Lowering the Total Coliform of Vermicompost From Solid Waste Materials Produced By African Night Crawler Worm Eudrilus Eugeniae

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Vermicomposting is an established ecological sanitation program of Bayawan City in Negros Oriental, Philippines that aims to produce low-cost organic fertilizers as an alternative to expensive commercial fertilizers. However, the vermicomposts from biodegradable and human sludge contain high total coliform levels above the limit set by WHO. The study developed coliform-reducing strategies such as agricultural and hydrated lime applications at three concentrations (1:1, 1:0.5, 1:0.25) in two types of vermicomposts. Prior to the experiment, a preliminary study for 15 months made use of air drying and direct sunlight exposure as methods to reduce total coliform levels. Results show that aeration and exposure to the environment did not reduce coliform levels in vermicompost produced by the African Night Crawler earthworm Eudrilus eugeniae. From an initial level of 54,000,000 cfu/100 mL, the levels of total coliform were 208,000 and 307,000 cfu/100 mL using these methods, respectively. Application of lime, either agricultural or hydrated, significantly reduced the levels of coliform beginning 30 days after treatment. Between Day 45 and Day 60, total coliform levels were reaching zero values and near zero values in both types of lime application. Post hoc analysis indicated higher total coliform levels in vermicompost applied with lower lime concentration. The study recommends the use of agricultural lime over hydrated lime as it reduced coliform levels without impairing the NPK levels of the vermicompost.

Keywords: African Night Crawler earthworm, Coliform, Escherichia coli, Vermicompost, solid waste, Bayawan City

INTRODUCTION

Vermicomposting in Bayawan City on Negros Island, Central Philippines is a large-scale composting program by the local government using African Night Crawler earthworm *Eudrilus eugeniae* to convert biodgradable waste material into humus-rich soil. Its main purpose is to provide a source of organic fertilizer for agricultural production such as corn, coffee, banana, and fruit trees as part of the local government drive to address food security. This makes the program congruent to the UN Sustainable Development Goals of promoting sustainable agriculture in Bayawan City, the agriculture capital of Negros Oriental province. The unique feature of vermicomposting in this city makes use of two types of substrate materials, namely biodegradable wastes from households and dried sludge from septic tanks; they are brought in and processed in a 10-hectare sanitary landfill facility.

Vermicomposting is a low cost biotechnology in fertilizer production that addresses the perennial need of farming communities for fertilizer input. There are three earthworm species commonly used to perform this function, namely Red Tiger worm *Eisenia fetida,* African Night Crawler *E. eugeniae,* and Indian Blue Worm *Perionyx excavatus* (Sinha, Bharambe & Chaudhari, 2008). The work of Joshi, Singh, and Vig (2014) underscores the advantages of vermicompost being an excellent soil additive, bio-control agent, and organic fertilizer, which makes it better than commercial fertilizers. The main problem with vermicompost material, however, is the high levels of bacterial coliform contamination which render fruits and vegetables unsafe for direct consumption due to pathogens such as E. coli, total coliform, fecal coliform, and enterococci bacteria (EPA, 2006; Paruch & Maehlum, 2012; Abakpa *et al.*, 2013). The study of Tura-Mutya, Relles, Jayme, and Guino-o (2013) documented coliform count of 54,000,000 cfu/gram of dried 30-day old vermicompost material. Depending on the substrate used, vermicomposts produced from human sludge contain high population levels of microbial pathogens. The studies of Monroy, Aira, and Dominguez (2009) and Lalander, Hill, and Vinneras (2013) indicated that Red Tiger worm *Eisenia fetida* significantly reduced total coliform level in a sludge that has undergone processing in its gut. However, pilot test of total coliform level using African Night Crawler pointed to contrasting results (Tura-Mutya *et al.*, 2013). These studies suggest the urgency to limit the pathogenic bacterial contamination from vermicompost which is the target of the waste management facility. Thus, this paper aims to develop strategies that lower bacterial coliform at an acceptable level based on the World Health Organization standards.

Specifically, the study has the following goals: a) conduct a preliminary study on the effects of aeration on total coliform; b) provide baseline data on total coliform levels in vermicompost; c) determine the NPK levels of vermicompost; and d) test the effects of bacterial reducing treatments involving lime application.

CONCEPTUAL FRAMEWORK

Bayawan City became the first city in the Philippines to utilize an integrated ecological sanitation (EcoSan) program through its constructed wetlands and sanitary landfill (SLF) to treat liquid and solid wastes into ecological products. The wastewater treatment facility of Bayawan City, otherwise known as constructed wetland, was built in September 2006. Guino-o, Aguilar and Oracion (2010) documented its technical efficiency and positive social acceptability in a study. Sometime later in 2010, the local government unit of Bayawan City embarked on a sanitary landfill that incorporates a biodigester and vermicompost production from biodegradable waste materials. These developments were responses to the Clean Water Act of 2004 (Republic Act 9275) and the Ecological Solid Waste Management Act of 2000 (Republic Act 9003). With the integrated waste management program, Bayawan was awarded the Presidential Lingkod Bayan during the 113th Philippine Civil Service Anniversary on October 24, 2013.

