IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402, p- ISSN: 2319-2399.Volume 19, Issue 8 Ser. 1 (August 2025), PP 15-25
www.iosrjournals.org

Sun-Drying As A Natural Intervention To Eliminate Pathogenic Bacteria From Composted Organic Waste.

Md Samim Hossain[1], Md Abdul Khaleque[2]*, Shahidul Ahmed Khan[3]
Stamford University Bangladesh, Department Of Microbiology, 51 Siddheswari Rd, Dhaka 1217, Bangladesh.Department Of Orthopedic Surgery, Daejeon St. Mary Hospital, College Of Medicine, The Catholic University Of Korea, Seoul, Republic Of Korea. 3 Department Of Applied Biology, Institute Of Environmentally Friendly Agriculture (IEFA), College Of Agriculture And Life Sciences, Chonnam National University, Gwangju, Republic Of Korea.
**Correspondence:abdulkhaleque.dream@gmail.com*

Abstract
Purpose: *Composted organic materials, although rich in nutrients and beneficial for the soil, may harbor pathogenic bacteria that can cause serious diseases in humans, animals, and plants. Therefore, compost is not always safe to apply directly as fertilizer. This study was conducted to isolate, identify, and quantify pathogenic bacteria present in compost and evaluate the effectiveness of sun-drying in reducing associated health risks.*
Materials and Methods: *Compost samples were collected after completion of the composting process. A 45-day sun-drying treatment was applied under natural conditions. Pathogenic bacteria, including total bacteria, coliforms, fecal coliforms, Shigella spp., Vibrio spp., Clostridium perfringens, and Salmonella spp., were isolated, identified, and enumerated using standard microbiological methods before and after sun drying treatment.*
Results and Discussion: *Before sun-drying, average bacterial counts were found for total bacteria (1.0 × 10), coliforms (1.6 × 10), fecal coliforms (2.5 × 10), and Salmonella spp. (1.8×10^2), Shigella spp. (1.2×10^6), Vibrio spp. (6.8×10^4), and Clostridium perfringens (3.7 × 10 CFU/g). After 45 days of sun-drying, these counts significantly decreased to (8.9×10^3), (4.8×10^2), (3.3×10^2), (1.2×10^3), (3.1×10^2), and 10 CFU/g, respectively. Salmonella spp. was completely absent in all sun-dried samples but was detected (2.4×10^3 CFU/g) in one wet sample. These results underscore sun-drying as a simple, eco-friendly, and cost-effective method for significantly reducing microbial contamination and enhancing compost safety.*
Conclusion: *The results demonstrate that sun-drying is an effective and environmentally friendly post-composting step, significantly reducing human pathogens in compost and thereby improving its safety for agricultural applications as a sustainable organic fertilizer.*
Keywords: *Compost, E. coli, Shigella spp., Vibrio spp., Clostridium perfringens, and Salmonella spp.*

Date of Submission: 26-07-2025 Date of Acceptance: 06-08-2025

I. Introduction

Composting is a controlled biological process in which microorganisms decompose organic materials, such as plant residues, kitchen waste, and animal manure, under aerobic conditions, resulting in a stable, humus-rich product known as compost. This process not only enriches soil fertility but also reduces landfill waste, suppresses odors, stabilizes organic matter, and helps eliminate many harmful pathogens [1, 2, 3, 4]. Composting generally proceeds through three stages: the mesophilic phase (20–40°C), where microbes break down simple compounds and raise the temperature; the thermophilic phase (>40°C), dominated by heat-tolerant organisms such as *Bacillus subtilis* and *Aspergillus fumigatus*, which degrade complex materials and eliminate most pathogens and weed seeds and the maturation phase, during which the compost cools, beneficial microbes recolonize, nutrients stabilize, and humus formation occurs[5, 6].

The environmental and agricultural benefits of composting are considerable. It is estimated that up to 75% of landfill waste could be diverted through composting [7]. Composting reduces waste volume by 30–50%, enables odor-free storage, stabilizes nitrogen to prevent leaching, suppresses soil-borne diseases, detoxifies harmful compounds, and enhances soil structure, water retention, and nutrient availability [8]. The efficiency of composting depends on key factors including temperature, aeration, and moisture content. Optimal thermophilic temperatures promote both the breakdown of organic matter and the destruction of pathogens [9]. Adequate aeration ensures oxygen supply to maintain aerobic microbial activity and thermophilic conditions; this can occur naturally via convection or be enhanced through forced aeration systems, depending on the setup

[10]. Moisture levels between 40–60% are ideal, as too little moisture slows microbial activity while excess water restricts oxygen, creating anaerobic conditions [11].

Various composting methods suit different scales and resource levels. Aerobic composting involves layering, moistening, and turning organic waste into oxygen, with compost ready in about four months. Anaerobic composting occurs without oxygen, producing methane-rich biogas that can serve as energy, though it needs aerobic curing afterward to stabilize the material [12,13]. Vermicomposting uses red worms to convert waste into nutrient-rich castings and can also remove heavy metals from wastewater [14]. Aerated static pile (ASP) composting pushes air through stationary piles with bulking agents, providing benefits in odor control and sludge treatment [15]. In-vessel composting encloses the process within controlled units, allowing for rapid composting in as little as four weeks but requiring higher capital costs [16]. Trench composting is a simple, low-tech method where organic waste is buried in soil trenches and naturally decomposes over several months, making it suitable for household use [17].

