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Enhancing agricultural sustainability through optimization of the slaughterhouse sludge compost for elimination of parasites and coliforms

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For a sustainable ecology, slaughterhouse sludge must be managed effectively in preview of the parasitic or coliforms' spill over to the community. In order to determine the effectiveness of a customized biological decomposer solution in lowering the parasitic eggs and coliform bacteria, three composting units (Unit 1, Unit 2, and Unit 3) were treated with its different amounts. Over a period of 60 days, pH, temperature, humidity, number of the parasitic eggs per gram (EPG) of faecal material, viability of eggs, and coliform counts were evaluated. By the fifth day of the composting process, pH had significantly ($P < 0.05$) increased across all the treatments and then decreased gradually. Also on the 5th day, all three units entered the thermophilic range (> 45 °C), which persisted for 20 days for Unit 3 and 15 days for Units 1 and 2. Humidity levels initially increased significantly ($P < 0.05$) in all three units (Unit 3 = 71%, Unit 2 = 64%, and Unit 1 = 55%) but then gradually decreased. On day 5, no decrease in EPG in Unit 1 was detected; however, a non-significant ($P > 0.05$) 12.5% decline in EPG in Unit 2 and Unit 3 was recorded. After that, a significant ($P < 0.05$) reduction in EPG was observed in all the three treatments until day 25. By day 5, decreased egg viability was significantly ($P < 0.05$) recorded in Unit 3 (21.43%); in Unit 1 and Unit 2, the decrease was 6.25% and 14.29%, respectively. Additionally, all units showed a significant ($P < 0.05$) decrease in total coliforms, meeting minimum allowable limit in Unit 2 and 3 on day 10 and on day 15 in Unit 1. The most substantial reduction in faecal coliforms was observed in Unit 3 (from 2.6 $\log_{10}$ to 1.3 $\log_{10}$), followed by Unit 2 (from 2.6 $\log_{10}$ to 1.5 $\log_{10}$), and then Unit 1 (from 2.6 $\log_{10}$ to 1.6 $\log_{10}$). The results of this study support recommendation of advanced composting techniques to eradicate or reduce the abundance of pathogens (parasites and coliforms). Hence, we endorse the value of careful composting procedures in environment-friendly abattoir waste management and agricultural practices through creating pathogen-free, eco-friendly fertilizers to promote both agricultural and environmental sustainability.

Keywords Slaughterhouse sludge, Composting, Parasites, Coliform, Waste management

Escalating global concerns over solid waste generation have been primarily fueled by rapid increases in population, industrialization, and urbanization, exerting immense pressure on both the environment and

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public health[1]. Alongside these concerns, contamination of groundwater with nitrates due to livestock waste has emerged as a critical issue[2]. Among the various sources of pollution, abattoirs or slaughterhouses stand out as significant contributors to land, water and air pollution on a global scale[3]. These abattoirs generate substantial amounts of waste, comprising animal faeces, blood, pieces of hides, hooves, hair, contaminated carcasses, and other byproducts, collectively referred to as abattoir waste. Inadequate management of such waste poses severe environmental and public health risks. Besides contaminating land, surface water and groundwater, untreated sludge from abattoirs can introduce pathogens and excess nutrients, thereby jeopardizing ecosystems and the safety of drinking water sources[4]. Improper disposal of wastes on land causes soil pollution due to heavy metals, pathogens, and excess of nutrients which results in comprosied soil quality and limited biodiversity[5]. The water runoff from these wastes introduces blood, pathogens, and organic matter, causing eutrophication, spread of waterborne diseases, and oxygen depletion, which threatens not only the aquatic life but also risk animal and human health through contaminated water supplies[6]. Emissions of greenhouse gases such as CH_4 and NH_3 by-products from decomposition of organic wastes not only responsible for air-pollution but also contribute to global warming and adversely affect the health of nearby residents especially through respiration[7]. These impacts can mainly be controlled via adequate waste management.

Slaughterhouse waste rich in pathogens, particularly parasites, demands proper disposal to prevent environmental degradation. Traditionally, this waste has been disposed of in landfills, incurring additional costs and wasting valuable resources[8]. In addition to environmental concerns, improperly managed abattoir waste can contribute to methane gas emissions, exacerbating the greenhouse effect. Methane is one of the greenhouse gases emitted into the atmosphere with the greatest potential to intensify climate change as it is mainly produced through the activity of methanogenic bacteria during anaerobic decomposition of organic matter[9]. A potential solution lies in harnessing slaughterhouse sludge for organic fertilizer production, enhancing soil fertility and promoting sustainable agriculture by treating the sludge. Organic fertilizers have demonstrated their effectiveness in improving soil health and crop yields[10]. While some waste materials have been utilized for the production of organic composts for fertilization, limited research exists on pathogen assessment in treated slaughterhouse sludge and its subsequent impact on agricultural productivity[11].

Efforts to address these issues have encompassed evaluating the efficacy of wastewater treatment plants in removing parasites, composting techniques for pathogen elimination, and the potential benefits of organic fertilizer production. For instance, studies conducted on urban and domestic wastewater treatment plants in Tehran province, Iran, highlighted the removal efficiencies of parasites from urban and slaughterhouse treatment plants[12]. A study on human excreta composting demonstrated a reduction in *Ascaris* eggs after 35 days, while *Entamoeba* cysts persisted beyond 60 days[13]. Temperature emerged as a reliable predictor for *Ascaris* inactivation in composting, and its efficacy was evident in various studies[14]. While current methods of composting organic waste are effective, they tend to be expensive because of the additional cost of infrastructure, manpower, and the length of time taken before the process is complete[15]. The efficiency of composting of organic waste varies with the location of the researchers, often hampered by the variable composition of the waste as well as contaminants such as pathogens[16]. Despite these efforts, comprehensive research specifically targeting the treatment of slaughterhouse sludge through composting for the reduction or elimination of parasites remains scarce. A prominent reason would be that people are less aware of the composting procedure itself and potential risks of contaminatns like parasites, chemicals and bacteria, which are quite popularly discussed in the context of public health[17]. Funding preference, lack of regulation, regional differences in parasitic testing of sludge, and similar factors create voids in research designs, making it impossible to conduct comparative studies[18].

In light of the substantial volume of slaughterhouse sludge generated, composting is a promising strategy for managing this waste and converting it into nutrient-rich fertilizers. The research reported here seeks to address the challenge of slaughterhouse waste management to control the spread of parasites and coliform bacteria. We treated abattoir sludge with customized biological decomposer solution and measured their effects on parasites and coliform bacteria in an effort to produce organic fertilizer with minimal pathogen contamination with the ultimate goal of promoting environment-friendly sanitation, hence, contributing to the sustainable agricultural practices.

Materials and methods

Study area

The study was conducted in Narowal, a district situated in the northeastern region of Punjab province, Pakistan (Fig. 1). Narowal district comprises three tehsils (subdivisions): Narowal, Shakargarh, and Zafarwal, encompassing a total of 74 Union Councils[19]. The current investigation was carried out between May and July 2023. The average low temperature during these months was around 25 °C, while the average high temperature was around 42 °C. The region typically experiences high levels of humidity at this time of the year. Additionally, the district experienced moderate to significant rainfall throughout these months.

