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Biodegradation Efficiency of Pla in Microbial Windrow Composting System with Organic and Invasive Species Waste

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ABSTRACT:

The rapid accumulation of plastic waste and invasive plant biomass poses serious environmental challenges in terrestrial and aquatic ecosystems. Biodegradable biopolymers such as polylactic acid (PLA), along with invasive weeds including Water hyacinth, Parthenium hysterophorus, Saccharum spontaneum, and Ipomoea carnea, generate substantial organic residues that are often poorly managed. This study evaluated the efficiency of windrow heap co-composting of invasive weed biomass and PLA biopolymer blended with vegetable waste and cow dung to produce nutrient-enriched organic fertilizer. Composting was conducted for 56 days under controlled aeration and moisture conditions with periodic turning. Changes in physicochemical parameters including temperature, moisture content, pH, electrical conductivity (EC), total organic carbon (TOC), C/N ratio, ash content, volatile organic compounds (VOCs), and macronutrients (N,P,K) were monitored. Microbial succession was assessed through culture-dependent isolation, biochemical profiling, and 16S rRNA gene sequencing. Results demonstrated rapid thermophilic activity during the initial phase, followed by stabilization and maturation, with final C/N ratios declining to 10–11.5, indicating compost maturity. Treatments containing moderate PLA (20–30%) and Parthenium biomass showed enhanced organic matter degradation and superior nutrient enrichment. Molecular identification revealed dominant bacterial taxa affiliated with Aeromonas caviae and Enterobacter hormaechei, highlighting active heterotrophic communities during biodegradation. The study demonstrates that windrow co-composting of invasive weeds and PLA biopolymer with organic wastes is an effective, scalable strategy for sustainable waste management, invasive species valorization, and production of high-quality organic fertilizer suitable for agricultural application.

Introduction

Plastic product production and consumption are growing due to its low cost, high efficiency, and ideal mechanical qualities. Plastic additives move into the soil because they are not chemically bonded to the polymeric chain. Many diseases are being caused by the leachates from plastic garbage that ends up in landfills. Pesticides, solvents, heavy metals, and petroleum hydrocarbons are the most often occurring compounds in soil (Rajesh Kumar Mishra, 2016). To escalating environmental concerns associated with conventional plastics underscore the need for innovative waste management strategies. Biodegradable and sourced from renewable materials biopolymers present a viable alternative to mitigate plastic pollution. The present study focuses on the recycling of biopolymer waste into organic fertilizer through the composting process as a sustainable approach. Types of Biopolymers, the method of waste management for biopolymers, and the composting process of biopolymers are discussed. Biopolymers, derived from renewable sources, offer a biodegradable alternative to traditional plastics and have emerged as a potential solution to plastic pollution (Jambeck *et al.,* 2015; Geyer *et al.,* 2017). The environmental impact of plastic waste emphasizes the urgency of transitioning to more eco-friendly alternatives and the benefits of utilizing biopolymer waste for organic fertilizer production. The composting process is monitored for key parameters such as temperature, moisture content, and microbial activity to ensure optimal conditions for biodegradation. The composting process involves the microbial breakdown of biopolymer waste over a

specified duration. Microorganisms play a crucial role in breaking down the biopolymers into simpler organic compounds, ultimately resulting in the production of organic fertilizer. The nutrient-rich compost is then analyzed for its chemical composition and suitability for agricultural applications. The working process provides insights into the efficiency and effectiveness of recycling biopolymer waste into organic fertilizer through composting. Plastic waste has emerged as a major source of environmental pollution, as its persistence in terrestrial and aquatic systems alters natural degradation pathways. In soils, the slow breakdown of plastics leads to long-term accumulation and emission of harmful by-products, while in aquatic ecosystems, fragmentation into microplastics disrupts ecological balance and threatens biodiversity. (Geyer *et al.,* 2013; Jambeck *et al.,* 2013; Borrell *et al.,* 2013). In response to these challenges, biodegradable polymers have emerged as a promising alternative to conventional plastic, with polylactic acid (PLA) attracting special attention due to its renewable appearance and environmentally friendly properties. Polylactic acid (PLA) is a biodegradable polymer made from renewable plant-based feeds such as cornstarch, sugarcane, sugarcane, and cassava. Its production includes the fermentation of these feedstocks to obtain lactic acid, which is then converted into polymer by ring-opening polymerization, giving it a versatile substance with applications in packaging, textiles, and biomedical devices. However, the increased use of PLA has led to an increase in PLA waste, which, if not managed effectively, can contribute to environmental pollution and waste management challenges (De Bie *et al.,* 2025; Jamshidian *et al.,* 2010). Some of the recent studies use composting method to reduce the PLA waste production. Composting PLA in combination with organic waste streams is becoming more widely acknowledged as a successful way of reducing its environmental impact. PLA undergoes microbial hydrolysis and biodegradation under regulated aerobic conditions, ultimately producing stable humic compounds, water, and carbon dioxide. This biodegradable approach lowers the possibility of secondary contamination creation, such as leachate pollution and microplastic fragments. PLA's role as a sustainable substitute for traditional plastics in waste management procedures is additionally supported by the fact that its incorporation into composting processes results in a cleaner aquatic and soil environment (Kale *et al.,* 2007; Yadav, Sharma, & Kumar, 2023). The integration of PLA into composting systems not only supports sustainable waste management but also helps to reduce dependency on fossil-based plastics, promoting a circular economy through the production of valuable end-products that can enhance soil fertility and support agricultural applications.