While composting reduces many pathogens, improperly processed compost may still contain harmful microorganisms such as *Escherichia coli, Salmonella spp., Shigella spp., Vibrio spp., and Clostridium perfringens* [18,19]. These pathogens can remain in the soil after compost application, posing potential health risks to humans, animals, and crops. Effective pathogen reduction is achieved by maintaining thermophilic temperatures (>55°C) during composting, where heat, microbial activity, and ammonia production help inactivate pathogens. Aeration, moisture control, and regular turning are essential to sustain these conditions [20, 21]. However, incomplete or uneven composting, especially with high-moisture or heterogeneous waste, may leave residual pathogens.

To further improve compost safety, sun-drying has become a cost-effective and environmentally friendly post-treatment method. By exposing compost to extended periods of sunlight and drying, sun-drying can decrease the number of surviving pathogens, complementing thermophilic composting, and enhancing microbial safety [22,23]. This method is especially useful in resource-limited settings where advanced treatment options are not available. The present study aims to isolate, identify, and measure pathogenic bacteria remaining in compost after traditional composting, evaluate the health risks these microbes may pose, and determine how effective sun-drying is at reducing pathogen levels. The results will support safer compost handling practices, promote sustainable agriculture, and improve waste recycling by ensuring the safe use of organic fertilizers across various farming environments.

II. Materials And Methods

Sample collection, media, and reagent preparation:

A total of 100 grams of decomposed human waste was aseptically collected from three distinct layers, upper, middle, and lower-of each pit using appropriate personal protective equipment, including boots, gloves, mask, and apron. Samples were obtained from five different pits located across various areas of Dhaka city (Table 1). Each sample was carefully transferred into sterile, tightly sealed containers and immediately transported to the Department of Microbiology Laboratory at Stamford University, Bangladesh, for further microbiological analysis.

All culture media and reagents were prepared following the manufacturer's guidelines. Dehydrated media, including Nutrient Agar (NA), MacConkey Agar (MA), Membrane Fecal Coliforms Agar (mFC), Salmonella-Shigella Agar (SS), Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS) , and Clostridium perfringens Agar (PC), were reconstituted with distilled water, sterilized by autoclaving at 15 psi for 20 minutes, and poured into sterile petri dishes under aseptic conditions. Enrichment broths such as Selenite Cystine Broth (for *Salmonella* and *Shigella*), Alkaline Peptone Water (for *Vibrio* spp.), and Fluid Thioglycolate Broth (for *Clostridium perfringens*) were also prepared similarly.

Biochemical test media, including Simmons' Citrate Agar, Triple Sugar Iron (TSI) Agar, and Motility-Indole-Urease (MIU) Agar, were prepared according to the manufacturer's instructions and stored at 4°C until use. All reagents and heat-sensitive chemicals were kept in a VEST frost refrigerator at 4°C. The pH of all media and solutions was measured and adjusted using a calibrated pH meter. Accurate weighing of media components and reagents was performed using a Mettler electronic balance.

Table -1

Name of the sample	Sample no.	Type of pit	Layers of the pit
Compost	1	Single	N/A
	2	Twin	Upper
			Middle
			Lower
	3	Twin	Upper
			Middle
			Lower
	4	Twin	Upper

			Middle
			Lower
	5	Twin	Upper
			Middle
			Lower

* N/A = Not applicable

Sample processing:

Serial Dilution

For microbial enumeration, ten 1-gram portions of compost samples were transferred into separate beakers containing 9 mL of normal sterile saline, depending on the target bacterial species, to obtain a 10^{-1} dilution. The mixtures were homogenized using a vortex mixer. Subsequently, 1 mL from the 10^{-1} dilution was transferred into 9 mL of sterile saline to achieve a 10^{-2} dilution. This 10-fold serial dilution process was continued up to 10

Microbiological Analysis

Culture Media

A variety of selective and differential media were used to isolate and identify target bacterial species:

Nutrient Agar

NA served as a general-purpose medium for the cultivation of non-fastidious bacteria. It comprises peptone, beef extract, and agar, and supports the surface growth of bacteria, forming visible colonies.

MacConkey Agar

This selective and differential medium is designed for the isolation of Gram-negative enteric bacteria and differentiation between lactose fermenters and non-fermenters. Bile salts and crystal violet inhibit Gram-positive bacteria, while lactose fermentation leads to the formation of pink colonies due to acid production, commonly by coliforms such as *Escherichia*, *Klebsiella*, and *Enterobacter*.

Membrane Fecal Coliform Agar

mFC agar is selective for fecal coliforms, particularly from the intestinal tracts of warm-blooded animals, and is incubated at 44.5 ± 0.2°C. Rosolic acid in the medium causes fecal coliform colonies to appear deep blue, while non-fecal organisms form grey to cream-colored colonies.

Salmonella-Shigella Agar

SS agar is utilized for isolating *Salmonella* and *Shigella* species. *Salmonella* typically forms colonies with black centers due to hydrogen sulfide production, whereas *Shigella* appears as colorless colonies.

Thiosulfate Citrate Bile Salts Sucrose Agar

TCBS agar is highly selective for *Vibrio* species, which appear as small yellow colonies. The alkaline pH and high bile salt concentrations inhibit most non-Vibrio organisms, particularly *Enterobacteriaceae*.

Clostridium perfringens Agar Base

These media form the basis for Tryptose Sulfite (TS), Tryptose Sulfite Cycloserine (TSC), or Shahadi-Ferguson Perfringens (SFP) agar. Sodium metabisulfite and ferric ammonium citrate act as indicators of sulfite reduction, producing black colonies. Egg yolk emulsion detects lecithinase activity, although not all *C. perfringens* strains express this trait. Both lecithinase-positive and lecithinase-negative black colonies were recorded as presumptive *C. perfringens*.

Pour Plate Technique

For the isolation and enumeration of *Clostridium perfringens*, 0.1 mL of the appropriately diluted sample was first dispensed into sterile Petri plates, followed by the addition of melted and cooled culture medium. The contents were gently mixed and allowed to solidify. This pour plate technique facilitated the growth of both surface and subsurface colonies.