Collection and transportation of gastrointestinal contents for composting

Sludge and rumen contents from slaughterhouses were collected from Narowal district. The composting procedure was then carried out at the KBCMA College of Veterinary and Animal Sciences, Narowal. An in-vessel approach was used to compost the leftovers from the abattoir. In particular, 200-liter-capacity oil drums were used as containers or bioreactors. These drums were altered for composting purposes by adding a central shaft that ran the length of the drum and was fitted with numerous longitudinal rods to make it easier to thoroughly mix the sludge. Additionally, a 50.0 × 40.5 cm door was built into each bioreactor for aeration, loading, unloading, sampling, and cleaning. The collected sludge was spread out on the ground and given three days to dry to lower the moisture level before composting. Each drum received about 100 kg of abattoir waste for

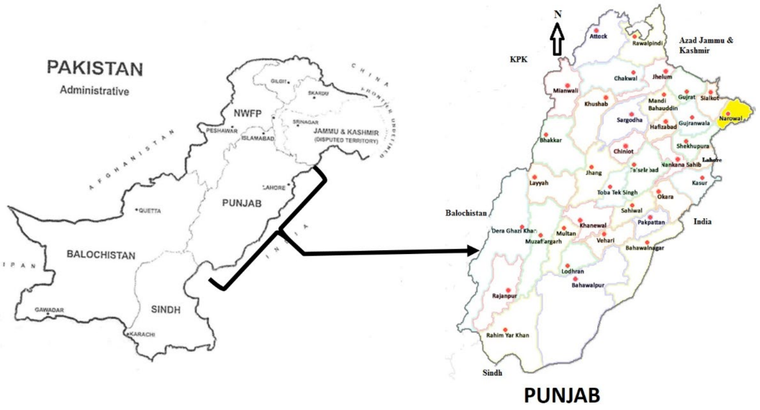

Fig. 1. Map of Pakistan showing the studied district Narowal located in Punjab, Pakistan.

the composting procedure. Digital thermo-hygrometers were used to measure temperature and humidity of the compost at three separate sites inside each drum during the composting process. Until the composting process was complete, these measurements were taken on the first day and then every five days[20].

Preparation of biological decomposer solution

Two hundred liters of water were carefully measured and placed into a drum to make an efficacious biological decomposer solution for composting. Two Kg of sugarcane, 500 g of broken rice, and two Kg of chickpea flour were added to this solution. This varied mixture of organic materials served as the compost's nutrient-rich base. One liter of biofertilizer[21] was bought from the Institute of Soil Science, Faculty of Agriculture, University of Agriculture, Faisalabad, to increase the efficacy of the biological decomposer solution. This biofertilizer served as the compost's natural decomposer because it was rich in necessary nutrients and beneficial microbes such as carbon fixers, phosphorus solubilizers, and zinc solubilizers. The solution was left in a cool, dry environment for 5 days so that fermentation could take place. To ensure that all ingredients were thoroughly mixed throughout this time, there was routine shaking of the mixture. The biological decomposer solution was utilized as a decomposer after preparation. Initially, 10 L of the biological decomposer solution were poured onto drum 1 (Unit 1), 15 L onto drum 2 (Unit 2), and 20 L onto drum 3 (Unit 3). Every fifth day, the drum door was opened to facilitate aeration, ensure thorough mixing of the compost material and allow collection of samples. Following this, the drum was tightly sealed with a plastic sheet to maintain optimal temperature and humidity levels.

Determination of pH

Using a pH meter, the pH of the composting material was measured. Initially, 20 g of thoroughly mixed composting material were taken from each drum. To ensure reliable measurements, the pH meter was calibrated using standard buffer solutions with pH values of 4.0, 7.0, and 10.0. To avoid contamination, the pH electrode was thoroughly cleaned and rinsed with distilled water before being gently inserted into the compost sample. The pH value of the composting material was recorded after stabilization. To assure accuracy, this process was carried out three times using fresh samples and the average pH value was determined[22].

Enumeration of parasitic eggs per gram of faeces

Every fifth day, three 15 g sludge samples were taken from each drum after full mixing to quantify the abundance of parasitic eggs. These samples were labelled and then transported to the lab at 4 °C. The modified McMaster technique, as described by Rizwan et al.[23], was used to quantitatively measure the parasite load, specifically the number of parasitic eggs per gram (EPG) of faecal material.

Egg viability test

The de Victoria and Galvan[24] method of safranin dyeing was used to determine the viability of helminth eggs. The sample was stained after centrifugation and removal of the supernatant by adding 50 mL of safranine O (2.5% in H_2O) to the sediment. The tubes were filled with water and centrifuged at 800 g for 5 min after waiting for at least 10 min. After pouring off the supernatant and re-centrifuging the tubes, the pellet was re-suspended in water. Eggs in the entire sample were analyzed on glass slides covered with glass coverslips after the sediment had been diluted with 0.1 N H_2SO_4. Eggs from helminths that displayed dye penetration were deemed to be non-viable.

The Trypan blue dyeing technique developed by Tan et al.[25] was used to assess viability of protozoa cysts. After centrifuging the sample and removing the supernatant, 50 mL of Trypan blue (0.4% in water) was added to the sediment. The supernatant was rinsed with 1X PBS every 30 min for 8 h after the initial 30 min. Tubes were centrifuged at 800 g for 5 min with the pellet re-suspended in water. On glass slides covered with coverslips, all cysts were counted. and reported as non-viable if the dye entered them.

Determination of total coliform bacteria and fecal coliform bacteria

The Membrane Filter (MF) technique was used to measure total and faecal coliform bacteria in compost samples. Suspensions were mixed thoroughly by adding 10 g samples of fresh compost to 90 mL distilled water that had been autoclaved at 121 °C for 15 min. The supernatant was then passed through membrane filters with 0.45 µm pore sizes, which were optimized to efficiently trap bacterial organisms. The filters were placed on m-Endo Agar medium to allow growth of total coliform bacteria for analysis. Colonies that developed on the filters after a 24-hour incubation period at 35 °C were counted. This number represented the total number of coliform bacteria in the sampled compost.

To identify faecal coliform bacteria, membrane filter method was used. Faecal coliform colonies appeared after the filters had been incubated for 24 h at 44.5 °C and were counted. The number of faecal coliforms per gram of fresh sample for each of the treatment samples was represented as log10 CFU (colony forming unit), and average values were determined[26].

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Statistix v. 8.1 software package (Analytical Software, 2005). The means were compared by least significant difference (LSD) test[27].

Results

pH

The pH increased on the fifth day after the process began in all treatments ($P < 0.05$) compared to the initial state. pH levels across composting treatments varied significantly ($P < 0.05$), according to the analysis of variance (ANOVA), from day 5 to day 50. The 55th and 60th day, however, revealed a non-significant change ($P > 0.05$) in pH between different treatments. From day 5 to day 15, pH in Unit 3 remained steady, while Units 1 and 2 had only small variations. pH thereafter gradually decreased, reaching virtually neutral levels between the 55th and 60th days in all three Units (Fig. 2).

