 ± 7.8
	Pb	0.141 ± 0.007	0.071 ± 0.008	0.093 ± 0.009	−49.6 ± 3.4
	Fe	23.4 ± 3.5	20.4 ± 4.1	23.8 ± 2.0	−10 ± 30
	Cr	0.079 ± 0.019	0.051 ± 0.008	0.065 ± 0.009	−31 ± 27
	Ni	0.024 ± 0.010	0.020 ± 0.003	0.033 ± 0.012	−10 ± 32
	Zn	0.297 ± 0.020	0.242 ± 0.007	0.296 ± 0.034	−18.2 ± 7.5
	As	0.150 ± 0.010	0.082 ± 0.015	0.089 ± 0.003	−45.5 ± 9.4
T2	Cu	0.202 ± 0.003	0.180 ± 0.004	0.194 ± 0.016	−10.7 ± 3.0
	Mn	1.601 ± 0.038	1.403 ± 0.024	1.439 ± 0.090	−12.4 ± 0.6
	Pb	0.159 ± 0.007	0.085 ± 0.005	0.084 ± 0.007	−46.4 ± 4.4
	Fe	47.9 ± 7.7	38.2 ± 7.4	36.8 ± 2.2	−18 ± 26
	Cr	0.138 ± 0.006	0.107 ± 0.014	0.106 ± 0.008	−22 ± 13
	Ni	0.042 ± 0.002	0.041 ± 0.005	0.044 ± 0.003	−4.1 ± 7.6
	Zn	0.408 ± 0.043	0.402 ± 0.020	0.414 ± 0.030	−0.9 ± 7.9
	As	0.182 ± 0.022	0.090 ± 0.007	0.093 ± 0.005	−50.0 ± 3.3
T3	Cu	0.134 ± 0.030	0.132 ± 0.007	0.114 ± 0.015	−1 ± 22
	Mn	1.444 ± 0.038	1.617 ± 0.189	1.520 ± 0.346	12 ± 11
	Pb	0.174 ± 0.011	0.116 ± 0.006	0.137 ± 0.076	−33.4 ± 6.8
	Fe	54 ± 22	41.16 ± 0.223	34.2 ± 5.1	−16 ± 29
	Cr	0.174 ± 0.031	0.122 ± 0.011	0.106 ± 0.017	−29 ± 11
	Ni	0.049 ± 0.015	0.048 ± 0.004	0.037 ± 0.013	−5 ± 29
	Zn	0.352 ± 0.081	0.406 ± 0.012	0.302 ± 0.037	20 ± 27
	As	0.189 ± 0.036	0.114 ± 0.010	0.108 ± 0.017	−38 ± 12

rate and feed quality. The results have proved that reproduction performance is better with appropriate ratio of bulking materials[14]. Earlier studies showed that greater N fractions enhanced cocoon production rates in epigeic earthworms, as it was observed in T2. Besides that cocoon production is highly dependent on food availability[16]. Several studies have indicated that cocoon production is one of the most sensitive biological responses in 28 day toxicity tests, with a strong decrease in cocoon production with increasing metal concentrations[17,18].

Heavy metal concentrations

After 90 days of vermiremediation, both the control sample and treatment in T1 showed the lowest final concentration for all the heavy metal elements compared to T2 and T3. This result contradicts the relationship between cocoon production and heavy metal reduction. However, this does not imply the vermicomposting process is put to a halt and affects the vermiremediation activity. The absence of cocoon production in T1 might be due to insufficient nutrient for cocoon to be developed or sustained until the 90 days[17], although it is sufficient for the growth of adult earthworm. In addition, T1 consisting of spent mushroom compost has added the advantage in mycoremediation which is explained in the later part. Table 3 shows the initial and final concentration for each heavy metal in each type of treatment. Different treatments showed variable reductions in heavy metals. The sequence, from highest to lowest, in reduction in heavy metals among treatments was T1, T2, T3.