Spread Plate Technique

In the spread plate method, sterile molten agar medium was first poured into Petri plates and allowed to solidify. Then, 0.1 mL of the diluted sample was aseptically spread on the surface using a sterile, bent glass rod to ensure even distribution of the microorganisms.

Incubation Conditions

All inoculated plates, except mFC agar, were incubated at 37°C for 24 to 48 hours. mFC agar plates were incubated at 45°C for 24 to 48 hours to favor the growth of thermotolerant fecal coliforms. During the enrichment process, selective broths were incubated at 37°C for 4 hours to allow sufficient growth of the target organisms before plating.

Colony Counting and Data Recording

Colony-forming units (CFU) were counted using a colony counter, and bacterial concentrations were calculated using the formula:
Number of cells per mL=(number of colonies x dilution factor)/sample volume used.

Enumeration of Total Bacteria

To determine the total bacterial load, 1 g of each sample was mixed with 9 mL of sterile normal saline. Then, 1 mL of the suspension was subjected to 10-fold serial dilutions ranging from 10^{-1} to 10^{-} . From the 10^{-} to 10^{-} dilution tubes, 0.1 mL of the suspension was spread onto NA plates and incubated at 37°C for 24 hours. Suspected bacterial colonies appeared off-white or colorless, entire, raised, circular, and smooth with either dry or gummy consistency. CFU were then counted.

Enumeration of Total Coliforms

To isolate total coliforms, 1 g of the sample was suspended in 9 mL of sterile normal saline and serially diluted up to 10^{-3}. From each 10^{-1} to 10^{-3} dilution, 0.1 mL was spread onto MA plates and incubated at 37°C for 24 hours. Suspected coliform colonies were identified as pink, entire, raised, circular, and smooth, with dry or gummy texture, and were subsequently enumerated.

Enumeration of Fecal Coliforms

For fecal coliform enumeration, 1 g of the sample was homogenized with 9 mL of sterile normal saline and serially diluted up to 10^{-3}. From each 10^{-1} to 10^{-3} dilution, 0.1 mL of the suspension was spread onto mFC agar plates and incubated at 44.5°C for 24–48 hours. Fecal coliforms were recognized by their characteristic deep blue, circular, raised, and smooth colonies.

Enumeration of *Salmonella* spp.

To isolate *Salmonella* spp., 1 g of the sample was inoculated into 9 mL of Selenite Cystine Broth and incubated at 37°C for 4 hours. After enrichment, 1 mL of the broth was serially diluted from 10^{-1} to 10^{-3}, and 0.1 mL from 10^{-1} and 10^{-3} dilutions was spread onto SS agar plates. The plates were incubated at 37°C for 24 hours. Colonies exhibiting black centers or colorless appearance were considered presumptive *Salmonella* and were counted accordingly.

Enumeration of *Shigella* spp.

For *Shigella* detection, 1 g of the sample was enriched in 9 mL of Selenite Cystine Broth and incubated at 37°C for 4 hours. Following enrichment, serial dilutions were performed from 10^{-1} to 10^{-3}, and 0.1 mL from 10^{-1} and 10^{-3} dilutions was spread on SS agar plates. After incubation at 37°C for 24 hours, colorless colonies were identified as presumptive *Shigella* spp. and enumerated.

Enumeration of *Vibrio* spp.

To isolate *Vibrio* spp., 1 g of the sample was inoculated into 9 mL of Alkaline Peptone Water and incubated at 37°C for 4 hours. Enriched cultures were serially diluted (10^{-1} to 10^{-3}), and 0.1 mL from 10^{-1} and 10^{-3} dilutions was spread onto TCBS agar plates. The plates were incubated at 37°C for 24 hours. Small, yellow, circular colonies were presumptively identified as *Vibrio* spp. and counted.

Enumeration of *Clostridium perfringens*

To enumerate *Clostridium perfringens*, 1 g of the sample was added to 9 mL of Fluid Thioglycolate Broth and subjected to heat treatment at 100°C for 30 minutes to destroy vegetative cells. The suspension was then incubated at 37°C for 4 hours to allow spore germination. Subsequently, 0.1 mL of the enriched culture was pour-plated onto *Clostridium perfringens* agar and incubated anaerobically at 37°C for 24–48 hours using a candle jar. Colonies appearing black, circular, and entire within the agar medium were considered presumptive *C. perfringens*.

Gram Staining

Gram staining was performed following the method described by Cappuccino et al. (1999). Briefly,

heat-fixed bacterial smears were stained with crystal violet (primary stain), followed by iodine treatment, alcohol decolorization, and counterstaining with safranin. The stained slides were observed under a bright-field microscope for Gram reaction and morphology determination.

Isolation of Pure Cultures

Pure cultures were obtained using the four-quadrant streaking method. Individual, well-isolated colonies from mixed cultures were carefully picked with a sterile inoculating loop and streaked onto fresh agar plates under aseptic conditions. Plates were incubated at 37°C, and isolated colonies were selected for further identification.

Observation of Colony Morphology

Colonial morphology was examined on various selective and differential media used throughout the study. Morphological characteristics such as colony color, shape, elevation, surface texture, and opacity were carefully recorded to aid in the presumptive identification of the isolated bacterial species.

Microscopic Observation of Isolated Microorganisms

The cellular morphology of the isolates, including size and shape, was assessed using simple staining with carbol fuchsin, following the guidelines of the *Manual of Methods for General Bacteriology* by the American Society for Microbiology (ASM, 1981). The stained slides were observed under a bright-field microscope.