Temperature

Throughout the composting period, significant ($P < 0.05$) temperature differences were observed among composting treatments. Temperature increased quickly over the first five days of the composting process and then gradually decreased until it reached ambient temperature. On the 5th day of the composting process, all three units entered the thermophilic range (> 45 °C), which persisted for 20 days for Unit 3 and 15 days for Units 1 and 2. Unit 3 regularly recorded significantly ($P < 0.05$) higher temperatures during this time. For Unit 3, Unit 2, and Unit 1, respectively, the day 5 saw high average temperatures of 56.6 °C, 55.7 °C, and 51.5 °C (Fig. 3). During the mesophilic phase (45–35 °C), Unit 3 consistently maintained a higher temperature compared to Unit 2 and Unit 1. This phase lasted from the 20th to the 25th day for Unit 1, from the 20th to the 30th day for Unit

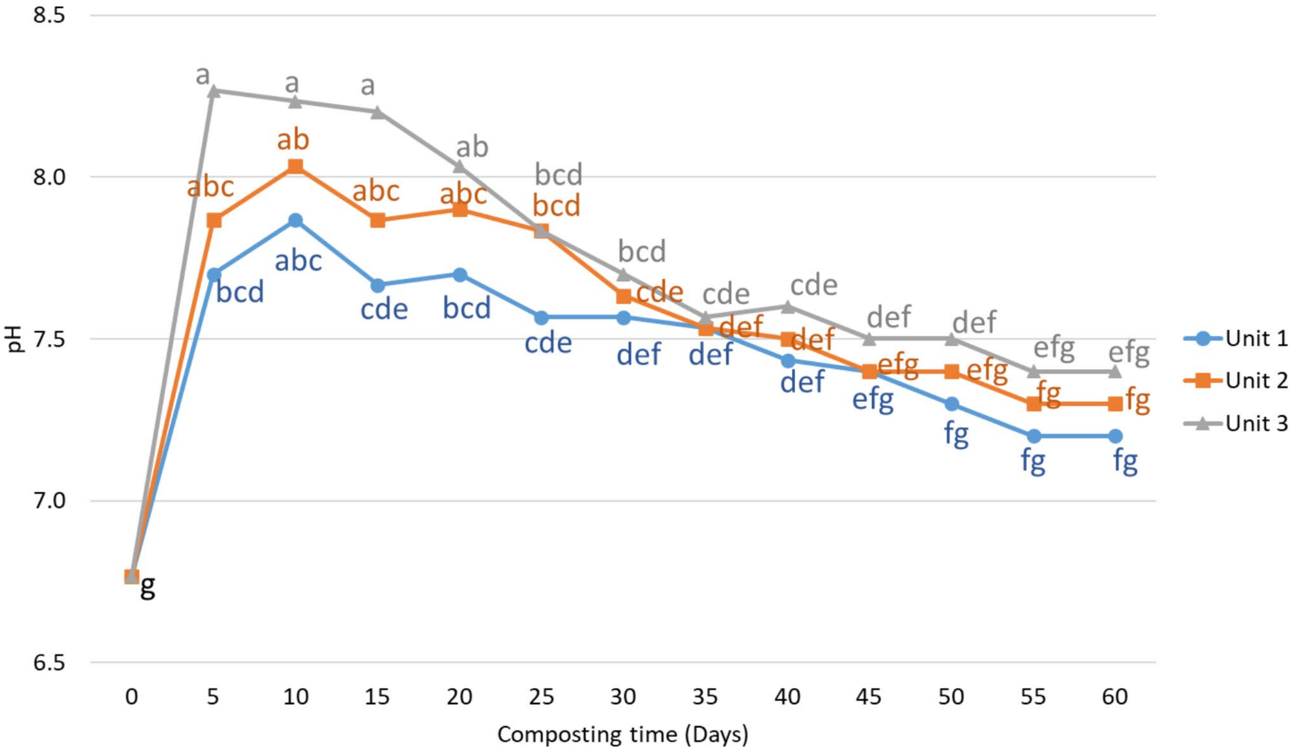

Fig. 2. Changes in pH in different composting treatments with time. Different letters indicate significant differences at $P < 0.05$.

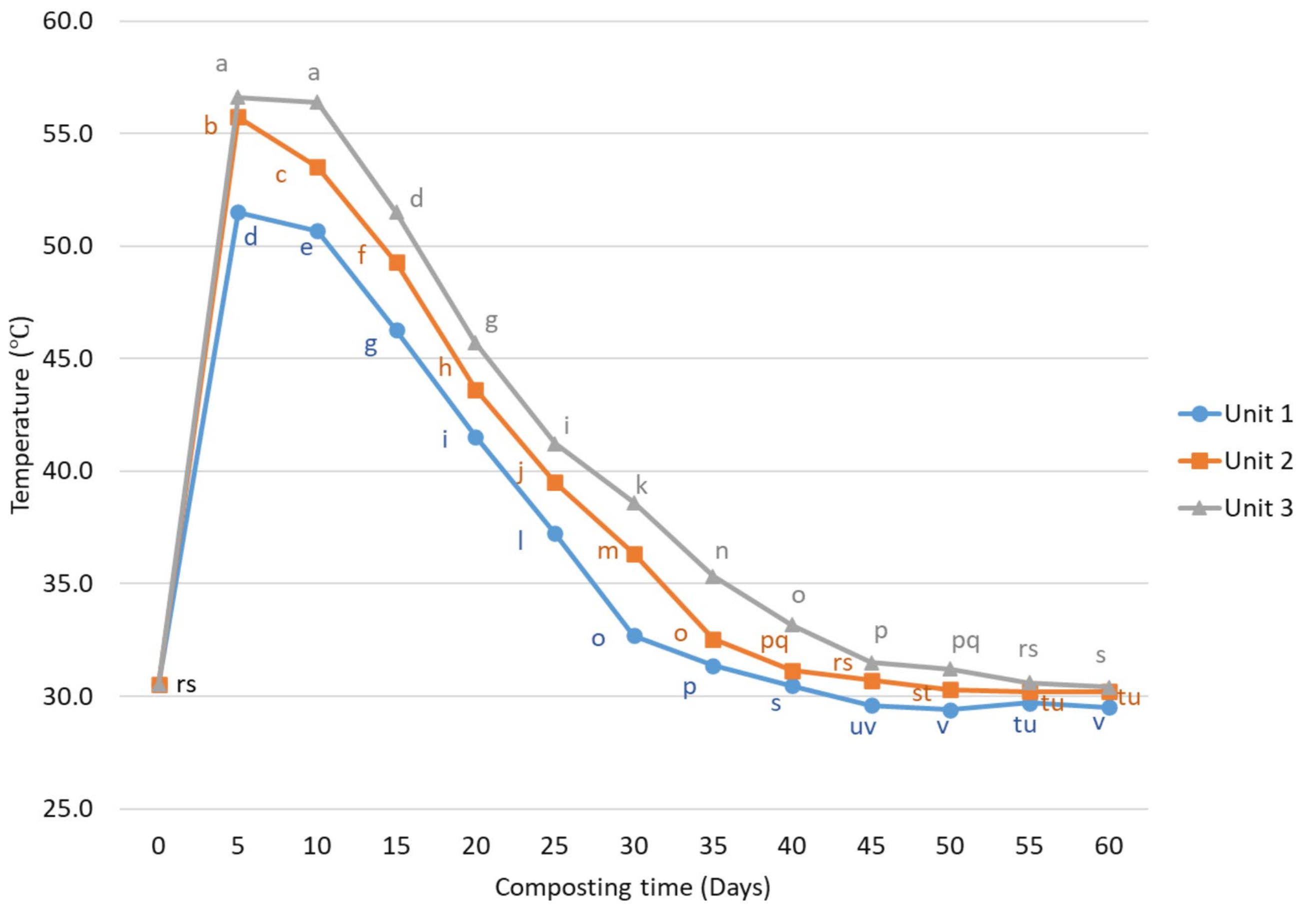

Fig. 3. Changes in temperature in different composting treatments with time. Different letters indicate significant ($P < 0.05$) differences of temperature among different units.

2, and from the 20th to the 35th day for Unit 3. Subsequently, starting from the respective days mentioned, the temperature values dropped below 35 °C and became very close to the ambient temperature for all composting treatments.

