

Reduction of Pathogens from Mixture of Cow Manure, Domestic Waste and Wastewater Treatment Plant Sludge by Vermicomposting Process

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ABSTRACT

Introduction: The present study aimed to investigate the microbial quality of the produced vermicomposts and compare them with the existing standards.

Materials and Methods: This experimental study was conducted as a pilot-scale one in the laboratory school. Some perishable domestic wastewater mixed in a reactor, including food waste, vegetables and fruits, cow manure and sludge from wastewater treatment plant. Tests to determine the microbial quality of the product were carried out at an early stage during the process of production and on the final product. These tests included determining the probable number of fecal coliform bacteria and parasite eggs.

Results: According to the results, a significant decrease was observed in the number of fecal coliforms in sludge manure and domestic waste, as the number of fecal coliforms reduced from 5000000 (MPN / g) in the raw sample to 1500 (MPN / g), eight weeks after the outset. Moreover, according to the obtained results, the mixture of manure, sludge, and domestic waste had some parasite eggs (20 number /gr) in the raw samples. This amount was fully removed by the process of vermicomposting during the third week.

Conclusion: Findings revealed that the earthworms have a high capability to reduce the pathogens without increasing in temperature, however, in order to standardize the number of coliforms (compost class A), the vermicomposting of the mixture of cow manure, domestic waste, and sludge of wastewater treatment plant is not appropriate.

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Introduction

A dramatic increase in the need for food ingredients, due to the population growth, has resulted in transforming traditional agriculture to industrial one and also leading to an increasing consumption of chemical fertilizers to accelerate the production of agricultural products. Therefore, the indiscriminate use of chemical fertilizers and chemical pesticides has led to the destruction of physical and chemical structures of soil, reduction of soil organic matter, environmental degradation, and human health problems. Using vermicompost can be considered a method to deal with such

harmful effects to reform the reinforcement of agricultural soils and prevent the degradation of agricultural lands^{1, 2}. Vermicomposting can be defined as the growing of earthworms in organic waste, its processing, and producing fertilizers from waste. Vermicompost, which is produced as a result of the conversion of organic wastes by earthworms, is used successfully to convert the waste such as sludge of wastewater treatment, dairy factory waste, food waste, municipal waste, paper waste, and animal waste to fertilizer. Vermicomposting is the processes of decomposing waste due to the interactions between earthworms and microorganisms. Although

microorganisms are primarily responsible for decomposition process, earthworms increase the contact surface lead to increase in microbial activity and change in biological activity³. The lack of sufficient information regarding the process of reducing human pathogens during the waste vermicomposting is a reason that why vermicomposting is not widely accepted. Since conventional composting process exploits in the thermophilic phase, vermicompost is exploited in mesophilic phase. In this phase, the temperature should be less than 35°C to prevent disorder in the process of vermicomposting as a result of the death of worms⁴. Several studies reported pathogen reduction in vermicomposting process under specific processing conditions to a safe level. The first reduction of pathogens was reported by USEPA. According to the report, the passage of biological solids of wastewater from the gut of the earthworms reduces pathogens⁵. Moreover, in 2005, a decrease of pathogens in the vermicompost of cow manure was reported by Contreras-Ramos⁶. In a study conducted in 2009 by Monroy et al. 85-98 percent reduction of total coliforms was reported on the vermicomposting process of pig manure⁷. Furthermore, Liu et al. reported some reduction in *Escherichia coli* O157: H7 in vermicomposting process of artificial soil⁸. In another study, Rodriguez et al. reported some reduction of pathogens in vermicomposting process of septic tank sludge⁹. Aira et al. reported a significant reduction of pathogens in the vermicomposting process of cow manure¹⁰. Similarly, Hila et al. in a study showed a decrease of coliforms in toilet vermicomposting¹¹. In another study, Hill reported the effect of vermicomposting on reducing the number of *Ascaris* eggs¹². The aim of this study was to investigate the effectiveness of vermicomposting in microbial quality improvement of the produced compost.

Materials and Methods

This pilot-scale experimental study conducted in the laboratory of Public Health School, Yazd. The work was done in a pilot with 50 (L) × 15(W) × 30(H) cm in size to produce vermicompost from perishable domestic waste, including food residue,

vegetables and fruit waste, cow manure, and sludge of wastewater treatment plant. These ingredients were mixed with equal proportions of cow manure, sludge of wastewater treatment plant, and domestic waste. The operational conditions of the pilots included a period of 8 weeks under aerobic conditions, and in the ambient temperature ($30 \pm 5^{\circ}\text{C}$) and 70 percent humidity. The type of worms used for vermicomposting was *Eisenia fetida*. Sampling was carried out duplicate during 57 days of the process. 50 g samples were collected at each sampling time. Finally, 68 samples were collected.

To determine the microbial quality of the product, some tests were carried out at an early stage during the process of production and on the final product. These tests included determining the probable number of fecal coliform bacteria and parasite eggs. Specific A₁ cultivating environments and Zinc were utilized for fecal coliform and the detection of parasite eggs, respectively¹³⁻¹⁴.

