Correlation Analysis of Bacterial Growth and Heavy Metal Concentration in Composting of Leachate Sludge and Municipal Sludge

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Abstract. The composting is an alternative solution that can be used to this environmental problem to control the increase in waste generation. The unused leachate waste and municipal sewage sludge could be treated and reused into compost that can be used for any beneficial purpose such as bio-fertilizer for plant growth. The objectives of this study were to analyze the trend of microbial growth and heavy metal concentration in compost from municipal sewage sludge and leachate sludge. The correlation between both parameters also was identified to determine the relationship between both parameters. Four design ratios were prepared to determine which ratio of compost has the most efficient compost in terms of microbial growth and heavy metals concentration. The result showed that mesophilic and thermophilic bacteria population were increases in ratio 1, 2 and 3. The compost of ratio 2 and ratio 3 were found to meet the Malaysian standard of organic fertilizer in terms of the heavy metal concentration. There is correlation between bacteria population and heavy metal concentration in both composts. This composting method could be developed and practised by any institutions or treatment plant so that the amount of sludge waste generated could be reduced and help decreased the environmental pollution crisis in this country.

1. Introduction

Sewage sludge production from wastewater treatment facilities is one of the crucial environmental issues in Malaysia due to the very rapid increase in development, where houses, industrial development have increase year by year. Increase development of wastewater facilities whether it is the new installations or improvements to existing wastewater facilities is one of the reasons that cause the increasing of sludge production in Malaysia. Thus, the increase in municipal sewage waste production contribute in high waste disposal at the landfill Based on the Malaysia Solid Waste and Public Cleansing Management Act 2007 (Act 672), the disposal of solid waste could be done by any method, which are degradation, incineration, recycling and decomposition [1]. Thus, there must be a solution that is rational, economic, and technical to solve the problem of high number of sewage generation.

Sewage sludge and leachate sludge containing high organic matter, macro-and micro-nutrients that may be used as a fertilizer / soil conditioner for feed, vegetable crops, horticultural plants and pastures, which can in most cases be recycled in a beneficial way [2]. The application of sewage sludge to agricultural fields may therefore be safe and economical due to the recycling of nutrients and the
disposal of sewage sludge [1]. Therefore, the composting method is the best economical auxiliary solution to solve this problem. Composting is a microbial breakdown of biodegradable materials and is regulated by physicochemical, physiological, and microbiological factors. Among the waste processing mode, composting specifically is a recycling approach represents the second most ideal technique comes after source reduction and reuse [3]. Thus, composting is one of the alternative methods in order to reduce the amount of waste which is municipal sewage and leachate sludge at landfill, so that the reduction of this waste could minimize the environmental pollution and toxicity to soil by heavy metals in Malaysia. This study aims to determine the population of bacteria (mesophilic and thermophilic) and heavy metals concentration during the composting of leachate sludge and municipal sludge. The relationship between the bacteria population and heavy metals also evaluated.

2. Experimental

2.1. Fermenting solution and seed compost preparation

The fermented solution that was used was the mixture of fermented foods (200g Tempeh) and sugared water (200ml of molasses). The fermented microorganisms in solution will increase rapidly in 3 to 5 days. Seed compost also was prepared by using the by-products such as rice bran and rice husk with the ratio of 1:1 with 500g: 500g [4]. The fermenting solution that was prepared earlier then mixed with these by-products. The water content of compost should be between 40 and 60 %. The conditions are right when no water drains out when the compost is squeezed, and when the hand released the compost, it forms a lump [4]. The seed compost was left for 3 to 5 days. The white mold formed on the surface of the seed compost where it indicates the completion of fermentation.

2.2. Material preparation and composting process

The materials for composting were landscape waste, seed compost and sludge. Firstly, the landscape waste was collected at Pusat Kompos UiTM Shah Alam. Meanwhile, the WHB leachate sludge (WLS) was collected at Worldwide Holding Berhad Company’s landfill site in Kuala Selangor and another UiTM municipal sludge sample (UMS) was taken at UiTM treatment plant. In this study, the compost was varied by extracting into four different ratios of compost as shown in table 1.