Humidity

Variations in humidity levels were observed in all units over the 60-day period. At day 0, all the three units had humidity levels of 27%. The first humidity reading, taken on the fifth day after the process began, showed a fast and significant ($P < 0.05$) rise in humidity relative to the initial state across all treatments. Humidity levels were highest on day 5, with readings of 71% for Unit 3, 64% for Unit 2, and 55% for Unit 1. Following that, humidity readings in all three units continuously declined until day 60 (Fig. 4). By day 60, Unit 1 had a humidity level of 35%, Unit 2 had 39%, and Unit 3 had 40%.

Egg per gram

At day 5, there was a non-significant ($P > 0.05$) reduction in EPG in all treatments. However, after that, a significant ($P < 0.05$) reduction was observed in all treatments until day 25, after which a non-significant reduction was observed in all treatments from day 30 to day 60. On day 10, a significant reduction (37.5%) in EPG in Unit 1 was noted. This was followed on days 15, 20, and 25 by additional reductions of 30.0%, 28.6%, and 20.0%, respectively. It is interesting to note that no further drop in Unit 1 was noted after day 30. On day 5, a non-significant 12.5% decline in EPG in Unit 2 and Unit 3 was recorded. Following that, significant decreases of 42.9%, 25.0%, 33.3%, and 25.0% were seen on days 10, 15, 20, and 25, respectively, in Unit 2. In Unit 3, Decreased in EPG of 50%, 42.9%, 25.0%, and 33.3% were recorded on days 10, 15, 20, and 25, respectively (Fig. 5).

Egg viability

The study also assessed viability of parasitic eggs over a 60-day period. On day 0, all Units exhibited 100% viability of eggs. However, by day 5, decreased egg viability was recorded in Unit 3 (21.43%); however, in Unit 1 and Unit 2 decreased egg viability were not significant (6.25% and 14.29%, respectively). This trend continued, and by day 10, Unit 3 experienced a further significantly higher ($P < 0.05$) reduction in egg viability to 57.14%, while Units 2 and 1 showed 25.00% and 10.00% reduction in viability. The decline in viability persisted, leading to 50.00% reduction in viability in Unit 3, 33.33% in Unit 2, and 14.29% in Unit 1 on day 15. By day 20, viability

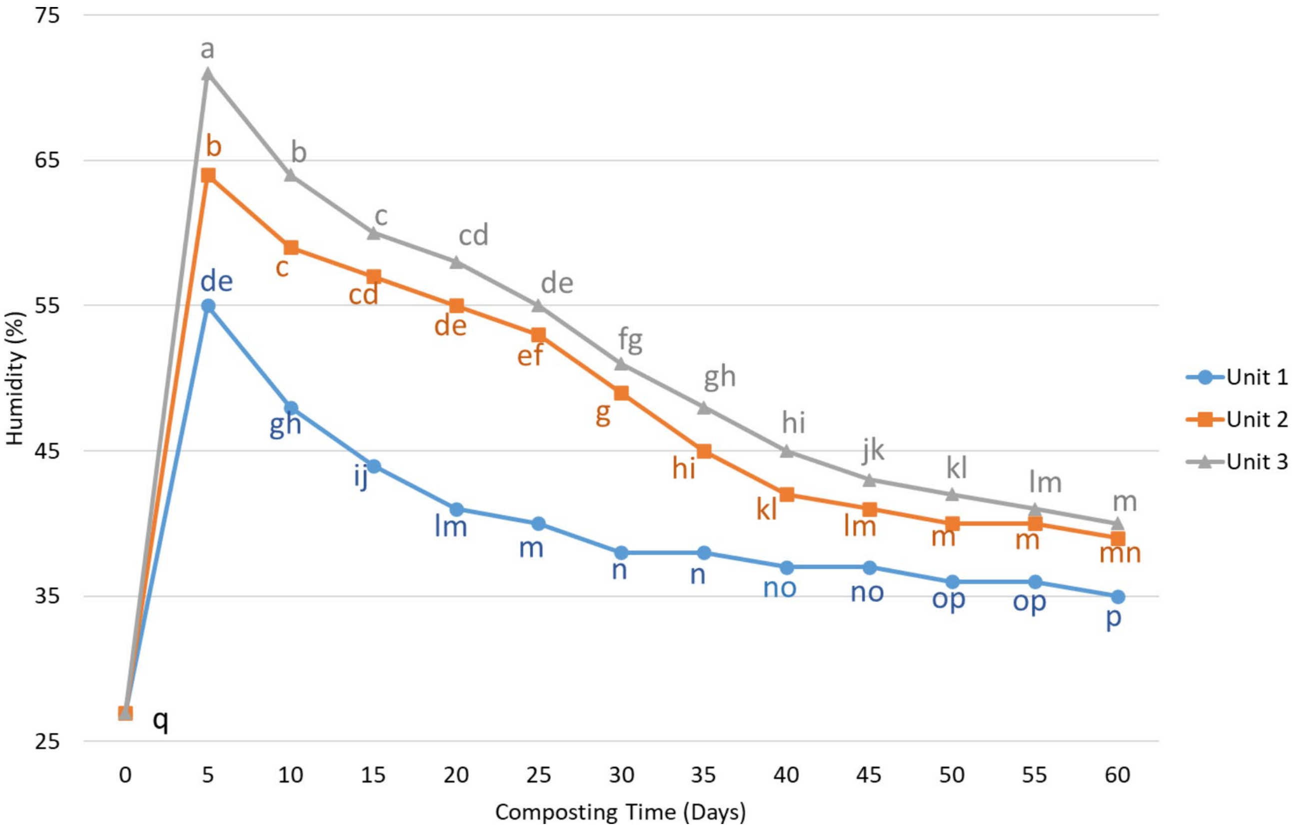

Fig. 4. Changes in humidity in different composting treatments with time. Different letters indicate significant ($P < 0.05$) differences of humidity among different units.

further decreased to 33.33% in Unit 3, 25.00% in Unit 2, and 0.00% in Unit 1. Notably, on days 25 to 60, there were no reduction in the viability of eggs in all three Units (Fig. 6).

Total coliforms

Both Unit 2 and Unit 3 demonstrated a substantial reduction in the total coliform population by day 10, maintaining counts below the minimum allowable limit of < 1000 cfu g^{-1}. However, Unit 1 achieved this limit slightly later, by day 15. The initial total coliform counts in Unit 1, starting at 3.3 $\log_{10}$ units, steadily decreased to 1.5 $\log_{10}$ units. Similarly, Unit 2 and Unit 3 exhibited consistent reductions, diminishing from 3.3 $\log_{10}$ units to 1.3 $\log_{10}$ units and 1.0 $\log_{10}$ units, respectively, by the end of the experiment. In Unit 1, a significant ($P < 0.05$) reduction in total coliforms was observed until day 40, whereas in Unit 2 and Unit 3, this reduction persisted until day 45. Between days 45 and 60, a non-significant reduction in total coliforms was noted in all three composting treatments (Fig. 7).

Faecal coliforms

In all three units, the numbers of faecal coliforms significantly ($P < 0.05$) decreased by day 5 and continued to decrease steadily until day 20. The most substantial reduction in faecal coliforms was observed in Unit 3 (from 2.6 $\log_{10}$ to 1.3 $\log_{10}$), followed by Unit 2 (from 2.6 $\log_{10}$ to 1.5 $\log_{10}$), and then Unit 1 (from 2.6 $\log_{10}$ to 1.6 $\log_{10}$). However, from days 20 to 60, a non-significant reduction of faecal coliforms was observed in all three composting treatments (Fig. 8).