The production of vermicompost is critical to input in agricultural activities. The success of vermicomposting lies on the bedding material of the earthworm, food source or substrate, moisture, adequate aeration, adequate temperature, and suitable pH (Garg, Gupta, & Yadav, n.d.). The substrates for the earthworms may come from various organic materials derived from agricultural or domestic wastes. In the case of Bayawan, the substrates used in vermicomposting include biodegradable solid wastes and human fecal matter from domestic and business establishments' septic tanks that have been decomposed for some time inside a biodigester tank. The use of vermicompost has the following advantages over chemical fertilizers. For example, the studies by Garg *et al*. (n.d.), Monroy *et al.* (2009), and Yadav, Tare & Ahammed (2011) have shown that vernicompost restores microbial population, which provides major and micronutrients to the plants. It also improves soil texture and water-holding capacity of the soil, provides good aeration to soil, and improves the structural stability of the soil, which helps in preventing soil erosion. Certain elements in vermicomposting help in improving root growth and proliferation of beneficial soil microorganisms, which decreases the use of pesticides for controlling plant pathogens. Vernicompost likewise enhances the quality of grains and fruits of plants or trees where it is applied due to increased sugar content.

There are noted disadvantages of vermicompost produced from human waste substrate in the form of high population of pathogens on the material. Nonetheless, Yadav, Tared and Ahmmed (2012) have provided some insights on how to control the bacterial levels of the vermicompost by experimentally subjecting it to high temperatures during the drying stage prior to vermicomposting. Yadav, Tared, and Ahammed (2010) concluded that pathogens were killed by *E. fetida* "earthworm actions involving intestinal actions, secretion of fluids and selective grazing." However, this is not the case of vermicompost produced by *Eudrilus eugeniae*, which is characteristically high in total coliform. Hence, this study aimed to come up with strategies to lower the coliform load of vermicompost to promote biosafety among the agricultural users and public consumers.

MATERIALS AND METHODS

Test Sites

Two areas in Bayawan City served as test sites, namely GK Multipurpose Hall of the Fishermen's Village in Bgy. Villareal and Bayawan Waste Management and Ecology Center (BWMEC) in Bgy. Anini-i. Each test site corresponds to a particular trial, which lasted for 90 days. The experiment took place from the month of June 2015 to August 2015 while laboratory tests lasted until February 2016.

Vermicompost

Two types of vermicomposts were produced by the local government unit of Bayawan City. The first type is a biodegradable-waste vermicomposts from domestic households that are mechanically sieved prior to being fed to the African Night Crawler worm *E. eugeniae*. The second type is human-sludge vermicomposts that have been stored from a biodigester tank at the BWMEC and then sundried and grinded before acted upon by *E. eugeniae*. The vermicomposting process took place in separate rectangular tanks in a roofed facility. The finished products are bagged and labelled in polyethylene sacks.

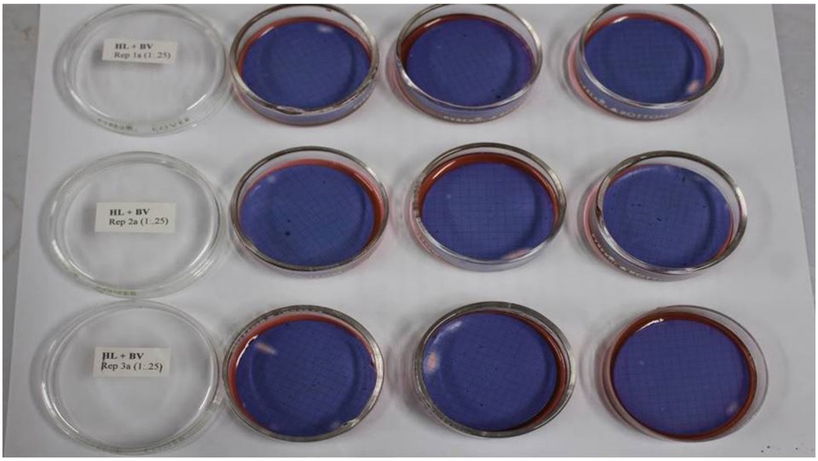

Figure 1. Membrane filtration results of hydrated lime to biodegradable vermicompost

Total Coliform Test

The total coliform test made use of the membrane filtration method as outlined by the World Health Organization. In this procedure, a gram of vermicompost from a composite sample was dissolved in 100 mL of distilled water, and then serially diluted between 10^4 and 10^5. The composite sample was taken from three areas of the treated vermicompost derived from the top, middle and bottom parts of a vermicompost sample.The same sample was filtered using a membrane filter of 0.45 µm pore size, 45 mm in diameter and with grid lines. The membrane was transferred aseptically to an EMB agar microplate and incubated at 37° C for 18 hours using Heraeus incubator. Bacterial colony count was expressed in terms of colony forming units per 100 mL (cfu/100 mL) using a Rocker-Galaxy 230 model colony counter.Total coliform test was performed every two weeks for a period of six weeks.

