Effect of Organic Matter on Pathogen Population During Composting of Municipal Sludge

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Abstract. Population growth in Malaysia is expected to increase rapidly. Every sector of industry is expected to have a revolution including in agriculture sector. The utilization of organic fertilizer in agriculture as a green movement is to replace the chemical fertilizer subsequently, reduce the municipal sludge waste. Hence enhancing the growth and supply of nutrient to plant. However, the pathogenic growth in the final product is the greatest concerns. Thus, this study aims to evaluate the pathogen population in the compost of municipal sludge mixed with landscape waste for 90 days. The pathogen dynamic and its correlation with organic matter in 3 different ratios were analysed and recorded. The result showed that the pathogenic trends was uniformly increased at the early stage of composting and decreased gradually after reached the peak. R4 which comprises of equal ratio between municipal sludge and seed compost showed positive correlation with organic matter. R3 and R4 showed better removal of pathogen at maturity stage. Pseudomonas aeruginosa and Salmonella were failed to comply with the standard for R3 and only Salmonella failed for R4. This study supports the achievement of the Sustainability Development Goal (SDG) as an alternative way to combat the environmental issue by leveraging the usage of the waste produced for the purpose of the waste reduction and sustain the environment.

1. Introduction
Population growth in Malaysia is rapidly increases year by year. Based on the press release by the Department of Statistics Malaysia (DOSM), Malaysia’s population in 2019 is estimated at 32.6 million, increased from 32.4 million as compared to 2018. The development in every sector of industry were expected to revolt together as well as the population growth. Today, agriculture has remains as an important sector of Malaysia's economy, by contributing 12 percent to the national GDP and providing employment for 16 percent of the population. In the agriculture system, the use of fertilizer is important. The most common use of fertilizers in agriculture is to promote growth and nutrient to plant. However, some chemical fertilizer is not only promoting growth to the plant, but also cause several harmful effects that may cause waterway pollution, chemical burn to crops, increased air pollution, acidification of the soil and mineral depletion.
The huge amount of municipal sludge waste was the current greatest concerns. According to Indah Water Konsortium (IWK), 3.2 million cubic metres of domestic sludge is estimated to be produced yearly. However, by the year 2005, Malaysia has producing 4.3 million cubic metres of domestic sludge annually which higher than it been expected. Municipal sludge is a product of municipal sewage treatment plant and contains many pollutants removed from the influent wastewater. Sludge is a concentrated suspension of solids, largely composed of organic matter and nutrient-laden organic solids and its consistency can range in form from slurry to dry solids, depending on the type of sludge treatment. The use of landscape waste as a composting material has been widely used now. Yard waste or landscape waste is vegetative waste resulting from the care and maintenance of landscaped areas, lawns, and gardens[1]. The composted yard waste can be used to produce fertilizer that returns nutrients to the soil. Thus, the pathogenic present and the correlation between the organic matter in the final product of composting is the main concerns of this study. Moreover, the effectiveness of the composting method to remove pathogen in the final product was also evaluated and compare with the Malaysian Standard of Organic fertilizer (MS 1517:2012). This study provides most economical and sustainable option for waste management because it can be operated easily and can be conducted in a confined space for proper management to produce a high-quality product[2].

2. Methodology

2.1. Preparation of municipal sludge
Municipal sludge (MS) was taken from a local municipal sewage treatment plant at Indah Water Konsortium (IWK) located in Shah Alam.

2.2. Preparation of landscape waste
The landscape waste (LW) was taken at the Compost Centre in Universiti Teknologi Mara (UiTM) Shah Alam. The leaves were cut approximately 3 cm in order to increase the rate of composting as particle size of materials affect the decomposition by the microorganisms [3].

2.3. Preparation of fermented solution
The type of fermentation solution used in this composting is sugar-based fermentation solution. This fermentation solution is necessary to produce native microorganisms that can enhance the composting process. The sugar-based solution is prepared by using the brown sugar and fermented foods such as yogurt, yeast, ‘tapai’, tempeh and mushroom as the substrate to be added into the solution. Tempeh (50% yellowish) was chosen in this study which was bought from local market. The fermentation solution from tempeh was prepared as follow: a 200 g brown sugar (molasses) was dissolved in 3 litres of water. Next, the brown sugar solution was mixed with 5 litres tempeh solution which contained of 200 g of chopped tempeh. The fermented solution was left for 5 days in room temperature. During the fermentation process, the lid of the bottle should be loosened once a day to release the gas. One of the criteria for successful fermented solution was the presence of sweet and sour smell.

2.4. Preparation of seed compost (SC)
There are several types of decomposing medium were being prepared. There are rice bran and rice husk. Basically, the rice husk is used to provide habitat for microorganisms to become active and the rice bran as the nutrients provider for the microbes to grow. The ratio for this medium is 1:1 with rice bran to rice husk by weight. After the mixing of the rice bran and rice husk was prepared, the fermentation solution was added into the medium with the right amount and was homogeneously mixed.