Terrestrial weeds are present all over the world. The major com ponent of terrestrial weed biomass is lignocelluloses which consist of lignin, hemicelluloses and cellulose (Sanchez, 2009). Lignocellu loses is considered as most abundant natural material which is released from different sources like food, agriculture, paper and pulp industry into the waste stream possessing serious problem of disposal. Cellulose and hemicelluloses component of lignocellu loses are the main source of energy in composting process as both are easily degradable by microbial activities whereas lignin is not (Birhanli and Yesilada, 2013; Hubbe, 2014). Lignocelluloses mate rial due to its bulky nature provides better access to aeration both during composting process and after the compost applied to the soil which makes it fit material for composting process. Complementing these developments, invasive aquatic species management presents another dimension of environmental complexity. Water hyacinth (Eichhorniacrassipes), recognized among the world's most problematic aquatic macrophytes, and exemplifies the intricate relationship between ecological disruption and resource potential (Villamagna *et al.,* 2010). Recent ecological studies show this rapidly proliferating species colonizes aquatic systems with remarkable speed, disrupting ecosystem balance and reducing biodiversity (Rafael L *et al.,* 2024). Paradoxically, the very characteristics that render it problematic explosive growth rates and high biomass productivity also position it as a valuable composting feedstock. Invasive plant species represent a major global environmental and socioeconomic challenge due to their rapid biomass accumulation, competitive displacement of native flora, alteration of ecosystem processes, and significant management costs across terrestrial and aquatic systems. Concurrently, these species produce vast quantities of biomass that, if not managed sustainably, contribute to waste accumulation and further spread. An emerging strategy in sustainable weed management is the bioconversion of invasive weed biomass into value-

added organic soil amendments via composting and vermicomposting, turning an ecological liability into an agronomic resource. (Adam Canning 2025). *Eichhornia crassipes* (water hyacinth) is one of the most aggressive aquatic invaders worldwide, choking waterways, altering nutrient cycling, and impeding irrigation and transport. Its high growth rate and biomass productivity have also made it a promising feedstock for compost and vermicompost production, with research showing nutrient-rich compost and potential to reduce reliance on mineral fertilizers without phytotoxicity in applied soils. *Parthenium hysterophorus* is a highly noxious terrestrial weed notorious for its allelopathic toxicity, negative effects on crop yields, human and animal health, and rapid colonisation of disturbed soils. Traditional control measures are costly and often ineffective, but vermicomposting and composting approaches have demonstrated the degradation of phytotoxic compounds and production of nutrient-enriched organic manure that can improve soil fertility while mitigating weed biomass. (Jha *et al* 2026). *Saccharum spontaneum* (wild sugarcane) is a perennial grass that can invade agricultural and marginal lands, forming dense stands that undermine biodiversity and land productivity. Although its use in composting has been less extensively studied than other weeds, recent investigations into the vermicomposting of its lignocellulosic biomass suggest that appropriate biological processing can reduce biomass waste and generate nutrient-rich organic amendments, providing a sustainable disposal route while valorising an otherwise problematic species. (Chaichi Devi and Meena Khwairakpam 2020). *Ipomoea carnea* is a fast-spreading invasive shrub with allelopathic and toxic properties that suppress native vegetation and livestock. Vermicomposting has been shown to degrade toxic compounds and transform its biomass into a productive organic fertiliser, offering an eco-friendly management alternative and contributing essential nutrients for soil enhancement. (Hussain *et al* 2016).

The objective of the present study is to evaluate the composting efficiency of different mixing proportions of invasive plant biomass (including *Saccharum spontaneum*, *Parthenium hysterophorus,* Water hyacinth and *Ipomoea carnea*) co-composted with vegetable waste and cow dung under windrow heap composting conditions. The study aims to identify the optimal proportion of invasive plant biomass in the composting mixture for producing nutrient-enriched and mature compost by evaluating changes in physico-chemical parameters such as pH, electrical conductivity, total organic carbon, C:N ratio, and macro-nutrient content (N, P, K) during the composting process. The novelty of the present work lies in the limited availability of earlier studies focusing on windrow co-composting of invasive plant biomass with vegetable waste and cow dung at varying proportions. Additionally, the study seeks to assess the biodegradation potential and stabilization efficiency of invasive plant residues in heap composting systems, thereby providing a sustainable waste management strategy for invasive weeds while converting them into value-added organic soil amendments.

Methodology

Composting Experimental setup and Feedstock preparation

The composting experiment was systematically designed utilizing vegetable waste (VW), cow dung (CD), water hyacinth (WH), *Parthenium heterosporous* (PH), Ipomoea cornea (IC), *Saccharum spontaneous* (SS) and polylactic acid (PLA) biopolymer as primary substrates. The combination of heaps (Heap 1 vegetable waste + cow dung), Heap 2 (Vegetable waste + cow dung + PLA), Heap 3(Vegetable waste + Cow dung+ PLA+ water hyacinth), Heap 4 (Vegetable waste + Cow Dung + PLA+ *Ipomoea carnea*), Heap 5(Vegetable waste +Cow dung +PLA + *Parthenium hysterophorus*) and Heap 6 (vegetable waste + Cow dung+ PLA+ *Saccharum Spontaneum)*

Vegetable waste was sourced from the Chidambaram municipality collection centre and mechanically shredded into uniform fragments (approximately 1-2 cm) to facilitate consistent decomposition while maintaining intrinsic nutrient integrity. Fresh cow dung, obtained from a local dairy farm, was utilized as a natural microbial inoculum to enhance biological activity and accelerate the composting process. Water hyacinth biomass was harvested from vellakulam pond, adjacent to the Annamalai University, Annamalai nagar, and Chidambaram campus. The biomass was sun-dried for 24 hours to reduce its moisture content and then mechanically shredded to achieve a consistent particle

size. *Parthenium hysterophorus* were collected from nearer to the university campus and chopped into small fragments used for the composting process. *Ipomoea carnea* were collected from local ponds nearby study area and using mechanical shredded it used to cut into small pieces used for preparation of compost heaps. *Saccharum Spontaneum* collected from crop field in surrounding agricultural lands and it also chopped into small fragments for composting process. Commercially available PLA (polyactic acid) bags, commonly used in food packaging, wear used as the second raw material. These bags wear mechanically fragmented into particles smaller than 2 mm without any chemical pre-treatment before being incorporated into the compositing mixtures. The mechanical shredding was performed using a [Mechanical shredder model no: SVIQ2401007, year-2024.