Discussion

In aerobic composting, maintaining proper air supply to the composting material is essential to facilitate the production of gases through biodegradation and provide oxygen for decomposer microorganisms. Adequate ventilation and regular turning of the compost material are critical for ensuring optimal oxygenation[28]. However, ventilation is often hindered by compaction, necessitating thorough mixing of materials to enhance accessibility and susceptibility to microbial attack. This promotes the restart of the biological process, leading to a rise in temperature, which explains the observed temperature fluctuations during different phases of composting[29]. In our experiments, each unit was treated with varying amounts of biological decomposer solution. Subsequently, air renewal and turning of the compost were conducted every 5th day to assess pH, temperature, and humidity levels within the composting units. These parameters were crucial in evaluating their impact on the reduction of EPG, total coliform bacteria, and faecal coliform bacteria in the slaughterhouse sludge before using as fertilizer.

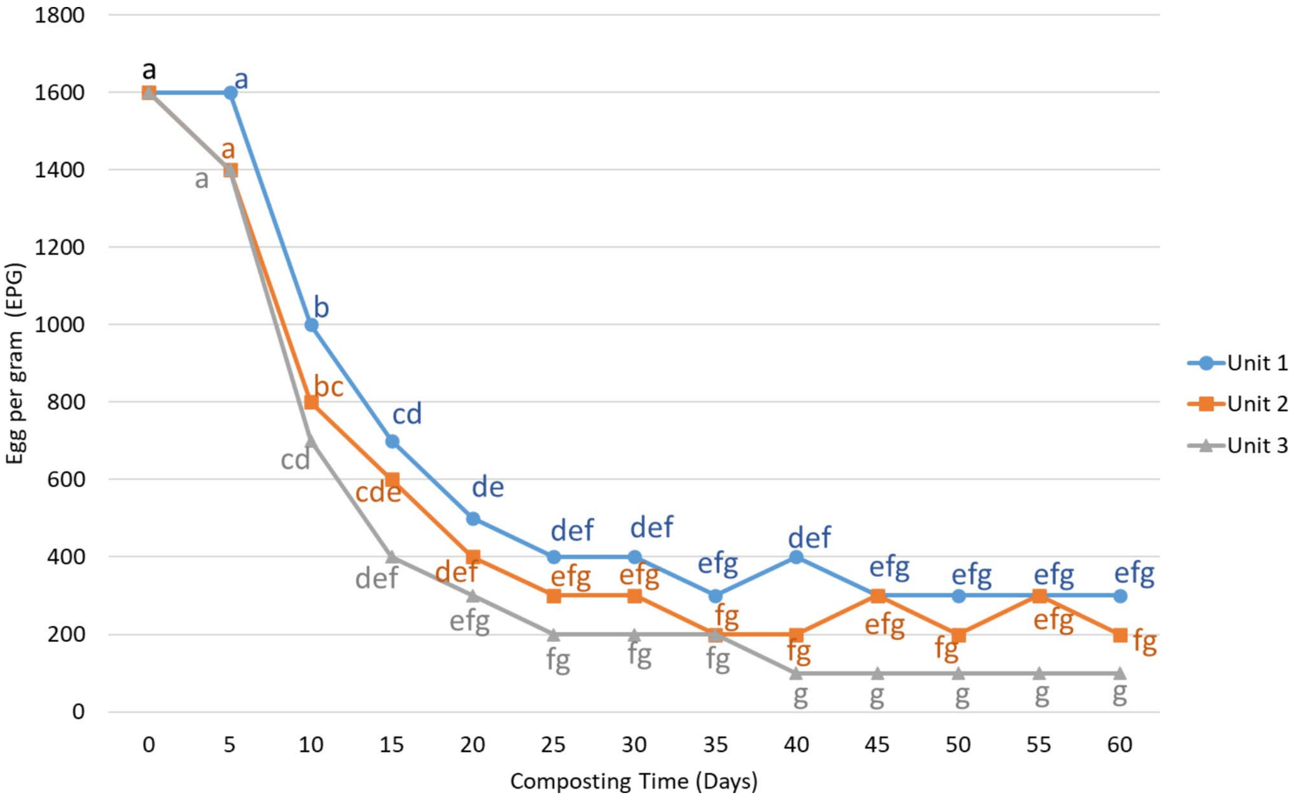

Fig. 5. Reduction in egg per gram in different composting treatments with time. Different letters indicate significant ($P < 0.05$) differences of egg per gram among different units.

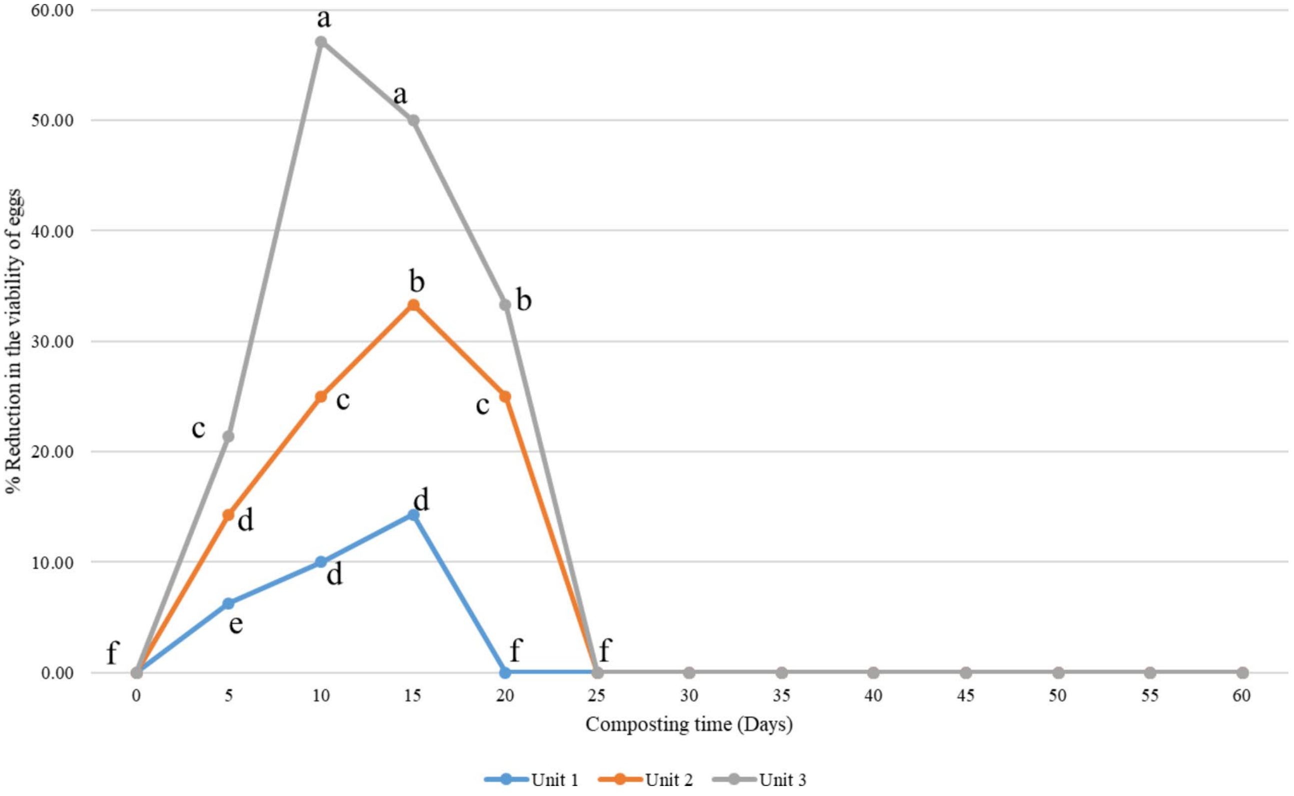

Fig. 6. Percentage reduction in the egg viability in different composting treatments with time. Different letters indicate significant ($P < 0.05$) differences of percentage reduction in the viability of eggs among different units.