To determine the optimum ratio, three (3) samples had been set up which were designated as R2, R3 and R4. Each of the samples consist of different ratio of municipal sludge, landscape waste and the seed compost but still have the same total weight of one kilogram in the total as tabulated in table 1.
Table 1. The percentage ratio of municipal sludge, landscape waste and seed compost.

| Compost | Percent Ratio (%)                     | Weight (kg) |
|---------|---------------------------------------|-------------|
| R 2     | 40 % LW: 10 % MS: 50 % SC             | 0.4: 0.1: 0.5 |
| R 3     | 30 % LW: 20 % MS: 50 % SC             | 0.3: 0.2: 0.5 |
| R 4     | 50 % MS: 50 % SC                      | 0.5: 0.5     |

The aim of this ratio was to determine the variation of nutrients characteristic between the composted municipal sludge and composted landscape waste. The original ratio that was taken from the previous study done by Dumistrescu et al. [4].

2.5. Material preparation to analyse the pathogen trends

A population of bacteria grown in the laboratory is referred as a culture. A pure culture contains only one single type, a mixed culture contains two or more different bacteria. The analysis of pathogen trends can be executed by applying plate count technique to determine the number of viable organisms.

2.6. Pathogen population determination

A 10 grams of the MS compost was harvested at day 3, 7, 14, 21, 30, 60 and 90 and homogenized with 90 ml of Phosphate Buffer Saline (PBS, OXOID) solution. Using sterile pipette, a serial dilution was prepared to count the viability of pathogen present in the compost. Different agar was used to determine the selected pathogen strain namely Eosin Methylene Blue (EMB, Himedia, India) for *Escherichia coli* spp., MacConkey agar (Himedia, India) for *Staphylococcus aureus* spp., Mannitol Salt agar (Himedia, India) for *Salmonella* spp. and Pseudomonas agar (Himedia, India) for *Pseudomonas aeruginosa* spp. The plate was incubated at 37 °C for 24 hours in the inverted position. The colony count was expressed as colony forming units per ml (CFU/ml).

2.7. Organic matter determination

The MS compost sample was collected for each ratio and weighed before left in the oven to dry. After at least 8 hours placing the sample in the oven at 105 °C, the sample was weighed again before placed in the muffle furnace for 30 minutes at 550 °C. The amount of organic matter was calculated as following equation:

\[
VSS \text{ (mg/g)} = \frac{B-A}{C}
\]  

Where A indicates weight of the sample before muffle furnace, B is the weight of the sample after muffle furnace and C is the weight of the MS compost sample used.

2.8. Statistical analysis

The correlations coefficient was analysed using ANOVA with positive sign of r indicates positive correlation and vice versa. Moreover, the significant different between pathogen population with organic matter was evaluated when \( P < 0.05 \).

3. Result and Discussion

3.1. The trends of pathogen population in municipal sludge composting

The analysis of the pathogen trends was analysed for R2, R3 and R4. *Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* are the strains of pathogen that been measured. During composting, pathogen reduction is achieved primarily through thermal destruction, but also through competitive interactions between microorganisms, nutrient depletion, by-product toxicity and natural die-off [5].
The trend of the pathogenic population in R2 is consists 100 g of municipal sludge, 400 g of landscape waste and 500 g seed compost has been plotted in the graph as shown in figure 1(a). The graph illustrates the trends in the pathogen population in R2. *E. coli* spp. was the earliest to achieve the peak at day 3 followed by *S. aureus* spp. and *Salmonella* spp. at day 7. While *P. aeruginosa* spp. takes about 14 days to attain this trend. This might happen due to the heat treatment used in the composting process takes longer time to activate the pathogen and that pathogen tolerates changes in temperature [6]. Overall, all the pathogen type was gradually decreasing at the end of day 90 of the observation.

![Graph](image1.png)

**Figure 1.** Trends of pathogen population in (a) R2, (b) R3 and (c) R4.

Meanwhile, the composition of R3 is consists of 200 g of municipal waste, 300 g of landscape waste and 500 g of seed compost. Graph of the pathogen population in R3 was illustrate in figure 1(b). The graph discloses that *Salmonella* sp. was reached the highest peak of the pathogen population at the earliest of day 3. Both *Salmonella* sp. and *E. coli* are very useful in defining the ecological quality composts products was establish in the European Commission Decisions [7]. *Salmonella* spp. in compost material will die within 60 and 20 minutes at a temperature of 55°C and 60°C [8]. From previous studies, the temperature plays an important role for inactivation of *Salmonella* spp. in solution especially in thermophilic conditions [9,10]. Next, *S.aureus* spp. and *E. coli* spp. also attain the peak at day 3 followed with *P. aeruginosa* spp. on day 7 of the observation.

The graph of ratio 4 that consisting 500 g of municipal sludge and 500 g of seed compost was plotted in Figure 1(c). The figure shows that *E. coli* spp. and *S. aureus* spp is the expeditious to achieve their peak at day 3 and gradually decreasing till day 90. In a laboratory study seeded with *E. coli*, Lemunier et al., (2005) [11] found that *E. coli* survived for only 4 weeks at 25°C (77°F) in a 3-month study. In other study on composted manure and other feedstocks in lab-scale bioreactors, *E. coli* populations went up during the first 24 hours of composting period and then decreased over the next 36 days[12]. At day 14, *P. aeruginosa* spp. and *Salmonella* spp. were both achieved their peak, respectively.