Preliminary Test

A preliminary test was conducted to determine the time for the total coliform bacteria from sludge-based vermicompost to decrease using air dry and direct exposure methods. In the air-dry method, the vermicompost material was spread in a clean working space and regularly turned. The second method placed the vermicompost in a pvc barrel and the sunlight exposed it to natural irradiance. Each treatment observed three replicates per vermcompost type while each replicate had three pseudoreplicates. A total of 18 replicates per month was observed for the preliminary test. Total coliform tests were performed on a monthly basis for a period of one year.

Baseline data for total coliform, and nitrogen, phosphorus, potassium (NPK)

Total coliform test was conducted on biodegradable-based vermicompost and sludge-based vermicompost prior to treatment application using WHO-approved membrane filtration method. Results of the test served as baseline data for comparison. Furthermore, nutrient analyses specifically on the levels of Nitrogen, Phosphorus, and Potassium (NPK) were analysed on the type of vermicompost using Kjeldahl method (AOAC International), spectrophotometry, and atomic absorption spectrophotometry, Flame method (Shimadzu AAS, AA-6300), respectively.

Treatments

Twelve treatments were tested for their effects in lowering total coliform in vermicompost. These treatments revolved around two kinds of lime application (agricultural lime and hydrated lime) in two kinds of vermicompost (biodegradable- and sludge-based) with three ratios of lime application relative to vermicompost volume (1 part vermicompost:1 part lime , 1 part vermicompost: 0.5 part lime, and 1 part vermicompost: 0.25 part lime). Each treatment had nine replicates including pseudoreplicates, which were observed every two weeks prior to total coliform determination at day 15, 30, 45 and 60. Meanwhile, each set-up was subjected to daily manual mixing using shovel specifically assigned for each treatment.

The set-ups were covered with a clean paper to prevent contamination from accidental droppings of birds or rodents or unnecessary exposure to heavy moisture when it rained.Table 1 shows a summary of the treatments in the experiment.

Table 1. Treatments used in lowering total coliform in vermicompost

Agri Lime (1:1) + SVC (treatment 1)	Hydrated lime (1:1) + SVC (treatment 4)	Agri Lime (1:1) + BVC (treatment 7)	Hydrated lime (1:1) + BVC (treatment 10)
Agri Lime (1:0.5) + SVC (treatment 2)	Hydrated lime (1:0.5) + SVC (treatment 5)	Agri Lime (1:0.5) + BVC (treatment 8)	Hydrated lime (1:0.5) + BVC (treatment 11)
Agri Lime (1:0.25) + SVC (treatment 3)	Hydrated lime (1:0.25) + SVC (treatment 6)	Agri Lime (1:0.25) + BVC (treatment 9)	Hydrated lime (1:0.25) + BVC (treatment 12)

Agrilime – agricultural lime; SVC – sludge vermicompost;
BVC – biodegradable vermicompost

Figure 2. Agricultural lime from site source (left); experimental set-up (right)

Statistical Treatment

Shapiro-Wilk's test was employed for normality test of the data followed by Levene's test for homogeneity test. In case of a non-normal distribution, data set was log transformed and then, proceeded to One-way Analysis of Variance. Post-hoc analysis used Tukey's HSD to determine where the significant difference lies among the 12 treatments. The level of significance was observed at $\alpha = 0.05$.

RESULTS

Preliminary Test

Figure 3 indicates trends of total coliform reduction in sludge-based vermicompost using air-dry method against direct exposure to sunlight. Initial total coliform population was 54,000,000 cfu/100 ml in the first month. By the end of 16 months, total coliform using air-dry method was 208,000 cfu/100 mL as compared to 307,000 cfu/100 mL using direct sunlight method. T-test showed no significant difference in either methods (p = 0.32).

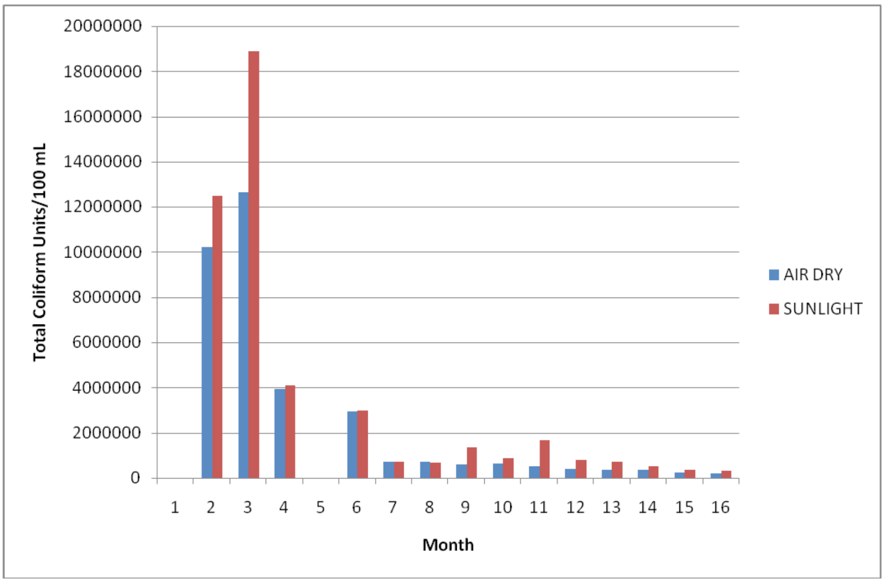

Figure 3. Coliform reduction strategies using air drying method versus direct sunlight exposure of vermicompostfrom August 2013-November 2014

Baseline Data of Total Coliform

Figure 4 shows that raw sludge contained the highest total coliform of 6,300,000 cfu/100 mL while raw biodegradables consisted of 2,550,000 cfu/100 mL of total coliform. When these base materials were processed in the gut of the African Night Crawler worm, sludge-based vermicompost contained 540,000 cfu/100 mL of total coliform while biodegradable vermicompost had 400,000 cfu/100 mL of total coliform. All four materials tested positive for *Escherichia coli*, which is a bacterium indicative of human fecal contamination.