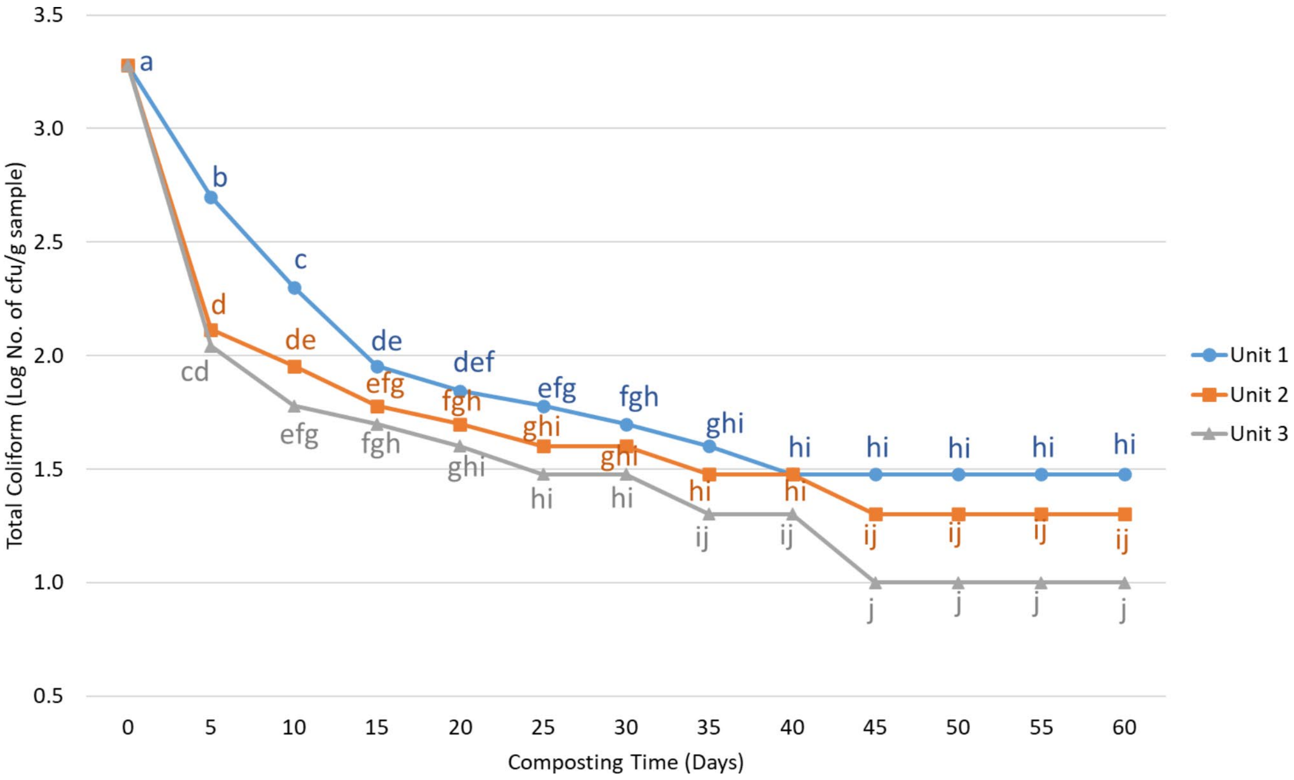

Fig. 7. Reduction in total coliforms in different composting treatments with time. Different letters indicate significant ($P < 0.05$) differences of total coliform among different units.

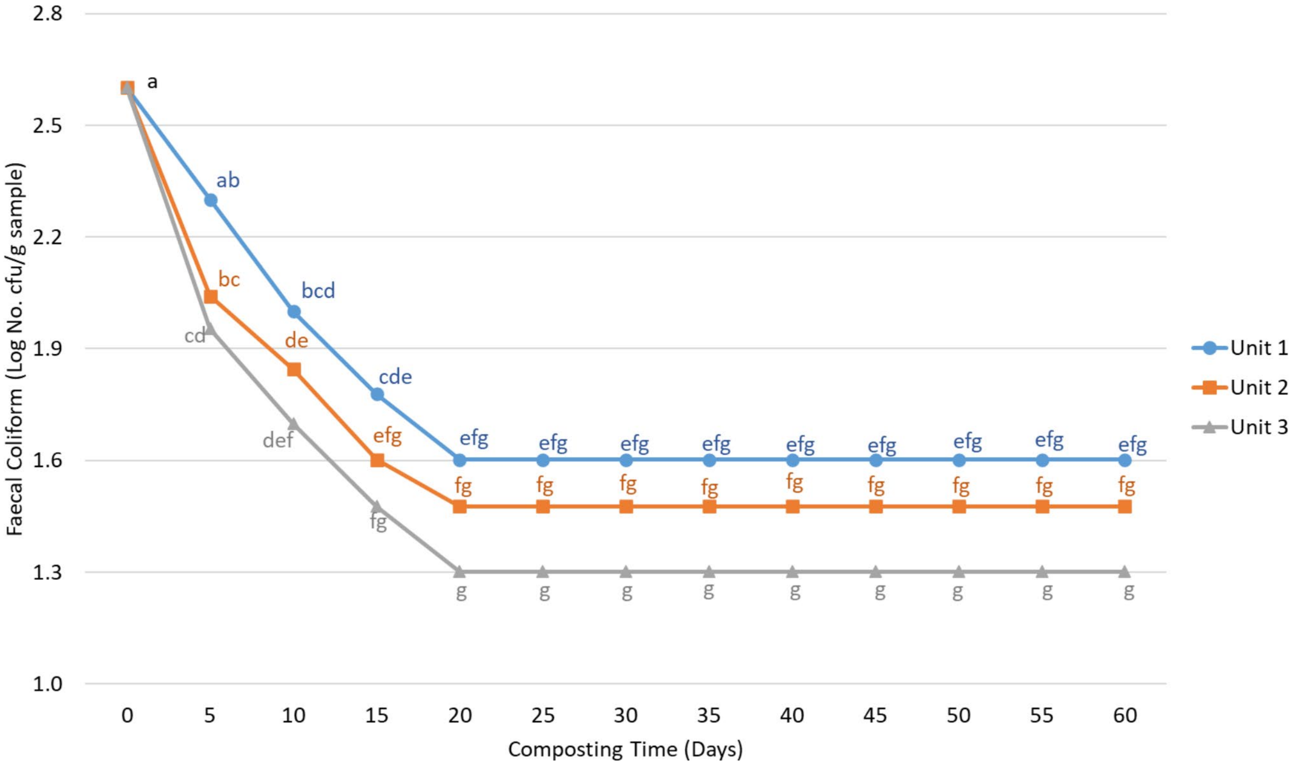

Fig. 8. Reduction in faecal coliform in different composting treatments with time. Different letters indicate significant ($P < 0.05$) differences of faecal coliform among different units.