Physicochemical analysis of Compost

The Compost maturity and degradation dynamics wear evaluated through comprehensive physicochemical analysis following standard methodologies. Samples wear collected at predetermined intervals (0, 7, 14, 21, 28, 42, 49 and 56 days) throughout the 56 days compositing period. Temperature monitoring was conducted daily using a compost sensor (Hanna HI9813-5). Moisture contact was determined gravimetrically by oven – drying at 105°C to constant weight, while ash content was quantified via ignition at 5500c in a muffle furnace. Total organic carbon (TOC) was analyzed using the Walkley-black wet oxidation and total Johan Kjeldahl nitrogen (TKN) methods, respectively. The corban-to-nitrogen (C/N) ratio was calculated from TOC and TKN values to assess compost stabilization. The pH and electrical conductivity (EC) were measured in 1:10 (w/v) aqueous extracts according to ISO 10390:2005 and IS 14767:2000, respectively. Phosphorus content was quantified using spectrophotometer and potassium concentration was determined via flame photometry. All analytical procedures were carried out in accordance in EN14045 (2003) fertilizer quality criteria.

Isolation of Biodegradation Microbes from Compost

Microbial analysis was conducted to isolate and identify bacterial species involved in the compositing process. The compost samples were serially diluted up to a 10^{-8} dilution; 0.1 mL of each dilution was inoculated in duplicate on plates of Nutrient Agar (NA) media for the isolation of bacteria using the spread plate technique. After inoculation, all plates were incubated at 37 °C in the incubator for 24 hours. The medium was supplemented with Nystatin at 50 µg/ml to inhibit fungal contamination. The selected colonies were isolated and purified by the streak plate technique using nutrient agar.

Genus level identification

Morphological characterization

The potential bacterial isolates were grown on nutrient agar medium at 37°C for 72 h. The plates were observed daily for bacterial growth, and their morphological characteristics were documented. The bacterial colony characteristics, like shape, colour, and margin of the colonies on plates, were observed.

Microscopical characterization

Gram's staining

The smear of the culture was made on a clean glass slide and heat fixed. It was flooded with crystal violet solution and allowed to remain for one minute. Then it was washed with water, flooded with iodine solution, and left for one minute. It was then drained and decolorized with 95% ethanol, washed gently in running water, and counterstained for one minute with Safranin and washed with distilled water. The water-drained smear was observed under the microscope. Purple colored cells that retain crystal violet were considered Gram-positive bacteria. Pink colored cells that have lost the primary stain and picked up safranin color and were considered Gram-negative bacteria.

Biochemical tests

The isolated strains are screened for Indole, Methyl red, Voges-Proskauer (VP), Citrate utilization test, Urease, Catalase and oxidase tests**.** Based on these tests the isolated strains were identified as genus level.

Molecular Profiling

Genomic DNA extraction

The genomic DNA of the bacterial strains was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel) following the manufacturer's instructions. A pure culture

is taken in a microcentrifuge tube. 180 µl of T1 buffer and 25 µl of proteinase K were added and incubated at 56 ºC in a water bath until they were completely lysed. After lysis, 5 µl of RNase A (100 mg/ml) was added and incubated at room temperature for 5 minutes. 200 µl of B3 buffer was added and incubated at 70 °C for 10 minutes. 210 µl of 100% ethanol was added and mixed thoroughly by vortexing. The mixture was pipetted into a NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000 x g for 1 minute. The NucleoSpin® Tissue column was transferred to a new 2 ml tube and washed with 500 µl of BW buffer. The Wash step was repeated using 600 µl of B5 buffer. After washing, the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube, and DNA was eluted using 50 µl of BE buffer.

Sequencing using Big Dye Terminator v3.1 and PCR amplification of 16S rRNA

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). Table 3 indicate the target and sequences priemers name nad direction. The sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) following manufactures protocol. The reverse complement sequence was constructed for both forward and reverse sequence files. Both sequences were aligned using the Pairwise alignment tool. The aligned sequence was used to create a consensus sequence (Hall, T.A, 1999). The created consensus sequence was used to BLAST in NCBI to retrieve similar sequences. The top 15 sequences from the hit table were chosen to construct a phylogenetic tree in MEGA software**.** (Madden, 2002)

Result and Discussion

Compost Preparation

The percentage distribution of vegetable waste used composting process was conducted using windrow methodology with standardized dimensions (l x b x h) (2m x 1.5m x 1.2m) over 56 days under controlled ambient conditions. Preparation of compost heaps were turned was performed weekly days (7, 14, 21, 28, 35, 42, 49 and 56) to maintain aerobic conditions and ensure mixture homogeneity. Figure 1 show the prepared compost heaps for windorw composting collection of vegetable waste, cow dung blended with Biopolymers PLA (polylactic acid) and invasive weeds used for the present study. Moisture content was regularly monitored and maintained at optimal levels (50-60%) through controlled water supplementation, with all experimental treatments conducted in triplicate to ensure statical reliability.

