Performance Evaluation of a Novel Two-Stage Biodegradation Technique for Composting of Diverse Biodegradable Wastes: Batch-Scale to Large-Scale Application

A thesis submitted

In partial fulfillment of the requirement for the degree of

Doctor of Philosophy

By

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Certificate

This is to certify that the thesis entitled "**Performance Evaluation of a Novel Two-Stage Biodegradation Technique for Composting of Diverse Biodegradable Wastes: Batch-Scale to Large-Scale Application**" submitted by Suryateja Pottipati (186104104), a Research Scholar in the Department of Civil Engineering, Indian Institute of Technology Guwahati, for the award of the degree of Doctor of Philosophy, is a record of an original research work carried out by him under my supervision and guidance. The thesis has fulfilled all requirements as per the regulations of the institute and, in my opinion, has reached the standard needed for submission. The results embodied in this thesis have not been submitted to any other University or Institute for the award of any degree or diploma.

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Dr. Ajay Kalamdhad

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ABSTRACT

A significant escalation of contamination hazards arises from environmentally incompatible treatment strategies for biodegradable organic waste. Detrimental environmental repercussions arise from the estimated 2 billion tons of organic waste generated annually worldwide, which are predisposed to accumulation and unregulated decomposition. The high-water content of organic wastes in particular (among other constraints) impedes existing treatment methods, with ensuing management hurdles, cost amplification, and resource depletion. A novel rapid treatment and recycling method is proposed in the present study to overcome impediments, involving rotary drum in-vessel composting, conversion of the resultant phyto-mass into organic fertilizers by vermicomposting, and final assessment of the vermicompost as a viable soil conditioner.

Organic waste feedstocks were first treated with various bulking agents prior to rotary drum in-vessel composting. This procedure accelerated the physiological thermophilic phase to less than 24 h. The same was maintained for 7 to 8 days, with optimisation of physicochemical parameters such as C/N ratio, pH, and moisture content. The thermophilic phase deconstructed considerable proportions of organic waste and simultaneously quickened the rate of mineralisation by the in situ native bacterial population. The final product of rotary drum composting, though nutrient-rich, fell short of being equivalent to vermicompost. Consequently, vermicomposting was adopted, driven primarily by *Eisenia fetida*, resulting in vermicompost with inherent high porosity, adsorption sites, and nutritional properties. The beneficial qualities of this organic fertiliser were evinced by improved soil fertility, augmented nutritional content, and lowered toxicity levels. Although the mandatory 15- to 20-day adaptation of vermiculture to fresh waste does seem to handicap the dependence on vermicomposting for fresh urban waste management,

Unfortunately, the individual capacities of the rotary drum composting and vermicomposting methods are not equipped to process large volumes or shorten the composting duration without affecting the final product quality. The magnitude of organic waste available for processing may have evinced from 2011, when one-third of world food production was wasted, or from 2019–2020 in India, when 99.07 and 191.77 million metric tons of fruit and vegetables, respectively, were produced. Thus, the primary objective of the current study was to evaluate the optimal composting period by adopting the best practices of rotary drum composting and vermicomposting.

Phase I of the study focused on assessing the effectiveness of the two-stage biodegradation process by testing a 550-liter rotary drum composter in addition to three 3-liter vermicomposters on diverse organic waste produced in the community. Its success was evident in attaining the shortest biodegradation period (27 days) with the selected earthworm species, *E. fetida*, in biodegrading the wastes. The total nitrogen in the end products of vegetable waste and sewage sludge was more than 4% and in weeds more than 3%. Further, the tested combined biodegradation process was found to be reliable in stabilizing sewage sludge quickly, vegetable waste and biodegrading tough biomasses like terrestrial and aquatic weeds.

In **Phase II** of the study, the vermicompost produced from Phase I was subjected to phytotoxicity tests, using pot studies to assess its safety as a soil conditioner before soil application. The seed germination index of the vermicompost produced through two-stage biodegradation was significantly more than 95%, which categorically endorses the conversion of the toxic terrestrial weed-based vermicompost into a nontoxic soil conditioner. In addition, the healthy growth of *Coriandrum sativum* provided additional support for the safety of the bioproduct. The results of our study, indicating that two-stage biodegradation was efficient in managing diverse organic wastes, are among the very few studies on pilot-scale biodegradation processes to be documented.

In **Phase III**, to understand the scale shift effect of the proven biodegradation process, a pilot-scale rotary drum composter (5000 L) and two different vermicomposting techniques, namely bag vermicomposting (3000 L) and stack vermicomposting (3000 L), were used. Challenging substrates like vegetable waste and *Eichhornia crassipes* (water hyacinth) were chosen to test the technique on a pilot scale, applying mono-substrate and co-composting. Waste was collected from vegetable markets and nearby water bodies; 250kg per day was fed to the pilot-scale reactor for 3 months for each substrate, i.e., vegetable waste, *E. crassipes*, and a combination of both wastes. Dry leaves were used to control moisture and as a potential carbon source. The reactor was commissioned using a substrate and cow dung mixture for 10 days before being subjected to a substrate and dry leaf mixture. The pilot-scale study demonstrated that the thermophilic peaks recorded were higher than the batch scale; 75°C during vegetable waste biodegradation and 63°C during *E. crassipes* biodegradation. The proven biodegradation was working much more effectively on a large scale, and the end product contained the desired levels of nutritional parameters (4.2% total nitrogen in vegetable waste biodegradation). During the study, the stack vermicomposting method was more efficient regarding land requirements and ease of work. The large-scale study demonstrated a 70% reduction in waste volume and a definite nutrient-rich vermicompost. A final in-situ field evaluation was conducted to confirm the nutrient-rich and approved safety parameters of the vermicompost generated by both methods.

In **Phase IV** of the study, 12 test areas measuring 1.5 × 2 m^2 were prepared on the IITG campus premises to evaluate vermicompost amendment on soil in natural conditions. The study involved three different plant models: okra, or *Abelmoscus esculentus* (an on-ground fruiting plant), radish, or *Raphanus sativus* (an underground fruit plant), and cilantro, or *Coriandrum sativum* (a leafy vegetable). An application of 10 tons per hectare was carried out, and the study was conducted for 100 days. The study demonstrated that the increase in soil organic carbon was due to the amendment. The growth of the plants was healthy compared to the control soil. The yield was also 3 times higher than that of soil without vermicompost amendment. It is noteworthy that the vermicompost generated through aquatic weed, *E. crassipes*, was remarkable in terms of all the vegetables growth and yield. Consequently, the study presented in this thesis has the potential to alter waste management scenarios in India and other nations across the globe.

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Chapter-1

INTRODUCTION

1.1 OVERVIEW

The current annual growth rates endorse the unprecedented surge in the global population and highlight the immense challenges affected by rapid urbanization and technological advancements. The capacity to simultaneously undertake and accomplish numerous objectives across domains has given rise to extraordinary challenges for the biosphere. The generation of vast quantities of waste materials in particular has emerged as a significant and crucial domain of management that can no longer be relegated to the backburner. According to Shah et al. (2022), swift urbanization is expected to result in a substantial increase in the generation of organic solid waste, threatening both sustainable development and the conservation of natural resources. The issue of waste disposal has become more complex due to the concurrent rise in population and improved living standards, as noted by Gupta et al. (2021). Organic waste has already touched approximately 46% of the total solid waste generated worldwide, or 2.01 billion metric tons, less than a decade ago in 2016 (Chavan et al., 2022).

According to the Food and Agriculture Organization (FAO, 2019), the worldwide post-harvest loss of fruits and vegetables has been documented to attain a maximum of 20%. This percentage is surpassed only by root, tuber, and oil-producing crops, which have a waste percentage of 25%. The rise in global population growth and the instability of supply chains have resulted in growing concerns about waste generation on a global level, as noted by Ganesh et al. (2022). The production of fruits and vegetables in India has exhibited a steady upward trend over the course of several decades, as evidenced by 88,977,000 MT/year in 2013–14 and 97,358,000 MT/year in 2018–19, Srivastava and Balakrishnan (2022). In urban settings, sewage sludge forms a significant byproduct of wastewater treatment processes, thus posing a significant threat to pollution and human health (Zhang et al., 2022). Prior to being disposed of on land, building materials, landfills, or incinerated, sewage sludge must undergo stabilization and reduction of its inherent pollution-causing constituents (Christensen et al., 2015). According to Gong et al. (2022), the disposal of sludge can potentially result in the accumulation and amplification of heavy metals (HMs) in the food supply chain, engendering health risks and carcinogenic effects, even in environments with naturally low levels of HMs.

Weeds form major sources of organic waste powered by their occupation of diverse physiological niches, infiltration, and dominance of novel habitats (Kleunen et al., 2010). Invasive weeds are the most dangerous as they establish dominance by effecting structural and compositional changes in the ecosystem and altering ecological processes (Gaertner et al., 2014). The evolutionary adaptability of weeds equips them to withstand wide-ranging exposure, invest in spore union and compelled migration, and successfully integrate into non-native environments (Gusain and Suthar, 2020). 21% of the total plant species are considered invasive in India, including 17 species categorized as the world's worst invasive weeds. Among the terrestrial weeds that have spread to 45 nations across the globe, *Ageratum conyzoides* (chickweed, billygoat-weed, goatweed, mantras, and whiteweed), *Mikania micrantha* Kunth (bitter vine, American rope, climbing hemp vine), *Parthenium hysterophorus*, and *Lantana camara* are considered major toxic herbaceous weeds of concern (Bajwa et al., 2016; Devi and Khwairakpam, 2020). *A. conyzoides*, is a branching annual tropical plant found predominantly in Africa, Asia, and South America; and especially abundant in agrarian dependant economies like India and China (Olowofolahan and Olorunsogo, 2021, Devi and Khwairakpam, 2020b). *M. micrantha*, native to South and Central America is considered a significant weed by the indegenous population (Kauser et al., 2022). The *M. micrantha* invasiveness is sparked by phytochemicals or allelochemicals that act as a powerful weapon for the biological invasion (Ma et al., 2020). Within a mere six weeks of germination, weeds like Parthenium show rapid growth, blooming, and reproduction (Cowie et al., 2022). The ability to deplete soil nutrients, and short circuit essential nutrient pathway to economically important plants makes an undesirable competitor (Oyewusi and Osunbitan, 2021). In addition, a substantial portion of their biomass is lignocellulosic containing allelochemicals, which critically impact detoxification and biodegradation processes (Anwar et al., 2021; Huang et al., 2010; Tuomela et al., 2000). Furthermore, their ability to hyperaccumulate heavy metals, could become a potent source of toxicity in the post biodegradation end product (Maturi et al., 2021). Chemical fertilizers have been employed to reduce this weed. However, this has resulted in significant ecological difficulties (Aryal et al., 2018; Devi and Khwairakpam, 2020).

Eichhornia crassipes, and *Hydrilla verticillata* (water thyme) dominate the list of other native plant species designated as significant and noxious among weeds documented by (Florêncio et al., 2021). *E. crassipes* poses a global hazard to marine systems because of its thick mat-like structure, outbreak within days of introduction, the susequent alteration in ecology, and reduction in biodiversity (Jawed et al., 2022, Ganorkar et al., 2022).The excessive expansion of *E. crassipes* produces economic catastrophes by diminishing fish populations, impacting other flora and fauna, obstructing rivers, and impeding irrigational operations (Ganorkar et al., 2022). Several biological, physical, and chemical approaches for controlling and eradicating *E. crassipes* have been tested, but none of these tactics has shown to be a lasting answer for weed management (Sindhu et al., 2017). The efforts have often proved expensive and labour intensive. Though, the utility of *E. crassipes* in phytoremidiation is a positive contribution via its inherent ability to hyperaccumulate heavy metals (Du et al.,2020). *H. verticillata*, an invasive aquatic weed, is particularly damaging due to its adaptability and competitive nature in its habitat (Shrivastava and Srivastava, 2021). The thick mat formation resulting from excessive growth reduces the dissolved oxygen in the water, thus affecting aquatic life (Shi et al., 2021). As an annual plant, the invasion process occurs year-round (Jain and Kalamdhad, 2018a). Physical, chemical, and biological treatments have been suggested by many authors to control *H. verticillata* growth (Jain and Kalamdhad, 2018b), but an efficient management technique is still under investigation.

1.2 COMPOSTING AND VERMICOMPOSTING IN BIODEGRADABLE WASTE MANAGEMENT

According to the literature aerobic biodegradation techniques are the most efficient in addressing the issue of toxic weeds (Blossey et al., 1994; Saha et al., 2017). Aerobic biodegradation can be accomplished using various techniques: windrow composting (Lopes et al., 2021), pile composting (Hernández-Lara et al., 2022), rotary drum composting (Kauser et al., 2020) and vermicomposting (Devi and Khwairakpam, 2020a). The annual estimated 2 billion tonnes of organic waste escalate global contamination hazards through environmentally incompatible treatment strategies (Thygesen et al., 2021). Consequently, the acknowledged benefits of composting are adopted to transform a variety of organic solid waste materials into humus-like compounds or compost through microbial activity (Wan et al., 2020). The resultant compost is an organic fertilizer that improves soil fertility and nutritional content (carbon, nitrogen, phosphorus, and sulfur), in addition to aiding the detoxification of polluted soil (Gandolfi et al., 2010). Urban organic wastes which are less than amenable to traditional time-consuming composting processes are treated with various bulking agents to reduce the duration of composting and by varying feedstock physicochemical parameters such as C/N ratio, pH, and moisture content (Liu et al., 2020). Among all the available techniques, biodegradation using a rotary drum composter may be accomplished in 20 days, resulting in a stable end product (Jain and Kalamdhad, 2019; Kauser et al., 2020). Since the thermophilic phase in the rotary drum composter may be achieved physiologically in less than 24 hours (Kalamdhad et al., 2009), these established thermophilic temperatures accelerate the substantial degradation of organic matter. Furthermore, the shift from thermophilic to mesophilic temperatures typically takes seven to eight days. The subsequent 13 days of biodegradation are required for cooling and stabilization of the feedstock in the reactor. Degradation in this phase was significantly less compared to the initial seven days. Rotary drum in-vessel composting (RDC) is an optimized and accelerated composting technique (Kalamdhad et al., 2009) that attains the physiological thermophilic phase in less than 24 hours and maintains it for 7 to 8 days (Kauser et al., 2020). The thermophilic phase deconstructs considerable proportions of organic waste, and simultaneously provokes the native bacterial population to mineralize organic materials at an enhanced rate (Kong et al., 2020). The final product of rotary drum composting, though nutrient-rich, falls short of being equivalent to vermicompost.

Vermicomposting is an alternative and more efficient method to degrade phyto mass into organic fertilizers (Mago et al., 2021). The resultant vermicompost, with its inherent high porosity, adsorption sites, and nutritional properties, is considered an excellent quality compost (Ravindran et al., 2021). Vermicompost can be produced by employing a variety of vermicultures, the most popular being *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus*. The mandatory requirement of an initial 15 to the 20-day period for adaptation of vermiculture to fresh waste, retards the vermicomposting-based processes for fresh urban waste management (Fernández-Gómez et al., 2010; Mago et al., 2021). The potential quantity of waste available for composting is evinced by wastage of one-third of world food production in 2011; and the potential for organic waste production from harvest of 99.07 and 191.77 million metric tons of fruit and vegetables respectively in 2019-2020 in India (Rena et al., 2020). Biodegradation of this humungous organic waste poses serious challenges that are beyond the individual capacities of the rotary drum composting and vermicomposting methods, with particular reference to the volume of waste to be handled and the time required to compost without affecting the final product quality (Hazarika and Khwairakpam, 2018; Jain and Kalamdhad, 2018a; Kalamdhad et al., 2009; Kauser et al., 2020; Sharma et al., 2018; Singh et al., 2012; Varma and Kalamdhad, 2016). It is also a well-known fact that in terms of the product's quality, the vermicomposting technique is the best option for producing nutrient-rich compost backed by the vital role of the earthworms (Varma et al., 2016). The combination of earthworms and microbes, accelerate the decomposition of organic waste, increase the circulation of carbon, nitrogen, and phosphorus, and enhance soil fertility (detritus-bound nutrient cycle, soil physical property release, and amelioration) (Devi and Khwairakpam, 2020b). Vermicomposting also contributes substantially to degradation by fine milling the substrates, resulting in enhanced porosity and surface area for microbial activity and this time-consuming procedure takes at least 45 days (Biruntha et al., 2020; Deepthi et al., 2021). Several studies have utilized vermicomposting as a maturation technique for predegraded organic wastes, and found that the partly degraded material was significant in reducing the degradation period with enhanced product quality (Kalamdhad et al., 2009; Varma et al., 2016; Varma and Kalamdhad, 2016). In addition, vermicomposting addresses the problems associated with heavy metal contamination. The heavy metal bioaccumulation of earthworms has been widely studied to determine whether they can be used as bioindicators of soil contamination and evaluate metal bioavailability and toxicity (Liu et al., 2005). During vermicomposting, earthworms accumulate heavy metals from the substrate and change the availability of metals in the soil by altering their characteristics and speciation distribution (Sun et al., 2020).

Numerous investigations have been conducted on composting, but most of them were done in batch mode, and there is no documentation of the technique's use in continuous or fed-batch operation. However, a study by Singh et al. (2009) showed that using demonstration-scale in-vessel rotary drum composting had a significant effect on the biodegradation of a mixture of vegetable waste, cow dung, and tree leaves. Industrial biodegradation of organic waste poses significant challenges due to the variety of feedstock materials, insufficient initial conditions, and unsuitable mixing methods (Estrella-González et al., 2020). The operation of the reactor also plays a crucial role in rotary drum composting at an industrial scale (Kalamdhad and Kazmi, 2009). Waste management facilities typically use windrow composting for mixed waste, producing a low-quality organic residue that is polluted with non-biodegradable materials, such as glass and plastic, posing a hazardous threat to the environment and public health (Wei et al., 2021;Mu et al., 2017). Large composting systems that process millions of tonnes of waste everyday can significantly impact the environment and public health if their sustainability is not considered (Makan and Fadili, 2020). However, most countries lack sufficient technology, infrastructure, and procedures for organic waste management and composting, necessitating regular amendment of the technologies used.

1.3 THESIS ORGANIZATION

The thesis organization is as follows:

Chapter 1 discusses global waste management trends, particularly for organic waste, and highlights the need for sustainable waste management practices in communities worldwide, including India. The chapter explores composting and vermicomposting as waste treatment options and their limitations in biodegradation. The scale of the study and the need for more efficient organic waste management techniques are also examined. Overall, Chapter 1 provides a comprehensive analysis of the context and motivation behind the research and sets the stage for the rest of the study.

Chapter 2 provides an in-depth review of the literature about organic solid waste. Specifically, it covers the challenges that arise when such waste is not managed effectively, the various strategies that have been implemented to address these challenges, the scaling up of in-vessel biodegradation processes, the potential for shortening the duration of such processes, the application of compost and vermicompost in pot studies and field studies, and the existing gaps in knowledge on this topic.

Chapter 3 serves as an introduction to the study's objectives, discussing the need for the research and its scope. The chapter outlines the specific goals of the study and the rationale behind them, highlighting the importance of addressing the challenges of organic waste management. By clearly defining the study's objectives and scope, Chapter 3 provides readers with a roadmap for the research, helping them understand the focus and direction of the investigation. This chapter is crucial for setting up the context and significance of the study, and it provides a solid foundation for the subsequent chapters

Chapter 4 presents a comprehensive overview of the experimental flowchart for each phase of this study, including the experimental setup employed for batch-scale and large-scale studies. Additionally, it discusses the phytotoxicity study that was conducted on the end products, as well as the metagenome sequencing procedures that were adopted. The chapter also delves into the procedures adopted for the field application of the produced vermicompost, along with the various physicochemical, biological, biochemical, and elemental parameters used to evaluate the quality of the waste at various stages of the biodegradation process.

Chapter 5 presents the experimental results obtained from the phase I study, which focused on analyzing various organic wastes in a batch-scale experimental setup. The results obtained from this study provide valuable insights into the characteristics and behavior of the organic wastes studied and serve as a foundation for the subsequent phases of the study. By analyzing the experimental results, gain a deeper understanding of the composition and properties of the organic wastes, and develop effective strategies for their management and conversion into value-added products.

Chapter 6 presents the results and discussions of Phase II, which involve the toxicity assessment of the compost produced through the biodegradation technique of various organic wastes discussed in Phase I. Additionally, the efficacy of the compost as a soil conditioner is evaluated through a lab-scale pot study, which utilizes various combinations of soil and the produced vermicompost. The chapter provides detailed insights into the effectiveness of compost as a soil conditioner and its impact on the growth and health of plants. By analyzing the results and discussions presented in this chapter, valuable insights are gained into the potential applications of compost as a sustainable solution for organic waste management and soil improvement.

Chapter 7 describes the results and discussions of phase III, which involved a study of the biodegradation technique studied in phase I at a larger reactor scale. The chapter presents detailed insights into the performance and efficiency of the biodegradation process and evaluates its potential for large-scale organic waste management. The metagenome study conducted at various stages of the large-scale biodegradation process is also discussed, providing a deeper understanding of the microbial communities involved in the process. The changes observed in the overall bulk of the waste are analyzed, and their implications for the potential applications of the biodegradation process are discussed. This chapter provides valuable insights into the scalability and effectiveness of the biodegradation technique and its potential for sustainable waste management.

Chapter 8 presents the results and discussions of phase IV, which involves the application of the vermicompost produced through the large-scale operation to soil for safety evaluation. Additionally, the chapter evaluates the impact of the vermicompost on plant morphology and changes in soil characteristics. Through an in-depth analysis of the experimental results, this chapter provides valuable insights into the potential applications of the vermicompost as a sustainable solution for soil improvement and organic waste management. The safety evaluation of the vermicompost provides important information about its potential impact on the environment and human health, while the analysis of changes in plant morphology and soil characteristics offers insights into the effectiveness of the vermicompost as a soil conditioner. By analyzing the results and discussions presented in this chapter, researchers and practitioners can gain a deeper understanding of the potential applications and limitations of the vermicompost produced through the large-scale operation, and its potential for sustainable waste management and soil improvement.

Chapter 9 gives the potential conclusions of the research work presented in this study and provides a comprehensive list of the overall conclusions that have been drawn from the study. It also outlines the future scope of research that can be undertaken based on the findings of this study. Through an in-depth analysis of the experimental results and data, the conclusions drawn in this chapter offer valuable insights into the potential applications and benefits of the research work and the areas that require further exploration and investigation. This chapter serves as a vital reference point for researchers and practitioners and provides a roadmap for future research.

Chapter 2

LITERATURE SURVEY

The present chapter is a comprehensive review of the extant literature on the efficacy vermicomposting of various biodegradable wastes using both laboratory and industrial-scale rotary drum composting, upscaling the reactor-based methodology and its potential in biodegradable organic waste management. The inadequate handling of organic waste across different regions, with a particular focus on India, control measures implemented to address these challenges by utilising organic waste feedstock and production of value-added bioproducts form vital segments of the literature review. The potential benefits of reactor-based versus the current composting techniques, optimisation of biodegradation techniques, and utilisation of compost toxicity tests for plant development have been cogently discussed.

2.1 BIODEGRADABLE ORGANIC WASTE

Biodegradable wastes encompass all organic matter present in waste that can decompose into carbon dioxide, water, methane, basic organic molecules, compost, and humus, facilitated by microorganisms and other living entities. The process of decomposition is aided by composting, aerobic digestion, anaerobic digestion, or analogous mechanisms. The present study primarily encompasses organic waste generated from culinary activities (viz. decomposed food, clippings, and non-consumable constituents), residue from combustion, earth, excrement, and additional botanical substances. In the domain of waste management, the purview extends to certain inorganic materials that are amenable to biodegradation by bacterial agents.

2.1.1 Major urban organic wastes

Food waste, including vegetable waste, is a significant contributor to the overall burden of waste. The United Nations Food and Agriculture Organization (FAO) (The State of Food Security and Nutrition in the World, 2022) estimates that approximately one-third of global food production is lost or wasted annually. In India, the second largest producer of fruits and vegetables worldwide, a substantial proportion of this produce, ranging from 18% to 58%, goes to waste. India's population, accounting for around 17.31% of the world's population, is expected to exceed 1.53 billion by 2030 due to rapid industrialisation, urbanisation, and population growth. This has generated a significant increase in municipal solid waste (MSW) production, with urban areas in India generating a staggering 68.8 million tonnes of MSW per year, equivalent to 500 grams per person per day. The composition of MSW in India differs from that of western countries, with a larger proportion of organic waste (40-60%), along with ash and fine earth (30-40%), and lower quantities of paper (3-6%), plastic, glass, and metals. The moisture content of urban MSW is approximately 47%, and the carbon-to-nitrogen (C/N) ratio ranges from 20 to 30. The average calorific value of MSW is 7.3 MJ/kg (1745 kcal/kg). To summarise, vegetable waste, along with other food waste, constitutes a significant portion of the overall waste burden. Inadequate management practices, insufficient infrastructure, and a lack of waste segregation contribute to environmental and health hazards. Some of the risks associated with vegetable waste disposal include greenhouse gas emissions and contamination via leachate, with subsequent adverse impacts on the environment and human health.

Sewage sludge is a significant by-product of wastewater treatment systems and constitutes a significant threat to human health by its potential for pollution. Therefore, proper management of sewage sludge is crucial prior to disposal on land or in building materials, landfilling, or incineration applications. Stabilisation of the sludge and reduction of its inherent sources of pollution are necessary steps in the disposal process. Research conducted by Gong et al. (2022) highlights the accumulation and amplification of heavy metals (HMs) in the food supply chain produced by improper disposal of sewage sludge. This poses serious health concerns and increases the risk of carcinogenic incidence, even in areas with low levels of heavy metals. Therefore, stabilisation not only reduces the potential for pollution but also improves the overall quality of the sludge, making it more suitable for various modes of disposal. Properly treated sludge can be safely used in land applications, where it can contribute to soil fertility and improved agricultural practices. Sludge can also be utilised in the production of building materials, such as bricks or cement, which can reduce the demand for virgin resources. Alternatively, if stabilisation alone is insufficient or not feasible, the treated sludge can be disposed of in a controlled manner through landfilling or incineration. However, it is crucial to ensure that these disposal methods comply with environmental regulations and incorporate measures to minimise potential negative impacts. By effectively managing sewage sludge, we can protect human health, preserve the environment, and utilise its potential as a valuable bioresource.

2.1.2 Weed biomasses

India possesses diverse geographical features, including towering mountains, riverine deltas, high-altitude forests, peninsular plateaus, and various other geological formations, resulting in a wide range of weather conditions (Chauhan et al., 2015). The country experiences a broad spectrum of temperatures, ranging from arctic cold to tropical hot, and rainfall patterns that vary from extreme aridity (less than 10 cm per year) to excessive humidity, with certain areas receiving the highest rainfall in the world (1,120 cm). The topography of India encompasses high plateaus, wide valleys, undulating highlands, plains, swampy lowlands, and arid deserts. Based on soil, bioclimate, and physiography, the country is divided into 20 Agro-eco regions and 60 agro-eco-subregions, which further classify into agro-eco-units at the district level to establish long-term land-use policies (Bhattacharyya et al., 2006).

Weed-related concerns vary with each agro-ecological location and crop. India has been invaded by varied alien invasive weeds, including *Lantana camara*, *Eichhornia crassipes*, *Salvinia molesta*, *Parthenium hysterophorus*, *Chromolaena odorata*, and *Mikania micrantha*. These weeds, except for the aquatic ones, have spread across extensive areas of woodland, grassland, wastelands, orchards, and plantation crops in certain locations. The extensive environmental impact is best exemplified by the humungous proliferation of *L. camara* post-introduction into the United States in 1908 (Varshney and Prasad Babu, 2008). *L. camara*, belonging to the Verbenaceae family, is an eye-catching yet hazardous plant found in temperate, tropical, and subtropical regions worldwide. Structurally, it is a highly branched shrub, reaching a length of 2-4 meters when young; it becomes woody and cylindrical and could reach a girth of up to 150 mm with age. Its square-shaped arc consists of pithy and short stumps that are reverse-hooked in cross-section. (Mishra, 2015; Ghisalberti, 2000). While *L. camara* is primarily native to subtropical and tropical America, some species are also native to Africa and Asia. It is cultivated in approximately 50 countries as numerous cultivars, with estimates of *L. camara* species ranging from 50 to 270, but the most reliable estimate is around 150 species. Initially developed as an ornamental plant by the British at the Calcutta Botanical Garden in 1809 (Nanjappa et al., 2005), *L. camara* has established itself indiscriminately in almost every region of India, except for the Thar Desert, with a strong ability to colonise fields, grasslands, fallow lands, and forests (Dobhal, 2011). The presence of allelochemicals in *L. camara* not only inhibits the growth of native plants but reduces their vitality (Sharma et al., 1988). The leachates from the *L. camara* stem, leaf, and fruit hinder the growth and seedling development of certain terrestrial plants (Quan et al., 2009). Managing *L. camara* effectively requires unremitting vigilance and control of new growth. The ability of *L. camara* varieties to hybridise with closely related species is a key factor contributing to its successful dispersal as a weed and the limited success of biocontrol methods. Biocontrol agents have shown more effectiveness when obtained from related *L. camara* species within the countries or derived from a wide host range.

2.2 EXISTING COMPOSTING TECHNIQUES

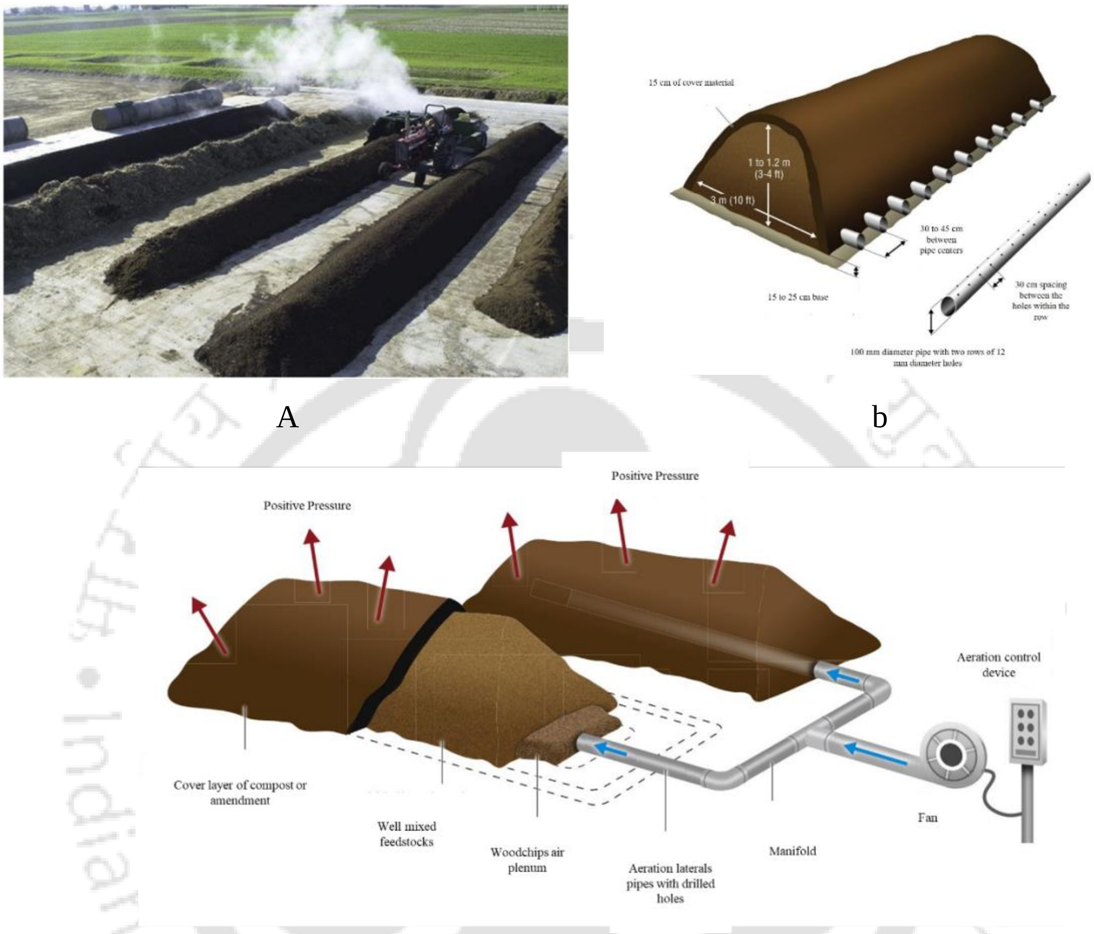
According to Haug (1993), the composting process can be achieved by different methods, as represented in Fig 2.1

2.2.1 Windrow composting

Piling of biodegradable wastes in long rows refers to windrow composting and is most suited for large volumes of waste. Windrows are used when improved aeration and porosity or removal of excess moisture content is necessary, often aided by the use of specific turners. The temperature of the windrows must be measured and logged constantly to determine the optimum turning time for quicker compost production. Typically, the shapes of the windrows are trapezoidal, measuring 90 to 360 cm in height and 300 to 600 cm in width. Windrow composting, which is a common farm-scale composting method, requires 90-270 days to yield good quality compost and is principally dependant on the type of waste (Michel et al., 2022c).

2.2.2 Passive aerated pile/wind-row method

In this method, open-ended perforated pipes are inserted under each pile or wind-row to allow the circulation of air and eliminate the need for turning. Air flowing into the pipes through their open ends builds up sufficient pressure to move upwards through the perforations, pushing out the hot gases accumulated in the composting feedstocks, thus creating a functional chimney effect. The pile should be 90-120 cm high. The FAO (2003) reported the holes drilled in the pipes should be about 1.27 cm in diameter. When the composting period is completed, the pipes are removed, and the base material is mixed with the compost. This method has been studied and used in Canada for composting seafood wastes with peat moss, manure slurries with peat moss, and solid manure with straw or wood shavings. The time required to achieve good quality compost depends on the type of waste, but usually, it takes 45-90 days to complete the process via the passive aerated pile/wind-row method (Michel et al., 2022).



A b

c

Fig. 2.1 Passive and active aeration during composting (Michel et al., 2022)

2.2.3 Active aerated pile/wind-row method

In this type of composting method, the aeration is forced or active in contrast to the former. The blended feedstock is placed on the perforated piping, and their open ends are connected to a blower. In large-scale composting systems, forced aeration is performed by the computerised aeration monitoring system. Depending on the substrate porosity, and the environmental conditions, the height of the aerated pile systems should be maintained between 150- 245 cm, whereas the width may vary between 300-490 cm and generally conforms to a triangular shape (Michel et al., 2022).

2.2.4 In-vessel composting technique (small-scale studies)

Rotating drum systems utilise a slightly inclined horizontal steel cylinder to mix, aerate, and transport compost throughout the system (Fig 2.2). The drum is mounted on massive bearings and rotated via a substantial external gear. A conveyor and/or hopper are used to transfer feedstocks at one end. As the drum gently rotates, the materials are flipped over and rolled toward the discharge end via continuous or intermittent rotation, as required. Some drums are equipped with blades or other protrusions for slitting open sacks and shattering materials as they descend. Warm, active compost is combined with fresh incoming feedstocks in the upper portions of the cylinder, which induces the decomposition process to commence rapidly.

The efficient incorporation of oxygen into the composting material is maintained by a fan placed at the drum's discharge end and reinforced by rotations of the drum. As the air travels in the opposite direction of the material being placed in the drum, the decomposed material close to the outlet is cooled by the fresh air. The material in the middle receives the warmed air, which promotes the process, and the newly inserted material receives the air that is the warmest, which initiates the process.

Certain compact barrels lack forced aeration. Instead, air exchange occurs via the tumbling action and exposed apertures at the extremities of the drum. Rotary drum composters (Fig 2.2) were among the earliest types of in-vessel composting systems to be designed. They were built with engineering techniques that were radically different from other traditional methods previously used (Michel et al., 2022a).

Tolvanen et al. (2005) measured bioaerosols (microbes, dust, and endotoxins) and volatile organic compounds (VOCs) in the working air of a drum composting plant that treated source-separated catering waste. Different composting activities occurred in the respective units, separated by modular design and construction in their Oulu drum composting plant. This is an important step forward in maintaining a generally clean working environment in the control room, with a lowered exposure to hazardous stimuli. The amount of viable airborne microorganisms, on the other hand, was high in both the biowaste received and the composting drum.

Aboulam et al. (2006) reported that any professional analysis of municipal solid waste composting plants is required to examine each piece of equipment used in the processing sequence. In composting plants, however, in-situ, analysis of working spinning drums is either limited or off-limits, mostly due to safety concerns. A spinning drum was built as part of a laboratory pilot system (1.5 m in length and 0.8 m in diameter). The output was compared to that of a rotary drum composting reactor in a working installation in Landerneau (France), using the same municipal solid waste input. This composting setup serves the dual purposes of microbial degradation and municipal solid waste size reduction.

Smith et al. (2006) evaluated mechanical rotating drum composting for food waste. Food scraps from a Texas prison were mixed with fine-textured soft-wood shavings in ratios of 2/1, 3/1, and 4/1 to yield 0.4 m3 of compost blend. During 15-day composting testing, all mixes showed volume and weight reductions. During the experiments, all blends reached thermophilic temperatures (> 45°C). The initial pH of all blends was low, reaching 3.55 before returning to compostable levels (6.0-7.5). Compost pH increased as temperature increased towards the thermophilic range. Most nutrients (P, K, Ca, Mg, S, Na, and Zn) rose in concentration during composting, except for N, which declined in some cases. The study concluded that mechanical in-vessel rotating drum composting might be a viable food waste disposal option. The mechanism of the composting process has been illustrated in Fig. 2.17.

Ripley et al. (2008) reported that in-vessel systems are typically employed in areas with fairly substantial amounts of organic waste generation with added issues of space crunch and odour exudation, particularly in bigger towns. Although in-vessel systems can be built to treat as little as 365 tonnes of organic waste per year, such low processing rates are frequently not economically feasible. Container in-vessel composting facilities in Germany and the Netherlands have shown that container in-vessel systems can treat between 3,000 and 20,000 tonnes of vegetable, fruit, and garden waste per year.

Kalamdhad and Kazmi (2009) stated that a substantial volume of waste material could be digested within an enclosed space in a short period if the conditions are well controlled. In this article, the authors discuss the decentralised processing of vegetable waste, which they believe is an effective and promising strategy. The stable final product of great quality is engendered by provisions for agitation, aeration, and uniform mixing of the compost material. Vertical towers, horizontal rectangular and circular tanks, and circular revolving tanks have all been applied in this process.

A b

Fig 2.2 (a) Small scale in vessel composter (Michel et al., 2022a)

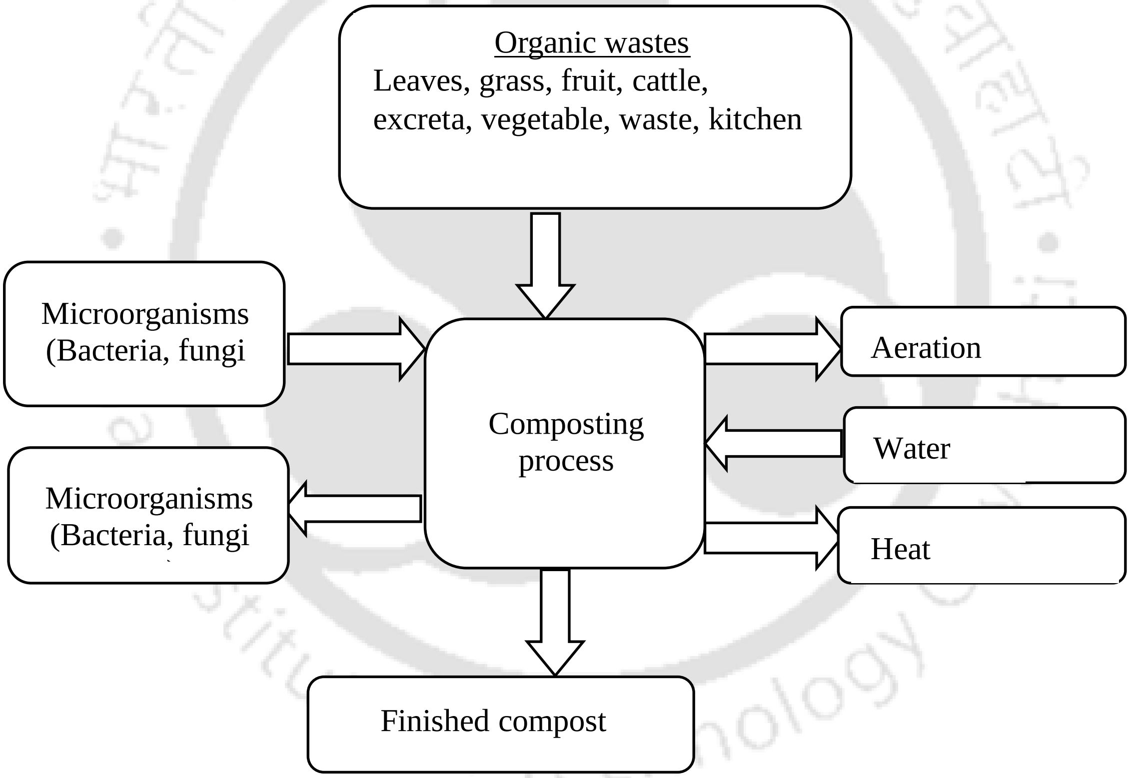


Fig. 2.3 Mechanism of the composting process

Hazarika et al. (2017)demonstrated the transformation of elemental hazardous heavy metals into immobile portions of paper mill waste using a rotating drum composter. The purpose of the study was to determine the variation in the bioavailable and leachable fraction of heavy metals (Cd, Cu, Fe, Ni, Pb, Cr, Zn, Hg, and Mn). The study also assessed the effect of temperature, pH, organic matter degradation, and humification during composting of paper mill sludge over 20 days of processing. The author further stated that the variation in bioavailability and leachability of heavy metals is influenced by organic matter breakdown and humification during composting, which can be maximised by using an optimum amount of cow dung during the rotary drum composting of paper mill waste.

Jain and Kalamdhad (2018) investigated the physical parameters of solid pulp and paper mill sludge composting in a 550 L rotational drum composter. During the composting process, physical factors such as bulk density, volatile solids, moisture content, free air space, void ratio, ash content, and particle density varied. The final product was characterised by an increase in bulk density, a decrease in free air space to 52%, an increased particle density of 610 to 680 kg m3, and a consistent increment in nutrient profile throughout the composting process.

Jain and Kalamdhad (2019) reported composting of the invasive aquatic weed H. verticillata using in-vessel composting technology. To achieve the objective, three carbon-rich agents (dry leaves in run A, grass clippings in run B, and wood chips in run C) were added in the ratio of 8:1:1. The control was optimised with a combination of H. verticillata, cow dung, and sawdust. Composting tests were carried out in a 550L rotational drum composter for 20 days to assess variations in physical, chemical, nutritional, and degradation kinetics. The study also concluded that the addition of dry leaves, grass clippings and wood chips as carbon-rich agents created compost with nutritional concentrations adequate for agricultural applications.

Ajmal et al. (2020) investigated and optimised varied temperature regimes on processing time evinced by the accelerated breakdown and mineralisation rates of agricultural waste in-vessel composting. Under the Taguchi technique, a total of nine experiments were carried out with three levels of temperature (55 °C, 65 °C, 75 °C) and time (15 h, 18 h, 22 h) in a pilot-scale composter. It was equipped with control systems for temperature, aeration, agitation, and humidity c for the composting of poultry manure (PM), vegetable waste (VW), and rice straw (RS) mixed at a ratio of 5.5:3.5:1.

Maturi et al. (2021)used the in-vessel composting technique to control the invasive weed Ageratum conyzoides. The research focused on composting diverse weeds by evaluating the heavy metal toxicity throughout the composting process. The levels of volatile solids were elevated, whereas the biological parameters of the substrate decreased in the final compost product. The dynamics of the composting process were assessed by application of the mass balance concept to the contents of the rotary drum. The results indicate specific dynamics of heavy metals in the rotary drum composting process and the proportions of each metal in several individual chemical fractions.

2.2.5 Vermicomposting process

Vermicomposting is a sophisticated bio-oxidative composting process that employs earthworms to transform organic wastes into high-quality compost. Different types of earthworm species used in the various studies for the degradation of organic waste have been shown in Fig. 2.4. Vermicomposting, unlike other composting processes, is not an exothermic process. The organic matter containing the majority of the nutrients is transformed into more accessible forms known as vermicast during this process. Initially, the substrate is split into small bits for swallowing by the earthworm. It then enters their gizzard, where mincing increases the surface for microbial action. Vermicomposting is the eco-friendliest recycling process to reduce organic waste. This recycling process not only converts organic debris into high-quality compost, but the chemical changes that the debris undergoes makes the nutrients easily available to the plants. Several epigeic (Eisenia fetida, *Eisenia andrei*, *Eudrilus eugeniae*, *Perionyx excavatus*, *Perionyx ceylanesis* and *Perionyx sansibaricus*) have been identified as potential candidates to decompose organic waste material (Suthar, 2007). Some attempts have also been made to biodegrade a variety of materials using vermicomposting (Sangwan et al., 2008). The improvement of plant growth with the use of vermicompost is attributed to its nutrients and biologically active substances (Warman and AngLopez, 2010). Many plant growth regulators, including auxins, gibberellins, humic acids (Atiyeh et al., 2000) and cytokinins of microbial origin, are found in vermicompost. Additionally, the activity of soil enzymes such as urease, phosphomonoesterase, phosphodiesterase and arylsulfatase increases significantly with vermicompost application (Albiach et al., 2000).

Vermicasts are earthworm excretory pellets, whereas vermicompost is a homogeneous mixture of vermicasts and humus-like decomposed organic materials in the vermibed (Fig. 2.5). Samal et al. (2019) thoroughly reviewed the ability of vermicasts to improve soil health, regulate soil texture, and the capacity of fresh vermicast's to maintain their stability for an extended period of time. The stability of the vermicasts is determined by the. Percentage of waste mixtures (waste materials + amendments) consumed by earthworms. The particles of the cast materials form strong bonds with a variety of microbial symbionts and enzymes. Dlamini and Haynes. (2004) confirmed that vermicasts ejected by epigeic earthworm species are more stable than vermicasts ejected by endogeic earthworm species.

Moisture content, pH, and temperature are all essential factors in vermicomposting since they directly impact cocoon development and earthworm growth, ultimately affecting composting stability. Most researchers have established that earthworms have well-defined tolerance limits to the aforementioned characteristics. As a result, if there is a significant difference in these limitations, the composting process may be slowed, and in the worst-case scenario, earthworms may die. It has been proposed that an average moisture content of 50 to 90 % be used to create a suitable environment for earthworms to function successfully in organic matter transformation (Domínguez et al., 2019). Furthermore, the optimal range of these factors is specific to the species used in the process. The earthworms are relatively sensitive to their ambient pH range and survive in alkaline to slightly acidic conditions but not below pH 4.5. Abduli et al. (2013) indicated a wide pH range ranging from 5.0 to 9.0 for maximum earthworm growth during the operations. They also reported that Lumbricus terrestris displayed much less sensitivity to pH among the other species cited during investigations. The C/N ratio is also crucial in the breakdown process during vermicomposting (Nayak et al., 2013). The mechanism of the vermicomposting process has been shown in Fig. 2.6.

A temperature range of 15 to 20°C was discovered to be optimal for the growth of *Eisenia fetida* (Edwards et al., 2010). This temperature variation has a significant impact on earthworm reproduction and activity. In a study on the effect of temperature on earthworms, low ambient temperatures evinced a much lower reproduction rate than moderate temperatures, which corroborated Neuhauser et al. (1980) results on the importance of temperature. Furthermore, as revealed by Jadia and Fulekar. (2008), in a study on vermicomposting of vegetable waste in a hydro-operating bioreactor, the thermophilic condition during the process favours the inactivation of many hazardous pathogens in the bedding materials. Earthworms are extremely susceptible to anaerobic environments, especially since they use their moist skin as a surface for the exchange of gases during respiration.

(a) (b) (c)

Fig. 2.4. Different types of earthworm species; (a) *Esenia fetida* (red wiggler worm), (b) *Eudrilus eugeniae* (African Nightcrawler), (c) *Perionyx excavatus*

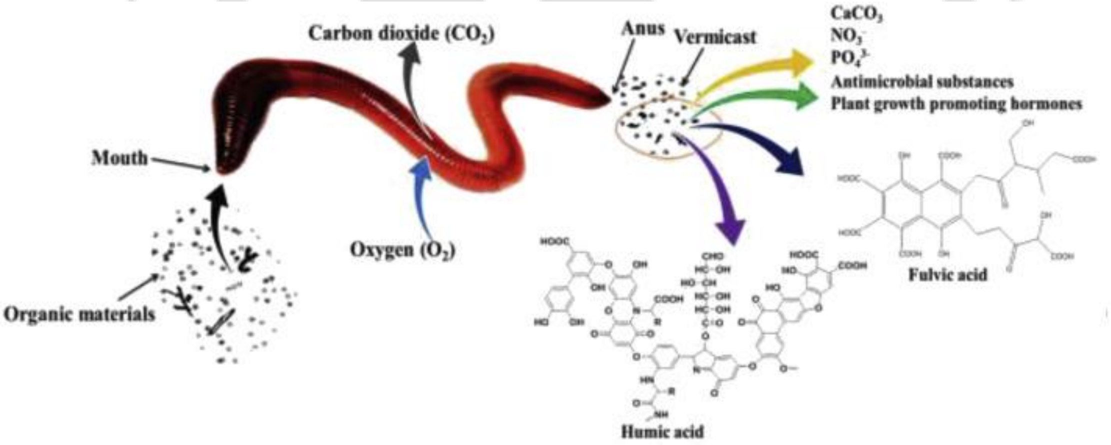


Fig. 2.5. The mechanism of conversion of organic waste into humified material (Samal et al., 2019)

According to Gunadi and Edwards. (2003), 55% to 65% respiration rates were reduced during anaerobic conditions, resulting in a fall in feeding rates. Individuals of *E. fetida* migrate in huge numbers from oxygen-depleted water-saturated substrates, according to Domínguez et al. (2000). Organic waste with a high concentration of inorganic salts and other cations causes earthworms to struggle in the compost.

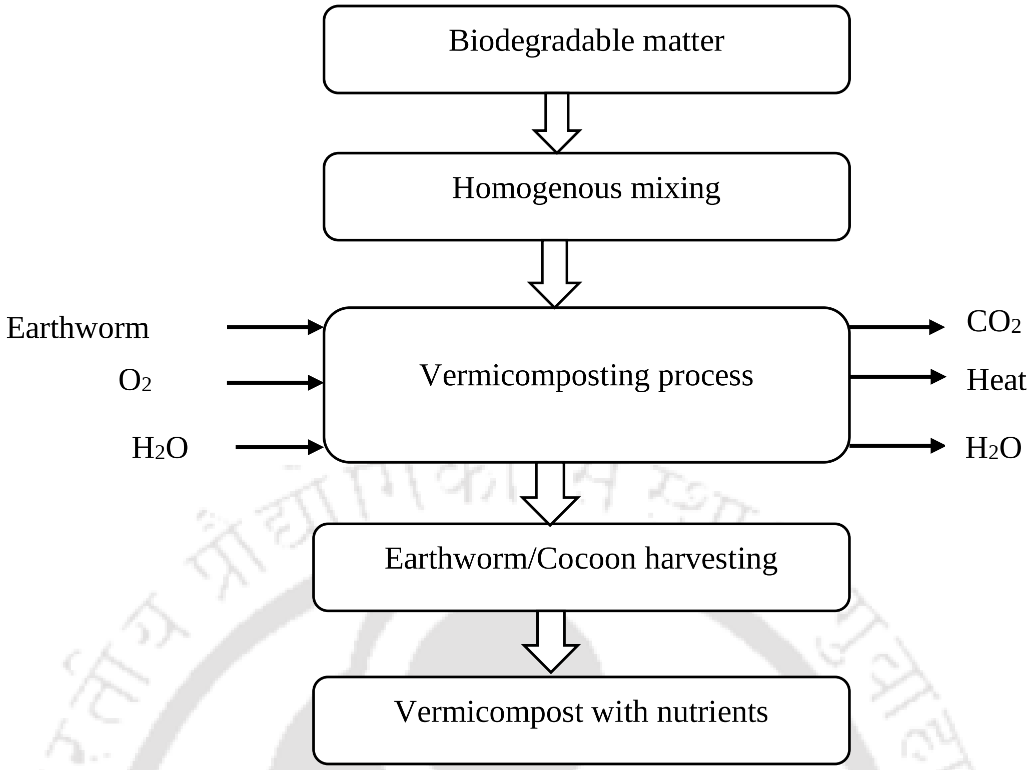


Fig. 2.6. Mechanism of vermicomposting process

In the final analysis, vermicompost is nutrient-rich, a source of helpful microbes, and displays enhanced utility as a soil conditioner or fertiliser to improve soil. Almost any sort of organic material, including agricultural, urban, or industrial organic material, can be vermicomposted; however, pre-processing such as washing, pre-composting, and macerating may be required to assist vermicomposting (Gajalakshmi and Abbasi, 2004). Fig. 2.7 depicts vermicomposting as a viable approach for degrading organic waste. Diversified research has been conducted on the degradation of organic matter using vermicomposting technology because it produces higher-quality compost than traditional composting techniques.

Shanthi et al. (1993) stated that the vermicomposting of organic waste occurs through the ingestion of earthworms, which results in the conversion of the waste material into worm castings. The investigation was carried out to determine the efficiency of vermicomposting of vegetable waste and the optimal levels of temperature and moisture during the process. Three commonly accessible species (Pheretima sp., Eisenia sp., and P.

excavatus) were used in the controlled laboratory experiments. Worms could survive in environments with moisture levels ranging from 20% to 80% and temperatures ranging from 20°C to 40°C. The results indicated P. excavatus is the most acceptable species for vegetable waste vermicomposting.

Elvira et al. (1998) reported vermicomposting Eisenia andrei with sludge sourced from a paper mill and mixed with cattle manure in a six-month pilot-scale experiment. A small-scale laboratory experiment was first conducted to investigate earthworm growth and reproduction rates in the various substrates studied. The number of earthworms increased 22-36-fold in the pilot-scale experiment, while total biomass increased 22-39-fold. The vermicomposts were nitrogen and phosphorus-rich, with good structure, low heavy metal levels, low conductivity, high humic acid concentrations, and good stability and maturity.

Benitez et al. (1999) studied the evolution of earthworm (Eisenia fetida) biomass and changes in enzyme activities during 18 weeks of sewage sludge vermicomposting. Hydrolase and dehydrogenase activities reduced throughout time as accessible organic molecules decreased. The resultant excellent correlation between all enzyme-related activities, individual parameters and the water-soluble carbon; it was concluded that both hydrolytic and dehydrogenase activities could be useful markers of the status and evolution of organic matter.

Bansal and Kapoor. (2000) conducted a 90-day composting experiment using *Eisenia fetida* to study vermicomposting of mustard residues and sugarcane garbage combined with calf manure using Eisenia fetida. After 90 days of composting, vermicomposting resulted in a considerable reduction in the C/N ratio and increased mineral N compared to treatments that were not inoculated with earthworms. As assessed by the dehydrogenase assay, microbial activity rose to 60 days after incubation and subsequently decreased after that period.

Jeyabal and Kuppuswamy, (2001), The interaction was investigated in a rice legume cropping system with different combinations of either coirpith or weeds mixed with i) cow dung, ii) sugarcane press mud or iii) bio-digested slurry. In terms of nutrient content and compost maturity duration, bio-digested slurry and weeds were determined to be an appropriate combination for vermicomposting, evinced by a drop in C/N ratio from 21–69:1 to 12–17:1. To compare the effect of vermicompost to bio digested slurry and farmyard manure (FYM) in rice, a pot culture study, based on an equal N basis with and without bio-fertiliser was set up. The combined application of vermicompost, fertiliser N, and biofertilisers such as Azospirillum and phosphobacteria boosted rice output by 15.9% above fertiliser N alone.

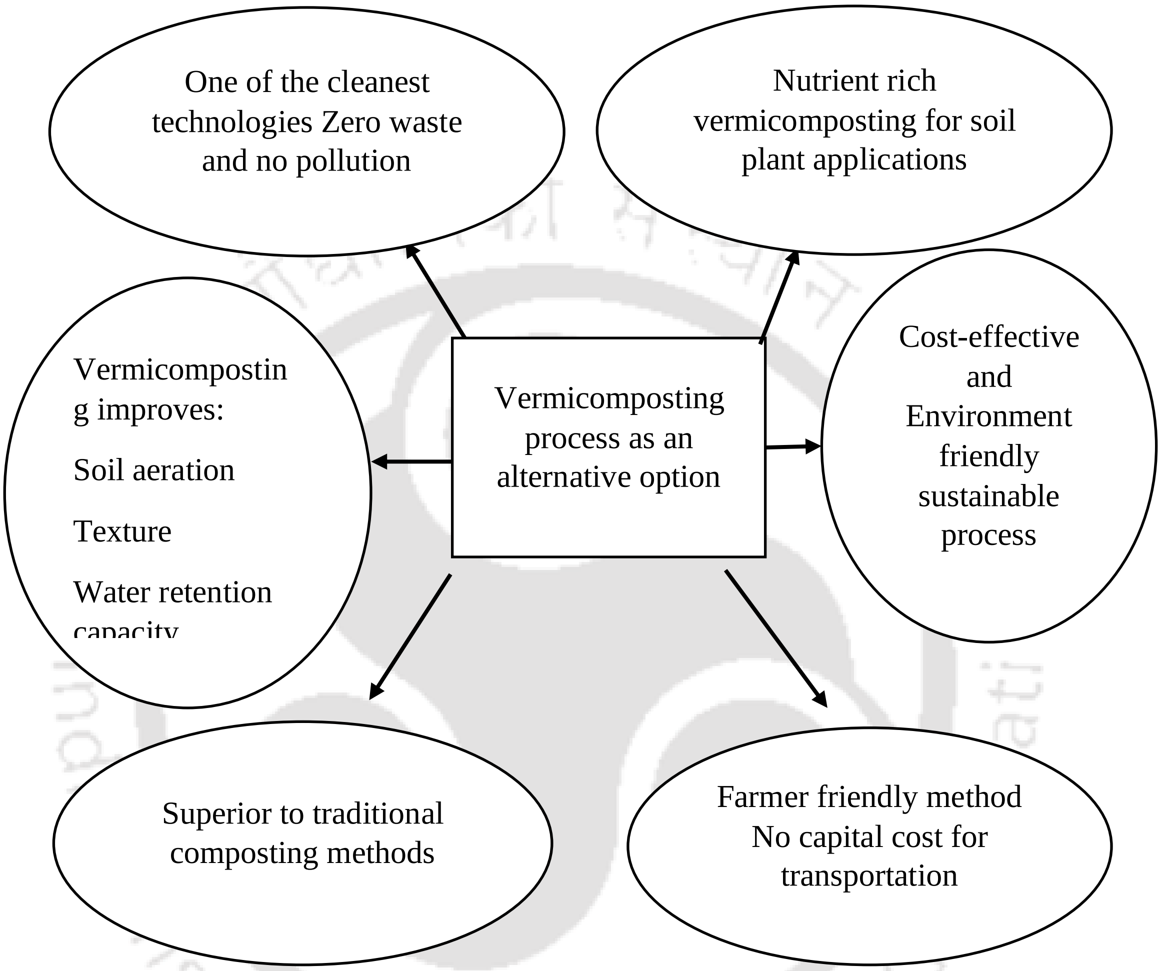


Fig. 2.7. Vermicomposting approach as an alternative approach for organic waste management

Fernández-Gómez et al. (2010) demonstrated the efficacy of vermicomposting as a recycling management strategy for bio-transforming greenhouse tomato-fruit wastes into an organic nutrient-rich product suitable for agricultural application. An experimental vermireactor was built using a manure layer, into which an initial population of *Eisenia fetida* was introduced and fed continuously at a high organic loading rate for 150 days. The final product was chemically stable and nutrient-rich, proving that tomato-fruit waste may be successfully vermicomposted into a beneficial soil amendment.

Soobhany et al. (2015) monitored the vermicomposting of organic solid waste using Eudrilus eugeniae as earthworm species. Regarding volatile solids reduction, the net degradation of the vermicompost pile was 80.58% for reactor 1, 61.03% for reactor 2, and 77.51% for reactor 3. C/N ratios showed an overall decrease of 41.5–48.4% in the final combinations. The E. eugeniae growth rates in the vermicomposting mixtures were 3.33 g day-1, 3.47 g day-1, and 1.67 g day-1 for reactors 1, 2, and 3. The findings revealed that vermicomposting was a suitable approach for the bioremediation of heavy metals from organic solid waste.

Taeporamaysamai and Ratanatamskul (2016) investigated the composting process by applying two composting systems. The first stage featured co-composting with a vermicomposting pile, and the second stage entailed developing a novel prototype vermicomposting reactor to improve the biodegradation activity of co-composting. The on-site system operation converted municipal solid waste via vermicomposting employing the earthworm Eudrilus engine. The compost was created using the developed prototype vermicomposting reactor. Due to the high efficiency of the vermiprocess, the developed reactor may be applied to the co-composting process and practically generate value-added benefits to residential areas.

Huang et al. (2017) studied the impact of earthworms on ammonia oxidation and the roles of ammonia-oxidising bacteria and archaea during fruit and vegetable waste (FVW) vermicomposting. For this, two individual systems, one with dry FVWs and another with fresh FVWs, were used and compared throughout 60 days of vermicomposting. Compared to controls, compost treated with earthworms had a higher amount of ammonia oxidisation and a more diverse population of bacteria and archaea in the end products. Ammonia-oxidising bacteria members Nitrosomonas and Nitrosospira, and ammonia-oxidising archaea groups Crenarchaeota and Thaumarchaeota were common in the final vermicompost products. Ammonia oxidising archaea, on the other hand, were the dominant members completing ammonia oxidisation during vermicomposting of dry and fresh fruit and vegetable waste. According to the findings, earthworms aid in the ammonia oxidation process by increasing the number and diversity of ammonia-oxidising bacteria and archaea during vermicomposting of fresh fruit and vegetable waste.

Bhat et al. (2018) reported that vermicompost is the final product involving the collective action of earthworms and microbes. By decreasing the detrimental impacts of garbage, the waste is turned into beneficial manure during the process. The study assessed the toxicity of industrial wastes using plant bioassays, specifically the Allium cepa and Vicia faba tests. For monitoring environmental contamination, these bioassays were both sensitive and cost-effective. The ability of earthworms to detoxify heavy metals in industrial wastes is due to their powerful metabolic system and the involvement of earthworm gut bacteria and chloragocyte cells.

Ganguly and Chakraborty (2019)used an indigenous earthworm species, Perionyx excavatus, to undertake vermicompost using wastes obtained from two paper mill effluents. FT-IR, GC-MS and TG analyses were used to assess the maturity and the stage of stability of the vermicompost produced. The study also demonstrates the amount of mineralisation by validating the tendency of complex compounds such as lignin, cellulose, and proteins to undergo biodegradation. According to TG spectral analysis, the mass loss in vermicompost produced from primary and secondary sludge was 80% and 71%, respectively. GCMS tests have also revealed the existence of numerous humic acids in the disintegrating compounds, such as octadecanoic acid, heptadecanoic acid, and others, which demonstrate the product's maturity level. The study also demonstrated a drop in the humification index, emphasising the cooperative action of earthworms and bacteria in the breakdown of organic wastes.