Biochemical Identification of Microorganisms

To further characterize and identify the bacterial isolates, a series of biochemical tests were performed in accordance with the *Manual of Methods for General Bacteriology* (ASM, 1981). These tests targeted specific enzymatic and metabolic activities to distinguish among bacterial species.

Oxidase Test

The oxidase test was conducted using 1% p-aminodimethylaniline oxalate solution impregnated onto Whatman filter paper. A colony of the test organism was picked with a sterile toothpick and touched onto the reagent-impregnated paper. The development of a dark purple coloration within 5–10 seconds indicated a positive result, confirming the presence of cytochrome oxidase. No color change was interpreted as a negative result.

Triple Sugar Iron (TSI) Agar Test

The TSI agar test was used to determine carbohydrate fermentation patterns and hydrogen sulfide (H S) production. TSI slants were inoculated by stabbing the butt and streaking the slant with a sterile inoculating needle, followed by incubation at 37°C for 24 hours. A yellow butt indicated acid production under anaerobic (fermentative) conditions, while a yellow slant indicated acid production under aerobic (oxidative) conditions. The presence of black precipitate in the medium signified H S production.

Motility-Indole-Urease (MIU) Test

Motility was assessed by stabbing the MIU medium vertically with a sterile straight wire, ensuring the bottom of the tube was not touched. Tubes were incubated at 37°C for 18–24 hours. Diffuse growth away from the stab line indicated motility. Urease activity was indicated by the development of a pink color in the medium; no color change was recorded as negative.

For the indole test, isolates were separately inoculated into T1N1 broth and incubated under the same conditions. After incubation, 3–4 drops of Kovac's reagent were added. A deep pink color formation at the surface of the broth within one minute confirmed indole production from tryptophan metabolism.

Citrate Utilization Test

Simmon's citrate agar was used to determine the ability of organisms to utilize citrate as the sole carbon source. This medium contains sodium citrate, ammonium ions, and the pH indicator bromothymol blue. Organisms capable of citrate utilization raised the pH of the medium, causing a color change from green to blue, which was considered a positive result.

Catalase Test

Catalase activity was tested by transferring a small amount of bacterial culture onto a slide containing a drop of 3% hydrogen peroxide (H O). The immediate formation of oxygen bubbles indicated a positive catalase reaction, demonstrating the organism's ability to decompose hydrogen peroxide into water and oxygen.

III. Results

Identification of microorganisms

In this study, bacterial isolates were initially identified based on their colony morphology (Figure 2) and cultural characteristics observed on various selective and differential media, including NA, MA, mFC Agar, SS Agar, TCBS Agar, and CP Agar. The term "cultural characteristics" refers to the macroscopic appearance of organisms when grown on different types of media. Descriptive terminology from Bergey's Manual of Determinative Bacteriology was used to record these characteristics. Additionally, Gram staining was performed on distinct colonies isolated from the various media to aid in their preliminary identification. A summary of the colony morphology and cultural characteristics of the different isolates is presented in the following Table 2

Table 2: Morphological characteristics and macroscopic appearance of bacteria on different agar media

Media	Isolate no	Cultural characteristics					Microscopic observation
		color	Form	Margin	Elevation	Size	
NA	1	Off white	Circular	Entire	Convex	Large	Short rod, Single and pair
	2	Off white	Circular	Entire	Convex	Large	Short rod, Single and pair
MA	1	Pink	Circular	Entire	Convex	Small	Short rod, single, pair and Gram negative
	2	Pink	Circular	Entire	Convex	Large	Short rod, single, pair and Gram negative
SS agar	1	Colorless colony	Circular	Entire	Raised	Small	Rod, single, pair and Gram negative
	2	Colorless colony	Circular	Entire	Raised	Large	Rod, single, pair and Gram negative
mFC	1	Deep blue	Circular	Entire	Convex	Small	Short rod, single, pair and Gram negative
	2	Deep blue	Circular	Entire	Convex	Large	Large rod, single, pair and Gram negative
TCBS agar	1	Yellow colony	Circular	Entire	Raised	Pin-point	Comma shape, single and Gram negative
	2	Yellow colony	Circular	Entire	Raised	Pin-point	Comma shape, single and Gram negative
CP agar	1	Black	Circular	Smooth	Raised	Large	Large rod, pair, or short chain, and gram-positive
	2	Black	Circular	Smooth	Raised	Large	Large rod, pair, or short chain, and gram-positive

Enumeration of total bacteria

Total bacterial count was determined by using the NA media. Colony counts of total bacteria are given below-

Table 3 Total bacterial Cunt on NA agar

Sample no.	Type of the pit	Layers of the pit	Total count (CFU/g) before sun-drying	Total count (CFU/g) after sun-drying
1	Single	N/A	2.0×10^5	1.7×10^4
2	Twin	Upper	3.0×10^5	2.0×10^4
		Middle	1.9×10^6	3.4×10^3
		Lower	3.0×10^6	3.1×10^3
3	Twin	Upper	9.5×10^4	2.5×10^4
		Middle	1.6×10^5	4.6×10^3
		Lower	1.8×10^4	3.5×10^3
4	Twin	Upper	4.6×10^7	1.0×10^4
		Middle	4.3×10^6	5.8×10^3
		Lower	3.8×10^5	2.7×10^3
5	Twin	Upper	6.5×10^7	1.3×10^4
		Middle	5.1×10^5	4.1×10^3
		Lower	3×10^5	3.9×10^3

Before sun-drying, the highest bacterial count was in Sample 5 (upper pit, 6.5×10^7 CFU/g), and the lowest in Sample 3 (lower pit, 1.8×10^4 CFU/g). After 45 days of sun-drying, all samples showed a significant reduction in bacterial load, ranging from 3.1×10^3 to 2.5×10 CFU/g. The highest post-drying count was in Sample 3 (upper pit), while the lowest was in Sample 2 (lower pit).