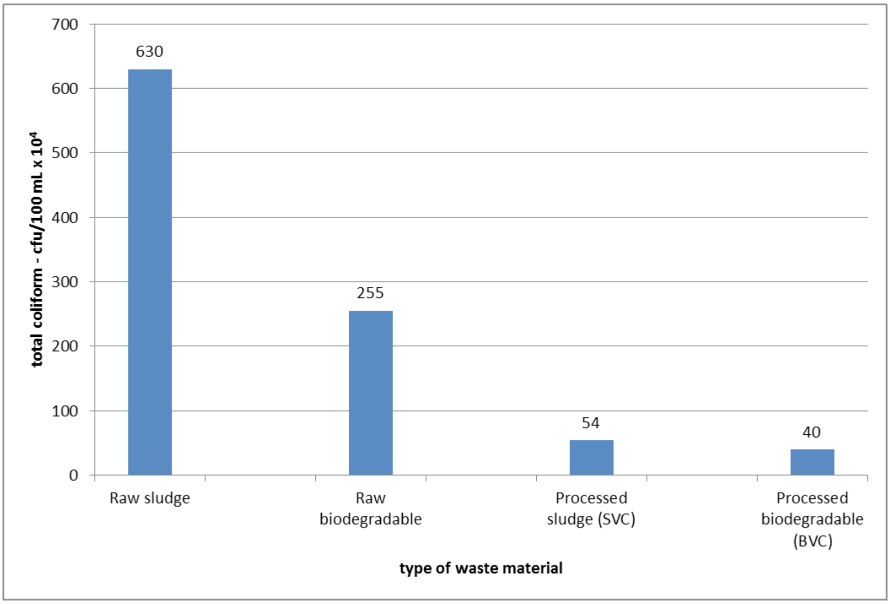

Figure 4. Baseline of total coliform levels of four types of vermicompost-related material

NPK Levels of Vermicompost Materials

When vermicompost materials were analysed for NPK, Nitrogen was highest in sludge-based vermicompost but lowest in the same vermicompost type that was added with hydrated lime. However, sludge-based vermicompost that was added with agricultural lime had intermediate levels of Nitrogen. Phosphorus was noted to be highest in raw sludge and lowest in sludge-based vermicompost that was added with hydrated lime. Those that were treated with agricultural lime showed intermediate values of Phosphorus as well. Lastly, Potassium was highest in biodegradable vermicompost and lowest in sludge-based vermicompost that was added with agricultural lime. The vermicompost with hydrated lime showed the lowest levels of NPK, indicating the nutrient-lowering effect of this lime as compared with agricultural lime.

Table 2. NPK Levels and pH of Raw Sludge, Biodegradable and Sludge-based Vermicomposts

Parameters	Raw Sludge	BVC	SVC	AL + SVC (1:1)	AL + SVC (1:1)	HL + SVC (1:1)
Nitrogen (%)	1.08 (+ 0.01)	0.64 (+0.08)	1.67 (+0.40)	0.92 (+0.05)	0.48 (+0.01)	0.28 (+0.05)
Phosphorus (%)	1.31 (+ 0.16)	0.34 (+0.04)	0.75 (+0.04)	0.65 (+0.03)	0.37 (+0.02)	0.20 (+0.04)
Potassium (%)	0.42	0.65	0.58	0.53	0.35	0.51

BVC – biodegradable-based vermicompost; SVC – sludge-based vermicompost; AL + SVC = Agricultural lime + Sludge-based vermicompost; AL + SVC = Agricultural lime + sludge-based vermicompost; HL + SVC = Hydrated lime + sludge vermicompost

Trends in Trial 1

Figure 5 shows that lime application hastened total coliform reduction in both types of vermicompost across 12 treatments. Generally, treatments with hydrated lime showed the faster reduction of total coliform within 15 to 30 days from application (1-B versus 1-A; 1-D versus 1-C of Figure 3, respectively). Meanwhile, those with agricultural lime showed slower reduction rate of total coliform in the same period. At the end of 60 days, however, total coliform was greatly reduced at levels below 1000 cfu/100 mL across all treatments.

Analysis of Variance test showed significant difference among 12 treatments in all observation periods: Day 15, Day 30, Day 45, and Day 60 (p=0.000). However, based on type of lime applied relative to their concentrations (i.e., agricultural lime, hydrated lime), Tukey's HSD indicated that lower lime concentrations (1:0.25>1:0.50>1:1) harbored higher total coliform population. The longer the day of treatment (>30 days), the more that the total coliform levels dropped significantly (p=0.000).

