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Research article

Management of swine mortalities through the use of a mixed composting-accelerating bio-inoculant

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ABSTRACT

A composting-accelerating bio-inoculant (*Bacillus subtilis*, *Talaromyces sayulitensis* (HC1), *Steinernema* sp., and *Heterorhabditis* sp.) was evaluated in a composting process made up of a different mix of wood chips, pig manure, urine, and swine mortality (raw material RM). Three different treatments (T1, T2, and T3) were assessed, and physicochemical, microbiological, and entomological evaluations were carried out at 0 and 45 days of the composting process. The highest organic nitrogen (1.34 %) concentration was detected in swine mortality, whereas the highest total oxidizable organic carbon (39.1 %) concentration was observed in wood chips. *Salmonella* spp., was not identified in any of the raw materials. *Clostridium* spp., count was 5.5, 2.0, and 1.0 Log10 unit, for pig manure, wood chips, and swine mortality, respectively. Pig manure, swine mortality, and wood chip total coliform count was 6.21, 5.32, and 1 Log10 unit, respectively. Helminth eggs were not detected in any of the RM and Cryptosporidium spp., oocysts were occasionally found in pig manure and wood chips. Several types of flies were identified, *Musca domestica*, *Muscina stabulans*, *Stomoxys calcitrans*, *Fannia canicularis*, *Sarcophaga* sp., and *Calliphora* sp. Treatment 3 (45.11 % swine mortality, 33.33 % wood chips, and 21.55 %, urine and bio-inoculant) had the greatest total oxidizable organic carbon availability, the highest carbon/nitrogen (C/N) ratio (20.67, $p < 0.05$), and the lowest dipterous larvae count. Moreover, *Salmonella* sp., was not observed and had only low *Clostridium* spp., and fecal coliform count. The bio-inoculant's effect on C/N ratio, cation exchange capacity, and electrical conductivity were beneficial, and resulted in production of a fertilizer complying with EPA 600/1-87-014, EPA 40 CFR Part 258, and NTC5167/11 norms. According to the characterization protocols used in this study the compost was apparently free from bacterial and parasitic pathogens and minimal dipteran counts. Last, maturation time was 15 days shorter compared with control (C4).

1. Introduction

With an increasing world population, where in 1990 the planet's population was estimated to be 5.3 billion people, yet today it has increased to more than 7.7 billion people; it entails an escalation in food demand, generating an growth in meat (source of minerals, proteins, and fats) production and consumption (https://www.un.org/es/sections/issues-depth/global-issues-overview/index.html).

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In 2017, the world's pig production was approximately 9.8 × 10^8. Despite efforts to reduce production chain mortality, it remains a problem affecting not only the productivity, but the final disposition of the mortality. Before weaning, 3 to 4 piglets per litter can die, approximately 20–25% of the population, representing a problem in production, profitability and mortality final disposition [1].

According to reports published by Pork Checkoff's US, piglet mortality (before weaning) in 2014 was 20.5 % and in 2016: 17.30 %. In the growing phase, the mortality was 5.47 and 4.58 % in 2014 and 2016 respectively. Regarding finishing stage pigs, in 2014 mortality was 5.78 % and in 2016: 5.34 % [2, 3, 4].

Colombian consumption of fresh pork increased in 2009 from 100,203 tons to 183,020 tons in 2018. Pork production almost doubled from 90,000 tons in 2009 to an estimated 173,194 tons in 2018. In 2016, 4,069.690 hogs were slaughtering for meat production. Only in the second quarter of 2018, 1,075.697 pigs were slaughtered [5].

Considering the number of pigs now being raised and slaughtered in Colombia, management of swine mortality (pig carcasses, fetal deaths, parts that are not marketed or consumed, pork viscera and placentas) has generated profitability in addition to an environmental management problem. In the production process, before slaughter, animals can die from natural causes or respiratory and gastrointestinal diseases, as well as from incidents related to the process, such as piglets trampled to death by a sow [6, 7]. In Colombia, in the last two decades, the number of piglets born per litter has increased, as well as the number of stillbirths [8]. Also, deaths occur during animal transportation, due to high animal density in trucks, long travel times, and climatic conditions [9]. Studies by the Colombian Pork Council (2016) on 27 pig farms reported on approximately 4.98 %, stillbirths. In addition, the number of mummified (small size, underweight dehydrated fetuses) was close to 4.16 %, while the mortality rate for unweaned piglets was 6.92 %. In Colombia, the percentage of miscarriages of pig fetuses is approximately 0.61 %, and the percentage of losses before weaning is 12.19 % [10].

From the environmental point of view, most pig producers worldwide, carry out mortality disposal through dangerous practices, such as incineration, landfill, and burial, among others (depending on the regulation of each country). Therefore, we propose composting processes, can become a feasible alternative to consider with less environmental impact [11, 12].

Both traditional methods (burial or incineration) are unsafe for the environment and human health. Buried carcasses contribute to the proliferation of dipterans; contaminate groundwater, surface water, and soils with compounds such as ammonium and chlorine [13], and can spread pathogenic microorganisms [14, 15]. On the other hand, when carcasses are incinerated at 850 °C or higher temperatures, dioxins and furans can be produced, due to incomplete organic matter combustion. These carcinogenic compounds affect the human immune system, as well as human reproduction and development. Incineration also releases greenhouse gases, such as polycyclic aromatic hydrocarbons, dioxins, and furans. In some cases, when ashes resulting from incineration are used as soil amendments, they contaminate the soil, affecting crops, and becoming a risk to human health, and the environment [16].