Enumeration of total coliforms

Total coliforms were determined by using MA media. Colony counts of total coliform bacteria are mentioned following table-4

Table 4 Total coliform count on MA

Sample no.	Type of the pit	Layers of the pit	Total count (CFU/g) before sun-drying	Total count (CFU/g) after sun-drying
1	Single	N/A	1.0×10^4	0
2	Twin	Upper	1.0×10^4	0
		Middle	1.9×10^5	0
		Lower	3.0×10^5	0
3	Twin	Upper	9.5×10^3	2.3×10^2
		Middle	1.6×10^4	0
		Lower	1.8×10^3	0
4	Twin	Upper	4.6×10^5	5.0×10^3
		Middle	4.3×10^5	7.0×10^2
		Lower	3.8×10^4	4.0×10^2
5	Twin	Upper	5.2×10^5	0
		Middle	0	0
		Lower	3×10^4	0

Before sun-drying, the highest total coliforms count was found in Sample 5 (upper pit, 5.2×10 CFU/g), while no coliforms were detected in its middle layer. After 45 days of sun-drying, coliforms remained in some samples but at significantly reduced levels. The highest post-drying count was in Sample 4 (upper pit, 5.0×10^3 CFU/g), whereas Samples 1, 2, and 5 showed no detectable coliforms, highlighting the effectiveness of sun-drying in reducing potential pathogens.

Enumeration of fecal coliforms

Fecal coliforms were determined by using mFC agar media. Colony counts of fecal coliforms bacteria are mentioned to the following table-5

Table 5 Fecal coliforms count on mFC agar

Sample no.	Type of the pit	Layers of the pit	Average count (CFU/g) before sun-drying	Total count (CFU/g) after sun-drying
1	Single	N/A	0	0
2	Twin	Upper	3.2×10^4	0
		Middle	0	0
		Lower	5.8×10^4	1.0×10^2
3	Twin	Upper	1.9×10^5	0
		Middle	2.6×10^4	3.0×10^3
		Lower	0	0
4	Twin	Upper	7.0×10^3	0
		Middle	1.6×10^4	1.3×10^3
		Lower	1.2×10^3	0
5	Twin	Upper	0	0
		Middle	0	0
		Lower	0	0

Before sun-drying, the highest total fecal coliforms count was found in Sample 3 (upper pit, 1.9×10^5 CFU/g), while no coliforms were detected in sample 5 of all layers. After 45 days of sun-drying, fecal coliforms remained in some samples but at significantly reduced levels. The highest post-drying count was in Sample 3 (middle pit, 3.0×10^3 CFU/g), whereas Samples 1 and 5 showed no detectable fecal coliforms, highlighting the effectiveness of sun-drying in reducing potential for the fecal coliforms.

Enumeration of *Salmonella* spp.

Salmonella spp. was determined by using SS agar media. Colony counts of *Salmonella* spp. are are mentioned to the following table-6

Table 6 Salmonella spp. counts on SS agar

Sample no.	Type of the pit	Layers of the pit	Average count (CFU/g) before sun-drying	Total count (CFU/g) after sun-drying
1	Single	N/A	0	0
2	Twin	Upper	0	0
		Middle	0	0
		Lower	0	0
3	Twin	Upper	0	0
		Middle	2.4×10^3	0
		Lower	0	0
4	Twin	Upper	0	0
		Middle	0	0
		Lower	0	0
5	Twin	Upper	0	0
		Middle	0	0
		Lower	0	0

Before sun-drying, *Salmonella* spp. was detected only in Sample 3 (middle pit, 2.4×10^3 CFU/g), while absent in other samples. Remarkably, after 45 days of sun-drying, *Salmonella* was completely eliminated from Sample 3, with all other samples maintaining their *Salmonella*-free status. This highlights sun-drying as a highly effective, natural method for eliminating *Salmonella* and enhancing compost safety.

Enumeration of *Shigella* spp.

Shigella spp. was determined by using SS agar media. Colony counts of total *Shigella* spp are are mentioned to the following table-7

Table 7 *Shigella* spp. counts on SS agar

Sample no.	Type of the pit	Layers of the pit	Average count (CFU/g) before sun-drying	Total count (CFU/g) after sun-drying
1	Single	N/A	0	0
2	Twin	Upper	1.4×10^5	1.2×10^3
		Middle	2.3×10^5	1.0×10^3
		Lower	2.0×10^6	2.0×10^2
3	Twin	Upper	1.4×10^5	0
		Middle	5.0×10^4	0
		Lower	1.0×10^4	0
4	Twin	Upper	6.6×10^6	2.7×10^3
		Middle	3.7×10^5	1.6×10^3
		Lower	3.4×10^5	1.5×10^2
5	Twin	Upper	4.4×10^6	3.2×10^3
		Middle	7.6×10^5	4.1×10^3
		Lower	6.2×10^5	2.9×10^2

Before sun-drying, *Shigella* spp. was most abundant in Sample 4 (upper pit, 6.6×10^6 CFU/g), while completely absent in Sample 1. After 45 days of sun exposure, all samples showed a dramatic decline in *Shigella* levels, ranging from 0 to 4.1×10^3 CFU/g. The highest remaining count was still in Sample 5, whereas Sample 1 remained *Shigella* spp.-free. These results underscore sun-drying as a powerful and eco-friendly approach for significantly reducing *Shigella* spp. and improving compost safety.

Enumeration of *Vibrio* spp.