Trends in Trial 2

Figure 6 confirms that lime application hastened total coliform reduction across 12 treatments. Like in Trial 1, hydrated lime application had faster reduction rate of total coliform bacteria within 15 to 30 days after application.

It was likewise noted that at the end of 60 days post lime application, the total coliform was reduced below 1,000 cfu/100 mL across all treatments. Regardless of type of lime that was applied, total coliform levels were reduced at acceptable levels two months after lime application.

Analysis of Variance test shows significant difference among the twelve treatments at all observation periods: Day 15, Day 30, Day 45, and Day 60. Similar to the results in Trial 1, agricultural lime application significantly reduced total coliform levels at Day 30 onwards, while hydrated lime showed faster reduction rate as early as Day 15 relative to lime concentrations.

DISCUSSION

Vermicompost fertilizers are organic materials that come from plant and animal sources. The top sources of organic fertilizers come from bat guano, crab and shrimp wastes, blood meal, bone meal, kelp, and chicken manure among others. Their NPK ratios can reach > 6: 4:1:5 as compared to commercial fertilizers, which contain >15:11:5 NPK ratios (OSU Extension Services, n.d.; EPA, 1999). Commercial fertilizers are noted for their rapid effects on soil nutrient improvement; however, long-term use renders the soil acidic and the cost of operation expensive. Organic fertilizers are economical in the long run and promote sustainable agriculture practices since they complement and balance microbial ecology and nutrient in the soil which the plants need (Monroy *et al.*,2009; Yadav *et al.*, 2011).

This study makes use of vermicompost from biodegradable waste materials and dried sludge from human wastes, which form the bulk material in the study site. Annually, a total of 1,200 tons of biodegradable material and 2,250 tons of septic materials were received by the sanitary landfill facility. From these volumes, only 56 and 48 tons were processed as biodegradable and sludge vermicompost products, respectively (BCWMEC, 2015). The rest were bagged as "garden soil" which served as enrichment material for fruit tree production. In effect, there was a great potential to produce vermicompost but was hindered because of the high coliform levels present in the vermicompost products.

The type of earthworm used is critical in the production of low coliform level in the vermicompost (Yadav *et al.*, 2010; 2011; & 2012). Unfortunately, the African Night Crawler earthworm does not possess this trait as seen in the preliminary test of this study. To reduce coliform pathogens, agricultural

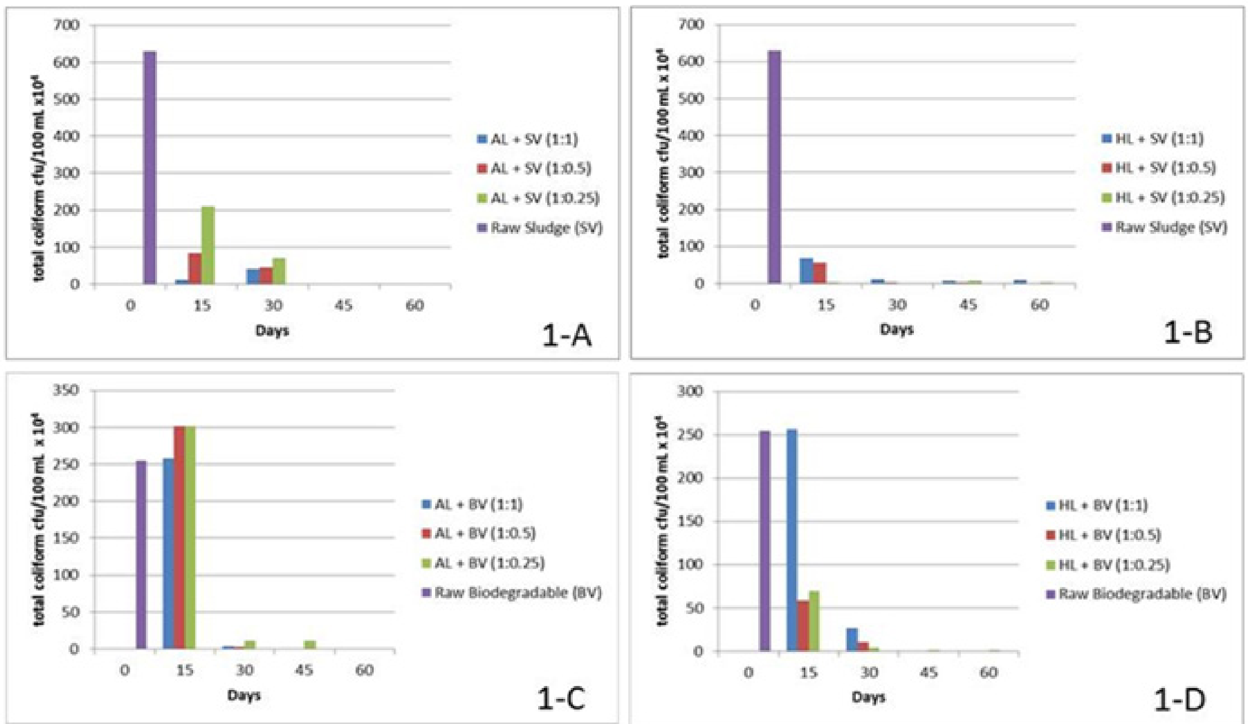

Figure 5. Trends in total coliform reduction in trial 1 using agricultural lime application + sludge vermicompost (1-A), hydrated lime application + sludge vermicompost (1-B), agricultural lime + biodegradable vermicompost (1-C) and hydrated lime + biogdegradable vermicompost (1-D). Each treatment had three kinds of ratio of lime application relative to vermicompost volume (1:1, 1:0.5 and 1:0.25)