It was determined in South Korea that most of the buried mortalities were not decomposed even after 3 years. Incineration can become a possibility, but transporting mortality to destined facilities is often undesirable. Portable incinerators are expensive, in some countries and require an operating air permit, and can generate infectious particulate aerosols, considered a problem [11, 12].

Therefore, pig producers use the composting process as an environmentally friendly method of disposing of swine mortality. This oxidative biological process is based on the biocatalytic activity of organisms present in the environment, including yeast, bacteria, fungi, parasites, insects, other invertebrates, and nematodes [17]. Many species work together in a synergistic and complementary manner to degrade animal and/or plant organic matter, providing the necessary substrates for development and growth of these organisms. Thus, accelerating mineralization and transformation of organic material into the humic acids and organic fertilizer, commonly known as compost [13, 18, 19]. Compost can be used as either soil amendment or organic fertilizer for crops [13, 20, 21]. Compost production requires time for organic matter degradation, a designated space set aside for the composting process, and human labor in every step of the process [22].

Furthermore, some organic waste native microorganisms do not have the capacity to produce the enzymes needed for degradation, or produce them in very small quantities; resulting in a slow and inefficient composting process. Bio-inoculants are added to provide the enzymatic capacity needed to reduce degradation time, improve compost quality, and increase available nutrient concentration [23, 24, 25].

In our work, most aspects described by other authors were considered [13, 16, 26]. Because organic matter degradation depends on microbial activity, our bio-inoculant was composed of thermophilic bacteria, a mesophilic fungus, and entomopathogenic nematodes. We also incorporated a combined aeration system (turning the composting every 8 days and simultaneous incorporation of perforated PVC pipes for aeration). This research was carried out under real conditions of an experimental farm. The objective was to evaluate the effect of compost-accelerating bio-inoculant, on composting of swine mortality.

2. Materials and methods

2.1. Study site and experimental treatments

This study was conducted at a modern swine production facility, located in the Department of Antioquia, Colombia. The farm is registered with the "*Instituto Colombiano Agropecuario* (ICA)". This research was ethically approved by the "Comité de Investigación y Ética de la Facultad de Ciencias de la Pontificia Universidad Javeriana, Bogotá D.C., Colombia". Approval code 18112016.

Wood chips, urine, porcine waste, and swine mortality were used as raw materials (RM), (to comply with regulations, animals that died from infectious diseases were not included in the composting processes). Composting-accelerating bio-inoculant was used in two experimental treatments. Treatments were (T1: Traditional proportion of raw materials + bio-inoculant, T2: Proportion of raw materials according to C/N without bio-inoculant, T3: Proportion of raw materials according to C/N + bio-inoculant and control C4: Experimental control, consisting of traditional waste management, including a proportion of raw materials, without bio-inoculant and only one turning after 30 days of the composting process), (Table 1). Each treatment was performed in triplicate in a 1 m^3 box (twelve boxes in total were used). To promote aeration, perforated PVC tubes were incorporated into T1, T2, and T3 boxes [26, 27].

2.2. Bio-inoculant preparation

Composting-accelerating bio-inoculant was prepared with two bacterial strains (consortium) from genus *Bacillus* spp., (with amylolytic and proteolytic activity), [28, 29] and a cellulolytic fungal strain, *Talaromyces sayulitensis* HC1. All three microorganisms were obtained from the Master Cell Bank [30] at the "*Laboratorio de Biotecnología Aplicada of the Pontificia Universidad Javeriana, Bogotá D.C., Colombia*". The other component of the composting-accelerating bio-inoculant consisted of infective juveniles (IJs) of entomopathogenic nematodes *Steinernema* sp., (Steinernematidae) and *Heterorhabditis* sp., (Heterorhabditidae).

Bacteria were batch-cultured for 18 h in a 10 L bioreactor with a 7 L work effective volume (WEV) at 55 °C, 155 r.p.m., and aeration of 0.5 vessel volumes per minute (v.v.m.), to attain 10^8 CFU mL^{-1}. Culture media consisted of 1.2 % (w/v) cornstarch, 1.2 % (w/v) milk, 1 gL^{-1} glucose, 2.5 gL^{-1} yeast extract, 1 gL^{-1} ammonium sulfate, 0.5 gL^{-1} calcium chloride, 0.5 gL^{-1} sodium monobasic phosphate and 0.5 gL^{-1} sodium dibasic phosphate. Bacteria count was performed in the same

Table 1. Treatments used for evaluation of mixed composting-accelerating bioinoculant.

Treatments	Swine mortality (% w/w)	Wood chips (% w/w)	Pig manure (% w/w)	Mixed composting-accelerating bioinoculant	Frequency of Turning the composting
C4 Control	54.99	20.26	24.75	Absent	At day 30
T1	54.99	20.26	24.75	Present (inoculated again after 15 days)	Every 15 days
T2	45.17	33.38	21.45	Absent	
T3	45.17	33.33	21.55	Present (inoculated again after 15 days)	

culture media used for bacteria production, supplemented with 15 gL^{-1} agar-agar. Evaluation of proteolytic and amylolytic enzyme activities were carried out by the trichloroacetic acid (TCA), [31, 32] and the 3, 5-dinitrosalicylic acid (DNS) assays [33], respectively.

Hundred mL of *T. sayulitensis* suspension were inoculated (10 % v/v) into 900 mL of modified starch broth (10 gL^{-1} starch, 1.26 gL^{-1} yeast extract, 0.5 gL^{-1} magnesium sulfate heptahydrate, 0.5 gL^{-1} calcium chloride, 0.5 gL^{-1} calcium carbonate, 1.0 gL^{-1} dipotassium phosphate). The culture was maintained for 10 days at 28 °C and 120 rpm to attain 10^8 conidia mL^{-1}. Conidia were quantified with a hemocytometer, and cellulolytic activity was determined using the 3–5, dinitrosalicylic acid (DNS) assay [28, 33, 34].