During first 10 days of composting, a rise in pH was noted, attributed to metabolic degradation of organic matter containing nitrogen, such as proteins and amino acids, leading to the formation of amines and ammonia salts through the mineralization of organic nitrogen, a process supported by previous studies[30]. This pH increase might also be due to the decomposition of organic acids, releasing alkali and alkali earth cations previously bound by organic matter, as suggested by Smith and Hughes[31] and Mupondi et al.[32]. Similar pH elevation during composting has been reported in various studies[30,33]. The subsequent decline in pH during the later stages of composting could be attributed to the nitrification process, releasing H + ions, a phenomenon supported by the significant increase in NO^{3-} observed during these stages. The pH levels ranged from 6.0 to 8.0 during composting, indicating a successful and fully developed process, and aligning with the literature[33]. Importantly, pH values attained in all treatments at the end of the experiment fell within the acceptable range for organic fertilizer, as recommended by Tognetti et al.[34].

The stabilization of pH especially in Unit 3 showed the presence of a suitable environment for the microbial process which is important for the decomposition of organic matter. The thermophile microorganisms responsible for the decomposition of highly complex organic matter thrive at the relative pH of 6.5 and 8.5 which promotes their activity hence improves the rate of decomposition[35]. Additionally, stable pH affords inactivation of pathogens that include parasitic eggs and disease-causing bacteria such as coliforms as they are less likely to survive in such conditions. This stability eventually enhances the composting process and pathogenic reduction resulting in production of better quality and safe compost[36]. Earlier works also support the statement that pH stability is necessary for battling pathogens and boosting microbial efficiency during composting[37].

The lack of pathogen (parasite eggs, coliforms) reduction during the last days of our study may relate to the stabilization of pH and the temperature returning to normal levels. High temperature (thermophilic phase) and high pH emerging during the initial phase of composting process assist in pathogen reduction. As the thermophilic temperatures range is reached and temperatures below thermophilic range (< 45 °C) are attained, pathogen degrading microorganisms may be slowed down tremendously[38]. It is well known that some parasitic eggs and coliform bacteria show special traits allowing them to survive changes in the environment temperature and pH among others. One line of bacteria parasites, for instance, is coliform that can tolerate neutral pH so it becomes impossible to completely eradicate them at the advanced stages of composting. In the same way, some impeded eggs contain tough shells which protect them from attacks due to the changes in environmental conditions and they will remain dormant unless there is adequate heat for a given period time[38,39].

There was an initial rapid increase in temperature during the first few days of composting, followed by a gradual decline over time, eventually reaching ambient temperature. These temperature fluctuations marked the thermophilic, mesophilic, and maturation phases of the composting process, respectively. The swift transition from the initial mesophilic phase to the thermophilic phase in the treated units indicated a high proportion of readily degradable substances and the waste's self-insulating capacity[33]. This temperature pattern aligns with other composting studies[30,40]. The rise in temperature within the composting mass occurred due to the accumulation of heat generated from the respiration and decomposition of sugar, starch, and protein by the microbial population, outpacing dissipation to the surrounding environment[41].

Variations in temperature among the composting treatments might have been influenced by factors such as the amount of material, initial moisture content, and aeration. Differences in the temperature profiles of the units, despite similar volume and aeration (air circulation), can be attributed to varying moisture content in the compost. Regular turning of the compost mass every 5th day in all three treatments likely facilitated air circulation, enhancing microbial activity in the oxidation process and thereby raising the temperature. Units 3 and 2, with a combination of aeration and relatively high moisture content, maintained a higher temperature for a more extended period than Unit 1. This finding is supported by Finstein et al.[42], who demonstrated a linear relationship between oxygen consumption and heat production during aerobic metabolism, corroborating the results of this study.

Fluctuations in humidity played a significant role in shaping the composting process. Higher humidity levels at the beginning likely facilitated initial decomposition stages by creating a favorable environment for microbial activity. Makan et al.[43] indicated that humidity ranging from 70 to 75% was associated with the most significant degradation. Huerta-Pujol et al.[44] noted that moisture content significantly influences biological activity, with a range of 40 − 60% recommended for effective composting. In a study conducted by Jain et al.[45], vegetable wastes with an initial moisture content of 89% were adjusted to 57% by adding bulking agents to maintain a suitable composting environment. However, as composting progressed, excessive humidity could have restricted aeration, creating anaerobic conditions. This might have slowed organic matter decomposition, impacting overall composting efficiency. Conversely, excessively low humidity levels in later stages could have hindered microbial activity, affecting organic material breakdown[46]. Furthermore, variations in humidity among units likely influenced the composition and quality of the final compost. Composting units with stable and optimal humidity likely produced compost with superior nutrient content and microbial diversity, essential for effective soil amendment. Therefore, regulating humidity levels during composting is a crucial factor for optimizing the process and ensuring production of high-quality compost.

When humidity levels changes, the behavior of the microorganisms present in the compost may alter, which may delay the rate of the composting process, or leading to death of pathogens. Excessive humidity may lead to compaction and reduced aeration, hindering aerobic conditions, while insufficient moisture may restrict the activities of micro-organisms. It has been reported that ranges of 50–60% humidity content in the mass are favorable for the effective composting[38,47]. In future studies, other approaches should be adopted such as increasing ventilation, turning the compost regularly and humidity control to avoid compaction and promote even aeration in order to improve the process of composting.

Our findings clearly showed that eggs of pathogenic parasites in faecal sludge are more effectively sanitized by the high temperature created in the thermophilic phase of composting. According to Koné et al.[48], high temperatures may make egg shells more permeable, facilitating movement of hazardous chemicals while also speeding up desiccation. Although many authors claimed that parasitic eggs were completely eliminated under thermophilic conditions[49], this was not the case in the current study. Helminth eggs were still present and viable despite the thermophilic condition (45 °C) being maintained for approximately 15 days in Units 1 and 2 and for approximately 20 days in Unit 3. The complete death of eggs may not be guaranteed because it is possible that the lethal temperature is not distributed uniformly throughout the compost biomass. Due to their exposure to the open air near the top of the compost, the substrates there may have been slightly cooler than those inside.

Diversity in variables may be a reason for EPG reduction after day 30, including processes like a decline in microbial activity and stabilization of certain environmental parameters such as temperature and pH. The thermophilic phase of composting (days 5 to 25) is often characterized by high temperature and active microorganisms that can significantly reduce pathogen loads, including the eggs of certain parasites. However up to this point, once the compost has warmed and shifted towards mesophilic conditions, this is followed by reduced pathogen destruction abilities because of drop in microbial activities. Furthermore, neutral or near neutral pH conditions probably does not favor the erosive degradation of parasite eggs anymore and therefore no further reduction of pathogens was noticed[35,36].

According to Strauch[50], composting assures material hygienization as long as all biomass is exposed to a high enough temperature (55 °C for 14 days). According to the temperature readings obtained in this study, Unit 3 only had a temperature >55 °C for an average of 10 days, during which time the compost was only rotated once, allowing it to reach that temperature. This would imply that if the compost feedstock had been turned more frequently, perhaps every two or three days, the biomass could have experienced the lethal high temperature evenly and for longer periods of time, leading to higher efficiency of helminth egg removal. This reasoning obviously conflicts with results by Koné et al.[51], who showed that turning frequency had no discernible impact on the effectiveness of inactivation of helminth eggs. It has been stated, however, that the amount of heat produced and the amount of time the thermophilic phase is sustained during composting depends on the volume of the compost feedstock. The amount of heat produced by a compost increases with volume, stays in the compost for longer, and can be turned less frequently due to the extended duration of the thermophilic phase. Smaller composts only experience a brief thermophilic phase; thus' unless they were stirred often, the biomass outside would not have the opportunity to experience the fatal high temperature. In the US, compost is considered hygienically acceptable if a temperature of >55 °C is maintained in windrows for no less than 15 days with an average of 5 turnings during the high temperature phase[52].