Figure 1. Compost heaps for windrow composting mixed with wastes

Changes in Physicochemical Characteristics During the Composting Process

Temperature and Moisture Content

During the initial stage of composting, the temperature in all treatments rapidly increased to the thermophilic range within the first week this was followed by a gradual decline to the mesophilic range (~50 °C) before decreasing further towards the maturation phase. Throughout the composting period, heap temperatures were consistently higher than ambient values, indicating active microbial metabolism, as also reported by (Dayanand Sharma and Yadav 2017). Similar thermophilic patterns have been documented in composting of various organic wastes by (Varma and Kalamdhad 2014; Singh and Kalamdhad 2014; Abdullah et al. 2013). The temperature increases during the thermophilic stage enhanced microbial degradation and possible PLA breakdown compare to invasive species. The temperature decline in the later composting stages was attributed to the depletion of readily degradable nutrient rich mature compost, as noted by (Awasthi et al. 2016). Temperature is one of the most frequently monitored parameters in composting facilities; in Europe, nearly 90% of composting plants monitor temperature regularly, although about half do so manually (López *et al.,* 2014). **Figure 2.** Illustrate the temperature variation observed during the composting process. Heap 1 as ambient temperature over 56 days of complete composting process. Heap 2 indicates the temperature decline throughout the final compost attain biopolymers; Heap 3 indicates the temperature range of water hyacinth species when compared with ambient temperature with all other set of heaps; Heap 4 show the range of thermophilic range of ipomoea carnea; Heap 5 indicates the parthenium Species temperature decline through the composting duration. Heap 6 Saccharum temperature indicate the increase and gradually decrease of temperature among with ambient range. Moisture content is equally critical, as water availability directly influences microbial activity. High organic matter content and adequate porosity facilitate microbial respiration (Carbó *et al.,* 2021). Moisture during composting is traditionally assessed through gravimetric analysis or by the hand-squeeze (fist) test. In recent years, soil humidity sensors have been increasingly employed for rapid and accurate in situ moisture monitoring (Guo *et al.,* 2012).

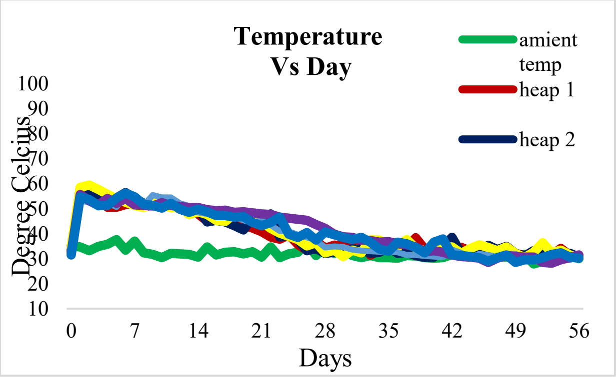

Figure 2: Temperature variation during composting period over 56 days with the different combination of compost heaps and ambient temperature

pH and Electrical Conductivity (EC)

The pH of the composting mixture plays a vital role in microbial activity. During the early stages of composting, microbial metabolism releases organic acids, lowering the pH (Chan *et al.,* 2016). As composting progresses, pH tends to shift from acidic to alkaline due to the bio-oxidation of organic matter and ammonia volatilization (Turan and Ergun, 2008). This increase can also result from the release of free NH_3 during organic matter degradation, as described by (Qian et al. 2014). Additionally, the nitrification process produces H^+ ions and NO_3^-, influencing pH fluctuations. In the present study, pH values across all treatments showed an initial acidic trend, followed by an alkaline shift as composting advanced. This pattern is consistent with the reported dynamics in other composting studies, where substrate decomposition and nitrogen transformations significantly influence pH evolution. Electrical conductivity (EC) reflects the concentration of soluble salts and is associated with the release of easily decomposable compounds into solution, serving as an indicator of compost suitability as a fertilizer (Vaverkova *et al.,* 2017). During aerobic composting, EC gradually decreased in all treatments, indicating microbial utilization of soluble salts. In EC values reduced from 1.58 to 1.28 mS/cm, while from the other heaps also gradually decreased from 1.54 to 1.14 mS/cm and 1.52 to 1.15 mS/cm, respectively. The decrease in EC during composting may also be due to ammonia volatilization and precipitation of mineral salts. All final EC values were within internationally accepted limits for agricultural application, confirming that the prepared composts are suitable for use as soil amendments.

Ash Content, Volatile Organic Compounds (VOCs), and Total Organic Carbon (TOC)

Ash content increased progressively during the composting process across all treatments, ranging from an initial 29.58% to 72.1% at maturity similarly from 30.12 % to 75.71%. This increase is consistent with the degradation of organic matter and the relative concentration of mineral residues, which also contributes to pH elevation and odour reduction. Volatile organic compounds (VOCs) were predominantly released during the initial composting stages as microorganisms decomposed easily degradable substrates. VOC emission rates are influenced by temperature, aeration, and feedstock characteristics. VOCs are embedded in the compost matrix and released upon wetting and heating. The results indicate that vegetable waste (VW) composting can emit hazardous VOCs, even when industrial or hazardous wastes are absent, as evidenced by emissions from VW components such as food scraps, mixed paper, and yard wastes. TOC decreased significantly in all treatments as composting progressed, reflecting the mineralization of carbon to CO_2. The highest TOC reduction occurred in Heap 3, likely due to its higher parthenium content, which provided abundant lignocellulosic material that served as a carbon source for microbial decomposers, especially actinomycetes. This observation aligns with reports of high cellulase activity in compost containing lignocellulosic feedstocks (Awasthi *et al.,* 2014).

C/N Ratio and Macronutrients (NPK)

The C/N ratio, a key indicator of compost maturity and microbial activity, declined steadily in all treatments. Initial values ranged from 26:1 to 22:1, falling to 10:1–11.5:1 by the end of composting, indicating well-stabilized compost. The ratio between three trails with control and 10,20,30,40, and 50 % C/N ratio of different proportion were noted on weekly basis. Figure .3 illustrate the progressive reduction in C/N ratio due to microbial degradation. The reduction is primarily due to carbon losses via microbial respiration, while nitrogen is conserved or moderately mineralized, resulting in a lower C/N ratio (Wang *et al.,* 2016). Ratios below 20 are widely accepted as indicators of compost maturity, and the values obtained in this study fall within the optimal range (8.8–19.8). Macronutrient concentrations (N, P, K) increased over time in all treatments, reflecting organic matter mineralization and nutrient concentration in the residual biomass. Nitrogen was increased from 1.53% initially to 2.97% at maturity, surpassing in all combination of heaps. Phosphorus and Potassium were also increased gro initial to final from 0.21% to 0.95%, and potassium increased from 0.32% to 0.97%, both higher than in the other treatments. This enhanced nutrient profile in Heap 4 is attributed to the combined effect of PLA and parthenium addition, which provided a favourable carbon-nitrogen balance and potentially stimulated microbial activity. Figure. a) Nitrogen is observed gradually increased due to mineralization. Phosphorus and Potassium also showed an increasing trend as illustrated in Figure. b) and c) Similar increases in NPK content during composting of vegetable waste blended with biopolymers and lignocellulosic plant matter have been reported by other studies, with nutrient enrichment linked to biomass loss and mineralization processes.