Inconsistent heavy metal reduction was found in all control samples (Table 3). All the treatments successfully reduced all heavy metal elements except T3, which experienced an increase in Mn and Zn of 12% and 20%, respectively. Thus it is undeniable that vermiremediation assists in reducing the concentration of heavy metals. The greatest reduction in heavy metals for T1, i.e., Cu, Mn, Pb, Cr, Ni, and Zn were 19%, 15%, 50%, 35%, 18%, and 19% lower, respectively (Table 3). However, for Fe and As, the greatest reductions were found in T3 and T2, respectively. It was observed that Pb and As, which are highly toxic heavy metals, were effectively removed, i.e., Pb levels were reduced by 50%, 47%, and 34% in T1, T2, and T3, respectively; and As levels were reduced by 46%, 50%, and 40% in T1, T2, and T3, respectively (Table 3).

Table 4 Comparison of heavy metal (HM) contained in vermicompost with EU and USA (biosolid) compost limits.

HM (mg/kg)	EU[a]	USA[b]	Vermicompost[c]
Chromium	70–200	1200	0.051–0.122
Copper	70–600	1500	0.067–0.180
Nickel	20–200	420	0.020–0.048
Lead	70–1000	300	0.071–0.116
Zinc	210–4000	2800	0.242–0.406

[a,b] Compost Quality Standard set by US and European countries[19].

[c] Vermicompost of T1, T2, and T3 from the experiment.

Most of the heavy metals showed reduction in T1, T2, and T3 except for Mn and Zn, which were increased in T3. However, the increased content of Mn and Zn would not have any detrimental effects on plant growth due to the small amounts (—0.406 mg/l and 1.617 mg/l for Zn and Mn, respectively), which are under the compost quality standards set by the EU and USA (Table 4). In Malaysia, Contaminated Land Management and Control Guidelines No. 1 was established in 2009 to allow for proper assessment and management of contaminated land. The quality standards of these guidelines (Table 4) indicate that the concentrations of Mn and Zn were below safety levels as well. Thus on-site application of vermiremediation should be conducted as trial to treat contaminated land. It has also been reported that all metals, including heavy metals, are generally found in the soil at low concentrations and are essential micronutrients for soil organism[20]. Small amounts of many of these elements may be essential for plant growth, and detrimental effects might be observed only at significantly higher concentrations[21].

T1 showed the highest reduction in most of the heavy metal elements compared to T2 and T3. This was partly due to the feed source in T1 including spent mushroom compost (SMC), which also resulted in remediation. Mycoremediation is a technique that utilizes the vegetative portion of a fungus to remove contaminants from a substrate, usually soil. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fibre. These organic compounds composed of long chains of carbon and hydrogen are structurally similar to many organic pollutants[22]. SMC is a newly found biosorbent of heavy metals, which has a vast sorption capacity for cadmium, lead, and chromium owing to the presence of hydroxyl, phosphoryl, and phenolic functional groups on its surface[23]. Fungi produce small amounts of biostatic or biocidal compounds, which can be employed in the process of mycoremediation. They have the ability to mineralize, release, and store various elements and ions and accumulate toxic materials. Fungi have been shown to modify soil permeability and soil ion exchange and to detoxify contaminated soil. Many saprophytic filamentous fungi can degrade compounds flowing with wastewaters into receiving waters and thus contribute to cleanup[24]. It has also been shown that the reduction in metal concentrations may be attributed to the dilution effect of the organic amendment or the formation of stable metal chelates by the organic matter present in SMC[23]. However, the reduction in heavy metal concentrations in all treatments was mostly due to the ability of earthworms to bioaccumulate heavy metals. Recently, many studies have shown the role of epigeic in bioremediation. A study on reducing heavy metal concentrations also reported that, at the end of the experiment, the high concentrations of Cd, Cu, and Zn found in *L. rubellus* that had been introduced to pollute soil provide direct evidence of the bioaccumulation of heavy metals by earthworms[25]. Apart from that it has been shown that the highest tissue concentrations of As are found in the middle sections of contaminated earthworms[26]. This likely occurred since this is the part of the body where the uptake of toxins, as well as nutrients, is maximal. Accordingly, lysosomes and chloragosomes in earthworms accumulate metals[27]; the worms survive due to metal detoxification involving the binding and storage of the metals in metallothionein and metal binding proteins. The action of heavy metals upon lysosomal membranes triggers structural and physiological changes that induce the lysosomes to release acid hydrolases into the cytoplasm. The breakdown of cytoplasmic components ultimately leads to cell death.