Singh et al. (2020) investigated the possibilities of bioconversion of several types of organic wastes using epigeic earthworm species. Waste could be blended with another organic material for optimal vermicomposting. It was shown that vermireactors containing 25% to 30% waste mixed with 70% to 75% other organic-rich material, such as cattle dung, could be quickly transformed into a useful product. However, the high concentration of organic waste caused mortality in earthworms.

Das et al. (2021) investigated the effect of rock mineral addition (i.e., rock phosphate, dolomite, and mica) on organic wastes (water hyacinth and paddy straw) in combination with microbial inoculums on vermicompost quality. During the vermicomposting process, several combinations of four microbial inoculums, Trichoderma viride, Azotobacter chroococcum, Bacillus polymixa, and Bacillus firmus, and three rock minerals were utilised to assess their effect on the quality of mature vermicompost. Each vermibed showed a significant decrease in pH, organic carbon, and C/N ratio, with a concomitant increase in humic acid, total nitrogen, accessible phosphorus, and exchangeable potassium.

Ameen and Al-Homaidan (2022) investigated vermicomposting of food waste altered with biochar and cow dung over a 90-day composting period. The addition of three mangrove fungal species as further amendments to the vermicomposting process was explored. The employment of the mangrove fungus Acrophialophora jodhpurensis as a bio-catalytic agent during vermicomposting favoured the final compost quality (available N, P, and K) and composting time. Heavy metal concentrations (Cd, Ni, Pb, Zn, Cu, and Cr) declined during the composting process. Vermicomposting using biochar, cow manure, and the mangrove fungus A. jodhpurensis can be used to remediate food waste. The finished vermicomposting product can be used in agriculture.

2.3 STUDIES ON LARGE-SCALE IN-VESSEL COMPOSTING

Rotating or rotary drum (horizontal flow reactor) utilises a receptacle with variable geometry and agitation method. In recent years, the number of companies offering rotary drum composters has increased substantially. But the majority of systems do not differ significantly. The following are the essential varieties of the rotary drum that vary in geometry and agitation method, as detailed by Haug (1993):

Dano

Dano Ltd. devised the Dano cylinder in Denmark around 1933 for decomposing waste. The reactor is a rotating cylinder with dispersed flow that is referred to as a "bio-stabiliser" and is 9 to 12 feet in diameter and up to 150 feet in length. The drum is maintained nearly full of trash and rotates at 0.1 to 1.0 revolutions per minute. As the cylinder rotates, the residues move in a helical path towards the outflow, where they are abrasion-mixed and granulated. Typically, 1 to 5 days are required for digestion in the cylinder, followed by sifting and windrow or static pile curing. It is possible to add water and nutrients to the cylinder and provide forced aeration. With several hundred installations, the Dano rotary cylinder has become one of the most prevalent reactor processes for municipal solid waste. In January 1992, the facility temporarily ceased accepting municipal solid waste due to a number of issues, including pollutants and the inability to generate an acceptable final product (Haug, 1993).

Fermascreen

The reactor is a hexagonal cylinder with three sides comprised of displays. Refuse is pulverised and transported in batches. Sealing the screens for initial compositing. When the drum is rotated with the screens open, aeration occurs. The length of waste detention in the reactor was approximately four days.

Eweson (Bedford Bioconversion Corporation)

Eric Eweson (1897-1987) was an active yeast industry fermentation technician. After reading Sir Albert Howard's opinion that modern fermentation technology could speed up the decomposition process, he became interested in composting. He conferred with Sir Howard in England and based the Eweson rotary drum on the Indore process devised by Sir Howard (Fig. 2.8). The Eweson drum was originally designed for decomposing refuse and sewage. The type of drum is cell-in-series. The waste is deposited in the rotating drum, which has a diameter of 11 to 12 feet, a length of 120 to 180 feet, and is slightly inclined from the horizontal. In most designs, the drum contains three compartments, but as few as one and as many as six have been used. Every 1 to 2 days, the mélange is transferred to the next compartment, resulting in a total digestion time of 3 to 6 days. Approximately 15% of the transferred material is retained in each cell to function as an inoculum for the incoming material. The rotational speed is approximately 1 rev per minute. The screened output is cured in static stacks or aerated channels.

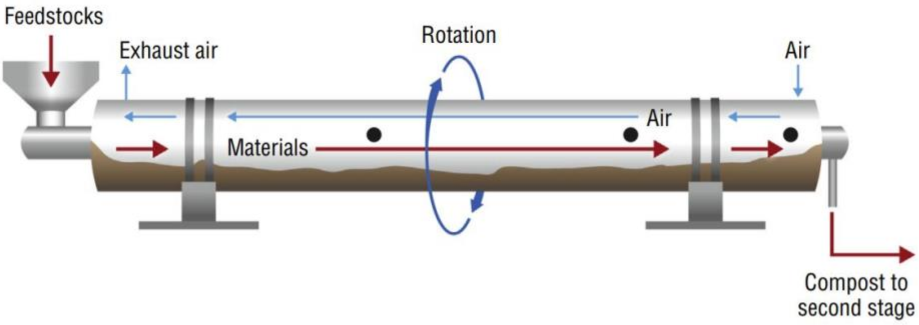


Fig 2.8 Typical large-scale rotary drum composter (Michel et al., 2022)

Ruthner

In Austria, the company Ruthner Industrieanlagen created this rotary drum system. The fermentation drum, in general, resembles a Dano drum. A refuse-sludge co-composting facility at Siggerwiesen (near Salzburg), Austria, began processing 400 mtpd of refuse and 120 mtpd of sludge in 1976 and is still in operation.

Voest-Alpine

The Voest-Alpine rotary drum/static pile system was devised in Australia by Voest-Alpine, in which the reactor resembles the Dano cylinder in general configuration. Typical pre-treatment consists of impact mill pulverising, magnetic separation, and trommel sifting to remove oversized materials. Some facilities use a rotary "homogenising drum" with a brief residence time in which liquid sediment or water is added and mixed. The mélange is deposited in an extended static pile for approximately 21 days, followed by two months of static pile curing. In the past, vibrating screens, ballistic separation, and additional magnetic separation were utilised.

Buhler Inc. (Formerly Buhler-Ming)

This recycling system was created in 1957 by the Buhler Brothers Company in Ifzwil, Switzerland, where Adolf Buhler established his foundry in 1860. Since then, Buhler Inc. and its subsidiaries have installed approximately one hundred composting facilities worldwide, primarily for refuse. In addition to the rotary drum, an aerated windrow process and a bin-type process known as the Wendelin system are also utilised either alone or after the rotary drum application (Fig. 2.7). Using the Buhler equipment, extensive pre- and post-processing is typically provided. Typical pre-treatment procedures include grinding, ferrous separation, sieving, and fine pressing. On average, windrow decomposition is conducted for three to four months using 15 to 18 m tall heaps. Typically, two to three turnings are performed during the cycle. In 1992, Reuter, Inc. commissioned a 667-tonne-per-day (5-day basis) MSW composting facility in Pembroke Pines, Florida, using the Buhler apparatus. The facility has 101 acres of enclosed space, including a 66-acre composting "hanger" with 24 windrows that are 720-foot-long, formed over oxygenation channels, and a 42-day designed residence time.

Aboulam et al. (2006), in their article "Use of a Rotating Drum Pilot Plant to Model the Composting of Household Waste on an Industrial Scale", discussed the construction and evaluation of a laboratory pilot plant that simulates the composting of household refuse. Their research investigated mass loss, gas release, and industrial-scale decomposition of plant products in a bioreactor or composter with a rotating cylinder. The authors observed rising household waste in the United States and Europe and the subsequent need for sustainable waste management. Recycling and composting are encouraged concurrently with landfilling. Composting reduces the amount of municipal waste and recycles organic materials. Waste from a French sorting-composting facility powered the prototype laboratory plant that accommodates a rotating cylinder of industrial scale. With continual rotation, the drum pulverised the garbage and biologically converted the organic material into compost. The drum was equipped with aeration and kept damp to enhance the decomposition process. The efficacy of the composting facility was assessed and analysed. The weight loss at 550 degrees Celsius determined organic matter losses and plant yield. The output of an operational rotary drum decomposition reactor was compared to that of the prototype plant. The experimental plant demonstrated the microbiological degradation and refuse reduction functions of decomposition. The pilot plant in the laboratory lost 1.5% of its organic matter daily, while the industrial unit lost 3.3%. These ratings were handicapped by less than accurate measurements of ash. The laboratory demonstration plant underscores industrial-scale drum decomposition, the requirement of rigorous equipment evaluation in municipal solid waste composting facilities and additional research to improve the accuracy and efficacy of decomposition.

Kalamdhad et al. (2009) focused on high-rate composting of institutional waste, specifically vegetable waste and tree leaves, using a demonstration-scale rotary drum composter (3.5 m^3). Physico-chemical and biological parameters were monitored to evaluate the changes during composting. The rotary drum composting process achieved higher temperatures in the inlet zone (60-70°C) and middle zone (50-60°C), resulting in efficient degradation within the drum. Consequently, all parameters, including TOC, C/N ratio, CO_2 evolution, and coliforms, decreased significantly within a few days of composting. Within a week, a compost of good quality with a total nitrogen content of 2.6% and a final total phosphorus content of 6 g/kg was obtained. However, relatively higher values of faecal coliforms and CO_2 evolution suggested further maturation was needed. To achieve maturation, two conventional composting methods, windrow composting and vermicomposting, were employed. As opposed to windrow composting, vermicomposting was found to be suitable for delivering fine-grained, high-quality matured compost within a maturation period of 20 days. In conclusion, the study demonstrated that institutional organic waste, such as vegetable waste and leaves from trees, can be successfully composted within a short period of 7 days using a rotary drum composter. The composting process maintained aerobic conditions through a passive air supply via an exhaust fan, even in cold weather conditions. The temperature gradient within the drum facilitated the transformation of waste material, and the mixing of previously added waste with incoming material contributed to higher degradation rates. The direct entry of incoming material into the thermophilic phase led to a rapid decrease in organic matter content.

Bhatia et al. (2013) conducted a study on the bacterial diversity in a full-scale rotary drum composter using biodegradable organic waste; the samples were analysed using two different approaches: culture-dependent and culture-independent techniques. The culture-dependent method enumerated indigenous bacterial isolates, including total heterotrophic bacteria (Bacillus species, Pseudomonas species, and Enterobacter species), Fecal Coliforms (Fecal Streptococci, Escherichia coli, Salmonella species, and Shigella species). Each population showed a reduction during the composting period. On the other hand, the culture-independent method involved PCR amplification of specific 16S rRNA sequences to identify bacterial species. The analysis revealed the presence of species of Acinetobacter, Actinobacteria, Bacillus, Clostridium, Hydrogenophaga, Butyrivibrio, Pedobacter, Empedobactor, and Flavobacterium. These species were identified through the clustering of sequences in a phylogenetic tree. Physico-chemical analysis of the compost samples was correlated with the bacterial diversity; they indicated that the bacterial communities changed throughout the composting process, most probably influenced by temperature variations and the availability of new metabolic substrates engendered at different stages of composting. Overall, the results showed the growth of significant microbial communities was responsible for the efficient degradation of organics. The bacterial diversity observed in the composting process underwent changes and probably was associated with temperature variations and the availability of new metabolic substrates at different stages of composting. Both the culture-dependent and culture-independent approaches provided insights into the major bacterial populations involved in the high-rate rotary drum composting process. Therefore, the rotary drum composter proved to be a viable, efficient, and appropriate technology for decentralised composting, successfully stabilising a majority of the organic waste within a 7-day period.

Karadag et al. (2013) conducted a full-scale study at a composting plant in Istanbul, monitoring various factors such as temperature, pH, moisture, C/N ratio, and bacterial community. The C/N ratio consistently decreased throughout the composting process, with the final mature compost products having a C/N ratio of less than 20. The temperature was mostly above 55°C during composting and gradually decreased to mesophilic conditions during the mature stages. Different types of bacteria dominated each stage of composting, and bacterial diversity was primarily influenced by temperature. Bacillus species were dominant in the early stages, while Acinetobacter and Sphingobacterium strains were detected in thermophilic and maturing stages. The bacteria were primarily involved in the degradation of cellulose and toxic organics, and some strains exhibited the capacity for denitrification. The thermophilic stages exhibited higher bacterial diversity compared to the mesophilic stages, while hyperthermophilic conditions significantly altered the bacterial community. The physicochemical characteristics and bacterial profiles were observed in the full-scale composting plant, leading to the following conclusions:

- Compost pH gradually increased to above 8.0 during active composting stages and then slightly decreased to a range of 7.5-8.0 during the maturing phase.
- Moisture content continuously decreased from 60% to approximately 25%, and high temperatures had a significant impact on moisture changes.
- The C/N ratio decreased below 20 in the final stage of composting.
- Microbial diversity increased with temperature, but a sudden change to hyperthermophilic temperatures (81°C) resulted in a decrease in bacterial diversity.
- Acinetobacter strains dominated the initial stages of composting, while mature compost was primarily dominated by bacteria belonging to the genera Sphingobacterium, Thermobacillus, and Citrobacter.
- These findings highlight the strong influence of temperature on bacterial dynamics in a composting plant.

Ali et al. (2014) demonstrated the impact of temperature, moisture, and pH on the breakdown and degradation kinetics of pesticides like aldrin, endosulfan (α), endosulfan (β), and lindane during vegetable waste composting in a full-scale continuous rotating drum composter (FSCRDC). Ultrasonication, silica gel column, and GC-MS analysis removed, concentrated, and quantified pesticides during composting. Aldrin, endosulfan α, β, and lindane were eliminated at 85.67%, 84.95%, 83.20%, and 81.36%, respectively. The composter's inflow zone reached 60–65°C, appropriate for the varied microbial life. After feeding and spinning, the temperature decreased to 38°C from 60-65°C but rebounded to optimal after 7-8 hours. After 10 hours, the pH recovered to 7.5 ± 0.3. Heterotrophic bacteria like Bacillus, Pseudomonas, and Lactobacillus fell from 4.4 × 103 to 7.80 × 102 CFU g^{-1} in 2 hours due to temperature and pH variations. Feeding and rotation did not affect middle and output zone temperatures. Aldrin, endosulfan α, β, and lindane had half-lives of 25.54, 18.43, 18.43, and 27.43 days from 1095, 60, 270, and 160 days. Pesticide degradation was of the first-order, as the vegetable waste was organochlorine pesticide-free after full-scale rotary drum composting. In 7-8 days, the FSCRDC eradicated 81.36% to 85.67% of aldrin, endosulfan alpha, and lindane pesticides. Turning, temperature, and pH affected composter inflow zone heterotrophic bacteria. Zone-specific pesticide degradation displayed shorter half-lives in comparison to. FSCRDC, which reduces vegetable waste organochlorine pesticide residues during full-scale composting.

Ali et al. (2016) assessed the decomposition of the pesticides aldrin and endosulfan in vegetable waste through rotary drum and windrow composting. The removal efficiencies, kinetics, and degradation pathways were assessed. In rotary drum composting, aldrin, endosulfan, and endosulfan removal percentages were 86.8%, 83.3%, and 85.3%. While in windrow composting, the removal percentages were 66.6%, 77.7%, and 67.6%, respectively. During rotary drum composting, the degradation rate constants for aldrin, endosulfan, and endosulfan ranged from 0.410 to 0.777 days, 0.057 to 0.076 days, and 0.009 to 0.061 days, respectively. The research identified degradation pathways for these pesticides during decomposition. Dieldrin and 1-hydroxychlorodene were indicative of aldrin's epoxidation and oxidation reactions. During endosulfan decomposition, the formation of chloroendic acid and chloroendic anhydride supported the occurrence of endosulfan sulfate and dehydration reactions. The study found that rotary drum decomposition was more effective at removing aldrin, endosulfan, and endosulfan than windrow composting. The degradation kinetics followed a first-order model, and the half-life of endosulfan decreased considerably during rotary drum decomposition from 14-25 days to 10-14 days. The study proposed pesticide degradation pathways involving isomerisation, hydroxylation, and oxidation based on the observed metabolites. Understanding the fate and degradation of these pesticides during decomposition is crucial, as composting remains a prevalent method for managing agricultural and vegetable refuse around the globe.

The study conducted by Tie et al. (2023) focused on a modified outdoor nutrient recycling system designed to compost organic sludge and recover clean nitrogen for cultivating high-value microalgae. The researchers investigated the effect of adding calcium hydroxide on enhancing ammonia (NH_3) recovery during thermophilic composting of dewatered cow dung. They prepared 350 kg of compost in a pilot-scale reactor over a 14-day period, observing thermophilic composting achieved through self-heating. The compost temperature reached up to 67 °C, indicating microbial activity. The high rate of CO_2 evolution in the initial days suggested active organic matter degradation. The addition of calcium hydroxide and increased aeration on day 3 resulted in the volatilisation of 9.83% of remaining ammonium ions, improving ammonia recovery. Geobacillus was identified as the dominant bacteria contributing to better NH_3 recovery under elevated temperatures. The study demonstrated that by thermophilic composting 1 ton of dewatered cow dung, up to 11.54 kg of microalgae could be produced. The addition of an alkaline agent in a large-scale composting reactor significantly enhanced ammonia recovery to 74%. The findings highlighted the potential of utilising dairy cow waste for ammonia recovery through thermophilic composting. Future research could explore supplying the CO_2 produced during composting to microalgae cultivation systems for photosynthesis and nutrient efficiency. The developed nutrient recycling system could also be applied to other organic waste biomass rich in nitrogen and organic matter. Overall, the study emphasised the economic and environmental benefits of actively recovering nutrients from organic waste and mass-producing high-value microalgae through thermophilic composting.

2.4 DURATION SHORTENING POSSIBILITIES USING IN-VESSEL COMPOSTING

Biodegradation is an ecologically continuing phenomenon because of the combined activity of various microbial communities, such as bacteria, actinobacteria, and fungi, which are related to successive environmental circumstances (Moreno et al., 2013). Microorganisms play critical and multifaceted roles in the bioconversion of organic substrates, with bacteria being the most significant due to their metabolic diversity (López-González et al., 2015). As a result, an in-depth understanding of the dynamics of bacterial communities would be beneficial for adequately managing and enhancing composting processes (Brown et al., 2008). The existing methods of composting need at least 2-3 months. Long residence duration and poor maturation efficacy have become crucial obstacles to the growth and promotion of organic wastes. In addition, specific composting systems, such as windrows, which work in the open-air ambience, encounter dramatic fluctuation of substrate and operational parameters in response to changing seasons and weather, making product quality management challenging (Wei et al., 2021). An increasing number of researchers have recently found that an optimised composting process, rotary drum composting (RDC), can stabilise various organic feedstock into nutrient-rich compost (Hazarika and Khwairakpam, 2018; Jain and Kalamdhad, 2018; Kauser et al., 2020; Nayak and Kalamdhad, 2015; Sharma et al., 2018; Singh et al., 2016; Varma and Kalamdhad, 2016). Further, the amendment of vermicomposting in series with rotary drum composting could enhance the product quality parameters. The working volume of these studies was limited to 550 L in batch mode. The efficacy of RDC on a large scale and in fed-batch or continuous feeding mode has received less documentation. The biodegradation of massive organic waste presents significant challenges that exceed the individual capacities of both the rotary drum composter and vermicomposting techniques, particularly in terms of the amount of waste to be managed and the time needed to compost without impacting the final product quality.

Varma and Kalamdhad (2016) compared the efficiency of drum composting followed by vermicomposting with the direct utilisation of vegetable waste for biomass production and carbon decomposition. Three trials were conducted using two species of earthworms: *E. fetida* (trial 1) and *E. eugenia* (trial 2). Trial 1 and trial 2 lasted for 45 days without pre-treatment of the vegetable waste, while trial 3 was subject to pre-stabilisation using a drum composter at thermophilic conditions. The results showed that trial 3, which involved the use of the drum composter followed by vermicomposting, yielded the highest earthworm population. Additionally, trial 3 exhibited the highest organic carbon reduction. This suggests that the combination of drum composting and vermicomposting was successful in achieving higher carbon decomposition rates, as well as promoting earthworm growth and reproduction in vermireactors. Furthermore, the drum composting method proved more efficient compared to the direct utilisation of vegetable waste for vermicomposting.

2.5 SAFETY EVALUATION OF THE COMPOST PRODUCED IN THE SHORTEST DURATION

Toxicity assessment is one of the most significant criteria for determining whether compost is suitable for agricultural use and avoiding environmental problems before recycling back to agricultural land. Phytotoxic chemicals, such as heavy metals, may cause toxicity in immature compost (Tam and Tiquia, 1994). Phytotoxicity can be characterised as a delay in germination, inhibition of plant growth, or any other detrimental effect induced by specific compounds (phytotoxins) or by insufficient growth circumstances in the presence of a plant (Barral and Paradelo, 2011). Excess salt accumulation, phenolic compounds, ethylene and ammonia, and organic acids are all examples of chemicals that can slow seed germination and plant growth(Tam and Tiquia, 1994). Acetic acid is probably the most harmful organic acid generated from immature compost, although there are additional substances that contribute to the phytotoxic effect of the immature compost as well (Ozores-Hampton, 1998). High levels of salt in the compost and the release of organic acids into the compost have been linked to the inhibition of germination and growth of plants. Germination and growth assays are frequently the most effective methods of determining phytotoxicity (Gariglio et al., 2002). Toxicity is one of the most important criteria to consider when evaluating the quality and suitability of compost for agricultural, landscaping, and environmental restoration. This is especially true when evaluating compost used in high-value horticultural applications, as it is in all of its potential applications.

Currently, there are no analytical methodologies that can be used to determine the effects of synergistic and antagonistic interactions between dangerous substances (Emino and Warman, 2004). However, biological tests are the most realistic and complete methods of determining whether composted materials are suitable for use with plants because they allow the evaluation of many phytotoxic variables present in the compost at the same time. In 1981, (Zucconi and Bertoldi 1981) published a paper describing a germination test or index based on cress. Warman (1999) conducted a comprehensive assessment of the literature on germination testing and concluded that the written process developed by Zucconi and Bertoldi (1981) is difficult to replicate (Lee et al., 2002). Following (Gariglio et al., 2002), who used lettuce as an indication in a plant growth bioassay, pinto bean and tomato as indicators in a plant growth bioassay starting in 1999 Fauci et al. (2002).

Smith and Hughes. (2001) conducted a study comparing cress germination and cellulolytic activity. During composting, the generation of phytotoxic chemicals is a fleeting phenomenon. It is highest during the first stage when the organic waste is rapidly destroyed and diminishes during the stability stage when "humification" and mineralisation predominate. In the last stages of composting, toxicity may decrease due to a variety of causes, primarily the metabolic breakdown of some phytotoxic organic compounds and the integration of certain phytotoxic chemicals into the 'humic acids' fraction (Zucconi, 1987). Several studies have been conducted to investigate the toxicity of compost/vermicompost using various plant models before applying it to agricultural areas.

Suthar and Sharma (2013) tested the phytotoxicity of vermicompost made from the invasive plant Lantana camara. The germination index test was performed, and the results showed that the GI % ranged between 47-83 % in all vermicomposts, as shown by the seed bioassay test (Zea mays seed). A GI of 50% was employed as an indicator of phytotoxin-free compost, and the results indicated that the vermicompost was suitable for agronomic uses.

Datta et al. (2018) reported that the A. cepa test is a valuable bioindicator of cytotoxicity and genotoxicity, besides acting as a warning for the population that utilises pesticides indiscriminately. According to the genotoxicity research of soil samples, the use of both inorganic and organic pesticides causes soil pollution and contamination. After 24 and 48 hours of exposure, the mitotic index was lowered to 10.3 and 9.7 in pesticide-treated soil and 24.4 and 25.4 in vermicompost-treated soil, respectively. Clastogenic aberrations were highest (54.5%) in pesticide-treated soil, which differed considerably from vermicompost-treated soil extract. Pesticide-treated soil extracts had considerably greater cytotoxic and genotoxic effects on *A. cepa* than vermicompost-treated soil. The results show that adding vermicompost to agricultural fields functions as a soil improver and plays an essential role in promoting cell division and proliferation, which is beneficial to plant health and crop productivity.

Khadra et al. (2019) evaluated the chemical, biological, and eco-toxicological parameters of compost made from dewatered primary sludge and date palm trash co-composting. The single and combined toxicity of antibiotics (ciprofloxacin, enrofloxacin, nalidixic acid, roxithromycin, and sulfapyridine) and the metal chromium were investigated. Although the final compost product significantly reduced genotoxicity, about half of the micronucleus frequency remained, which might be explained by the persistence of certain refractory chemicals such as chromium and several antibiotics. Overall, the presence of antibiotics and chromium demonstrated that certain combinations of pollutants pose an ecological risk to soil health and ecosystems, even at environmentally insignificant concentrations.

Wang et al. (2021) investigated the vermicomposting of spent drilling fluid (SDF) from the nature-gas industry mixed with cow dung employing *E. fetida* under a 6-week trial. In terms of growth and reproduction, *E. fetida* performed better in the first three vermireactors, but mortality was higher (40%) in the vermireactors that included more wasted drilling fluid. The lower phototoxicity, cytotoxicity, seed germination, mitotic index, and chromosomal abnormality displayed on testing using cowpea indicated that the vermicomposts are appropriate for agricultural usage.

2.6 THE BENEFIT OF COMPOST SOIL APPLICATION AND PLANT GROWTH

A related concern is the vast accumulation of organic waste by human activities, which has prompted the development of several alternatives to landfilling and accelerated recycling processes. Composting is among the most well-known and well-established processes which stabilise and sanitise organic waste by accelerating aerobic decomposition under regulated circumstances, resulting in compost production. It has been shown that the use of compost can significantly reduce the use of ammonia-type fertilisers, which account for approximately 2% of total natural gas consumption in the United States. Ammonia-type fertilisers are manufactured using approximately 2% of total natural gas consumption in the United States (Schanfeld et al., 2003). Since the compost is predominantly comprised of NPK and other micronutrients, it can be utilised as a fertiliser in various situations. If the amount of ammonia lost during the composting process is decreased, the majority of the nitrogen can be trapped in the compost. The addition of compost to the soil nourishes it and increases the number of important nutrients and organic matter in the soil. As a result, soil physical qualities such as bulk density and porosity are improved, as well as cation exchange capacity, the presence of various chemicals and improvement in biological characteristics (Tits et al., 2014; Weber et al., 2007). In addition to lowering the financial burden of acquiring chemical fertilisers, compost can also assist in mitigating the negative environmental impacts connected with the manufacture of chemical fertilisers and their use (Jain and Kalamdhad, 2020). Recent research has explored the significance of compost application in the soil (Goswami et al., 2017; Ren et al., 2018), though an appreciable amount of further research is still awaited. According to many authors, compost-treated soils had statistically significant positive effects on the overall health of the soil when compared to untreated soils in the study. For example, Willekens et al. (2014) reported an increase in the accessible potassium contents of the soil following the application of compost to the soil. By preventing nutrients from seeping into the groundwater, compost has also been beneficial to the soil environment (Grey and Henry, 1999).

Using compost/vermicompost made from a pernicious weed such as water hyacinth, Gajalakshmi and Abbasi (2004) investigated the effect of it on kitchen gardens containing lady's finger (*Hibiscus esculentus*), brinjal (*Solanum melongena*), cluster bean (*Cyamopsis tetragonoloba*) chilli (*Capsicum annum*), and tomato (*Lycopersicon esculentum*). Hussain et al. (2020) reported the possibility of using the invasive weed Ipomoea carnea as an organic fertiliser. The effect of Ipomoea vermicompost at four different levels (0, 2.5, 3.75, and 5 tonnes/ha) on the germination, growth, and fruition of a lady’s finger (*Abelmoschus esculentus*) was reported. The vermicompost was found to promote the germination and growth of a lady’s finger at all levels, with the highest results coming from 5 t/ha treatments. The beneficial effect was seen at every step of *A. esculentus* cultivation, from seed germination to vegetative growth phases and fruit output. Due to the vermicompost application, the quality of fruits improved in terms of mineral, protein, and carbohydrate content increased versus a decrease in disease incidence and pest attacks. According to (Hussain et al., 2016), vermicompost obtained only from the action of the epigeic earthworm *E. fetida* on *Parthenium* (*Parthenium* *hysterophorus*) had a favourable effect on the green gram (*Vigna radiata*), ladies finger (*Abelmoschus esculentus*), and cucumber (*Cucumis sativus*).

2.7 INFERENCES FROM THE LITERATURE

Several conclusions can be derived from the literature regarding the administration of biodegradable organic waste:

- Composting is the most viable option for mitigating organic waste management problems in urban and industrial contexts. Composting expedites the decomposition of organic waste in a relatively efficient manner.
- Composting techniques involving rotary drums have the advantage of producing high-quality compost output. This technology can contribute to better waste management practices and facilitate greater comprehension of waste management processes.
- There is a need for additional research and study in the field of waste management, particularly the investigation of a larger number of reactors and the expansion of their capacity. Composting processes at a larger scale have been the subject of few studies, which hinders the development of effective waste management strategies.
- The combination of rotary drum composting and vermicomposting has the potential to increase the compost's quality while reducing production time. This comprehensive strategy has the potential to address waste management issues in cities with a high production of organic refuse.
- While efforts have been made to optimise the duration of composting by modifying traditional processes, there is a paucity of research on incorporating various composting techniques as pre- or post-composting methods. This indicates a possible area for future research and innovation.
- Existing research focuses primarily on bulk analysis and small-scale vermireactors with limited capacity. In the context of waste management in India, however, research on decomposition methods employing larger reactors, such as those with a higher capacity, is lacking.
- The literature emphasises the need for sophisticated composting techniques, such as rotary drum composting, and the integration of complementary methods, such

as vermicomposting, to address the challenges associated with organic waste management in urban and industrial settings. Further research is required to investigate the scalability and optimisation of these waste management techniques.

2.8 GAP OF KNOWLEDGE

According to the literature, implementing decomposition processes is the most effective way to resolve the challenges associated with organic waste management. Composting techniques, such as rotary drum composting, offer a promising solution because they ensure the rapid decomposition of organic waste. To gain a deeper comprehension of waste management, it is necessary to examine a larger number of reactors and increase their capacity to manage larger waste volumes. However, there are very few available studies on large-scale decomposition processes particulate to Indian conditions.

The innovative concept of combining rotary drum decomposition with vermicomposting can serve as an effective alternative for municipal corporations in Guwahati, Mumbai, Delhi, and other major cities where waste management has become difficult. The implementation of rotary drum decomposition techniques in waste management has the potential to address significant waste management challenges, particularly in countries with high organic waste production. The combination of rotary drum composting and vermicomposting can improve the quality of compost produced in a time-efficient manner, making this technology applicable to a variety of organic waste management applications.

While many authors have focused on optimising composting duration by modifying traditional composting processes, very few have investigated various pre- and post-composting techniques. In addition, no authors have attempted to combine two distinct techniques into a singular decomposition method to manage the high organic burden. Existing research focuses primarily on bulk analysis and vermi-reactors with a capacity of less than 0.5 m^3. In the context of waste management in India, there is a dearth of research on decomposition techniques involving large-scale reactors. Consequently, this study on rotary drums followed by vermicomposting presents a novel concept for implementing large-scale reactors in both technologies.

Chapter 3

OBJECTIVES OF THE STUDY

This chapter mainly deals with the knowledge gap, research objectives, the need of the study, the scope of the study.

3.1 OBJECTIVES OF THE STUDY

Based on the literature survey on rotary drum composting and vermicomposting, the main objective of this study is to effectively manage a large volume of organic waste by combining these two processes in series. The aim is to identify the most efficient approach for reducing various types of organic waste through composting, as an alternative to open dumping in landfills or improper disposal methods. The scope of the present study is defined as follows:

1. Efficacy study using a batch mode: The research involves assessing the effectiveness of a batch mode composting reactor with a capacity of 550 L in series with vermicomposters of 3 L capacity of various organic wastes. This step focuses on evaluating the performance of the rotary drum composter followed by vermicomposting in decomposing the organic waste.
2. Scaling up to a 5000 L rotary drum reactor and 3000 L vermi reactor: Based on the efficacy studies conducted in the batch reactors, the study progresses to a larger-scale operation. This step aims to observe the performance and efficiency of the larger reactors in managing significant amounts of organic waste.
3. Application of produced compost through pot and field studies: The study includes the assessment of the compost produced by the combined rotary drum and vermicomposting process. Pot studies and field studies will be conducted to understand the impact and effectiveness of applying the compost to soil. This step aims to evaluate the quality and suitability of the produced compost as a soil amendment.

By focusing on these specific objectives, the research aims to contribute to the understanding of combined rotary drum and vermicomposting processes for managing large volumes of organic waste. The outcomes of the study will provide insights into the efficacy, scalability, and applicability of this approach in waste management practices.

3.2 NEED FOR THE STUDY

Adopting composting techniques, such as the rotary drum composter, is necessary to achieve a comprehensive solid waste management system, especially for urban communities where a variety of biological waste-generating units operate daily. To manage masses of waste produced by communities, the waste management system cannot avoid viable options like composting. This study is required to determine the best alternative to disposal in landfills or burning at the community level for managing the large quantities of organic waste, vegetation, and sludges produced in urban areas. To manage the enormous amounts of waste produced by the community, it is necessary to have a deeper comprehension of the community's routines, including the materials involved, the primary categories of waste to be managed, and the quantity of waste in different seasons. To effectively manage waste, it is necessary to gain a deeper understanding of each individual process and, if possible, to employ a combination of processes. The finest example is the combination of rotary drum and vermicomposting, in which the drawbacks of one procedure provide a highly adaptable alternative for another. By combining two distinct composting techniques, one of which produces a high quantity (rotary drum) and the other of which produces a high quality (vermin composting), it is possible to manage massive volumes with a product that is economically viable. For optimal use of soil fertility, natural or biofertilizers are necessary due to the increased use of artificial or chemical fertilizers, which are destroying the soil bacterial burden, which is the root cause of soil fertility. On a daily basis, the news reports numerous instances of a large quantity of soil becoming infertile and the loss of many lives. It is time to examine the long-term effects of any fertilizer applied to agricultural soil.

3.3 SCOPE OF THE STUDY

The scope of this work incorporates a number of important facets associated with the implementation of a rotary drum composter for organic waste management:

- Optimization of composting duration and final product quality: The objective of this research is to optimize the input ratio of organic waste and inoculum in the rotary drum composter in order to accomplish an effective composting duration of various organic biomasses. Additionally, the quality of the produced compost will be evaluated with the aim of producing a high-quality final product.

- Fabrication of a 5000 L capacity rotary drum reactor: The research concentrates on the fabrication of a 5000 L capacity rotary drum composter. This involves designing and constructing the reactor in accordance with established requirements and standards.
- Operation of the reactor with various categories of organic waste: The fabricated rotary drum reactor will be used to treat various types of organic waste. The purpose of this study is to evaluate the efficacy and efficiency of the reactor in decomposing these waste materials and producing compost.
- Understanding the behavior of the reactor under heavy organic loads: The research centers on analyzing the efficacy and limitations of the scaled-up rotary drum reactor when subjected to substantial amounts of organic waste. This investigation seeks to determine the reactor's capacity to efficiently manage and process large amounts of refuse.
- Analysis of decentralized waste management the study examines: The use of rotary drum composters to address the need for decentralized waste management facilities. The objective of the research is to develop alternative methods for managing organic waste and to reduce reliance on centralized waste management systems.
- Contribution to waste management knowledge: The purpose of this study is to contribute to the extant corpus of waste management knowledge by shedding light on the practical application and scalability of rotary drum decomposition technology. The research findings may inform future waste management practices and provide guidance for the application of large-scale composters in various settings.

Chapter 4

MATERIALS AND METHODS

This study utilized a range of experimental techniques to meet the defined objectives. The research was conducted across multiple phases and involved testing different combinations of raw materials. A comprehensive methodology for the study is presented in this chapter.

4.1 EXPERIMENTAL FLOWCHART

The study was conducted in four phases to complete the objectives of this thesis. In Phase-I Batch-scale reactors were used to assess the combined efficiency of rotary drum composting and vermicomposting of the screened and selected biodegradable waste (substrates). Feasibility tests revealed *Eisenisa fetida* and *Eudrilus eugeniea* vermicultures to be the most effective. Phase II evaluated the phytotoxicity of the vermicomposts produced on the seed model *Vigna radiata*, followed by soil application of the tested vermicompost and compost in pots containing alluvial soils with *Coriandrum sativum* model. Phase III witnesses scale shift from batch to pilot-scale, using a 5 m^3 rotary drum composter, equipped with a 3 m^3 vermicomposting facility with the selected *Eisenia fetida* to assess feasibility of bag and stack composting. In Phase IV, a field scale assessment of vermicompost was carried out and analyzed via changes in soil and plant morphology in *Coriandrum sativum*, *Okra* and *Rhaphanus sativum* Fig. 4.1 depicts the comprehensive research methodology.

4.2 PHASE-I: BATCH SCALE STUDIES ON ROTARY DRUM COMPOSTING FOLLOWED BY VERMICOMPOSTING

4.2.1 Substrates considered in the study

A wide range of feedstocks were included to analyze in a batch reactor. The major wastes and invasive weeds include

1. Vegetable waste.
2. Sewage sludge.

3. Terrestrial weeds.
4. Aquatic weeds.

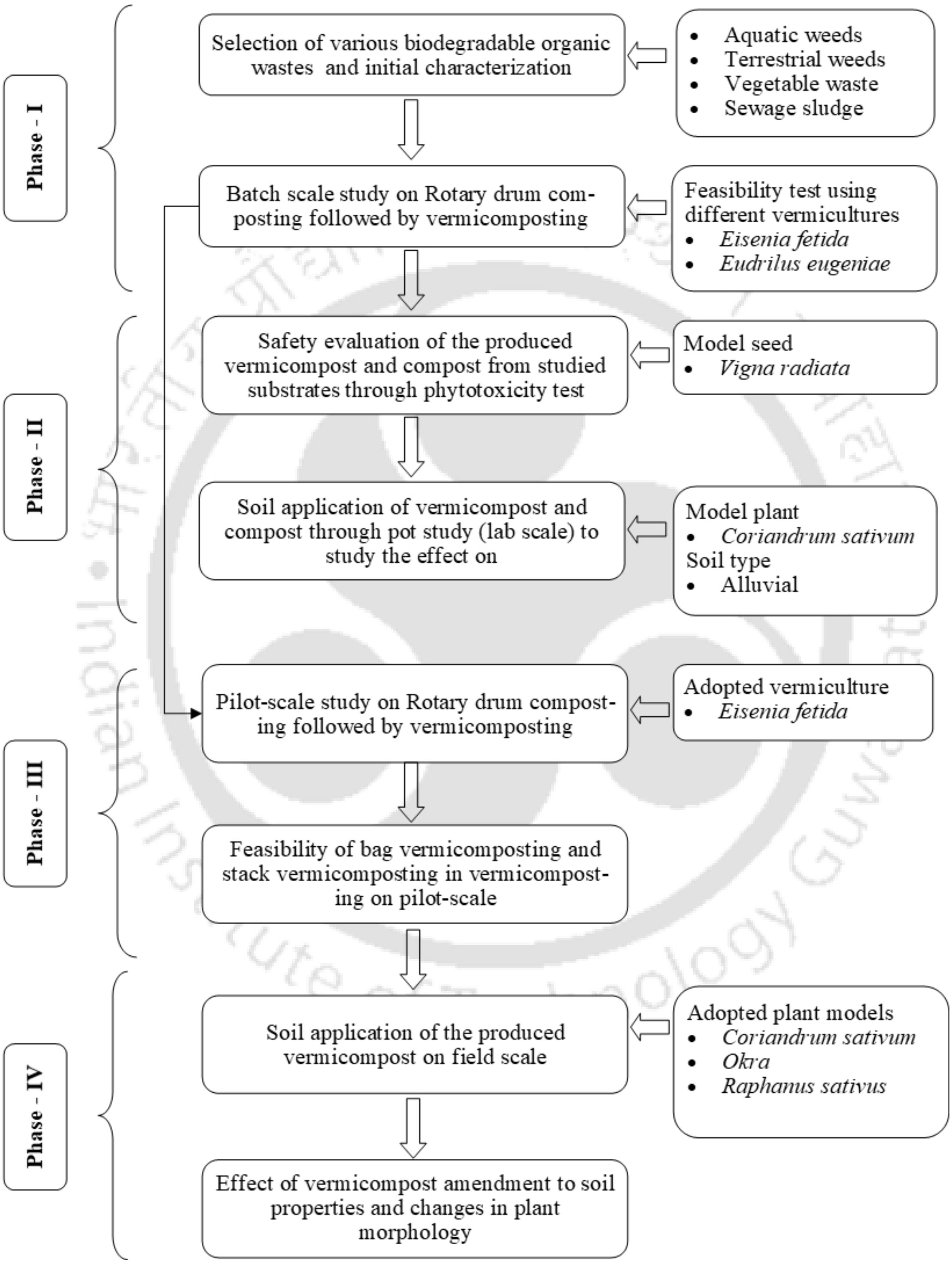


Fig. 4.1 Experimental flow chart

All the feedstocks were sourced from the IIT Guwahati campus, in Assam, India. Aquatic weeds (*Pontederia crassipes* and *Hydrilla verticillata),* terrestrial weeds (*Mikania micrantha*, *Parthenium hysterophorus*, *Lantana camera*, and *Azaridam conoids)* were collected from the ponds, lakes and terrestrial areas of the campus (Fig. 4.2). Vegetable waste was collected from campus hostels, and sewage sludge was collected from the wastewater treatment plant behind the Kameng hostel. Only cow dung and sawdust, and collected from Amingaon, Guwahati adjoining the campus were used to mix wastes shredded to 2-3cm.

Urban organic wastes of concern

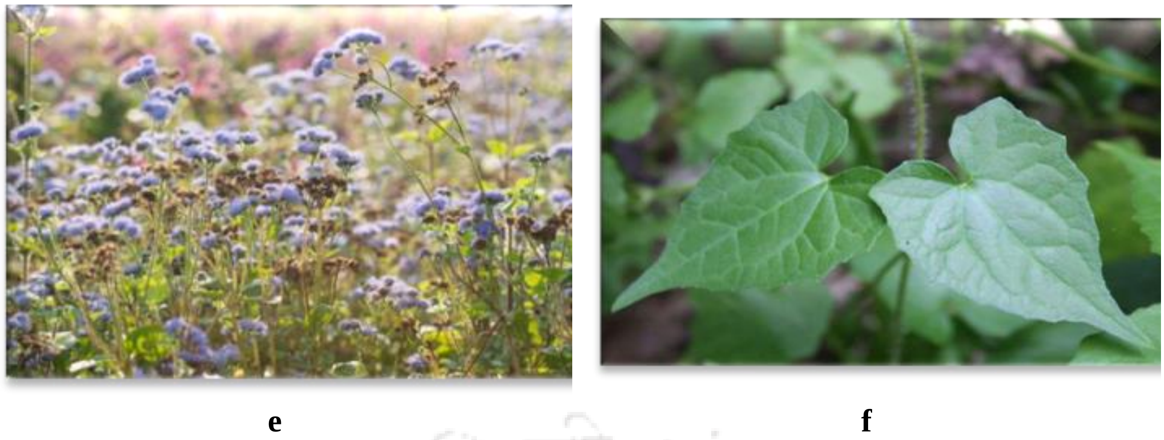
a

b

Terrestrial weeds

c

d



Aquatic weeds

Fig. 4.2 Substrates studied in the study a) vegetable waste, b) sewage sludge, c) *Parthenium hysterophorus* d) *Lantana camera* e) *Azaridam conoids* f) *Mikania micrantha* g) *Hydrilla verticillate* and h) *Pontederia crassipes*

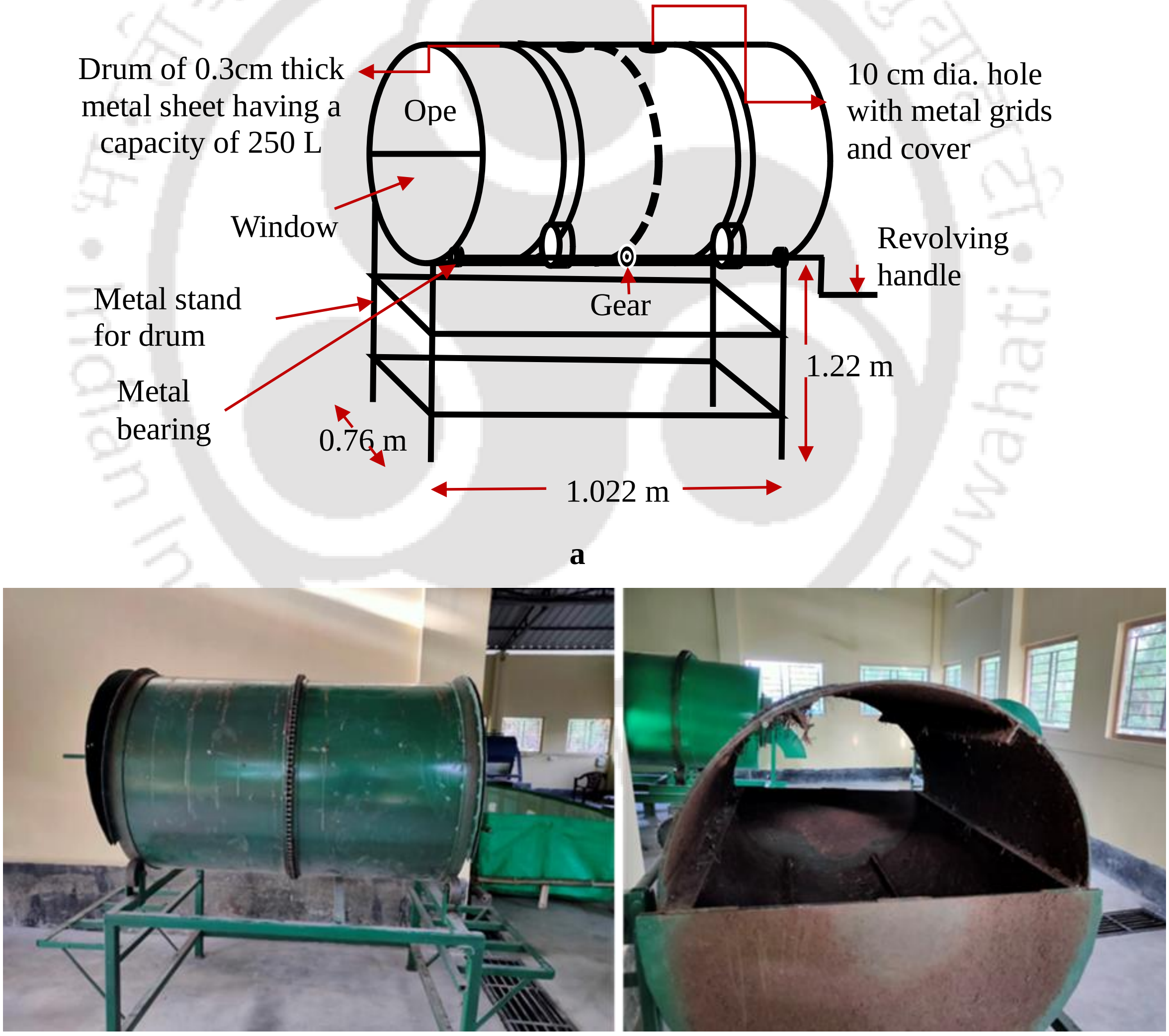
4.2.2 Initial characterization

The waste substrate, inoculum, and sawdust were initially characterized by analyzing essential physicochemical, biological, and biochemical parameters. Initial characterization demonstrated that aquatic weeds had higher moisture content than other feedstocks. In addition, their high volatile solids and the initial nitrogen content were constraints for efficient composting. While, the moisture content of terrestrial weeds was in the optimum range for composting, and the C/N ratio suited the composting process. Vegetable wastes, characterized by high oxygen uptake rate and below par C/N ratio; lead to higher rates of degradation in the initial days of composting, and necessitated bulking with sawdust and garden cuttings for effective composting.

4.2.3 Experimental setup

Rotary drum composter

The schematic representation of a batch rotary drum composter is shown in Fig. 4.3. The 550 L capacity drum was mounted on four rubber rollers, attached to a metal stand and rotated mechanically by a revolving handle facilitated batch mode operation. The length and diameter of the central unit of the drum were 1 m and 0.76 m, respectively, and the thick metal sheet of 4 mm were welded longitudinally at 40×40 mm angles inside the drum to ensure the appropriate mixing, agitation, and aeration of the wastes during rotation. The interior of the drum was quoted with anti-corrosive material.



a

b

Fig. 4.3 a) Schematic diagram of the reactor b) Batch scale rotary drum composter

Table 4.1 Initial characterization of raw materials used in the study

Parameters	***Eichhornia crassipes***	***Hydrilla verticillata***	**Vegetable waste**	**Sewage sludge**	***Lantana camara***	***Ageratum conyzoid***	***Mikania micrantha***	***Parthenium hysterophorus***	**Saw dust**	**Cow dung**
Volatile Solids (%)	76.8 ± 1.1	70 ± 3.5	77 ± 3.1	42.69 ± 1.24	65.19 ± 1.43	70.19 ± 1.63	77 ± 3.1	72.05 ± 2.1	86.6 ± 1.3	87.8 ± 1.4
pH	5.94 ± 0.3	7.5 ± 0.2	5.63 ± 0.02	6.63 ± 0.18	6.92 ± 0.08	6.36 ± 0.11	8.1 ± 0.2	6.68 ± 0.07	6.1 ± 0.01	6.5 ± 0.01
Electrical Conductivity (mS/cm)	4.5 ± 0.2	3.3 ± 0.2	2.35 ± 0.04	2.62 ± 0.11	4.83 ± 0.12	5.07 ± 0.08	4.3 ± 0.4	5.1 ± 0.05	0.6 ± 0.03	3.4 ± 0.02
Moisture Content (%)	92 ± 1.7	93 ± 1.4	90.12 ± 2.22	86.15 ± 0.21	72.1 ± 0.87	74.2 ± 0.28	87 ± 2.4	69.2 ± 1.34	13.2 ± 1.1	85.3 ± 1.9
CO_2 evolution rate (mg/gVS/day)	1.66 ± 0.2	3.2 ± 0.2	22.12 ± 1.32	5 ± 0.83	2.88 ± 0.17	4.42 ± 0.09	2.32 ± 0.2	4.91 ± 0.08	-	-
OUR (mg/gVS/day)	1.89 ± 0.3	5.85 ± 0.5	26.55 ± 0.6	4.04 ± 0.12	3.21 ± 0.12	3.22 ± 0.25	6.83 ± 0.5	3.81 ± 0.32	-	-
TKN (%)	2.38 ± 0.1	3.4 ± 0.2	2.66 ± 0.2	1.68 ± 0.073	1.4 ± 0.07	1.36 ± 0.05	2.5 ± 0.2	1.12 ± 0.09	0.35 ± 0.1	1.5 ± 0.1
Total phosphorus (g/kg)	2.3 ± 0.3	4.8 ± 0.5	6.68 ± 0.20	2.59 ± 0.34	3.083 ± 0.34	3.93 ± 0.27	12.18 ±0.1	2.71 ± 0.12	2.2 ± 0.3	5.0 ± 0.3
C/N ratio	17.92	11.4 ± 1.1	16.10 ± 2.3	14.12 ± 0.34	22.72 ± 1.34	24.93 ± 1.78	17.11 ± 2.1	25 ± 1.02	137.45 ± 12.8	32.51 ± 1.5

Vermicomposter

A plastic container was used to avail adequate aeration for the compost. The reactor was first lined with paper followed by material from dry banana trunks to provide a conducive environment for growth of earthworms. Three different earthworm species, i.e., *Eisenia fetida*, *Eudrilus eugeniae* and *Perionyx cylarences* were then applied to degrade the compost. All reactor runs were conducted in triplicate for each earthworm species and the same parameters were maintained for analysis. As earthworms eat half their body weight per day, the number of earthworms was optimized to 180 earthworms per species/ in total per reactor and supplied with 2.5 kg of feed. In the control reactor no earthworms were introduced.

Fig. 4.4 Batch scale vermicomposting unit experimental setup

4.2.4 Sampling and Analysis

A 500 g sample was collected from the rotary drum composter and vermicomposts for biological, physicochemical, and biochemical analysis. The sample was prepared by taking representative/grab samples from 9 different points, mainly from the mid-span and end terminals of the pilot-scale rotary drum composter, after drum rotation, to ensure a homogenized sample. These homogenized samples were collected at two-day intervals for drum composting. Finally, all the representative/grab samples were mixed thoroughly to make a homogenized sample. Triplicate samples were collected, immediately air-dried, ground through a 0.2 mm sieve, and stored for physicochemical and biochemical analysis. The sub-samples were either used or stored at 4°C for biological analysis of the wet sample within 2 days.

i) Physicochemical analysis

Relevant experimental procedures used for physicochemical analysis of the substrate, cow dung, and sawdust were conducted in the Environmental engineering laboratory of the Civil Engineering Department, IIT Guwahati.

Temperature

The substrate temperature was monitored during batch and pilot-scale composting, every 6 hours using a digital thermometer. While temperature measurement at 12 different locations every 24 hours was carried using an analog thermometer.

Moisture content

The gravimetric method was used to determine compost moisture content moisture by weighing it before and after drying it at 105°C for 24 hours.

pH and electrical conductivity (EC)

The pH and electrical conductivity were measured by stirring sample of compost in distilled water and measuring the pH with a calibrated pH meter. Subsequently, the electrical conductivity was measured using a conductivity meter after filtering the mixture through Watman filter paper No. 42. The EC and pH were measured in filtered supernatant (BIS: 10158-1982).

Volatile solid (VS) and ash content (AC)

Volatile solid (VS) and ash content were also measured according to APHA (2012). The initial weight of the crucible was measured as W1. About 10 ± 0.1g of the sample was weighed and heated in hot oven air for 24 hours at 100 ± 5°C. The weight of the crucible after 24 hours was noted as W2. Crucibles were then placed in a muffle furnace at 550°C for 2 hours. The final weight of the crucible was designated as W3. VS was expressed as a percentage of total solids, as given in equation (4.1.)

$$(W2-W3)/(W2-W1) = VS\ (\%) \qquad (4.1)$$

Total nitrogen (TN or TKN) and ammonical nitrogen (NH_4-N)

Total nitrogen (TN) was analyzed using the Kjeldahl method and NH_4-N (KCl extraction) (Tiquia and Tam, 2000). For TN analysis, 0.2 g of sample (passed through 0.22 mm sieve) was taken, and 3g of catalyst mixture (potassium sulfate and cupric sulfate, 5:1) o was added and digested with 10 mL conc. H_2SO_4 using digestion equipment at 400°C for 4 h. The end color of the digested sample was green which was reconstituted to 100 ml. From the diluted sample10 mL was distilled using a distillation unit (Pelican Equipment, Chennai, India) with 40% NaOH and distilled water; the distillate was collected in 25 mL boric acid with a mixed indicator. Collected distillate displaying clear green cooler, was titrated with 0.02 N H_2SO_4 till a purple endpoint was achieved.

Nutrients and trace elements

The Flame photometer (Systronic 128) was used to analyze Na, K, and Ca concentrations. Mg concentration was measured by atomic absorption spectrometer (AAS) (Varian Spectra 55B) after the digestion of 0.2 g sample with 10 mL of H_2SO_4 and $HClO_4$ (5:1) mixture, using a block digestion system (Pelican equipment, Chennai, India) for 2 h at 300°C.

ii) Biological analysis

Soluble Biochemical Oxygen Demand (sBOD) (APHA, 2012)

About 10 ± 0.1g of fresh compost was taken in a conical flask and dissolved in 100 ml of distilled water. The flask was kept in a horizontal shaker for 2h, and subsequently filtered using What man filter paper (Grade no. 42). The supernatant of samples was taken and analyzed by the BOD_5 test and equation as given in Eq. 4.2.

Calculation:

$$BOD_5,\ mg/L=(D1-D2)/P \quad (4.2)$$

Where

D1= initial DO of sample in mg/l,

D2= final DO of sample after 5-day incubation in mg/l,

P = sample volume (in ml) diluted to 300 ml with dilution water.

Soluble Chemical Oxygen Demand (sCOD) (APHA, 2012)

About 10 ± 0.1g of fresh compost was taken in a conical flask and dissolved in 100 ml of distilled water. The flask was kept in a horizontal shaker for 2h. Then it was filtered using Whatman filter paper. The sample supernatant was taken and analyzed by the closed reflux method; where 1.5mL K2Cr2O7, 2.5mL of the sample, and 3.5mL of COD acid were added to the COD vials and shaken well. The mixture was digested in a COD digester at 150°C for 2 hours, and then left to cool to room temperature. Using the Ferroin indicator, the mixture was then titrated against Ferrous Ammonium Sulphate (FAS). The requisite colour change was from yellow to wine red. The sCOD was calculated using the following equation 4.3.

$$\text{sCOD (mg/L)} = ((A-B) \times \text{Molarity of FAS} \times \text{Dilution factor} \times 8000)/(\text{Volume of sample}) \qquad (4.3)$$

CO_2 evaluation by Soda-Lime method ((Kalamdhad et al., 2008)**)**

About (25 ± 0.1g) of fresh compost sample was taken in a 1-litre PVC airtight container. 10g of soda lime oven-dried at 105°C and grinded to 1.5 - 2.0 mm size mesh was taken in a 100ml beaker and placed in the above container. The initial weight of the soda-lime was taken as (W1) g. The container with a soda-lime beaker was kept in an incubator, set at a temperature of 25°C. After 20 - 24 h, the soda-lime was taken out and oven-dried again, and the final weight noted as (W2) g. The difference in mass of soda-lime will give the amount of CO_2 absorbed, vide equation 4.4.

Calculation:

$$CO_2 \text{ evolution rate ("mg/g VS/day")} = ((W2-W1))/(W \times T) \times 1000 \qquad (4.4)$$

Where, W1 = Initial weight of the soda-lime (g), W2 = Final weight of the soda-lime (g), W = Weight of compost sample taken (g), T = Time duration of incubation (h)

Oxygen uptake rate (OUR) (Kalamdhad et al., 2008)

The oxygen uptake rate (OUR) was performed according to the method described by (Lasaridi & Stentiford, 1996). The OUR was measured in a liquid suspension of compost (5-8g in 500 ml of distilled water mixed with $CaCl_2$, $MgSO_4$, $FeCl_3$, and phosphate buffer at pH 7.2). The solution was suspended by placing it on the magnetic stirrer at constant temperature and keeping the whole assembly in the water bath at 30°C. During this time, the dissolved O_2 of the suspension was continuously observed through the attached digital DO meter. The oxygen consumption rate or OUR in mg O_2/gmVS/hour was calculated from the change in DO at the designated time intervals.

Coliform analysis (APHA, 1995).

The Coliform testing aimed to identify: total coliforms (TC), fecal streptococci (FS), and fecal coliforms (FC). A sample of 10 g was blended with 100 ml of deionized water; and the resultant liquid formed on top of the mixture was used for testing. Subsequently, this liquid was added to culture tube media containing Lauryl tryptose broth, Azide dextrose broth, and EC medium respectively, using the Most Probable Number (MPN) method as outlined in APHA's 2012 guidelines.

iii) Biochemical analysis

According to the National Renewable Energy Laboratory procedure, the difference between acid-soluble lignin and acid-insoluble lignin was taken as lignin (Ehrman, 1996; Templeton, 1995). Cellulose was determined by the acetic/nitric reagent extraction method, as reported by (Varma et al., 2017), and hemicellulose determined by the protocol explained by (Varma et al., 2017). Lignin measurement was conducted by taking 0.3 g of powdered sample, recorded as W1, digested using 72% H_2SO_4, and filtering the extract. The acid-soluble lignin was measured from the filtered sample by measuring the absorbance at 205 nm.

Earthworm growth

In batch reactors, the earthworm count was done manually in all the triplicated reactors on day 10 and day 20 of the composting. In pilot-scale reactors, the study was conducted initially on day 0 and finally on day 20 (Meena and S, 2011). The growth in earthworm culture was based on the increase in number and the number of cocoons produced in regular interval.

Spectroscopic analysis of compost

Fourier transform infrared spectra (FTIR) were obtained by mixing dried KBr powder with a pre-desiccated sample to analyze the functional groups involved in the composting process. The mixture was then compressed under a pressure of 10 MPa for 3 minutes to obtain translucent disc samples. The FTIR spectra were recorded over 400 to 4000 cm-1 wavenumbers with a 4 cm-1 resolution and 16 scans (Paul et al., 2020).

Statistical analysis

The reported results were obtained from three separate replicates, and the statistical analysis was aimed at the possible significant differences among the parameters analyzed during the composting process. In this study, determined averages of three independent samples and their standard deviations were presented. For statistical analysis, SPSS-20 was employed, and $P<0.05$ (significant) was plotted. All of the Fig.s were created using the OriginPro-22 software.