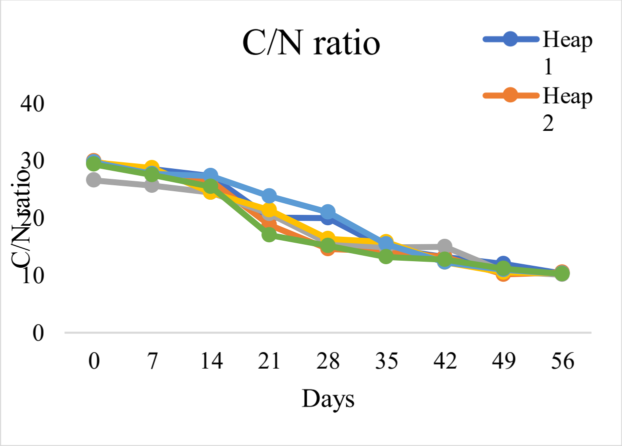

Figure .3. Variation of C/N ratio during 56 days of windrow composting process for the different combination of compost heaps from (1-6)

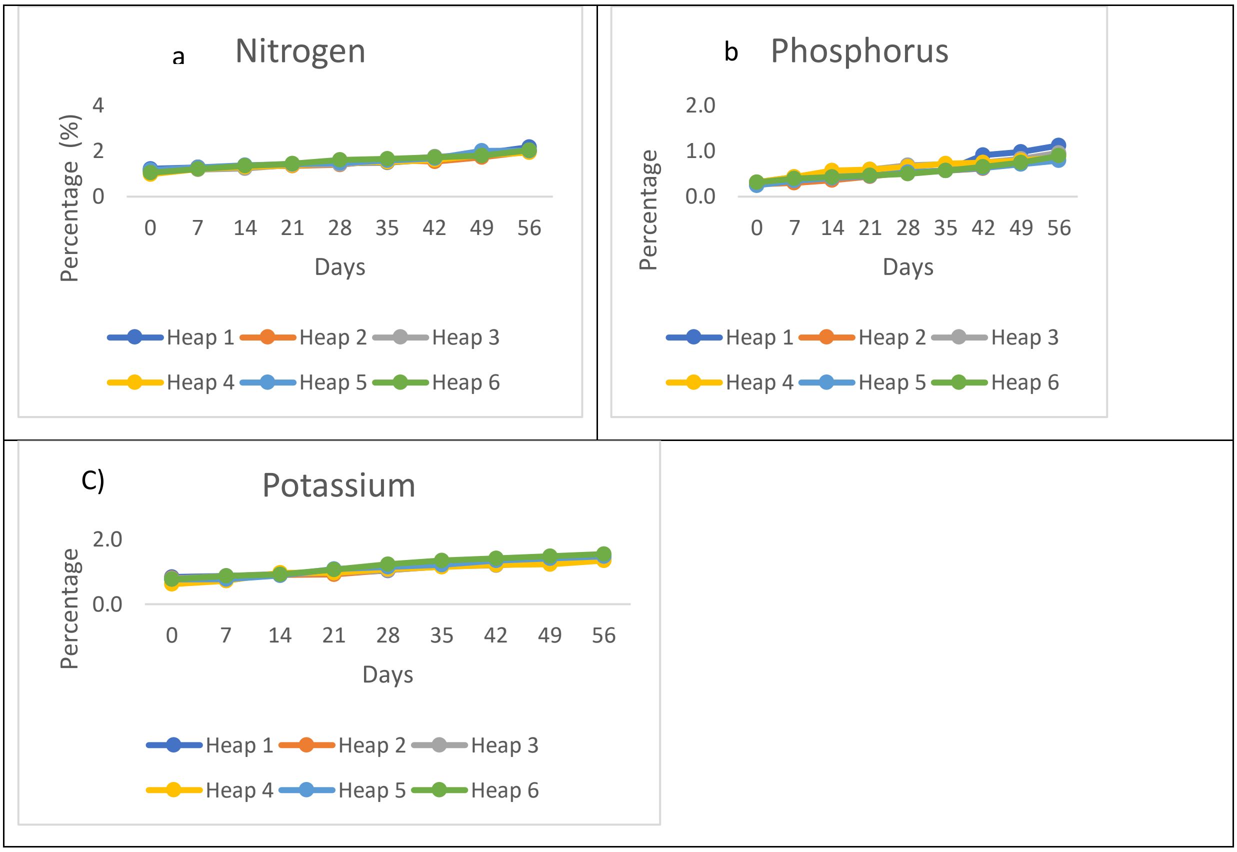

Figure .4.Temporal variation of Macronutrients during windrow composting of nitrogen, Phosphorous and potassium content in different compost heaps over 56 days.

Isolation and identification of microbes involved in biodegradation Process

All the samples were serially diluted and plated on Nutrient agar to isolate bacteria. The selected colonies were purified by the quadrant streaking method. The pure culture of a total of 6 isolates was inoculated on NA plates and incubated at 37ºC for 24 - 72 hours. After the incubation, pure cultures were obtained by repeated streaking. Figure.5 shows the different bacterial colonies Present in the 10^{-6} and 10^{-7} dilution taken from tubes.