The different degrees of heavy metal reduction are described in Table 3. This can also be explained as a result of the earthworm selective consumption pattern. Selective consumption by earthworms can result in clear differences in residual concentrations in the bulk soil and ingesta, in the case of both trace organics and heavy metals[28]. Additionally, each type of metal exhibits specific physiological mechanisms of assimilation during digestion in the earthworm gut[29]. Sherameti and Varma suggested that another factor that contributes to the differential reduction between treatments could be explained

Table 5 Initial and final nutrients concentration in three types of treatment (means ± SD, $n = 3$).

Treatment	Nutrient	Concentration (%)	
		0 days	90 days
T1	Nitrogen	0.37 ± 0.06	3.70 ± 0.46
	Carbon	14.9 ± 0.6	37.8 ± 2.4
	Phosphorus	0.40 ± 0.04	0.33 ± 0.05
	Potassium	1.36 ± 0.46	1.58 ± 0.08
T2	Nitrogen	2.27 ± 0.21	5.70 ± 0.40
	Carbon	17.3 ± 1.0	41.2 ± 1.1
	Phosphorus	0.29 ± 0.03	0.29 ± 0.04
	Potassium	1.23 ± 0.12	1.29 ± 0.14
T3	Nitrogen	0.27 ± 0.06	1.60 ± 0.10
	Carbon	3.5 ± 0.5	39.4 ± 1.1
	Phosphorus	0.16 ± 0.04	0.16 ± 0.04
	Potassium	1.16 ± 0.09	1.11 ± 0.07

by the affinity of metals for soil constituents[21]. The distribution of metals among the soil phases is important for bioaccumulation in earthworms, as the main pathways for chemical absorption are the skin for soluble elements, gut transit, and digestion.

Nutrient analysis

For nutrient concentrations, significant differences using ANOVA analysis were found in nitrogen ($F = 183.195$, $p = 0.00$), carbon ($F = 470.532$, $p = 0.00$) and phosphorus ($F = 17.703$, $p = 0.00$) except potassium ($F = 1.981$, $p = 0.15$). The initial and final nutrient compositions of the treatments are listed in Table 5.

Phosphorus showed a reduction in T1 while T2 and T3 remained at the same concentration after 90 days. It was observed that earthworms affect phosphorus mineralization if reared for long periods[14]. As organic matter passes through the gut of earthworm, some amount of phosphorus is converted and become available to plants. The release of phosphorus in available form is performed partly by the earthworm gut phosphatases, and further release of phosphorus might be attributed to the P-solubilizing microorganisms present in worm casts. Increase in phosphorus concentration during vermicomposting is probably the result of mineralization and mobilization of phosphorus due to bacterial and faecal phosphatase activity of earthworms[30]. Additionally, this increase in phosphorus is the direct action of worm gut enzymes and is indirectly stimulated by the microflora[31]. However, this contradicts the results of experiment in which phosphorus showed a reduction in T1 while remained at the same concentration in T2 and T3.

Reduction of total potassium by the end of the vermicomposting process occurred in T3. The reduction might due to high water solubility and leaching by the excess water that drained through the feed mixtures[32]. In contrast, T1 and T2 showed an increment in potassium. Additionally, the enhancement of potassium was probably due to physical decomposition of organic matter of waste passing through the gut, coupled with enzymatic activity in the worm gut, which may have caused its increase[33]. The microorganisms present in the worm gut probably converted insoluble K into the soluble form by producing microbial enzymes[34].

Increased in nitrogen has been observed in the treatment of T1 and T2. Earthworms have a great impact on nitrogen transformations in manure. By enhancing nitrogen mineralization, nitrogen is retained in the nitrate form[35]. Moreover, that increased in nitrogen concentration is due to the addition of nitrogen in the form of mucus, nitrogenous excretory substances, growth stimulating hormones, and enzymes from earthworms[36]. Nitrogen rich substances were not originally present in the feed material and hence might have contributed to the additional nitrogen content. Apart from that the rise of nitrogen concentration is due to inoculation of lignolytic fungi that might enhance decomposition of the organic matter by fungi and the extent of N fixed by free-living nitrogen-fixing bacteria[37].