Entomopathogenic nematodes *Steinernema* sp., and *Heterorhabditis* sp., were produced "*in vivo*" following previously published recommendations [35].

Final composting-accelerating bio-inoculant consisted of (1 L Ton^{-1} (10^7 conidia mL^{-1}) *T. sayulitensis*; 1 L Ton^{-1} (10^7 CFU mL^{-1}) of bacterial consortium and 10^5 IJs m^{-2} of *Steinernema* sp., and 10^5 IJs m^{-2} *Heterorhabditis* sp.

2.3. Composting system

T2 and T3 raw material proportions were determined according to their physicochemical characterizations. The process initiated with a C/N ratio between 15:1 and 25:1, respectively. At the beginning of the composting process (T1 and T3) the bio-inoculant was sprayed over the RM mixture, and thereafter every 15 days after turning the compost (Table 1). In addition, 100,000 IJs were sprayed on decaying matter, and 700,000 IJs were sprayed around each box to contain immature dipterous states present in the leachate. To control entomopathogenic nematode immature stages, each box was covered with adhesive plastic four days before turning the compost to count the number of adult dipterans that emerged from each box. Adult flies around the facilities, where the treatment boxes were kept were managed with 18 colored plastic glue strips (6 yellow, 6 blue, and 6 white) plus six-wire mesh and Macphail traps. These traps were installed with pheromones to attract males and adults, to disturb the insect's life cycle. The number of flies caught in each trap was recorded weekly. Additionally, relative humidity, temperature, and precipitation data reported on the farm were recorded for each sampling time [36].

2.4. Physical, chemical, microbiological and entomological characterization

Physicochemical analyzes were carried out for both raw materials during the composting process (at 0 and 45 days). Physico-chemical characterization was carried out in AGRILAB S.A. Laboratory (https://agrilab.com.co/), certified by the "*Instituto Colombiano Agropecuario (ICA)*". Samples were tested for moisture % (gravimetric method AGRILAB), ashes % (gravimetric method NTC 5167), volatilization losses % (gravimetric method AGRILAB), oxidizable organic carbon % (colorimetric method NTC 5167), pH (saturation paste, pH meter method AGRILAB), density (Dry Base - 20 °C) g/c.c. (gravimetric method AGILAB), electrical conductivity dS/m (using a conductivity meter AGRILAB), moisture retention % (gravimetric method ADRILAB), cation-exchange capacity (CEC) me/100g (volumetric method NTC 5167), C/N, total organic nitrogen % (NTC 370), total phosphorus pentoxide (P_2O_5) % (colorimetric method NTC 5167), total potassium oxide (K_2O) % (atomic absorption method NTC 5167), total calcium oxide (CaO) % (atomic absorption method NTC 5167), total magnesium oxide (MgO) % (atomic absorption method NTC 5167), total sulfate ($S–SO_4$) % (turbidimetric method NTC 5167), total iron (Fe) % (atomic absorption method NTC 5167), total manganese (Mn) p.p.m. (atomic absorption method NTC 1369), total copper (Cu) p.p.m. (atomic absorption method NTC 5167), total zinc (Zn) p.p.m. (atomic absorption method NTC 1369), total boron (B) p.p.m. (colorimetric method NTC 1860), total sodium (Na) % (flame emission method NTC 1146), total solid silicon dioxide (SiO_2), soluble in hydrogen fluoride (HF) % (atomic absorption method AGRILAB), and insoluble acid residue % (gravimetric method NTC 5167). The composting temperature was measured daily, and the pH was measured at each time the composting was turned, by the saturation paste method [37, 38, 39, 40, 41, 42].

Trap captured flies were identified [43]. Immature stages were controlled by entomopathogenic nematodes; limiting the number of adults emerging during each treatment. To evaluate the management of adult flies, their numbers around the facilities were counted weekly.

Salmonella spp., density was estimated by the Most Probable Number (MPN) method following method 1682 of the Environmental Protection Agency of the United States (EPA). On the other hand, to count *Clostridium* sp., 1 mL serial dilutions of homogenized sample was plated on SPS Agar (Sulfite Polymyxin Sulfadizine Agar), 0.5 gL^{-1}, polymyxin B sulfate, 0.01 gL^{-1} and sodium sulfadiazine, 0.12 gL^{-1}. To test for total coliforms, samples were plated on Chromocult® Coliform Agar (CCA). Petri dishes were incubated at 35 ± 2 °C for 24 h under anaerobic and aerobic conditions, respectively. After incubation, sulfite reducing colonies were counted (black color, with or without precipitate formation). To confirm *C. perfringens* presence, motility and indole confirmatory tests were performed.

The RVC/FAO guide 21 was used to quantify helminth and protozoan eggs. For each method, 2 g of sample was used. For helminth eggs, the sample was resuspended in a sodium chloride and glucose saturated solution, so parasitic structures could float for 30 min. For sedimentation, the sample was resuspended in distilled water for 45 min. The end result of each technique was microscopically analyzed at 10X and 40X magnifications. Results were reported by employing an occasional, moderate, and abundant quantitative scale [44].

Cryptosporidium spp., was detected by the modified Ziehl-Neelsen method [45]; preparations were analyzed under light microscopy at 40X and 100X.

2.5. Statistical analysis

Descriptive statistics and analysis of variance among treatments were used to determine significant differences, SPSS 18 software was used to carry out analyses. The F-test allowed to determine the model that best fitted the data. To compare data sets a one-way ANOVA test was used. Similarities between treatments was identified with lowercase letters; the letter "a" was used to indicate the treatment with the lowest result.