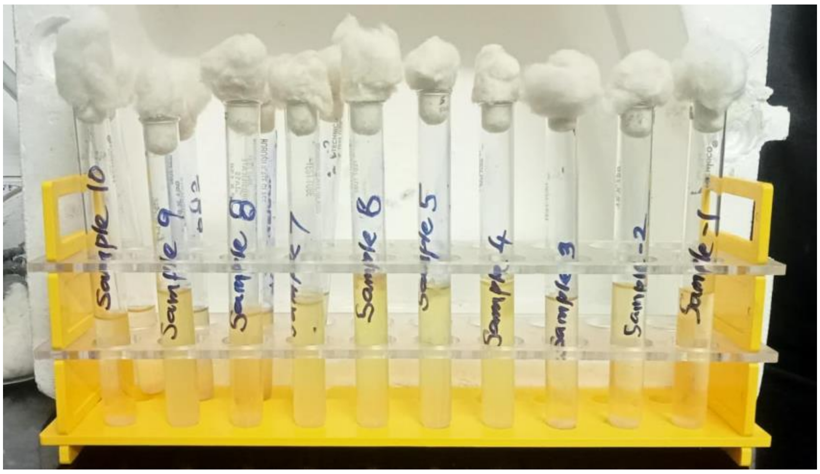

Figure 5.Serial Dilution tube method for isolating bacteria

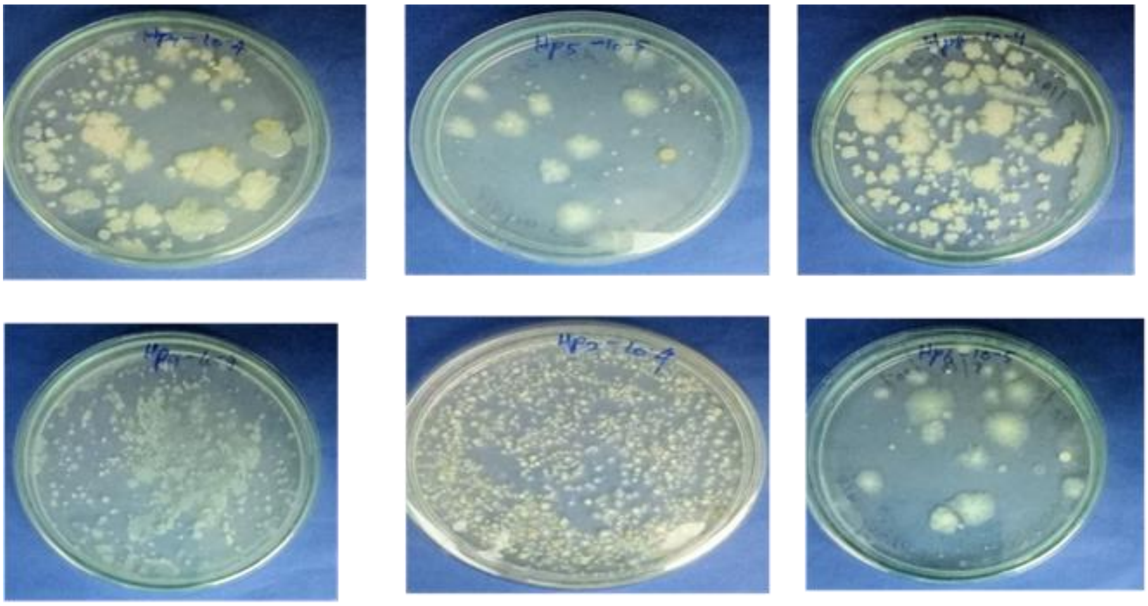

Figure.6. Spread Plate technique for isolating the microbes from compost Sample

Genus level identification

Based on biochemical screening, isolate 2 & 5 were selected for molecular identification due to their distinct and reproducible phenotypic profiles. Molecular characterization using 16S rRNA gene sequencing remains the gold standard for bacterial identification, owing to the conserved nature of the gene interspersed with hypervariable regions that allow taxonomic discrimination (Janda & Abbott, 2007). Phylogenetic analysis of isolate Isolate-2 revealed closest similarity to *Aeromonas caviae*, with a sequence similarity of 86.97%. Although this similarity value is below the commonly accepted species-level threshold of ~98.7%, it strongly suggests affiliation with the genus *Aeromonas*, indicating that may represent a divergent strain or potentially a novel taxon within this genus (Stackebrandt, 2006).

All the isolates exhibited distinct biochemical reactions. The isolates were biochemically characterized using Gram's staining, Motility Test, Spore staining, Catalase Test, Oxidase test, MR, VP, and Colony Morphology color. Table 1 shows the distinct biochemical profiles among the isolates, enabling their preliminary identification and differentiation. Figure 7 results are determined the gram staining results, The Isolate-2 and Isolate-5 both are shows the Gram-negative rod-shaped Bacilli bacteria observed under the light microscopy after the staining Procedure. Figure 8.Shows the Pure Culture Plates of *Aeromonas caviae & Enterobacter hormaehei.*

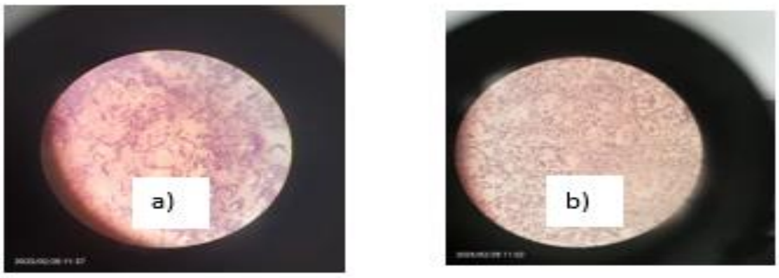

Figure.7. Gram Staining observed 100X View (Isolate 2 & Isolate 5) showing distinct cell morphology and gram reaction characteristics

Table 2: Identification of Microbes by Genus level

Samples	Gram's staining	Motility test	Spore staining	Catalase test	Oxidase test	MR	VP	Colony Morphology Colour
Isolate-1	+ve rod	Motile	Spore forming	+ve	Variable	Variable	+ve	White cream
Isolate-2	-ve rod	Motile	Non-spore-forming	+ve	-ve	-ve	+ve	White to orange
Isolate-3	-ve rod	Non motile	Non-spore-forming	+ve	-ve	-ve	+ve	Creamy white
Isolate-4	+ve cocci	Non motile	Non-spore-forming	+ve	-ve	+ve	+ve	White to golden yellow
Isolate-5	-ve rod	Motile	Non spore forming	+ve	+ve	-ve	+ve	Greyish White

Molecular Identification of Potential Strains

Identification of the isolates 2 and 5 carried out by sequencing the variable regions of the 16S rRNA gene.