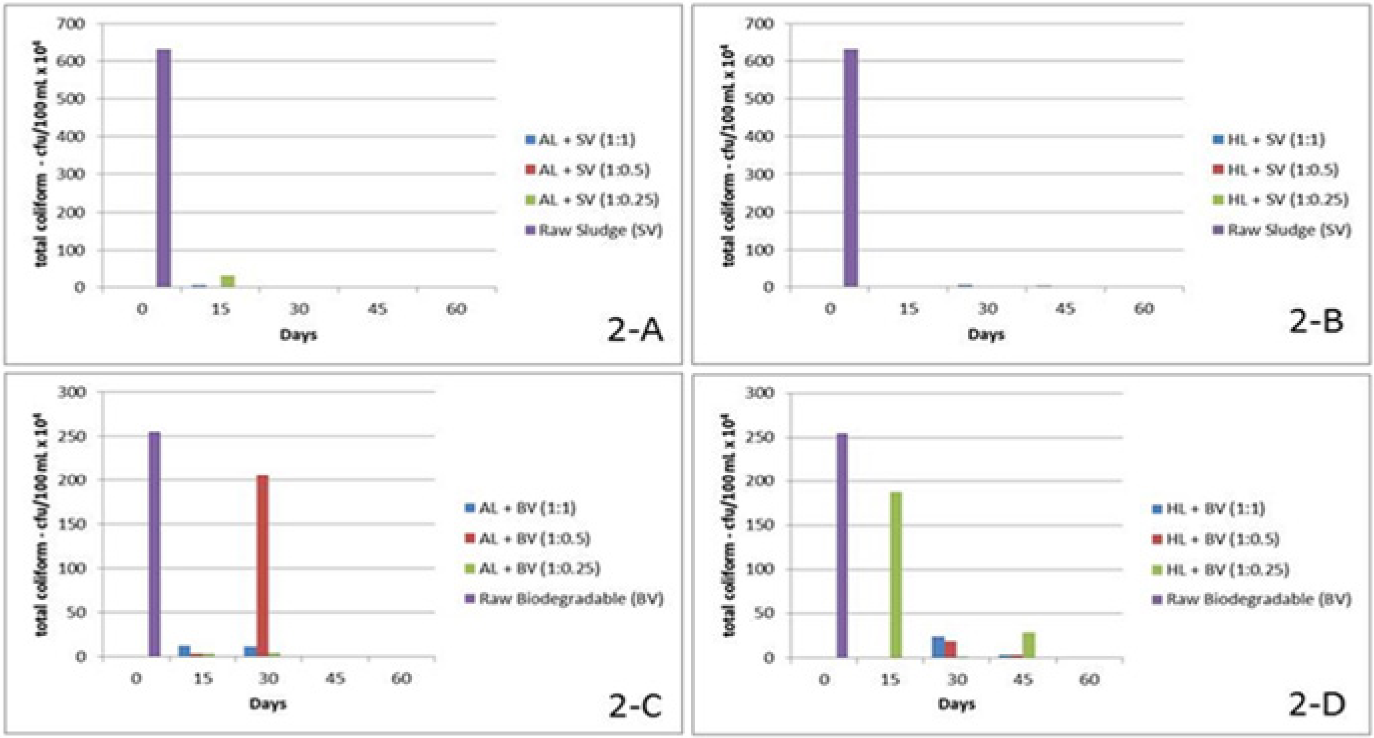

Figure 6. Trends in total coliform reduction in trial 2 using agricultural lime application + sludge vermicompost (2-A), hydrated lime application + sludge vermicompost (2-B), agricultural lime + biodegradable vermicompost (2-C) and hydrated lime + biogdegradable vermicompost (2-D); each treatment had three kinds of ratio of lime application relative to vermicompost volume (1:1, 1:0.5 and 1:0.25)

and hydrated lime were applied from low to high concentrations. In Trial 1, agricultural lime application to sludge vermicompost (Treatments 1, 2, and 3) showed no significant variations in the levels of coliform at Day 30 ($p = 0.898$). This was because total coliform levels were still high at this period; however, Day 45 and Day 60 showed significant coliform variations where Treatment 3, which had the lowest lime concentration, showed higher coliform level although at an acceptable level by WHO.