4.3 PHASE-II: PHYTOTOXICITY STUDY OF THE END PRODUCTS AND SOIL APPLICATION THROUGH LAB-SCALE POT STUDY

4.3.1 Phytotoxicity using *Vigna radiata* seed model

The phytotoxicity test was conducted following a modified procedure based on Haq and Kalamdhad (2021) and Kauser et al. (2020). The samples contained either fresh waste or compost produced from the rotary drum process or vermicompost from the two-stage biodegradation process. The preparation of samples involved the combination of a 100g single sample, with 300 mL of dH_2O, followed by mechanical shaking in a rotary shaker for 24 hours at 120 rpm to attain a homogenous mixture. After 24 hours of uninterrupted agitation, the samples were subject to filtration procedures, and the resultant filtrates were subsequently utilized to assess toxicity. Various dilutions (0, 25, 50, 75, and 100% v/v) were employed to formulate solutions of the substrate, rotary drum compost, and rotary drum composting, followed by vermicompost extract, utilizing dH_2O. Fig. 4.5 depicts the utilization of *Vigna radiata* (Mung bean) as a test plant for toxicity assessment. The Mung bean was procured from a certified vendor in Assam's Guwahati region. Before the commencement of the experiment, Mung bean seeds underwent appropriate sterilization by immersion in a 0.1% w/v $HgCl_2$ solution for 10 minutes. Subsequently, the seeds were thoroughly washed to eliminate any residual traces of $HgCl_2$. The study involved the selection of healthy seeds, which were then transferred to Petri dishes for evaluation and sprout length inhibition test. This methodology follows the guidelines established by Haq et al. (2016). The experiments were performed in triplicate, and the plates were incubated at 25 ± 1°C for 4-5 days. Following a 5-day incubation period, an analysis was conducted on the growth and biomass of seedlings, with a light cycle lasting 8 hours and a dark cycle lasting 16 hours. Equation (4.5) shows how the phytotoxicity of various dilutions was tested using a seed germination index (SGI %).

$$\text{SGI (\%)} = \frac{\text{Number of germinated seeds} \times \text{Average plant length in the sample}}{\text{Number of germinated seeds in control} \times \text{Average plant length in control}} \times 100 \quad (4.5)$$

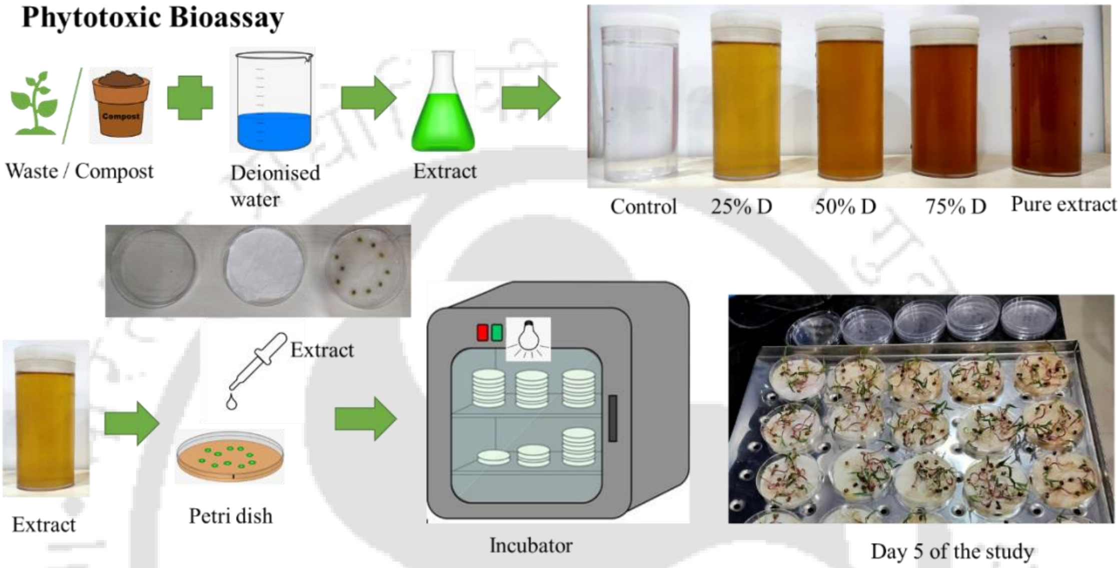


Fig. 4.5 Phytotoxicity study

4.3.2 Pot study using *Coriandrum sativum*

Pots of size 10L were chosen with a height of about 28 cm & an upper diameter of 30 cm, as shown in Fig. 4.6. The material of the pot was hard plastic, with a few holes drilled at the bottom for excess water drainage and a 2-3 cm thick gravel layer placed as the base. Seeds of *C. sativum* were purchased from a certified local seed vendor in Guwahati, Assam, and alluvial soil was obtained from the adjacent area of the IITG campus. The soil weight was kept constant (4kg) (Jain & Kalamdhad, 2020), and compost was applied in the proportion (0%, 10% and 25%). *C. sativum* is a warm-season crop that thrives when watered during the summer or rainy season, but it may be grown all year in tropical, subtropical, and temperate climates. It can flourish in a wide range of soil types as long as the soils are well-drained. The experimental setup was carried out on the terrace of the Civil Engineering Department and was protected from rainfall for the entire study duration. The two replicates of each treatment pot were used for the experiment. Details of the pot study have been illustrated in Fig. 4.6. Pots were placed in the open space to ensure 8-9 hours of direct sunlight. Seven seeds were potted in each pot and watered twice daily (morning and evening). The experiment was carried out for 30 days to ensure optimal growth and the development of appropriate morphological characteristics in the plant. The length of the fruit was measured immediately after collection and rinsed with distilled water before being oven-dried for 24h at 105°C. For additional investigation, dried samples were ground and sieved through a 0.2 mm mesh screen. Plant morphology was taken in to consideration to evaluate the effect of soil amendment with compost/vermicompost in comparison to control soil.

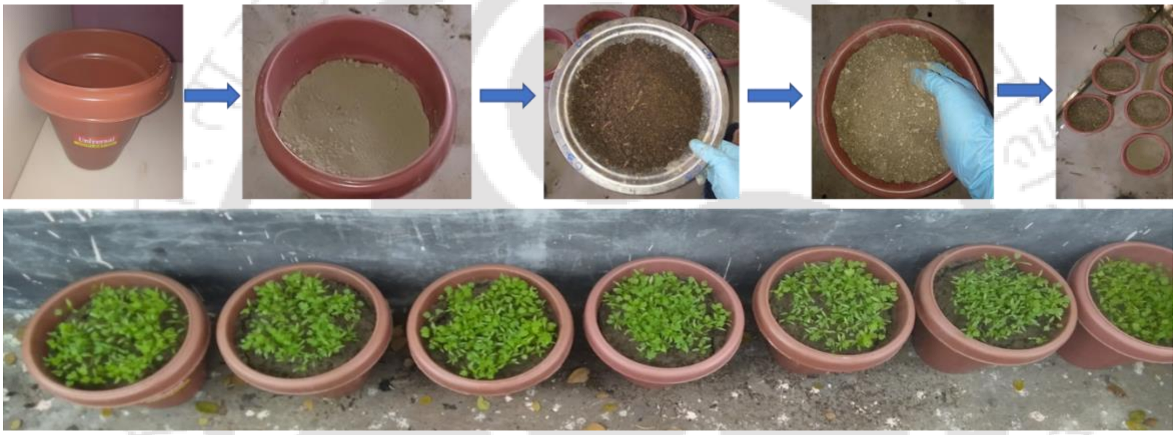


Fig. 4.6 Pot study setup using alluvial soils

4.4 PHASE-III: PILOT-SCALE STUDIES OF TWO-STAGE BIODEGRADATION TECHNIQUE

4.4.1 Experimental setup

i) Rotary drum composter

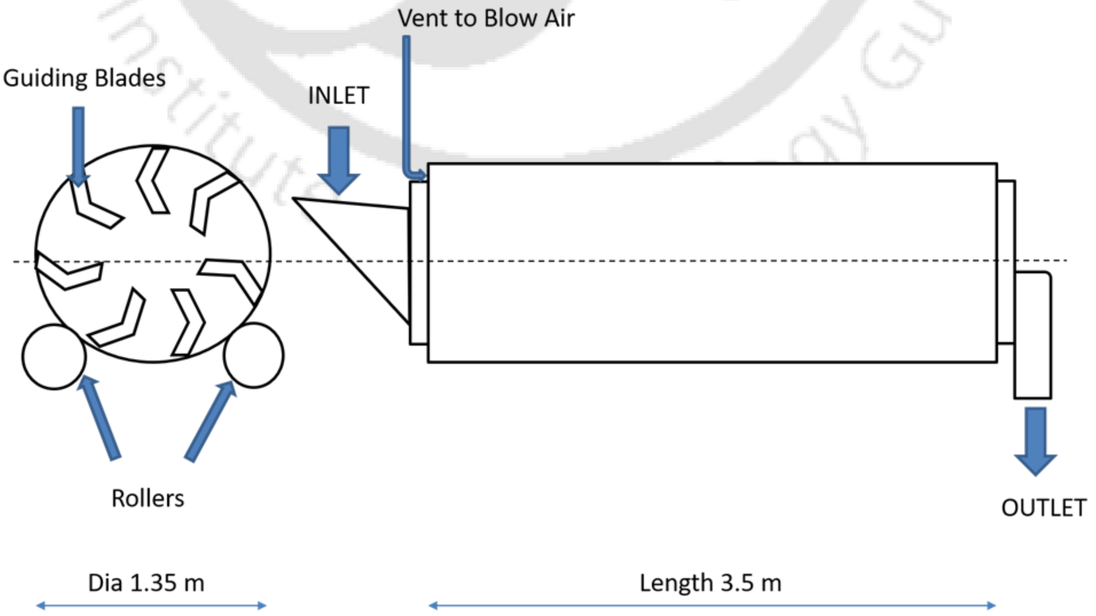
A large-scale rotary drum composter with a capacity of 5000 L was established at the solid waste laboratory of the Indian Institute of Technology, Guwahati (IITG), in compliance with all safety standards.

. The reactor's installation was facilitated by a cast iron frame and rollers for easy operation. A 7-horsepower motor was incorporated to facilitate the rotational motion of the drum. The feed biomass was aerated using a 2-horsepower air blower, as illustrated in Fig 4.8. The composter was utilized to facilitate active thermophilic degradation during the initial seven-day period, as per the findings of phase I. The drum had a volumetric capacity of 5000 liters, with a length of 3.5 meters, a diameter of 1.35 meters, and a metal thickness of 6 millimeters. An anti-corrosive material was applied to the interior of the drum in order to prevent moisture from coming into contact with the metal. The reactor was equipped with a steering mechanism powered by a motor, enabling the rotation of the drum to meet the daily production quota. An additional air blower with limited capacity had been affixed to facilitate forced aeration. The continuous drum offers the benefit of eliminating the need for material removal from the reactor. The slope and guiding blades within the reactor facilitate the movement of the waste toward the reactor outlet, from where it can be conveniently collected in a container. The rotation of the drum was facilitated by eight rollers distributed across the reactor supports. The rotation process was facilitated by welding the guide wheel to the drum, and set in motion by the motor.

ii) Vermicomposter

Bag vermicomposter

In the study, commercially available bag vermicomposters were used to hold the vegetable waste substrate. The daily designated amount of substrate was placed in regular unbroken succession from one end till one bag at a time was full and necessitated using the next bag as displayed in Fig. 4.9.

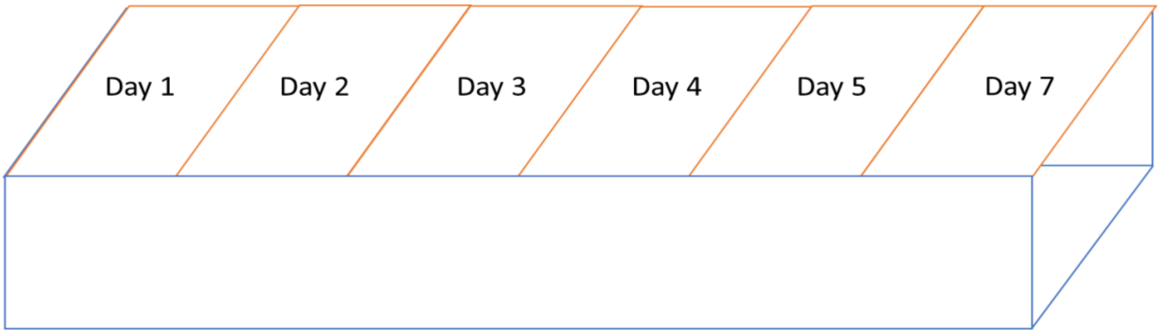


4.7 Schematic diagram of a pilot-scale rotary drum composter

Fig. 4.8 Pilot-scale rotary drum composter

a

b



C

Fig. 4.9 Pilot-scale vermicomposter used in the study

A total of four bags were used for the study, each one with a capacity of 1000 kg. *Eisenia fetida* vermiculture was obtained from the state Krishi Vigyan Kendra (KVK) Guwahati used in the study. 10 mature earthworms per kg of substrate were used throughout the study. Growth in earthworm culture was studied on the 10th and 20th day of vermicomposting. Parameters like the earthworm numbers, the weight of the earthworms, and the number of cocoons were documented and analyzed.

Stack vermicomposting

A stack vermicomposting approach has been adopted to vermicompost the partially degraded material of rotary drum composting (RDC).

a

b

c

Fig. 4.10 Stack vermicomposting unit installed at laboratory

The iron-made frames with 100 subunits of 2000 kg capacity were manufactured, 20 kg of RDC material was fed to each unit, and 200 adult earthworms were released into the material. The stacking of 10–11 units per day was carried out daily, to reach a total weight of 220 kg per day, in one stack vermicomposting frame. The fed units remained undisturbed for 20 days before being subject to vermiculture separation, drying, and sieving of the material. Fig. 1 illustrates the setup and the feeding procedure of one unit of the frame. *Eisenia fetida* vermiculture obtained from the state Krishi Vigyan Kendra (KVK) Guwahati was used in the study. Growth in earthworm culture was studied on the 10th and 20th days of vermicomposting. Parameters like the earthworm numbers, the weight of the earthworms and the number of cocoons were examined.

4.4.2 Feedstocks selected for the study

The selection of three substrates (namely, Vegetable waste, *Water hyacinth*, and *Hydrilla verticillata*) for the pilot-scale operation of a two-stage biodegradation process was based on their availability and accessibility from the IITG campus. The vegetable market yard of Guwahati city in the Fancy Bazar area was chosen since it meets the city's significant vegetable needs. The waste collection was carried out in the morning (04:00 to 08:00 IST), including waste generated during later market hours, all of which was brought to IITG solid waste laboratory facility. Before further processing, the garbage was rigorously checked, and non-biodegradable wastes, such as plastic coverings, were physically separated. Weed biomass was collected from the water bodies near the campus for the entire study duration. The segregated waste was then mechanically shredded for a maximum size reduction of 2 cm. Dry leaves were gathered daily at IITG and utilized as a potential carbon source to lower the feedstock's moisture level throughout the investigation. Saw dust was also utilized to absorb moisture produced during the shredding process. The sawdust was gathered from Amingaon village adjacent to IITG. Further, the feedstock was homogeneously mixed and supplied into the reactor. The experiments for this investigation were carried out using the 5:1 ratio, (i.e., x amount of waste mixed with 20% of x (w/w) bulking agent); the desired moisture of 65-70% was maintained on a daily basis throughout the study. A total of 250 kg of feedstock was applied daily for three months for vegetable waste and water hyacinth. The same amount of feedstock was utilized from co-composting (40% VW, 40%WH, and 20% Dry leaves with water hyacinth and vegetable waste) and *Hydrilla verticillata* for two and one month respectively.=

4.4.3 Sampling and Analysis

1 kg sample was collected every two days from the rotary drum composter inlet and outlet zones. During the process of vermicomposting, partially degraded feedstock samples were collected every 10 days to facilitate the growth of vermiculture. The physicochemical, elemental, stability parameters, and phytotoxicity of these samples were evaluated as outlined in phase I studies. Additionally, in the pilot-scale operation, metagenome sequencing and bulk density measurements of the feedstock at the inlet zone, outlet zone, and final vermicompost were also conducted.

a) Metagenome sequencing of the samples

Sampling was done on day 1 (Inlet of the reactor, thermophilic zone), day 7 (outlet of the reactor) and day 27 (final day of the vermicompost) of the composting process. Sample from each phase was taken and outsourced for metagenomics study so we could develop a diversified bacterial community.

A commercially available Nucleospin kit was used to isolate metagenomic DNA from outsourced samples. Nano Drop was used to quantify the properties of the isolated metagenomic DNA sample. The Nextera XT Index Kit (Illumina Inc.) was used to create the amplicon libraries, which followed the 16S Metagenomic Sequencing Library preparation methodology (Part # 15044223 Rev. B). The bacterial 16S V3-V4 region was amplified using specific forward and reversed primers, GCCTACGGGNGGCWGCAG and ACTACHVGGGTATCTAATCC, respectively.

Fig. 4.10 Large scale reactor feeding process

The amplicon libraries were purified using AMPure XP beads and measured using a Qubit Fluorometer after the amplified PCR product was resolved on 1.2% agarose gel. According to the manufacturer's recommendations, the amplified libraries were examined using an Agilent Technologies 4200 Tape Station system using D1000 Screen tape. Libraries were loaded onto MiSeq at a suitable concentration (10-20 pM) for cluster formation and sequencing once the mean peak sizes from the Tape Station profile were obtained. Paired-End sequencing using MiSeq allows template fragments to be read in forward and backward orientations. On a paired-end flow cell, the kit reagents bind materials to complementary adapter oligoes. The adapters were created to enable selective cleavage of the forward strands following reverse strand re-synthesis during sequencing. The cloned reverse strand was then sequenced from the fragment's opposite end.

b) Variation in the bulk density and volume reduction during the biodegradation

Using Equation 4.6, the material's bulk density (BD), both on the first day of the RDC, on the last day of the RDC, and after the vermicomposting process, was determined (Zhang et al., 2021). The bulk density values were used to determine the volume changes during the RDC and vermicomposting processes. Additionally, the volume of the material inhabited after the operation was considered.

$$BD = \text{mass of the material} / \text{volume of the container} \qquad (4.6)$$

4.5 PHASE-III: FIELD STUDIES ON COMPOST AMENDMENT TO SOIL

4.5.1 Field soil preparation

The field study area is located in the IITG campus, located state of Assam, India. The plain-river-valley landform is characteristic of Brahmaputra river basin located at 24°39′N–28°15′N and 89°42′E–97°25′E in this state. The cultivated land area has increased from 5.78 million hectares in 2000-01 to 6.05 million hectares in 2018-19. Climatically Assam has a humid subtropical climate, with high rainfall in the monsoon season and moderate temperatures throughout the year, with average annual temperature ranging from 19°C to 27°C, depending on the location. While the average annual rainfall is about 2300 mm, with significant regional variation, the average annual evaporation reaches about 1500-2000 mm. The Brahmaputra River and its tributaries principally contribute to average annual surface water runoff of 620 billion cubic meters. The surface water varies widely within a year, with monsoon season accounting for 70-80% of the total runoff and the dry season accounting for only 20-30%.

Fig. 4.11 Field prepared for the study

Twelve plots of 2×2 m^2 were prepared with an elevation of 0.15 m in the IITG campus premises. The soil was plowed numerous times, and the required elevation was maintained in all the plots (Duong et al., 2012). Application of 20% (w/w) of vermicompost to the plot area (as per the results of the pot study) and similar to studies conducted by Al-Sayed et al. (2022) and Wang et al. (2021), Vermicompost produced from three mono substrates biodegradation (Vegetable waste, *Water hyacinth*, and *Hydrilla verticillata*) through the rotary drum composting followed by vermicomposting were used.

Comparing the soil amended only by soil, it can be seen that the water-holding capacity (WHC) treatments (WHVC + SOIL and HVVC + SOIL) have higher values for almost all parameters, excluding the Control and VWVC + SOIL treatments. The WHVC + SOIL treatment has the highest values for soil organic matter, total organic carbon, available phosphorus, and total phosphorus, indicating that it is the most suitable treatment for improving soil fertility.

4.5.2 Soil Initial Characteristics post vermicompost amendment

Table 4.2 Initial characterization of soil and spil amendments studies

Parameter	Control	VWVC +SOIL	WHVC+SOIL	HVVC+SOIL
Moisture content (%)	21.50 ± 0.56	22.40 ± 0.25	25.60 ± 0.50	24.30 ± 0.55
Soil Organic matter (%)	1.60 ± 0.20	13.20 ± 0.15	10.30 ± 0.10	12.30 ± 0.12
pH	6.24 ± 0.11	6.71 ± 0.10	7.23 ± 0.10	7.32 ± 0.10
EC (mS/cm)	0.56 ± 0.01	1.53 ± 0.10	1.40 ± 0.01	1.79 ± 0.01
Total organic carbon (%)	1.20 ± 0.25	3.12 ± 0.22	3.42 ± 0.25	4.02 ± 0.10
Bulk density (kg/m^3)	1380 ± 10	1180 ± 15	1150 ± 10	1130 ± 20
Specific Gravity	2.42 ± 0.05	2.30 ± 0.04	2.15 ± 0.05	2.07 ± 0.05
Porosity (%)	42.97 ± 0.50	48.69 ± 0.55	47.32 ± 0.50	45.41 ± 0.45
WHC (%)	13 ± 0.50	30 ± 0.50	29 ± 0.55	29 ± 0.50
TKN (%)	0.23 ± 0.10	1.02 ± 0.11	0.84 ± 0.20	0.98 ± 0.10
Available phosphorus (g/kg)	0.61 ± 0.01	1.91 ± 0.05	1.43 ± 0.03	1.56 ± 0.05
Total phosphorus (g/kg)	1.73 ± 0.20	5.82 ± 0.15	6.12 ± 0.10	5.27 ± 0.15
Sodium (g/kg)	1.42 ± 0.05	1.79 ± 0.05	2.12 ± 0.05	2.18 ± 0.05
Potassium (g/kg)	3.21 ± 0.03	6.70 ± 0.02	5.48 ± 0.01	5.10 ± 0.01

4.5.3 Selected plant models

Three different model plants were selected for cultivation *Coriandrum sativum* (leafy vegetable), *Okra* (on-ground fruiting plant), and *Raphanus* sativus (off-ground fruiting plant). The seeds were purchased from certified vendor in the market area of Guwahati City. *Coriandrum sativum* is a leafy vegetable commonly known as coriander or cilantro. It is an annual herb widely used in cooking for its distinct flavor and aroma. Coriander leaves are rich in vitamins and minerals such as C, K, and potassium. It is also used in traditional medicine for its anti-inflammatory and antibacterial properties. *Okra* is a warm-season vegetable known as lady's fingers or bhindi. *Okra* is a popular vegetable in many cuisines, including Indian cuisine. Okra is also a good source of vitamins and minerals such as C, K, and magnesium.

a b c

Fig. 4.12 Chosen plant models a) Coriandrum sativum b) Okra and c) Raphanus sativus

Raphanus sativus: It is commonly known as radish and is a member of the Brassicaceae family. Radish is a root vegetable. It is rich in vitamins and minerals such as vitamin C, potassium, and calcium. Radishes are often consumed raw in salads or as a garnish, but they can also be cooked and used in various dishes. The three selected plants, *Coriandrum sativum* (leafy vegetable), *Okra* (on-ground fruiting plant), and *Raphanus sativus* (off-ground fruiting plant), were chosen to cover a range of vegetable types that may benefit from vermicompost-amended soil. Leafy vegetables, such as coriander, are typically harvested for their leaves and require high nitrogen levels for growth. Fruit vegetables, such as *okra*, produce their fruit above the ground and require well-drained soil with high levels of potassium and phosphorus. Root vegetables, such as radish, require deep and loose soil for proper root development. By selecting these three plants, the study aimed to investigate the potential benefits of vermicompost-amended soil for various vegetable types.

4.5.4 Sampling and Analysis

a) Sampling

500gr of soil sample homogenously collected from various parts of the plot area was collected every 10 days and was subjected to drying in a hot air oven at 105 ◦C for 24 h and subsequently ground to pass through 212 µm IS sieve.

b) Soil bulk density

Measuring soil bulk density involved placing approximately 50 grams of the sample in a container with a known volume. The container was then tapped 15-20 times on a table, to produce the same packing as would occur naturally in the field. The volume of the packed sample is equal to the volume of the container. The mass of the sample is determined by oven drying it and then weighing it; and the bulk density of the soil is calculated by dividing the mass of the dried sample by the volume of the container.

c) Cation exchange capacity (EPA 9081)

4 g of soil with a medium or fine texture or 6 g of soil with a coarse texture was transferred into a narrow-necked, round bottomed, 50 ml centrifuge tube. The soil was first washed thrice by adding 33 mL of 1.0 Normality Sodium Acetate (NaOAc) solution, firmly sealed with a stopper, subject to mechanical shaking for 5 min followed by centrifugation till a clear supernatant was obtained for decantation. The next wash was repeated twice with addition of 33 mL of 99% concentration isopropyl alcohol followed by the previous steps to get a clear supernatant which was decanted. The final wash with 33 mL of ammonium acetate solution was also carried out twice and subject to the previous procedure to get a clear supernatant which was also decanted. The resulting laundry or wash solution was then transferred into a volumetric flask with a capacity of 100 mL, and diluted twice with ammonium acetate solution up to the 100-mL mark. The sodium concentration was determined using atomic absorption, emission spectroscopy, or a comparable technique (Mazumder et al., 2021).

d) Specific Gravity (IS: 2720:1980)

To determine the specific gravity of soil, a clean, dry pycnometer was used by the procedures outlined in IS: 2720:1980. The pycnometer, including its stopper, was weighed using a weighing balance of 0.001 gm (W_1). Approximately one-third of the pycnometer volume was then filled with an air-dried soil sample that had been passed through a 0.22 mm sieve. The pycnometer, filled with soil and stoppered, was weighed again (W_2). Distilled water was added to the pycnometer until the soil was completely soaked, and the pycnometer was wiped clean and dried. The weight of the pycnometer was then determined (W_3). The pycnometer was emptied, cleaned, filled with distilled water, and weighed again (W_4). The specific gravity of the soil was calculated using Eq 4.7:

$$\text{Specific gravity of soil} = (W_2 - W_1) / (W_3 - W_4) \tag{4.7}$$

To determine the total porosity of a soil sample, the equation (4.8) can be used:

$$\text{Total Porosity} = (1 - \text{Bulk density} / \text{Particle density}) \tag{4.8}$$

e) Water holding capacity

The study determined the soil's water-holding capacity based on the method described by Priha and Smolander in 1999. The procedure involved soaking a soil sample for two hours and draining it for another two hours. The amount of water retained by the soil after draining was used to calculate the water-holding capacity (Jain and Kalamdhad, 2020).

f) Soil organic carbon

The determination of soil organic carbon was carried out using the Walkley and Black oxidation method. 0.5 to 1gm of dried sample, passed through a 0.22 mm sieve, was taken in a 500 ml conical flask, with the addition of 10 ml of $K_2Cr_2O_7$ and swirled. Next, 20 ml of concentrated sulfuric acid was added and the contents carefully swirled until the soil and reagent are mixed. The mixture was left to cool for 30 minutes and then diluted to 200 ml (Wolka and Melaku, 2015). To the diluted solution, 10 ml of 85% H_3PO_4, 0.2 g of NaF, and 15 drops of diphenylamine indicator were added. The resultant solution was titrated with 0.5 N FAS, and the soil organic carbon is calculated using Eq. (4.9):

$$\text{Organic carbon (\%)} = (B - S) \times N \times 0.003 \times (100/ \text{ weight of dry soil}) \tag{4.9}$$

Where,

B is the ml of standard 0.5 N ferrous ammonium sulfate required for the blank,

S is the ml of standard 0.5 N ferrous ammonium sulfate required for the blank sample, and

N is the normality of standard ferrous ammonium sulfate (0.5N).

The correction factor of 1.3 is multiplied, as according to the Walkley and Black oxidation method, only estimated 77% carbon.

Soil organic Matter (%) = Organic carbon (%) × 1.80. (4.10)

g) Plant growth parameters: During the entire study of the plants, two parameters were considered, which are as follows.

i) Duration for germination: This refers to the time difference between the seeding and seed germination duration. This was recorded for each seed to determine the average germination time for the crop.

ii) Crop yield: The crop yield refers to the crop produced per unit of land area and was measured at the end of the growth period. This parameter assessed the success of the crop and the effectiveness of the growing conditions.

4.5.5 Instruments used in the study

Table 4.3 instruments used in the study

Parameter tested	Instrument used	Model/Manufacturer
pH	µ pH system 361	132, Systronics, India
EC	Digital conductivity meter	VSI-04-Deluxe
$Na+$, $K+$ & $Ca2+$	Flame Photometer 128	Systronics
Heavy Metals	Atomic Absorption Spectrophotometer	Varian Spectra 55B
Nitrite, Ammonia, chloride, fluoride, sulphate	Spectrophotometer	MRC spectro V-110
Nitrate	UV-Spectrophotometer	CARY 50 Bio, VARIAN
TKN	Kelpus distillation unit	Pelican kelpus – Digital EM VA
Weight	Weighing balance	SL-234, Denver Instrument
Drying	Hot air oven	ICT, Calcutta, India

VS	Muffle furnace	ICT, Calcutta, India
Sample preservation	Refrigerator	MRC scientific instruments, India
Functional groups	FTIR	Remi Model: Autosorb-IQ MP Perkin elmer spectrum version 10.4.3

Chapter 5

BATCH SCALE STUDIES OF ROTARY DRUM COMPOSTING FOLLOWED BY VERMICOMPOSTING

5.1 MAJOR URBAN WASTES

5.1.1 The effect of a two-step biodegradation process on utilizing vegetable waste as a source of feedstock

The different combinations prepared for the experimental study are R1: RDC of the substrate, inoculum, and bulking agent in a 5:4:1 ratio (w/w), respectively; R2: RDC followed by VC using *E. eugeniae*; R3: RDC followed by VC using *E. fetida*; R4: RDC followed by VC using *P. excavatus*. The biodegradation duration in RDC was 27 days for R1 (for comparison) and 7 days each for R2, R3, and R4 followed by 20 days of VC. The vermicomposting of these combinations was studied in triplicates.

i) Physicochemical examination

Variation in temperature of feedstock mass

Composting is influenced by the microenvironment and is notably constrained by ambient temperature (Jiang et al., 2021). The increase in temperature during composting is attributed to a variety of pores in the material, which increase the aeration of the compost, creating a more favorable environment for microbial activity (Yin et al., 2021). In most composting processes, the initial rise in temperature and attainment of the thermophilic stage is followed by the mesophilic stage, where ambient temperatures are attained. In the current study, feedstock temperature increased from 37 °C to 51.5 °C in the initial 24 hours of composting. This increase is attributed solely to microbial activity and the effective operation of the rotary drum composter, as no additional heat source was provided for the reactor. The reactor volume remained in the thermophilic range for 7 days before gradually descending to the mesophilic range.

The temperature shift during composting has been attributed to the rapid breakdown of readily available biodegradable organic materials by microbial activity (Zhou et al., 2018). The indigenous microbial communities in the composts that consist of bacteria and fungus, display varying degrees of thermotolerance, and evince a series of community successions, during various thermophilic stages. The elevated temperature of the thermophilic phase kills pathogens, and destroys phytotoxic components (Zhu et al., 2021a) ($P<0.01$).

Changes in moisture, volatile solid, ash content, and total organic carbon

The influence of MC is crucial to the process of composting process. To avoid leachate production, organic waste with a greater moisture content must be combined with bulking agents such as sawdust, wood chips, dry leaves, or grass cuttings. During the composting process, the material could lose moisture due to elevated temperature and aeration. Optimal water content is known to promote microbial activity and organic matter decomposition during composting (Wan et al., 2020). In this study, the moisture content in RDC was reduced from 77 to 67 % (Fig. 5.1(a)). A significant reduction occurred in the initial 7 days of RDC (4% out of 12 %). Since moisture is mandatory for the for the healthy growth of earthworms, a moisture content of 70 ± 2 % was maintained by sprinkling waster on the material during vermicomposting. Subsequently, the final vermicompost was subject to measured and optimum drying, to reduce MC for improved workability and handling, mandatory in mature compost (Mandpe et al., 2021).

VS is a measure of the organic content of a substance. All combinations reflected drop in VS throughout the degradation process, specifically due to ammonia gas volatilization during the overall conversion of organic matter to humic compounds, (Wan et al., 2020). An average VS decrease of 38 to 40 % occurred in the current study, with a maximum decrease of 42 % in *E. fetida* (R3) (Fig. 5.1(d)). The ash content increased from 30 to 60 % during the procedure, and the *E. fetida* reactor demonstrated a maximum increase of 51 % in ash content value ($P<0.05$).

Effect on pH and electrical conductivity

The finished compost demonstrated a basic pH range (6.5 to 8.2). A highly alkaline end product resulted across all experiments, with *E. fetida* earthworm culture. pH variations are associated with the production of organic acids during the composting process by bacteria, nitrification, and ammonification processes. The rise in pH values is ascribed to ammonia release and accumulation in the composting heaps, from the breakdown of nitrogen-containing compounds (Wan et al., 2020). Furthermore, earthworms can neutralize pH in their intestine by producing calcium and ammonia, which control humic acid synthesis (particularly carboxylic and phenolic groups), during the vermicomposting conversion process (Deepthi et al., 2021). The end products are well stabilized if the pH levels remain between 6.5 and 8 (Wang et al., 2021) (P<0.001).

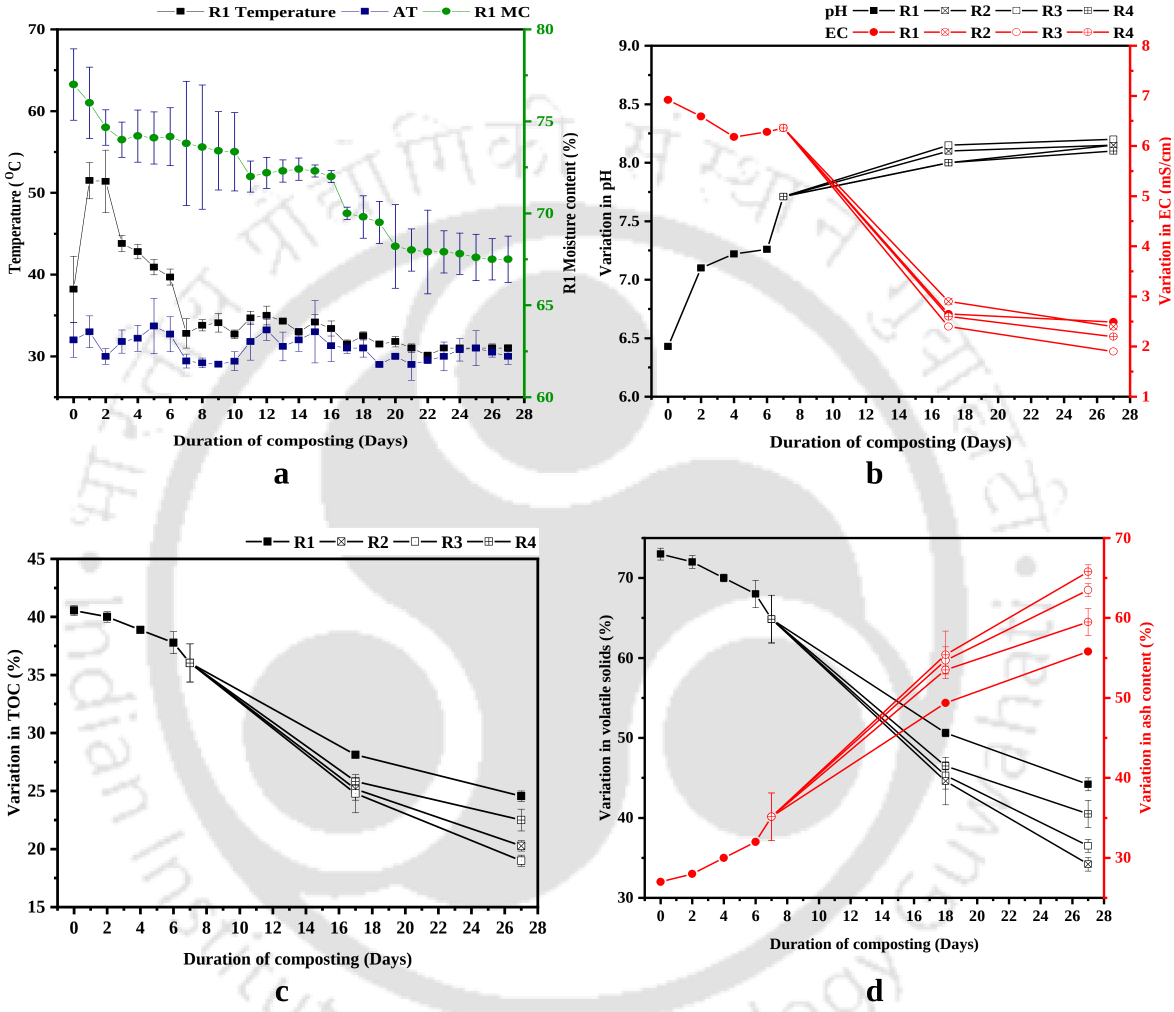


Fig. 5.1 Variation in (a) Temperature and moisture content (b) pH and electrical conductivity (c) Total organic carbon (d) Volatile solids and ash content.

AT* Ambient temperature

MC* Moisture content

The combined composting process significantly reduced the EC value or salt concentration from 8.2 to 2 ± 1 mS/cm on an average in all the 3 trials; the *E. fetida* culture in the reactor (R3) displayed a noteworthy 50% reduction of EC. Since the final product evinced no inhibition, the EC value was deemed fit for plant growth (P<0.01). A high concentration of soluble salts in the compost product reported to inhibit seed germination (Yin et al., 2021).

Composting and vermicomposting involve various biological, physical, and chemical processes, resulting in a change in organic carbon. In all the combinations, a decrease in TOC was observed during composting and vermicomposting. The significant TOC reduction in R3 (53.15 %) is ascribed to the robust earthworm development in the reactor versus R1(35.15%), where earthworms were excluded. The former evinced earthworm-induced fast organic matter breaks down in contrast to the slow deterioration process in the absence of earthworms. The initial 40.5% TOC in feedstock eventually dropped to 24.5 %, 20.27 %, 19 %, and 22.5 % in R1, R2, R3, and R4, respectively, by the end of 27 days; with the *E. fetida* culture in R3 scoring the maximal drop. Similar changes in total organic carbon is well documented (Devi and Khwairakpam, 2020a; Jain and Kalamdhad, 2019). It is also noted that overall organic carbon content varies considerably ($P<0.05$) among the reactors. TOC loss is critical for determining the degradation rate since it indicates organic carbon absorption into the worm biomass and CO_2 evolution during the decomposition process (Kumar et al., 2017).

ii) Elemental analysis

The increase in nitrogen content is proportional to the original nitrogen content of the feedstock and earthworm activity during vermicomposting. Numerous explanations have been cited for the increase in nitrogen concentration during vermicomposting. These include a reduction in TOC, nitrogenous secretions (growth hormones and enzymes, mucus, and other excretory products) produced throughout the process by earthworms (Mago et al., 2021). In this study, the nitrogen content of vermicompost was considerably enhanced as compared to RDC compost. The primary explanation for this might be the presence of nitrogen-fixing bacteria in the earthworm intestines. The growth rate of the *E. fetida* culture was faster than that of other earthworm cultures, which may account for the *E. fetida* reactor accumulating more nitrogen. The nitrogen concentration in R3 increased from 1.8 to 4.15 %, while it increased to only 2.3 % in R1, indicating the efficacy of combined composting over RDC ($P<0.001$).

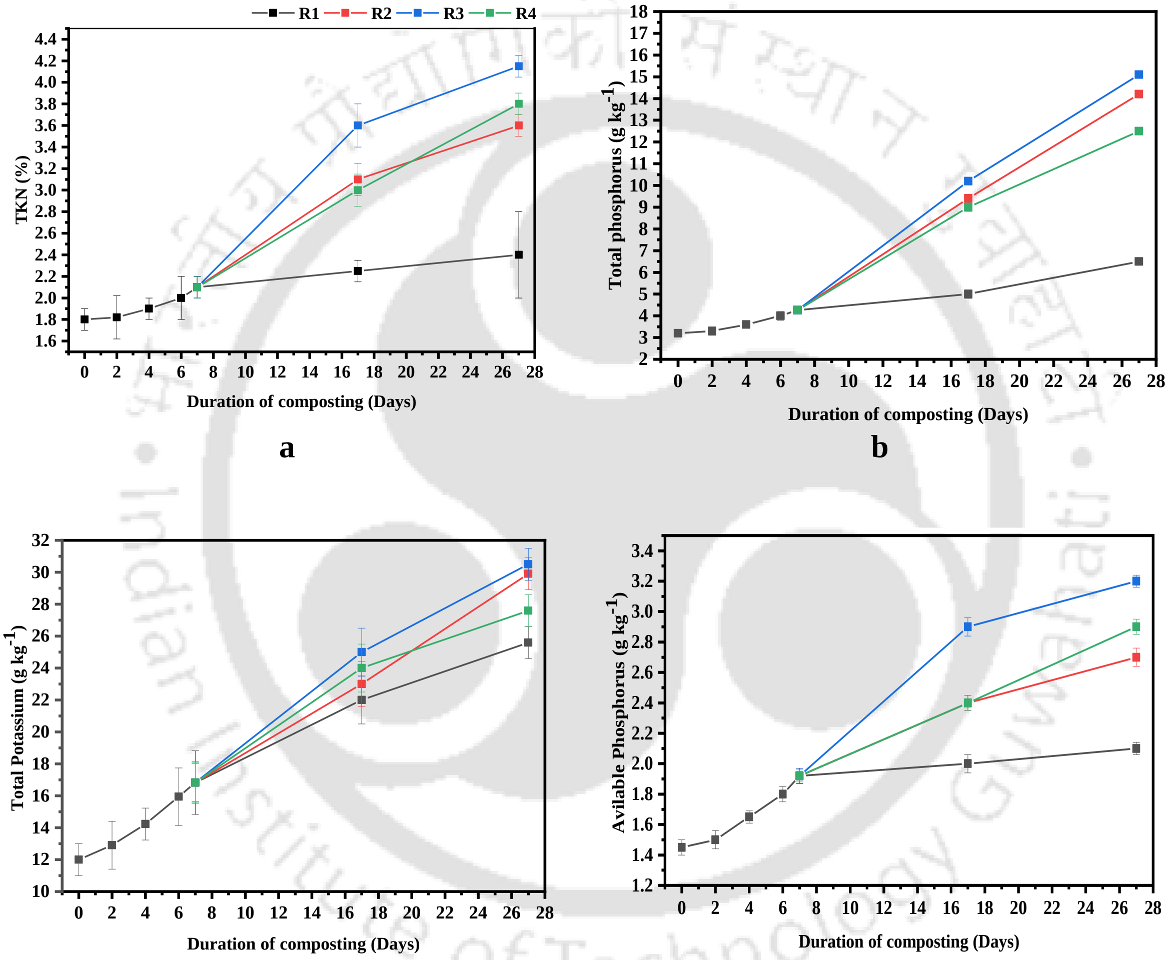
TP is a critical nutrient for crop development and growth. Consequently, many P-containing fertilizers are used in agriculture to compensate for soil P deficiency (Zhan et al., 2021). In the present study, TP and AP were increased. The *E. fetida* culture significantly increased the TP of R3 from 3.25 g kg^{-1} to 15.1 g kg^{-1}, and AP from 1.4 to 3.2 g kg^{-1}. In comparison, in R1, the TP increase was significantly lower, registering values of 6.5 from 3.25 g kg^{-1} and to 2.2 g kg^{-1} from 1.4 for AP. In R2 and R4, the increase in TP and AP was significant compared to R1, but their values were distinctly less than R3 by the end of the 27-day period. TP change during the composting process is most likely due to mineralization of organic phosphorus, consumption by microbes, organic matter decomposition, and mass loss during rotary drum composting. The P dynamics of the current study demonstrated that the mineralization was significantly higher in R3 compared to R2 and R4, indicating the competence of *E. fetida* vermiculture. Since the reactors amended with vermicultures, all had higher TP and AP values compared to R1 ($P<0.001$), the superiority of the combined composting is evident in comparison to RDC.

Potassium, along with nitrogen and phosphorous, forms the indispensable trinity of critical plant nutrients. After 27 days of composting, the increase of Na, Ca, and K in all experimental trials were evinced by time-bound patterns, displayed in Fig. 5.2. TK values displayed a two-fold increase from 12 g kg-1 to 25.6 g kg-1 in R1, and an even higher increase from 12 g kg-1 to 30.5 g kg-1 in R3. The higher value of R3 can be attributed to the robust growth of *E. fetida*, evinced by high density of earthworms, which subsequently enabled increased food stock mineralization in comparison to R1, R2 and R3. The increased activity of earthworms, accelerates mineralization, which in turn alters the distribution of potassium between exchangeable and non-exchangeable forms (Mago et al., 2021). ANOVA analysis indicated significant differences in the concentrations of all macronutrients between days ($P < 0.01$) and across mix proportions ($P < 0.01$).

iii) Analysis of compost stability

Oxygen uptake rate

The OUR rate is directly linked to microbial activity and forms a precise indicator for biodegradation of organic matter during composting, since an adequate supply of oxygen is an essential prerequisite for aerobic biodegradation (Zhang et al., 2021). The bulk organic matter was aerated by drum rotations, as the baffles in the rotary drum composter reduced the formation of lumps by breaking up the material during every rotation. The OUR values for the combinations decreased with time, indicating microbial degradation. Once again, enabled by increased earthworm activity, R3 exhibited the most substantial reduction in OUR from 19.35 to 4.2 mg gVS^{-1} day^{-1}, In contrast, R1 registered a reduction from 19.35 to only 6.1 mg gVS^{-1} day^{-1}, demonstrating lower stability in comparison to R3. A similar trend has been reported by Jain and Kalamdhad (2018). This remarkable difference of OUR in R1 versus R3 demonstrates that combined composting was significant and faster in degrading the organic matter when compared to RDC, indicating that the assumed hypothesis was proven correct in optimization ($P<0.01$).



a

b

c

d

Fig. 5.2 Variation of (a) Total nitrogen content (b) Total phosphorus content and (c) Total potassium and d) Available phosphorus content.

Carbon dioxide evolution

CO_2 emissions from the feedstock of all the reactors were measured regularly throughout the duration of composting to monitor carbon mineralization, perceived as a direct indication of organic matter breakdown and microbial activity. The significantly higher CO_2 emissions in all reactors during the initial stages of composting, owing to aeration, allowed elevated microbial oxygen consumption, ensuing good levels of organic decomposition. The progressive stabilization of the compost is marked by the final lower rate of CO_2 (Xiong et al., 2021).

In this study, the CO_2 evolution in R1 decreased from 13.25 to 8.34 mg gVS^{-1} day^{-1} in the initial 7 days of RDC, and another 20 days would lapse before 3.5 mg was attained. In contrast, the presence of *E. fetida* in R3 allowed an impressive reduction from 8.34 to 2.5 mg gVS^{-1} day^{-1}. The huge reduction was a decisive indicator of higher degradation of organic matter, backed up by the lowest CO_2 evolution in R3 on day 27; a testimony to the highly significant role of *E. fetida* in the early stabilization of feedstock ($P<0.01$).

Kinesis of ammonium nitrogen (NH_4-N)

NH_4-N component of active nitrogen is converted to NO_2-N and then to NO_3-N during composting (Zhang et al., 2021). Consumption of NH_4-N microorganisms was evinced by the overall decrease of NH_4-N content in all four combinations during composting. The maximal loss of NH_4-N during the first 20 days is attributed to the initial high nitrogen content of the fresh organic waste. The subsequent and rapid mineralization of readily available organic material can be attributed to the initial thermophilic temperatures in the composting process (Zhang et al., 2021). The same decreasing trend has been recorded in this study (Fig. 5.3).

NH_4-N is also considered a compost stability parameter. Thus, the higher the reduction in NH_4-N, the more mature the compost (Zhang et al., 2021). Thermophilic degradation in RDC encourages the reduction of NH_4-N. In R1, the overall reduction was observed to be 46%, out of which 37% occurred in the initial 7 days, when the thermophilic temperatures prevailed in the feedstock. Further, vermicomposting stabilized the feedstock, wherein R3 29% reduction was observed in the next 20 days, in comparison to only 9% in RDC. From the results, it was evident that RDC in the initial thermophilic phase reduced the greater amount of NH_4-N. Only a minor effect was observed in the subsequent mesophilic stage. The further reduction of NH4-N in the pre-degraded waste was comparatively homogenous in vermicomposting, when compared to RDC. Overall, combined composting process stabilizes the feedstock comparatively earlier. Similar trends were reported in previous studies (Devi and Khwairakpam, 2020a) ($P<0.005$).

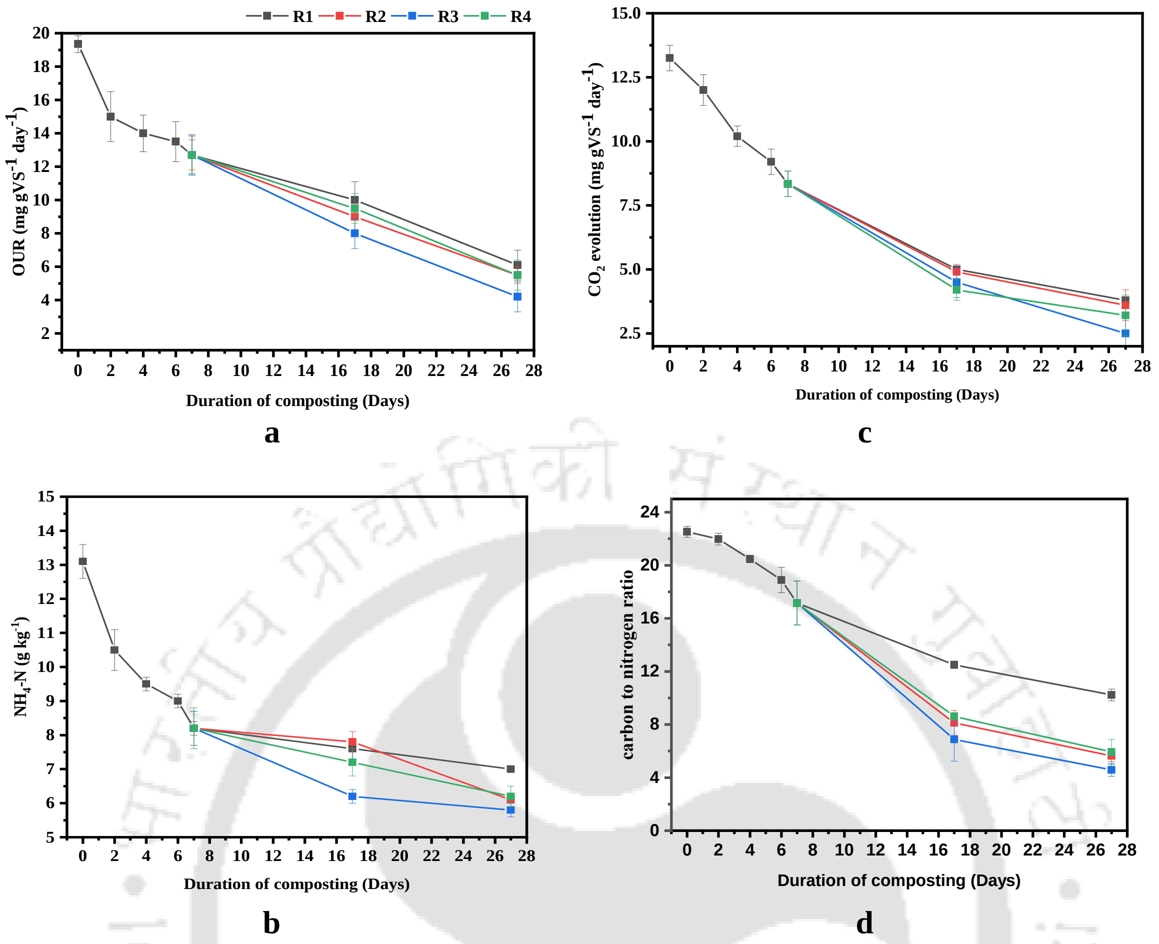


Fig. 5.3 Variation in (a) Oxygen uptake rate (b) Ammonia nitrogen and (c) CO_2 evolution rate and (d) C/N ratio

Change in Carbon nitrogen ratio

The ratio of carbon to nitrogen in a compost mixture determines its quality and speed of decomposition. All four experiments were started with 22.53 C/N. The C/N ratio drops when composting decomposes organic materials into stable humus. The C/N ratio decreases with composting time, indicating that more organic matter is converted into stable humus. The C/N ratio drops significantly after 17 and 27 days of composting, especially in trials R2, R3, and R4, demonstrating that these composting combinations break down organic matter better than experiment R1. The lower the C/N ratio, the greater the compost quality and plant nutrient content. The table shows the percentage decrease in C:N ratio after 7 and 27 days of composting for each experiment. The C/N ratio dropped 24.1% in all four studies after 7 days. By day 27, R2, R3, and R4 had lower C/N ratios than R1. After 27 days, experiment R2 showed the greatest C/N ratio decrease of 75.0%, followed by R3 at 77.5%, R4 displayed 72.2%, and R1 was at 54.5%. This shows that R2, R3, and R4 broke down organic materials more efficiently than R1.

iv) Observations of odour and colour in the composting process

Decomposition of organic matter and the subsequent ammonification by the bacterial community, releases ammonia from the compost; causing the characteristic pungent odor during the first two days of composting. Once the feedstock reaches thermophilic temperatures, the pungent odour decreases steadily. There was a minimal odour when the feedstock was fed to the vermiculture, and the finished vermicompost smelt earthy. The feedstock was green in colour for the first two days, then was light brown for 11 consecutive days before turning black. The colour shift in the feedstock is caused by the degradation of chlorophyll, bacterial activity, thermophilic temperatures, and moisture loss. In vermicomposting, the changes occur as the feedstock ages and consumption of feed by the vermiculture. Stable and mature vermicompost will be black in colour, smooth in texture and possess zero unpleasant odour.

v) Growth of earthworm culture

The earthworm population is a significant indicator of vermicomposting (Cao et al., 2021). The average biomass of the experimental earthworm was 0.16 ± 0.02 g at the start of the experiment and 0.4 ± 0.01 g at the conclusion (Table 3). The number of cocoons was also significant in the *E. fetida* vermiculture compared to other vermicultures, enabled by feeding the earthworms on the degraded material. The 7-day RDC period prior to vermicomposting acted as a pretreatment for the vermicomposting of the feedstock. The study conducted by Ning et al. (2021) adopted a similar combined composting technique, and in addition, achieved a significant reduction of chromium. For the combined composting technique adopted in the current study, it was observed that *E. fetida* was best suited in the enriching the nutrient content of feedstock. The healthy growth and the higher cocoon production of *E. fetida* signify the early acclimatization and enhanced feedstock degradation.

Notably, earthworms can promote bacterial degraders like *Lysobacter*, *Kaistobacter*, *Flavobacterium*, *Arenimonas*, *Aquicella*, *Aeromonas*, *and Algoriphagus*. According to most reports, these 7 promising degraders, supplemented by earthworms, play a critical role in decomposing organic contaminants (Pu et al., 2020). Among the bacterial degraders, *Aeromonas* is found abundantly in the guts of earthworms (Hong, 2011).

Earthworms may enhance the oxygen level of the soil material and supply nutrients via feces and burrowing, thus promoting the development of indigenous aerobic degrading bacteria (Han et al., 2021). It is opined that various types of worms used in the vermicomposting process degrade waste, remove heavy metals, and their effectiveness mainly relies on earthworm density and mass (Ning et al., 2021; Sharma and Garg, 2018).

Table 5.1. Growth in earthworm population.

Day	**R2** **(*Eudrillus euginae)***			**R3** **(*Eisenia fetida*)**			**R4** **(*Perionyx excavates*)**		
	Adults	**Juveniles**	**Cocoons (per 100 g)**	**Adults**	**Juveniles**	**Cocoons (per 100 g)**	**Adults**	**Juveniles**	**Cocoons (per 100 g)**
7	180	0	0	180	0	0	180	0	0
17	185	8	4	193	9	2	182	6	6
27	199	20	18	201	12	22	188	15	11

The vales are the average of triplicate study

vi) Correlation of analysed compost parameters

A correlation matrix was constructed using all the studied data related to composting parameters to evaluate the relationship between them (Table 3 for R3). The reactor temperature (RT) correlated positively with the stability parameters OUR, CO_2 evolution (CE), and NH_4-N nitrogen (AN). Consequently, the correlation was interpreted as the impact of the reactor temperature on the stability of the compost material, borne out by the faster stability attained under higher temperatures maintained. The ambient temperature had a negligible impact on the rotary drum composting process, as shown by the correlation of AT with other parameters in Table 4. The TN, TP, and TK contents are favourably associated with each other, while being negatively linked with C/N, NH_4-N, VS, and MC, reinforcing the comparable findings by Alburquerque et al. (2004). The biological maturity parameters of CO_2 evolution rate and OUR are positively linked with the physicochemical maturity parameter NH_4-N (Varma and Kalamdhad, 2014). All the stability metrics are positively associated with VS, MC, and C/N, demonstrating the dependence of biological activity on the volatile fraction of material in all four trials. Similar connections were observed between *E. euginea* and *P. excavates* earthworms and

RDC. this data can be found in the Annexture II of this thesis. The coefficient value is significantly higher in R3, and allied reactors, with the exception of R1 where it is considerably lower. The higher correlation between the parameters in R3 proves that VC post RDC has a positive impact on the stability and increment of nutritional values of vermicompost in comparison with only RDC.

vii) Summary

The novel combination of RDC thermophilic biodegradation and *E. fetida* based vermicomposting yielded nutrient-rich vermicompost from substantial vegetable waste in the shortest feasible period (27 days). In-depth optimization of process parameters combined with the reduction in EC, CO_2 evolution, increased SGI (%) validated the technique and product. The combined technique is capable of handling sizable quantities of organic waste in an environmentally compatible mode to deescalate wide scale contamination hazards and produce an excellent soil conditioner. The novel process set out in this paper could potentially reconfigure organic waste treatment facilities across the world.

5.1.2 The effect of a two-step biodegradation process on utilizing sewage sludge as a source of feedstock

The experimental trials of S1, S2 and S3 were used in the current study. The sludge in S1 was subject to only rotary drum composting without any application of VC. For S2 and S3 composting of the sludge in a rotary drum was followed by *E. eugeniae* and *E. fetida* administration, respectively. The biodegradation time for S1 was 27 days, whereas, for S2 and S3, the same period was divided into 7 days of rotary drum composting, followed by 20 days of VC. The *P. excavates* culture evinced mortality during the initial 1 week of introducing so the data was not considered.

i) Changes in the physicochemical qualities of the feedstock

Variation in temperature and moisture content

Changes in the temperature profile of feedstock during composting indicate degradation of organic matter due to microbial activity and aid in pathogen destruction (Song et al., 2021). The rise in temperature was recorded in S1, with a recorded maximum of 51ºC on the 3rd day, which shifted to the mesophilic range by the 5th day (Fig. 5.4a). Results published by Jain et al., 2019 lend support to this temperature profile ($P < 0.001$). Further, the mesophilic range prevailed in composting mass throughout the composting period (Kauser et al., 2020) The addition of sawdust at the beginning of the composting process improved the thermophilic conditions by increasing porosity, which enhanced microbial activity (Song et al., 2021). The vermireactors used for S2 and S3 were established on day 7 when the temperature of feedstock mass was at < 35ºC, which favored the vermicomposting process. Subsequently, the feedstock exhibited the ambient mesophilic temperature profile.

MC is critical to the circulation of air and nutrients required for the physiological and metabolic cycles of bacteria and microorganisms in the feedstock. As anaerobic conditions increase significantly, excessive moisture may affect the breakdown pathway. The initial MC of the substrates utilized dictates the ultimate moisture content of feedstock (Kauser et al., 2020). In the present research, a moisture loss of 11% was observed by the end of the 7th day ($P < 0.001$). Further, in S1, the MC was reduced to 55% (from 69%). Evaporation causes moisture loss throughout the composting process when the feedstock gains a physiologically developed thermophilic phase in the RDC (Maturi et al., 2021). Over 20 days, moisture reduction from 58% to 52% was recorded in the vermicomposting reactors S2 and S3. Since lower MC increases workability and is suitable in the final vermicompost, no further addition of water would not be required (Mandpe et al., 2021).

Variation in pH and volatile solid fraction

The pH of the original raw material has a significant impact on the composting and vermicomposting processes. pH levels rose from 6.55 to 7.69 in S1, to 7.92 in S2, and touched 7.85 in S3 ($P < 0.001$) by the end of 27 days (Fig. 5.4). The increased pH level was attributed to organic matter breakdown, which aided the release of organic acids and nitrogen and phosphorus mineralization. pH levels ranging from slightly acidic (pH 6.50) to neutral (pH 8.00) are reported to be ideal for composting and vermicomposting (Kalamdhad et al., 2009; Garg et al., 2012).

The EC of the material is an indicator of salinity. The salinity affects soil fertility and is phytotoxic to seed germination and plant growth. According to previous studies, the maximum limit for the products of any biodegradation processes should be less than 4 mS/cm to be accepted for land application (Kauser and Khwairakpam, 2022). In the current study, the EC for sludge increased in the S1 trail from 1.39 mS/cm to 1.55 mS/cm and in S2, there was a decrease to 1.22 mS/cm; while a decrease to 1.11 mS/cm was seen in S3 derived end products (Fig.5.4). The significantly lower values for S2 and S3 end products are a clear testimonial of the decrease in EC values due to earthworm activity ($P < 0.001$). According to Hazarika and Khwairakpam (2018), the reduction of the EC value during composting is ascribed to ammonium volatilization, inorganic (mineral) salt precipitation, and water-insoluble humic compounds.

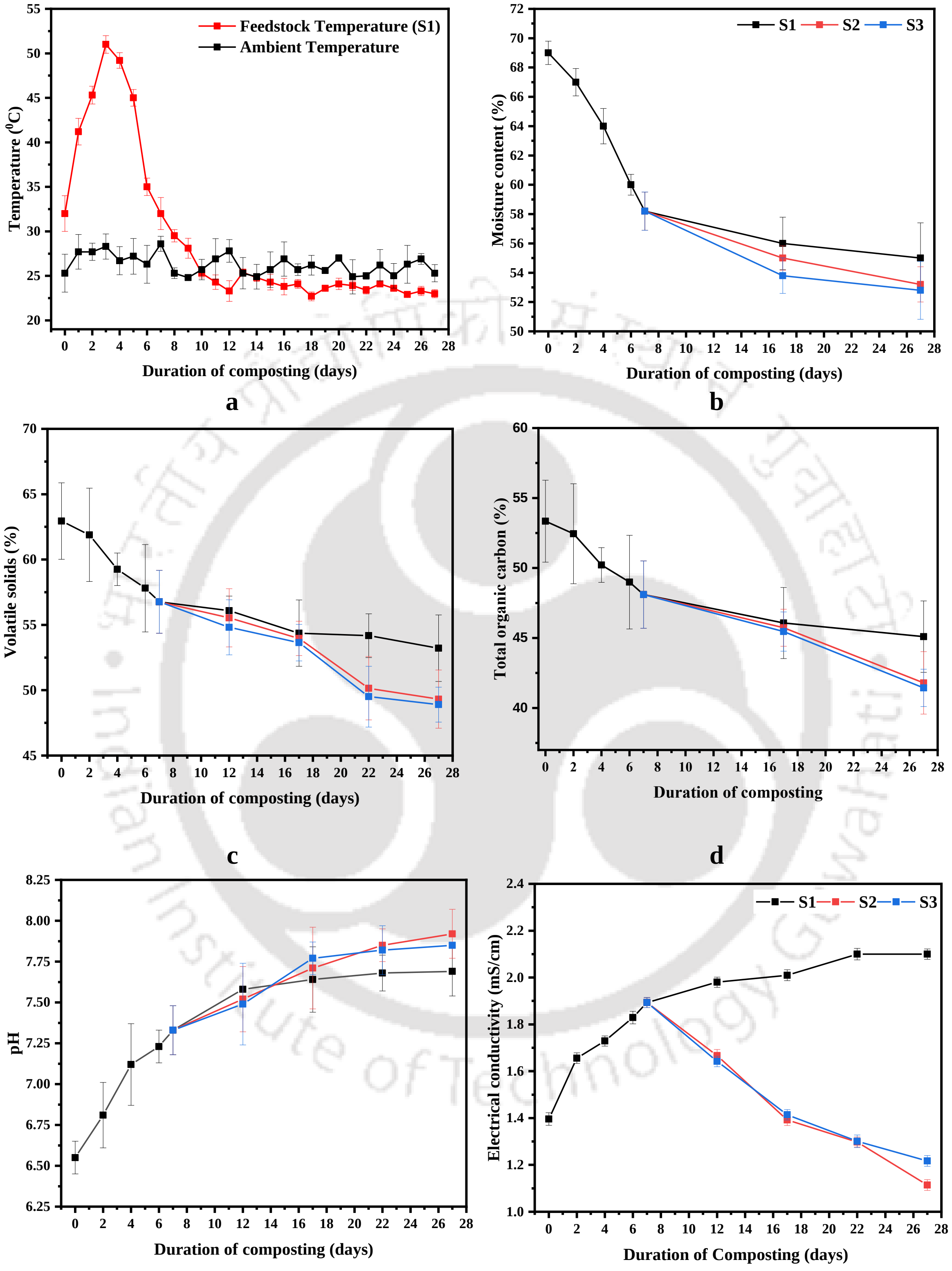
Effective thermal breakdown of organic waste in the rotary drum is engendered by microorganisms and thermophilic bacterial activity. The decrease in volatile solids of feedstock is assessed as the breakdown of organic components throughout the stabilization process (Kauser et al., 2020). The initial 10% VS reduction in the current study is attributed to the physiologically developed thermophilic temperature in the rotary drum composter (S1), and a further reduction of 12% in vermicomposting is ascribed to vermiculture consumption microbial degradation (S2 and S3).