The obtained sequences and their similarity percentages are summarized in Table 3, and the evolutionary relationships are illustrated in the phylogenetic tree (Figures 9 & Figure 10).

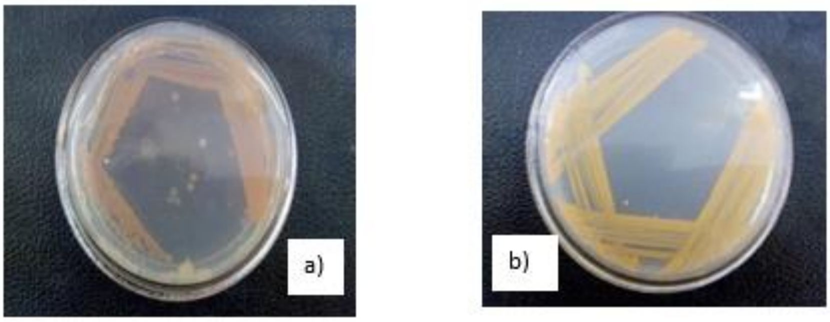

Fig.8. Pure culture Plate of a)*Aeromonas caviae & b)Enterobacter hormaehei* illustrating colony morphology growth characteristics and isolation under laboratory conditions

Table 3: Sequence similarity of the isolates HP1E, HP2B

Isolates Codes	Sequence Similarity in %	Accession Number
Isolate-2 (*Aeromonas hydrophila*)	**86.97**	OM877013.1
Isolate-5 (*Enterobacter hormaehei*)	**99.73**	CP039384.1

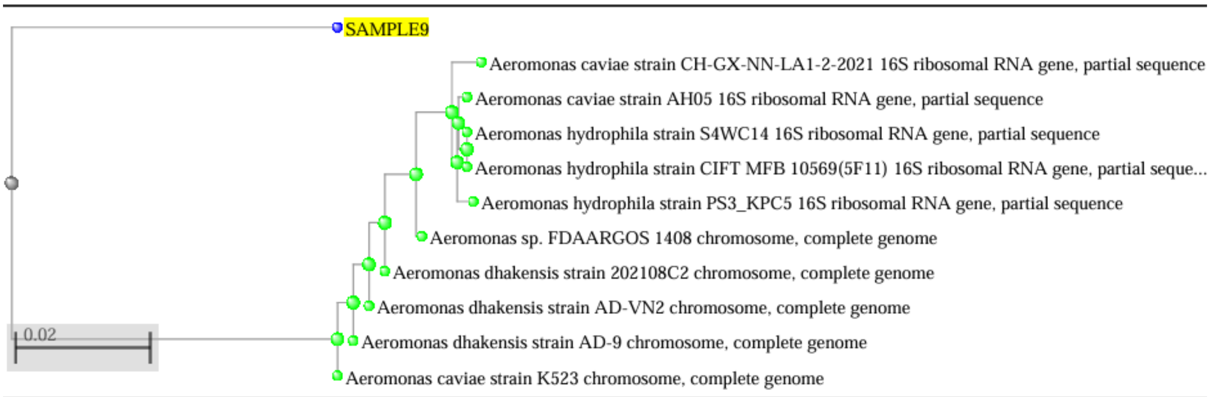

Figure 9. Phylogenetic tree of isolate 2. The phylogenetic tree analysis showed the isolate showed sequence similarity with *Aeromonas caviae* (86.97 %). The sequenced 16S rRNA gene sequence was submitted to NCBI Genbank, and the Accession number for the sequence was retrieved (OM877013.1).

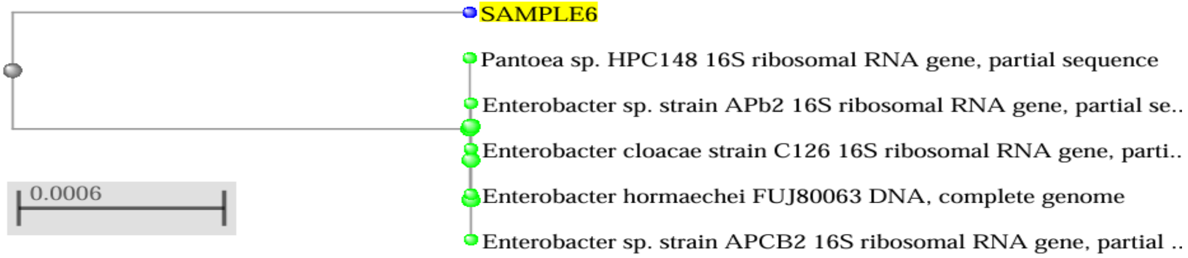

Figure 10. Phylogenetic tree of isolate 5. The phylogenetic tree analysis showed the isolate showed sequence similarity with *Enterobacter hormaehei* (99.73%). The sequenced 16S rRNA gene sequence was submitted to NCBI Genbank, and the Accession number for the sequence was retrieved (CP039384.1).