Vibrio spp. was determined by using TCBS agar media. Colony counts of *Vibrio* spp. are are mentioned to the following table-8

Table 8 *Vibrio* spp. counts on TCBS agar

Sample no.	Type of the pit	Layers of the pit	Average count (CFU/g) before sun-drying	Total count (CFU/g) after sun-drying
1	Single	N/A	0	0
2	Twin	Upper	3.3×10^5	0
		Middle	1.2×10^5	0
		Lower	3.2×10^5	0
3	Twin	Upper	7.0×10^4	2.3×10^3
		Middle	2.0×10^4	1.1×10^3

		Lower	8.9×10^3	6.5×10^2
4	Twin	Upper	1.0×10^2	0
		Middle	2.0×10^2	0
		Lower	0	0
5	Twin	Upper	9.9×10^3	0
		Middle	5.9×10^3	0
		Lower	7.0×10^2	0

Before sun-drying, *Vibrio* spp. was most abundant in Sample 2 (upper pit, 3.3×10^5 CFU/g) and undetectable in Sample 1. Following 45 days of sun exposure, all samples showed a sharp decline in *Vibrio* levels, ranging from 0 to 2.3×10^3 CFU/g. The highest residual count appeared in Sample 3, while *Vibrio* spp. remained undetectable in Sample 1 and others. These findings highlight sun-drying as a highly effective, low-cost, and eco-friendly method for minimizing *Vibrio* contamination and enhancing compost hygiene.

Enumeration of *Clostridium perfringens*

C. perfringens was determined by using CP agar. Colony counts of *C. perfringens* are are mentioned to the following table-9

Table 9 *C. perfringens* count on Perfringens agar

Sample no.	Type of the pit	Layers of the pit	Average count (CFU/g) before sun-drying	Total count (CFU/g) after sun-drying
1	Single	N/A	0	0
2	Twin	Upper	4.1×10^3	25
		Middle	6.8×10^3	0
		Lower	1.0×10^4	0
3	Twin	Upper	1.3×10^3	75
		Middle	0	0
		Lower	9×10^2	0
4	Twin	Upper	4.8×10^3	0
		Middle	2.5×10^4	30
		Lower	0	0
5	Twin	Upper	2.0×10^3	0
		Middle	0	0
		Lower	0	0

Before sun-drying, *Clostridium perfringens* was highest in Sample 4 (middle pit, 2.5×10 CFU/g) and undetectable in Sample 1. After 45 days of sun exposure, all samples exhibited a dramatic reduction, with levels ranging from just 0 to 75 CFU/g. The highest post-drying count was in Sample 3, while no growth was detected in Samples 1 and 5. These results emphasize the remarkable effectiveness of sun-drying as a natural, low-cost solution for reducing *C. perfringens* and improving compost safety.

Biochemical tests for the identification of isolates

After initial growth on Nutrient Agar, MacConkey Agar, mFC Agar, SS Agar, and TCBS Agar plates, the isolates were further identified using a series of biochemical tests. These included the Triple Sugar Iron (TSI) test, Motility Indole Urea (MIU) test, Simmons' Citrate test, Catalase test, and Oxidase test, which were performed on the selected isolates. The organisms were identified based on the results of these tests, following the guidelines outlined in the *Microbiology Laboratory Manual* by G. Cappuccino and Natalie Sherman (1983). The biochemical characteristics of the bacterial isolates are summarized in the following table.

Table 10 Biochemical Chart for Isolates found in Bio-compost-

Type of Organisms	Name of the isolates	TSI			MIU			Citrate Utilization	Catalase	Oxidase
		Slant	Butt	Gas	Motility	Urease	Indole			
Coliforms and Fecal Coliforms	*E. coli*	Ac	Ac	(+)	(+)	(-)	(+)	(-)	(+)	(-)
	Klebsiella spp.	Ac	Ac	(+)	(-)	(+)	(-)	(+)	(+)	(-)
	Enterobacter spp.	Ac	Ac	(-)	(+)	(-)	(-)	(+)	(+)	(-)
	Citrobacter spp.	Ac	Ac	(+)	(+)	(-)	(+)	(+)	(+)	(-)
Shigella	*Shigella spp.*	Al	Ac	(+)	(-)	(-)	(-)	(-)	(+)	(-)
Vibrio	*V. cholerae*	Al	Ac	(-)	(+)	(-)	(-)	(-)	(+)	(+)
Clostridium	*Clostridium perfringens*	Ac	Ac	(+)	(+)	(-)	(-)	(-)	(-)	(-)

All biochemical tests were repeated several times with different isolates from the same sample or

plates, and values are from representative data. Here, Ac=Acid (Yellow); Al=Alkaline (Red); (+) = Positive; (-) = Negative.

Comparison between the average count (CFU/g) of different pathogens and total bacterial count

The average counts (CFU/g) of total bacteria, total coliforms, fecal coliforms, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., and *Clostridium perfringens* were calculated in compost samples before and after forty-five days of sun-drying. A comparative analysis revealed that the average counts of these pathogenic bacteria were reduced by approximately 60–70% following sun-drying. Notably, *Salmonella* spp. was absent in all samples after the sun-drying period.