3. Results

3.1. Physical, chemical, microbiological and entomological characterization

3.1.1. Raw materials (RM)

Swine mortality had the highest water content (85.9 %). Wood chips had the highest percentage of total oxidizable organic carbon (39.1 %) and the highest C/N ratio (138). Swine mortality had the highest proportion of organic nitrogen (1.34 %).

Identified dipterans included *Musca domestica* (housefly), *Muscina stabulans* (false stable fly), *Stomoxys calcitrans* (stable fly), *Fannia canicularis* (lesser house fly), *Sarcophaga* sp., (arrow fly), and *Calliphora* sp., (blue fly).

Salmonella spp., was not identified in any of the raw materials. Pig manure had the highest *Clostridium* spp., (5.5 Log_{10} units) count, whereas in wood chips and swine mortality concentration was 2.0 and 1.0 Log_{10} units, respectively. Coliforms total-concentration was 6.21, 5.32, and 1 Log_{10}, in pig manure, swine mortality, and wood chips, respectively. Helminth eggs were not detected in any of the RM, but occasional

Table 2. Results of physical and chemical analyses at 0 and 45 days of composting.

Parameter	Unit	C4 (Control) value ± SD	(T1) value ±SD	(T2) value ±SD	(T3) value ±SD
Water content at day 0	%	61.40 ± 1.91	66.93 ± 2.61	56.43 ± 7.06	60.30 ± 0.95
Water content at day 45		38.33 ± 10.02	29.93 ± 6.18	30.77 ± 5.70	26.17 ± 5.56
Ashes at day 0		4.03 ± 0.92	3.32 ± 0.70	3.98 ± 1.14	4.05 ± 1.08
Ashes at day 45		5.16 ± 0.25	8.12 ± 2.01	6.93 ± 4.21	5.81 ± 1.73
Lost due to volatilization at day 0		34.53 ± 1.68	29.77 ± 2.85	39.63 ± 6.40	35.63 ± 0.15
Lost due to volatilization at day 45		56.50 ± 10.05	61.93 ± 8.08	62.30 ± 2.01	68.00 ± 7.35
Oxidizable organic carbon at day 0		14.47 ± 2.17	12.97 ± 3.12	17.63 ± 3.44	15.77 ± 0.96
Oxidizable organic carbon at day 45		27.73 ± 5.75	27.83 ± 3.69	28.63 ± 1.96	31.33 ± 2.96
pH ± 0.2 (saturation paste) at day 0		6.21 ± 0.08	5.94 ± 0.21	6.01 ± 0.10	6.16 ± 0.07
pH ± 0.2 (saturation paste) at day 45		5.98 ± 0.33	6.05 ± 0.33	5.78 ± 0.31	6.04 ± 0.28
Density (Dry Base - 20 °C) at day 0	g/c.c.	0.33 ± 0.03	0.37 ± 0.04	0.31 ± 0.05	0.30 ± 0.07
Density (Dry Base - 20 °C) at day 45		0.22 ± 0.06	0.24 ± 0.02	0.20 ± 0.06	0.21 ± 0.05
Electrical conductivity at day 0	Ds/m	7.70 ± 1.01	9.58 ± 1.40	7.96 ± 0.68	7.30 ± 2.13
Electrical conductivity at day 45		4.53 ± 0.82	5.51 ± 0.73	4.37 ± 1.48	4.16 ± 1.22
Humidity retention at day 0)	%	98.63 ± 13.37	63.27 ± 3.91	118.83 ± 31.43	118.00 ± 34.07
Moisture retention at day 45		296.33 ± 106.45	254.0 ± 34.77	342.67 ± 90.34	382.33 ± 109.92
Organic nitrogen (N) at day 0		1.22 ± 0.06	1.11 ± 0.17	1.14 ± 0.16	1.04 ± 0.23
Organic nitrogen (N) at day 45		1.50 ± 0.46	1.86 ± 0.21	1.43 ± 0.16	1.55 ± 0.17
Total phosphorus (P_2O_5) at day 0		1.35 ± 0.65	1.17 ± 0.28	1.36 ± 0.27	1.36 ± 0.50
Total phosphorus (P_2O_5) at day 45		1.83 ± 0.55	3.02 ± 0.63	2.45 ± 1.85	1.78 ± 0.62
Total calcium (CaO) at day 0		1.08 ± 0.25	1.04 ± 0.11	1.27 ± 0.50	1.42 ± 0.64
Total calcium (CaO) at day 45		1.39 ± 0.29	2.63 ± 0.34	2.28 ± 2.10	1.55 ± 0.41
Total magnesium (MgO) at- day 0		0.50 ± 0.17	0.33 ± 0.06	0.41 ± 0.11	0.42 ± 0.16
Total magnesium (MgO) at day 45		0.57 ± 0.25	0.67 ± 0.21	0.47 ± 0.29	0.52 ± 0.24
Total sulfur ($S–SO_4$) at day 0		0.15 ± 0.02	0.14 ± 0.03	0.16 ± 0.03	0.11 ± 0.01
Total sulfur ($S–SO_4$) at day 45		0.19 ± 0.02	0.24 ± 0.05	0.18 ± 0.03	0.19 ± 0.02
Total iron (Fe) at day 0		0.05 ± 0.02	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
Total iron (Fe) at day 45		0.06 ± 0.04	0.04 ± 0.02	0.06 ± 0.05	0.03 ± 0.01
Total manganese (Mn) at day 0	ppm	99.00 ± 13.11	57.33 ± 14.57	78.67 ± 39.27	89.33 ± 36.30
Total manganese (Mn) at day 45		0.57 ± 0.25	0.67 ± 0.21	0.47 ± 0.29	0.52 ± 0.24
Total copper (Cu) at day 0		24.33 ± 4.16	22.33 ± 9.29	20.33 ± 7.57	24.00 ± 9.17
Total copper (Cu) at day 45		23.33 ± 9.45	26.00 ± 9.64	19.17 ± 10.54	23.00 ± 9.17
Total zinc (Zn) at day 0		235.33 ± 118.94	158.00 ± 20.07	187.00 ± 33.72	186.67 ± 51.62
Total zinc (Zn) at day 45		266.67 ± 106.61	257.00 ± 84.51	200.33 ± 108.01	237.33 ± 89.53
Total silica SiO_2 (solid soluble in HF) at day 0	%	0.49 ± 0.14	0.35 ± 0.05	0.36 ± 0.08	0.37 ± 0.04
Total silica SiO_2 (solid soluble in HF) at day 45		0.50 ± 0.12	0.43 ± 0.17	0.31 ± 0.16	0.62 ± 0.28
Insoluble residue in acid at day 0		0.71 ± 0.09	0.57 ± 0.20	0.68 ± 0.45	0.56 ± 0.21
Insoluble residue in acid at day 45		0.66 ± 0.12	0.48 ± 0.18	0.40 ± 0.17	0.80 ± 0.27
Number of flies at day 0		12.33 ± 2.52	9.33 ± 0.58	10.33 ± 2.08	6.33 ± 2.08
Number of flies at day 45		8.33 ± 0.58	5.33 ± 0.58	6.33 ± 0.58	4.00 ± 0.00
Number of flies around the composter at day 0		35.33 ± 25.79	61.33 ± 23.03	66.00 ± 5.57	73.67 ± 15.18
Number of flies around the composter at day 45		24.67 ± 3.06	13.67 ± 1.53	15.00 ± 1.00	3.33 ± 1.15
Clostridium spp., at day 0	Log_{10} units	5.86 ± 0.22	5.90 ± 0.12	5.52 ± 0.44	5.72 ± 0.21
Clostridium spp., at day 45		1.89 ± 1.00	3.07 ± 0.80	2.66 ± 0.96	2.63 ± 0.45
Coliforms at day 0		6.08 ± 0.66	6.73 ± 0.45	6.36 ± 0.40	5.83 ± 0.15
Coliforms at day 45		4.34 ± 0.19	1.46 ± 2.53	2.28 ± 2.02	2.45 ± 2.37
Salmonella spp., at day 0	NMP/4g	0.54 ± 0.03	0.72 ± 0.05	0.57 ± 0.05	0.47 ± 0.08
Salmonella spp., at day 45		0.38 ± 0.04	0.39 ± 0.04	0.35 ± 0.01	0.34 ± 0.01