In comparison to S1, 2 times and 2.5 times higher reduction of the volatile solid fraction of the feedstock was attributed to the vermiculture degradation in S2 and S3 trails, respectively. The results of Nayak and Kalamdhad (2015) support the present study results.

ii) Stabilization of sewage sludge

Variation in oxygen uptake rate

Composting is an aerobic biodegradation activity that needs oxygen to stabilize organic wastes and adequate MC for the proliferation of microorganisms (Puyuelo et al., 2010). OUR represents the absorption of oxygen by bacteria during the decomposition of organic matter and can also be used to identify the stability of compost (Said-Pullicino et al., 2007). OUR for sludge decreased from 6.25 mg/gVS/day to 1.54 mg/gVS/day for S1, to 1.15 mg/gVS/day for S2, and reached 1.09 mg/gVS/day for S3 (Fig. 2a). The value near 1 represents the adequate stabilization of feedstock for safe application on the soil. The S3 trail was notable for attaining stability sooner than the other experimental trials and was attributed to increased earthworm activity. Jain and Kalamdhad (2018) observed similar rates of reduction in OUR.



a b

c d

e f

Fig. 5.4 Variation in a) Temperature, b) Moisture content, c) VS d) TOC e) pH and f) EC

Feedstock CO_2 evolution rate

CO_2 evolution determines the degree of degradation throughout the composting process (Yang et al., 2020). During composting, organic matter is degraded into tiny molecules such as polyphenols, polysaccharides, and amino acids, which may contribute to the synthesis of humic substances through polymerization or condensation (Wang et al., 2021). Furthermore, microbes use these compounds as energy sources, releasing a high quantity of carbon dioxide (CO_2). Consequently, the CO_2 evolution rate can be used as a parameter of maturity in determining the stability of composting feedstock. In the current study CO_2 evolution rate decreased from 5.95 mg/gVS/day to 2.13 mg/gVS/day in S1, to1.71 mg/gVS/day for S2 and attained 1.59 mg/gVS/day for S3 trials (Fig. 5.5). In the production of humic substances, CO_2 emissions must be reduced, and the participation of additional precursors encouraged (Wang et al., 2021).

Change in C:N ratio

A decrease in the C:N ratio due to the concurrent consumption of carbonaceous organic material indicates an excellent composting process, and compost with a C:N ratio < 20 is considered mature (Song et al., 2021). The production of high levels of CO_2 during organic matter results in carbon loss (Wang et al., 2021). In the present study, a 38% reduction occurred in the preliminary 7 days due to the thermophilic degradation in rotary drum composting (S1). During mesophilic degradation over a20 day period, 13% reduction was seen in S1, 24% in S2 and 27% in S3. Overall, a 50% reduction in C:N ratio was recorded in S1, 62% in S2 and 65% in S3. After 27 days, the C:N ratio for the S1 trail is 9.13, 7.21 for the S2, and 6.65 for S3 trails (Fig. 5.5). This is a clear testimony of the effect of the thermophilic phase on feedstock and the efficiency of *E. fetida* in stabilizing the sludge.

Ammonium nitrogen (NH_4-N) dynamics

Temperature and pH may alter the equilibrium of NH_4^+-N and NH_3 throughout the composting process (Song et al., 2021). Due to enhanced emissions of NH_3 and its transformation to the gaseous state, there is a decrease in the amount of NH_4^+-N. Furthermore, the reduction in NH_4^+-N may be attributed to bacteria immobilizing nitrogenous substances such as amino acids, nucleic acids, and proteins (Sanchez-Monedero et al., 2001).

In the present study, the initial ammonium nitrogen content decreased from an average of 157 mg/kg to 23 mg/kg for S1, to 19 mg/kg for S2 and reached a low of 17 mg/kg for S3 trail. A reduction of 85% in S1, 87% in S2 and 88.6% in S3 were seen; of which 71% documented the initial 7 days, might be due to NH_3 volatilization at high temperatures and pH rise. Further, the S3 trail demonstrated a maximum of 17% reduction in the mesophilic degradation stage, concluding that *E. fetida* is particularly efficient and unique to the process (Fig. 5.5).

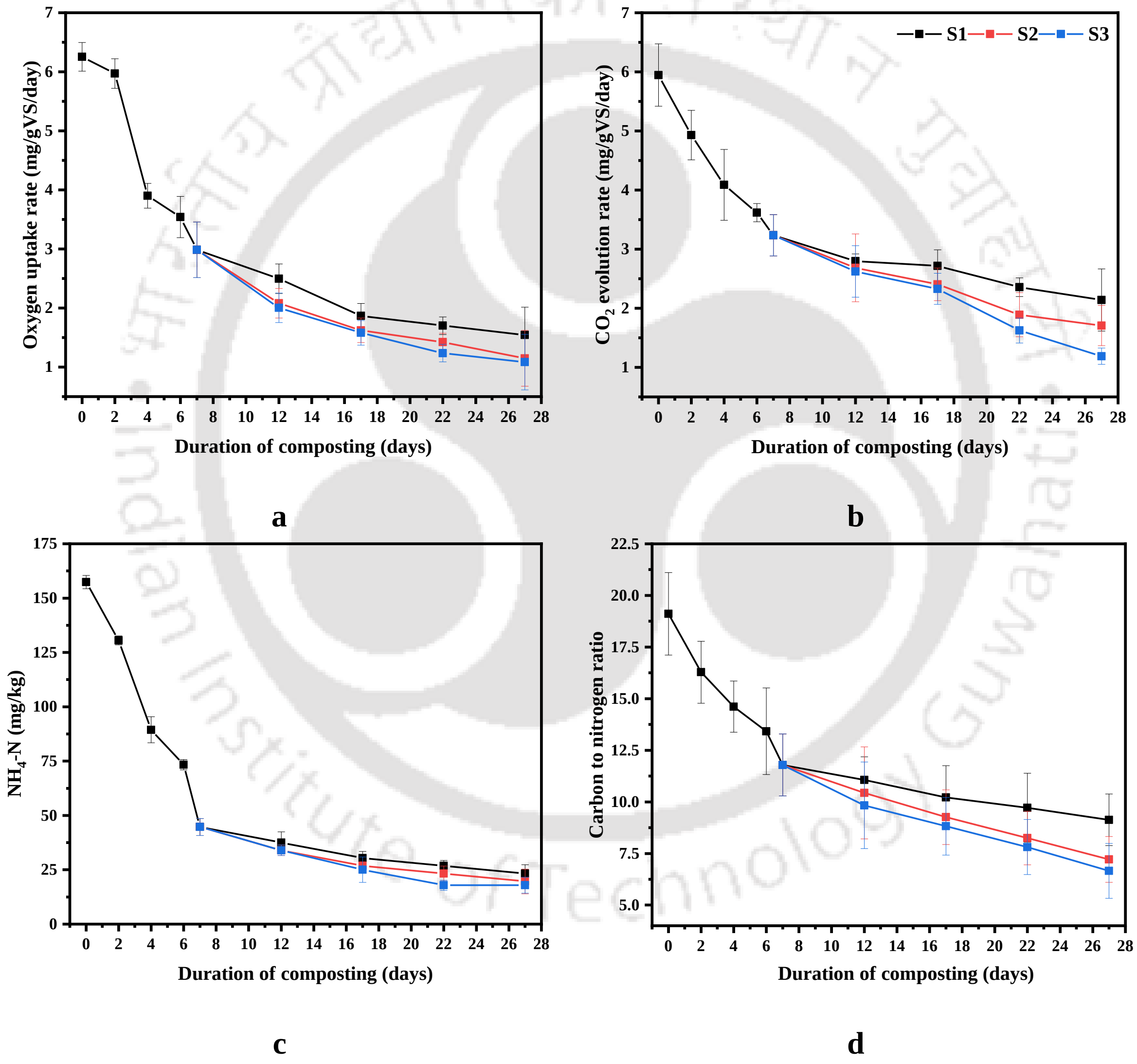


Fig. 5.5 Changes in the a) Oxygen uptake rate, b) CO_2 evolution rate, c) Ammonium nitrogen (NH_4-N) and d) Carbon to nitrogen ratio of feedstock

iii) Nutrient augmentation during the stabilization

Total Kjeldahl nitrogen (TKN) in composting often increases owing to loss in organic matter following CO2 evolution, and the activity of nitrogen-fixing bacteria (Nakasaki et al., 2005; Kalamdhad et al., 2009). The nitrogen content in the S3 trail grew significantly in the current study, rising from 1.82% to 4.06% by the end of the 27-day timeframe (Fig. 5.6a). The nitrogen increase in S1 was 3.22%, and attained 3.78% in S3. The findings show that the difference in nitrogen concentration between S2 and S3 end products compared to S1-derived compost can be related to the influence of vermiculture, with *E. fetida* vermiculture playing a substantial role in the S3 trail ($P < 0.001$).

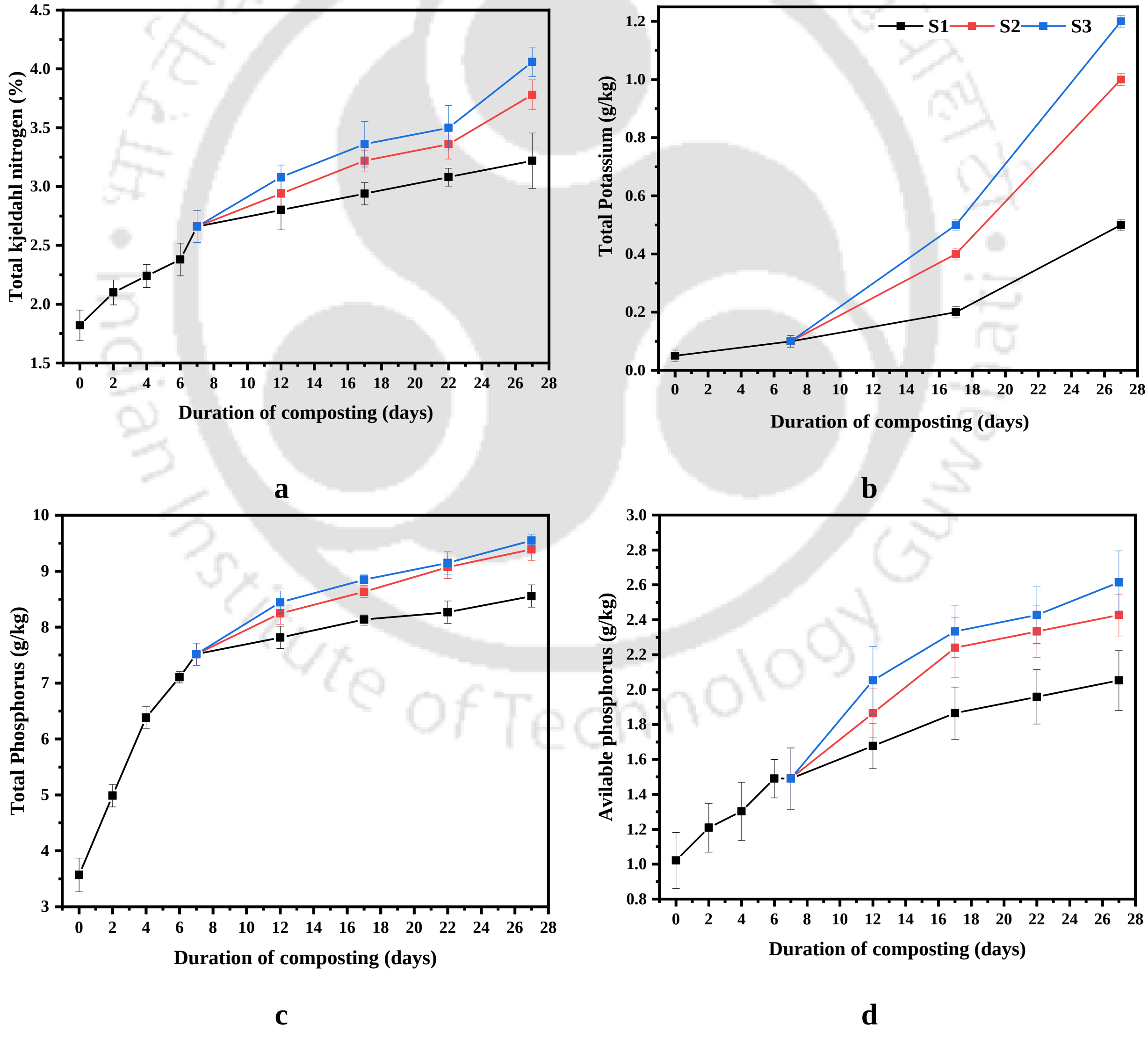


Fig. 5.6 Augmentation of a) Total Kjeldhal nitrogen, b) Total potassium, c) Total phosphorus and d) Available phosphorus during stabilization

The rise in macronutrients can be attributed to earthworm mineralization, which converts a fraction of macronutrients from bound to free forms, resulting in enrichment (Yadav and Garg, 2011). The TK of the feedstock increased from 0.05 g/kg to 0.6 g/kg in S1, to 1g/kg in S2 and a final level of 1.2 g/kg in S3. Total potassium, the significant micronutrient in the end products of biodegradation, also evinced an increasing trend in the current study (Fig. 5.6b).

Phosphorus content in the final compost/vermicompost is a necessary nutrient for plant photosynthesis and is accessible to plants as inorganic ions. During vermicomposting, even inaccessible forms of phosphorous may be transformed into available forms in the final vermicompost, which is easily available to plants (Trap et al., 2021). The present study results reveal that the TP concentration increased from 3.57 g/kg to 8.56 g/kg in S1, to 9.39 g/kg in S2 and reached 9.55 g/kg in S3. Further, AP increased from 1.02 g/kg to 2.05 g/kg in S1, to 2.43 g/kg in S2 and attained 2.615 g/kg in S3 (Fig 5.6). The rise in TP and AP may be attributed to the breakdown of organic matter into organic acids, which further solubilizes the insoluble phosphorous and raises the phosphorous content in the vermicompost (Nayak et al., 2013).

iv) Earthworms growth and coliform bacterial precedence

Growth in earthworm culture

The growth of earthworm species used in VC organic waste is a good indicator of a successful VC procedure (Kauser and Khwairakpam, 2022). Earthworm species increased abundance throughout the vermicomposting process, with *E. fetida* generating many adults and cocoons. The partial decomposition of biomass in the rotary drum thermophilic stage benefited the feeding and growth of earthworm cultures. The mesophilic hydrolytic stage of the vermicomposting process is defined by a rise in bacterial metabolic activity in the earthworm gut and followed by an increase in earthworm biomass growth (Vivas et al., 2009). The notably higher worm count in the case of *E. fetida*, naturally resulted in a higher nitrogen content end product, in comparison to *E. eugeniae*. Table 5.2. provides the details of the growth during the vermicomposting period. The research findings of Kauser and Khwairakpam (2022) support the results of the earthworm growth patterns of this study.

Table 5.2 Growth in earthworm cultures

	***Eudrilus Eugeniae* (S2)**			***Eisenia Fetida (S3)***		
Days	Earthworms	Juveniles	Cocoons	Earthworms	Juveniles	Cocoons
7	180 ± 0	-	-	180 ± 0	-	-
17	256 ± 8	45 ± 8	112 ± 5	311 ± 4	52 ± 10	189 ± 5
27	345 ± 10	51 ± 10	176 ± 2	532 ± 3	64 ± 15	233 ± 2

*cocoons were reported per 100 g of sample

Coliform precedence

Since the substrate under consideration is sewage sludge, which contains an abundance of pathogens, it is critical to determine if the composting process alters the pathogen count. Pathogens are effectively eliminated throughout the composting process since the thermophilic phase of the composting is known to destroy pathogens (Song et al., 2021).

In the present study, the total coliform reduced from 9.3×10^8 MPN/g to 4.5×10^3 MPN/g in S1, while reduced level of 1.1×10^3 MPN/g was attained by S2 and S3. Further, the fecal coliform reduced from 3.5×10^6 MPN/g to 4.5×10^2 MPN/g in S1 and identical levels 3.6×10^2 MPN/g were obtained for both S2 and S3 (Table 5.3). The presence of coliform bacteria is often utilized to measure the overall hygiene status of the compost. Further, Khwairakpam and Bhargava (2009) reported that fecal coliform is lowered during VC when the earthworm feeding chain is reached. Similar results were found in the current study, where the vermicompost recorded lower levels of fecal coliform than compost, validating the positive outcome elicited vermiculture.

Table 5.3 Changes in total and fecal coliform during stabilization

	S1		**S2**		**S3**	
Duration (days)	TC	FC	TC	FC	TC	FC
0	9.3×10^8	3.5×10^6	-	-	-	-
7	4.5×10^8	4.5×10^2	4.5×10^8	4.5×10^2	4.5×10^8	4.5×10^2
27	2.1×10^4	1.8×10^3	1.1×10^3	3.6×10^2	1.1×10^3	3.6×10^2

*TC: total coliform; FC: fecal coliform

v) Monitoring total heavy metals concentrations and bioaccumulation in earthworm cultures

According to Yadav and Garg (2011), the increase in HMs in completed compost is driven by CO_2 emissions, which decreases the overall mass of waste, while concurrently boosting its metal content. Furthermore, Sun et al. (2020) concluded the heavy metal bioaccumulation of Cr, Pb, and Zn during vermicomposting biogas residues in 8 days of vermiculture exposure to the substrate.

Table 5.4 Change in total heavy metal concentration

Day	S1	S2	S3	S1	S2	S3	S1	S2	S3
	Zn			**Pb**			**Cr**		
0	51.57 ± 1.21			72.10 ± 0.30			39.25 ± 1.21		
7	56.52 ± 1.05	56.52 ± 1.05	56.52 ± 1.05	79.25 ± 0.25	79.25 ± 0.25	79.25 ± 0.25	42.10 ± 1.02	42.10 ± 1.02	42.10 ± 1.02
17	60.00 ± 1.11	51.20 ± 1.11	50.10 ± 1.00	80.95 ± 0.21	77.14 ± 0.15	75.20 ± 0.12	49.20 ± 1.00	33.21 ± 1.11	32.40 ± 1.20
27	68.85 ± 1.02	48.60 ± 1.21	48.27 ± 1.04	85.12 ± 0.22	75.42 ± 0.10	72.10 ± 0.11	57.50 ± 1.10	21.25 ± 1.21	20.75 ± 1.00
	Cd			**Cu**					
0	5.22 ± 0.12			19.60 ± 0.12					
7	5.97 ± 0.01	5.97 ± 0.01	5.97 ± 0.01	20.42 ± 0.15	20.42 ± 0.15	20.42 ± 0.15			
17	6.32 ± 0.02	5.74 ± 0.02	5.42 ± 0.01	20.44 ± 0.11	20.35 ± 0.12	20.32 ± 0.13			
27	6.57 ± 0.01	5.62 ± 0.02	5.17 ± 0.01	20.47 ± 0.10	19.95 ± 0.11	19.50 ± 0.17			

*All the concentrations are in mg/l

In the current study, the S1 process recorded increased the total heavy metal concentration, while a decrease in S2 and S3 processes were recorded from day 7 to day 27 due to vermiculture. The total heavy metal concentration in S1 derived final product displayed an increase of 33% Zn, 18% Pb, 46% Cr, 26% Cd, and 4.5% Cu. The bioaccumulation of Zn and Cr concentrations in S2 and S3 derived vermicompost was an average of 14% Zn and 50% Cr. Heavy metals like Pb, Cd, and Cu were highly bioaccumulated (9%, 13%, and 4.5%, respectively) in final product of S3 compared to S2

(4.8%, 5.8%, and 2.3%, respectively) ($P < 0.001$). The concentrations and the reduction during the process have been listed in Table 5.4. S3 trail was shown to produce a safer end product than S2. The end products of S2 and S3 were safer than S1 in terms of heavy metal presence.

vi) Summary

This study references stabilizing freshly dewatered sewage sludge over an optimized duration through the dual-stage biodegradation process. The study of stability parameters revealed expeditious sludge stabilizing potential through a 7-day thermophilic degradation of rotary drum composter, followed by 20-day mesophilic degradation using *E. fetida*-based vermicomposting (S3), proved positive and significant among the experimented trials. This process reduces the time required for sludge stabilization and feedstock retention time in the RDC and VC acclimation time in the feedstock mass. The study of heavy metal concentration throughout the stabilization process revealed the bioaccumulation of toxic metals like Zn, Pb and Cr during vermicomposting. Further, compared to the soil amendmended with compost, the vermicompost amended soil, significantly and positively affected overall plant growth. In a biologically appropriate mode, the biodegradation process validated in this study, could help sludge management facilities stabilize sludge faster, and produce a marketable end product, in an optimized time frame.

5.2 AQUATIC WEED BIOMASS

WH_{RDC} (*E. crassipes*) and HV_{RDC} (*H. verticillata*) were used in the rotary drum composting experiments. WH_{RVF} (*E. crassipes*) and HV_{RVF} (*H. verticillata*) studies were conducted utilizing *E. fetida* in the RDVC method and using *E. eugeniae* WH_{RVE} (*E. crassipes*) with HV_{RVE} (*H. verticillata*).

5.2.1 Physicochemical analysis

According to Song et al. (2021), composting is a process in which organic waste breaks down through microbial activity, with varying temperatures in the feedstock assisting in the elimination of pathogens. This temperature increase may be due to pores in the material that promote aeration and provide a favorable environment for microbes (Yin et al., 2021). Typically, composting involves a thermophilic phase in the feedstock for 7-10 days, followed by a maturation and cooling phase. However, the present study replaced the cooling and maturation phase with vermicomposting. The feedstock in the study reached a maximum temperature of 47°C within 24 hours (WH_{RDC}), with the thermophilic phase shifting to the mesophilic range on day 7 (<45°C), at which point the sample was collected for vermicomposting. Temperature is essential in the biodegradation of organic waste during vermicomposting, and achieving the thermophilic range is crucial for destroying pathogens and biodegradable carbon (Bao et al., 2021). The study observed a high temperature of 57°C within the first 24 hours (HV_{RDC}), and the feedstock remained in the thermophilic range until day 6 before shifting to the mesophilic range. The temperature profile matches previous reports by Jain and Kalamdhad (2018). The study found the temperature profile to be statistically significant ($P < 0.05$) through single-factor ANOVA analysis.

The moisture content of the compost was minimally affected by the environmental conditions. The elevated temperature observed in the rotary drum composting system can be attributed primarily to the process of moisture evaporation during aeration. The initial moisture content of the feedstock was recorded at 87%. However, it declined to 80% within the first week and further decreased to 74% over the subsequent 20-day period. In order to promote optimal growth conditions for earthworms, the moisture levels within the vermi-reactors were carefully regulated within the 75-80% range. Comparable patterns were noted during additional examination of aquatic vegetation as substrates for composting. The absence of leachate generation during the entire composting procedure was another positive outcome of this investigation. Fig. 5.7(b) displays the mean values of the moisture content along with their corresponding standard deviation. A statistical analysis using one-way ANOVA revealed a significant variation in the moisture content over time, with a p-value of less than 0.05.

The measurement of the organic content of a substance is referred to as volatile solids (VS). During the degradation process, organic materials are transformed into humic chemicals, resulting in a decrease in VS. This study found a 15-21% average decrease in VS, with the most significant reduction of 21% observed in *E. eugeniae* (WH_{RVE}) (Fig. 5.7(b)) ($P<0.05$). In addition, a decreasing trend in total organic carbon (TOC), with the highest reduction observed in WH_{RVE} was observed. *E. fetida* also showed a strong capacity for reducing TOC by 17.72% within 20 days of vermicomposting. The amount of organic matter degraded during composting can be determined by the VS content of the substrate. Higher VS values indicate higher organic matter and *vice versa*. The microbial inoculum in the composting material obtains its food from the substrate, resulting in a significant loss of organic matter by microbial activity. A reduction of 4% in VS content in the initial 7 days of RDC, a maximum of 12.61% reduction in HV_{RVF}, and 10.66%, and 4.43% in HV_{RVE} and HV_{RDC}, respectively was documented. The release of CO_2 during composting is due to microbial utilization of the substrate, which reduces the TOC content. 60 to 70% of organic carbon is converted to CO_2, while the remaining carbon is present as microbial biomass. Fig.5.7 illustrates the decrease in TOC during the composting process, with the most substantial reduction of 11.12% observed in the $HV_{\mathbf{RVF}}$ reactors. One-way ANOVA analysis showed a significant difference in the data regarding composting days and the different vermicultures ($P<0.05$).

During water hyacinth biodegradation the final compost generated exhibited a neutral pH range of 7.5 to 8.5. The present research reveals cultivation of *E. fetida* earthworms resulted in the generation of a residual substance that was highly basic in nature. The process of composting involves the breakdown of nitrogenous substances, resulting in the release of ammonia and consequent elevation of pH levels due to microbial activity. A decrease in electrical conductivity was observed across all reactors, with a significant decline in the *E. fetida* reactor (WH_{RVF}). The presence of elevated levels of soluble salts in compost has the potential to impede the process of seed germination. Compost typically exhibits pH levels ranging from 6 to 8, with a pH of 8 being the most favorable for thermophilic composting. The pH values recorded during the composting process are presented in Fig. 5.7.

Further, during *H. verticilata* biodegradation the compost pH level at the outset was below 6, with an increase to 6.5 by the conclusion of the first week. It persisted within the standard range for composting for the subsequent 20 days. Regular rotation of the compost drum to enhance aeration has the potential to decrease CO_2 concentrations, resulting in an elevation of pH levels. The pH level observed in the vermi-reactor was found to be relatively alkaline in comparison to the control. The process of ammonification in composting is known to have a significant impact on the pH levels, leading to an increase in alkalinity. The pH values recorded for RDC and vermicomposting were within the range of pH 5.7 to 6.5 and pH 6.5 to 7.7, respectively. The ANOVA analysis revealed significant variation, with a P-value of less than 0.001.

The EC value of the material can be used to understand the degree of salinity. In the initial days of composting, the EC value of the substrate was 4.15 dS/m, 4 dS/m by the end of 7 days of RDC, and was an average of 3.3 dS/m in the end product of all the vermicomposters. The compost produced through HV_{RVF} culture reduced the EC value to 2.55 dS/m engendering a reduction of 36%. The EC of the compost was decreased by 19.51% through the combined composting process. Compost with an EC of more than 4 dS/m is unacceptable for application on agricultural soils and indicates increased salinity in the final product, which is phytotoxic to exposed seeds and plants (Singh and Kalamdhad, 2014).

5.2.2 Elemental analysis

Research shows that both conventional composting and vermicomposting lead to a rise in total nitrogen concentration (Shan et al., 2023). Gut bacterial products contributed by earthworm's impact vermicomposting, and primarily drive the increase in nitrogen content (Tong et al., 2019). In the present study, the *E. fetida* mediated reactor ($WH_{\mathbf{RVF}}$) demonstrated higher nitrogen content by the end of the vermicomposting, touching a high of 1.7, up from 0.5% in 27 days of biodegradation (Fig. 5.8).

Total Kjeldahl nitrogen and ammonium nitrogen were calculated for the duration of composting and depicted in Fig. 5.8(c). The total nitrogen content showed an increasing trend in composting, but the application of vermiculture made the significant difference compared to the control. A maximum of 3.56, 3.12, and 3.1% was observed in HV_{RVF}, HV_{RVE} and HV_{RDC} reactors, respectively, and thus higher than the findings by (Jain and Kalamdhad, 2019). It is understood that earthworm gut contains nitrogen-fixing bacteria, which help increase the nitrogen content of the vermicompost. In comparison to other earthworm cultures, *E. fetida* cultures elicited a more significant impact on nitrogen-rich end products, supporting results obtained by Devi and Khwairakpam (2020b). The ANOVA analysis showed a significant variation in the data between the composting and vermiculture processes ($P<0.05$). The ammonium nitrogen concentration decreased throughout the entire duration of the composting. The single-factor ANOVA showed significant variation in the data ($P<0.05$).

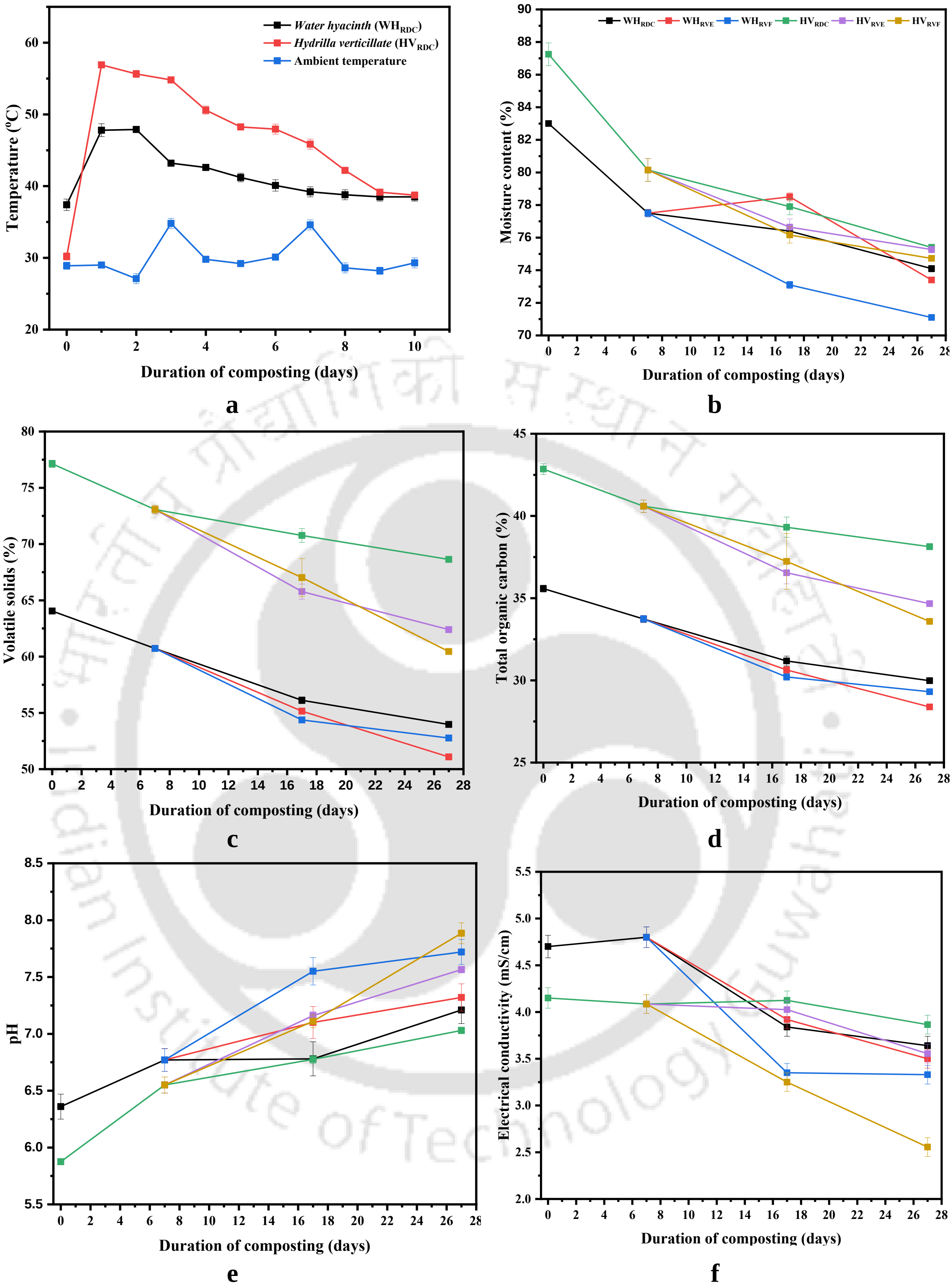
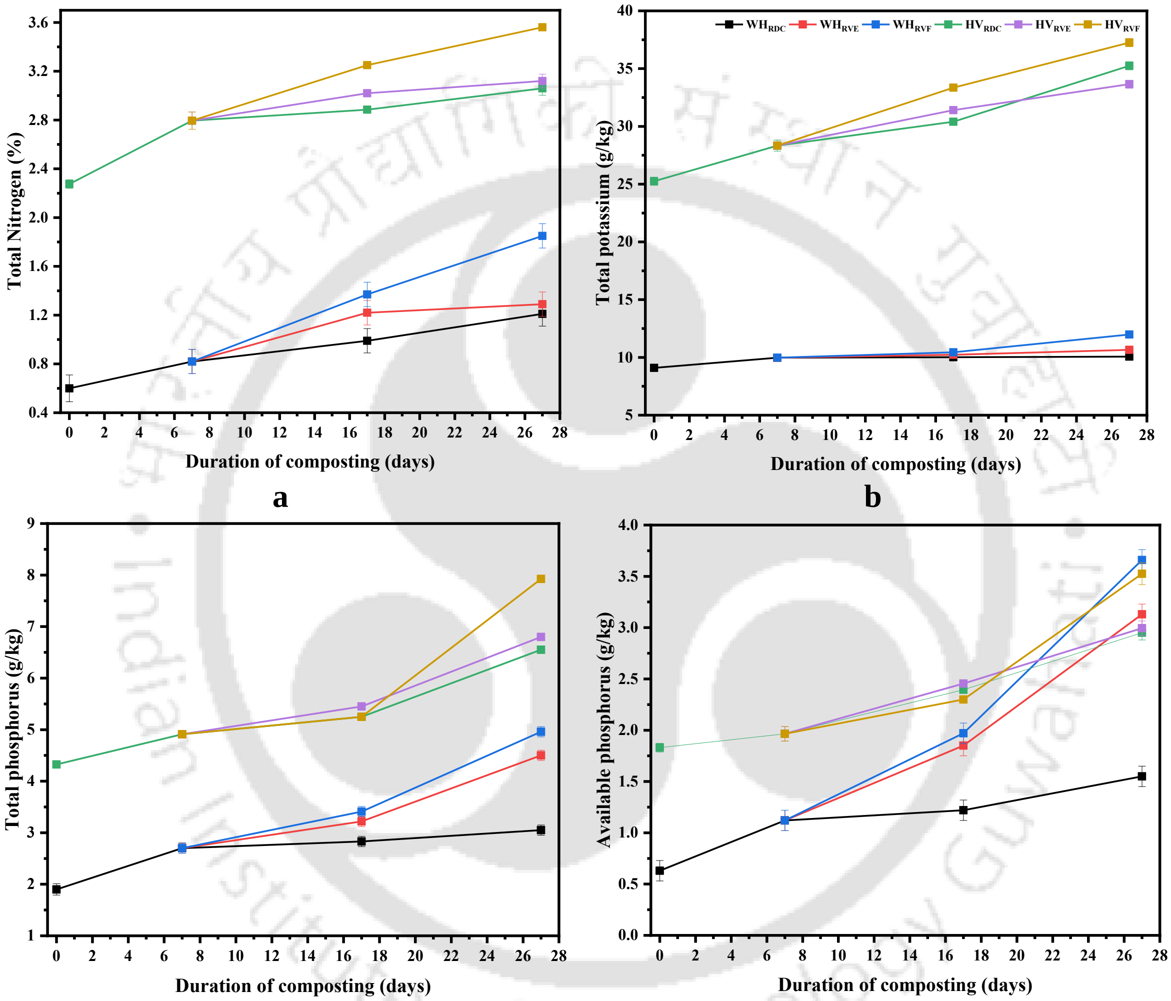


Fig. 5.7 Variation in a) Temperature, b) Moisture content, c) VS d) TOC e) pH and f) EC

The higher TP levels seen during composting may be attributed to several factors, including phosphorus mineralization, bacterial consumption, organic matter decomposition, and mass loss (Kauser et al., 2022). In the current research, the initial concentration of phosphorus was near 2 g/kg, but by the end of degradation, 5 g/kg was recorded in $\mathrm{WH_{RVF}}$. The proven efficiency of *E. fetida* in attaining higher nitrogen values attributed to the robust growth of worms in the substrate. The increasing phosphorus trend was seen in all the reactors.



a

b

c

d

Fig. 5.8 Augmentation of a) Total Kjeldhal nitrogen, b) Total potassium, c) Total phosphorus and d) Available phosphorus during stabilization

The increase in TP and AP can be attributed to the breakdown of organic matter into organic acids, which further solubilizes the insoluble phosphorus, consequently increasing the phosphorus content in the vermicompost. The TP rose from 4.32 to 7.93 g/kg in $\mathrm{HV_{RVF}}$, to 6.8 g/kg in $\mathrm{HV_{RVE}}$, to 7.66 g/kg in $\mathrm{HV_{RVC}}$, and was 7.1 g/kg in $\mathrm{HV_{RDC}}$. The AP rose from 1.83 to 3.5 g/kg, 2.9 g/kg, 3.15 g/kg, and 2.95 g/kg in $\mathrm{HV_{RVF}}$, $\mathrm{HV_{RVE}}$, $\mathrm{HV_{RVC}}$ and $\mathrm{HV_{RDC}}$, respectively (Fig. 5.8). This result is due to the earthworm gut enzymes that transfer phosphorus to available forms (acid phosphatases and alkaline phosphatases). Similar increasing trends of AP were observed in studies conducted by (Sharma and Garg, 2018). The one-way ANOVA performed on the data showed a significant variation in the nutrients for the days of composting ($P<0.05$).

TK in the present study was 9.1 g/kg initially and reached a maximum of 12 g/kg attributed to volume change and *E. fetida* vermi growth in $WH_{\mathbf{RVF}}$. Further vermicomposting of the feedstock was aided by the increased activity of earthworms, which hastens the mineralization phase and changes the potassium distribution (Mago et al., 2021). One of the essential nutrients for plant growth is phosphorous. TK is a significant consideration in all organic fertilizers, increasing soil fertility and enhancing plant growth. A similar trend was observed by earlier researchers (Singh and Suthar, 2012). The single-factor ANOVA done on the data showed a significant variation in the nutrients for the days of composting ($P<0.05$).

5.2.3 Stability analysis

The results of the study demonstrate that an increase in composting duration led to a consistent reduction in the rate of CO_2 evolution across all reactors. This finding suggests that the composting process became more stable over time. An observed reduction in the CO_2 evolution rate was noted for the WH_{RDC} reactor, decreasing from 6.55 mg/gVs/day on day 0 to 1.56 mg/gVs/day on day 27. The biodegradation process, which involved two stages (WH_{RVF}), yielded a CO_2 evolution rate of 0.66 mg/gVs/day at the conclusion of a 27-day period. This rate was the lowest among all reactors and indicated that the biomass remained stable. The single-stage HV_{RDC} exhibited the highest CO_2 evolution rate after 27 days (Fig. 5.9), with a recorded value of 1.43 mg/gVs/day, when compared to other systems. The observed correlation between composting duration and CO_2 evolution rate, as well as the variations between reactors, underscores the significance of meticulous management of the composting process to attain a stable and effective compost.

At the onset of the composting procedure, all six trials exhibited comparable oxygen uptake rate OUR values, which varied between 5.05 and 9.52. Throughout the composting process, the observed OUR values exhibited a decrease across all six experimental trials, thereby suggesting a transition towards a more mature state. The rate of decline exhibited variability across the experiments, with certain combinations manifesting a more rapid decrease than others. Following a seven-day composting period, all six experiments exhibited comparable OUR values, which ranged from 3.87 to 5.36. Nevertheless, upon the completion of 17 and 27 days of composting, discernible variations in the OUR measurements across various composting blends were noted. At the 27th day of the experiment, the OUR values for $WH_{\mathbf{RVF}}$ and $HV_{\mathbf{RVF}}$ composting mixtures were comparatively lower than the other mixtures, which suggests a greater level of stability.

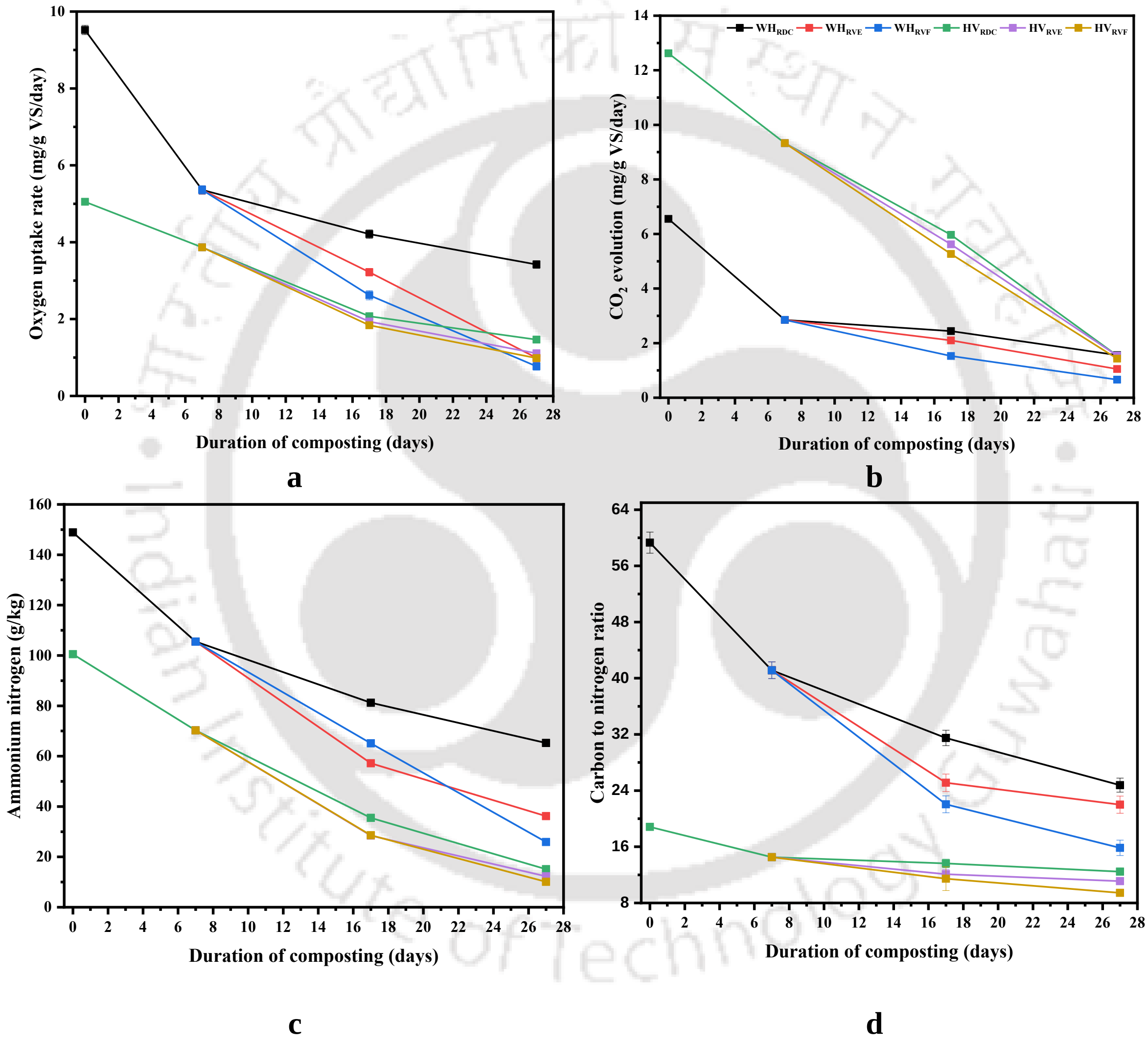


Fig. 5.9 Augmentation of a) Total Kjeldhal nitrogen, b) Total potassium, c) Total phosphorus and d) Available phosphorus during stabilization

The presented data depicts the levels of ammonium nitrogen (NH_4-N) concentration in six distinct composting trials, namely WHRDC, WHRVE, WHRVF, HVRDC, HVRVE, and HVRVF, across varying composting periods (0, 7, 17, and 27 days). The experiments were conducted with NH_4-N solutions that exhibited a relatively high initial concentration, varying between 100.50 and 148.90 mg/kg. The decline in NH_4-N concentration during the progression of composting signifies the conversion of organic nitrogen to ammonium nitrogen through mineralization. Following a 27-day composting period, a notable reduction in NH_4-N concentration was observed. Specifically, HV_{RDC}, HV_{RVE}, and HV_{RVF} exhibited lower NH_4-N concentration compared to WH_{RDC}, WH_{RVE}, and WH_{RVF}. The data indicates that the composting blend comprising of HV_{RDC}, HV_{RVE}, and HV_{RVF} exhibited greater efficacy in transforming organic nitrogen into ammonium nitrogen in comparison to the blend of WH_{RDC}, WH_{RVE}, and WH_{RVF}. The tabulated data suggests that the duration of the composting process and the composition of the composting mixture are significant determinants of the NH_4-N concentration, a key factor in the production of compost with superior quality. The observed decrease in NH_4-N concentration as composting duration increases implies the stabilization of the compost, thereby indicating its maturity and quality.

The results of the study demonstrate that an increase in composting duration led to a consistent reduction in the rate of CO_2 evolution across all reactors, suggesting that the composting process became more stable over time. The $WH_{\mathbf{RDC}}$ reactor exhibited a reduction in the CO2 evolution rate from 6.55 mg/gVs/day on day 0 to 1.56 mg/gVs/day on day 27. The biodegradation process, which involved two stages ($WH_{\mathbf{RVF}}$), yielded a CO_2 evolution rate of 0.66 mg/gVs/day at the conclusion of a 27-day period (Fig. 5.9). This rate was the lowest among all reactors and suggests that the biomass remained stable. The single-stage $HV_{\mathbf{RDC}}$ exhibited the highest CO_2 evolution rate after 27 days, with a recorded value of 1.43 mg/gVs/day, when compared to other systems. The observed correlation between composting duration and CO_2 evolution rate, as well as the variations between reactors, underscores the significance of meticulous management of the composting process to attain a stable and effective compost.

5.2.4 Earthworm population dynamics

Table 2 presents data pertaining to the proliferation of distinct earthworm populations throughout the vermicomposting procedure. The tabular data illustrates the quantities of adult earthworms, juveniles, and cocoons per 100 grams of the ultimate compost for two distinct species of earthworms, namely *E. fetida* ($WH_{\mathbf{RVF}}$ and $HV_{\mathbf{RVF}}$) and *E. eugeniae* ($WH_{\mathbf{RVE}}$ and $HV_{\mathbf{RVE}}$). According to the table, the initial population of adult earthworms in each of the four cultures was 120. The *E. fetida* culture in the WH_{RVF} exhibited a rise in the number of adult earthworms from 120 to 130 following a 10-day period of vermicomposting, which further increased to 143 after 20 days. Within this particular cultural context, it was observed that the population of juvenile organisms experienced a numerical increase from 17 to 27 over a period of 20 days. Additionally, the number of cocoons present per 100 g of compost increased from 17 to 27. In comparison to the WH_{RVE} culture of *E. eugeniae* exhibited a modest rise in the population of mature earthworms, with a recorded count of 120 increasing to 125 after 10 days, and further increase to 136 after 20 days. Over a period of 20 days, there was an observed increase in the population of juvenile organisms from 2 to 12. Additionally, the number of cocoons per 100 g of compost increased from 5 to 10.

Table 5.5. Growth in the vermi culture population during the vermicomposting duration

	Adults			**Juvenules**		**Cocoons**	
Day	0	17	27	17	27	17	27
WH_{RVF}	120 ± 1	130 ± 2	143 ± 6	4 ± 2	10 ± 2	5 ± 2	12 ± 1
WH_{RVE}	120 ± 1	125 ± 2	136 ± 10	2 ± 1	12 ± 2	5 ± 2	10 ± 2
HV_{RVF}	120 ± 1	133 ± 1	155 ± 1	6 ± 2	24 ± 2	5 ± 2	32 ± 3
HV_{RVE}	120 ± 1	136 ± 1	148 ± 1	4 ± 2	18 ± 2	4 ± 3	22 ± 3

*cocoons in the table refer per 100 grams of the final compost

The *E. fetida* (HV_{RVF})culture exhibited a statistically significant rise in the population of mature earthworms, increasing from 120 to 155 individuals following a 20-day period of vermicomposting. Among all four cultures, this particular culture exhibited the highest count of juveniles (24) and cocoons (32 per 100 g of compost). The culture of *E. eugeniae* (HV_{RVE}) exhibited a rise in the population of mature earthworms from 120 to 136 over a period of 10 days, followed by a subsequent increase to 148 after 20 days. Over a period of 20 days, t increase in the number of juveniles from 4 to 18, and a corresponding increase in the number of cocoons from 4 to 22 per 100 grams of compost was observed. The findings presented in Table 2 indicate that *E. fetida* cultures exhibited a favorable acclimation to the vermicomposting process, and demonstrated superior growth and reproductive rates in comparison to *E. eugeniae* cultures. During the 20-day vermicomposting process, the *E. fetida* HV_{RVF} culture exhibited a significant rise in population size, accompanied by a greater production of juveniles and cocoons. The results indicate that *E. fetida* may be a more appropriate species for vermicomposting operations on a larger scale.

In comparison to the acclimatized *E. fetida* culture that exhibits high reproductive growth, the observed increase in total nitrogen within the reactors serves as an indicator of the rapid degradation of substrate by the *E. fetida* culture. The present study underscores the significance of selecting an appropriate earthworm species to attain maximum efficacy in the process of vermicomposting. The species *E. fetida* has been identified as the most efficient in decomposing organic matter and generating compost of superior quality.

5.2.5 Summary

The efficacy of the RV biodegradation technique in decomposing marine vegetation has been demonstrated to occur within a period of 27 days. The methodology comprises of two distinct stages, namely RDC and VC. The active thermophilic phase of the RDC phase was achieved within the initial 24-hour period, which plausibly facilitated the growth of earthworm species in the substrate during the VC phase lasting 20 days. The (TOC) exhibited a notable reduction as the feedstock underwent further degradation. The efficacy of the RV method in breaking down lignocellulose compounds in the feedstock was validated through spectroscopy-based investigations. The investigation on the phytotoxicity of compost extract exhibited a favorable influence on the index of germination. Furthermore, the vermicompost additives were found to fulfil the soil's nutritional demands and enhance its NPK composition, thereby promoting robust plant development. In general, the ability of the RV decomposition technique to reduce composting time has the potential to mitigate the burden on vegetation management practices globally.

5.3 TERRESTRIAL WEED BIOMASS

5.3.1 The effect of a two-step biodegradation process on utilizing *Mikania micrantha* and *Ageratum conyzoides* as a source of feedstock

MM_{RDC} (*M. micrantha*) and AC_{RDC} (*A. conyzoides*) were the experimental combinations for RDC. The experiments utilizing *E. fetida* in the RV approach were MM_{RVF} (*M. micrantha*) and AC_{RVF} (*A. conyzoides*). In the case of MM_{RVE} (*M. micrantha*) and AC_{RVE} (*A. conyzoides*), *E. eugeniae* were used.

i) Physicochemical examination

Temperature

The temperature of composting is crucial for organic matter breakdown by microorganisms (Wang et al., 2023). The usual composting process is subject to mesophilic, thermophilic, cooling, and maturation phases. In rotary drum composting, the initial mesophilic phase lasts only for hours, and the thermophilic phase lasts for the initial 7 days, as shown in Fig.5.10. The thermophilic temperatures were observed in both MM**RDC** and AC**RDC** within 24 h after feeding the rotary drum composter (56.90°C in MM**RDC** and 55.70°C in AC**RDC**). The maximum temperature of 56.90°C in MM**RDC** and 63.80°C were observed in AC**RDC** by 1st and 2nd day, respectively, during the RDC of feedstock.

Moisture content and Volatile solids

During composting, MC influences microbial metabolic activity and material transformation (Tang et al., 2023). The microbial decomposition of organic materials yields water (Zhao et al., 2023). During the thermophilic degradation, the moisture thus produced turns into vapor and subsequently released into the atmosphere when the rotary drum is rotated for aerating the biomass during composting (Kauser et al., 2022). Fig 5.10 illustrates the moisture content profile of the degradation process. MM**RDC** observed a reduction of 10% in MC during thermophilic degradation, and the value was only 3.5% in AC**RDC** since the weed was lignocellulosic, whereas *M. micrantha* is a creeper weed and contained less lignocellulosic material. Further, 18.5% and 7% were reduced overall during 27 days of RDC in MM**RDC** and AC**RDC**.

In rotary drum biodegradation, microorganisms and thermophilic bacterial activities efficiently break down organic content (Wan et al., 2020). Compost maturity is indicated by a considerable drop in volatile solids (VS). A more significant drop in VS suggests a more reliable product, endorsing that earthworms are crucial in waste decomposition (Zhong et al., 2023). The destruction of volatile solids was observed as 14.27% and 14.33% in MM**RDC** and AC**RDC** during the initial 7 days, and the further widespread destruction figures of 23.90% and 20.14%, respectively were subsequently documented. Further, vermicomposting feedstock post thermophilic degradation resulted in 40.02% (MM**RVE**)and 43.89% (MM**RVF**) reduction in VS; similar trend was also observed in *A. conyzoides* reactors, 26.45% and 29.75% in AC**RVE** and AC**RVF**, respectively.

Changes in Total organic carbon

The availability of organic carbon in the composting mixture affects metabolic activity since it serves as the primary energy source for bacteria. A more significant metabolic activity uses the available substrate material better, leading to a more efficient breakdown. As a result, increased breakdown reduces the amount of organic carbon (Devi and Khwairakpam, 2020b). There was higher TOC reduction during vermicomposting due to the action of earthworms. In *M. micrantha* fed reactors, post-RDC, 24.74% and 29.62% TOC were reduced in MM_{RVE} and MM_{RVF}, whereas it was observed to be only 9.63% in MM_{RDC}. Similarly, in *A. conyzoides* fed reactors, 5.81, 12.12 and 15.42% in AC_{RDC}, AC_{RVE} and AC_{RVF}, respectively.

Variatuion in pH and electrical conductivity

The release of ammonium ions from the breakdown of organic substances like proteins, peptides or amino acids caused the pH level to increase. This pH shift was not uniform and may exhibit divergent behavior dictated by the amount of feedstock, the kind of amendment, and the earthworm species (Devi and Khwairakpam, 2020b). The presence of earthworms reduced the pH, indicating that earthworms hastened the breakdown of organic materials into humic/fulvic acid (Zhong et al., 2023). In the present research, the pH of the feedstock evinced a change from neutral to alkaline pH in all the experimented trials. The trials where vermicomposting was included exhibited higher alkaline rage (> pH 8.5), whereas, in control, it was between pH 8.10-8.35.

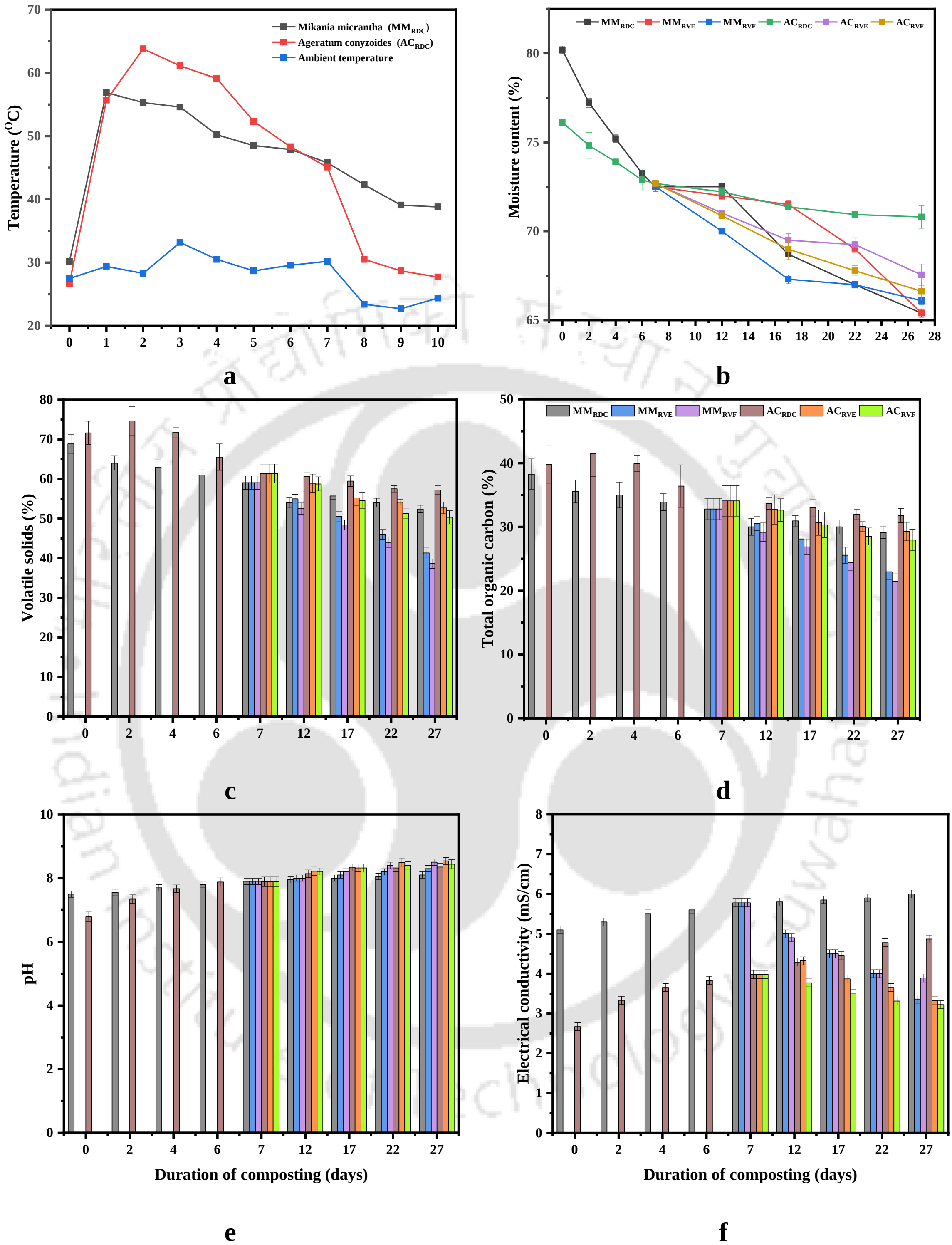
Furthermore, there is a negative impact on soil fertility and plant development due to the high salt levels in the soil. The EC value of the trials that include only RDC exhibited an increasing trend, whereas the trials where vermicomposting was incorporated exhibited a degreasing trend, attributed to the mineralization of the organic matter by earthworms. The EC values changed from 5.10 to 6 mS/cm in MM_{RDC} and from 2.67 to 4.87 mS/cm in AC_{RDC}. Further, due to vermicomposting, the EC value decreased from 5.78 to 3.36 and 3.89 mS/cm in MM_{RVE} and MM_{RVF}, respectively (Fig. 5.10). Similarly, EC values decreased from 3.98 to 3.32 and 3.22 mS/cm in AC_{RVE} and AC_{RVF}, respectively. Previous research indicates that biodegradation by-products with a value of less than 4 mS/cm must be considered suitable for land application, which underscores that the compost produced from RDC is specifically unfit for soil application.

ii) Elemental analysis

The significance of microbial activity during the composting is validated by quick decomposition of organic matter, generation of nitrogenous compounds, and increase in the TKN concentration (Liu and Zhang, 2023). Conversely, the nitrogen concentration of vermicompost was much higher than the RDC end product in this research and attributed to the occurrence of the nitrogen-fixing bacterium in the earthworm intestines. Further, the *E. fetida* culture multiplied faster than other earthworm cultures, evinced by higher nitrogen accumulation the *E. fetida* reactors. The final TKN was found to be 2.21% (MM$_{RDC}$) and 2.52% (AC$_{RDC}$) via RDC, versus RVC 2.68, 2.86, 3.50, and 3.92% in MM$_{RVE}$, MM$_{RVF}$, AC$_{RVE}$ and AC$_{RVF}$, respectively. In addition, the change was more than the initial TKN values of feedstock mass 1.45% (*M. micrantha*) and 1.54% (*A. conyzoides*) (Fig. 5.11).

Phosphorus is required for photosynthesis in plants and is accessible through inorganic ions known as orthophosphates. Mineralization of organic substances raises TP and AP content. In the second stage of RV, the increase in TP and AP was attributed to a thermophilic breakdown in RDC and worm gut enzyme activity (Devi and Khwairakpam, 2020a). Whereas in single-stage RDC, it can be said that only the influence of thermophilic breakdown played a significant role. By the end of 27 days, the TP increased from 7.18 to 9.52, 10.35 and 14.20 g/kg in MM$_{RDC}$, MM$_{RVE}$ and MM$_{RVF}$ (*M. micrantha* reactors) respectively; and from 5.24 to 9.20, 11.75 and 12.76 g/kg in AC$_{RDC}$, AC$_{RVE}$ and AC$_{RVF}$ (*A. conyzoides* reactors), respectively (Fig. 5.11). Further, AP increased from 2.15 to 3.01, 3.68 and 3.94 g/kg in MM$_{RDC}$, MM$_{RVE}$ and MM$_{RVF}$ (*M. micrantha* reactors) and from 2.20 to 3.65, 4.40 and 4.77 g/kg in AC$_{RDC}$, AC$_{RVE}$ and AC$_{RVF}$ (*A. conyzoides* reactors), respectively.

Total potassium increased during the process and was attributed to the bulk change (Maturi et al., 2021). After biodegradation, TK amplified from 18.22 to 22.14, 22.20 and 26.71 g/kg in MM$_{RDC}$, MM$_{RVE}$ and MM$_{RVF}$ (*M. micrantha* reactors), and from 0.70 to 1.70, 2.30 and 2.40 g/kg in AC$_{RDC}$, AC$_{RVE}$ and AC$_{RVF}$ (*A. conyzoides* reactors), respectively. Among the micronutrients, the increasing availability of TK is due to the action of organic acids generated during the biodegradation of organic materials (Mago et al., 2021).