Table 11 Average counts (CFU/g) of total bacterial count and different pathogens found in compost before and after sun-drying

Name of the bacteria	Average counts before sun-drying (cfu/g)	Average count after sun-drying (cfu/g)
Total bacteria	1.0×10^7	8.9×10^3
Total coliforms	1.6×10^5	4.8×10^2
Fecal coliforms	2.5×10^4	3.3×10^2
Salmonella spp.	1.8×10^2	0
Shigella spp.	1.2×10^6	1.2×10^3
Vibrio spp.	6.8×10^4	3.1×10^2
C. perfringens	4.2×10^3	10

IV. Discussion

The escalating global burden of waste generation demands urgent and sustainable management approaches. Among these, composting stands out as a promising, eco-friendly solution that not only reduces waste volume but also recycles essential nutrients [24]. Nevertheless, compost produced from human fecal matter poses serious microbial safety concerns due to the potential survival of harmful pathogens. Composting is a biologically intensive process driven by diverse microbial communities that facilitate organic matter decomposition and determine compost quality [25]. However, despite the benefits, pathogens such as *Salmonella*, *Shigella*, *Vibrio* spp., and *Clostridium perfringens* may survive adverse conditions or re-emerge if compost is not adequately treated or is mishandled after processing [26,27].

In this study, the impact of sun-drying on microbial pathogen reduction in human feces-derived compost was systematically evaluated. Samples collected from five different compost pits were subjected to 45 days of natural sun-drying. The results demonstrated a consistent and substantial declined in bacterial counts across all examined microbial groups.

Strikingly, *Salmonella* spp. was completely eliminated in all dried compost samples, while significant reductions were observed for total coliforms, fecal coliforms, *Shigella* spp., *Vibrio* spp., and *C. perfringens*. The highest bacterial loads detected post-drying were: total bacteria (2.5×10 CFU/g), total coliforms (5.0×10^3 CFU/g), fecal coliforms (3.0×10^3 CFU/g), *Shigella* spp. (4.1×10^3 CFU/g), *Vibrio* spp. (2.3×10^3 CFU/g), and *C. perfringens* (75 CFU/g). In contrast, the wet (untreated) compost samples showed significantly higher contamination levels, with *Salmonella* spp. reaching 2.4×10^3 CFU/g and *Shigella* spp. as high as 6.6×10^6 CFU/g.

On average, sun-dried compost exhibited a marked reduction in bacterial counts: total bacteria (8.9×10^3 CFU/g), total coliforms (4.8×10^2 CFU/g), fecal coliforms (3.3×10^2 CFU/g), *Shigella* spp. (1.2×10^3 CFU/g), *Vibrio* spp. (3.1×10^2 CFU/g), and *C. perfringens* (10 CFU/g). These findings strongly support the effectiveness of solar drying in reducing microbial loads, particularly enteric pathogens.

Corroborating earlier findings by Hassen et al. (2002), which reported untreated compost harboring fecal coliforms up to 1×10 CFU/g, *Shigella* spp. up to 9×10 CFU/g, and *C. perfringens* up to 6×10 CFU/g, our results suggest a 2–4 log reduction post sun-drying. This significant decline underscores the potential of sun-drying as a low-cost, accessible method for enhancing compost microbiological safety.

The mechanism behind this pathogen reduction can be attributed to the prolonged exposure to solar radiation, which reduces moisture content and promotes thermophilic microbial activity. The resulting elevated temperatures and desiccation stress create an unfavorable environment for pathogenic survival and proliferation [28]. Although *Salmonella* spp. was fully eradicated, the presence of low levels of *Shigella* spp. and *C. perfringens* indicates that sun-drying alone may not guarantee complete decontamination and might require complementary treatments for highly sensitive applications.

It is crucial to highlight that improper handling during or after the composting process can lead to pathogen reintroduction. Residual organisms may also persist or multiply under conducive environmental conditions. Therefore, ensuring proper composting techniques, hygienic handling, and effective post-treatment strategies is essential for safe application [29,30]. Particularly for compost intended for use on raw-consumed

produce such as fruits and leafy vegetables, regular microbiological assessments using indicator organisms remain a critical safety measure [31].

V. Conclusion

This study confirms that sun-drying is an effective, low-cost method for significantly reducing pathogenic bacterial loads in compost. A 45-day sun-drying period was sufficient to eliminate *Salmonella* spp. and markedly lower the presence of other harmful bacteria. For safer agricultural applications, particularly on crops consumed raw, further research is recommended to determine the optimal duration and environmental conditions for complete pathogen inactivation.

References:

[1] Bernal MP, Alburquerque JA, Moral R. Composting Of Animal Manures And Chemical Criteria For Compost Maturity Assessment. A Review. Bioresour Technol. 2009 Nov;100(22):5444-53.

[2] Suzanne Donn, Ron E. Wheatley, Blair M. Mckenzie, Kenneth W. Loades, Paul D. Hallett, Improved Soil Fertility From Compost Amendment Increases Root Growth And Reinforcement Of Surface Soil On Slopes, Ecological Engineering, Volume 71, 2014, Pages 458-465.

[3] Lucimar Lopes Fialho, Wilson Tadeu Lopes Da Silva, Débora M.B.P. Milori, Marcelo Luiz Simões, Ladislau Martin-Neto, Characterization Of Organic Matter From Composting Of Different Residues By Physicochemical And Spectroscopic Methods, Bioresource Technology, Volume 101, Issue 6,2010, Pages 1927-1934.

[4] Pahriya Ashrap And Amber Cathey, Trash To Treasure: The Incredible Benefits Of Composting School Of Public Health, University Of Michigan, September 19, 2019.

[5] Strom, P.F. (1985). Effect Of Temperature On Bacterial Diversity In Thermophilic Solid-Waste Composting. Appl. Environ. Technol. 50, 906-913.

[6] Alexander, M. (1999). Biodegradation And Bioremediation (2nd Ed.). Academic Press.

[7] Maryland Cooperative Extension. (2000). Home Composting: A Guide For Maryland Residents. University Of Maryland Extension.

[8] Brady, N.C. And Weil, R.R. (2002) The Nature And Properties Of Soil. 13th Edition, Prentice Hall, Upper Saddle River, New Jersey.