Cryptosporidium spp., oocysts were observed in pig manure and wood chips.

3.2. Composting process (45 days)

Table 2 shows physicochemical parameters from the beginning (day 0) and at 45 days of the composting process.

At day 45, moisture content ranged between 23 and 37 %, but the greatest decrease was observed in T1 (37 %) and T3 (34.13 %) treatments, both containing bio-inoculant (Table 2). Furthermore, ash greatest percentage was observed in T1, whereas T3 had greater availability of oxidizable organic carbon. On the other hand, T1 displayed the highest organic nitrogen percentage, followed by T3. The highest moisture retained percentage was evidenced in T3.

Some physicochemical parameters with significant differences ($p < 0.05$) are presented in Figure 1; among these are T3's C/N ratio with a value of 20.67, cation exchange capacity of 50.93 me/100g in T1, followed by cation exchange capacity of 43.47 me/100g in T3. It is also noteworthy that potassium (K) showed significant differences ($p < 0.05$) in T1 with 0.89 %, followed by T3 with 0.66 %, both at day 45. Sodium (Na) in T1, also displayed a significant difference ($p < 0.05$), with 0.26 %; representing 0.15 % higher availability compared to day 0. On the other hand, although total boron (B) concentration decreased throughout the 45 days, a significant difference ($p < 0.05$) was only recognized in T1, where it was 9.97 ppm.

In the number of flies quantified, no significant differences were observed among treatments during the evaluation period ($p > 0.9743$), or in the types of traps used to capture adult flies ($p > 0.4312$). Furthermore, spraying of entomopathogenic nematodes in treatment boxes did not generate significant differences in the number of adult flies that emerged from the boxes; as determined by counting flies in the colored plastic strips placed in each box ($p > 0.1496$). In each count, fly number ranged between 4 and 10 flies, although the highest number was distinguished in the C4 control. Treatment 3 had the lowest fly number in each count and after turning the compost (Figure 2).

Microbiological analyses (Table 2) revealed neither RM nor the compost contained *Salmonella* spp. On the other hand, *Clostridium* spp., decreased by almost 3.0 Log_{10} units during the composting process. The greatest reduction was obtained in C4, followed by T3. Total coliform count decreased in all treatments compared to C4, but it decreased the most in T1 (Table 2).

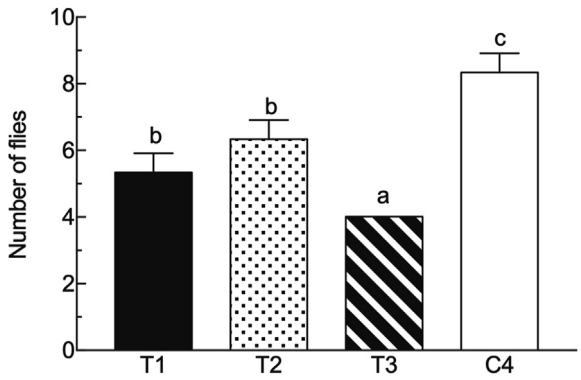

Figure 2. Adult dipterans in each treatment at day 45.