The cessation of the egg viability reduction between day 25 and day 60 can suggest that there are certain constraints in the composting mechanism, most likely limited microbial activity and/or microclimatic conditions e, g., temperature dropping below thermophilic range causing killing of the pathogens are hindering the process. Some eggs of the parasites may be more tough and may not be destroyed easily unless exposed to higher temperature continuously afterwards[8,35,38]. Prolonging the thermophilic phase or finding other means to enhance the effectiveness of egg viability reduction can increase the efficiency of the composting process in the longer-term.

In a study conducted by Topal et al.[53], total coliforms were decreased to 78.2 −99.9%, whereas faecal coliforms were decreased to 72.5 −99.9% following the thermophilic stage. They used six different aeration rates during in-vessel aerobic composting of vegetable and fruit wastes to eliminate total and faecal coliform bacteria. In addition to high temperatures, the decline in organic matter led to a reduction in coliforms. Because they cannot multiply on complicated compounds like lignin and humic materials, coliforms typically use highly degradable materials. When there are sufficient nutrients, coliforms can multiply. However, they are killed when the nutrients are depleted and inappropriate circumstances arise.

Hassan et al.[54] used municipal solid waste in a semi-industrial pilot plant and applied a moderate aeration during the composting process, to eliminate the coliform bacteria. Similar to our findings, they found that throughout the thermophilic phase, the average number of faecal coliforms declined significantly from 2.5×10^7 bacteria/g waste at the start of the procedure to 7.9×10^7 bacteria/g waste. The high temperature (60–65 °C) and the adverse conditions created during the thermophilic phase are likely to be responsible for this drop. High coliform eradication efficiencies have been reported previously, consistent with our findings. Pathogen inactivation in four compost piles was researched by Pereira-Neto et al.[55] using the aerated static pile technique. Using common indicator organisms, they assessed bacterial inactivation. Similar to our findings, *E. coli* and faecal streptococci decreased from around 10^7 org/g to less than 10^2 org/g. Similarly, Larney et al.[56] found that $>99.9\%$ of total coliforms and *E. coli* were removed in the first 7 days during open-air windrow composting of cattle feedlot manure. *Salmonella*, faecal coliforms (*E. coli*), and faecal streptococci were found in samples collected after 1, 30, 60, and 90 days of composting in the study by Banegas et al.[57]. Similar to our findings, they observed that the majority of bacteria were eliminated at the high temperatures (57–61 °C) reached during composting, making the resulting composts safer for agricultural use.

In contrast to our findings, Cekmecelioglu et al.[58] composted food waste along with cow dung and bulking materials during a 12-day period and reported a faecal coliform decrease of 59.3% with a maximum temperature of 56.6 °C. It is generally accepted that the high temperatures (>55 °C) created by microbial activity along with aeration rates, feedstock composition, and composting methods are principally responsible for inactivating bacteria during composting. A huge surface-to-volume ratio of the mass, however, could prevent it from reaching such lethal temperatures because a significant amount of the heat produced is lost to the environment. Additionally, microbial communities require the right ratios of carbon and nitrogen to develop and produce an adequate amount of heat. A substantial amount of heat may also be lost from the composted substrate to the environment at low ambient temperatures[36]. By comparing the results with other studies on coliform bacteria reduction, it is evident that variable findings might be reported presumably due to variety of composting materials, methods, and other microclimatic factors. The studies of Topal et al.[53], Hassan et al.[54], and Larney et al.[56] stressed that even though high temperatures are fundamental in achieving pathogen destruction, other factors like oxygen supply, feedstock addition, and composting methods are also important for the decrease of coliforms. It should be done in future research regarding these variables in more detail to get the most efficient composting deformation on pathogenic eradication.

Under the limited budget of the project, standardization of methods relative to lowering the burden of parasites and coliforms, were used as significant measures for risk assessment of the pollution monitoring and public health threat. Although factors such as heavy metals and pharmaceuticals contaminants are of significances in further studies, this is not an area to be tackled in this specific case. This study made use of the membrane filter method, however, we are of the opinion that application of more sophisticated techniques in subsequent studies will improve the quality and breadth of studies of the microbial communities. This aspect of additional completion will be accomplished in future works urgent for those additional aspects. It is true that core temperatures (> 55 °C) are essential for the inactivation of pathogens, nevertheless, we recognized that thermophilic phase did not overtly last in all the units of this study particularly regarding the more tolerant helminths' eggs sanitization. Reliance on temperature alone is not optimal, to say the least. Optimizing turning frequency in future studies to achieve better heat distribution and isolating the bio heat for longer periods could be fruitful in extending the thermophilic phase thus potentially improving pathogen kill within the entire compost mass.

The compost derived from slaughterhouse sludge can serve as an organic soil modifier to upgrade soil quality and fertility. This compost can also be practiced in the agricultural fields in order to minimize the use of synthetic fertilizers and enhance soil characteristics. In addition, the parameters of the composting process can be modified to include biological decomposer solutions, adding feedstock, adjusting moisture contents, and turning frequencies to ensure maximum pathogen kill and nutrient retention. Implementing these measures would also improve the waste management and decreas environmental pollution fostering healthier environmental practices.

Conclusions

Significant changes were seen in a number of metrics during the 60-day composting process in each of the three units (Unit 1, Unit 2, and Unit 3). By day 5, pH levels in every unit rose quickly and then began to gradually fall towards neutral values. Temperature fluctuations showed a rapid transition into the thermophilic phase, which persisted for varying lengths of time in different units. After initially rising, humidity levels decreased during the course of the research period across all units. EPG ccounts were significantly lower in all units, with a steady fall in Unit 3 and considerable reductions in Units 2 and 3 throughout the experiment. Egg viability testing revealed a range of effects, with Unit 3 having a sharp drop. Following safety guidelines, each unit successfully reduced the total and faecal coliform counts.

This study demonstrates how composting can effectively lower parasite egg and coliform concentrations in abattoir sludge. The discrepancies seen between the units highlight the significance of composting techniques in pathogen eradication. Unit 3 showed quick coliform reduction and improved efficacy in maintaining low EPG levels. These findings highlight the value of precise composting methods for controlling abattoir waste and emphasize the need for customized strategies for the best outcomes. This research bridges the gap between agricultural improvement and environmental preservation by offering insightful information about sustainable waste management practices. By converting slaughterhouse sludge into compost, one has remarkable agricultural and ecological benefits can be achived since the sludge can be utilized in soil amendments improving soil structure and fertilit0y. This process potentially saves the environment by recycling precious organic wastes that would have led to excessive use of chemical fertilizers negatively influencing the crop farming. Composting has a positive impact on the environment since it decreases the methane and other greenhouse gas emissions by decreasing the wastes rather than dumping it to the land under more polluting practices. Nutrient stabilization and pathogen reduction are effectively managed through the compositing process hence aiding in the strengthening of the agriculture sector and protection of the environment.

Data availability

All the data can be found in the main text.

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Author contributions

Conceptualization, HMR; methodology, MN and MSS; software, MM and MR; formal analysis, NN and MHK; investigation, MHK; writing—original draft preparation, H.M.R. And M.M.; writing—review and editing, M.Y, F.S.A, and D.F.; visualization, MSS, MY, and MN; project administration, HMR, MY and MSS. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Institutional review board statement

This study was approved by the Research Ethics Committee, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan and University of Veterinary and Animal Sciences, Lahore, Pakistan. The standard guidelines for institutional animal care and use (IACU), University of Agriculture, Faisalabad, Pakistan, were followed.

Additional information

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