### 3.2. Analysis on the correlation between organic matter to the pathogen dynamic composting in municipal sludge

There are many factors that affect the decomposition of organic matter in the composting process[13]. To estimate the effect of a single factor on the rate of organic decomposition is not a straightforward procedure because composting process is very complex. However, several factors were generally recognized as primary factors affecting the composting process such as nutrient content, moisture content, oxygen concentration, microbial community, temperature and pathogenic effect [7]. These factors contribute to an optimum environment for the microbial process in composting. The correlation between the pathogen dynamic are summarized in table 2.
Table 2. Summary of correlation between the pathogen population and organic matter.

| Compost | Pathogen Colonies       | Correlation |
|---------|-------------------------|-------------|
| R2      | *Pseudomonas aeruginosa* | Positive    |
|         | *Salmonella*             | Positive    |
|         | *Staphylococcus aureus*  | Positive    |
|         | *Escherichia coli*       | Negative    |
| R3      | *Pseudomonas aeruginosa* | Negative    |
|         | *Salmonella*             | Negative    |
|         | *Staphylococcus aureus*  | Negative    |
|         | *Escherichia coli*       | Negative    |
| R4      | *Pseudomonas aeruginosa* | Positive    |
|         | *Salmonella*             | Positive    |
|         | *Staphylococcus aureus*  | Positive    |
|         | *Escherichia coli*       | Positive    |

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. All the pathogenic germs in R4 which is 0.5:0.5 ratio of municipal sludge to the seed compost have a positive correlation to organic matter.

3.3 Analysis of the effectiveness of the composting method on removing pathogenic bacteria in municipal sludge

The amount of pathogen during the composting does have an effect to the success of composting method. The final product of composting should be sufficiently stable for storage and application to land without adverse environmental effects [7]. The analysis of the effectiveness of composting method on the permissible pathogen is tabulated in table 3. R2 which contains 0.4:0.1:0.5 ratio of landscape waste (LW) to municipal sludge (MS) to seed compost (SC) failed to comply with the Malaysia Standard of Organic Fertilizer (MS1517:2012)[14]. However, in R3 which have 0.3:0.2:0.5 of LW:MS:SC, *P. aeruginosa* spp. and *Salmonella* spp. are the pathogen that failed while *S. aureus* and *E. coli* were allowed. The pathogen in R4 were observed below the permissible limit except for *Salmonella* spp. which is still exist.
Table 3. Comparison of allowable number of pathogen colonies with the standard.

| Compost | Pathogen Colonies             | Percentage Removal (%) | Allowable Number of Pathogen in The Standard | Pass / Fail to Comply with The Standard |
|---------|-------------------------------|------------------------|---------------------------------------------|----------------------------------------|
| R2      | *Pseudomonas aeruginosa*      | 0                      | < 10 cfu/g                                  | FAIL                                   |
|         | *Salmonella*                  | 99.59                  | Absent                                      | FAIL                                   |
|         | *Staphylococcus aureus*       | 0                      | < 10 cfu/g                                  | FAIL                                   |
|         | *Escherichia Coli*            | 11.11                  | < 10 cfu/g                                  | FAIL                                   |
| R3      | *Pseudomonas aeruginosa*      | 99.96                  | < 10 cfu/g                                  | FAIL                                   |
|         | *Salmonella*                  | 99.99                  | Absent                                      | FAIL                                   |
|         | *Staphylococcus aureus*       | 100                    | < 10 cfu/g                                  | PASS                                   |
|         | *Escherichia Coli*            | 100                    | < 10 cfu/g                                  | PASS                                   |
| R 4     | *Pseudomonas aeruginosa*      | 100                    | < 10 cfu/g                                  | PASS                                   |
|         | *Salmonella*                  | 99.99                  | Absent                                      | FAIL                                   |
|         | *Staphylococcus aureus*       | 100                    | < 10 cfu/g                                  | PASS                                   |
|         | *Escherichia Coli*            | 100                    | < 10 cfu/g                                  | PASS                                   |

4. Conclusion
This study successfully investigated the effect of organic matter on pathogen in a compost of municipal sludge as an initiative for waste minimization and producing organic fertilizer. As the result shown, the composting of municipal sludge has a very high number of pathogenic bacteria during the active phase of composting. The correlation between pathogen population and organic matter contained in the compost showed positive and negative correlation depending on the strains. R4 was observed to have positive correlation for all selected strains. Meanwhile, for the effectiveness of the composting method, R3 and R4 show better removal of pathogen but still not comply with the standard for certain pathogen and require extra treatment. In summary, to use the composting of municipal sludge, the composted should be i) Stabilize the raw municipal sludge during composting without further treatment plant which can reduce the cost of operation; ii) High in temperature produce during the thermophilic stage can kill the pathogens; iii) Stored the mature compost properly to avoid pests and can be used without expensive equipment.

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