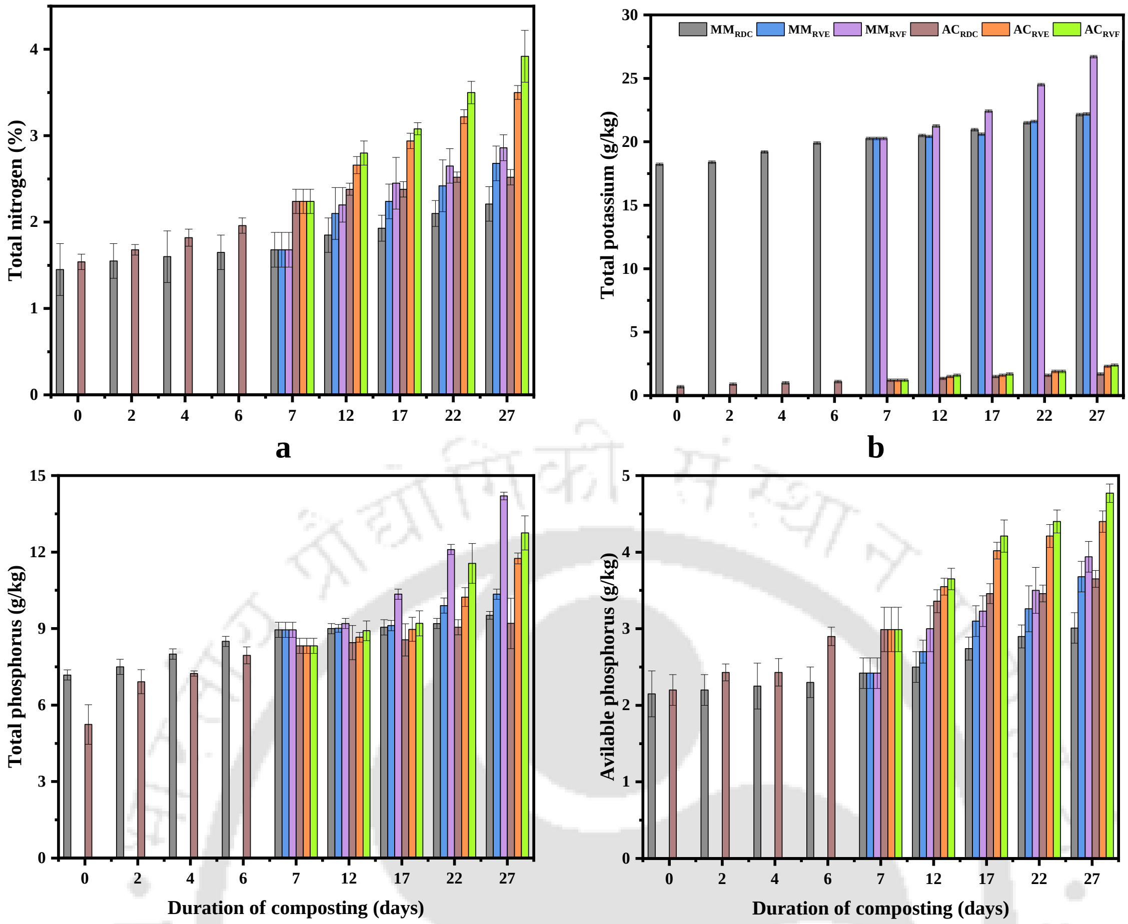


a b

c d

e f

Fig. 5.10 Changes in a) Temperature profile, b) Moisture content, c) Volatile solids fraction, d) Total organic carbon content, e) pH of the feedstock and d) Electrical conductivity throughout the study.



a b c d

Fig. 5.11 Changes in a) Total nitrogen, b) Total potassium, c) Total phosphorus and d) Available phosphorus throughout the study.

iii) Stability analysis of the feedstock

When the supply of oxygen (O_2) is inadequate, microbial metabolism available for composting is reduced, the biomass is not entirely decomposed, and numerous anaerobic microsites develop in the feedstock, emitting greenhouse gases such as hydrogen sulphide and methane (Ge et al., 2015). Whenever aeration is high, energy consumption rises, and the physiological thermophilic phase is hampered, with low levels of pathogen destruction. (He et al., 2013). OUR has been extensively investigated and modelled as a vital indicator of actual O_2 consumption and degradation (Tremier et al., 2005). The current study has a maximum of 48.45, 87.66, 92.21, 69.54, 81.19, and 83.02 % OUR reduction in MM$_{RDC}$, MM$_{RVE}$, MM$_{RVF}$, AC$_{RDC}$, AC$_{RVE}$ and AC$_{RVF}$ were recorded, of which 14 and 46 % was due to the thermophilic phase in reactors MM$_{RDC}$ and AC$_{RDC}$. The RVDC approach recorded the maximum drop.

The rate of CO_2 evolution is connected to the breakdown of volatile compost material and reflects the presence of quickly degradable composting material in the compost sample (Mishra and Yadav, 2022). As a consequence, the rate of CO_2 release was maximum through the thermophilic phase. The CO_2 release reduced steadily and stabilized, suggesting gradual compost maturation (Xiong et al., 2021). In the present study, a maximum of 63.45, 73.33, 78.85, 77.54, 78.53 and 78.18 % reduction in MMRDC, MMRVE, MMRVF, ACRDC, ACRVE and ACRVF were logged, of which 40.69 and 38.33 % was due to the thermophilic phase in reactors MMRDC and ACRDC. Further, the end products of RDCVC exhibited a CO_2 evolution rate near 1 mg/gVS/Day, which is optimal for soil application.

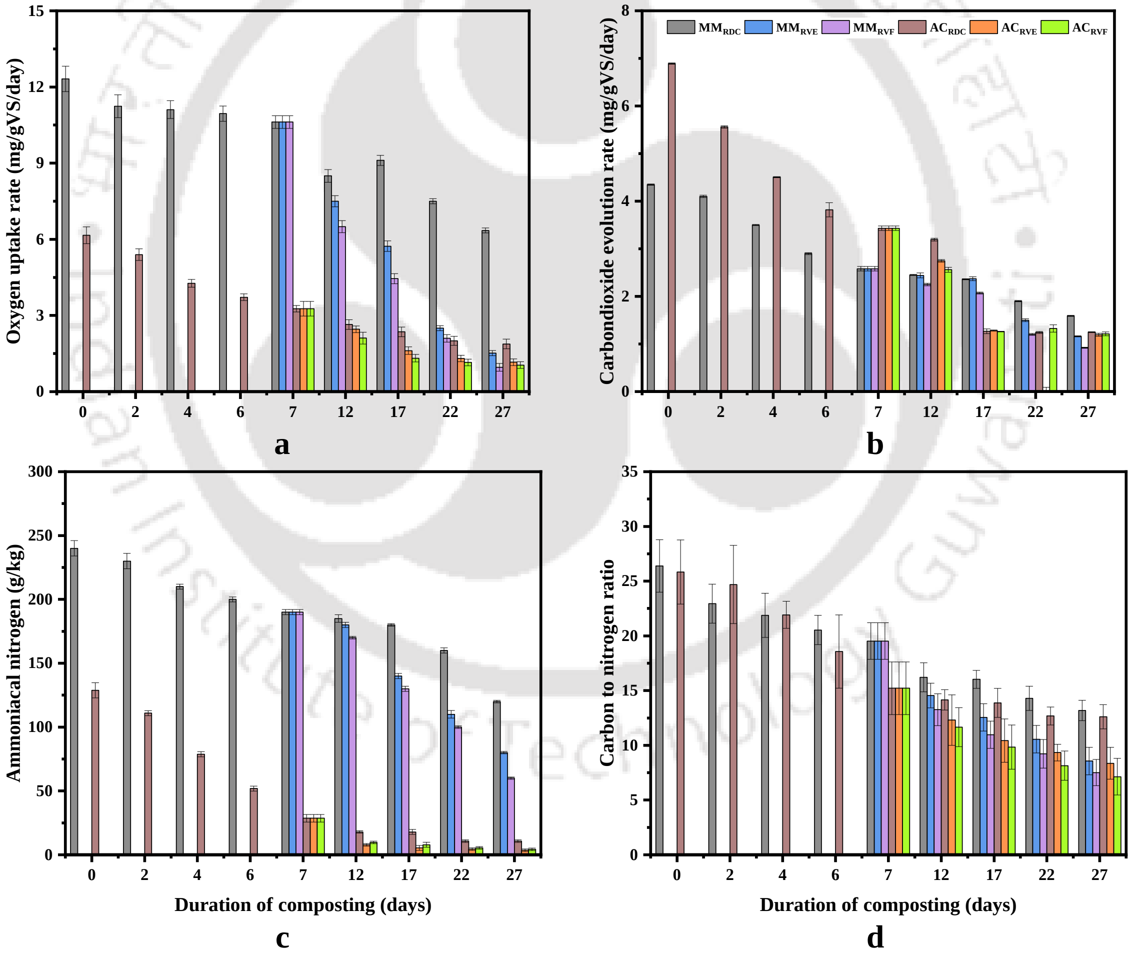


Fig. 5.12 Changes in a) Oxygen uptake rate, b) Carbon dioxide uptake rate, c) Ammoniacal nitrogen content and d) Carbon to nitrogen ratio throughout the study.

During thermophilic degradation, the NH_4-N concentration decreased substantially in composting processes. Further, during the maturation stage, excessive aeration, a significant rise in pH, and CO_2 loss in the process will result in a drop in ammoniacal nitrogen (Kauser et al., 2020). In the present study, the reduction in NH_4-N throughout the composting was seen as higher in *A. conyzoides* reactors compared to *M. micrantha* mediated reactors. Thermophilic biodegradation in the RDC *A. conyzoides* (AC_{RDC}) reactor experienced a 77.77% reduction in NH_4-N, whereas in the *M. micrantha* (MM_{RDC}) reactor only a 20.83% reduction was exhibited. Further vermicomposting was efficient in NH_4-N removal in MM_{RVE} and MM_{RVF} with 66.67 and 75% overall reduction in NH_4-N. Fig.5.12 illustrates the trends of NH_4-N removal efficiency of RDC and RV. Furthermore, RV displayed higher proven efficiency in NH_4-N removal versus RDC in the weed bioconversion process. Reduction in NH_4-N indicates the maturation progression of the feedstock (Kauser et al., 2020). In the present study, the C/N ratio was observed to be decreasing throughout the biodegradation both in RDC and RV trials. The trials with *A. conyzoides* as feedstock exhibited higher content of TKN (>3%), which is also a reason for the higher reduction in the C/N ratio for the trials. A maximum of 50.07, 67.55, 71.55, 51.20, 67.64 and 72.41% overall reduction was observed in MM_{RDC}, MM_{RVE}, MM_{RVF}, AC_{RDC}, AC_{RVE} and AC_{RVF}, out of which 26 % (MM_{RDC}) and 41.10% (AC_{RDC}) are due to thermophilic degradation due to RDC.

iv) Growth in the earthworms during vermicomposting

The mechanisms through which the addition of partially degraded material enhances vermicomposting effectiveness may be as follows: (1) changing temperature, humidity, and ionic strength, all of which reduce the amount of alcohol, ammonia, and methane; besides promoting respiration and regular physiological activity in earthworms; (2) increasing feedstock consumption by improving palatability for earthworms; in addition to (3) controlling the C/N proportion of the material, and generating favorable conditions for earthworms (Zhong et al., 2023). The number of earthworms by the end of 20 days of vermicomposting the partially degraded material has been presented in Table 5.6. *E. fetida* displayed robust degradation of the phyto-biomass, enabled by inherent faster growth rate and replicating capacity, which engenders successful survival and multiplication on a broad range of substrates and environmental circumstances. Based on the literature, *E. fetida* is the most often employed epigamic earthworm, with *E. eugeniae* coming in a close second (Varma et al., 2016). The growth in *E. fetida* was higher with weeds and vermicomposting. Further, the earthworm count was much higher (nearly 2 times) in *A. conyzoides* reactors. The TKN and NH_4-N trends can be correlated with the earthworm growth dynamics. Also, cocoon production was significant in *E. fetida*.

Table 5.6 Growth in the earthworms during vermicomposting

Day	Worms	Juveniles	Cocoons (per 100 g)	Worms	Juveniles	Cocoons (per 100 g)
	MMRVE			**MMRVF**		
7	180 ± 0	0	0	180 ± 0	0	0
17	210 ± 2	55 ± 12	6 ± 2	208 ± 3	35 ± 10	9 ± 3
27	249 ± 5	42 ± 10	33 ± 4	269 ± 6	26 ± 12	48 ± 5
	ACRVE			**ACRVF**		
7	180 ± 0	0	0	180 ± 0	0	0^c
17	270 ± 6	25 ± 3	90 ± 5	310 ± 5	24 ± 10	122 ± 3
27	310 ± 10	22 ± 2	170 ± 10	567 ± 6	20 ± 12	198 ± 5

v) Change in total heavy metal concentration

Heavy metals (HMs), non-biodegradable and indestructible, can only be transmitted from one source to another (Khan et al., 2023). Emissions of CO_2 and organic material biodegradation, which decreases the overall bulk of waste while raising its metal content, all contribute to increased HMs in the end product (Yadav and Garg, 2011). During rotary drum composting, it was evident that due to changes in the bulk of the waste, the concentrations of HMs exhibited an increasing trend (Wang et al., 2016). Further, the bioaccumulation of HMs during vermicomposting is documented in various studies, and it has been concluded that vermicomposting reduces heavy metal contamination in soil and biomass (Sun et al., 2020).

During only RDC, the concentration was in the order of Pb>Ni>Cu>Mg for MMRDC and in the order of Mg>Cu>Ni>Pb for ACRDC. The higher concentration of heavy metals in *A. conyzoides* is one of the reasons for the change in the order of the heavy metal increment and can be attributed to changes in bulk during the biodegradation process. In RV trials of *M. micrantha*, the order in the initial 7 days was Pb>Ni>Cu>Mg and Pb>Ni>Cu>Mg for MMRVF and MMRVE, respectively. By the end of 20 days of vermicomposting, the order changed to Pb>Cu>Ni>Mg and Pb>Ni>Cu=Mg for MMRVF and MMRVE, respectively. In RV trials of *A. conyzoides*, the order in the initial 7 days was Cu>Mg>Ni>Pb and Cu>Mg>Ni>Pb for ACRVF and ACRVE, respectively.

Table 5.7. Change in total heavy metal concentration

	Duration of composting (days)								
	0	7	17	27	17	27	17	27	MoEF
	MM$_{\mathrm{RDC}}$		**MM$_{\mathrm{RVE}}$**		**MM$_{\mathrm{RVF}}$**				
Pb	16 ± 0.52^{ac}	25 ± 0.14^{ac}	26 ± 0.14^{ac}	27 ± 0.35^{ac}	24 ± 0.50^{ac}	23 ± 0.60^{ac}	24 ± 0.15^{ac}	22 ± 0.12^{ac}	100
Cu	50 ± 0.23^{b}	55 ± 0.25^{b}	58 ± 0.25^{b}	59 ± 0.12^{b}	54 ± 0.96^{b}	52 ± 0.14^{b}	54 ± 0.26^{b}	50 ± 0.15^{b}	300
Ni	25 ± 0.12^{a}	29 ± 0.21^{a}	32 ± 0.12^{a}	33 ± 0.15^{a}	28 ± 0.98^{a}	27 ± 0.45^{a}	28 ± 0.69^{a}	27 ± 0.14^{a}	50
Mg	45 ± 0.52^{b}	48 ± 0.75^{b}	49 ± 0.25^{b}	50 ± 0.12^{b}	48 ± 0.11^{b}	46 ± 0.14^{b}	47 ± 0.52^{b}	46 ± 0.25^{b}	NI
	AC$_{\mathrm{RDC}}$		**AC$_{\mathrm{RVE}}$**		**AC$_{\mathrm{RVF}}$**				
Pb	900 ± 10^{ac}	952 ± 11^{ac}	978 ± 16^{ac}	985 ± 10^{ac}	946 ± 12^{ac}	910 ± 10^{ac}	921 ± 15^{ac}	899 ± 10^{ac}	100
Cu	70 ± 15^{b}	82 ± 10^{b}	88 ± 15^{b}	92 ± 05^{b}	81 ± 11^{b}	79 ± 14^{b}	80 ± 12^{b}	78 ± 10^{b}	300
Ni	1450 ± 13^{a}	1552 ± 15^{a}	1590 ± 10^{a}	1621 ± 15a	1520 ± 10^{a}	1489 ± 05^{a}	1425 ± 05^{a}	1324 ± 12^{a}	50
Mg	1420 ± 15^{b}	1659 ± 11^{b}	1865 ± 10^{b}	1925 ± 11^{b}	1596 ± 05^{b}	1582 ± 06^{b}	1524 ± 05^{b}	1359 ± 15^{b}	NI

NI: Not included. The values are averages with standard deviations (n = 3). According to Tukey's test, the measures reveal statistically significant variations ($P < 0.05$). All the values are in mg kg^{-1} with mean ± standard deviation

By the end of 20 days of vermicomposting, the order changed to Mg>Ni>Pb>Cu and Mg>Pb>Ni>Cu for AC$_{\mathrm{RVF}}$ and AC$_{\mathrm{RVE}}$, respectively. The total heavy metal concentration dynamics throughout the biodegradation of all the trials are presented in Table 5.7, and the standards of the Ministry of Environment, Forest and Climate Change (MoEF) are tabulated as a reference (Swati and Hait, 2017). The reactors with only RDC treatment exhibited increasing trends of total heavy metal concentration, whereas the vermicomposting-mediated trials exhibited a reduction in the metal concentration by the end of the biodegradation process. The heavy metal content investigation validates the utility of the RV in reducing metal contamination of the end product.

Bivariant correlation of heavy metals during biodegradation

Pearson’s bivariant correlation was studied for the concentrations of metals obtained during biodegradation in all trials. It is evident from the results that the amendment of vermicomposting significantly impacted the metals and their correlation with time, during the biodegradation process. The correlation coefficient of metals with the duration of biodegradation was significant in MM$_{\mathbf{RDC}}$ ($r > 0.84$) and AC$_{\mathbf{RDC}}$ ($r > 0.91$). The r values were insignificant with regard to duration and metal concentration in vermicomposting amended trials ($r \approx 0$). Further, the effect of vermiculture growth (Annexure II) can be better understood in the correlation matrix of AC$_{\mathbf{RVF}}$, where the correlation was insignificant and negative for all metal concentrations. Furthermore, the study shows that the correlation between metals is highly significant in RDC trials ($r > 0.95$) but weak in RV trials ($r > 0.73$). It is statistically evident that vermicomposting process positively affected metal bioaccumulation during the vermicomposting of the feedstock.

5.3.2 The effect of a two-step biodegradation process on utilizing *Parthenium hysterophorus* and *Lantana camara* as a source of feedstock

The experimental combinations for RDC were P$_{\mathbf{RDC}}$ (*P. hysterophorus) and* L$_{\mathbf{RDC}}$ (*L. camara*). In the RV technique, the trials using *E. fetida* were P$_{\mathbf{RVF}}$ (*P. hysterophorus*) and L$_{\mathbf{RVF}}$ (*L. camara*). Using *E. eugeniae* P$_{\mathbf{RVE}}$ (*P. hysterophorus*) and L$_{\mathbf{RVE}}$ (*L. camara*).

i) Physicochemical analysis

Variation in temperature and moisture content

The temperature profile provides a clear indication of the different stages of the biodegradation process. On the 2nd day of the RDC, the recorded maximum temperatures in the P$_{\mathbf{RDC}}$ and L$_{\mathbf{RDC}}$ were 55.90°C and 62.20°C (Fig. 5.13(a)). The effective functioning of the rotary drum composter, the amended inoculums, and the bulking agent accelerated early attainment of the thermophilic phase in feedstock mass (Karadag et al., 2013). The critical bacterial population during this thermophilic phase is known to help in the breakdown and mineralization of organic waste rapidly (Kong et al., 2020). The resultant self-heating of the feedstock is also attributed to the thermophilic and mesophilic microbial consortia (Antunes et al., 2016), followed by subsequent progressive cooling towards the mesophilic phase. The temperature shift in the rotary drum is usually caused by impeded heat-related moisture evaporation. However, heat loss is produced by the sensible heat of the feedstock, the ambient temperature, latent heat of evaporation, heat loss via the reactor wall, surface convection, and the rotation of the rector for feedstock aeration (Jain and Kalamdhad, 2019). Consequently, the temperature difference between the ambient and reactor temperatures after the 8th day was insignificant. Similar temperature patterns were documented when the rotary drum was used in the biodegradation of organic materials (Hazarika and Khwairakpam, 2018; Jain and Kalamdhad, 2019; Kalamdhad et al., 2008; Kauser et al., 2020). Subsequently, the reactors P_{RVF}, P_{RVE}, L_{RVF}, and L_{RVE} were established on the 8th day to vermicompost the partially degraded feedstock from the rotary drum. As these reactors were kept in ambient atmospheric conditions in the laboratory, the ambient temperature profile followed.

MC is crucial in sustaining efficient aerobic biodegradation process (Shen et al., 2015). A low MC hinders microbial activity, resulting in an unstable and immature product, while a high MC restricts mass oxygen transfer (Liang et al., 2003). The optimum MC varies according to the type of waste and the degradation procedure (Gurusamy et al., 2021). Numerous studies suggest that a 60-75 % MC is optimal depending on the type of waste used in the biodegradation process (Guo et al., 2012; Gurusamy et al., 2021). The MC of the feedstock in this study was maintained between 70 and 75 % as the feedstock consisted of lignocellulosic weeds. There was no evidence of leachate formation throughout the biodegradation process utilising RDC. During the thermophilic degradation of RDC, the temperature increase impacted the moisture content of the feedstock, resulting in a decrease of 7 %, while from the 8th to the 27th day, the change in moisture content of the feedstock remained negligible ($P<0.05$).

Changes in the volatile solids fraction and Total organic carbon

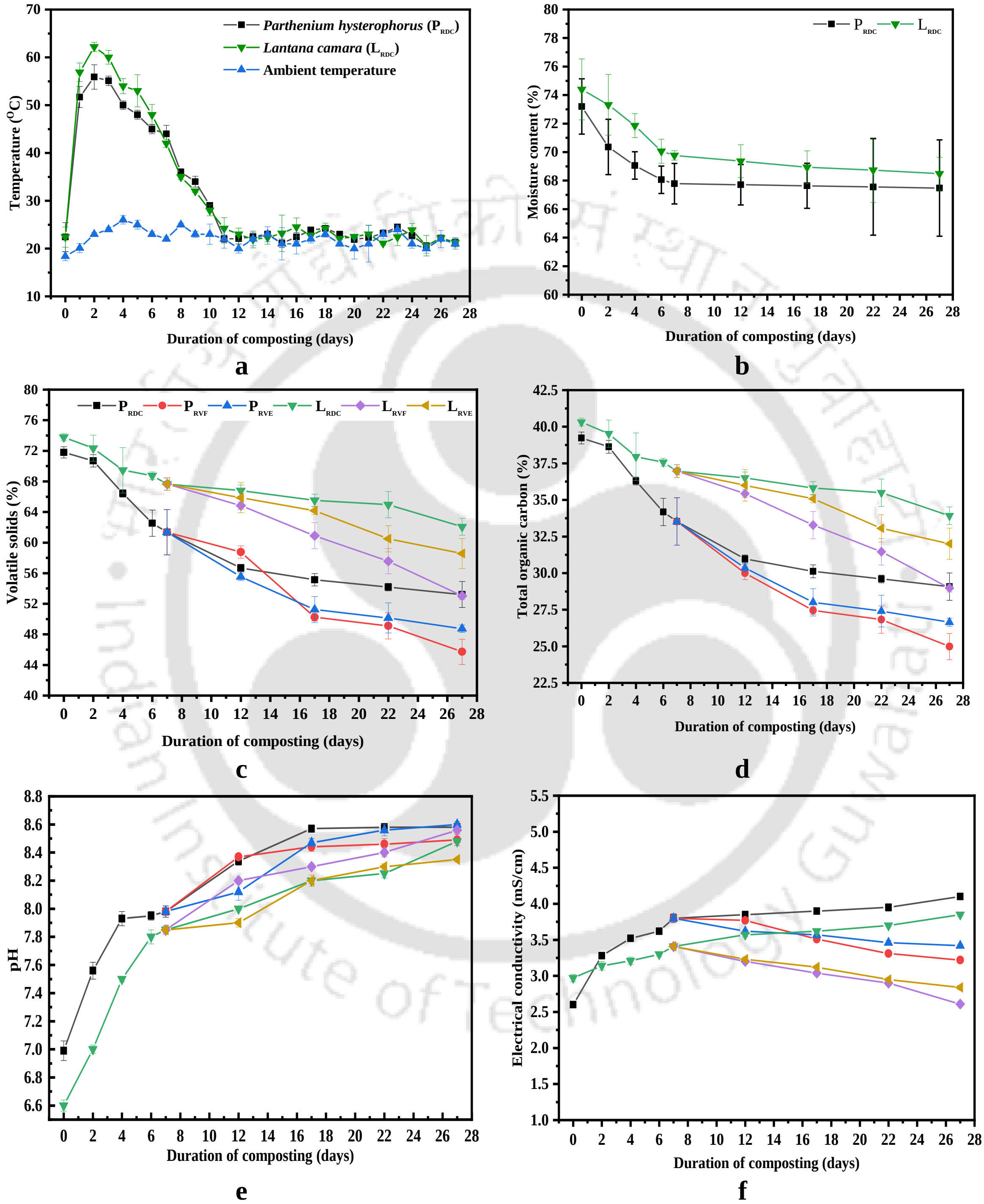
VS content is used to quantify the organic content in feedstock and is advantageous for evaluating physiologically inert organic materials such as lignin, hemicellulose, and ash content (Jain and Kalamdhad, 2018). The VS concentration of P_{RDC}, P_{RVF}, P_{RVE}, L_{RDC}, L_{RVF}, and L_{RVE} experienced a drop of 25.89, 36.32, 32.10, 15.85, 28.13, and 20.61 %, respectively, by the end of examined degradation period, which also resulted in the increased ash content. The decrease in VS may be attributed to the microbial decomposition of organic materials (Fig. 1(c)). During the initial 7 days of thermophilic degradation in P_{RDC} and L_{RDC}, the most significant decrease in VS occurred, characterized by the rapid breakdown of readily available organic materials by thermophilic bacteria (Kauser et al., 2020). In the case of P_{RVF}, P_{RVE}, L_{RVF}, and L_{RVE}, the significant decrease occurred on the 20-day of the second stage of RV, due to the earthworm activity and high utilization of volatile solids, while degrading the mass (Devi and Khwairakpam, 2020b). One-way ANOVA was used to evaluate the data, which revealed that volatile solids changed substantially across days ($P<0.01$) and between combinations ($P<0.01$).

Carbon is the fundamental building block of all living organisms and is the primary component of organic substrates. Carbon is used as a source of energy during various physiological functions such as respiration during the vermicomposting process, resulting in a decrease in organic carbon and the release of CO_2. The most significant reduction in TOC was observed in RDC by the 7th day for P_{RDC} (14.50%) and L_{RDC} (8.30%); while values of P_{RVF} (36.32%), and L_{RVF} (28.13%) were obtained in the RV technique (Fig. 5.13(d)). Previous studies have also reported a similar decreasing trend in TOC (Jain et al., 2018; Paul et al., 2020; Varma and Kalamdhad, 2016). Earthworm-mediated substrates achieve the conditions necessary for carbon loss as CO_2 and organic compound mineralization (Devi and Khwairakpam, 2020b). The observed TOC reduction was higher in the RV technique than in RDC in the current study. TOC concentrations varied significantly ($P<0.05$) across all reactors.

Variation in the pH and electrical conductivity during the study

In RDC, pH increased rapidly over the initial 7 days, then gradually increased until the 27th day. However, in RV similar increase was found in the initial and later stage. In both RDC and RV, the compost remained alkaline. This rise in pH was due to the breakdown of organic components such as proteins, amino acids, or peptides and the subsequent release of ammonium ions (Lopez et al., 2021). Similar pH trends were reported in the RDC of various feedstocks (Jain and Kalamdhad, 2019; Kauser et al., 2020). The pH change was not constant and exhibited distinct patterns based on feedstock mass, kind of amendment utilized and the type of earthworm culture applied (Devi and Khwairakpam, 2020a) ($P<0.05$).

EC dynamics depend inherently on the degree of mineralization of organic matter during the biodegradation process, and it is also an indicator of phytotoxicity or probable phytotoxic inhibitory effects (Meng et al., 2019). EC was utilised as a salinity indicator to test the compost as a positive soil supplement. The EC should be < 4 mS cm^{-1} to be tolerated by plants (Lopez et al., 2021).



a

b

c

d

e

f

Fig. 5.13. Variation in a) Reactor and ambient temperatures b) Moisture content c) Volatile solids d) Total organic carbon content e) pH and d) Electrical conductivity of the trails

Though an increasing trend of EC was observed in both RDC and in RV over the initial 7 days, the formation of complex compounds from ions lead to a decreasing trend during the second stage of the RV process. The EC of the products of RV trials was < 4 mS cm^{-1} ($P<0.05$), indicating the remarkable safety of the product for soil application, which contrasted with the RDC compost, exhibiting values of 4 mS cm^{-1}.

ii) Elemental analysis

TKN is the combination of organic nitrogen, NH_3, NH_4^+ (Kauser et al., 2020). In the current study, the final TKN was found to be 2.10% (P_{RDC}) and 2.52 % (L_{RDC}) through RDC., Whereas, the values for RV were 3.50%, 3.08%, 3.64%, and 3.36% in P_{RVF}, P_{RVE}, L_{RVF}, and L_{RVE} respectively. These were change more significant than the initial values of feedstock mass 1.12% (*P. hysterophorus*) and 1.50% (*L. camara*) reactors. Different enzymatic actions contribute to the vermicompost's overall nitrogen increment depending on the characteristics of the adopted vermicultures (Devi and Khwairakpam, 2020a). The change of TKN in the final 10 days of the RV technique signifies that the density of earthworm culture has been hugely impacted. Reactors amended with *E. fetida* vermiculture in the RV technique had shown a significant increase in nitrogen content, indicating the increased possibility of process optimization.

Phosphorus plays an essential role in plant photosynthesis and is available to plants in inorganic ions as orthophosphates (Devi and Khwairakpam, 2020a). After degradation, TP increased from 5.43 to 10.14, 13.69, and 12.60 g kg^{-1} in P_{RDC}, P_{RVF}, and P_{RVE}, in *P. hysterophorus* reactors, and from 4.86 to 11.30, 15.40, and 14.91 g kg^{-1} in L_{RDC}, L_{RVF}, and L_{RVE}, in *L. camara* reactors (Fig. 5.14). Organic matter mineralization increases TP. The rise in TP was ascribed to thermophilic degradation in RDC and worm gut enzyme activity in the second stage of RV. Nutrient release from chemical components in organic materials is also reported to increase TP soluble form (Devi and Khwairakpam, 2020a). During the second stage of the RV process, the unavailable forms of phosphorus are majorly converted into available forms since several microorganisms work to solubilize phosphorus (Kauser et al., 2020) ($P<0.01$).

TK is one of the micronutrients, followed by sodium and calcium that have become increasingly accessible owing to the activity of organic acids released during the breakdown of organic materials. After biodegradation, TK increased from 0.90 to 1.70, 2.90, and 2.30 g kg^{-1} in P_{RDC}, P_{RVF}, and P_{RVE} in *P. hysterophorus* reactors and from 0.80 to

1.30, 2.10, and 2 g kg^{-1} in L_{RDC}, L_{RVF}, and L_{RVE} in *L. camara* reactors. During the RV degradation, Na increased from 2.21 to an average of 2.81 g kg^{-1} in *P. hysterophorus* reactors and from 1.60 to 1.80 g kg^{-1} in *L. camara* reactors after biodegradation. Ca increased from 17.04 to an average of 18.74 g kg^{-1} in *P. hysterophorus* reactors and from 16.32 to 18 g kg^{-1} in *L. camara* reactors after biodegradation (Fig. 5.14). The micronutrient change was minor compared to the nitrogen and phosphorus content in the biodegradation process ($P<0.05$).

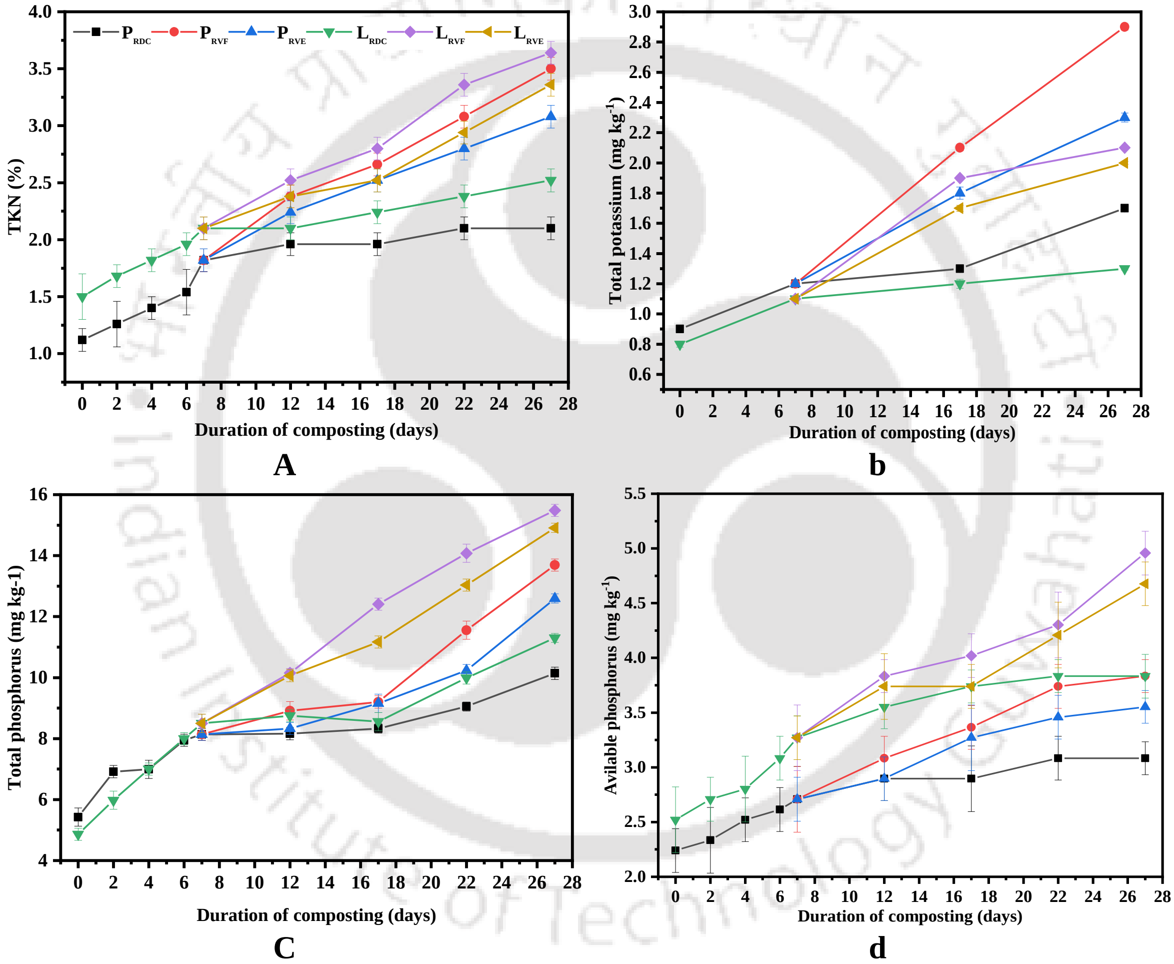


Fig. 5.14 Variation in a) TKN b) NH_4-N c) Total phosphorus d) Available phosphorus e) Total potassium and d) carbon to nitrogen ratio in studied trails

iii) Stability analysis

CO_2 emissions are a crucial indicator of microbial activity throughout the process. Microorganisms use oxygen rapidly during the initial thermophilic stages, digesting readily decomposable material and maximizing the quantity of CO_2 produced. The rate of CO_2 emission gradually decreased and eventually stabilised, indicating that the progressive evolution of the compost (Xiong et al., 2021). In this study, a maximum of 82.35, 82.86, 83.14, 62.31, 71.67 and 76.49 % reduction in P_{RDC}, P_{RVF}, P_{RVE}, L_{RDC}, L_{RVF}, and L_{RVE} were recorded, of which 51.56 and 37.77 % was due to the thermophilic phase in reactors P_{RDC} and L_{RDC}. The higher reduction was recorded in the RV technique. The direct relation of oxygen uptake rate (OUR) with microbial activity, makes OUR an accurate monitor and reflection of organic matter breakdown during the degradation processes. Consequently, aerobic biodegradation requires a sufficient oxygen supply to proceed (Zhang et al., 2021). A higher reduction of OUR was observed in 7 day RDC thermophilic phase (66.45 % in P_{RDC} and 55.36 % in L_{RDC}). A maximum of 79.81, 94.26, 93.81, 66.23, 83.80, and 83.78 % was observed in P_{RDC}, P_{RVF}, P_{RVE}, L_{RDC}, L_{RVF}, and L_{RVE}, respectively (Fig. 5.15) ($P<0.05$).

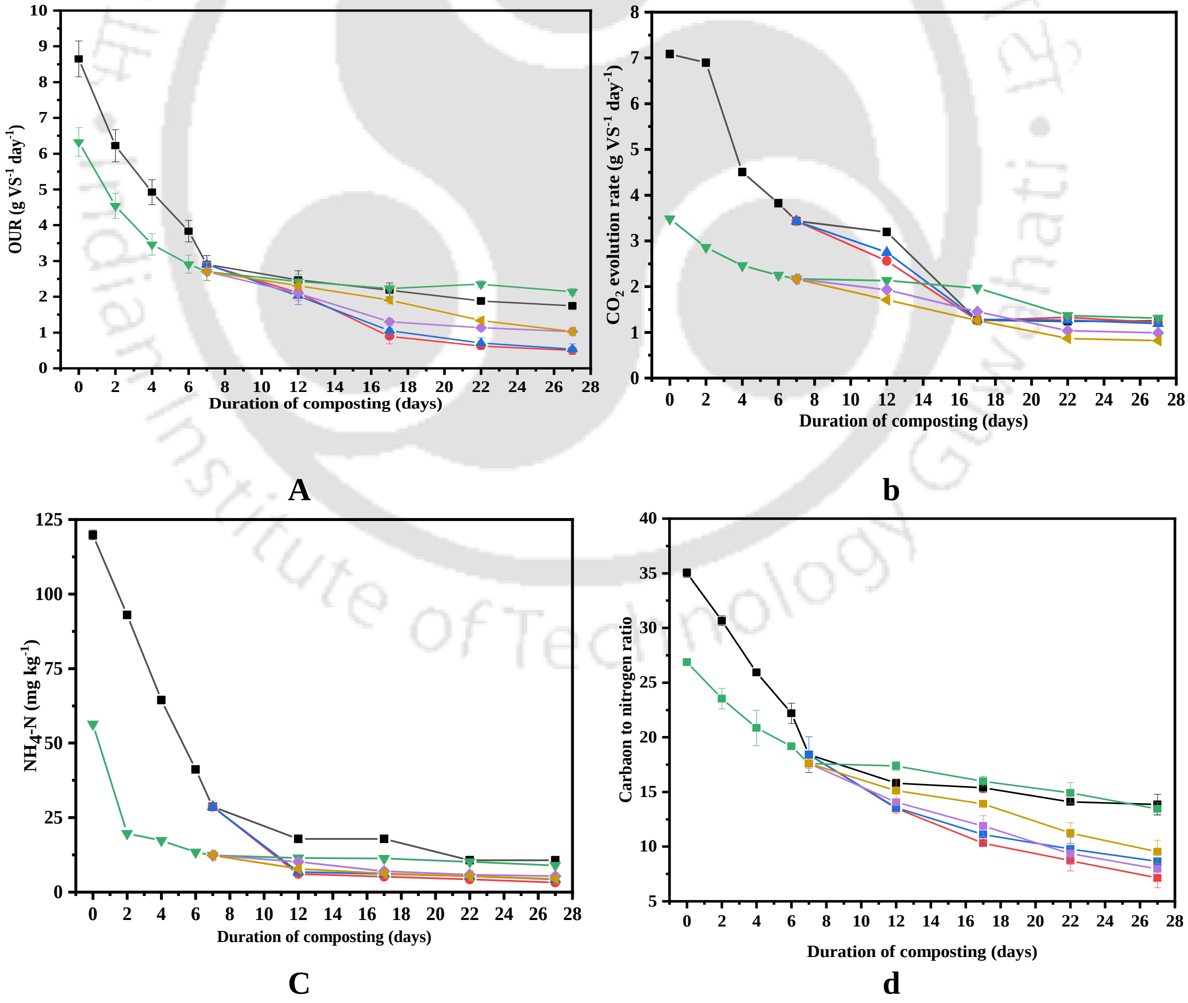


Fig. 5.15. Variation in a) Biochemical oxygen demand b) Chemical oxygen demand c) Oxygen uptake rate and d) CO_2 evolution in studied trails

The carbon to nitrogen ratio is one of the parameters to monitor the stability and maturity of compost (Jain and Kalamdhad, 2019). The C:N ratio of the final product must be less than 20 (Devi and Khwairakpam, 2020a). The substrate’s initial C:N ratio is critical for natural nourishment for earthworm growth in vermireactors, since carbon and nitrogen are basic requirements for cell synthesis in all living creatures (Ndegwa and Thompson, 2000). The reduction in the C:N ratio can be attributed to the earthworm growth (Devi and Khwairakpam, 2020a) in the VC process since a significant reduction was seen in reactors employing the RV technique (79.62 % in P_{RVF} and 70.39 % in L_{RVF}) compared to the RDC (47.42 % in P_{RDC} and 34.51 % in L_{RDC}). A maximum reduction of 61.24 % and 54.78 % was recorded in P_{RVF} and L_{RVF}. The C:N ratio for all the experimented trials was less than 20 by the end of 27 days of biodegradation (Fig. 5.15) ($P<0.05$).

Nitrification is critical in increasing ammonium nitrogen conversion to nitrite and nitrate and reducing NH_3 volatilization (Wang et al., 2019). Nitrifying bacteria and ammonia-oxidizing microbes may efficiently decrease nitrogen loss throughout biodegradation (Wang et al., 2019). Microorganisms quickly break down and use organic nitrogen compounds during the thermophilic phase of degradation, resulting in the production of a significant quantity of NH_4^+, and the concomitant rise in pH promotes the transition of NH_4^+ to NH_3 (Shan et al., 2021). The substantial drop in the NH_4-N concentration during the procedure was attributed to the ammonium to ammonia conversion. After degradation, NH_4-N decreased from 119 to 10.73, 3.22, and 4.29 mg kg^{-1} in P_{RDC}, P_{RVF}, and P_{RVE} of *P. hysterophorus* feedstock; and from 56.34 to 8.94, 5.37, and 4.47 mg kg^{-1} in L_{RDC}, L_{RVF}, and L_{RVE} of *L. camara* feedstock. A higher reduction was observed in the initial 7 days of the rotary drum thermophilic phase. Further recorded reduction was higher in RV than RDC, indicating early stabilization of feedstock ($P<0.01$).

iv) Earthworm growth

In all reactors, *E. fetida* gained more biomass than *E. eugeniae*. By the end of the 20 day period in the vermicomposting process, the net biomass gain was found to be 3 times more compared to the initial day value in P_{RVF} and L_{RVF}, with *E. fetida* (Table 5.8). In all reactors, earthworm growth and cocoon output varied significantly ($P<0.05$). *E. fetida* produced more cocoons than *E. eugeniae*. The reactor P_{RVF} produced the most cocoons at

0.02 (cocoons/worm/day), which was 50 % more than the cocoon output of *E. eugeniae*. The quantity and quality of substrate consumed are critical in determining the earthworm population, and higher nitrogen ratios stimulate faster growth and cocoon creation. The partially degraded material before vermicomposting seemed beneficial, attributing to the biomass change in the reactors.

Though it is reported earthworms require an acidic pH of less than 6, but most species prefer a neutral pH range (Gajalakshmi and Abbasi, 2004). Although the earthworms demonstrated significant biomass change and performed well in the current investigation, the pH remained alkaline throughout the vermicomposting period in the reactors mediated by *E. fetida* and *E. euginae*. In terms of growth and reproduction, *E. fetida* outperformed *E. euginae* in the current investigation. *E. fetida* is highly adaptable to various types of organic substrates and can survive a wide range of abiotic environmental fluctuations as reported in earlier studies (Devi and Khwairakpam, 2020a). The quicker growth rate and the higher rate of reproduction enable survival of this species on a wide variety of substrates and climatic conditions. According to the literature, the most frequently employed epigamic earthworm is the red worm, *E. fetida*, whereas the second most frequently used species is *E. eugeniae* (Varma et al., 2016).

Table 5.8 Growth in earthworm population

	Adult earthworms			**Juveniles**			**Cocoons**		
	Day 7	Day 17	Day 27	Day 7	Day 17	Day 27	Day 7	Day 17	Day 27
PRVF	180 ± 0	223 ± 10	452 ± 15	0	95 ± 14	128 ± 9	0	110 ± 4	210 ± 7
PRVE	180 ± 0	190 ± 07	259 ± 14	0	25 ± 12	114 ± 4	0	45 ± 8	86 ± 4
LRCF	180 ± 0	256 ± 15	464 ± 12	0	93 ± 14	124 ± 7	0	109 ± 9	188 ± 9
LRVE	180 ± 0	210 ± 09	244 ± 18	0	20 ± 18	121 ± 6	0	22 ± 4	98 ± 6

All the values are within mean ± standard deviation from conducted triplicated study

v) Biochemical analysis

The biodegradation of organic matter during degradation is greatly influenced by the lignocellulosic fractions of the waste materials and the initial waste composition. Microorganisms convert organic matter such as lignin, cellulose, and hemicellulose into a stable humic substance during degradation. The evolution of lignocellulose components is critical for the maturation and stabilisation of organic matter (Jiang et al., 2021). The primary carbon sources for microbial metabolism are oligosaccharides and simple sugars, which can be produced from cellulose and hemicellulose (Zhu et al., 2021b). The enhanced microbial reproduction and enzyme secretion generated by the readily available organic components in the raw materials may cause cellulose and hemicellulose degradation early in the degradation process (Zhu et al., 2021c).

Hemicellulose (HC) is the second most prevalent polysaccharide in plant biomass, occurring as cross-linking fibers between cellulose and lignin. It is mainly composed of sugars such as mannans and glucans (Harindintwali et al., 2020). HC is the first fraction utilized as a carbon and energy source by microorganisms during degradation, followed by cellulose. In comparison to cellulose, HC polymers are easily hydrolyzed and therefore capacity to degrade hemicellulose is l more prevalent than the ability to degrade cellulose; though microbes that produce hemicellulose frequently produce cellulase (Varma et al., 2017). A maximum of 23.95 and 14.89% reduction was recorded by the 7th day, and further reduction of 17.18 and 11.57% was seen in the subsequent 20 days of the RDC. Degradation of hemicellulose was higher in the RV than RDC, which can be attributed to the degradation by microbial mass of the feedstock during the thermophilic phase. A maximum of 36.04, 27.64, 42.85, and 34.95% was recorded in P_{RVF}, P_{RVE}, L_{RVF}, and L_{RVE}, respectively ($P<0.01$) (Fig. 5.16).

Cellulose is a crystalline polymer composed of 1,4-linked d-glucose molecules that are joined together via a hydrogen bond. White-rot fungi produce various hydrolysing enzymes (cellobiose dehydrogenase, lytic polysaccharide monooxygenases, and cellulases) that oxidize cellulose by breaking the glycosidic bonds (Harris et al., 2010). It has been reported that higher degradation temperatures resulted in more significant degradation of cellulose (Xiao et al., 2009). However, in this study, the higher degradation in cellulose was recorded in the RV technique, with, a maximum of 41.70, 33.66, 64.40, and 37.91 % reduction seen in P_{RVF}, P_{RVE}, L_{RVF}, and L_{RVE}, respectively. Whereas a maximum of 1.82 and 5.90 % was recorded in P_{RDC} and L_{RVC} reactors in the thermophilic condition, and post thermophilic degradation, a maximum of 18.20 and 22.20 % further reduction was recorded in RDC. *E. fetida* has been the efficient degrader with higher reduction percentages in both the weeds in the RV technique ($P<0.01$).

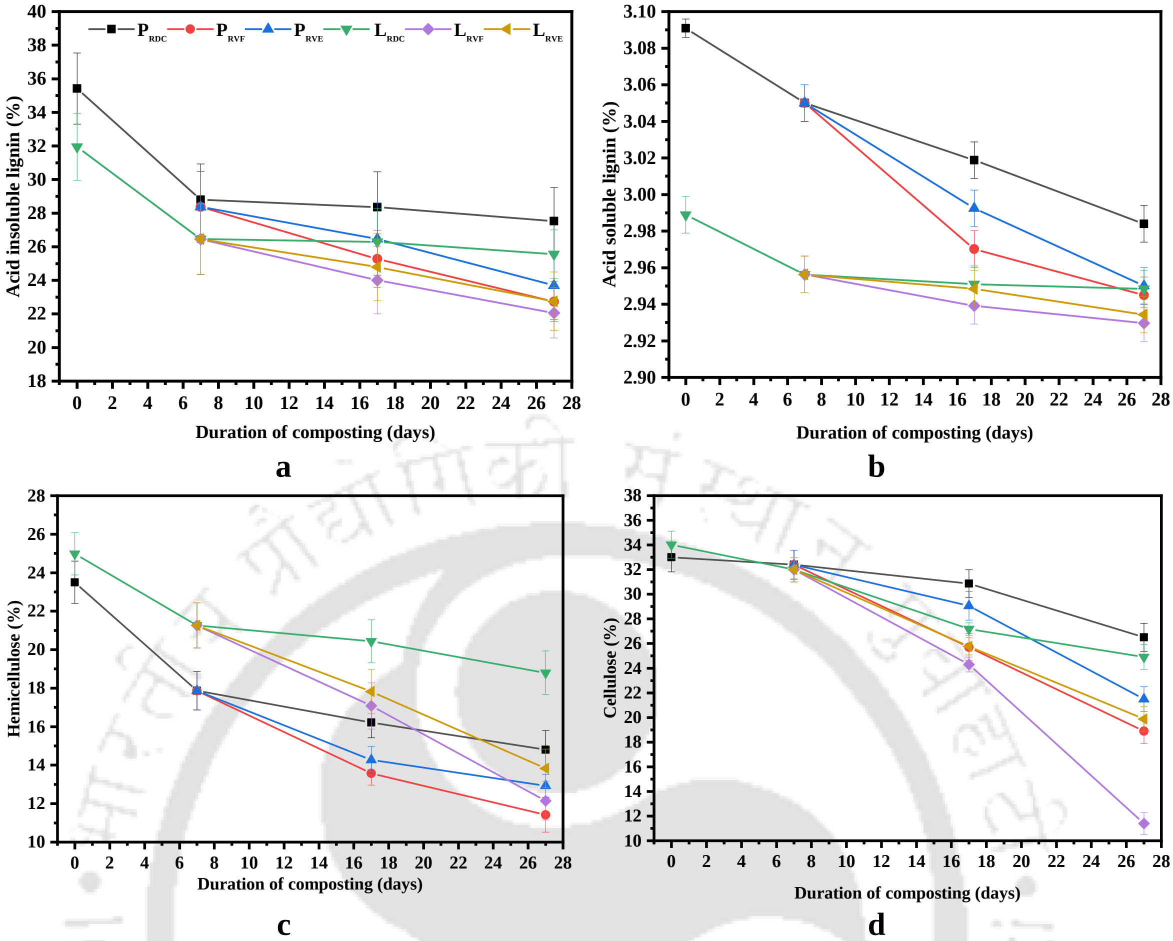


Figure 5.16 Variation in a) Acid insoluble lignin b) Acid soluble lignin c) Hemicellulose content and d) Cellulose content of the studied trails

Lignin biodegradation is crucial during the biodegradation process because of its involvement in humification (Huang et al., 2008). The percentage degradation of the hemicellulose and cellulose is higher than that of lignin as the chemical structure of lignin is very complex. The degradation of lignin results in the production of carbon dioxide, water, and humus. Although lignin degradation does not generate energy for microorganisms, it enables the efficient utilisation of carbohydrates (Varma et al., 2017). In the present study, the majority of the lignin degradation happened in the initial 7-day RDC. A maximum of 19.90 and 17.20 % reduction Acid insoluble lignin (AIL) was recorded in P_{RDC} and L_{RDC}. A further reduction of 2.98 and 3.38 % was recorded in the subsequent 20 days, which can be related to the thermophilic conditions prevalent in the reactor in the initial stages of biodegradation. A similar trend was observed in the degradation of agricultural waste by Varma et al, (2017). In P_{RVF}, P_{RVE}, L_{RVF}, and L_{RVE}, a maximum reduction in AIL of 19.85, 16.49, 16.59, and 14 % respectively were recorded, attributed to earthworm activity. Overall, the reduction of Acid soluble lignin (ASL) was

3 % in the P_{RDC}, P_{RVF}, and P_{RVE} reactors of *P. hysterophorus*. A 2 % average reduction in ASL was recorded in the L_{RDC}, L_{RVF}, and L_{RVE} reactors of the *L. camara*. ($P<0.01$).

vi) Changes in heavy metals concentration

The increase in metal concentration may be attributed to mass loss throughout the degradation process owing to organic matter breakdown, CO_2 and moisture escape, and calcification processes (Amir et al., 2005). Additionally, vermicomposting addresses the problems associated with heavy metal contamination. The heavy metal bioaccumulation of earthworms has been widely studied to determine whether they can be used as bioindicators of soil contamination and evaluate metal bioavailability and toxicity (Liu et al., 2005). During vermicomposting, earthworms accumulate heavy metals from the substrate and change the availability of metals in the soil by altering their characteristics and speciation distribution (Sun et al., 2020). Concentration of heavy metals has been increased in the P_{RDC} and L_{RDC} reactors of RDC, whereas an increasing trend was observed in the P_{RVF}, P_{RVE}, L_{RVF}, and L_{RVE} reactors of RV until day 7, when they tend to decrease due to earthworm amendment. When *P. hysterophorus* was degraded in P_{RDC}, the metal content increased significantly in the order: Zn>Pb>Cd>Fe>Cu>Cr, and in *L. camara* rector L_{RDC} was Pb>Zn>Cr>Cd>Fe>Cu. After introduction of the RV technique, the heavy metal content reduced in both cultures (P_{RVF} and P_{RVE}) in the order: Pb>Zn>Cr>Cd>Cu>Fe. The decrease in heavy metals in reactors L_{RVF} and L_{RVE} was in the order: Pb>Zn>Cd>Cr>Fe>Cu. Earthworm cultures have accumulated a more significant amount of Pb and Zn and a lesser amount of Fe. Similar heavy metal content reduction trends have been reported in previous studies when the feedstock was exposed to vermicultures (Khan et al., 2019; Sun et al., 2020). The heavy metal content study results demonstrate the efficiency of the RV technique in reducing the heavy metal contamination in the end product.

Table 5.9 Changes in total heavy metal concentration

Duration of composting (days)	**Day 0**	**Day 7**	**Day 17**	**Day 27**	**Day 17**	**Day 27**	**Day 17**	**Day 27**
	P_{RDC}				P_{RVF}		P_{RVE}	
Zn	38.75 ± 5.20	71.72 ± 2.60	90.25 ± 4.50	158.45 ± 2.80	56.20 ± 5.80	39.475 ± 8.20	51.05 ± 4.60	39.52 ± 5.10
Pb	29.90 ± 4.70	76.17 ± 4.80	82.12 ± 9.20	97.36 ± 7.20	49.35 ± 8.60	32.30 ± 7.10	45.67 ± 9.50	33.82 ± 4.50
Cr	3444.10 ± 26	4070.30 ± 28	4782.50 ± 62	4896.70 ± 85	2227.10 ± 29	1611 ± 85	3401.20 ± 45	3100.70 ± 36
Cd	881.22 ± 15	1396.32 ± 18	1520.40 ± 48	1690.20 ± 42	916.85 ± 52	806.23 ± 50	966.25 ± 41	831.25 ± 35
Cu	2214.45 ± 100	3173.92 ± 152	3220 ± 124	3423.40 ± 142	2136.20 ± 126	2105.90 ± 145	2138.70 ± 172	2050.30 ± 184
Fe	1126.26 ± 54	1468.58 ± 59	1655.20 ± 47	1758.20 ± 75	1325.80 ± 84	1268.90 ± 35	1368.80 ± 29	1294.50 ± 51
	L_{RDC}				L_{RVF}		L_{RVE}	
Zn	54.70 ± 8.90	83.02 ± 9.20	124.20 ± 3.90	148.73 ± 5.90	73.75 ± 7.20	55.90 ± 9.70	67.75 ± 6.40	52.92 ± 6.70
Pb	12.95 ± 2.60	29 ± 2.80	39.12 ± 3.10	46.47 ± 3.90	19.60 ± 6.40	15.80± 5.80	22.46 ± 8.40	18.20 ± 6.40
Cr	3184.02 ± 25	4805.07 ± 50	5900.90 ± 26	5923.70 ± 38	4152.30 ± 48	3227 ± 96	4256.40 ± 28	3356.90 ± 34
Cd	656.50 ± 70	694.37 ± 42	742.88 ± 42	818.63 ± 62	558.03 ± 22	464.60 ± 62	585.80 ± 57	462.08 ±47
Cu	2009.90 ± 157	2020 ± 184	2045 ± 195	2082 ± 174	1999.80 ± 252	1952.30 ± 184	1997.30 ± 167	1964.50 ± 122
Fe	530 ± 44	550 ± 60	590 ± 57	600 ± 53	501 ± 58	456 ± 32	541 ± 28	432 ± 26

All the values are in mg kg^{-1} within mean ± standard deviation

Correlation of total heavy metals

The Pearson's correlation coefficient matrix contains all the validated relationships between total heavy metals. The generated matrix for the P_{RDC} and L_{RDC} trails showed a significant positive correlation between the total heavy metals, 1 for Cd and Pb, 0.99 for Cu and Pb, and a near 1 coefficient value for many other metals many others. This relationship has broken in P_{RVF}, P_{RVE}, L_{RVF}, and L_{RVE}, which can be attributed to the heavy metal accumulation in earthworms. In L_{RVE}, very few metals significantly correlated Cr with Zn and Pb and Cu with Cd, while other coefficients were positive but insignificant (Table 5.9). The change in correlation coefficients may be attributed to the amendment of earthworm cultures and the heavy metal accumulation in their cells.

vii) Summary

The research demonstrates that the utilization of the RV methodology, which comprises of a sequential approach of rotary drum composting and vermicomposting, is efficacious in transforming weed biomass into a soil amendment that is abundant in nutrients. During the initial seven-day period of thermophilic temperatures andsubsequent vermicomposting, the presence of pathogens is reduced due to the toxic nature of weeds. This process leads to an increased concentration of TN, TP, and TK in the final product. According to the research findings, the *E. fetida* worm culture exhibits strong capabilities in breaking down hazardous weeds and demonstrates a high degree of adaptability towards weed biomass. The application of vermicompost produced through the RV method, utilizing *E. fetida*, had a significant impact on plant growth in alluvial soils. This was confirmed by the observation of a higher SGI (%) in the final product, exceeding 80%. The RV methodology is distinct in its approach in managing lignocellulosic weeds that are toxic, and it yields a final product of superior quality and safety within a compressed timeline of 27 days. The novel approach for weed management holds promise for restoring nutritional vigor to areas affected by weeds across the globe.

5.4 MAJOR FINDINGS OF THE STUDY

- Active degradation in the RDC takes one week.
- Partially degraded material is the best suited feed for earthworms.

- The technique of RDC followed by VC has the potential to be the novel and fastest biodegradation technique (27 days).
- RV technique is consistent in bio-converting various biodegradable organic wastes into vermicompost.
- Among the earthworm cultures used, *E. fetida* was proven to be a robust degrader.
- Along with the degradation strategy, the technique was also significant in reducing the metal content of the substrates like sewage sludge, *P. hysterophorus* and *L. camera*.

Chapter 6

SAFETY EVALUATION OF THE PRODUCED BIOPRODUCTS

The utilization of compost or vermicompost as beneficial soil conditioners is imperative to successful plant development in real-world scenarios (Tahir and Tian, 2021). The mandatory assessment of phytotoxicity and maturity of biodegradation byproducts via the seed germination index (SGI %), prior to field application is well documented (Haq et al., 2016; Wang et al., 2021). The suitability of stabilized compost as a soil supplement is acceptable when the SGI rating exceeds 60 % (Haq et al., 2016). Compost products securing an SGI rating that exceeded 80%, indicate biodegradation has reached a mature phase, free of phytotoxic components and can be endorsed as good, and healthy (Wang et al., 2021). In addition, an auxiliary assessment of potential environmental risks can be achieved by utilizing the phytotoxicity bioassay detailed by Wang et al. (2022). Phytotoxicity is defined as the detrimental outcomes caused by specific compounds called phytotoxins, evinced by delay seed germination, and/or suppression of plant dynamics of development and growth (Blok et al., 2019). Since the weeds chosen for the current study are toxic weeds of concern, the identification of phytotoxicity is vital for end product safety validation. In the assessment of the toxicity of compost, the utilization of plants is a dependable approach, especially in cases where the feedstock comprises of toxic terrestrial weeds (Li et al., 2020). In the present investigation lab scale pot studies were carried out with *Vigna radiata* and *Coriandrum sativum* as a representative seed models, based on well-established phytotoxicity responses and plant growth profiles respectively.

6.1 PHYTOTOXICITY BIOASSAY USING *Vigna Radiata*

Utilizing *V. radiata* seeds for phytotoxicity bioassays is a highly sensitive method, rendering it a favorable option for evaluating phytotoxicity. The study conducted by Kauser and Khwairakpam (2022), reported the growth of *V. radiata* after a five-day incubation period. The results indicated that even when tap water was used as a control, there was an increase in root and shoot length, suggesting toxicity levels had decreased during the composting process. In this chapter, the outcomes of seed germination, followed by inhibition of *V. radiata* after being exposed to different dilutions of compost extracts and weed extract are discussed. The impact of weed toxicity on the growth of root, shoot, and biomass in *V. radiata* seedlings was documented by analyzing percentage of seed germination inhibition at varying dilutions (0%, 25%, 50%, 75%, and 100%). The results indicate that employing phytotoxicity bioassay and seed germination index as metrics is crucial in assessing the ecological risk and appropriateness of the compost as a soil amendment.

a

b

Fig. 6.1 Effect of vermicompost extract vs fresh waste on the seed germination on a) day 2 and b) day 5

6.1.1 Major Urban Wastes

i) Phytotoxicity bioassay using vegetable waste biodegradation products

In the initial stages of composting, the meager values of SGI and the rotting of some seeds by the second-day end were attributed to phytotoxic volatile fatty acids produced by the rapid degradation of organic matter (Barje et al., 2013). Later, the reactors evinced seed germination values above 60% for *V. radiata* when the produced compost and vermicompost from vegetable waste were tested. In R2 and R3, the seed germination values were higher than in R1 and R4, possibly due to their individual earthworm cultures. R3 and R4 reactors showed higher SGI values at 50% sample dilution, whereas R1, and R2 displayed comparable results at 75% dilution. The effect of composting can be understood from the results when compared to raw vegetable waste (Table 6.1). The higher seed germination values obtained can be correlated to reduction in EC and NH_4-N values (Luo et al., 2018) ($P<0.001$).

ii) Application of sewage sludge biodegradation products in bioassay for phytotoxicity

When the final composts derived from sewage sludge were tested for phytotoxicity, the seed germination index (SGI %, root length, shoot lengths) of *V. radiata* was considerably higher and enhanced in the S2 and S3 vermicompost in comparison to compost derived from S1 ($P< 0.01$). According to Wang et al. (2022), composting and vermicomposting can reduce phytotoxicity by degrading, transforming, and aggregating harmful chemicals and decreasing toxin bioavailability., The results of the present study supported this contention, when high levels of toxins curtailed seed germination at 75% dilution of untreated raw sludge. Therefore, in all three treated sludge experiments (S1, S2 and S3), germination was above 88%, while in S3, it touched 95%. Therefore, treated sludge produced more biomass as compared to raw sludge (Table 6.2). The higher SGI (%) in the end products of S2 and S3 endorsed the significantly higher efficacy of vermicompost over the compost. The lower efficiency of compost maybe attributed to heavy metals, inorganic nitrogen and organic acids, present in most aqueous compost extracts, responsible for phytotoxicity and inhibition of seed germination (Chen et al., 2021).

6.1.2 Aquatic Weed Biomass

i) Phytotoxicity bioassay using vermicompost extract from Water hyacinth

The presented data in Table 6.3 illustrates the impact of varying concentrations of vermicompost extract derived from water hyacinth on the parameters of seed germination and plant growth in plant model *V. radiata*, including shoot length, root length, and biomass. Concentrations of 25%, 50%, 75%, and 100% vermicompost extract were taken and evaluated against the control which contained zero extract.