T1 and T2 contained eggs compatible with *Strongylus* spp., (2 per preparation) and *Trychostrongyllus* spp., (1 per preparation). Some oocysts were compatible with *Cryptosporidium* spp., (quantitative scale: occasional) found in most samples from some of the treatments during the composting process. Cysts were occasionally found in all samples, without a discernible pattern, which included *Balantidium coli* cysts (10.3 %), *Iodamoeba* spp., cysts (5.1 %), unidentified Coccidia oocysts (17.9 %), and *Eimeria* spp., oocysts (17.9 %).

4. Discussion

Swine mortality has an important potential as a source of organic matter that can be composted. Therefore, this compost can become an organic fertilization alternative for farmers, representing a reduction in chemical fertilization use and costs. Thus, producing improved organic crops and nutrient-rich soils.

In our work, most aspects (particle size, aeration, temperature, C/N ratio, porosity, gas emission, humidity, and pH, among others) mentioned by other authors were taken into account [13, 16, 26]; since degradation of organic matter depends on microbial activity.

Table 2 suggests that during the composting process bio-inoculant microorganisms allow degradation of otherwise difficult-to-decompose materials, such as keratin, collagen, and elastin, present in hair, skin, ligaments, meat, and cellulose content of carcasses [16, 46, 47]. Table 2 demonstrates degradation of these materials was achieved. In addition,

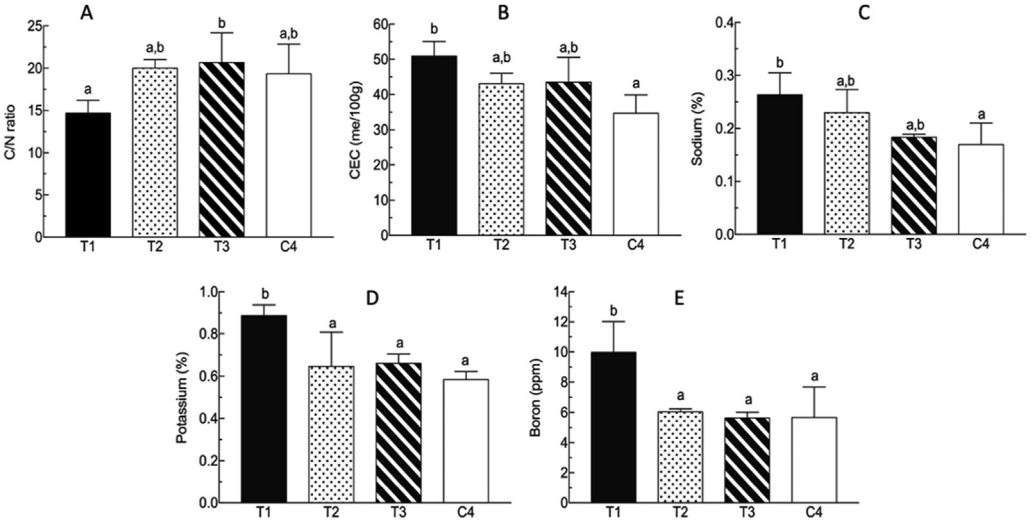

Figure 1. Physical and chemical parameters with statistically significant differences ($p = 0.05$). A: C/N ratio, B: CEC me/1000g, C: Sodium %, D: Potassium %, E: Boron ppm. Average of 3 replicas of each Treatment and Control. Differences among mean ± SD are represented by letters as established by Tukey *post hoc* test (significant differences, $p < 0.05$). Error bars correspond to SD.

to increase microorganism's metabolic activity at the beginning of compost assembly, a favorable C/N ratio of raw materials resulted in an acceleration the process [48]. When initiating the composting process, the appropriate C/N ratio should be between 15:1 and 35:1, because at a lower ratio there is excess nitrogen, while at a higher ratio there is excess carbon. When the C/N ratio is greater than 35:1, the transformation rate slows down and required composting time increases [50]. Additionally, nitrogen excess can result in proliferation of microbial growth, triggering odor problems, due to NH_4^+ gas production. Furthermore, the high O_2 demand can lead to generation of anaerobic conditions in the process [17, 50]. In contrast, low C/N values result in nitrogen loss and ammonia (NH_4^+) production, which would decrease protein production, since microorganisms generally use 30 parts of carbon to one part of nitrogen, because carbon makes up about 50 % of the microbial biomass content [49].

Swine mortality contains an extreme amount of nitrogen. Consequently, to achieve an appropriate C/N ratio requires adding sufficient total oxidizable organic carbon with a similar structure of lignin, cellulose, and hemicellulose found in wood chips, sawdust, stems, crushed corn, and hay, mixed into the compost [51, 52, 53]. Addition of carbon-rich organic materials with different textures allow absorbing moisture and leachates generated in the process. This in turn, increases the compost's porosity, which facilitates aeration, essential for the composting process, since it is an aerobic system [19, 29, 47, 54, 55]. Humidity greater than 70 % can generate anaerobiosis and limit RM microbiological degradation. Hence, slowing down the composting process, and producing unpleasant odors as a result of compound production, such as dimethyl disulfide, dimethyl trisulfide, dimethyl sulfide, carbon disulfide, ammonia, trimethylamine, acetone, and methyl ethyl ketone [22, 26, 56, 57].