The agricultural lime application to biodegradable vermicompost (Treatments 7, 8 and 9) showed significant variations in the levels of coliform at Days 30 and 60 ($p=0.002$, $p=0.000$, respectively). The significant difference lies in the treatment with the lower concentration of agricultural lime which resulted to a higher total coliform level (Treatment 9 > Treatment 7 = Treatment 8). Nevertheless, at Day 60, total coliform levels were at acceptable levels (0-1000 cfu/100 mL) across all three concentrations of agricultural lime. In contrast, hydrated lime application (Treatment 4, 5, and 6) to sludge vermicompost resulted in significant variations at Day 15 ($p=0.000$)and Day 30 ($p=0.000$) while no significant variations were noted at Days 45 and 60. This suggests that 45 days after treatment, hydrated lime in Treatments 4, 5 and 6 greatly reduced, if not eliminated, coliform bacteria present in the sludge vermicompost. Hydrated lime application to biodegradable vermicompost (Treatments 10,11 and 12) showed significant variations at Days 15, 30, 45 and 60($p=0.000$, $p = 0.000$, $p=0.000$, *respectively)* where the lowest concentration of hydrated lime showed the highest coliform levels although at an acceptable level set by WHO.

Trial 2 confirmed the results in Trial 1; agricultural lime application to sludge vermicompost (Treatments 1,2, and 3) showed no significant variations in the levels of coliform at Days 15, 30, 45 and 60 ($p = 0.171$, $p = 0.132$, $p = 0.151$ *and* $p=0.383$, respectively). This was because total coliform levels were high at the early days after treatment. The same treatments showed very low levels of total coliform after 45 and 60 days. However, the agricultural lime application to biodegradable vermicompost showed significant variations in the levels of coliform at Days 15, 30 and 60 ($p=0.004$, $p=0.000$, $p=0.039$, respectively). The significant difference lies in the treatment with the higher concentration of agricultural lime which resulted in the lowest total coliform level (Treatment 7 < Treatment 8 = Treatment 9). Nevertheless, at Day 45 and 60, total coliform levels were at acceptable levels (0-1000 cfu/100 mL).

In contrast, hydrated lime application (Treatment 4, 5, and 6) to sludge vermicompost showed significant variation at Day 30 (*p=0.016*) while no significant variations were noted at Days 45 and 60. It was apparent that 30 days after treatment, hydrated lime in Treatments 4, 5 and 6 greatly reduced, if not eliminated, coliform bacteria present in the sludge vermicompost. Hydrated lime application to biodegradable vermicompost (treatments 10, 11 and 12) showed significant variations at Days 15, 30, and 45*(p=0.000, p = 0.000, p=0.001)*. The significant difference lies in the treatment with the lowest concentration of hydrated lime, which likewise contained higher coliform bacteria although at an acceptable according to WHO standards. No variation was noticed at Day 60 (*p=0.123)*, indicating that coliform levels were zero to near zero values.

The study of Lalander *et al.* (2013) showed that pH contributed the highest impact in reducing pathogens present in vermicomposted human wastes. Since the optimum pH range of *E. coli* is between 6 and 7 (Desmarchelier & Fegan, 2003 as cited by Yates, n.d.), this serves as a basis for the coliform-reducing activity of lime application. However, the interaction between NPK and high lime application (Table 2) suggests that hydrated lime reduces NPK levels of vermicompost which impairs optimal plant growth. The study of Jensen (2010) supports this negative interaction where high pH causes precipitation of phosphorus, thus, making it less available to plants.

It is, therefore, practical to use agricultural lime rather than hydrated lime to target good plant growth and low coliform levels in the vermicompost. Based on economics, hydrated lime is costlier than agricultural lime; however, both lime materials reduce the total coliform level effectively within 60 days after treatment across three concentrations. Thus, this study suggests that lowest concentration (1:0.25) of agricultural lime offers the best option in lowering coliform while maintaining nutrient availability when using vermicompost produced by African Night Crawler earthworm.

CONCLUSION

Aeration and exposure to the environment for 15 months did not reduce coliform levels in vermicompost produced by the African Night Crawler earthworm *E. eugeniae*. From an initial level of 54,000,000 cfu/100 mL, the levels of total coliforms were 208,000 and 307,000 cfu/100 mL using aeration method and exposure to sunlight method, respectively. Application of lime, either agricultural or hydrated, significantly reduced the levels of coliform beginning 30 days after treatment. Between Day 45 and Day 60, total coliform levels were reaching zero values and near zero values in both types of lime application. It was clear that hydrated lime reduced the coliform levels faster than agricultural lime as early as Day 15 and Day 30. In terms of concentration of lime and its effects on coliform, higher agriculture lime application to biodegradable vermicompost (1:1) resulted in lower coliform levels ($p=0.000$).

Meanwhile, when agriculture lime was added to sludge vermicompost, no significant variations of coliform were noticed regardless of agriculture lime concentrations ($p=>0.05$). Hydrated lime application to sludge vermicompost showed significant variation where high concentration lowers coliform levels 30 days after treatment ($p = 0.016$), but then at Day 45 onwards, no significant difference was noted ($p=>0.05$) in the treatments as coliform levels were reduced to zero values. Hydrated lime application to biodegradable vermicompost showed no significant difference at Day 15, Day 30, Day 45 as coliform levels were greatly reduced regardless of concentrations of hydrated lime ($p=>0.05$).