| Ratio | UMS sludge (kg) | WLS sludge (kg) |
|-------|----------------|-----------------|
| Ratio 1 | LW:SC = 0.5:0.5 | LW:SC = 0.5:0 |
| Ratio 2 | LW:SS:SC=0.4:0.1:0.5 | LW:LS:SC=0.4:0.1:0.5 |
| Ratio 3 | LW:SS:SC=0.3:0.2:0.5 | LW:LS:SC=0.3:0.2:0.5 |
| Ratio 4 | SS:SC = 0.5:0.5 | LS:SC = 0.5:0.5 |

LW: Landscape waste  SS: Sewage sludge  LS: Leachate sludge  SC: Seed compost

2.3. Microbial growth analysis

The compost was sacrificed on day 0, 3, 7, 14, 21, 28, 60 and 90. The sample from each ratio of compost was weighed by using analytical balance at 10 g and then blended with 90 ml of Phosphate buffer saline solution (PBS, OXOID) to convert the solid compost into homogenize solution. A serial dilution technique was prepared to count the viability of bacterial presents in the compost. Nutrient agar (NA, HiMedia, India) plate was used for growing the bacteria. Thermophilic bacteria plate was placed inside the incubator of 50 °C while mesophilic bacteria plate was incubated at 35 °C. Colonies of bacteria was express in units colony forming unit (CFU) and formed on the surface of the agar plate after 1 to 3 days of incubation period.
2.4. Heavy metals concentration analysis

Heavy metal was measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The sample was oven dried for 24 hours at the temperature of 550 °C to ensure no moisture content present in the samples. Then, the dried sample was blended into powder and extracted using Digesdahl Digestor. The dilution was syringe filtered by using 0.45 µm filter paper and acidified before it was tested using (ICP-MS).

2.5. Correlation between heavy metals and microbial growth

The relationship between bacteria growth and heavy metals content in compost was identified to identify whether the bacteria growth does affect the heavy metals reduction during composting process. The correlation between bacteria growth and heavy metals concentration is identified by using ANOVA with positive correlation coefficients indicate that when the value of one variable increases, the value of the other variable also tends to increase while negative correlation coefficients represent cases when the value of one variable increases, the value of the other variable tends to decrease.

3. Results and Discussion

3.1. Bacterial growth in compost

The population of the mesophilic bacteria in ratio 1 decreased linearly until day 90 which recorded the value of 3x10⁷ cfu/g as shown in figure 1 (a). Thus, this study was in line with the previous study with the range from 1.7 × 10⁷ cfu/g to 3.84 × 10⁷ cfu/g [1]. At the final day of composting for ratio 4, the WLS compost has higher population of mesophilic bacteria at day 90 with the value of 1.74x10¹⁰ cfu/g compared to UMS compost which has the value of 6x10⁹ cfu/g. This explain that the temperature does affect the microbial population [1]. As shown in figure 1 (b), ratio 1 of thermophilic bacteria decreased from 4x10¹⁰ cfu/g to 1x10⁸ cfu/g on day 90. Based on the previous study, the population range of thermophilic bacteria in sewage sludge compost was (10⁶-10⁹ cfu/g) [5]. At day 90 of composting for ratio 2, UMS compost recorded 3.9 x10⁷ cfu/g population of thermophilic bacteria meanwhile WLS compost recorded 1.0x10⁷ cfu/g of thermophilic bacteria. The population of the bacteria in both composts of ratio 3, UMS and WLS have the value of 5x10⁶ cfu/g and 1.78x10⁹ cfu/g respectively.

![Figure 1](image1.png)

**Figure 1.** Number of mesophilic bacteria (a) and thermophilic bacteria (b) in UMS and WLS compost.

3.2. Heavy metals concentration in compost

For Cd analysis as presented in figure 2 (a), the ratio for UMS and WLS compost that has the lowest amount of Cd concentration was ratio 3 and ratio 2, respectively. Meanwhile, for Cu analysis (figure 2 (b)), the ratio for UMS and WLS compost that has the lowest amount of Cu concentration was ratio 2
which has the value of 64.3 mg/kg and 15.73 mg/kg respectively. For Pb analysis (figure 2 (c)), the ratio for UMS and WLS compost that has the lowest amount of Pb concentration was ratio 3 (1.792 mg/kg) and ratio 2 (12.56 mg/kg) respectively. Lastly, For Zn analysis (figure 2 (d)), the ratio for UMS and WLS compost that has the lowest amount of Zn concentration was ratio 3 (957.39 mg/kg) and ratio 2 (322.13 mg/kg) respectively. Bożym & Siemiątkowski [6] stated that heavy metals will be increased during the maturation phase. The increasing of heavy metals was due to the mineralization of organic matter during composting process of sewage sludge. This is the reason why heavy metals increase in the compost in some ratios.