Table 6.1 Effects of using vegetable waste degradation products on phytotoxicity bioassay

Test sample (%)	**Day 0**	**Day 27**			
	Raw vegetable waste (%)	**R1 (%)**	**R2 (%)**	**R3 (%)**	**R4 (%)**
0	79 ± 0.1	78 ± 0.8	80 ± 0.1	78 ± 0.3	78 ± 0.3
25	< 60	83 ± 0.3	85 ± 0.1	86 ± 0.6	85 ± 0.1
50	< 60	90 ± 0.1	93 ± 0.3	98 ± 0.3	96 ± 0.6
75	< 60	98 ± 0.3	96 ± 0.6	93 ± 0.3	93 ± 0.3
100	< 60	91 ± 0.6	93 ± 0.3	90 ± 0.1	91 ± 0.6

All values are the average ± standard deviation

Table 6.2. Effects of using sewage sludge bioproducts in phytotoxicity bioassay

Material	**Concentration (v/v)**	**SGI (%)**	**Shoot length (cm)**	**Root length (cm)**	**Biomass (g)**
Control	0	100	9.34 ± 0.53	4.98 ± 0.22	0.52
Raw sludge	25	72	6.25 ± 0.15	3.21 ± 1.50	0.31
	50	< 60	4.21 ± 0.11	3.25 ± 1.20	0.25
	75	< 60	-	-	-
	100	< 60	-	-	-
S1 (Compost)	25	100	10.89 ± 0.32	5.52 ± 0.42	0.56
	50	90	10.17 ± 0.29	6.18 ± 0.23	0.57
	75	88	8.75 ± 0.12	5.32 ± 0.33	0.49
	100	80	6.93 ± 0.56	4.71 ± 0.45	0.46
S2 (Vermicompost)	25	100	14.23 ± 0.65	10.23 ± 0.66	0.75
	50	100	15.69 ± 0.40	12.32 ± 0.76	0.89
	75	90	11.92 ± 0.33	8.32 ± 0.48	0.60
	100	85	9.63 ± 0.28	6.72 ± 0.21	0.54
S3 (Vermicompost)	25	100	13.23 ± 0.13	6.67 ±0.63	0.65
	50	96	14.57 ± 0.47	8.09 ± 0.32	0.78
	75	95	10.88 ± 0.41	7.19 ± 0.44	0.70
	100	90	9.82 ± 0.43	5.89 ± 0.42	0.62

The control and 75% concentration groups exhibited a complete seed germination rate of 100% and 98.3% respectively. However, the 25%, 50% and 100% concentration groups displayed marginally lower rates of 83.3%, 90% and 91.6%, respectively. The experimental findings indicate that the control group exhibited the greatest shoot length (9.34 ± 0.53 cm) and root length (4.98 ± 0.23 cm) in comparison to the remaining groups. A significant reduction in shoot and root lengths was observed as the concentration of the extract increased. A marginal increase in biomass of 0.62 and 0.60 at concentrations of 75% and 100% were observed over the control (0.521). Therefore, the extracts may potentially elicit favorable outcomes on the process of seed germination, albeit within specific concentration ranges. Additional research may be required to determine the most effective concentration of the extract in stimulating plant growth and improving seed germination.

Table 6.3 Effects of using water hyacinth and vermicompost extracts on phytotoxicity bioassay

	Control	***Water hyacinth* vermicompost extract**			
Conc (v/v)	-	25%	50%	75%	100%
Germination index (%)	100%	83.3	90	98.3	91.6
Shoot Length (cm)	9.34 ± 0.53	2 ± 0.01	4.1 ± 0.02	3.3 ± 0.01	2 ± 0.01
Root Length (cm)	4.98 ± 0.23	2.5 ± 0.03	3.3 ± 0.30	5.2 ± 0.30	4.1 ± 0.02
Biomass (g)	0.521	0.255	0.324	0.621	0.6

ii) Phytotoxicity bioassay with *Hydrilla verticillate* vermicompost extracts

Table 6.4 presents the recorded duration of seed germination and *V. radiata* inhibition resulting from exposure to varying extract dilutions of *Hydrilla verticillate* vermicompost extract. SGI of *V. radiata* touched 79%, when tap water was used in the control. It is noteworthy that increase in concentration of *H. verticillata* raw extract from 25, 50, 75, to 100%. inhibited seed germination to values below 60%. If the seed germination index is less than 60 %, the compound can be categorized as toxic for the seeds (Haq et al., 2016). Therefore, the SGI results establish the toxic nature of the raw weed and caution against direct application. At this juncture, it is noteworthy that existing physical management norms involve direct dumping land after removal from the water body. Thus, awareness must be created regarding the ensuing toxicity generated from this practice. The suppression of seed germination observed in the weed extract may be ascribed to elevated levels of salinity and carbon content, as suggested by (Radelyuk et al., 2019). On the other hand, addition of vermicompost led to comparatively better germination and growth on day 27, which could be attributed to the reduction in the impact of EC and TOC.

Table 6.4. Effects of using water *H. verticillate* and vermicompost extracts on phytotoxicity bioassay

Test sample	**Germination Index (%)**		**Vermicompost extract**	
Dilution (%)	Day 0 Fresh extract	Day 27 Vermicompost extract	Day 27 Root length (cm)	Shoot length (cm)
Control	79.00	80.00	1.90 ± 0.2	1.50 ± 0.3
25	< 60%	85.00	3.70 ± 0.1	1.60 ± 0.5
50	< 60%	93.30	4.00 ± 0.1	2.80 ± 0.1
75	< 60%	96.60	4.10 ± 0.1	5.20 ± 0.2
100	< 60%	93.30	3.40 ± 0.2	3.30 ± 0.3

6.1.3 Terrestrial weeds

i) Phytotoxicity bioassay using *A. conyzoides* and *M. micrantha* biodegradation products

Table 6.5 summarizes the findings of germination percentage and *V. radiata* inhibition after exposure to different dilutions of extracts of weeds and end products. When the final bioproducts were tested for phytotoxicity in this study, the SGI %, stem and root, shoot lengths were much higher and improved in vermicompost created by RDVC compared to compost derived from RDC (P<0.01). Aqueous compost extracts include phytotoxic chemicals that inhibit seed germination, including toxic substances, inorganic nitrogen, and organic acids (Chen et al., 2021). Table 6.5 shows that all the experimented trials exhibited SGI (%) greater than 60%. The greater SGI (%) in the final products of MMRVE, MMRVF, ACRVE and ACRVF supported the far superior effectiveness of vermicompost over compost. Furthermore, *A. conyzoides* vermicompost exhibited higher biomass growth than *M. micrantha* vermicompost. Results also show that the root length was higher in vermicompost extracts than in compost extracts in all the experimental trials.

Table 6.5. Effect of using *A. conyzoides* and *M. micrantha* on the phytotoxicity bioassay

Experimental trial	**Concentration (v/v)**	**SGI (%)**	**Shoot length (cm)**	**Root length (cm)**
Control	0	100	9.34 ± 0.53	4.98 ± 0.22
MMRDC	25	100	2.00 ± 0.02	1.70 ± 0.03
	50	78	1.80 ± 0.01	1.50 ± 0.02
	75	70	1.60 ± 0.02	1.10 ± 0.02
	100	60	1.10 ± 0.03	1.00 ± 0.01
MMRVE	25	93	4.00 ± 0.01	3.20 ± 0.02
	50	97	3.00 ± 0.01	4.40 ± 0.02
	75	88	3.50 ± 0.01	5.50 ± 0.01
	100	85	3.00 ± 0.10	4.90 ± 0.20
MMRVF	25	93	3.00 ± 0.11	4.00 ± 0.10
	50	96	4.60 ± 0.12	4.50 ± 0.02
	75	86	2.90 ± 0.20	3.10 ± 0.01
	100	78	1.50 ± 0.10	2.00 ± 0.12
ACRDC	25	100	10.11 ± 0.32	6.12 ± 0.49
	50	90	10.87 ± 0.29	6.98 ± 0.31
	75	88	8.45 ± 0.12	4.32 ± 0.73
	100	80	7.23 ± 0.56	4.11 ± 0.34
ACRVE	25	100	10.73 ± 0.23	6.67 ± 0.63
	50	95	12.17 ± 0.54	7.09 ± 0.32
	75	90	9.88 ± 0.41	5.79 ± 0.44
	100	85	8.82 ± 0.54	4.89 ± 0.42
ACRVF	25	100	11.76 ± 0.35	6.70 ± 0.76
	50	97	14.56 ± 0.76	10.98 ± 0.89
	75	93	10.32 ± 0.83	8.32 ± 0.12
	100	87	7.98 ± 0.23	4.32 ± 0.39

ii) Phytotoxicity bioassay using *P. hysterophorus* and *L. camara biodegradation* products

The highest level of inhibition was observed at a 100 % concentration of weed extract, with no seed germinating (Table 6.6). Whereas in vermicompost, the germination and growth were comparatively far more because of the minor effect of the EC and TOC. Based on this outcome of the study, it is clear that the raw weed is toxic if applied directly to the land, which usually happens in the physical management of weeds. After degrading the feedstock, the seed germination capacity was significantly increased in all the combinations. The detrimental effect on SGI (%) evinced post administration of 25 % dilution raw weed extract, displayed maximum germination was seen after application of final vermicompost extract. This is a clear indication of the shift to a nontoxic zone post vermicompost application SGI (%) is a strong indicator of both plant germination percentage and root length, as roots not only transport bioavailable toxic compounds to the plants via shoots but also alter the composition, moisture retention, and mineralogy of soil (Merkl et al., 2005). A significant difference in the root and shoot lengths was observed in the different dilutions of the RV end product. At 50 % dilution concentration, it was observed that the root length and shoot length were significant in P_{RVF}, P_{RVE}, L_{RVF}, and L_{RVE}. In P_{RDC} and L_{RDC}, the shoot exhibited greater length in comparison to root.

6.2 ASSESSMENT OF BIOPRODUCT AMENDMENTS VIA POT STUDIES OF *Coriandrum Sativum* GROWTH PARAMETERS

Application of compost to the soil is known to reduce the demand for chemical fertilizers, resulting in a cleaner and more sustainable environment. Compost amendment enhances soil texture; moisture retention capacity in sandy soil and drainage capacity of clay soil, consequently acting primarily as a soil conditioner.

These improvements are achieved by the production of tiny particles of matter which provide open paths for water and air, post compost amendment. Improved soil structure provides for better air circulation and water drainage. Soil amendments of compost and vermicompost not only encourage crop growth, root growth, and enhance nutrients; but even help minimize crop disease severity (Santos et al., 2021). Compost and vermicompost amendment to soil can stimulate plant growth, root development, and nutrient absorption. Humic materials, the main components of soil organic matter in the compost, may enhance shoot biomass via hormonal effects on root elongation and plant growth (Duong et al., 2012). In the present investigation, bioproducts (compost and vermicompost) derived from major urban wastes (vegetable matter and sewage sludge), aquatic weed biomass (*W. hyacinth* and *H. verticillata*) and terrestrial weed biomass (*A.*

conyzoides and *M. micrantha*) were applied to soil as 10% and 20% by weight. *Coriandrum sativum* was selected as plant model for cultivation in pot studies to assess the effect of these amendments on plant growth dynamics via root, shoot and plant length changes versus control with no amendments.

6.2.1 Major urban wastes

i) Effect of vegetable waste bioproducts on the growth of *C. sativum*

Pot study control without addition of compost or vermicompost derived from vegetable waste was maintained against pots with defined percentages of compost or vermicompost as displayed in Table 6.7. The SDG was recorded after two weeks, followed by documentation of change in plant length (root+ shoot) after 30 days from initiation of the experiment. The experimental group R1 consisting of soil + 10% compost and soil + 20% compost exhibited doubling of root length, shoot length, and total plant length in relation to the control group. Among the R2, though root length was almost the same as in R1, but in pots amended with 20% vermicompost, the shoot and total plant length exhibited a distinct increase to 12.50 ± 0.33 and 19.40 ± 0.39 respectively.

The experimental results of R3 indicate that the application of soil + 20% vermicompost treatment resulted in a statistically significant improvement in root length, shoot length, and total plant length when compared to both the control group and the soil + 10% vermicompost treatment. The experimental results of R4 indicate that the treatments involving soil + 10% compost and soil + 20% compost exhibited a noteworthy reduction in root length, shoot length, and total plant length in comparison to the control group. To summarise, the incorporation of compost or vermicompost into the soil has the potential to enhance plant growth. However, the ideal proportion may differ based on the specific compost variety and the plant species under cultivation. The findings indicate that the application of soil + 20% vermicompost treatment may exhibit the highest efficacy in enhancing plant growth. Conversely, the soil + 10% compost and soil + 10% vermicompost treatments demonstrated varied outcomes, while the soil + 20% compost treatment resulted in detrimental effects on plant growth.

Table 6.6 Effects of using *P. hysterophorus* and *L. camara biodegradation* products on Phytotoxicity bioassay

Combination	**Concentration (v/v) %**	**SGI (%)**	**Shoot length (cm)**	**Root length (cm)**	**Biomass (gm)**
Control (Tap water)	0	100	9.34 ± 0.53	4.98 ± 0.22	0.52 ± 0.02
P_R	25	58	1.96 ± 0.10	0.40 ± 0.10	0.06 ± 0.01
	50	25	0.70 ± 0.10	0.25 ± 0.01	0.03 ± 0.01
	75	18	0.30 ± 0.10	0.10 ± 0.01	0.01 ± 0.01
	100	10	0.10± 0.01	0.10 ± 0.01	0.01 ± 0.01
L_R	25	45	1.30 ± 0.10	0.60 ± 0.10	0.04 ± 0.01
	50	20	0.30 ± 0.10	0.20 ± 0.10	0.01 ± 0.01
	75	16	0.10 ± 0.01	0.10 ± 0.01	0.01 ± 0.01
	100	9	0.10 ± 0.01	0.10 ± 0.01	0.01 ± 0.01
P_{RDC}	25	100	10.11 ± 0.32	6.12 ± 0.49	0.55 ± 0.06
	50	90	10.87 ± 0.29	6.98 ± 0.31	0.59 ± 0.04
	75	88	8.45 ± 0.12	4.32 ± 0.73	0.51 ± 0.05
	100	80	7.23 ± 0.56	4.11 ± 0.34	0.46 ± 0.02
P_{RVF}	25	100	12.43 ± 0.43	8.93 ± 0.45	0.62 ± 0.06
	50	95	13.98 ± 0.32	10.22 ± 0.76	0.65 ± 0.02
	75	88	9.22 ± 0.12	4.28 ± 0.67	0.56 ± 0.03
	100	85	5.10 ± 0.54	2.39 ± 0.23	0.48 ± 0.09
P_{RVE}	25	100	10.73 ± 0.23	6.67 ± 0.63	0.57 ± 0.08
	50	95	12.17 ± 0.54	7.09 ± 0.32	0.61 ± 0.07
	75	90	9.88 ± 0.41	5.79 ± 0.44	0.56 ± 0.04
	100	85	8.82 ± 0.54	4.89 ± 0.42	0.49 ± 0.05
L_{RDC}	25	100	10.11 ± 0.32	5.12 ± 0.23	0.53 ± 0.09
	50	90	10.87 ± 0.29	5.68 ± 0.31	0.55 ± 0.09
	75	88	8.45 ± 0.12	4.92 ± 0.47	0.51 ± 0.05
	100	80	7.23 ± 0.56	4.54 ± 0.56	0.43 ± 0.09
L_{RVF}	25	100	12.22 ± 0.45	7.63 ± 0.23	0.52 ± 0.06
	50	95	14.54 ± 0.54	7.40 ± 0.63	0.61 ± 0.02
	75	90	9.23 ± 0.67	5.65 ± 0.29	0.50 ± 0.01
	100	80	6.12 ± 0.23	3.45 ± 0.12	0.49 ± 0.06
L_{RVE}	25	100	10.73 ± 0.23	5.87 ± 0.43	0.57 ± 0.02
	50	92	11.17 ± 0.27	6.69 ± 0.32	0.63 ± 0.09
	75	88	10.38 ± 0.41	5.79 ± 0.44	0.56 ± 0.04
	100	85	8.34 ± 0.45	4.89 ± 0.42	0.53 ± 0.02

*P_R and L_R are the raw weed extracts of *P. hysterophorus* and *L. camara*

Table 6.7 Pot study using vegetable waste bioproducts on plant growth dynamics of *C. sativum*

	Composition (w/w)	**SGD**	**Plant length in cm on Day 30**		
			Root length	**Shoot length**	**Total**
Control	Soil	Day 16	3.45 ± 0.15	4.60 ± 0.30	8.05 ± 0.23
R1	Soil + 10% compost	Day 14	7.50 ± 0.11	7.80 ± 0.24	15.30 ± 0.18
	Soil + 20% compost	Day 14	6.80 ± 0.45	10.30 ± 0.36	17.10 ± 0.41
R2	Soil + 10% vermicompost	Day 14	6.30 ± 0.61	11.50 ± 0.49	17.80 ± 0.55
	Soil + 20% vermicompost	Day 14	6.90 ± 0.44	12.50 ± 0.33	19.40 ± 0.39
R3	Soil + 10% vermicompost	Day 14	7.10 ± 0.43	13.20 ± 0.53	20.30 ± 0.48
	Soil + 20% vermicompost	Day 14	9.40 ± 0.29	15.40 ± 0.75	24.80 ± 0.52
R4	Soil + 10% compost	Day 14	4.50 ± 0.20	5.30 ± 0.10	9.80 ± 0.15
	Soil + 20% compost	Day 14	7.50 ± 0.15	9.30 ± 1.72	16.80 ± 0.94

ii) Effect of Sewage sludge bioproducts on the growth of *C. sativum*

Pot study control without the addition of compost or vermicompost derived from sewage sludge was maintained against pots with defined percentages of compost or vermicompost as summarized in Table 6.8. The final product of S3 had a substantial influence on plant growth, and at 20% amendment, the increase in plant length was observed to be the greatest in all the trials tested. When vermicompost derived from sewage sludge was applied on the soil, it resulted in longer root lengths as seen in S2 and S3, in comparison to S1, where compost was utilized. Compared to the control soil, the plants remained healthy throughout the research, which signifies that the supplied vermicompost did not have any harmful effects and indicates that the product is safe. It is noteworthy that seed germination was accelerated when the soil was supplemented with vermicompost.

Table 6.8 Pot study using sewage sludge bioproducts on plant growth dynamics of *C. sativum*

	Composition (w/w)	**Seed germination**	**Plant length in cm on Day 30**		
			Root length	Shoot length	Total
Control	Soil	Day 14-16	3.50 ± 0.50	4.50 ± 0.50	8.00 ± 0.50
S1	Soil + 10% compost	Day 12-14	7.50 ± 0.11	7.80 ± 0.24	15.30 ± 0.15
	Soil + 20% compost	Day 12-14	6.80 ± 0.45	10.30 ± 0.36	16.90 ± 0.39
S2	Soil + 10% vermicompost	Day 12-13	6.30 ± 0.61	11.50 ± 0.49	17.80 ± 0.55
	Soil + 20% vermicompost	Day 12-13	6.90 ± 0.44	12.50 ± 0.33	19.40 ± 0.35
S3	Soil + 10% vermicompost	Day 12-13	7.10 ± 0.43	13.20 ± 0.53	20.30 ± 0.49
	Soil + 20% vermicompost	Day 12-13	9.40 ± 0.29	15.40 ± 0.75	24.80 ± 0.52

6.2.2 Aquatic weed biomass

i) Effect of Water hyacinth and *H. verticillata* based bioproducts on the growth of *C. sativum*

Pot study control without addition of compost or vermicompost derived from *W. hyacinth* and *H. verticillata* biomass was maintained against pots with defined percentages of compost or vermicompost as summarized in Table **6.9** The findings indicate that the plant growth was positively impacted by the compositions that included vermicompost ($\mathrm{WH_{RVE}}$, $\mathrm{WH_{RVF}}$, $\mathrm{HV_{RVE}}$, and $\mathrm{HV_{RVF}}$) in comparison to the control group without soil amendment. Overall, a positive correlation was observed between the proportion of vermicompost incorporated into the soil and the resultant increase in plant length. The experimental groups (denoted as $\mathrm{WH_{RVF}}$ and $\mathrm{HV_{RVF}}$), which incorporated 20% proportion of vermicompost, exhibited the greatest plant length. Additionally, both shoot length and root length surpassed 12 cm and 8 cm, respectively.

By contrast, the compositions that solely consisted of compost ($\mathrm{WH_{RDC}}$, $\mathrm{HV_{RDC}}$) did not exhibit a statistically significant enhancement in plant length when compared to the control group. The study found that the addition of vermicompost to compost ($\mathrm{WH_{RVE}}$, $\mathrm{WH_{RVF}}$, $\mathrm{HV_{RVE}}$, and $\mathrm{HV_{RVF}}$) resulted in a significant increase in plant length. This suggests that vermicompost may have a more favourable impact on plant growth compared to compost alone. In general, the findings indicate that the utilisation of vermicompost as a soil amendment can be a viable strategy for enhancing plant growth. The ideal proportion of vermicompost to soil could potentially vary based on the specific plant species and additional ecological variables. Additional research may be required to determine the most advantageous mixture for particular plant varieties and environmental circumstances.

Table 6.9 Pot study using aquatic weed bioproducts on plant growth dynamics of *C. sativum*

	Composition (w/w)	**SGD**	**Plant length in cm on Day 30**		
			Root length	**Shoot length**	**Total**
Control	Soil	Day 16	3.45 ± 0.15	4.60 ± 0.30	8.05 ± 0.23
$\mathrm{WH_{RDC}}$	Soil + 10% compost	Day 14	6.50 ± 0.20	8.00 ± 0.50	14.50 ± 0.35
	Soil + 20% compost	Day 14	6.80 ± 0.71	9.30 ± 0.55	16.10 ± 0.63
$\mathrm{WH_{RVE}}$	Soil + 10% vermicompost	Day 14	6.20 ± 0.50	9.00 ± 0.22	15.20 ± 0.36
	Soil + 20% vermicompost	Day 14	6.90 ± 0.32	10.90 ± 0.35	17.80 ± 0.34
$\mathrm{WH_{RVF}}$	Soil + 10% vermicompost	Day 14	7.03 ± 0.21	9.12 ± 0.24	16.15 ± 0.23
	Soil + 20% vermicompost	Day 14	7.40 ± 0.64	12.60 ± 0.37	20.00 ± 0.51
$\mathrm{HV_{RDC}}$	Soil + 10% compost	Day 14	5.40 ± 0.60	6.50 ± 0.20	11.90 ± 0.40
	Soil + 20% compost	Day 14	6.80 ± 0.24	9.30 ± 0.72	16.10 ± 0.48
$\mathrm{HV_{RVE}}$	Soil + 10% vermicompost	Day 14	5.21 ± 0.21	8.12 ± 0.24	13.33 ± 0.23
	Soil + 20% vermicompost	Day 14	6.90 ± 0.24	11.50 ± 0.55	18.40 ± 0.40
$\mathrm{HV_{RVF}}$	Soil + 10% vermicompost	Day 14	8.10 ± 0.41	12.40 ± 0.50	20.50 ± 0.46
	Soil + 20% vermicompost	Day 14	8.40 ± 0.42	14.60 ± 0.35	23.00 ± 0.39

*SGD: Seed germinated day

Upon comparing the outcomes of plant growth between *W. hyacinth* based bioproducts and *H. verticillata* based bioproducts, it can be inferred that *W. hyacinth* based bioproducts may have a marginally superior impact on plant growth in comparison to *H. verticillata* based bioproducts. In the study, two groups were compared: $\mathrm{WH_{RVE}}$ and $\mathrm{WH_{RVF}}$, which utilised 10% and 20% water hyacinth vermicompost, respectively, and $\mathrm{HV_{RVE}}$ and $\mathrm{HV_{RVF}}$, which employed household waste vermicompost. The results indicated that the plant length in the former groups was consistently greater than that in the latter groups. It is important to acknowledge that the outcomes cannot be exclusively ascribed to the specific variety of vermicompost employed. Plant growth may be influenced by various factors, including but not limited to the composition of the soil, the species of the plant, and the conditions under which it grows. Therefore, it is crucial to exercise prudence in interpreting the outcomes and consider various variables when drawing comparisons among distinct interventions.

6.2.3 Terrestrial weed biomass

i) Effect of *A. conyzoides* and *M. micrantha* based bioproducts on the growth of *C. sativum*

Pot study control without addition of compost or vermicompost derived from *A. conyzoides* and *M. micrantha* biomass was maintained against pots with defined percentages of compost or vermicompost as summarized in Table 6.10

Table 6.10 Pot study using terrestrial weed *A. conyzoides* and *M. micrantha* based bioproducts on plant growth dynamics of *C. sativum*

	Composition (w/w)	**SGD**	**Plant length in cm on Day 30**		
			Root length	Shoot length	Total
Control	Soil	Day 16	3.45 ± 0.15	4.60 ± 0.30	8.05 ± 0.20
MMRDC	Soil + 10% compost	Day 14	5.50 ± 0.21	8.10 ± 0.25	13.30 ± 0.15
	Soil + 20% compost	Day 14	5.30 ± 0.51	8.50 ± 0.90	13.80 ± 0.55
MMRVE	Soil + 10% vermicompost	Day 13	6.30 ± 0.61	8.5 ± 0.34	14.80 ± 0.55
	Soil + 20% vermicompost	Day 13	6.00 ± 0.45	10.00 ± 0.36	16.00 ± 0.39
MMRVF	Soil + 10% vermicompost	Day 13	7.10 ± 0.43	8.0 ± 0.25	15.30 ± 0.50
	Soil + 20% vermicompost	Day 13	9.50 ± 0.50	6.60 ± 0.15	16.10 ± 0.50
ACRDC	Soil + 10% compost	Day 14	8.80 ± 0.46	7.90 ± 0.26	16.70 ± 0.25
	Soil + 20% compost	Day 14	9.30 ± 0.72	6.80 ± 0.24	16.10 ± 0.24
ACRVE	Soil + 10% vermicompost	Day 13	10.50 ± 0.61	6.30 ± 0.39	16.80 ± 0.50
	Soil + 20% vermicompost	Day 13	11.50 ± 0.55	6.90 ± 0.24	18.40 ± 0.15
ACRVF	Soil + 10% vermicompost	Day 13	12.90 ± 0.61	7.10 ± 0.44	20.00 ± 0.24
	Soil + 20% vermicompost	Day 13	14.60 ± 0.35	8.40 ± 0.42	23.00 ± 0.29

*****SGD: Seed germinated day

The produced vermicompost significantly impacted *C. sativum* growth, with the most considerable increase in plant length found at 20% amendment in all experiments evaluated. The results revealed that when the MMRVF and ACRVF end products were added to the soil, they resulted in more extensive root lengths than the MMRDC and ACRDC end products. It was found that adding vermicompost to the soil improved seed germination. Since the plants did not show any signs of distress throughout the investigation, it can be concluded that the vermicompost used in the experiment was both practical and harmless.

ii) Effect of *P. hysterophorus* and *L. camara* based bioproducts on the growth of *C. sativum*

Pot study control without addition of compost or vermicompost derived from *P. hysterophorus* and *L. camara* biomass was maintained against pots with defined percentages of compost or vermicompost as summarized in Table 6.12 Germination of the *C. sativum* seeds was seen on the 14th day of the study in control and on the 10th day in compost/vermicompost amended soils. The root and shoot lengths were measured on the 20th and 30th days of the study (Table 6.11)

Table 6.11 Pot study using terrestrial weeds *P. hysterophorus* and *L. camara* based bioproducts on plant growth dynamics of *C. sativum*

	Composition (w/w)	**Overall plant length in cm (root length and shoot length)**		
		Day 10	Day 20	Day 30
Control	Soil	Germinated on day 14	4.10 ± 0.35	8.45 ± 0.25
PRDC	Soil + 10% compost	Germinated	4.41 ± 0.28	12.90 ± 0.38
	Soil + 20% compost	Germinated	5.92 ± 0.69	16.10 ± 0.63
PRVF	Soil + 10% vermicompost	Germinated	6.58 ± 0.85	18.30 ± 0.91
	Soil + 20% vermicompost	Germinated	6.05 ± 0.38	17.80 ± 0.44
PRVE	Soil + 10% vermicompost	Germinated	5.86 ± 0.94	15.80 ± 0.68
	Soil + 20% vermicompost	Germinated	6.36 ± 0.83	17.40 ± 0.97
LRDC	Soil + 10% compost	Germinated	4.87 ± 0.17	13.10 ± 0.54
	Soil + 20% compost	Germinated	5.62 ± 0.25	16.30 ± 0.81
LRVF	Soil + 10%	Germinated	7.36 ± 0.36	20.30 ± 0.39

	vermicompost			
	Soil + 20% vermicompost	Germinated	7.45 ± 0.54	20.10 ± 0.71
LRVE	Soil + 10% vermicompost	Germinated	5.52 ± 0.84	15.40 ± 0.78
	Soil + 20% vermicompost	Germinated	6.71 ± 0.55	18.30 ± 0.65

The root and shoot lengths in the vermicompost amended soils were higher compared to control soil. The effect of vermicompost can be seen from day 20 to day 30, where higher increments in the lengths were recorded. The plants amended with vermicompost were free from pest attacks throughout the study and showed healthier plant growth. The root lengths were higher in the vermicompost amended soils on day 30 than compost amended soil. Higher values were recorded for the vermicompost amendment than compost on *C. sativum*. This study proved that the vermicompost amended to the soil helped in the early germination of seeds and healthier growth in plant root and shoot lengths and signifies that the RV technique was efficient in producing safer end products compared to RDC.

6.3 MAJOR OUTCOMES OF THE STUDY

- The two-stage biodegradation process was expeditors in stabilizing the biomass.
- The toxic terrestrial weed-based vermicompost was recorded as an SGI% >95%.
- The root lengths were higher in the germinated plants during the seed germination test.
- The pot study demonstrates that the added 20% (w/w) vermicompost amendment to soil showed a higher plant growth of *C. sativum*.
- *E. fetida*-based vermicompost was coherent in higher plant growth.

Chapter 7

PILOT SCALE OPERATION OF THE TWO-STAGE BIODEGRADATION PROCESS

The present chapter expounds upon a pilot-scale operation of a two-stage biodegradation process that employs vegetable waste, water hyacinth, and a synergistic combination of both substrates as feed biomass. The present investigation, conducted at the pilot scale, seeks to determine the feasibility and efficacy of the process, with the ultimate goal of upscaling to an industrial-scale operation. The utilization of vegetable waste and water hyacinth as feed biomass is a fascinating avenue of research, owing to the abundance and renewability of these resources. This approach holds the potential to mitigate waste and extract value from otherwise underutilized resources.

7.1 VEGETABLE WASTE AS FEED BIOMASS

Vegetable waste pollutes air, water, and greenhouse gases and makes up a large fraction of landfills worldwide. Vegetable waste in landfills produces methane, a strong greenhouse gas that contributes to global warming. Vegetable trash must be separated from other garbage at residences and restaurants. Composting minimizes organic waste and methane emissions, making waste management sustainable. Composting vegetable waste reduces organic waste in landfills, produces nutrient-rich fertilizers, and reduces methane gas emissions, supporting a clean and healthy environment.

7.1.1 Physico-chemical analysis

Variation in temperature and moisture content

Composting shows the decomposition of organic matter owing to microbial activity by changing the temperature profile of feedstock, which aids in pathogen eradication (Song et al., 2021). Because of the artificial regulation of process factors such as moisture content, C:N ratio, and aerating conditions, thermophilic aerobic composting has a greater temperature range (>40°C) than heap composting. Thermophilic biodegradation can produce safe mature compost efficiently (Wang et al., 2022). The present study's temperature profile is shown in Fig.7.1(a). During the commissioning period, there was a rising trend in 24 h from the ambient to thermophilic phase (Varma and Kalamdhad, 2016), and the temperature profile at the inlet zone was thermophilic throughout the study period. According to the graph, ambient temperatures have less of an impact on the development of thermophilic circumstances, but they affect the thermophilic temperature as they rise. In the early weeks, while the ambient temperature was 20°C, the thermophilic temperatures were more than 50°C, demonstrating that the rotating drum composter can develop a thermophilic phase in the feedstock regardless of weather conditions. Vermicomposting was initiated after 7-10 days when the feedstock temperature was favourable for vermicomposting (<35ºC). The feedstock then followed the mesophilic temperature profile of the ambient environment.

The initial physicochemical qualities of the raw material are frequently connected to the success of compost. Among them, moisture content (MC) is essential for effective composting (Ge et al., 2022). Moisture is also necessary for microbial activity, and the moisture level should be maintained and monitored during composting, with specific emphasis paid to a large sum of organic content and the material's porosity (Moncks et al., 2022). In the current study, the initial moisture content of the feedstock was maintained between 70-75%. The outlet moisture was recorded between 70-79% in the winter. Further, in spring, ambient and reactor temperature rise and higher moisture loss was seen, and the average outlet moisture content was recorded between 60-70%. The average moisture content of the outlet was 65-70% which was suitable for vermicomposting. During vermicomposting, a moisture level of 70% was maintained.

Variation in volatile solids and total organic carbon

VS provides the measurement of a substance's organic content. Organic matter is critical in understanding microbial activity and the maturity of compost products (Jiang et al., 2019). In the current research, the volatile solid fraction differed according to the seasonal variation, the availability of vegetables differed in the different seasons, and the range of volatile solids in the feedstock was 45 to 57% throughout the reactor loading. There was a 17% reduction in average VS in the initial 7 days and a further 53% reduction overall by the end of 27 days. The quick declines in VS during the initial phase of the composting process result from organic matter mineralization, indicating that early microbial activity was robust (Zhu et al., 2022), which can also be understood by the physiological thermophilic range developed in the rotary drum reactor. According to reports, the reduction in volatile solids was reported to be higher in the rotary drum composter's active thermophilic phase (Kauser and Khwairakpam, 2022).

Conventional composting and vermicomposting include a multistep process involving biological, physical, and biochemical changes that ultimately convert the organic carbon into the starting material. In the current research, a decreased trend was observed in the TOC. 18% reduction was seen in the RDC, and a further 44% reduction was observed in vermicomposting. A drop-in TOC content is essential in measuring the biodegradation rate because it indicates organic carbon uptake by the vermi biomass and Carbon dioxide evolution during biodegradation (Kumar et al., 2017)

Variation in pH and electrical conductivity of feedstock during the process

The more considerable fluctuation in pH throughout the composting process suggests a higher rate of organic content biodegradation (Cao et al., 2022). The final products are well-stable if the pH stays near 8. In the current investigation, an average inlet pH of 5.6 increased to 8 during RDC. Further, during vermicomposting, the change was less, and the material attained a maximum of pH 8.5 ($P<0.05$). The RDC thermophilic degradation has a significant effect on pH fluctuation. The increase in pH is attributed to the release and build-up of ammonia in compost material, a by-product of the decomposition of nitrogen-containing substances (Wan et al., 2020).

The degree of mineralization of organic materials throughout the process greatly influences EC dynamics, which is an indication of likely phytotoxic inhibitory effects (Meng et al., 2019). The EC values had experienced a decreasing trend, accounting for a 46% overall reduction during the process. According to studies, a high concentration of soluble salts in the compost product restricts seed germination (Yin et al., 2021). The end product of the current study exhibited an average value of 2.43 mS/cm and was found safe upon soil use ($P<0.01$).

7.1.2 Elemental analysis

It is evident from the literature that the total nitrogen content increases during composting and also significantly during vermicomposting. This effect is primarily driven by the decomposition of organic matter, which concurrently produces soluble nitrogen and reduces material moisture during composting (Tong et al., 2019) and during vermicomposting, where earthworms gut bacterial effects are added to the process and

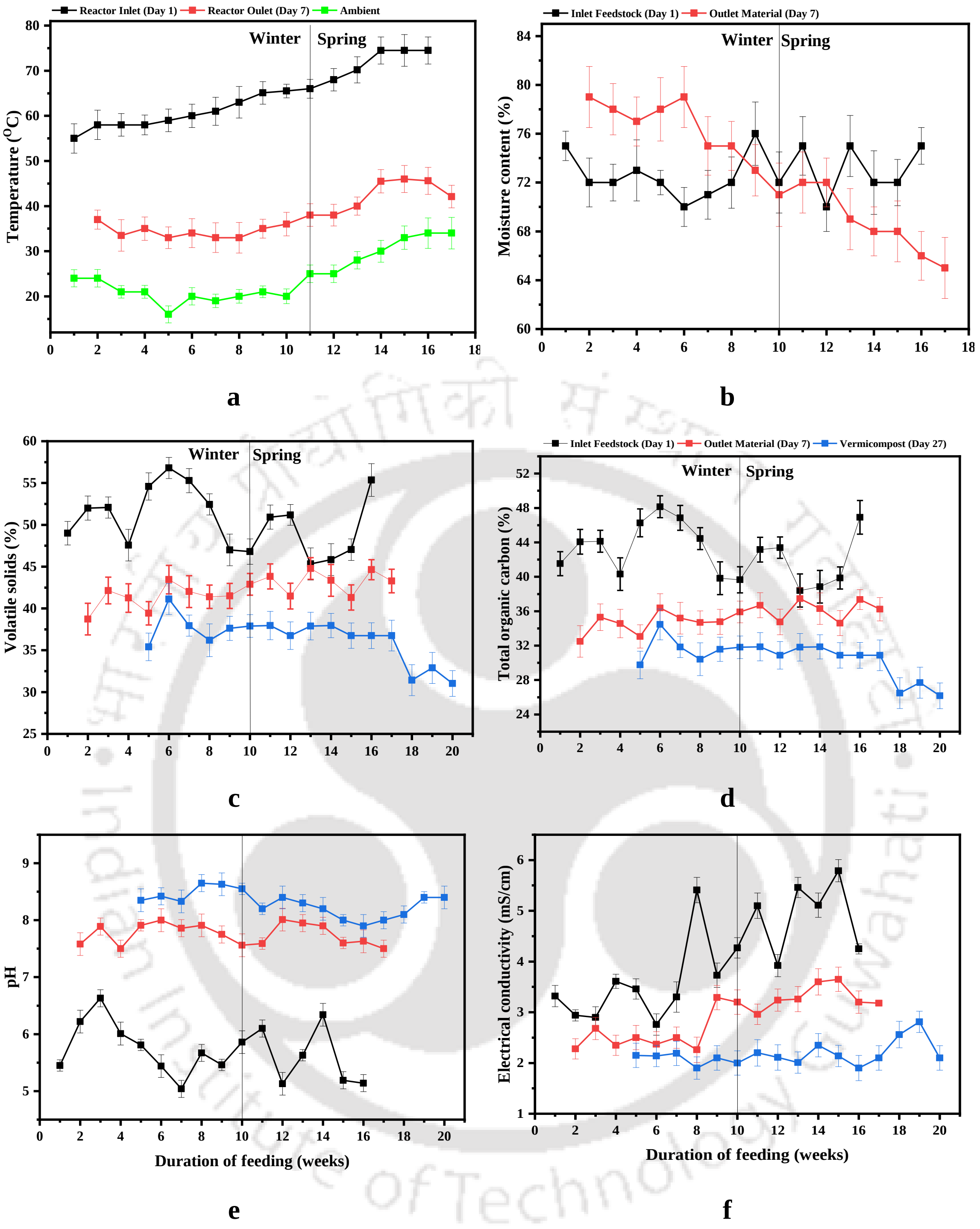


Fig. 7.1 Variation in a) Temperature b) Moisture content c) Volatile solids d) Total organic carbon e) pH and f) Electrical conductivity

produce nitrogen-rich vermicomposting (Saha et al., 2017). In the current study, both processes were employed, resulting in higher nitrogen content in the end product. In the winter, the process could make 3.5% TN; in the spring, when the reactor's thermophilic range went above 70°C, the process made 4.2% TN in total (Fig.7.2(a)). A clear testimony of the efficiency of two-stage composting can produce nitrogen-rich vermicompost.

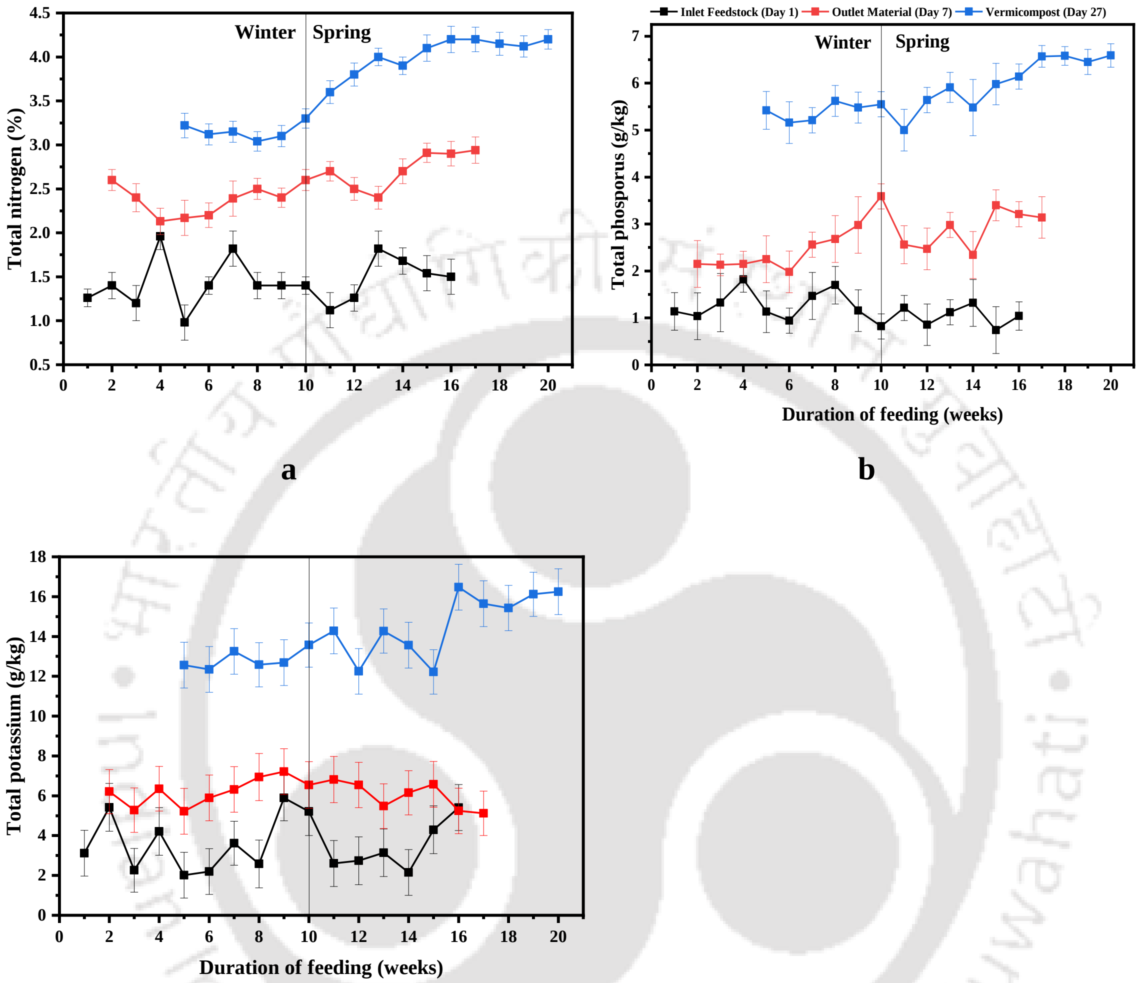
In agroecosystems, phosphorus is one of the critical nutrients for crop development (Alewell et al., 2020). Composting organic waste generates effective fertilizer and relatively high amounts of phosphorus (Rehman and Qayyum, 2020). Organic phosphorus mineralization, microbial consumption, organic matter breakdown, and mass loss during composting are the driving forces behind the increased TP levels seen during the composting process (Kauser et al., 2022). The TP content in the present study increased significantly in RDC (40%) and overall 50% during the two-stage process. Further, the expeditious increment in the TP can be attributed to the thermophilic degradation in the initial 7 days of the composting process ($P<0.001$).

TK also increased along with TN and TP during the process, and a significant increase was seen in RDC (90% of increased value), and overall the increment was 38% ($P<0.001$). This phenomenon can be attributed to mineralization and mass loss during biodegradation. The expanded action of earthworms accelerates the mineralization process and modifies the potassium dispersal in further vermicomposting the feedstock (Mago et al., 2021). Previous studies employing RDC observed similar trends of increased nutritional properties (Kauser and Khwairakpam, 2022).

The increasing trend of micronutrients in the RDC can be attributed to the change in bulk (Jain and Kalamdhad, 2018b). The impact of pH shift and microbial activity on feedstock mass as it was consumed by the earthworms may be ascribed to an increase in macronutrients (Kouba et al., 2018). In the present research, the micronutrients were observed to be increasing throughout the loading period. Na content increased by 33% initially and 35% when the reactor attained more than 70°C. Ca content increased by 39-41%, and Mg content increased by 10-12% during the biodegradation (Table 2).

7.1.3 Heavy metals dynamics

The rise in HMs in finished compost is caused by CO_2 emissions, Organic matter degradation, which reduces the total bulk of trash while increasing its metal concentration (Yadav and Garg, 2011). Thus, there was an increasing trend of heavy metal concentration during the rotary drum composting. Further, there was a reduction in the course of vermicomposting. According to the studies conducted by (Sun et al., 2020), there was bioaccumulation of heavy metals concentration due to vermiculture amendment. Various studies documented this mechanism of heavy metal accumulation (Kheir et al., 2021; Saqib Rashid et al., 2022; Xu et al., 2022).



a

b

c

Fig. 7.2. Progression of a) Total Nitrogen b) Total Phosphorus and c) Total Potassium throughout the feeding time

In the current study, the detectable heavy metals were tabulated. The total heavy metal concentrations of Fe, Mn, Pb and Cu increased by 65%, 93%, 160% and 60%, respectively. Further reduction during vermicomposting was 13% for Fe,7% for Mn and 100% for Pb and Cu (P<0.001). The reduction of Pb and Cu was also due to significantly low quantities in the feedstock mass (Table 7.1).

Table 7.1 Variation in the micronutrients and the total heavy metal concentration during biodegradation

Micronutrients			
Duration of Composting (days)	**Sodium (Na)**	**Calcium (Ca)**	**Magnesium (Mg)**
Feedstock (day 0)	265.00 ± 53.15	650.00 ± 72.47	135.34 ± 36.88
Reactor outlet material (day 7)	302.50 ± 45.26	910.00 ± 70.17	153.25 ± 14.40
10 days of vermicomposting (day 17)	310.00 ± 23.80	916.00 ± 48.71	153.00 ± 24.45
Vermicompost (day 27)	360.00 ± 14.14	920.00 ± 35.62	152.00 ± 28.28

Heavy metals				
	Iron (Fe)	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
Feedstock (day 0)	88.73 ± 36.17	8.45 ± 2.91	1.25 ± 0.25	1.30 ± 0.18
Reactor outlet material (day 7)	146.80 ± 5.39	16.38 ± 7.15	3.25 ± 0.22	2.08 ± 0.68
Vermicompost (day 27)	126.59 ± 1.12	6.45 ± 0.78	ND	ND

ND: Not in detectible concentrations

7.1.4 Worm growth and cocoon production

Earthworms promote *Algoriphagus*, *Aeromonas*, *Aquicella*, *Arenimonas*, *Flavobacterium*, *Kaistobacter* and *Lysobacter*. *Aeromonas* populates earthworm intestines (Hong, 2011). These potential degraders, together with earthworms, decompose organic pollutants. Earthworms increase soil oxygen and deliver nutrients by excrement and burrowing, supporting aerobic decomposing bacteria, thus decomposing waste and removing heavy metals (Ning et al., 2021). In the present investigation, the employed *E. fetida* degraded the feedstock robustly (Table 7.2). Two thousand earthworms were inoculated to 1000 kg of partially degraded material (through rotary drum composting) with an earthworm density of 2 worms/kg on the initial day. The population increased significantly to 8 worms/kg of material in 20 days. Further, the number of juveniles and cocoons was reported to be 15 worms/kg and 32/kg on average. *E. fetida* was proven suitable for feedstock nutication. Healthy growth and more outstanding cocoon production indicate early adaptation and feedstock bioconversion. This study also signifies the large-scale vermicomposting possibility through fed-batch feeding.

Table 7.2. Worm growth during the biodegradation

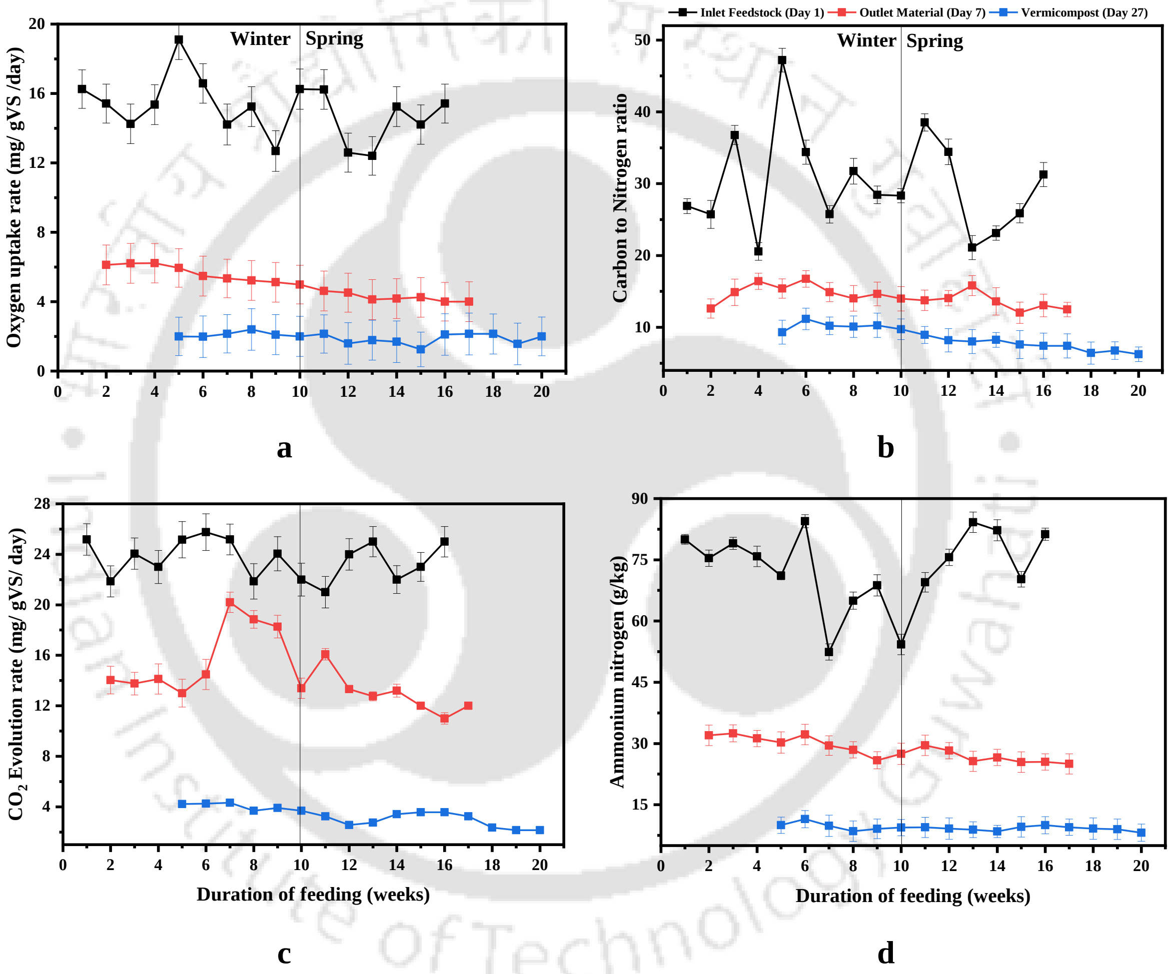
Earthworms	**Initial day (Day 7)**		**Final day (Day 27)**	
	Numbers (per kg)	Biomass (in g)	Numbers (per kg)	Biomass (in g)
Adults	2 ± 1	0.65 ± 0.1	8 ± 1	6.4 ± 0.2
Juveniles	0	0	15 ± 2	9.6 ± 0.15
Cocoons	0	0	32 ± 5	3.5 ± 0.5

7.1.5 Maturation and stability analysis

A substantial amount of oxygen is necessary for aerobic biodegradation (Zhang et al., 2021). In the current research, the oxygen uptake rate of the biomass was evaluated to understand the rate of oxygen utilization by feedstock mass at different stages of composting. The results revealed that the feedstock had a higher OUR value of 15 mg/gVS/day at the start of the biodegradation, which gradually decreased to 5 mg/gVS/day on average by day 7. A 60% decrease was observed in the rotary drum composting, and further, the final product exhibited a value of 1.95 mg/gVS/day ($P<0.05$). The reduction in OUR values of the biomass is a clear testimony of the feedstock stabilization during the biodegradation ($P<0.05$). Further, the variation of thermophilic temperatures during RDC affected the feedstock OUR values significantly (Fig.7.3a).

The rate of CO_2 release reduces steadily and ultimately stabilizes, suggesting that the compost gradually stabilizes (Xiong et al., 2021). CO_2 evolution must be minimized, and other precursors must be allowed to contribute in producing humic compounds (Wang et al., 2021). In the present study, a 39% reduction in CO_2 evolution rate was observed during the RDC, and further in vermicomposting, a reduction of 77% was recorded on average.

Further, a reduction of 86% was seen overall during the biodegradation process ($P<0.01$). The results show that a greater decrease was found during the vermicomposting of the material. Microorganisms use oxygen quickly in the early stages, digesting easily decomposable material and creating considerable CO_2. It is also evident that the amended vermicomposting was significant in stabilizing the feedstock mass in the present investigation.



a

b

c

d

Figure 7.3. Variation in a) Oxygen uptake rate b) C/N ratio c) CO2 evolution and d) Ammonium nitrogen during the biodegradation.

The high temperature during the initial phase and alkaline environment created in further phases of composting lower ammonia nitrogen (NH_4^+–N) solubility inside the composting mass, resulting in a decreased pattern during composting (Chen et al., 2022). In the present study, a reduction of 87% was observed, of which 61% was due to the active thermophilic phase of the rotary drum composter ($P<0.001$). NH_4^+–N is also considered a compost maturity characteristic (Fig. 7.3d). Thus, the more significant the NH_4^+–N decrease, the higher the compost maturity (Zhang et al., 2022).

A reduction in the C/N ratio caused by the degradation of carbonaceous-containing organic substances also indicates high nitrogen content, which indicates effective composting, and compost with a C/N ratio of 20 is considered mature (Song et al., 2021). Further, during vermicomposting, the expeditious increase in TN affects the C/N ratio (Devi and Khwairakpam, 2020). In the present study, the C/N ratio was significantly reduced to 8.51 from 52 on average by the end of composting and a minimum value of 6.26 during higher temperatures in thermophilic composting ($P<0.05$) (Fig. 7.3b). The substrate's initial carbon to nitrogen (C:N) ratio is critical for earthworm development in vermireactors since these elements are required for cell production in all organisms (Ndegwa and Thompson, 2000).

7.1.6 Variation in bulk and the changes in volume throughout the operation

The bulk density of the organic material depends on the size shredded during the composting process and the initial moisture content (Jain et al., 2020). In the present study, the moisture content was reduced during composting, affecting the material's bulk density. Furthermore, the particle size was reduced during biodegradation due to the release of CO_2 and NH_4. The initial 350 kg/m^3 was increased to 594 kg/m^3. According to (Jain et al., 2019), the final bulk density of the compost should lie between 500-900 kg/m^3 (Fig.7.4). The particulate density varies according to the VS of the material. The current study's particle densities varied from 625-868 kg/m^3. Similar increasing trends were observed in studies conducted by (Jain et al., 2019). The reduction of bulk influences the volume of the mass. In the current study, a volume reduction of 15% was observed by rotary drum composting. Furthermore, an overall 70% volume reduction was observed by the end of the 27-day biodegradation. Higher volume reduction was observed during vermicomposting of the feedstock due to reduced particles.

7.1.7 Heavy metals dynamics

The rise in HMs in finished compost is caused by CO_2 emissions, Organic matter degradation, which reduces the total bulk of trash while increasing its metal concentration (Yadav and Garg, 2011). Thus, there was an increasing trend of heavy metal concentration during the rotary drum composting. Further, there was a reduction in the course of vermicomposting. According to the studies conducted by (Sun et al., 2020), there was bioaccumulation of heavy metals concentration due to vermiculture amendment. Various studies documented this mechanism of heavy metal accumulation (Kheir et al., 2021; Saqib Rashid et al., 2022; Xu et al., 2022).

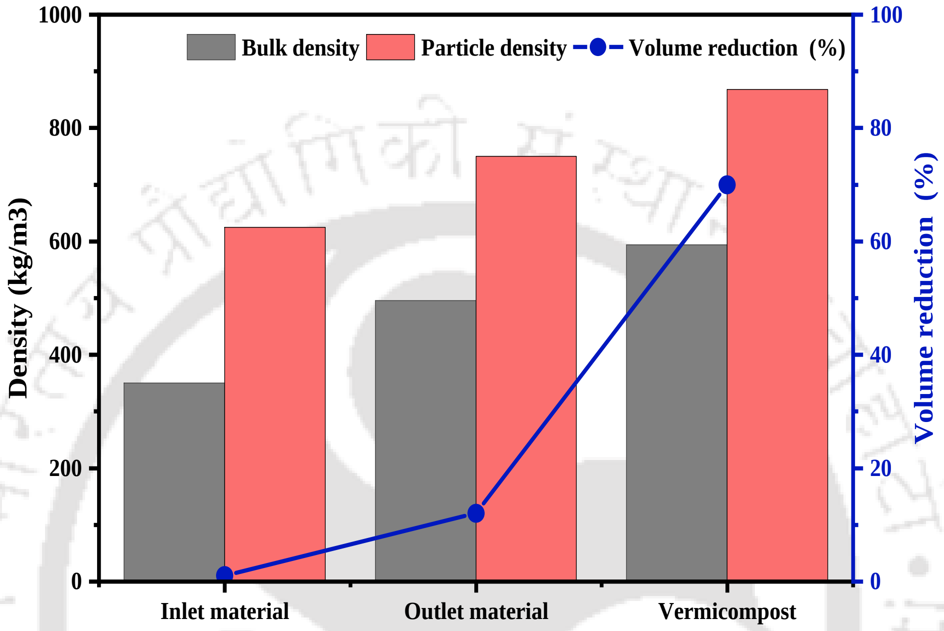


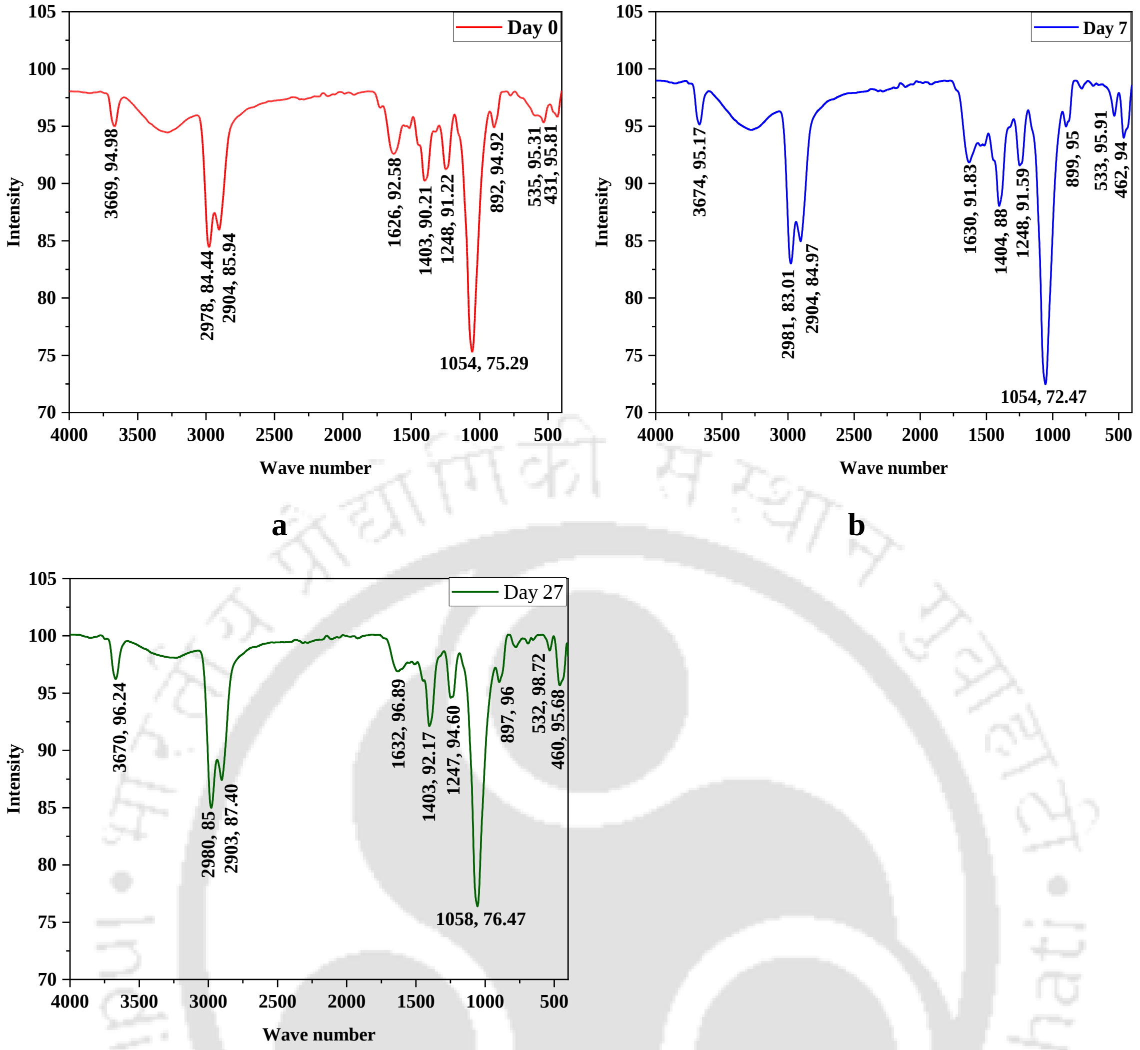
Fig. 7.4. Changes in the bulk density and Volume reduction through biodegradation

7.1.8 Spectroscopic instrumental analysis

FESEM imaging is mainly used to investigate the surface morphological properties of materials (Pandit, 2022). Fig.7.6 illustrates the imaging of the sample taken on the initial and final days of the biodegradation. The surface morphology has changed and increased the surface area of the particle. This is evident in the increased moisture holding capacity and also the pour space when compost is amended to soil (Mazumder et al., 2021)

7.1.9 Metagenomic analysis

Composting and vermicomposting have successfully biodegraded vegetable waste resulting in a nutrient-rich fertilizer (Varma and Kalamdhad, 2016). However, significantly less is known about microbial composition variation and succession during composting and vermicomposting vegetable waste. This investigation offers new insight into the dynamics of bacterial population fluctuation and succession during the composting of plant matter.



a

b

c

Fig. 7.5. FTIR spectra of a) Feedstock on initial day b) Partially degraded feedstock and c) Vermicompost

The bacterial population's phylum, class, order, and family (Fig 7.7) composition changed dramatically during the decomposition of vegetable waste (day 1 to day 27). The phylum Bacteroidetes predominated in the inlet zone (day 1) bacterial population. However, Bacteroidetes (40.6%) were the most common types of bacteria present in compost, followed by Proteobacteria (31.4%), Firmicutes (27.2%), and minor contributions of Actinobacteria (0.3%). Between 7 and 27 days, there were notable shifts in the overall bacterial population makeup (Annexure II). Bacterial communities between 0, 7 and 27 days of the composting show an increase of operational taxonomic units (OTUs), i.e., 569, 734 and 1246, respectively, and the Shannon alpha diversity observed were 6.05, 6.84 and 8.10, respectively. The operational taxonomic units have been summarized in Annexure II for the samples of days 0, 7 and 27. The abundant taxonomy identified in the samples of days 0, 7 and 27 at the phyla level, class, order, family, genus and species is depicted in Table 7.3.