Physicochemical results demonstrated when raw material nutritional content is higher (carbon, nitrogen, microelements, salts, and others), association of microorganisms and flies to the decomposition of organic matter also increases. Insects remove more organic matter than vertebrates and other terrestrial organisms. Flies, and especially fly larvae, consume large quantities of wood, grasses, humus, fungi, manure, and all kinds of decomposing organic materials. Moreover, *Musca domestica*, *Muscina stabulans*, *Stomoxys calcitrans*, *Fannia canicularis*, *Sarcophaga* sp., and *Calliphora* sp., were identified in this study [57].

Raw materials used for compost attracted Diptera and microorganisms, such as *Penicillium* sp., *Pleurotus* sp., *Trichoderma* sp., *Aspergillus* sp., *Pseudomonas* sp., *Serratia* sp., and *Bacillus* sp. These wastes represent a natural habitat for these microorganisms, as they are directly involved in the biotransformation of organic substrates. Moreover, fungi produce lignocellulosic enzymes that degrade various plant residues [20, 23, 54, 58, 59, 60, 61].

The highest *Clostridium* spp., count was found in pig manure, certainly because this microorganism makes part of swine's intestinal microbiota. Species such as *C. perfingens* are responsible for causing enteric infections [62]. In contrast, *Clostridium* spp., concentration in wood chips was low, due to low protein concentration necessary for bacterium, development this in this raw material. As expected, pig manure contained the highest total coliform count, followed by pig mortality and wood chips.

It is important to highlight that parasite identification, particularly *Cryptosporidium* spp., oocysts in pig manure and wood chips can affect compost quality. Considering this compost can be utilized in food crops, ornamental plants, or grasses; detection of these pathogens is essential. Moreover, recognition of these zoonotic pathogens in swine production generates economic limitations, a serious production, and public health problem [63]. Never the less, in this study, *Cryptosporidium* spp., presence was occasional.

Total oxidizable organic carbon and total organic nitrogen reached their maximum percentages in T1 and T3 (Table 2), supported by the bio-inoculant's presence [32]. Enzymatic activity of cellulolytic fungi degrades cellulose and carboxymethyl cellulose-rich compounds, present in wood chips, while proteolytic microorganisms break down nitrogenous compounds present in pig mortality, and pig waste. All of these microorganisms increase the availability of assimilable carbon and nitrogen. These enzymatic activities are reinforced by substrate availability and the aeration process, which was of great importance for T3 [64, 65, 66]. Collectively, the C/N ratio has been used as a stability and maturity index of the compost. When this ratio is close to 25:1, the compost has reached maturity [19].

Another indicator of organic matter transformation is pH. Even though, no significant differences were observed among treatments ($p < 0.05$); pH can be used to demonstrate effectiveness in transforming organic matter during the composting process. In this study, low pH values suggest secondary metabolite production, particularly organic acids. Subsequent values, around 6.0 ± 0.2 may be associated with protein decomposition, ammonification, and CO_2 release [27].

According to Ballesteros Trujillo et al. (2018), *Bacillus* spp., produce amylases and proteases, whose optimum activities are in a pH range between 3.5 and 10.6 ± 0.2 [67]. At pH 7.0 ± 0.2 stimulation of amylase enzymatic synthesis occurs and reaches optimal levels between pH 6.0 and 6.5 ± 0.2. Taking this into account, T1 was within the optimum range for amylolytic and proteolytic activity; in contrast, T2 and C4 both had pH levels below 6.0 ± 0.2. Under these conditions, appropriate organic matter mineralization took place in T1 and T3; accelerating the degradation process, thus reducing the required composting time. This mineralization consists of the release of essential inorganic forms. Through a humification process, during and after composting, organic materials are solubilized until they become humus. When the compost is applied to crops humus generates higher quality soils, and increases fertilization for plants [57].

Cation exchange capacity is a fundamental physicochemical aspect required for improving soil fertility, since it allows balance between pH, water, and salts [25]. Cations such as ammonium NH_4^+ remain close to the plant's roots, making nutrients available for longer periods than nutrients in the anion form, such as NO_3^-, because cations are not leached while anions do. Plants supply other cations like H^+ that can be exchanged with other soil nutrients and then become absorbed. As shown in Figure 1, there were significant differences ($p < 0.05$) in the cation exchange capacity between T1 and T2, T3, and C4; indicating that compost was mature for use in the field.

Furthermore, ashes in the composting process are beneficial, as they absorb odors, as shown in T1, with the highest ash amount [26]. Elements such as boron, potassium, and sodium, are essential for plant growth and development. Plants can absorb potassium in equal amounts or even greater than nitrogen [68].

In all treatments evaluated water content decreased below initial levels; which is an important indicator of degradation. Excess moisture reduces oxygen transfer and produces anaerobiosis [69]. Low levels of moisture indicate that the compost is mature and that organic matter has been degraded. In the present study, very low water content percentages were recorded in the last stage of the composting process, which was especially notable for T1 and T3, whose water content decreased by 37.0 and 34.13 %, respectively.

Because bio-inoculant was used in both T1 and T3 treatments, it can be considered that moisture decrease was the result of organic matter accelerated degradation. Therefore, increased microbial metabolic activity led to faster compost maturity [19, 60].

Aeration allows for evaporation to occur more rapidly and completely; hence, water content decreases during the composting process [49]. In T1, T2, and T3, aeration was provided in two ways: by turning the composting every 15 days and by incorporating perforated PVC pipes into the compost. In this study, moisture content results are in line with these factors. Regulations limit allowed moisture percent in mature compost. The Chilean Standard, NCh 2880, requires for it to be below 25 %, the European regulation obliges for moisture content below 40 %, and the Colombian Standard, NTC 5167/11, mandates it be below 35 %. T1 and T3 met all three standards [60].