### 3.3. Relationship between bacterial growth and heavy metals concentration

The relationship of both parameters was identified by interpreting the correlation of number of bacteria and heavy metals content. Composting treatment induced the microbial growth development that cause the changes of physico-chemical in compost [6]. Based on the result from data analysis, some ratio has bacteria changed proportionally to heavy metals content, and some have an inverse relationship between both parameters. The concentration of Pb was inversely proportional with the mesophilic bacteria population which is presented in negative sign in all ratios as shown in table 2. Vela et al., (2014)[7] found that there was a higher reduction of Pb by *Rhodococcus* sp. which was 63% of reduction as the bacteria population increases. For thermophilic bacteria, most of the heavy metal concentration is positively correlated in both composts as presented in table 3.

![Graphs of heavy metals concentration in UMS and WLS composts](image-url)

**Figure 2.** Heavy metals concentration in UMS and WLS composts.
Table 2. Correlation value between mesophilic bacteria and heavy metal in ULS & WMS compost.

| Ratio | Bacteria          | Content of heavy metals in compost |
|-------|-------------------|-------------------------------------|
|       |                   | Cd       | Cu       | Pb       | Zn       |
| Ratio 1 | ULS Mesophilic  | -0.63    | 0.25    | -0.45    | 0.28    |
|        | WMS Mesophilic   | -0.63    | 0.25    | -0.45    | 0.28    |
| Ratio 2 | ULS Mesophilic  | 0.30     | 0.23    | 0.53     | 0.37    |
|        | WMS Mesophilic   | 0.12     | 0.47    | -0.25    | 0.48    |
| Ratio 3 | ULS Mesophilic  | 0.16     | 0.08    | -0.31    | 0.21    |
|        | WMS Mesophilic   | -0.40    | 0.27    | -0.44    | 0.41    |
| Ratio 4 | ULS Mesophilic  | -0.25    | -0.63   | -0.32    | -0.49   |
|        | WMS Mesophilic   | -0.07    | 0.62    | -0.31    | 0.81    |

Table 3. Correlation value between thermophilic bacteria and heavy metal in ULS & WMS compost.

| Ratio | Bacteria          | Content of heavy metals in compost |
|-------|-------------------|-------------------------------------|
|       |                   | Cd       | Cu       | Pb       | Zn       |
| Ratio 1 | ULS Thermophilic | 0.58     | 0.06    | 0.28     | -0.35   |
|        | WMS Thermophilic | 0.60     | 0.06    | 0.52     | -0.35   |
| Ratio 2 | ULS Thermophilic | -0.31    | -0.21   | 0.01     | -0.52   |
|        | WHB Thermophilic | 0.02     | 0.44    | 0.51     | 0.51    |
| Ratio 3 | ULS Thermophilic | -0.15    | 0.37    | 0.18     | -0.29   |
|        | WMS Thermophilic | 0.68     | -0.31   | 0.14     | -0.32   |
| Ratio 4 | ULS Thermophilic | 0.26     | 0.41    | 0.24     | 0.44    |
|        | WMS Thermophilic | 0.10     | 0.04    | 0.30     | -0.20   |

4. Conclusion
In conclusion, the best product for UMS and WLS of mesophilic bacteria among the ratios were ratio 3 and ratio 1, respectively. Meanwhile, the best ratio for UMS and WLS compost of thermophilic bacteria were ratio 2 and ratio 3, respectively. In the final compost, the overall metal content was suitable for the usage as a decent soil fertilizer that compared with Malaysian Standard for Organic Fertilizer (MS 1517:2012) [8]. Thus, the best ratio for UMS and WLS was ratio 3 and ratio 2 respectively. This is because both ratios have the most minimum amount of heavy metals content (Cd, Cu, Pb and Zn) for UMS and WLS. Therefore, the bacterial growth was strongly correlated with the heavy metal concentration in both UMS and WLS compost.

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