[9] J. I. Boulter, Greg J. Boland, J. T. Trevors (2000).Compost: A Study Of The Development Process And End-Product Potential For Suppression Of Turfgrass Disease. World Journal Of Microbiology And Biotechnology.

[10] Hellmann B, Zelles L, Palojarvi A, Bai Q. Emission Of Climate-Relevant Trace Gases And Succession Of Microbial Communities During Open-Windrow Composting. Appl Environ Microbiol. 1997 Mar;63(3):1011-8.

[11] Epstein, E. (1997). The Science Of Composting. CRC Press. ISBN: 9781566764780.

[12] Bhattacharyya R, Ghosh BN, Mishra PK, Mandal B, Rao CS, Sarkar D, Das K, Anil KS, Lalitha M, Hati KM, Soil Degradation In India: Challenges And Potential Solutions. Sustainability. 2015; 7(4):3528-3570

[13] Abu Zahrim Yaser, Junidah Lamaming, Emma Suali, Mariani Rajin, Sariah Saalah, Zykamilia Kamin, Nurliyana Nasuha Safie, Nur Aqeela Syuhadah Aji, Newati Wid, Composting And Anaerobic Digestion Of Food Waste And Sewage Sludge For Campus Sustainability: A Review. International 2022(9).

[14] J.I. Nirmal Kumar, Hiren Soni, Rita N. Kumar, And Narendra Patil. Growth And Reproduction Of Eisenia Foetida In Various Industry Waste Sludge During Vermicomposting: A Laboratory Investigation, International Journal Of Environment And Waste Management. 5, No. 3-4, March 5, 2010, Pp. 379-391.

[15] Environmental Control Systems, Inc. (ECS). (2010). Aerated Static Pile Composting Systems: Design And Operation Manual. Environmental Control Systems, Inc.

[16] Socolofsky, T. (2008). In-Vessel Composting Systems: An Overview Of Designs And Performance. Composting Science & Utilization, 16(2), 113–118.

[17] Elizabeth Waddington (2024).Trench Composting: Effective Methods For Expert Gardeners, Https://Blog.Firsttunnels.Co.Uk/Trench-Composting/.

[18] Sidhu JP, Toze SG. Human Pathogens And Their Indicators In Biosolids: A Literature Review. Environ Int. 2009 Jan;35(1):187-201.

[19] Hay, J.C. (1996). Pathogen Destruction And Bio-Solids Composting, Biocycle, J. Waste Recyc. 46, 67-76.

[20] Hussong, D. And Burge, W.D. (1985). Occurrence, Growth And Suppression Of Salmonellae In Zuberer, D.A. (1994). Recovery And Enumeration Of Viable Bacteria. In Methods Of Soil Analysis, Part 2. Microbiological And Biochemical Properties. Soil Science Society Of America. Madison, WI 53711, Book Series No 5, 119-144.

[21] Redlinger T, Graham J, Corella-Barud V, And Avitia R (2001). Survival Of Fecal Coliforms In Dry-Composting Toilets. Applied And Environmental Microbiology. Volume 67, No. 9, 4036-4040.

[22] Al-Ghazali MR And Al-Azawi SK (1990). Listeria Monocytogenes Contamination Of Crops Grown On Soil Treated With Sewage Sludge Cake. J Appl Bacteriol. 69 : 642-647.

[23] Ahn, H. K., Smith, M. C., Kondrad, S. L., & White, J. W. (2008). Evaluation Of Biothermal Drying Of Dairy Manure And Food Waste Mixtures Through Laboratory-Scale Experiments. Bioresource Technology, 99(17), 8441–8449.

[24] Steineck, S., Stintzing, R., Rodhe, L., Elmquist, H. & Jakobsson, C. (1999). Influence Of Composting Techniques On Microbial Succession, Temperature And Ph In A Composting Municipal Solid Waste. African Journal Of Biotechnology. 3(4) : 239-243

[25] Iranzo, M., I. Sainz-Pardo, R. Boluda, J. Sánchez And S. Mormeneo (2001). The Use Of Microorganisms In Environmental Remediation. Ann. Microbiol., 51 : 135-143

[26] Piet, J.L., X. Derik, J.M. Huub, C.V. Drift, J.L.D. Leo And D. Vogel (1990). Biomass And Biological Activity During The Production Of Compost Used As A Substrate In Mushroom Cultivation. Appl. Environ. Microbiol., 56(10) : 3029-3034

[27] Hassen, A., Belguith, K., Jedidi, N. And Cherif, M. Et A.L (2002). Microbial Characterization During Composting Of Municipal Solid Waste. EPCOWM, P.357-368.

[28] Mihial, D.J., T. Viraraghavan And Y.C. Jin. 2006. Bioremediation Of Petroleum Contaminated Soil Using Composting. Pract. Periodical Of Haz. Toxic And Radioactive Waste Mgmt. 10(2) : 108-115.

[29] Madigan, T.M. & Martinko, M.J. 2006. Brock Biology Of Microorganisms. International Edition. 11th Edition. Published By Pearson Prentice Hall. Pearson Education Inc., Upper Saddle River, United States Of America. ISBN 0-13-196893-9.

[30] Stenström, Thor & Schonning, C. (2004). Guidelines For The Safe Use Of Urine And Faeces In Ecological Sanitation Systems..
[31] WHO (2006). Guidelines For The Safe Use Of Wastewater, Excreta And Greywater. Excreta And Greywater Use In Agriculture. ISBN 92 4 154685 9. Vol. 4.
[32] Oluwadara Alegbeleye, Olumide Adedokun Odeyemi, Mariyana Strateva, Deyan Stratev, Microbial Spoilage Of Vegetables, Fruits And Cereals,Applied Food Research,Volume 2, Issue 1,2022.