Figure 3. Photographic observation of the composting process. A: piglet carcasses are observed before weaning; representing a large part of the mortality rate that requires proper management. B: the mortality weighing process is observed, to incorporate exact quantities in the assembly of the drawers, since they must be in the appropriate proportions along with the other components of the RM, to achieve the C/N ratio required to start the composting process. C: in C-1, C-2, C-3, the mortality that will be treated by the composting system is observed. In these drawers, different layers of organic material (shavings, swine and mortality) are incorporated to form the drawer, so that the production of leachates and the balance in textures of the materials can be controlled. Likewise, the disposition of the PVC pipes with perforations that allowed to improve the aeration of the system that is clearly aerobic is observed, accelerating the degradation process. D: in D-1 and D-2, the aspersion of the mixed compost-accelerating bio-inoculant is observed, the capacity of the boxes was 1 m^3. E: in E-1 the state of decomposition is observed at 45 days in control C4 compared to E-2, in which T3 is observed at 45 days, where RM has already been completely degraded.

On day 45, the control treatment had the highest water content. Most likely, this was due to two factors: less aeration during the process, and bio-inoculant absence. The higher moisture content contributed to a greater presence of dipterans in two ways. According to Morales Mira et al., (2010), *Stomoxys calcitrans* development is favored by humidity and temperature in the system. However, they are also attracted by volatile compounds such as dimethyl trisulfide, butanoic acid, and p-cresol, which can be generated by excess moisture and lack of aeration. For C4, these two aspects were present, as the composting was not turned and no perforation with PVC tubes promoted aeration. Additionally, it did not contain any bio-inoculant with entomopathogenic nematodes for Diptera control.

In contrast, the lowest number of flies was observed in T3, with the lowest moisture percentage. In addition, it was treated with bio-inoculant containing entomopathogenic nematodes. The nematodes' life cycles occur in the substrate within the fly larvae, killing the larvae and allowing for nematodes to proliferate. Addition of 100,000 IJ of each *Steinernema* sp., and *Heterorhabditis* sp., (1:1) before each composting turning, allowed to reduce the emergence of adult flies from each of the boxes. Infective juveniles' behavior involves searching for fly larvae and pupae, infecting them; thus, interrupting the insect's life cycle by preventing adult emergence and reproduction. Application of 700,000 IJ around the compost reduced insect populations on the walls, floor, and leachate.

In T3, the dipteran population decrease correlated with a *Clostridium* spp., reduction by nearly 3.0 Log_{10} units during the composting process. According to Morales Mira et al., (2010), as organic matter becomes pathogen-free, the insect population decreases. Turning the composting decreases the *Clostridium* spp., population in two ways: First, it allows for oxygenation, favoring microorganism proliferation present in this treatment bio-inoculant. Second, oxygen supplied by the aeration process is toxic to *Clostridium* spp., which is an anaerobic microorganism [57]. However, this process alone does not destroy all the bacterial population, because some of them can be sporulated. Additionally, compost temperatures cannot guarantee their complete elimination [70].

Several microbiological aspects related to our results are worth highlighting. First, compost free of *Salmonella* spp., can be safely used for short-stemmed fruit and vegetables. According to our findings, there is a small risk of cryptosporidiosis, due to *Cryptosporidium* spp., Helminths and protozoa, since cryptosporidiosis is a zoonotic disease with a diverse distribution and mainly affects people who interact daily with farm animals [71]. In general, protozoan infect domestic pigs at early ages and has only mild symptoms, while adult animals are usually asymptomatic. The importance of detecting these pathogens is that they constitute a serious reproductive health problem, an economic limitation, and are also involved in zoonotic diseases that cause public health problems. These protozoa are pathogenic for both humans and pigs, but the quantities detected do not alter the quality of compost obtained and does not represent a risk to public or animal health [63, 72].

The physical appearance of the material to be treated (day 0) and material once composted (day 45) is shown in Figure 3. A video that summarizes the composting process proposed in this article, using the mixed composting-accelerating bio-inoculant is shown in Supplementary Video “Composting swine mortalities”.

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e05884.

5. Conclusions

Although the study design did not allow to determine whether the composting methodology had any effect on helminth and/or protozoan pathogen population, use of the mixed compost-accelerating bio-inoculant and the proportion of selected raw materials (T3) were very useful in managing swine mortality. At 45 days, the physicochemical parameters, the quality and maturity of the compost were high; reducing by 15 days the composting process in comparison with the

control (60 days). Also, less flies were observed in T3 traps after turning the composting. *Clostridium* spp., counts decreased by almost 3.0 Log_{10} units in T3 and C4 (control). The obtained compost had appropriate parasitological quality, complying with EPA 600/1-87-014, EPA 40 CFR Part 258 and NTC5167/11 norms. Considering several countries are pork producers and most likely encounter the same problems with mortality and other waste management, our proposal for a mixed composting-accelerating bio-inoculant becomes a promissory and more environmentally friendly strategy, capable of being implemented in different countries.

Declarations

Author contribution statement

Adriana Matiz-Villamil, Adriana Sáenz-Aponte: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Iliana C. Chamorro-Tobar, Andrea M. Sánchez-Garibello, Irina A. Barrientos-Anzola: Performed the experiments; Analyzed and interpreted the data.

Adriana Pulido-Villamarín, Ana K. Carrascal-Camacho, Ivonne S. Gutiérrez-Rojas: Conceived and designed the experiments; Wrote the paper.

Diana C. Zambrano-Moreno: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Raúl A. Poutou-Piñales: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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