All nitrogen, phosphorus, potassium, and carbon are macronutrients required by plant, which indicates these nutrients are needed in greater quantity. Concentration level of these nutrients is therefore essential for nutrients uptake in plant. Nitrogen is important for normal plant development while phosphorus is essential for photosynthesis, energy transfer within plants, and for good flowering and fruit growth. It is more important for plant maturation than plant growth. Other than that addition of phosphorus to vermicompost also prevents nitrogen loss through ammonia volatilization[38]. Potassium alternatively involved in plant growth, which includes manufacturing and movement of sugars and cell division.

According to Table 6, both control ($F = 17.581$, $p = 0.00$) and treatment ($F = 31.598$, $p = 0.00$) were significantly different for C:N ratio. The C:N ratio demonstrated increment in the control for both T2 and T3 while only T1 showed reduction of C:N ratio. However, in the treatment, only T3 alone showed increment of C:N ratio. Thus it can be proved that the introduction of earthworm helped to lower the C:N ratio. The overall decrease in

Table 6 Initial and final C:N ratio between control and treatment in T1, T2, and T3.

C:N	Control		Treatment	
	0 day	90 days	0 day	90 days
T1	59 ± 17	10.2 ± 1.2	41 ± 8	10.3 ± 1.1
T2	6.9 ± 0.8	8.4 ± 0.5	7.7 ± 1.1	7.3 ± 0.7
T3	15 ± 10	22.0 ± 0.9	14.1 ± 5.6	24.7 ± 0.9

C:N ratio was associated with an increased in TKN of final vermicompost. This increased in TKN is due to the nitrogen fixation and the conversion of ammonium-nitrogen to nitrate by earthworm. Earthworms stimulate non-symbiotic nitrogen fixation in the substrate by modulating the microbial community. Besides, earthworms can boost the nitrogen levels of the substrate during digestion in their gut by adding their nitrogenous excretory products, mucus, body fluid, enzymes, and even decaying dead tissues to the vermicomposting subsystem[39]. The reduction in C:N ratio resulted from the decomposers using the carbon compounds as their energy source[40]. The addition of cow dung also improved the process which is supported by the statistical analysis that showed significant different of C:N ratio in all types of treatment. The C:N ratio is traditionally considered as a parameter to determine the degree of maturity of the compost. C:N ratio below 20 is indicative of acceptable maturity, while a ratio of 15 or lower is being preferable for agronomic used of the composts. In addition, C:N ratio of the substrate also reflects the organic waste mineralization and stabilization during the process of vermicomposting. Higher C:N ratio indicates that the process of substrate degradation is slow and thus the period of vermicomposting need to be extended. Hence a lower C:N ratio implies a higher level of mineralization[15]. At the end of the experiment, T1 and T2 showed C:N ratio below 15 which were 10.30 and 7.2 6, respectively. However, T3 showed high C:N ratio which was 24.66.

A high C:N ratio means a poor fertilizer as the process of decomposing is still in process without reaching the level of maturity. Addition of carbon will lead to insufficient nitrogen being obtained from the residue and indirectly increasing the microorganism activity. Consequently, the microbes absorb the plant-available sources of nitrogen in the soil. This process probably would cause a nitrogen deficiency in the plant in which immobilization occurred. Nevertheless, if the organic residue has a low C:N ratio, then the microorganisms will obtain adequate nitrogen for their needs and will convert excess organic nitrogen to ammonium. The optimum ratio in soil organic matter is about 10 carbons to 1 nitrogen, or a C:N ratio of 10:1.

CONCLUSIONS

This study showed that the reduction in the concentrations of heavy metals was most effective in the substrate containing spent mushroom compost and organic soil (T1). T1 offered the greatest reduction in the overall heavy metal element concentration as well as the optimum end product (vermicompost) with a C:N ratio of 10:1. It was observed that an increase in the total number of earthworms occurred in T1, which implied that contaminated conditions did not inhibit the survival of earthworms. Thus vermicomposting is useful for bioremediation and nutrient recovery from leachates of urban waste as well as agricultural waste.

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