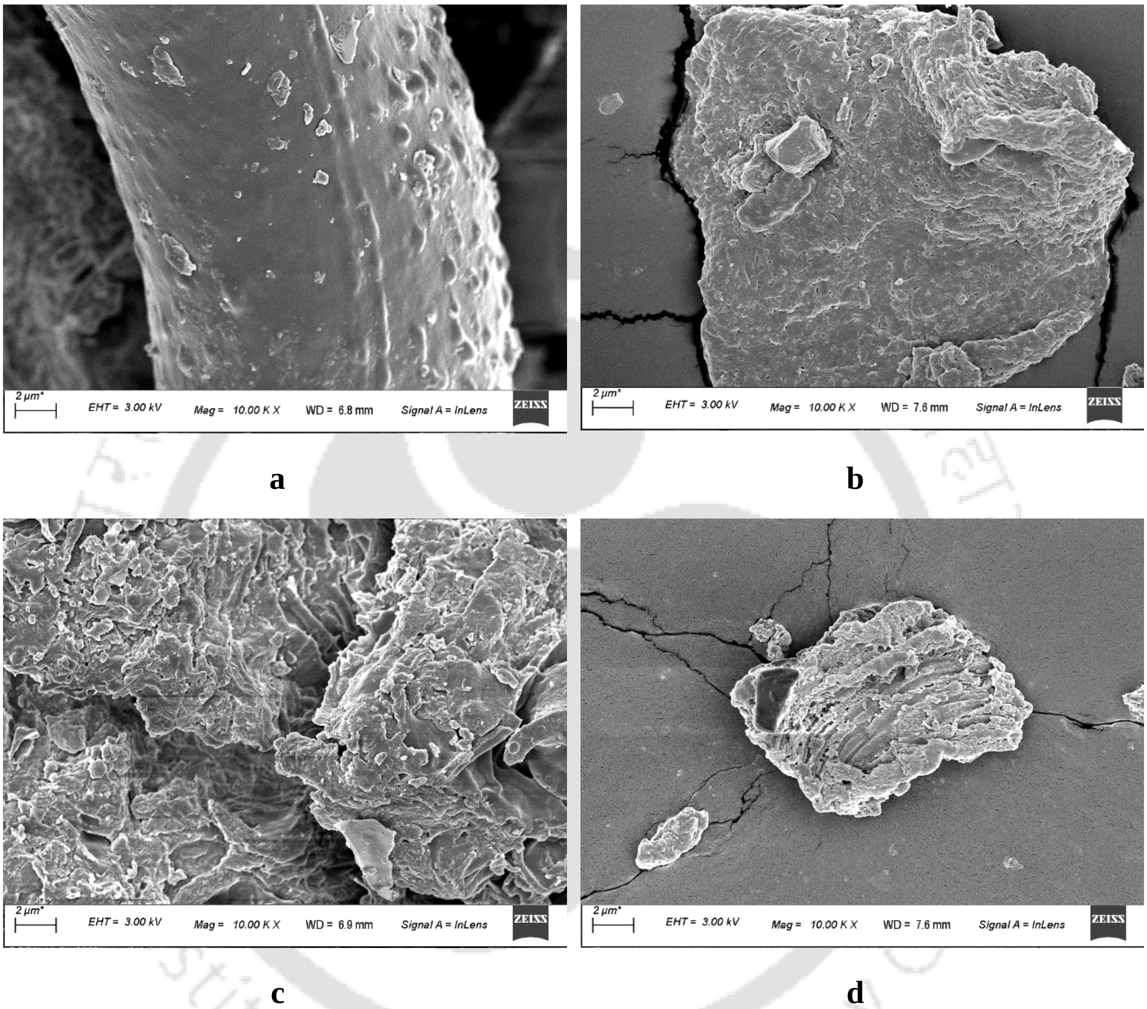


Fig. 7.6. Feed stock (a and b) and vermicompost (c and d)

Previous studies have described clear bacterial succession in thermophilic composting (Antunes, Martins, Pereira, Thomas, Barbosa, Lemos, Silva, Moura, Epamino, Digiampietri, Lombardi, Ramos, Quaggio, De Oliveira, et al., 2016; López-González et al., 2015; Lv et al., 2015), as well as changes in bacterial and fungal diversity (Franke-Whittle et al., 2009). Few studies have examined how the bacterial composition changes over time during composting and vermicomposting (Cai et al., 2018; Gopal et al., 2017; Lv et al., 2015, 2018). Lv et al. (2015) compared composting and vermicomposting of cattle manure and sewage sludge. They discovered an increase in bacterial diversity during vermicomposting, consistent with the findings of (Huang et al., 2013), who compared 2 months old vermicompost to control with no earthworms. (Cai et al., 2018) discovered more bacterial abundance and variety in vermicompost compared to compost samples during green waste vermicomposting. The combination of numerous periods throughout the active phase of vermicomposting by (Cai et al., 2018) and our work provides a full picture of bacterial succession. Vegetable waste composting follows a similar pattern of fast breakdown, consistent with previous findings of vegetable waste vermicomposting (Huang et al., 2013).

The study findings show that variations in the organic carbon supply cause bacterial succession. The bacterial community composition may be divided into three major types during composting. The first group consists of microorganisms (Bacteroidetes, Proteobacteria, and Firmicutes) found in inlet zone waste and the other components mentioned above (day 1). On day 7, the second group was apparent when the community composition comprised bacteria (Bacteroidetes, Proteobacteria, Euryarchaeota and Firmicutes with minor amounts of other bacteria) that had recently progressed out through the reactor (7 days of retention duration). The third group of microbial communities comprises (Bacteroidetes, Proteobacteria, Euryarchaeota, Acinitobacteria, Planctomycetes and Firmicutes, with other bacteria) in vermicompost. The rise in Proteobacteria (Table 7.3) on day 27 is most likely related to earthworm gut-associated activities. Later on, as microbial succession progresses, the amount and quality of accessible nutrition sources will gradually change, which will be linked to the emergence of bacterial taxa specialized in metabolizing the remaining more resistant substrates.

Bacterial diversity rose dramatically at taxonomic and phylogenetic levels, with the latest succession group reaching a high point (day 27). Cai et al. (2018) discovered an immense bacterial richness and diversity during vermicomposting of green waste in the last stages of the process, days 120 and 150. After 75 days of vermicomposting, Gopal et al. (2017) discovered a high bacterial diversity of coconut leaves combined with cow dung slurry after 75 days of vermicomposting, followed by a reduction at the end of the trial on the 105th day. A decrease in the last phases of the process might be due to a drop in the moisture level. Our results are also similar to the previous vermicomposting studies of (Domínguez et al., 2019).

Table 7.3. Most abundant taxonomy identified in the samples at different taxonomic levels

Sample	Day 1 (%)	Day 7 (%)	Day 27 (%)
Phylum	Bacteroidetes (40.55%)	Bacteroidetes (25.91%)	Proteobacteria (22.16%)
Class	Bacilli (22.38%)	Bacteroidia (14.69%)	Alphaproteobacteria (15.30%)
Order	Bacteroidales (21.35%)	Bacteroidales (14.69%)	Methanosarcinales (15.30%)
Family	Moraxellaceae (17.76%)	Porphyromonadaceae (10.67%)	Methanosarcinaceae (15.28%)
Genus	Acinetobacter (17.65%)	Unclassified Genus from Porphyromonadaceae Family (10.59%)	Methanosarcina (15.28%)
Species	Unclassified Species from Acinetobacter Genus (15.46%)	Unclassified Genus from Porphyromonadaceae Family (10.59%)	Unclassified species from Methanosarcina Genus (14.15%)

7.1.10 Phytotoxicity analysis

Phytotoxicity Plant bioassay was employed as an extra safety measure to assess environmental danger and quantify its impacts on plant development (Wang et al., 2022a). In the present study, the *Vigna radiata* model seed was used to understand the toxic effect of the extracts prepared using feedstock mass and vermicompost produced with tap water as the control. The SGI of the waste before treatment was less than 60% because of the seed-inhibitory effect of inorganic nitrogen (Peng et al., 2022). In addition, the influence of EC and high salt and organic acid levels may contribute to this low SGI (%) (Wang et al., 2022c). Further, after the treatment, the SGI (%) increased to a maximum of 98.3% in a 50% dilution of the raw extract (Table 7.4). From previous studies, we know that biodegradation processes like composting and vermicomposting can reduce the phytotoxic effect of the mass. In the current investigation, the reduction was seen by the end of 27–30 days of degradation, which is not in the potential of other biodegradation procedures (Wang et al., 2022a). The adopted biodegradation technique effectively produced safe vermicompost in an optimized 27 days of composting.

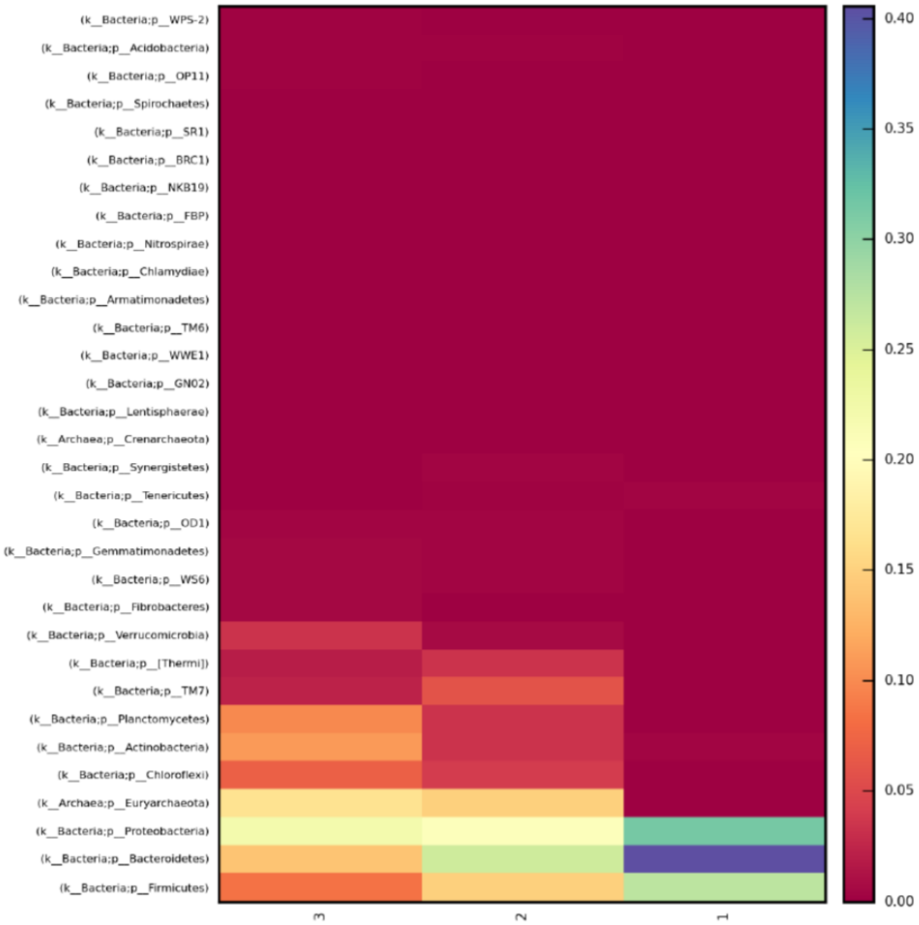


Fig. 7.7. Heatmap to visualize the OTUs table at Phyla Level

Table 7.4. Phytotoxicity bioassay

Phytotoxicity	Concentration (v/v)	SGI (%)	Shoot length (cm)	Root length (cm)
Tap water (Control)	0	90	2.6 ± 0.1	2.5 ± 0.1
Inlet feedstock	25	72	1.5 ± 0.1	2.0 ± 0.1
(Day 1)	50	<60	0.9 ± 0.1	1.2 ± 0.1
	75	<60	0	0
	100	<60	0	0
Vermicompost	25	85	1.4 ± 0.1	3.5 ± 0.1
(Day 27)	50	98	4.2 ± 0.1	4.3 ± 0.1
	75	93	3.1 ± 0.1	3.4 ± 0.1
	100	91	1.8 ± 0.1	4.0 ± 0.1

7.1.11 Bivariant correlation between total heavy metal concentration during biodegradation

Pearson's correlation coefficient matrix includes all verified associations between total heavy metals. The matrix for the studied heavy metals revealed a considerable positive correlation between Pb and Mn (r = 0.98) and Cu and Pb (r = 0.96). The studied heavy metals were negatively correlated with the duration of composting except Fe (r = 0.39). The concentration of Fe saw only an increasing trend during the process as other metals increased during RDC and reduced during vermicomposting (Table 7.5). The shift in correlation coefficients might be related to earthworm culture amendment and heavy metal build-up in their cells.

Table 7.5 Pearson correlation coefficient matric of total heavy metal concentrations

	Duration of composting	**Fe**	**Mn**	**Pb**	**Cu**
Duration of composting	1.00				
Fe	0.39	1.00			
Mn	-0.47	0.63	1.00		
Pb	-0.64	0.47	0.98	1.00	
Cu	-0.82	0.21	0.89	0.96	1.00

7.1.12 Summery

This study supports the effectiveness of large-scale in-vessel RDC in conjunction with vermicomposting as a successful organic waste management strategy. The study's biodegradation process efficiently lowered the heavy metal content and seed inhibitory toxic nature (SGI-98%). Furthermore, the two-stage composting consistently produced a nutrient-rich soil conditioner from waste (4.2% total nitrogen). At 1.95 mg/gVS/day of OUR, the end-product displayed stable and mature enough to apply to the soil. Taxonomic hit distribution at the domain level indicated that bacteria accounted for 83.17% of the sequences in the vermicompost sample, while archaea accounted for 16.7%. Increased microbial activity was discovered due to the two-stage composting of vegetable waste, leading to greater waste stabilization within a 27-day time frame. The large-scale reactors validated in the study can be a potential tool (70% volume reduction) in urban waste management.

7.2 WATER HYACINTH AS FEED BIOMASS

The water hyacinth, an invasive aquatic plant, is known to pose a significant threat to aquatic ecosystems due to its ability to form dense mats on the water surface. This, in turn, obstructs water transport, reduces oxygen levels, and promotes disease-transmitting insects. The utilization of composting as a sustainable measure for water hyacinth management has garnered significant attention in recent times. The process involves the conversion of water hyacinth and other organic waste materials into compost, leading to a reduction in water hyacinth mat density, enhancement of water quality, and stimulation of aquatic plant growth. The composting of water hyacinth results in the production of organic matter that is rich in nutrients and can be utilized as a natural fertilizer for plants. This practice has the potential to reduce reliance on chemical fertilizers and promote sustainable agricultural practices. The employment of water hyacinth for composting objectives represents a noteworthy stride towards waste minimization and the dissemination of sustainable waste management methodologies. The utilization of composting as a sustainable approach for the management of water hyacinth has been identified as a promising solution. This approach not only contributes to the restoration of aquatic ecosystems but also aids in waste reduction.

7.2.1 Changes in physicochemical analysis

In the pilot scale study, the temperature attained a thermophilic range within 6 h of feeding post-commissioning. The highest of 64°C was recorded on the 80th day of the daily feeding process. For the entire duration of the daily feeding study, the temperatures at the inlet zone were in the thermophilic range. The outlet temperatures varied between the mesophilic and thermophilic ranges. Both the inlet and outlet zone temperatures were higher than ambient temperatures for the entire duration of the study (Fig. 7.8). The higher temperatures were attributed to the mass of the feedstock fed daily and the aeration of the reactor using a mechanical blower and the number of rotation (in the current study 1 rotation per day).

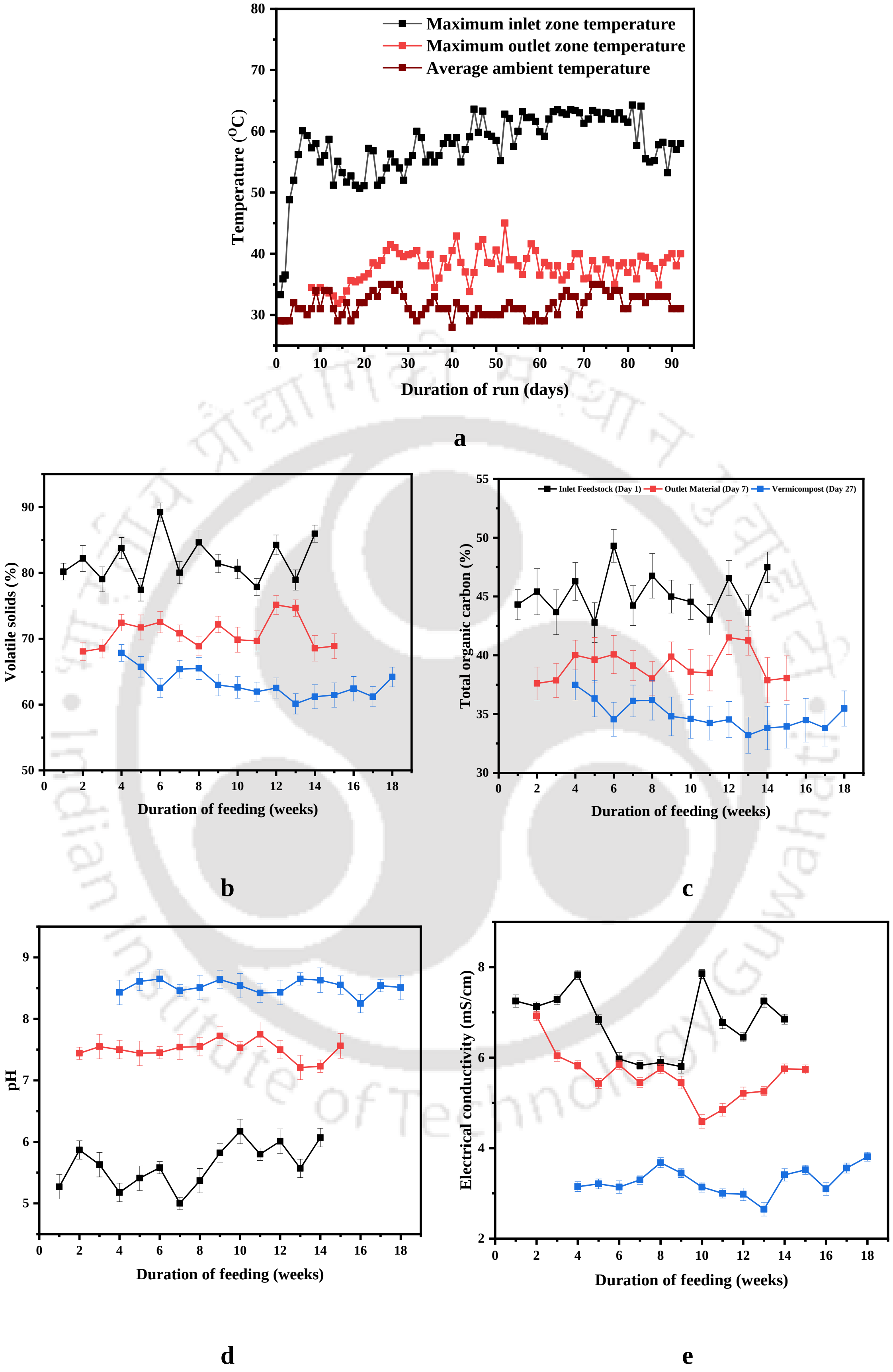
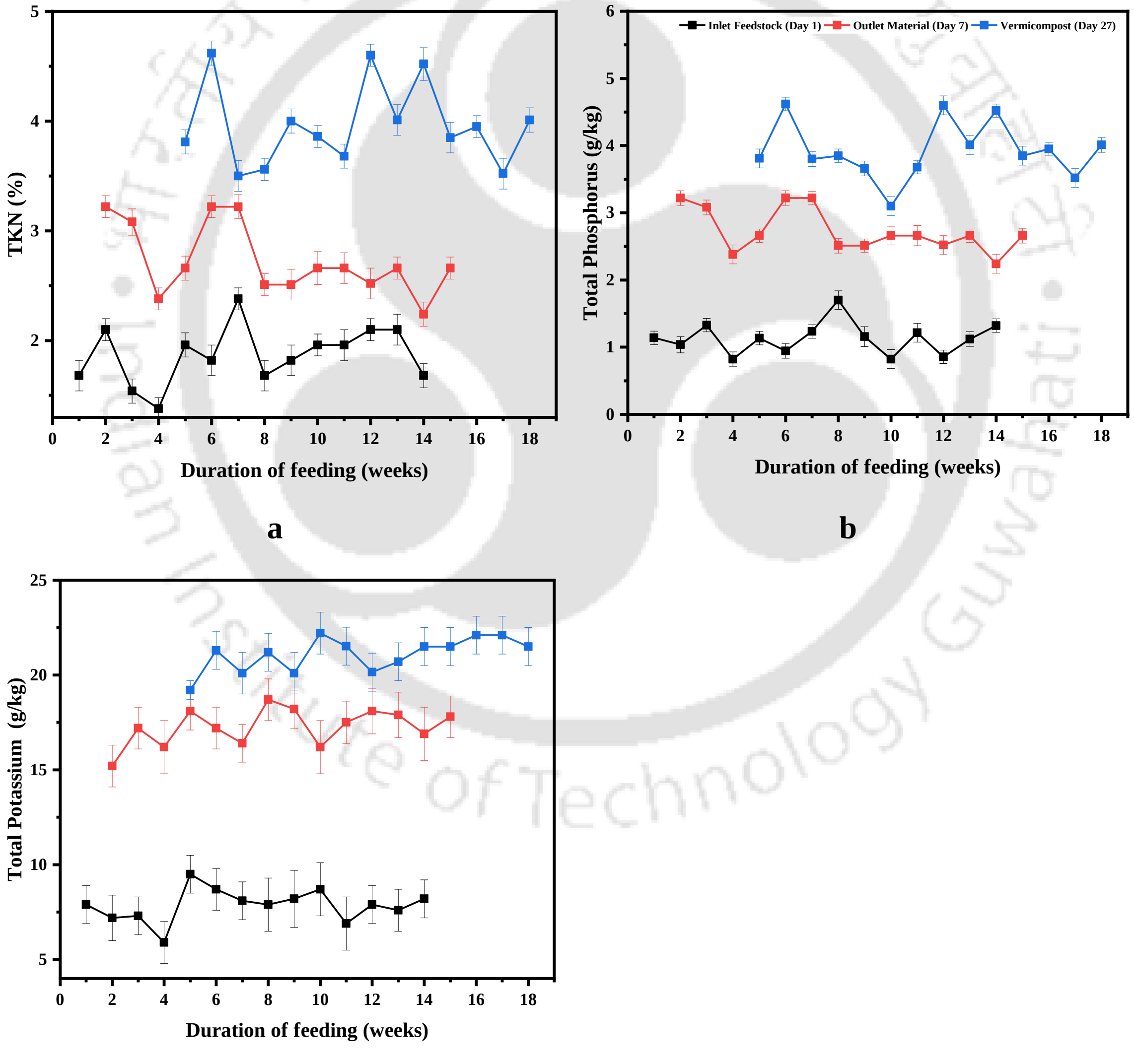


Fig. 7.8. Variation in a) Temperature profile, b) Volatile solids, c) Total organic carbon, d) pH and e) Electrical conductivity during the pilot scale study

The VS content of the fed material varied daily but was in a range of 76-89% for the duration of the run. The two-stage composting efficiently reduced the VS content by 22-24% during the study. Similarly, the toc content was significantly decreased, attributed to the bacterial mineralization and vermiculture influence on the substrate. The initial pH of the substrate varied between pH 5 and pH 6 at the inlet zone. By the end of 7 days, the value shifted from pH 7.2 to pH 7.8 and eventually, after vermicomposting, attained a value of pH 8.2 to pH 8.7. The EC varied significantly in the inlet feed material, and the reduction was higher on the 11th week of feeding. The final EC of vermicompost was in the range of 1.5- 3.5 mS/cm, which can safely apply to the soil.



a

b

c

Fig. 7.9. Changes in a) Total nitrogen, b) Total phosphorus and c) Total potassium during the pilot scale study

7.2.2 Changes in elemental analysis

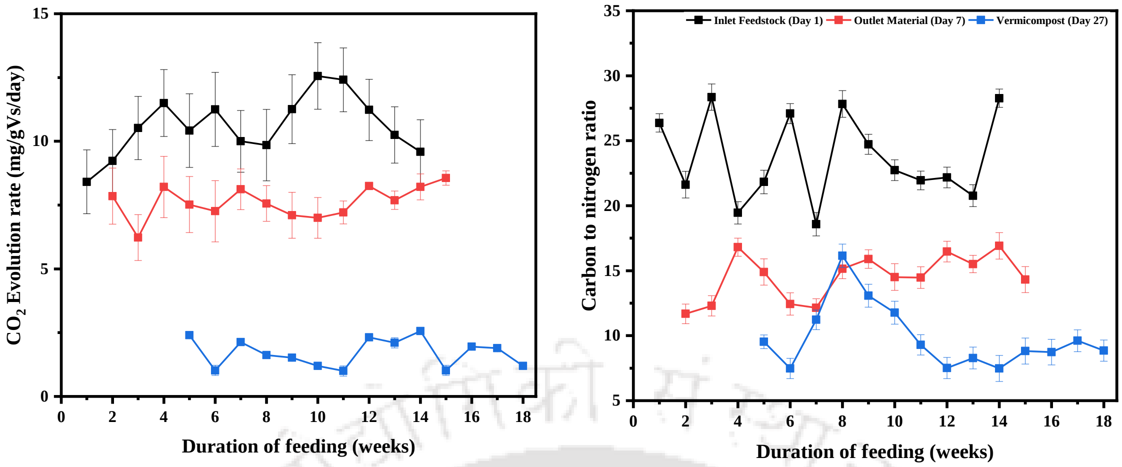
The TN content of the feedstock was undesirable to be directly used in the soil. The bioconversion process increased the TN content to 3.5-4.6% by the end of 27 days of biodegradation for the entire duration of the study. TP significantly increased from 1-2 g/kg to 3.5-4.5 g/kg, which can be attributed to mineralization and mass loss during biodegradation. Previous studies employing RDC observed similar trends of increased nutritional properties but in batch mode (Kauser et al., 2022). Further TK also followed an increasing trend and reached a maximum of 20-23 g/kg for the duration of the study (Fig. 7.9). The two-stage composting enhanced the nutritional strength of the vermicompost within 27 days much higher than the batch process, which can be attributed to the thermophilic biodegradation of pilot scale in the feedstock.

7.2.3 Changes in feedstock stability

The C/N ratio significantly reduced during the process, attributed to the increment in the nitrogen value and reduction in the total organic carbon. A higher degree of thermophilic degradation was observed in the pilot scale process compared to the batch scale, which can also be one of the reasons for the early stabilization of the feedstock. Fig. 7.10 depicts the changes in the C/N during the entire study period. Further, the CO_2 evolution experienced a reduction during the process for the entire study. The changes from a maximum of 12.56 mg/gVs/day to a minimum of 1.02 mg/gVs/day were observed, which signifies that the two-stage composting mediated using *E. fetida* worm culture was proven efficient both in batch and pilot scale studies.

7.2.4 Vermiculture growth dynamics

Vermicomposting endorses the production of the Bacteroidota population in the earthworm excreta, which further increases the degradation process (Hong, 2011). By excreting and burrowing, earthworms provide oxygen to the soil and provide nutrients that aerobic decomposition bacteria may use to break down organic matter and chelate metals (Ning et al., 2021). The current investigation inoculated three prospective degraders at a batch size and examined the differences in product quality after RDC. The initial analysis found that *E. fetida* was the most resilient of the used earthworm cultures. In the pilot scale also used this worm as a vermicomposting worm. The culture expanded faster in the pilot scale setup than in the batch setup (Srivastava et al., 2023). The kinetics of earthworm development throughout both phases are shown in Table 7.6.



a **b**

Figure 7.10. Changes in a) CO2 Evolution rate and b) C/N ratio during the pilot scale study

Table 7.6. Vermiculture growth dynamics

	E. fetida		
Day	**Adults**	**Juveniles**	**Cocoons (No's per 100 grams)**
7	200	0	0
27	250 ± 29.25	44 ± 5	30 ± 10

7.2.5 Phytotoxicity diminishing during the pilot-scale run

A phytotoxicity plant assay corroborated the noticeable effects on plant growth, and a bioassay was performed as an enabling factor of environmental hazards (Wang et al., 2022). The model seed *Vigna radiata* was used in this experiment to learn about the harmful effects of extracts made from feedstock material and vermicompost made with regular water as a control. The SGI of weed biomass was less than 60%, which indicates the seed-inhibitory nature of the weed, which transformed to more than 90% by the end of the biodegradation process (Table 7.7). According to (Wang et al., 2022) of the SGI, more than 80% of biomass-based products are safe to apply to the soil. In the study, the SGI was observed to be 97% in the raw vermicompost extract produced from the two-stage biodegradation on a pilot scale, which can be considered safe for soil application as a soil conditioner.

Table 7.7. Phytotoxicity diminishing during pilot scale study

Phytotoxicity	Concentration (v/v)	SGI (%)	Shoot length (cm)	Root length (cm)
Tap water (Control)	0	90	2.5 ± 0.2	2.6 ± 0.2
Inlet feedstock	25	65	1.5 ± 0.1	2.0 ± 0.1
(Day 1)	50	<60	0.5 ± 0.2	1.0± 0.2
	75	<60	0	0
	100	<60	0	0
Vermicompost	25	90	2.4 ± 0.2	2.5 ± 0.5
(Day 27)	50	92	3.2 ± 0.5	3.3 ± 0.5
	75	95	3.8 ± 0.5	3.7 ± 0.5
	100	97	4.9 ± 0.5	4.5 ± 0.5

7.2.6 Metagenomic study of the samples at various stages

The combined biodegradation technique in the current study efficiently bio-converted weed biomass into soil conditioning vermicompost. Although biodegradation was effective, significantly less was known about microbial succession during the process. This study provides a comprehensive understanding of the fluctuations and succession of the bacterium during the process.

According to the hypothesis, the sampling was done at the inlet and outlet zones, emphasising the effect of RDC. Further, the outlet zone and final vermicompost sample were used to understand the vermicomposting effect. The succession of the bacterial communities in the study can be majorly attributed to the changes in the organic carbon during the process. Accordingly, the succession can be divided into 3 groups, 1st group consists of microorganisms (Proteobacteria (45.16%), Firmicutes (39.30%) and Bacteroidetes (14.73%)) found at the inlet zone of the Rotary drum composter. After 7 days, the 2nd group appaired and majorly comprised of bacteria (Euryarchaeota (32.52%), Bacteroidetes (25.33%), Chloroflexi (10.465%), Proteobacteria (9.03%) and Firmicutes (7.93%)). The 3rd group of bacterial communities (Proteobacteria (36.60%), Bacteroidetes (15.18%), Firmicutes (11.02%), Planctomycetes (9.85%), Euryarchaeota (9.18%) and Acinitobacteria (8.2%)) appaired in end-product (vermicompost). Between 7 and 27 days, there were notable shifts in the overall bacterial population makeup (Fig.7.11).

Table 7.8. Metagenome study of the samples at various stages of pilot-scale biodegradation

Sample	**Inlet zone (%)**	**Outlet zone (%)**	**Vermicompost (%)**
	Day 1	**Day 7**	**Day 27**
Phylum	Proteobacteria (45.16)	Euryarchaeota (32.52)	Proteobacteria (36.60)
Class	Bacilli (36.13)	Methanomicrobia (26.30)	Gammaproteobacteria (19.08)
Order	Pseudomonadales (32.21)	Methanosarcinales (25.49)	Methanosarcinales (8.01)
Family	Maraxellaceae (30.47)	Methanosarcinaceae (24.65)	Methanosarcinaceae (8.01)
Genus	Acinetobacter (30.40)	Methanosarcina (24.65)	Methanosarcina (8.01)
Species	Unclassified species from the genus Acinetobacter (29.79)	Unclassified species from the genus Methanosarcina (23.08)	Unclassified species from the genus Methanosarcina (7.31)

The abundant taxonomy identified in the samples of days 0, 7 and 27 at the phyla level, class, order, family, genus and species is depicted in Table 7.8. On day 27, the increase in Proteobacteria is most likely due to earthworm gut-associated activities. Later, as microbial succession advances, the quantity and quality of available nutrition supplies will progressively alter, which will be associated with the rise of bacterial taxa specialised in metabolising the remaining more resistant substrates. Bacterial diversity increased considerably at taxonomic and phylogenetic levels, with the most recent succession group reaching a peak (day 27). Several observations revealed a significant rise in the total bacterial count of actinomycetes and bacteria in compost treated with worms (Antunes, Martins, Pereira, Thomas, Barbosa, Lemos, Silva, Moura, Epamino, Digiampietri, Lombardi, Ramos, Quaggio, de Oliveira, et al., 2016; Danon et al., 2008; Gopal et al., 2017; López-González et al., 2015; Lv et al., 2015; Peters et al., 2000).

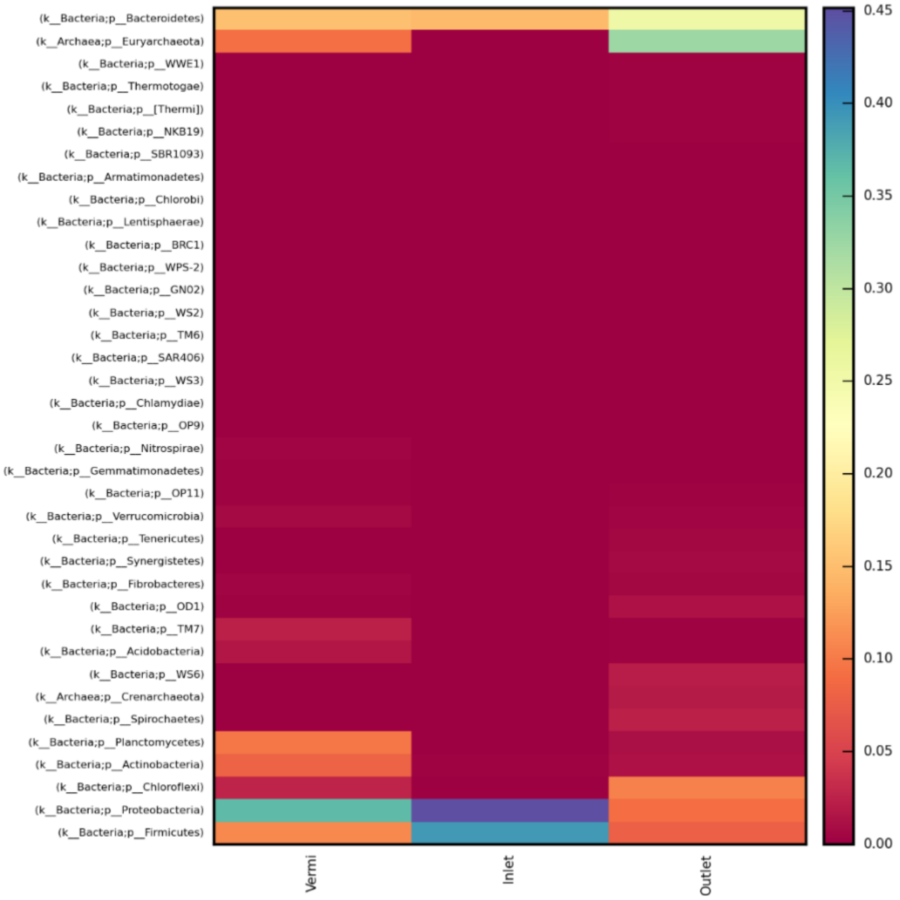


Fig. 7.11. Heat map

The rise in microbial strength may be attributed to the favourable conditions for microbe development inside the earthworm's digestive system, as well as the absorption of nutrient-rich organic wastes that give energy and function as a platform for the growth of microbes. During green waste vermicomposting, (Cai et al., 2018) observed increased bacterial abundance and diversity in vermicompost compared to compost samples. Similarly, our study presents a complete picture of bacterial succession by combining many times during the active phase of vermicomposting. Composting and vermicomposting of cattle dung and sewage sludge were compared by (Lv et al., 2015). They observed an increase in bacterial diversity during vermicomposting, which agrees with Huang et al. (2013), who compared 2 months old vermicompost to a control with no earthworms. Weed biomass composting follows a similar pattern of fast breakdown, which is consistent with previous findings of vegetable waste vermicomposting (Huang et al., 2013).

7.2.7 FTIR spectroscopy

Infrared spectroscopy describes organic compounds and functional groups during the biodegradation process. The current study's major peaks were 463, 531, 1020, 1622, 2918 and 3305 cm^{-1}. The wavenumber 3000 to 2852 cm^{-1} represents the CH_2 methylene and CH_2 methyl groups, and 3000 to 3700 cm^{-1} represents hydroxile groups of lignin cellulose and hemicellulose inter-molecular hydrogen bonds. Previous reports identified the C-O vibration of the alcohol group (C_3-O_3H) (Biyada et al., 2020). Peak, 1020-1030 cm^{-1}, are ascribed to polysaccharide C-O-C rings (Fig 7.12). All the function groups were in decreasing order of their intensities during the biodegradation process. The decrease in functional group intensities and the removal of specific peaks signify the stabilisation process and the bioconversion of biomass to mature vermicompost (Kauser and Khwairakpam, 2022).

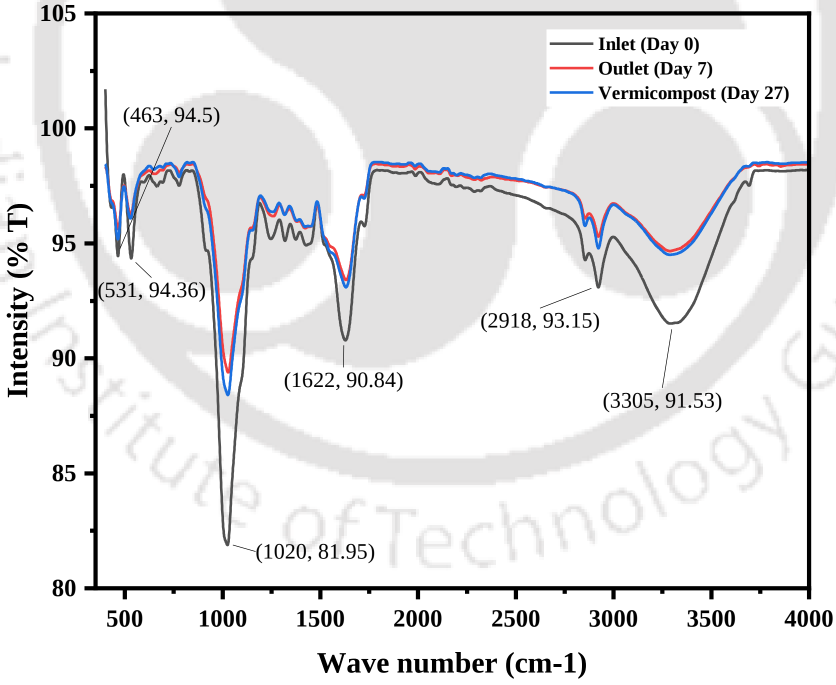


Fig. 7.12. FTIR spectra

7.2.8 Change in bulk throughout the biodegradation

The bulk density of organic material is heavily influenced by the shredded material's size and the feedstock's initial moisture level (Jain et al., 2020). The moisture significantly lowered during the composting process in the current research, altering the substance's bulk density. Furthermore, the particle size of the feedstock was decreased during biodegradation owing to the emission of CO_2 and NH_4. The previous 235 kg/m^3 was raised to 595 kg/m^3. According to Jain et al. (2019), the ultimate bulk density of the compost should be between 500 and 900 kg/m^3. Jain et al. discovered similar rising tendencies in their research (2019). The volume of the mass is affected by the bulk reduction. Rotary drum composting resulted in a 15% volume decrease in the present research. Furthermore, towards the conclusion of the 27-day biodegradation, an overall volume decreases of 71% was observed. Because of the smaller particles, the volume decrease during worm composting of the feedstock was more significant.

7.2.9 Practical implications of the study

The present study uses an optimised biodegradation technique on a batch scale to understand the process feasibility and further apply the same on a pilot scale. The study was primarily conducted in batch mode in a 550-litre reactor, and further, the scale has been shifted to 5000 litres for rotary drum composting. Vermicomposting was conducted in 3 kg and 20 kg capacity reactor trays at batch and pilot scales, respectively. The batch-scale study results demonstrate the combined technique's feasibility in managing the noxious weed *E. crassipes*. The pilot-scale study was conducted for 18 weeks, with 14 weeks daily feeding weed biomass mixed with horticultural material to a maximum of 250 kg per day. A total of 25 tonnes of waste have been managed using the reactors in the laboratory. Further, the biodegradable material was sieved using a 4 mm sieve to get the finely powdered vermicompost. The total amount of vermicompost generated was 7 tons. The study's primary goal is to propose reactor-based technology, and its feasibility to run a daily-fed pilot scale has thus been successfully demonstrated.

The process difference in both the rotary drum composter and vermicompost is combinely utilising the active degradation phases of each reactor in series to overcome the limitations of traditional composting in terms of product end quality and process duration. Rotary drum composters' thermophilic phase extensively impacts the composter's degradability in the entire composting duration, which only lasts for 7 days. Further, the reactor will only be used to aerate and cool the feedstock. That is the primary reason for choosing the 7-day degradation in the rotary drum composter. Furthermore, the partially degraded material, which is best suited in terms of the moisture content for vermicomposting, was fed with different vermicultures in the batch study and the efficient and robust degrader *E. fetida* was adopted in the pilot scale. The results showed that the combined two-stage degradation process could potentially manage the troublesome aquatic weed water hyacinth. This study provides the waste management sector with the shortest and most effective way of managing weed biomass.

7.2.10 Summary

This study supports the effectiveness of large-scale in-vessel rotary drum composting in conjunction with vermicomposting as a successful organic waste management strategy. The process efficiently increased the SGI to 98%. Furthermore, the technique consistently produced a nutrient-rich soil conditioner from weed biomass. The end-product was stable and mature, and increased microbial activity was discovered (bacteria; 83.17% and archaea; 16.7% in vermicompost) due to the two-stage composting of biomass, leading to more excellent waste stabilization within a 27-day time frame. The large-scale reactors validated in the study can be a potential tool (71% volume reduction) in urban waste management.

7.3 CO-COMPOSTING OF VEGETABLE WASTE AND WATER HYACINTH A COMPARATIVE STUDY

Co-composting vegetable waste and water hyacinth might reduce waste. A nutrient-rich compost may be made by carefully mixing two ingredients. Co-composting may effectively control water hyacinth, an invasive aquatic plant that threatens aquatic environments. Vegetable waste and water hyacinth may be co-composted via in-vessel biodegradation. "In-vessel biodegradation" is the controlled breakdown of organic materials in closed vessels. The procedure requires proper temperature, moisture, and aeration. In-vessel biodegradation reduces odor, greenhouse gas emissions, and produces high-quality compost. In-vessel biodegradation of vegetable waste and water hyacinth has several advantages. The procedure produces nutrient-rich compost that might replace synthetic fertilizers. This compost might reduce chemical fertilizer use, promoting sustainable agriculture. Co-composting helps reduce water hyacinth growth, which can help restore aquatic habitats. In-vessel biodegradation technologies for co-composting vegetable waste and water hyacinth may provide several ecological and agricultural benefits.

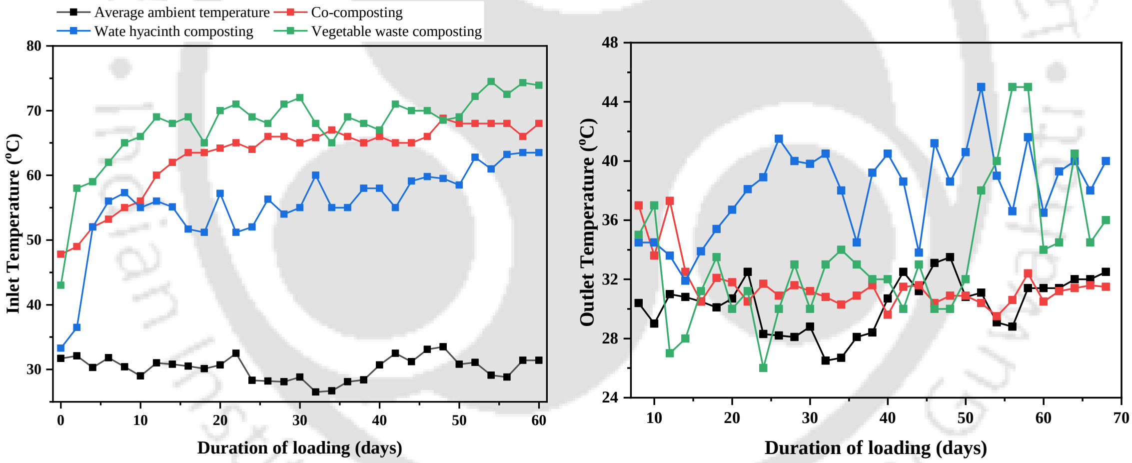
7.3.1 Changes in physicochemical parameters

To assess the operational efficiency of the adopted biodegradation procedure, physicochemical parameters of the decomposition process, such as temperature, pH, EC, TOC and VS, were measured.

The rise in the temperature profile of the feedstock is a clear sign of microbial activity on the feed mass; additionally, the higher the temperature range produced biologically, the greater the biodegradation of the substrate (Sun et al., 2022). The degradation process with temperature regulation is distinguished by its controlled temperature and brief composting period, high bioconversion effectiveness, and high final product quality (Rochaeni et al., 2021). In the current research, it has been observed that the thermophilic temperature in RDC is biologically developed without any external heat source, aeration of the mass using a blower and the timely rotations of the drum were the only means of supplying oxygen to the feedstock and microbes, as well as removing evaporated moisture from the reactor. Thermophiles are a collection of heat-resistant microorganisms that can survive at high temperatures, typically above 45ºC (Xin et al., 2023). The physiologically developed temperatures attained a maximum of 74.3ºC in vegetable waste composting (VWC), 63.5ºC in water hyacinth composting (WHC), and in co-composting (CC), the value was recorded as 68.8ºC for a prolonged period during the study period (Fig. 7.13). The thermophilic range in feedstock was observed from the very beginning of the study (in CC). In contrast, in WHC, the thermophilic range was observed from the 3rd week of the loading period. However, as the waste retention time was 7 days in RDC, partially degraded waste was removed from the outlet daily after the 2nd week, and the temperature profile of the feedstock was recorded as 30 - 41ºC in CC, VWC and WHC. Further, it was also observed that CC was beneficial for degrading the weed biomass effectively through thermophilic biodegradation when compared to WHC, whereas thermophilic biodegradation can efficiently create safe mature compost (Wang et al., 2022).

pH and EC variations are helpful factors for tracking the decomposition process. Furthermore, pH and EC are essential to compost factors because they can influence the end product's quality and fit for plant development (Kauser et al., 2020). The pH values of the experimented trials in the current study experienced an increasing trend. The pH of 5-

6 in the initial feedstock has changed to a pH of 8-8.65 by the end of biodegradation (Fig. 7.14). Studies have shown that a high concentration of soluble salts in the compost product reduces seed germination (Yin et al., 2021). In the current study, the pH of the material was changed from alkaline to acidic during the first 7 days of biodegradation ($P<0.05$). Furthermore, the shift in pH of VWC has been seen to transform into alkaline sooner than WHC. The vermicompost from the three testing experiments was found to be greater than 8.1 in almost all of the samples. In the first seven days, the EC was reduced by 39% in CC, 13% in VWC, and 27.41% in WHC. In total, CC has decreased by 66.39%. 52.05% for WHC and 38.27% for VWC. Furthermore, the EC value of CC and WHC had decreased by 44%, especially in 20-day vermicomposting. A pH of 5.5 to 8.5 is deemed acceptable in compost, and an EC value between 1.5 dS/m and 4.0 dS/m, respectively, are considered for growth substrates and manageable by medium-sensitivity plants (Nakhshiniev et al., 2014).



a **b**

Fig. 7.13. Temperature profile a) at inlet zone b) at outlet zone of the rotary drum composter

Part of the organic matter in the biomass is changed to H_2O, CO_2, and energy during the biodegradation process, while the rest is ultimately converted to stable organic matter (Nakhshiniev et al., 2014). VS measures organic matter in biomass (Jain et al., 2018). In the present study, it was found that the VS content was decreased in all 3 experiments. Particularly in CC, there was a decrease of 65.65% in the initial 7 days, while in 20 days of vermicomposting, it was only 20.45%, bringing the total reduction to 72.67%. Whereas in

WHC and VWC, there was only a 9.8 and 8.7% decrease in VS during vermicomposting, respectively, and altogether 22% and 28.32%, respectively, in 27 days ($P<0.05$). The TOC of the material was decreased by an average of 87.05 to 23.79% during CC, with further overall reductions of 22 and 28% in WHC and VWC, respectively. The drop in total carbon can be explained by both the process of mineralization and the way CO_2 evolves during the biodegradation process (Fig. 7.14).

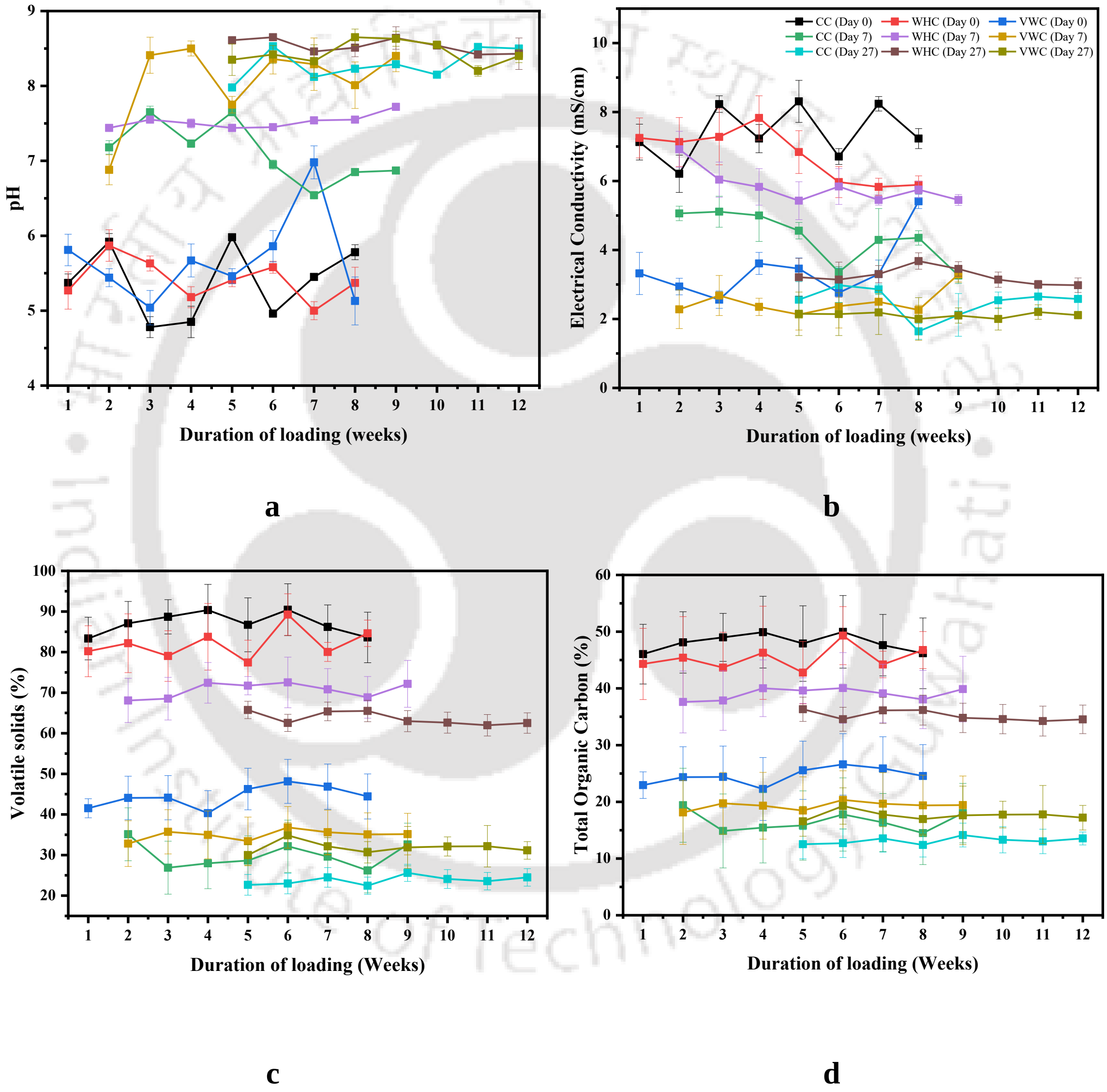


Fig. 7.14. Temperature profile a) at inlet zone b) at outlet zone of the rotary drum composter

7.3.2 Changes in elemental parameters

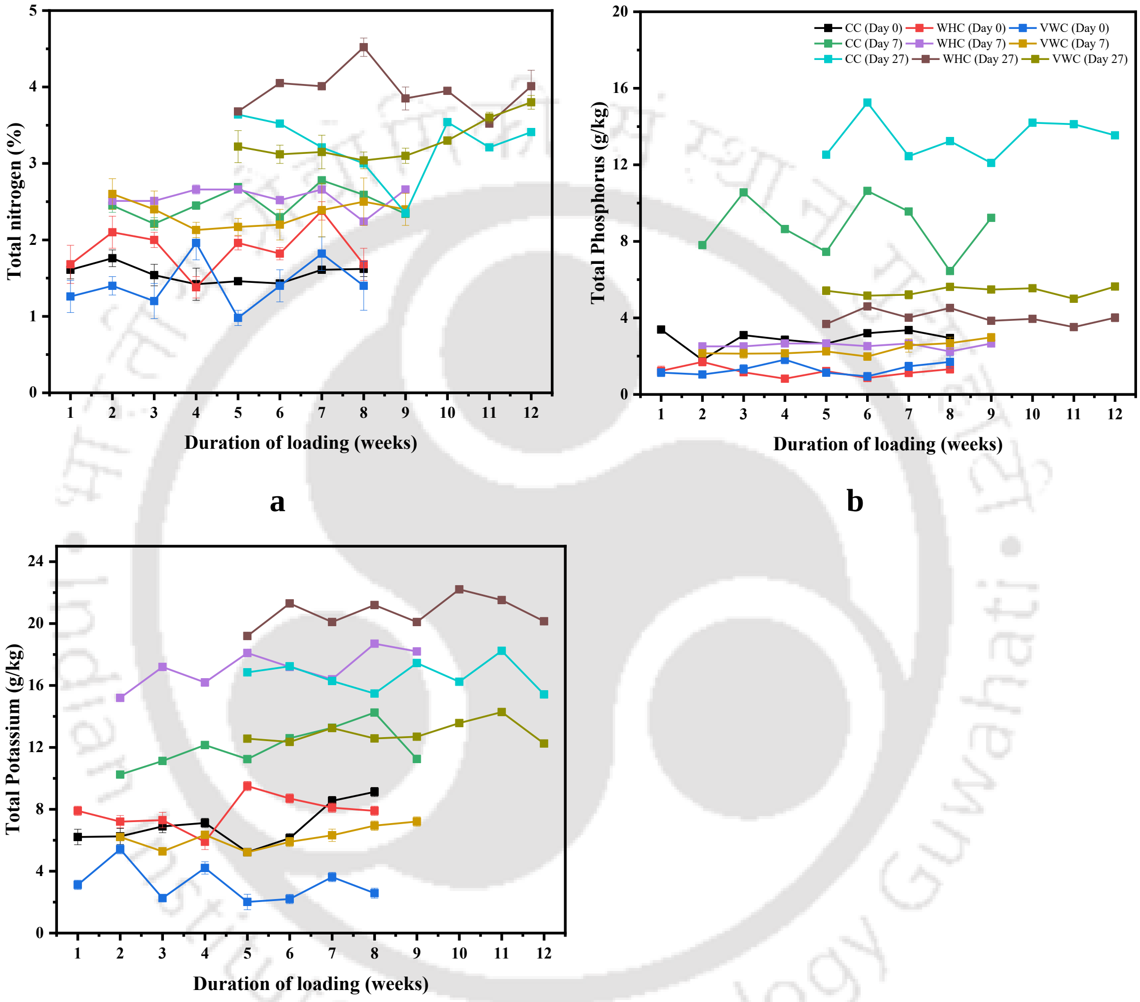
The TN content of the material bulk grew throughout the decomposition process, with WHC rising considerably from 1.8% to 3.95% on average for the entire research. Furthermore, compared to WHC, there has been a 59% rise in total nitrogen content in CC. The VWC process increased total nitrogen concentration by 64.54% in the first 7 days of the RDC (Fig. 7.15), and vermicomposting increased total nitrogen concentration by 40.13% on average for the study period ($P<0.01$). According to the research, overall nitrogen concentration rises considerably during decomposition and vermicomposting. This effect is mainly caused by organic matter breakdown, which creates readily available nitrogen while also reducing material wetness during composting and vermicomposting, where worms' stomach flora influences the process and results in nitrogen-rich vermicomposting (Tong et al., 2019).

The TP concentration in the material has increased in all the experiments. The TP concentration in the CC has increased fourfold higher than the initial value; in WHC, it is 3.41; in VWC, it is 4.07 folds higher. There has been a significant increase in that TP concentration, especially in CC, where 2.91% on average has increased to 13.43% by the end of the 27th day, whereas in WHC, it is 1.18–4.02 g/kg, and in VWC it is 1.32–5.39 g/kg (Fig. 7.15). TP is a crucial component of plant development in agroecosystems (Alewell et al., 2020). The study results of (Kauser et al., 2022) are in support of the current study ($P<0.05$).

TK concentration has increased from 6.94 to 16.65 g/kg in CC, which is 2.4 times higher than the initial value. In WHC, the increase was 2.65 times, whereas, in VWC, it was 4.07 times the initial value on average during the loading period. It was also observed that in CC, there had been a 73.20 % increase in TP concentration only in the initial seven days of RDC; in WHC, it is 119 %, and in VWC, it is 94.41% ($P<0.01$). During vermicomposting, it was also found that VWC had a higher rise in total potassium concentration, which can be attributed to the warm growth in VWC vermicomposting reactors. The increase in nutritional properties can also be attributed to the mineralization process due to the microbial activity and thermophilic temperature ranges that prevailed during the biodegradation process (Fig. 7.15).

7.3.3 Changes in stability parameters

In in-vessel composting, the rapid heating of the feedstock enhances thermophilic bacteria that produce humus compounds and bones through oxidation, thereby speeding up the humification process (Xin et al., 2023).



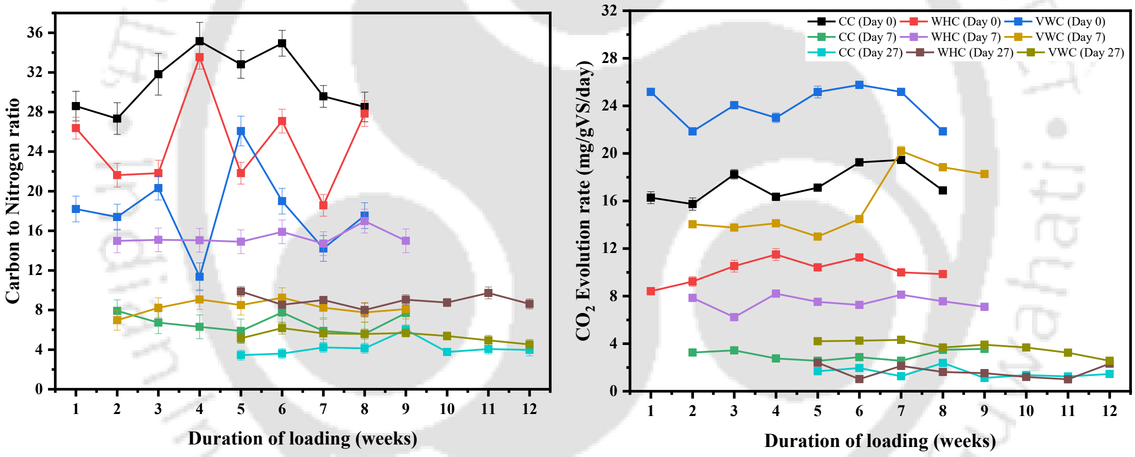
a

b

c

Fig. 7.15 Change in elemental parameters a) Total nitrogen, b) Total phosphorus, c) Total potassium

In the present study, during the co-composting, the C/N has decreased; in the initial 7 days, there was a 78.39% reduction; further, in the next 20 days, it was recorded as 38.24%; overall, it stands at 86.65% in a 27-day duration. Whereas in WHC, it has been recorded as 38.30% in the initial 7 days and 41.63% in the next 20 days of the vermicomposting, becoming a further 54.14% in the VWC initial seven days and 34.85% in vermicomposting overall at 70.12% on average ($P<0.05$). In the current study, CC showed a higher reduction in the C/N ratio (Fig. 7.16), which can be attributed to the reduction in volatile solids, which reduced the feedstock's carbon content. Further, there was an increase in nitrogen, contributing to the reduction in the C/N ratio of the feedstock. From the results, it can be suggested that co-composting is more beneficial than mono-substrate composting. In the current study, during CC in the initial 7 days, there has been an 82.43% reduction in the CO_2 evolution when compared to WHC, where it is 26.27 under 28.86 during VWC, further during vermicomposting, a 49.18% reduction in CO_2 evolution has been recorded, and in WHC it is 77.93%, and in VWC it is 78% overall during CC a 91.07% reduction has been recorded ($P<0.05$), which is higher than VWC and WHC individually for the entire study duration on average for the entire study duration It signifies that the carbon dioxide evolution was higher in the initial RDC for CC.



a **b**

Fig. 7.16 Changes in a) Carbon to nitrogen ratio and b) CO_2 evolution rate

7.3.4 Vermiculture growth dynamics

Vermicomposting promotes the growth of the Bacteroidetes community in earthworm excreta, which speeds up the decomposition process (Deepthi et al., 2021). Earthworms provide air to the soil and minerals that aerobic breakdown microbes can use to break down organic matter and bind metals by excreting and tunnelling (Ning et al., 2021). The present study used a potential degrader in a pilot scale and looked at the variations in product quality after RDC. The study revealed that *E. fetida* was the most robust of the earthworm and accounted for a significant reduction of VS and an increase in TN. This worm was used as a robust degrader in the experimental stage. The culture spread quickly in the bulk setup (Srivastava et al., 2023). Table 7.9 depicts the dynamics of earthworm growth during both stages ($P<0.05$).

7.3.5 Changes in bulk during the process

The material underwent a significant reduction of volume and weight due to the thermophilic degradation followed by the activity of earthworms. The two-stage process in all the experimented trials resulted in quality vermicompost. The end products exhibited a bulk density of 595 - 650 kg/m^3 by the end of 27 days, according to (Jain, 2019) The compost's final mass density should range between 500 to 900 kg/m^3. The current study results in a favourable end material for soil use. Further, in the opinion of waste volume reduction, due to the thermophilic degradation for 7 days, the waste volume in the experimented trials was reduced by 12-15% in WHC and VWC. Further, it has been noticed that the reduction was higher in CC, counting to 19%. The vermicomposting post-RDC has substantially reduced the volume in all the experimented trials. An overall reduction of 69 -71% reduction in volume was observed ($P<0.5$). The results of the current study demonstrate the possibility of bio-product generation out of substantial waste generated from various sectors of urban spaces.

Table 7.9. Changes in the vermiculture growth during the biodegradation.