After preparing the intended cultivating environments, 5 g of the sample was inoculated in liquid enrichment environment of non-selective peptone water. After putting the sample in an incubator shaker at the temperature of 25°C, for 5 minutes, it was placed in an incubator at the temperature of 37°C, for 16-20 hour.

Coliform identification was done using 9 Tube fermentation method and A₁ environment. Moreover, at testing period, coliform was used in three dilutions (0.01, 0.1, and 1) under the Serological Ben Murray condition with a temperature equal to 41.5 °C for about 20-24 hours.

In order to evaluate the effect of treatment on the number of coliforms, one-way analysis of variance (ANOVA) was used. The amount of 0.05 is considered the level of significance. As a result, lack of normality in data and due to coliform rapid growth, mathematical conversion logarithm was used on the number of coliforms. This conversion could smooth the way for using ANOVA.

Results

The results of this study demonstrated a significant reduction in the number of fecal coliforms in sludge,

manure, and domestic waste, as the number of fecal coliforms reduced from 5000000 (MPN / g) in the raw sample, to 1500 (MPN / g), eight weeks after the outset (Figure1). According to the results listed in figure 2, a mixture of manure, sludge and waste domestic had some parasite eggs (20 number/g) in the raw samples. This amount of eggs was fully removed by the process of vermicomposting during

the third week. Physico-chemical properties of raw samples after performing the relevant tests are shown in tables 1 and 2. These properties include moisture, nitrogen, organic carbon, pH, and electrical conductivity (EC). Domestic waste moisture was significantly different from that of the manure and sludge whereas other parameters revealed a slight difference.

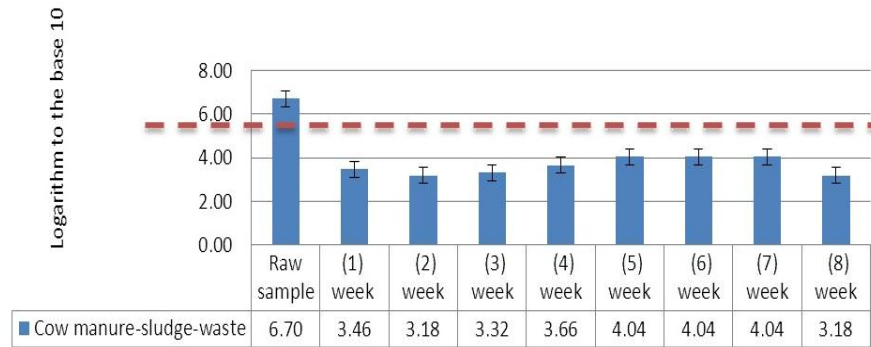


Figure 1: Changes in the number of fecal coliforms in stabilized Cow manure-sludge-Waste after 8 weeks

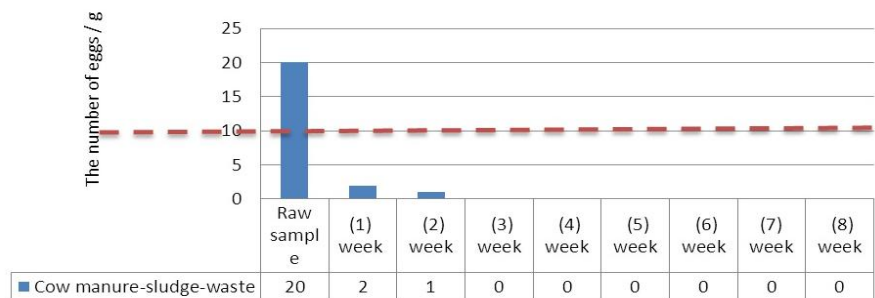


Figure 2: Changes in the number of parasite eggs in vermicompost produced from the mixture of cow manure and sludge of wastewater treatment and Waste after 8 weeks

Table 1: Physico-chemical parameters, sludge of wastewater treatment plant and cow manure in the raw samples

| parameters | Sludge of wastewater treatment plant | | | Cow manure | | |
|--------------------|--------------------------------------|---------|---------|--------------|---------|---------|
| | Mean ± SD | Maximum | Minimum | Mean ± SD | Maximum | Minimum |
| Moisture (%) | 17.25 ± 2.21 | 20 | 15 | 35.75 ± 4.34 | 40 | 30 |
| EC (mmhose/cm) | 2.17 ± 0.69 | 2.9 | 1.4 | 1.9 0.09 | 1.9 | 1.2 |
| Organic carbon (%) | 70.26 ± 8.06 | 79.8 | 40.24 | 64.4 ± 11.42 | 75.14 | 50.21 |
| Nitrogen (%) | 5.41 ± 0.65 | 7.9 | 1.2 | 2.11 ± 0.15 | 2.26 | 1.91 |
| pH | 7.06 ± 1.09 | 8.11 | 5.86 | 7.1 ± 0.75 | 7.5 | 6.2 |

Table 2: Physico-chemical parameters, raw samples of domestic waste

| | Moisture (%) | EC (mmhose/cm) | Organic carbon(%) | Nitrogen (%) | pH |
|---------|--------------|----------------|-------------------|--------------|-----|
| Minimum | 70 | 1.46 | 53.88 | 1.68 | 6.2 |
| Maximum | 73.7 | 1.46 | 54.16 | 1.7 | 6.4 |

Data analyzed by one-way ANOVA test, it was revealed that the average of the minimum fecal coliform for one of the weeks was significantly different from the others ($p < 0.001$). Duncan test

was used to check the details. The results of Duncan test is shown in table 3. The results show that there is a significant difference.