Lime application is an effective way of reducing vermicompost produced by African Night Crawler Earthworm, *E. eugeniae*. It is recommended that the SLF of Bayawan City consider the following options to improve the status of its vermicompost production targets:

1. Perform cost analysis of each treatment related to coliform reduction strategies in the vermicompost of Bayawan City;

2. Optimize the production of vermicompost as yearly biodegradable vermicompost production of the facility is 56 tons from a raw biodegradable material of 1,200 tons. Sludge vermicompost production, in like manner, has to be optimized as yearly barely reached 50 tons from a raw sludge material of 2,250 tons;

3. Do trial application of lime-treated vermicompost to identified agricultural farms. This can be undertaken by Bayawan LGU to serve as baseline production data;

4. Compare total coliform levels in the vermicompost produced by alternative earthworms such as Red Tiger worm Eisenia fetida, and Indian Blue Worm Perionyx excavates;

5. Explore other ways of reducing total coliform such as addition of ash from burned rice hulls and sugar waste, which are abundant in the agricultural landscape of Bayawan City.

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APPENDIX

Appendix - A. T-test Comparing Air Dry Method Versus Direct Sunlight

t-Test: Two-Sample Assuming Equal Variances		
	Air dry Method	**Direct Sunlight**
Mean	2,459,000	3,316,357.143
Variance	1.58218E+13	3.01353E+13
Observations	14	14
Pooled Variance	2.29786E+13	
Hypothesized Mean Difference	0	
Df	26	
t Stat	-0.473204947	
P(T<=t) one-tail	0.320007919	
t Critical one-tail	1.70561792	
P(T<=t) two-tail	0.640015838	
t Critical two-tail	2.055529439	

Appendix - B. Summarized ANOVA values in Trial 1 and Trial 2 with Tukeys HSD in parenthesis

Trial 1

Treatments 1-3 Trt 1 – AL + SVC (1:1) Trt 2 – AL + SVC (1:0.5) Trt 3 – AL + SVC (1:0.25)	**Treatments 4-6** Trt 4 – HL + SVC (1:1) Trt 5 – HL + SVC (1:0.5) Trt 6 – HL + SVC (1:0.25)
Day 15 p = 0.000 (Trt 1 < Trt 2 < Trt3) Day 30 p = 0.898 Day 45 p = 0.032 (Trt 3 > 1=2) Day 60 p = 0.005 (Trt 3 > 1=2)	Day 15 p = 0.000 (Trt 6 < Trt 4 = Trt 5) Day 30 p = 0.000 (Trt 4 > Trt 5 = Trt 6) Day 45 p = 0.440 Day 60 p = 0.062
Treatments 7-9 Trt 7 – AL + BVC (1:1) Trt 8 – AL + BVC (1:0.5) Trt 9 – AL + BVC (1:0.25)	**Treatments 10-12** Trt 10 – HL + BVC (1:1) Trt 11 – HL + BVC (1:0.5) Trt 12 – HL + BVC (1:0.25)
Day 15 p = 0.06 Day 30 p = 0.002 (Trt 9> Trt 7 = Trt8) Day 45 p = 0.000 (Trt 9> Trt 7 = Trt8) Day 60 p = 0.853	Day 15 p = 0.000 (Trt 10 > Trt 11 = Trt 12) Day 30 p = 0.000 (Trt 10 > Trt 11 = Trt 12) Day 45 p = 0.000 (Trt 12 > Trt 10 = Trt 11) Day 60 p = 0.000 (Trt 12 > Trt 10 = Trt 11)

AL – Agricultural lime; BVC – Biodegradable vermicompost; HL – hydrated lime; SVC – Sludge-based vermicompost

Trial 2

Treatments 1-3 Trt 1 – AL + SVC (1:1) Trt 2 – AL + SVC (1:0.5) Trt 3 – AL + SVC (1:0.25)	**Treatments 4-6** Trt 4 – HL + SVC (1:1) Trt 5 – HL + SVC (1:0.5) Trt 6 – HL + SVC (1:0.25)
Day 15 p = 0.171 Day 30 p = 0.132 Day 45 p = 0.151 Day 60 p = 0.383	Day 15 – no variation in the data Day 30 p = 0.016 (Trt 4 > Trt 5 = Trt 6) Day 45 p = 0.090 Day 60 – no variation in the data
Treatments 7-9 Trt 7 – AL + BVC (1:1) Trt 8 – AL + BVC (1:0.5) Trt 9 – AL + BVC (1:0.25)	**Treatments 10-12** Trt 10 – HL + BVC (1:1) Trt 11 – HL + BVC (1:0.5) Trt 12 – HL + BVC (1:0.25)
Day 15 p = 0.004 (Trt 7 > Trt 8 = Trt 9) Day 30 p = 0.000 (Trt 7 > Trt 8 = Trt 9) Day 45 p = 0.653 Day 60 p = 0.039 (Trt 7 < Trt 8 = Trt 9)	Day 15 p = 0.000 (Trt 12 > Trt 10 = Trt 11) Day 30 p = 0.000 (Trt 12 < Trt 10 = Trt 11) Day 45 p = 0.001 (Trt 12 > Trt 10 = Trt 11) Day 60 p = 0.123

AL – Agricultural lime; BVC – Biodegradable vermicompost;
HL – hydrated lime; SVC – Sludge-based vermicompost