Discussion

The present study systematically investigated cultivable bacterial diversity through culture-dependent isolation, biochemical characterization, and molecular identification using 16S rRNA gene sequencing. The successful isolation of 6 bacterial strains following serial dilution and plating on nutrient agar demonstrates the effectiveness of conventional microbiological techniques in recovering diverse heterotrophic bacterial populations. Nutrient agar, a non-selective medium, is widely employed for primary isolation as it supports the growth of a broad spectrum of bacteria without imposing selective pressure, thereby allowing a representative recovery of cultivable microorganisms (Atlas, 2010; Madigan *et al.,* 2021). Incubation at 37 °C for 24–72 hours favored the growth of mesophilic bacteria, which constitute a dominant fraction of environmental and host-associated microbial communities. The variation in incubation duration ensured the recovery of both fast and slow growing organisms, highlighting the importance of extended incubation periods for comprehensive bacterial isolation (Tortora *et al.,* 2024). The repeated quadrant streaking method proved effective in obtaining pure cultures by progressively diluting the bacterial load on the agar surface, thereby minimizing contamination and ensuring genetic homogeneity of the isolates. The maintenance of pure cultures on nutrient agar slants at 4 °C is a standard short-term preservation strategy that retains bacterial viability and phenotypic stability for subsequent analyses (Cappuccino & Welsh, 2017). Biochemical characterization revealed marked diversity among the isolates, as evidenced by variations in Gram reaction, motility, spore formation, enzymatic activities, and carbohydrate metabolism patterns. The predominance of Gram-negative rods among the isolates aligns with previous reports indicating their widespread distribution in diverse ecological niches due to their metabolic versatility and adaptive capabilities (Shoaib *et al.,* 2020). The presence of Gram-positive rods, particularly spore-forming isolates, suggests the

occurrence of *Bacillus* like organisms, which are well known for their ability to withstand adverse environmental conditions through endospore formation (Madigan *et al.,* 2021). Catalase positivity observed across all isolates indicates the ability to detoxify reactive oxygen species, a characteristic commonly associated with aerobic and facultative anaerobic bacteria. Oxidase test variability further differentiated the isolates, with oxidase-positive strains suggesting the presence of cytochrome c oxidase, a feature typical of genera such as *Aeromonas* and *Pseudomonas* (Bergey, 1994). Methyl Red (MR) and Voges–Proskauer (VP) test results revealed distinct fermentation pathways among the isolates, reflecting metabolic diversity and enabling preliminary differentiation at the genus level. Colony morphology and pigmentation, including creamy-white, yellow, and green pigmented colonies, provided additional phenotypic markers that supported biochemical differentiation. Pigment production, particularly green pigmentation, is often associated with secondary metabolites that may confer ecological advantages such as antimicrobial activity (Atlas, 2010). Based on biochemical screening, isolates HP1E and HP2B were selected for molecular identification due to their distinct and reproducible phenotypic profiles. Molecular characterization using 16S rRNA gene sequencing remains the gold standard for bacterial identification, owing to the conserved nature of the gene interspersed with hypervariable regions that allow taxonomic discrimination (Janda & Abbott, 2007). Phylogenetic analysis of isolate Sample A revealed closest similarity to *Aeromonas caviae*, with a sequence similarity of 86.97%. Although this similarity value is below the commonly accepted species-level threshold of ~98.7%, it strongly suggests affiliation with the genus *Aeromonas*, indicating that Sample A may represent a divergent strain or potentially a novel taxon within this genus (Stackebrandt, 2006). Members of the genus *Aeromonas* are ubiquitous aquatic bacteria known for their ecological significance and biotechnological potential, as well as their role as opportunistic pathogens. The identification of an *Aeromonas* related isolate underscores the ecological adaptability of this genus and its ability to thrive under diverse environmental conditions (Parker & Shaw, 2011). The deposition of the 16S rRNA gene sequence in the NCBI GenBank database (Accession No. OM877013.1) ensures data transparency and facilitates future comparative and phylogenetic studies. In contrast, isolate Sample B exhibited a high sequence similarity of 99.73% with *Enterobacter hormaehei*, confirming its taxonomic identity at the species level. The genus *Enterobacter* comprises facultative anaerobic bacteria commonly found in soil, water, and the intestinal tract of humans and animals. *E. hormaehei* is recognized for its metabolic versatility and environmental resilience, enabling survival in diverse habitats (Yeh *et al.* 2022). The strong phylogenetic clustering of Sample B with reference *E. hormaehei* strains further validates the accuracy of biochemical and molecular identification approaches used in this study. The sequence submission under Accession No. CP039384.1 adds to the growing molecular database of this clinically and environmentally relevant species.

Conclusion

The present investigation demonstrates that windrow co-composting of invasive weed biomass with vegetable waste, cow dung, and PLA biopolymer is an efficient and environmentally sustainable strategy for converting problematic organic residues into nutrient-rich compost. All composting treatments exhibited a clear thermophilic phase followed by stabilization, confirming active microbial degradation and effective process performance. The progressive decline in TOC and C/N ratio, together with increases in N, P, and K concentrations, indicates successful compost maturation and nutrient enrichment suitable for agricultural use. Among the tested mixtures, moderate PLA incorporation (20–30%) combined with Parthenium biomass yielded superior compost quality, with enhanced temperature profiles, accelerated biodegradation, and higher macronutrient content. Final EC values remained within permissible limits for soil application, indicating low salinity risk. The isolation and molecular identification of dominant composting bacteria, including species affiliated with *Aeromonas caviae* and *Enterobacter hormaechei*, confirm the role of diverse heterotrophic microbial communities in organic matter mineralization and biopolymer degradation. Overall, this study provides a practical framework for integrating invasive weed management with biopolymer waste recycling through windrow composting. The approach supports circular economy principles by reducing environmental pollution, mitigating invasive plant biomass accumulation, and producing value-added

organic fertilizer. The findings highlight the potential for large-scale adoption in municipal and agricultural waste management systems, contributing to sustainable soil fertility enhancement and ecosystem

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