Table 3: Output of Duncan test

| Duncan ^a | | Subset for alpha = 0.05 | | | | | |
|---------------------|---|-------------------------|-------|-------|-------|-------|-------|
| Time | N | 1 | 2 | 3 | 4 | 5 | 6 |
| 2.00 | 2 | 3.17 | | | | | |
| 8.00 | 2 | 3.17 | | | | | |
| 3.00 | 2 | | 3.33 | | | | |
| 1.00 | 2 | | | 3.47 | | | |
| 4.00 | 2 | | | | 3.67 | | |
| 5.00 | 2 | | | | | 4.04 | |
| 6.00 | 2 | | | | | 4.04 | |
| 7.00 | 2 | | | | | 4.04 | |
| 0.00 | 2 | | | | | | 6.7 |
| Sig. | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

Discussion

Removing pathogenic agents from waste such as cow manure and sludge of wastewater treatment plants, that may be used to amend the soil, can be so significant in preventing the transmission of the disease. Vermicomposting has the ability to remove a significant number of pathogens. Passing different treatments through the gut of the earthworm can affect the microbial population. Reduction in the number of pathogens in vermicomposting depends on various factors such as the action of enzymes in the gut of the earthworm, colonic fluid secretion, which has antibacterial properties, as well as competition between different groups of microorganisms^{4, 15}. Monroy et al. conducted a study in 2008 to explore the reduction in density of nematodes, protozoa, and fecal coliforms as a

result of passing through the gut of the earthworm. The results showed that the nematode and fecal coliforms have significantly reduced. These results are with the same as the findings of this study. Reduction in the number of nematodes may be due to the fact that nematodes are part of earthworms' food. It may also be due to the point that the enzymic activity of proteolytics eliminates pathogens and nematodes¹⁶. In this study, the highest removal of coliform happened in the second week. The results of the study matches the findings of the one conducted by Monroy et al. in 2009 in which the greatest reduction of coliforms in pig manure, as a result of passing through the gut of the earthworm, was reported after the second week. Moreover, in this study, the number of coliforms increased after the 4th week. This

increase may happen as a result of moisture or worms running out of food⁷. There are some other studies, the results of which are with the same as the findings of this study, for example, Lui et al. in 1982 reported the reduction of more than 98 percent of pathogens within 17 days in vermicomposting process⁴. The results of a study conducted by Rodriguez, in 2010, on reducing the number of pathogens existing in septic tanks sludge in the process of vermicomposting showed that the number of pathogens had significantly reduced. These findings are consistent with the results of the present study⁹. Moreover, in a study carried out by Aira et al., in 2011, on the reduction of pathogens in cow manure, it was revealed that the number of fecal coliform did not achieve the EPA standard level, which is in line with the results of this study¹⁰. In another study by Contreras in 2005, the results, which are consistent with the ones presented in this study, showed that after 60 days, the number of parasite eggs was not detectable, and fecal coliforms had significantly reduced⁶. In another study in 2011, Hait reported that in the final product of vermicomposting derived from primary sludge of wastewater, pathogens had significantly reduced, and they were lower than the standard level set by the USEPA, which is consistent with the present results which may be due to differences in the type of treatment or test conditions¹⁵. Monroy et al., in a study conducted in 2008 and Eastman et al., in 2001, showed that the number of fecal coliforms in the process of vermicomposting had significantly reduced during 7 days. The results of above-mentioned study are consistent with the present study. The results of that research revealed a significant reduction in the number of parasitic worms after three days of vermicomposting which are in line with the results of the present study^{16,17}. In a study, in 2006, by Bowman et al. on the impact of vermicomposting process on the removal of the parasite eggs, the results revealed that vermicomposting is not capable of eliminating the parasite eggs. These findings are

different from the results of the present study, which may be due to differences in vermicomposting circumstances or laboratory settings¹⁸.

Conclusion

The results indicated that vermicomposting can be used as an appropriate method to convert perishable waste to fertilizer and also is usable in agriculture. Earthworms have a high ability to reduce pathogens without the rise in temperature; however, in order to minimize the number of coliforms, in vermicomposting, to the standard level of compost class A, the mixture of cow manure, domestic waste and sludge of wastewater treatment plant may not be appropriate.

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Conflict of interest

We have no competing interests.

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