Duration of composting	**CC**		
	Adults	**Juveniles**	**Cocoons (No's per 100 grams)**
7	200	0	0
27	310 ± 15	35 ± 15	26 ± 15

7.3.6 Diminishing of phytotoxic nature in feedstock

The SGI is an essential parameter in determining the phytotoxicity and safety of biodegradable waste-derived bioproducts (Xin et al., 2023). The SGI values of the 3 treatments progressively increased and surpassed the standard value of 70% on the 27th day of CC, VWC, and WHC, deemed non-phytotoxic and sufficiently mature (Nakhshiniev et al., 2014). Also, the finished compost's SGI surpassed 90%, indicating an outstanding organic fertilizer grade (Xin et al., 2023).

Table 7.10. Changes in the phytotoxic nature of the feedstock

Extract concentration (v/v)	**Seed germination index (%)**		**Plant length (cm)**	
	Fresh material (Day 0)	**Vermicompost (Day 27)**	**Fresh material (Day 0)**	**Vermicompost (Day 27)**
	Co-composting (CC)			
25	68 ± 0.50	92 ± 1.0	4.10 ± 1.30	5.20 ± 1.10
50	<60	95 ± 0.9	2.10 ± 1.10	7.10 ± 1.20
75	<60	98 ± 1.2	0	8.50 ± 1.50
100	<60	98 ± 0.8	0	6.40 ± 1.20
	Water hyacinth composting (WHC)			
25	65 ± 0.10	90 ± 0.50	3.20 ± 1.20	4.90 ± 1.10
50	<60	92 ± 0.90	1.50 ± 1.50	6.50 ± 1.60
75	<60	95 ± 0.80	0	7.40 ± 1.50
100	<60	97 ± 0.70	0	9.40 ± 1.90
	Vegetable waste composting (VWC)			
25	72 ± 0.90	85 ± 0.60	3.50 ± 1.10	5.1 ± 1.20
50	<60	98 ± 0.90	2.10 ± 1.20	8.5 ± 0.90
75	<60	93 ± 1.00	0	6.10 ± 1.80
100	<60	91 ± 0.90	0	5.80 ± 1.20
Control	92 ± 1.5		5.1 ± 2.1	

All the parameters are expressed in mean ± standard deviation of triplicates

In the current study, *Vigna radiata* was used as a model seed to assess the phytotoxicity of compost and vermicompost derived from various stages of biodegradation of 3 experimented trials. The study revealed that *V. radiata* seeds exhibited high responsiveness levels, even in tap water as a control. The findings also indicated that the two-stage composting process effectively reduced the toxicity of the sludge, as evidenced by the significant increase in the root and shoot lengths of *V. radiata*. Upon evaluating the final composts for phytotoxicity, it has been found that the seed germination index (SGI %) was notably higher in vermicompost produced through CC, WHC, and VWC compared to compost derived from control. The results also revealed that composting and vermicomposting could mitigate phytotoxicity by transforming and aggregating harmful chemicals and reducing toxin bioavailability. Notably, an SGI (%) of 98% was observed in the end products of CC in 75% and 100% extracts. It indicates that the vermicompost produced through CC outperformed the products of WHC and VWC (Table 7.10) ($P<0.01$).

7.3.7 Changes in the functional groups

During biodegradation, organic compounds and functional groups are described using Fourier transform infrared spectroscopy. Fig.7.17 depicts the FTIR spectra of samples taken at the inlet, outlet regions of RDC and vermicompost of the 3 experimented trials. In the current study, during the WHC process, major peaks were 3300- 3350, 2910-2920, 1620-1625, 1020-1025, 530-540, and 460-470 cm^{-1}. During the VWC process, significant peaks were observed in the samples 3660-3675 cm^{-1}, 2900-2910 cm^{-1}, 1620-1640 cm^{-1}, 1400-1410 cm^{-1}, 1240-1250 cm^{-1} and 430-470 cm^{-1}. Further, during CC, the most dominating peaks were 1025-1030 cm^{-1}, 1630-1635 cm^{-1}, 1730-1740 cm^{-1}, 2920-2930 cm^{-1}, and 3280-3290 cm^{-1}.

The wavenumber range of 3000 to 2852 cm^{-1} is commonly associated with CH_2 methyl and methylene groups, while the range of 3000 to 3700 cm^{-1} is associated with hydroxyl groups of cellulose, hemicellulose, and lignin intermolecular hydrogen bonds. The C-O bonds of the alcohol group (C_3-O_3H) can be observed at 1020-1030 cm^{-1}, while the polysaccharide C-O-C rings are ascribed to the peak at 1730-1740 cm^{-1}. The intensity of these functional groups decreases during the twos step composting process (Biyada et al., 2020). Other peaks observed in the spectral data include C-O stretching, O-H bending of carboxyl groups, and C-N and C double bond N oscillation in amide, which are associated with the peaks at 1590 and 1220 cm^{-1} (Zhu et al., 2021). Alcohols and phenols can be observed at 3590-3650 cm^{-1}, while the alkane compounds' C-H bonding is often between 2850-2970 cm^{-1}. C=C bonds of alkenes can be observed at 1610-1680 cm^{-1}, while C-O-H distortion and asymmetric carboxyl group (C-O) stretch are observed at 1407 cm^{-1} (Mandpe et al., 2021). The C-N strong bonding of amides and amines is observed at wavenumbers between 1180-1360 cm^{-1}. The firm (C-O) bonds of ethers and alcohols can be observed between 1050-1300 cm^{-1}, while the strong C-H bonds or aromatic rings can be observed between 690-900 cm^{-1} (Wang et al., 2017). The reduction in intensities of functional groups and the elimination of some peaks may be ascribed to the maturation process and the development of a mature end product. (Kauser and Khwairakpam, 2022). These observations can provide insights into the chemical changes occurring in the material under study and help identify the functional groups present.

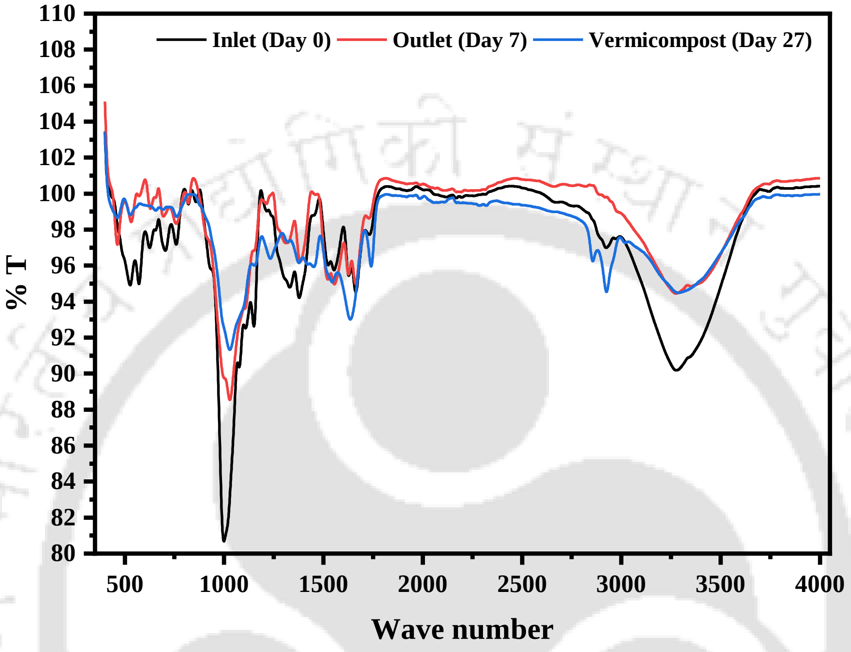


Fig. 7.17 FTIR spectra of the compost samples

7.3.8 Changes in the metal concentrations during co-composting

Researchers are convinced that heavy metals connected with various fractions have varying environmental effects and that their phytotoxicity is linked to particular molecular forms rather than the overall quantity (Xu et al., 2022). According to (Maturi et al., 2021), metal concentrations that are water soluble were the troublemakers in the end product; further, the RDC process efficiently converted the water-soluble fractions into water-insoluble fractions in heavy metals, which did not influence the plant growth process.

Further, studies were conducted on water hyacinth metal speciation during biodegradation by (Singh and Kalamdhad, 2013) and also concluded similar results. In the current study, the total metal concentration of Iron (Fe), Manganese (Mn), Copper (Cu), Cobalt (Co), Cadmium (Cd) and Nickel (Ni) were evaluated during co-composting (Table 7.11). The results of the 8 weeks of inlet material, outlet material and final vermicompost were considered to comment on the metal dynamics during the process. It was observed that metals like Mn and Cd were recorded to be reduced by the end of 27 days. Due to the breakdown and oxidation of organic matter, the release of CO_2, and water loss, the weight of substances decreases during composting, which could raise the heavy metal concentrations (Xu et al., 2022). Also, according to (Sun et al., 2020), vermicomposting is effective in heavy metal removal for metals like Cd, Pb, and Zn. In the current study, similar trends on Cd were observed.

7.3.9 Bivariant correlation of metal concentrations during co-composting

Correlation matrices were generated using the average metal concentrations of the study. It was observed that a strong correlation was observed between the duration of the composting with Fe (r=0.98) and Ni (r=0.99) (P<0.005).

Table 7.11. Bivariant correlation of metal concentration during co-composting (CC)

	Duration	**Iron**	**Manganese**	**Copper**	**Cobalt**	**Cadmium**	**Nickel**
Duration	1.00						
Iron	0.98	1.00					
Manganese	0.18	0.38	1.00				
Copper	1.00	0.95	0.08	1.00			
Cobalt	0.64	0.79	0.87	0.56	1.00		
Cadmium	-0.83	-0.69	0.40	-0.88	-0.10	1.00	
Nickel	0.99	1.00	0.32	0.97	0.74	-0.74	1.00

Metals other than Fe and Ni exhibited an insignificant correlation with the composting duration. Further, Cr exhibited a negative correlation with all other metals, including the duration of composting, higher with Cr (r=-0.88). Fe exhibited a strong positive correlation with metals like Cu (r=0.95) and Ni (r=1). Furthermore, a strong correlation was observed between Ni and Cu (r=0.97) (Table 7.12).

Table 7.12. Heavy metal concentration during the loading period

		Duration of feeding (weeks)								
Metal Concentration (g/kg)	**Duration of the process (days)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Average**
Iron	**Day 0**	150.56	141.89	256.63	224.47	120.64	130.74	154.67	207.67	**173.41 ± 46.34**
	Day 7	164.24	246.65	257.36	263.17	170.65	236.15	185.24	201.52	**215.62 ±37.37**
	Day 27	244.62	256.24	312.58	321.45	259.24	235.48	264.52	245.26	**267.42 ±29.98**
Manganese	**Day 0**	55.61	52.16	48.35	35.61	39.26	61.23	51.24	62.31	**50.72 ± 8.92**
	Day 7	58.26	64.23	44.23	57.62	57.24	54.26	55.59	62.15	**56.70 ± 5.62**
	Day 27	58.12	56.47	57.48	48.95	46.27	49.75	51.24	58.47	**53.34 ± 4.51**
Copper	**Day 0**	46.82	45.63	41.36	38.25	43.27	42.56	38.74	25.61	**40.28 ± 6.21**
	Day 7	49.24	32.17	37.19	40.28	47.28	47.25	46.28	36.45	**42.02 ± 5.91**
	Day 27	49.35	48.25	47.25	49.56	48.75	52.45	57.18	54.26	**50.88 ± 3.21**
Cobalt	**Day 0**	16.58	15.26	11.37	8.62	10.86	17.29	14.89	13.85	**13.59 ± 2.83**
	Day 7	24.56	36.21	29.56	22.47	24.56	26.85	24.59	27.36	**27.02 ± 4.03**
	Day 27	24.85	26.45	25.18	31.45	27.15	23.12	25.18	23.25	**25.83 ± 2.49**
Cadmium	**Day 0**	54.98	65.24	34.75	49.26	59.23	43.24	57.98	61.24	**53.24 ± 9.53**
	Day 7	58.65	54.67	58.47	49.85	52.14	54.87	52.48	58.26	**54.92 ± 3.10**
	Day 27	48.65	56.23	48.26	51.36	52.56	52.45	47.68	42.86	**50.01 ± 3.78**
Nickel	**Day 0**	27.69	35.12	18.24	26.19	15.26	33.45	24.65	26.47	**25.88 ± 6.32**
	Day 7	29.65	30.45	29.26	28.45	21.65	21.58	27.48	31.24	**27.47 ± 3.55**
	Day 27	31.58	29.45	28.75	31.28	32.59	35.18	28.59	22.56	**30.00 ± 3.48**

7.3.10 Summary

The present investigation underscores the efficacy of large-scale rotary drum composting and stack vermicomposting techniques in generating vermicompost from vegetable waste and water hyacinth, utilizing mono and co-composting approaches. The results indicate that CC could offer a superior alternative to conventional aerobic composting and landfill disposal methods for organic waste, as evidenced by the lower carbon-to-nitrogen ratio, electrical conductivity, and the resulting pH and nutrient content products compared to WHC and VWC. Upon conclusion of the study, it was observed that during CC, there was an 80% reduction in the carbon-to-nitrogen ratio compared to the WHC and VWC. Also, the EC values of compost are less than 4ms/cm, which suggests that the compost can be utilized in agricultural fields without any adverse consequences. The findings indicate that using two-stage biodegradation holds considerable promise as a viable strategy for managing organic waste within urban areas through CC.

7.4 MAJOR OUTCOMES OF THE STUDY

- The temperature profile during the pilot-scale study was significant in comparison to the batch study a maximum of 74°C and 65°C was recorded in the biodegradation of vegetable waste and *water hyacinth* biodegradation.
- The process efficiently increased the SGI to 98%.
- The study supports the large-scale operation of two stage biodegradation technique.
- Cd bioaccumulation was observed in the vermicomposting of the partially degraded waste.
- Co-composting was proven efficient in producing high quality output in comparison to mono substrate composting of *water hyacinth*.

7.5 SPACE REQUIREMENT FOR A TWO STAGE BIODEGRADATION VS INDIVIDUAL ROTARY DRUM COMPOSTER OR VERMICOMPOSTER

The space required for the individual system will be higher compared to two-stage biodegradation system. The following are the limitations of the individual system

Limitations of rotary drum composting system

- The retention time in the rotary drum composter will be for minimum of 20 days, thus increased working volume of the rotary drum composter will be needed.
- The same 250 kg per day will be needing two times more reactor space to manage the waste with compromised end product quality.

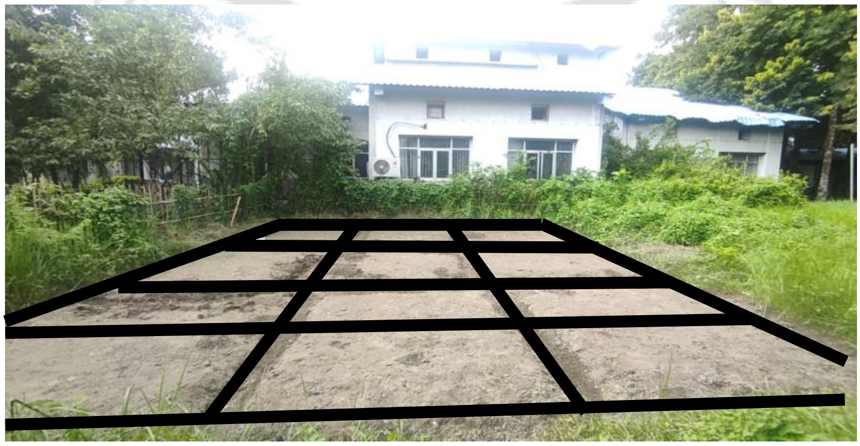
Limitations of the vermicomposting system

- The stack vermicomposting units required will be for minimum of 45 days and the number of units will be 3 times more than the two-stage biodegradation system.
- Per reactor vermicompost generation will be half compared to two-stage system.
- Furthermore, the vermicomposting with fresh waste may trigger the mesophilic ranges to turn thermophilic and there can be mortality of worm culture.

Chapter 8

SOIL APPLICATION OF VERMICOMPOST FROM VARIOUS BIODEGRADABLE WASTE SOURCES TO SOIL ON FIELD SCALE

It is crucial to acknowledge the importance of soil organic matter in a variety of aspects, such as nutrient retention, soil structure maintenance, and plant water availability. Significantly influenced by agricultural management practices, soil organic matter is frequently depleted when intensive cultivation is implemented. A renewed interest in using organic soil amendments such as plant residues, manures, and composts has emerged in response to the costs and environmental dangers associated with the use of chemical fertilizers. Composts offer several advantages over plant residues, including a smaller volume, delayed decomposition rates, and the potential for waste recycling (Bernal et al., 2009). Composts have a variety of properties that can be modified to accomplish the desired effects on soil properties and plant growth, depending on the specific feedstocks and processing methods used. Nevertheless, it is essential to establish a clear understanding of the relationship between compost properties and their influence on soil and plant health. When waste driven bioproducts is used as agricultural fertilizer, the antibiotics contained in it may stay in soil and be immediately utilized by plants, infiltrating the food chain thus, eventually, endangering people's health. To eliminate detrimental environmental effects, organic waste and other kinds of animal dung must be appropriately disposed of (Wang et al., 2022).



a

b

Fig. 8.1 a) Selected field and b) plants growth by the end of cultivation period

This chapter will be about agricultural or horticultural studies involving the sowing, development, and yield analysis of various plant species in various test locations. In addition to monitoring pH levels in the growing environment, the focus of the study appears to be on germination rates, plant survival, biomass production, and crop production (Fig. 8.1).

8.1. EFFECT OF VERMICOMPOST AMENDMENT ON THE PLANT GROWTH

8.1.1 *Okra* cultivation

Seed germination

The germination rates vary across the different tested areas. C3 had the highest germination rate at 95%, indicating favourable conditions for seed germination and growth. On the other hand, C2 had the lowest germination rate at 56%. This discrepancy suggests that there may have been variations in environmental factors such as soil quality, temperature, light, or water availability among the different areas, which affected the germination process. The number of plants that survived varied across the tested areas. C3 had the highest number of surviving plants with 190 out of 200 seeds, indicating a relatively high survival rate. C1 and C4 had lower survival rates, with 136 and 120 surviving plants, respectively. C2 had the lowest number of surviving plants at 112 (Table 8.1). This discrepancy in survival rates suggests that factors such as soil conditions, nutrient availability, pests, diseases, or other environmental factors might have influenced the overall plant health and survival. When comparing the germination and survival rates across the tested areas, it is evident that C3 performed the best, having both a high germination rate (95%) and a high survival rate. C2, on the other hand, had the lowest values for both germination (56%) and survival. C1 and C4 had intermediate values (Fig. 8.2). These differences could be due to variations in environmental conditions, management practices, or other factors specific to each area.

Fig. 8.2 Germination of *okra* seeds

Table 8.1 Germination of the *okra* seeds

Tested area	Total number of planted seeds	Survived plants	Germination (%)
C1	200	136	68
C2	200	112	56
C3	200	190	95
C4	200	120	60

Plant morphology

The weight of fruits harvested varied across the tested areas. C1 had the lowest fruit weight at 0.08 kg, while C2 had the highest at 0.01 kg. This indicates that C2 had a more productive outcome in terms of fruit yield compared to C1. However, it is worth noting that the fruit weight for all areas is relatively low. The average weight of fruits per plant also varied among the tested areas. C3 had the highest average weight per plant at 0.017 kg, followed by C2 at 0.016 kg. C1 had the lowest average weight per plant at 0.005 kg. These values suggest that C3 and C2 had comparatively larger and heavier fruits per plant than C1 (Table 8.2).

The weights of leaves, stems, and roots indicate the biomass distribution in each tested area (Fig. 8.3). C3 had the highest biomass across all categories, followed by C2, C4, and C1. This suggests that C3 had the most extensive overall plant growth, while C1 had the least biomass. When considering both fruit production and average weight per plant, C3 emerges as the most productive area, having both a higher fruit weight and average weight per plant compared to the other areas. C2 had a relatively high fruit weight and average weight per plant but a lower overall biomass compared to C3. C1 had the lowest fruit weight, average weight per plant, and biomass among the tested areas.

Fig. 8.3 Plants removed from field for testing

Table 8.2 Plant morphology of *Okra* on Day 40

Tested area	**Tested Plants for biomass**	**Weight of fruits (kg)**	**Average weight per plant (kg)**
C1	68	0.08	0.01
C2	56	0.01	0.02
C3	95	0.03	0.02
C4	60	0.00	0.01
	Weight of leaves	**Weight of stems (kg)**	**Weight of roots (kg)**
C1	0.17	0.18	0.02
C2	0.34	0.51	0.03
C3	0.66	0.87	0.07
C4	0.24	0.34	0.04

Plant yield

C4 had the highest total fruit yield in both the first and second harvests, with 1.272 kg and 0.402 kg respectively (Fig.8.4). This indicates that C4 had the highest overall fruit production among the tested areas. C3 had the second highest total fruit yield, followed by C2 and C1. C4 also had the highest average number of fruits per plant, with 3.03 fruits. This suggests that C4 had the most fruitful plants on average. C2 and C3 had slightly lower average fruit counts, while C1 had the lowest average fruits per plant. C4 had the highest fruit biomass per plant at 0.027 kg, indicating that the plants in this area produced the most fruit mass (Table 8.3). C3 had the second highest fruit biomass per plant, followed by C2, and C1 had the lowest value. Overall, C4 stands out as the most productive area in terms of fruit yield, average fruits per plant, and fruit biomass per plant. C3 also performed well, showing a relatively high fruit yield and biomass. C2 had moderate results, while C1 had the lowest fruit production and biomass.

Table 8.3 Yield collected during the study (on Day 82)

Tested area	**Weight of First yield (kg) (No. of fruits)**	**Weight of Second yield (kg) (No. of fruits)**	**Average fruits per plant**	**Fruit biomass per plant (kg)**
C1	0.06 (13)	0.33 (64)	1.13	0.01
C2	0.37 (57)	0.73 (97)	2.75	0.02
C3	1.19 (123)	0.97 (124)	2.60	0.02
C4	1.27 (129)	0.40 (53)	3.03	0.03

Fig. 8.4 Yield collected during the study

Inferences

C3 exhibited the highest germination rate (95%) and the highest number of surviving plants (190 out of 200 seeds). This indicates that C3 had favorable conditions for seed germination and a high rate of plant survival. C2, on the other hand, had the lowest germination rate (56%) and the lowest number of surviving plants (112). C1 and C4 had intermediate values. These differences suggest variations in environmental factors and management practices among the tested areas. When considering fruit yield, average weight per plant, and fruit biomass per plant, C3 emerges as the most productive area. It had higher fruit weight, average weight per plant, and overall biomass compared to the other areas. C2 had relatively high fruit production and average weight per plant but a lower overall biomass compared to C3. C4 showed the highest total fruit yield and average fruits per plant, indicating a productive outcome. C1 had the lowest fruit weight, average weight per plant, and biomass among the tested areas. C3 had the highest biomass across all categories (leaves, stems, and roots), followed by C2, C4, and C1. This suggests that C3 had the most extensive overall plant growth, while C1 had the least biomass. Overall, C3 consistently performed well across multiple factors, including germination rate, plant survival, fruit production, and biomass. C4 showed high fruit yield and average fruits per plant. C2 had relatively high fruit production but lower overall biomass. C1 had lower performance in terms of fruit production, average weight per plant, and biomass compared to the other areas.

8.1.2 *R. sativus* cultivation

Seed germination

The germination rates for all tested areas ranged from 34% to 50%. This indicates that there was variation in the success of seed germination among the different areas (Fig. 8.5). C3 and C4 had relatively higher germination rates of 49% and 50% respectively, suggesting favorable conditions for seed germination. C1 and C2 had lower germination rates at 34% and 34.5% respectively, indicating a lower success rate in seed germination. The number of plants that survived varied across the tested areas. C4 had the highest number of survived plants with 100, closely followed by C3 with 98. C2 had 69 surviving plants, while C1 had the lowest number of survived plants at 68. The differences in survival rates could be attributed to various factors such as environmental conditions, soil quality, pest infestation, or other factors that may have affected plant health and survival. C3 and C4 performed relatively better in terms of both germination rate and survival of plants. They had higher germination rates and a greater number of surviving plants compared to C1 and C2. C2 and C1 had lower germination rates and slightly lower numbers of survived plants, indicating potential challenges in seed germination and plant survival.

Fig. 8.5 Germination of *R. sativus* seeds

Table 8.4 Germination of *R. sativus* seeds

Tested area	Total number of planted seeds	Survived plants	Germination (%)
C1	200	68	34
C2	200	69	34.5
C3	200	98	49
C4	200	100	50

In summary, there are variations in germination rates and survival of plants among the tested areas. C3 and C4 showed better performance in terms of higher germination rates and a greater number of surviving plants. C1 and C2 had lower germination rates and slightly lower numbers of survived plants (Table 8.4). Further investigation and consideration of environmental and management factors are needed to understand the underlying reasons for these variations and to improve seed germination and plant survival in each tested area.

Plant yield

The number of *R. sativus* fruits harvested varied across the tested areas. C3 and C4 had the highest number of *R. sativus* fruits with 98 and 100 respectively, indicating a relatively abundant harvest. C1 and C2 had slightly lower numbers with 68 and 69 *R. sativus* fruits respectively. The weight of *R. sativus* fruits also varied among the tested areas. C3 had the highest weight at 4.51 kg, followed by C2 with 3.167 kg. C4 had a weight of 1.55 kg, while C1 had the lowest weight at 0.620 kg. These values indicate the overall mass of *R. sativus* fruits harvested in each area. C1 had an average of 4-6 leaves per plant, indicating healthy leaf growth. The specific number of leaves per plant was not provided for C2, C3, and C4. The weight of leaves was given for C1 (1.02 kg) and C2 (4.79 kg), indicating a significant difference in leaf biomass between these two areas. The total biomass per plant, which includes the weight of leaves, stems, and roots, varied among the tested areas. C2 had the highest total biomass at 0.12 kg, followed by C3 with 0.09 kg. C1 had a total biomass of 0.02 kg, while C4 had the lowest value at 0.03 kg. These values appear that C3 and C2 had relatively higher yields in terms of both the number and weight of *R. sativus* fruits (Table 8.5). C1 and C4 had lower values for both parameters (Fig. 8.6). However, it's important to note that the number of leaves and leaf weights were not provided for C2, C3, and C4, limiting our understanding of leaf growth and overall plant development in these areas.

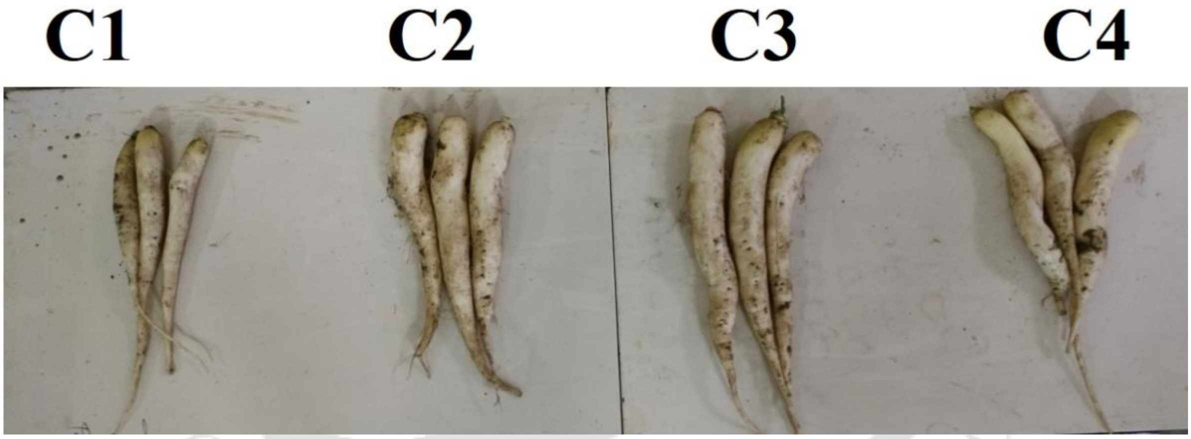


Fig. 8.6 yield comparison of *R. sativus*

Table 8.5 Yield of *R. sativus* on Day 70

Tested area	**No. of *R. sativus* fruits**	**Weight of fruit (kg)**	**Leaves per plant**	**Weight of Leaves (kg)**	**Total biomass per plant (kg)**
C1	68	0.620	Average 4-6 per plant	1.025	0.02
C2	69	3.167		4.793	0.12
C3	98	4.513		3.987	0.09
C4	100	1.546		1.500	0.03

Further analysis and consideration of factors such as soil quality, nutrient availability, watering practices, and environmental conditions would be necessary to fully assess the factors contributing to the observed differences in *R. sativus* fruit production and overall plant biomass in each tested area. Additionally, it would be valuable to analyse the root weights to gain a more comprehensive understanding of the plant's growth and yield potential.

8.1.3 *C. sativum* cultivation

Plant yield

The same number of seeds, 100 g, were planted in each tested area (C1, C2, C3, and C4). This allows for a fair comparison between the areas and ensures that any differences observed in biomass and dry mass are not due to variations in the initial seed count. The biomass generated varied across the tested areas (Fig. 8.7). C3 had the highest biomass at 1.4 kg, followed by C4 with 1.6 kg. C2 had a biomass of 1.2 kg, while C1 had the lowest biomass at 0.6 kg.

Table 8.6 Yield of *Coriandrum* on Day 50

Tested area	Total number of planted seeds (g)	Biomass generated (kg)	Dry mass (kg)
C1	100	0.60	0.09
C2	100	1.20	0.20
C3	100	1.40	0.25
C4	100	1.60	0.19

Fig. 8.7 Yield of *Coriandrum*

These values indicate the total mass of plant material produced in each area. The dry mass of the plants was measured in each tested area, representing the weight of the plant material after removing moisture content. C3 had the highest dry mass at 0.253 kg, followed by C2 with 0.196 kg. C4 had a dry mass of 0.192 kg, while C1 had the lowest dry mass at 0.090 kg (Table 8.6). Based on the data, it appears that C3 had the highest biomass and dry mass, indicating successful growth and development of the plants in that area. C4 also had relatively high biomass and dry mass values, suggesting significant plant growth. C2 had intermediate values, while C1 had the lowest biomass and dry mass among the tested areas.

8.2 CHANGE IN SOIL PHYSICOCHEMICAL PARAMETERS DUE TO VERMICOMPOST AMENDMENT

8.2.1 Change in soil pH

Based on the pH values recorded over the course of the trials, some preliminary conclusions can be drawn from the table. Firstly, among the plants in set C1, *Okra* displayed a relatively stable pH range throughout the 100-day period, maintaining values between 6.34 and 6.50. *R. sativus* (*R. sativus*) and Cilantro (*C. sativum*) in the same set exhibited slight variations in pH, but overall, they remained within a similar range. In set C2, all three plants showed some fluctuations in pH, with *Okra* displaying a gradual increase from 6.78 to 6.95. *R. sativus* and Cilantro, however, experienced both increases and decreases in pH during the trial period. In set C3, *Okra* demonstrated a consistent decline in pH from 7.32 to 7.02, while *R. sativus* and Cilantro maintained relatively stable pH values, albeit with slight decreases. Lastly, in set C4, all three plants showcased similar pH trends, gradually decreasing from the initial values. Overall, these observations suggest that different plants may exhibit distinct pH patterns over time, with some plants demonstrating more stability while others undergo fluctuations or gradual changes. Many intermediate organic substances are released into the soil system due to the biodegradation of complex organic compounds. These alkaline humates and phosphates are frequently released throughout earthworm-mediated degradation(Yagi et al., 2003) , which is known to cause pH changes.

8.2.2 Change in soil EC

In Trial C1, the EC values for *Okra*, *R. sativus* (*R. sativus*), and *C. sativum* (Coriander) are relatively similar throughout the experiment. There are slight variations between the plant varieties, but no significant differences stand out. In Trial C2, the electrical conductivity values also show similar trends across the different plant varieties. Again, there are no clear distinctions between *Okra*, *R. sativus*, and *C. sativum*. In Trial C3 and C4, the electrical conductivity values exhibit some variations between the plant varieties. For example, in Trial

C3, *R. sativus* shows higher conductivity values compared to *Okra* and *C. sativum* on day 20. Similarly, in Trial C4, *R. sativus* displays slightly higher conductivity values compared to *Okra* and *C. sativum* on day 40. For *Okra*, the electrical conductivity values generally decrease over time in Trials C1, C2, and C3, but they remain relatively stable in Trial C4. However, there is no significant difference in the conductivity values of *Okra* between the trials. *R. sativus* (*R. sativus*) shows some variations in conductivity values between the trials. For instance, in Trial C3, *R. sativus* exhibits a spike in conductivity on day 20, which is not observed in the other trials. Additionally, in Trial C4, *R. sativus* demonstrates higher conductivity values compared to Trials C1 and C2. *C. sativum* shows consistent electrical conductivity values across the trials, with no substantial differences. The electrical conductivity values for all plant varieties tend to vary slightly over time, but no consistent patterns emerge. While there are some minor differences between plant varieties within the same trial, there is no clear indication of a significant impact on electrical conductivity. Comparisons between trials show that variations in electrical conductivity values can occur for the same plant variety. Factors such as different experimental conditions, growth stages, or plant health could contribute to these variations.

8.2.3 Change in soil cation exchange capacity

In Trial C1, there are noticeable variations in cation exchange capacity between the plant varieties. *C. sativum* consistently shows higher CEC values compared to *Okra* and *R. sativus* throughout the experiment. In Trial C2, *Okra* displays a significant increase in CEC values from day 0 to day 20, followed by relatively stable values. *R. sativus* and *C. sativum* also show some variations, but no clear pattern emerges. In Trial C3, *Okra*, *R. sativus*, and *C. sativum* exhibit increasing CEC values over time. *Okra* has the lowest CEC initially but surpasses the other varieties by day 80. In Trial C4, *C. sativum* consistently demonstrates higher CEC values compared to *Okra* and *R. sativus*, especially on day 40 and beyond (Table 8.6). *Okra* generally shows increasing CEC values over time in Trials C2, C3, and C4. However, in Trial C1, *Okra*'s CEC values remain relatively stable throughout the experiment. *R. sativus* exhibits variations in CEC values across the trials. In Trial C1, *R. sativus* shows a decreasing trend, whereas in Trials C2, C3, and C4, the CEC values generally increase. *C. sativum* demonstrates varying CEC values between the trials. In Trial C1, CEC values are relatively stable, while in Trials C2, C3, and C4, there is an increasing trend. CEC values generally show fluctuations over time, with some plant varieties exhibiting increasing trends, while others display variations or stability. *C. sativum* consistently demonstrates higher CEC values compared to the other plant varieties in most trials. Comparisons between trials indicate variations in CEC values for the same plant variety, suggesting the influence of different experimental conditions or other factors.

In Trial C1, all three plant varieties (*Okra*, *R. sativus*, and *C. sativum*) show similar TKN values throughout the experiment. There are no significant differences observed between the varieties. In Trial C2, *Okra* and *R. sativus* exhibit comparable TKN values, with some variations between different days. *C. sativum* also shows similar TKN values, but with slightly lower values compared to *Okra* and *R. sativus*. In Trial C3, *Okra* and *R. sativus* display similar TKN values, with some fluctuations over time. *C. sativum* generally has higher TKN values compared to the other two varieties. In Trial C4, *Okra* and *R. sativus* show comparable TKN values, while *C. sativum* has slightly higher TKN values throughout the experiment. *Okra* exhibits varying TKN values across the trials. In Trial C1, C2, and C4, *Okra* shows decreasing TKN values over time. However, in Trial C3, *Okra*'s TKN values increase initially and then decrease. *R. sativus* demonstrates variations in TKN values between the trials. In Trial C1, C2, and C4, *R. sativus* generally shows decreasing TKN values. However, in Trial C3, *R. sativus* exhibits an increasing trend in TKN values. *C. sativum* also displays variations in TKN values between the trials. In Trial C1, C2, and C4, *C. sativum* shows relatively stable TKN values. In Trial C3, there is a decreasing trend in TKN values. TKN values show variations over time within each trial and across different plant varieties. Comparisons between plant varieties reveal some similarities in TKN values, while others show variations. *C. sativum* generally exhibits higher TKN values compared to *Okra* and *R. sativus* in most trials. There are variations in TKN values for the same plant variety across different trials, indicating the influence of experimental conditions or other factors. Pramanik et al. (2009) report that the substitution of organic anions by organic compounds in soil can inhibit the P adsorption to the positively charged margins of soil aggregates. On the other hand, K and N bioavailability in vermi-treated soil were significantly influenced by microbial activity (Goswami et al., 2013). Increasing microbial biomass C and soil respiration in soils amended with vermicompost promoted nitrogen mineralization. However, the reduction in the nutrients during the study in all the tested areas can be attributed to plant uptake during cultivation.

Table 8.8 Changes in pH and EC of soil during cultivation

		pH						**Electrical conductivity (mS/cm)**					
Trial	**Days**	**0**	**20**	**40**	**60**	**80**	**100**	**0**	**20**	**40**	**60**	**80**	**100**
C1	***Okra***	6.34 ± 1.25	6.44 ± 2.10	6.40± 1.10	6.40 ± 0.70	6.45 ± 1.20	6.50 ± 0.24	42.97 ± 2.56	43.38 ± 1.50	43.38 ± 1.25	42.56 ± 1.15	42.14 ± 0.81	42.14 ± 2.00
	R. sativus	6.24 ± 1.30	6.27 ± 0.90	6.33 ± 1.45	6.40 ± 0.68	6.41 ± 1.25	6.45 ± 0.35	42.97 ± 2.15	43.38 ± 2.10	44.21 ± 1.44	42.56 ± 2.15	42.21 ± 0.25	42.21 ± 1.32
	C. sativum	6.38 ± 1.10	6.44 ± 0.95	6.44 ± 0.22	6.44 ± 071	6.44 ± 1.15	6.50 ± 0.61	42.97 ± 1.12	43.83 ± 2.00	43.38 ± 2.02	42.56 ± 1.45	42.14 ± 1.88	42.14 ± 0.58
C2	***Okra***	6.78 ± 1.50	7.05 ± 0.85	7.07 ± 0.15	7.01 ± 0.77	6.93 ± 1.50	6.95 ± 0.53	48.69 ± 0.89	47.82 ± 1.56	47.39 ± 1.98	47.39 ± 1.05	46.96 ± 1.47	46.96 ± 1.25
	R. sativus	6.71 ± 1.90	6.79 ± 0.70	6.74 ± 0.95	6.81 ± 0.74	6.77 ± 1.22	6.81 ± 0.11	48.69 ± 2.41	48.26 ± 0.99	47.82 ± 0.25	47.39 ± 1.15	46.95 ± 1.30	46.95 ± 1.10
	C. sativum	6.76 ± 1.52	6.80 ± 0.87	7.09 ± 1.52	7.03 ± 0.88	7.00 ± 1.20	6.90 ± 0.15	48.69 ± 0.85	47.38 ± 1.45	47.26 ± 0.64	47.39 ± 1.52	46.54 ± 2.04	46.54 ± 1.90
C3	***Okra***	7.32 ± 1.11	7.27 ± 1.58	7.22 ± 1.17	7.16 ± 0.85	7.14 ± 1.62	7.02 ± 0.22	47.32 ± 1.84	46.40 ± 1.25	45.58 ± 1.24	45.65 ± 0.25	47.90 ± 1.25	47.90 ± 2.26
	R. sativus	7.23 ± 1.48	7.20 ± 1.64	7.14 ± 1.67	7.10 ± 0.14	7.00 ± 1.87	6.89 ± 0.78	47.32 ± 1.05	52.26 ± 0.11	45.53 ± 2.15	45.11 ± 1.25	44.65 ± 1.10	44.65 ± 2.41
	C. sativum	7.44 ± 1.52	7.32 ± 1.95	7.18 ± 1.23	7.14 ± 0.85	7.07 ± 0.99	7.00 ± 0.72	47.32 ± 1.69	47.32 ± 0.87	46.51 ± 1.24	46.05 ± 2.00	44.59 ± 1.45	44.59 ± 1.68
C4	***Okra***	7.23 ± 0.92	7.19 ± 1.24	7.14 ± 1.25	7.09 ± 0.95	7.02 ± 1.11	6.92 ± 0.15	45.41 ± 2.59	44.97 ± 2.50	44.83 ± 1.45	44.05 ± 1.25	42.99 ± 0.95	42.99 ± 1.55
	R. sativus	7.21 ± 1.20	7.20 ± 1.35	7.12 ± 0.89	7.09 ± 1.10	7.02 ± 1.02	6.90 ± 0.16	45.41 ± 2.50	44.92 ± 2.20	46.61 ± 1.26	44.44 ± 1.22	43.47 ± 1.25	43.47 ± 0.95
	C. sativum	7.31 ± 1.15	7.28 ± 2.00	7.20 ± 0.95	7.09 ± 1.58	7.05 ± 0.40	7.01 ± 0.18	45.41 ± 1.50	44.93 ± 1.52	43.96 ± 1.20	43.96 ± 2.11	42.51 ± 2.05	42.51 ± 0.99

Table 8.8 Changes in CEC and TKN of soil during cultivation

		Cation exchange capacity (meq/100g)						**Total Kheldal Nitrogen (%)**					
Trial	**Days**	**0**	**20**	**40**	**60**	**80**	**100**	**0**	**20**	**40**	**60**	**80**	**100**
C1	***Okra***	13.10 ± 1.75	13.10 ± 1.28	13.23 ± 1.71	13.18 ± 1.23	13.70 ± 1.61	14.10 ± 1.24	0.23 ± 0.09	0.21 ± 0.02	0.15 ± 0.03	0.15 ± 0.13	0.14 ± 0.03	0.14 ± 0.04
	R. sativus	12.91 ± 1.12	11.90 ± 1.95	12.45 ± 1.89	12.00 ± 1.45	11.45 ± 1.87	13.12 ± 1.86	0.23 ± 0.06	0.20 ± 0.10	0.14 ± 0.06	0.14 ± 0.07	0.12 ± 0.10	0.12 ± 0.05
	C. sativum	12.45 ± 1.98	12.34 ± 1.14	14.50 ± 1.33	15.60 ± 1.76	16.60 ± 1.23	16.60 ± 1.12	0.23 ± 0.11	0.21 ± 0.13	0.15 ± 0.12	0.15 ± 0.09	0.15 ± 0.08	0.15 ± 0.04
C2	***Okra***	24.57 ± 1.45	25.14 ± 1.72	32.45 ± 1.57	31.90 ± 1.89	34.12 ± 1.52	34.12 ± 1.77	0.72 ± 0.07	0.64 ± 0.08	0.80 ± 0.10	0.64 ± 0.05	0.42 ± 0.14	0.28 ± 0.10
	R. sativus	25.98 ± 1.23	26.18 ± 1.36	28.14 ± 1.96	30.12 ± 1.11	35.13 ± 1.76	36.09 ± 1.33	0.72 ± 0.13	0.68 ± 0.07	0.84 ± 0.09	0.64 ± 0.14	0.42 ± 0.06	0.38 ± 0.09
	C. sativum	26.78 ± 1.57	28.14 ± 1.83	31.12 ± 1.28	32.45 ± 1.67	35.12 ± 1.34	34.54 ± 1.55	0.78 ± 0.14	0.72 ± 0.05	0.64 ± 0.07	0.60 ± 0.08	0.42 ± 0.13	0.29 ± 0.08
C3	***Okra***	29.67 ± 1.89	32.12 ± 1.45	35.12 ± 1.64	38.90 ± 1.34	41.90 ± 1.91	42.14 ± 1.92	0.88 ± 0.10	0.92 ± 0.09	0.92 ± 0.13	0.78 ± 0.11	0.56 ± 0.07	0.32 ± 0.05
	R. sativus	28.98 ± 1.34	29.45 ± 1.62	33.34 ± 1.42	37.02 ± 1.98	40.20 ± 1.08	42.19 ± 1.07	0.84 ± 0.03	0.80 ± 0.14	0.90 ± 0.04	0.72 ± 0.06	0.56 ± 0.12	0.30 ± 0.05
	C. sativum	30.12 ± 1.67	30.50 ± 1.29	34.00 ± 1.75	38.90 ± 1.55	42.30 ± 1.45	43.16 ± 1.69	0.92 ± 0.12	0.84 ± 0.06	0.78 ± 0.08	0.70 ± 0.12	0.56 ± 0.04	0.35 ± 0.06
C4	***Okra***	24.20 ± 1.06	26.78 ± 1.53	28.90 ± 1.12	30.12 ± 1.78	35.12 ± 1.96	34.87 ± 1.45	0.80 ± 0.05	0.84 ± 0.11	0.74 ± 0.15	0.48 ± 0.10	0.28 ± 0.11	0.30 ± 0.23
	R. sativus	22.30 ± 1.81	24.50 ± 1.76	28.45 ± 1.81	29.07 ± 1.23	33.40 ± 1.12	35.90 ± 1.28	0.72 ± 0.08	0.70 ± 0.12	0.64 ± 0.05	0.48 ± 0.03	0.32 ± 0.05	0.24 ± 0.05
	C. sativum	28.89 ± 1.28	27.18 ± 1.11	32.45 ± 1.68	33.67 ± 1.99	31.12 ± 1.69	34.43 ± 1.35	0.78 ± 0.04	0.72 ± 0.04	0.68 ± 0.11	0.52 ± 0.04	0.50 ± 0.09	0.37 ± 0.15

8.3 SOIL ORGANIC MATTER AND ORGANIC CARBON

Soil organic matter is crucial for nutrient retention, soil structure maintenance, and plant water availability. Agricultural management practices have a significant impact on the organic matter content of soil, with intensive cultivation frequently resulting in a loss of organic matter. Cost and environmental risk associated with the use of chemical fertilisers have rekindled interest in the use of organic soil amendments, including plant residues, manures, and composts. Composts have several advantages over plant residues, including reduced volume, delayed decomposition rates, and waste recycling (Duong et al., 2012).

Table 8.9 Changes in organic matter of soil during cultivation

Trial	**Days**	**0**	**20**	**40**	**60**	**80**	**100**
C1	***Okra***	2.20 ± 0.02	2.15 ± 0.03	1.63 ± 0.03	1.43 ± 0.02	1.27 ± 0.05	1.00 ± 0.01
	R. sativus	2.16 ± 0.03	1.97 ± 0.05	1.65 ± 0.04	1.43 ± 0.09	1.27 ± 0.02	0.81 ± 0.02
	C. sativum	2.29 ± 0.04	2.20 ± 0.02	1.68 ± 0.02	1.49 ± 0.08	1.25 ± 0.08	0.90 ± 0.05
C2	***Okra***	3.83 ± 0.05	3.74 ± 0.04	3.47 ± 0.04	3.11 ± 0.02	2.60 ± 0.05	1.45 ± 0.05
	R. sativus	3.82 ± 0.03	3.81 ± 0.10	3.54 ± 0.02	3.15 ± 0.04	2.95 ± 0.04	1.63 ± 0.10
	C. sativum	3.82 ± 0.01	4.98 ± 0.01	3.76 ± 0.03	2.99 ± 0.08	2.17 ± 0.06	1.56 ± 0.06
C3	***Okra***	5.19 ± 0.05	5.08 ± 0.02	4.65 ± 0.04	3.79 ± 0.07	3.19 ± 0.07	1.75 ± 0.04
	R. sativus	5.14 ± 0.04	4.93 ± 0.03	4.55 ± 0.03	3.54 ± 0.05	3.15 ± 0.09	1.86 ± 0.08
	C. sativum	5.42 ± 0.02	5.30 ± 0.04	4.94 ± 0.02	3.97 ± 0.06	2.76 ± 0.05	1.63 ± 0.07
C4	***Okra***	3.24 ± 0.01	3.17 ± 0.01	2.52 ± 0.04	2.36 ± 0.03	2.06 ± 0.08	1.38 ± 0.05
	R. sativus	3.22 ± 0.05	3.06 ± 0.05	2.60 ± 0.02	2.51 ± 0.01	2.31 ± 0.09	1.56 ± 0.01
	C. sativum	3.37 ± 0.03	3.17 ± 0.02	3.01 ± 0.03	2.60 ± 0.07	2.52 ± 0.01	1.45 ± 0.01

Based on the Soil Organic Matter and Soil Organic Carbon values provided in the table, here are the conclusions and comparisons. Comparing different plant varieties within the same trial (C1, C2, C3, C4), there are variations in the Soil Organic Matter values over time. The values generally decrease as the days progress. In Trial C1, all three plant varieties (*Okra*, *R. sativus*, and *C. sativum*) show similar trends in Soil Organic Matter, with decreasing values over time. In Trial C2, *Okra*, *R. sativus*, and *C. sativum* display decreasing Soil Organic Matter values as the days progress. *Okra* consistently has higher Soil Organic Matter values compared to the other two varieties. In Trial C3, all three plant varieties (*Okra*, *R. sativus*, and *C. sativum*) exhibit decreasing Soil Organic Matter values over time (Table 8.7). *Okra* generally has the highest values among the three varieties. In Trial C4, all three plant varieties (*Okra*, *R. sativus*, and *C. sativum*) show decreasing Soil Organic Matter values over time. *Okra* has relatively higher Soil Organic Matter values compared to *R. sativus* and *C. sativum*.

Table 8.10 Changes in organic carbon of soil during cultivation

Trial	**Days**	**0**	**20**	**40**	**60**	**80**	**100**
C1	***Okra***	1.23 ± 0.05	1.20 ± 0.03	0.91 ± 0.02	0.80 ± 0.07	0.71 ± 0.15	0.56 ± 0.02
	R. sativus	1.20 ± 0.10	1.10 ± 0.04	0.92 ± 0.08	0.80 ± 0.05	0.71 ± 0.10	0.45 ± 0.04
	C. sativum	1.27 ± 0.01	1.23 ± 0.02	0.94 ± 0.09	0.83 ± 0.04	0.70 ± 0.20	0.50 ± 0.06
C2	***Okra***	2.14 ± 0.06	2.09 ± 0.04	1.94 ± 0.07	1.74 ± 0.03	1.45 ± 0.10	0.81 ± 0.05
	R. sativus	2.12 ± 0.20	2.13 ± 0.03	1.98 ± 0.05	1.76 ± 0.01	1.65 ± 0.55	0.91 ± 0.04
	C. sativum	2.12 ± 0.22	2.78 ± 0.08	2.10 ± 0.01	1.67 ± 0.05	1.21 ± 0.41	0.87 ± 0.11
C3	***Okra***	2.90 ± 0.05	2.84 ± 0.04	2.60 ± 0.05	2.12 ± 0.08	1.78 ± 0.05	0.98 ± 0.10
	R. sativus	2.86 ± 0.20	2.75 ± 0.07	2.54 ± 0.70	1.98 ± 0.09	1.76 ± 0.06	1.04 ± 0.20
	C. sativum	3.01 ± 0.15	2.96 ± 0.03	2.76 ± 0.07	2.22 ± 0.07	1.54 ± 0.08	0.91 ± 0.24
C4	***Okra***	1.81 ± 0.05	1.77 ± 0.05	1.41 ± 0.06	1.32 ± 0.05	1.15 ± 0.07	0.77 ± 0.21
	R. sativus	1.78 ± 0.01	1.71 ± 0.02	1.45 ± 0.60	1.40 ± 0.15	1.29 ± 0.09	0.87 ± 0.15
	C. sativum	1.87 ± 0.01	1.77 ± 0.03	1.68 ± 0.05	1.45 ± 0.10	1.41 ± 0.05	0.81 ± 0.10

Comparing different plant varieties within the same trial (C1, C2, C3, C4), there are variations in the Soil Organic Carbon values over time. The values generally decrease as the days progress. In Trial C1, all three plant varieties (*Okra*, *R. sativus*, and *C. sativum*) exhibit decreasing Soil Organic Carbon values over time. *Okra* has higher values compared to the other two varieties. In Trial C2, *Okra*, *R. sativus*, and *C. sativum* show decreasing Soil Organic Carbon values as the days progress. *Okra* generally has higher values compared to *R. sativus* and *C. sativum*. In Trial C3, all three plant varieties (*Okra*, *R. sativus*, and *C. sativum*) display decreasing Soil Organic Carbon values over time. *Okra* generally has higher values compared to the other two varieties. In Trial C4, all three plant varieties (*Okra*, *R. sativus*, and *C. sativum*) demonstrate decreasing Soil Organic Carbon values over time. *Okra* has relatively higher Soil Organic Carbon values compared to *R. sativus* and *C. sativum*.

Both Soil Organic Matter and Soil Organic Carbon values show decreasing trends over time within each trial and across different plant varieties. Comparisons between plant varieties reveal variations in Soil Organic Matter and Soil Organic Carbon values, with *Okra* generally having higher values compared to *R. sativus* and *C. sativum*. It's important to consider additional information about the experimental setup, soil management practices, and specific objectives of studying Soil Organic Matter and Soil Organic Carbon to provide a more comprehensive interpretation.

8.4 SOIL AGRICULTURAL PARAMETERS

8.4.1 Change in soil bulk density

Bulk Density values remain relatively consistent over time within each trial and across different plant varieties. In Trial C1, all three plant varieties (*Okra*, *R. sativus*, and *C. sativum*) show similar Bulk Density values, which are slightly above 1.3 g/CC. In Trial C2, *Okra*, *R. sativus*, and *C. sativum* also display similar Bulk Density values, which range from 1.18 g/CC to 1.23 g/CC (Table 8.6). In Trial C3 and C4, all three plant varieties within each trial have comparable Bulk Density values, with slight variations. The vermicompost amendment enhanced soil structure by decreasing bulk density, according to the results. Organic matter contributed to an increase in soil porosity. It ultimately increased the soil's water retention capacity (Song et al., 2015).

Table 8.11 Changes in bulk density of soil during cultivation

Trial	**Days**	**0**	**20**	**40**	**60**	**80**	**100**
C1	***Okra***	1.37 ± 0.05	1.37 ± 0.03	1.37 ± 0.06	1.39 ± 0.02	1.40 ± 0.11	1.40 ± 0.16
	R. sativus	1.38 ± 0.10	1.37 ± 0.01	1.35 ± 0.02	1.39 ± 0.20	1.40 ± 0.20	1.40 ± 0.12
	C. sativum	1.37 ± 0.12	1.37 ± 0.02	1.37 ± 0.01	1.39 ± 0.40	1.40 ± 0.14	1.40 ± 0.11
C2	***Okra***	1.19 ± 0.04	1.20 ± 0.04	1.21 ± 0.03	1.21 ± 0.05	1.22 ± 0.15	1.22 ± 0.12
	R. sativus	1.18 ± 0.05	1.19 ± 0.06	1.20 ± 0.09	1.21 ± 0.19	1.22 ± 0.10	1.22 ± 0.10
	C. sativum	1.19 ± 0.06	1.21 ± 0.05	1.21 ± 0.04	1.21 ± 0.17	1.23 ± 0.14	1.23 ± 0.12
C3	***Okra***	1.15 ± 0.08	1.08 ± 0.15	1.17 ± 0.05	1.19 ± 0.18	1.12 ± 0.11	1.12 ± 0.11
	R. sativus	1.15 ± 0.07	1.10 ± 0.15	1.17 ± 0.02	1.18 ± 0.17	1.19 ± 0.10	1.19 ± 0.12
	C. sativum	1.15 ± 0.02	1.14 ± 0.19	1.15 ± 0.00	1.16 ± 0.14	1.17 ± 0.16	1.17 ± 0.13
C4	***Okra***	1.13 ± 0.01	1.14 ± 0.10	1.14 ± 0.06	1.16 ± 0.11	1.18 ± 0.19	1.18 ± 0.14
	R. sativus	1.13 ± 0.03	1.14 ± 0.14	1.14 ± 0.05	1.15 ± 0.10	1.17 ± 0.18	1.17 ± 0.16
	C. sativum	1.13 ± 0.05	1.14 ± 0.11	1.16 ± 0.10	1.16 ± 0.15	1.19 ± 0.17	1.19 ± 0.15

8.4.2 Change in soil water holding capacity

Field capacity is synonymous with the terms water retention capacity and water holding capacity. Field capacity is the amount of water retained in pervious soils of uniform structure and texture after precipitation or irrigation, when the rate of downward movement has reduced significantly. This typically happens within two to three days (Rai et al., 2017). WHC values show variations over time within each trial and across different plant varieties. In Trial C1, *Okra*, *R. sativus*, and *C. sativum* exhibit different WHC values, with some fluctuations over time. In Trial C2, *Okra* generally has higher WHC values compared to *R. sativus* and *C. sativum*. The values increase over time. In Trial C3 and C4, there are variations in WHC values among the plant varieties, with fluctuations over time.

Table 8.12 Changes in WHC of soil during cultivation

Trial	**Days**	**0**	**20**	**40**	**60**	**80**	**100**
C1	***Okra***	12.00 ± 1.72	11.70 ± 1.32	13.00 ± 1.47	13.00 ± 1.27	13.10 ± 1.37	15.61 ± 1.57
	R. sativus	13.00 ± 1.36	13.60 ± 1.76	14.00 ± 1.62	14.00 ± 1.85	13.00 ± 1.82	14.12 ± 1.28
	C. sativum	13.00 ± 1.98	14.20 ± 1.12	15.00 ± 1.86	15.00 ± 1.42	14.56 ± 1.09	14.56 ± 1.85
C2	***Okra***	29.00 ± 1.13	29.89 ± 1.95	30.12 ± 1.28	32.00 ± 1.11	33.14 ± 1.64	34.80 ± 1.42
	R. sativus	30.00 ± 1.84	32.34 ± 1.64	30.00 ± 1.57	31.12 ± 1.76	32.14 ± 1.23	33.45 ± 1.79
	C. sativum	29.00 ± 1.25	33.41 ± 1.47	35.13 ± 1.93	34.02 ± 1.61	33.72 ± 1.98	33.45 ± 1.61
C3	***Okra***	31.00 ± 1.57	33.00 ± 1.87	36.13 ± 1.34	34.57 ± 1.33	36.89 ± 1.52	35.80 ± 1.93
	R. sativus	27.12 ± 1.41	30.12 ± 1.28	35.00 ± 1.72	34.18 ± 1.96	34.78 ± 1.17	36.32 ± 1.36
	C. sativum	30.15 ± 1.68	32.45 ± 1.53	37.18 ± 1.41	35.15 ± 1.18	36.71 ± 1.71	34.57 ± 1.71
C4	***Okra***	30.00 ± 1.29	32.00 ± 1.71	33.09 ± 1.89	33.78 ± 1.52	31.25 ± 1.32	29.90 ± 1.23
	R. sativus	28.18 ± 1.93	29.08 ± 1.39	32.00 ± 1.68	32.46 ± 1.71	33.12 ± 1.87	34.90 ± 1.97
	C. sativum	29.67 ± 1.07	35.34 ± 1.84	36.14 ± 1.23	33.90 ± 1.29	34.14 ± 1.06	29.39 ± 1.68

8.4.3 Porosity

Porosity values show variations over time within each trial and across different plant varieties. In Trial C1, all three plant varieties (*Okra*, *R. sativus*, and *C. sativum*) have similar

Porosity values, which remain relatively consistent over time. In Trial C2, *Okra*, *R. sativus*, and *C. sativum* exhibit similar Porosity values, which decrease slightly over time. In Trial C3 and C4, there are variations in Porosity values among the plant varieties, with fluctuations over time.

Table 8.13 Changes in porosity of soil during cultivation

Trial	**Days**	**0**	**20**	**40**	**60**	**80**	**100**
C1	***Okra***	42.97 ± 3.62	43.38 ± 4.57	43.38 ± 2.56	42.56 ± 4.12	42.14 ± 3.42	42.14 ± 3.78
	R. sativus	42.97 ± 2.85	43.38 ± 3.28	44.21 ± 4.11	42.56 ± 3.53	42.21 ± 4.11	42.21 ± 3.29
	C. sativum	42.97 ± 4.21	43.83 ± 2.85	43.38 ± 3.98	42.56 ± 3.01	42.14 ± 3.23	42.14 ± 4.59
C2	***Okra***	48.69 ± 3.08	47.82 ± 3.42	47.39 ± 2.78	47.39 ± 2.92	46.96 ± 2.89	46.96 ± 2.66
	R. sativus	48.69 ± 2.46	48.26 ± 4.79	47.82 ± 4.95	47.39 ± 4.77	46.95 ± 3.76	46.95 ± 3.45
	C. sativum	48.69 ± 4.73	47.38 ± 2.61	47.26 ± 2.34	47.39 ± 4.31	46.54 ± 4.58	46.54 ± 2.37
C3	***Okra***	47.32 ± 2.35	46.40 ± 3.93	45.58 ± 3.45	45.65 ± 3.28	47.90 ± 2.65	47.90 ± 4.22
	R. sativus	47.32 ± 3.97	52.26 ± 4.36	45.53 ± 4.65	45.11 ± 4.64	44.65 ± 4.93	44.65 ± 4.87
	C. sativum	47.32 ± 4.57	47.32 ± 3.71	46.51 ± 2.89	46.05 ± 2.56	44.59 ± 2.34	44.59 ± 3.63
C4	***Okra***	45.41 ± 2.93	44.97 ± 2.23	44.83 ± 3.12	44.05 ± 2.17	42.99 ± 4.27	42.99 ± 2.91
	R. sativus	45.41 ± 4.12	44.90 ± 4.97	46.61 ± 4.23	44.44 ± 3.89	43.47 ± 3.89	43.47 ± 2.54
	C. sativum	45.41 ± 3.26	44.93 ± 2.68	43.96 ± 3.76	43.96 ± 4.45	42.51 ± 2.72	42.51 ± 4.12

8.5. SUMMERY

- The produced vermicompost post-amended soil increased the seed germination rate and yield in all the tested areas, particularly C3.
- Bulk density values remain relatively consistent throughout the trials and do not show significant variations.
- Comparisons between plant varieties reveal differences in water holding capacity and porosity values, indicating variations in soil water holding capacity and pore space.

- Soil organic matter and soil organic carbon increased post-amendment and, in comparison, were highly depleted due to the cultivation effect in C2, C3, and C4, indicating the requirement for further soil amendment with vermicompost.
- In comparison to vegetable waste-based vermicompost, vermicompost derived from aquatic weed biomass showed promising results.

Chapter 9

OVERALL CONCLUSIONS AND FUTURE RECOMMENDATIONS

This chapter demonstrates the summery of the outcomes of the study.

9.1 OVERALL CONCLUSIONS

The novel combination of RDC thermophilic biodegradation, followed by *E. fetida* based VC or dual-stage biodegradation process, yielded nutrient-rich vermicompost from batch scale studies using vegetable waste, sewage sludge, aquatic and terrestrial weeds. It could potentially reconfigure organic waste treatment facilities across the world.

- Prior to rotary drum in-vessel composting, the impediment of high-water content of organic wastes was successfully overcome by the addition of bulking agents, which accelerated the physiological thermophilic phase to <24 h.
- The continuation of the physiological thermophilic phase for 7 to 8 days, facilitated the optimization of physicochemical parameters like C/N ratio, pH, and moisture content for individual biodegradable organic wastes.
- Extensive portions of the organic waste were broken down in the thermophilic phase, which stimulated faster mineralization by the *in situ* native bacterial population. The nutrient-rich final RDC product required VC to match levels of nutrients in vermicompost.
- The handicap of the mandatory 15 to 20-day adaptation of vermicultures to fresh waste, notwithstanding, the *Eisenia fetida* driven VC yielded vermicompost with inherent high porosity, adsorption sites, and nutritional properties. The resultant organic fertilizer evinced positive results when tested for soil fertility, augmented nutritional content, and lowered toxicity levels.
- The four individual phases of investigations adopted in the present study has projected faster decomposition, with the production of a tested and marketable end product, in an optimized time frame, without affecting the final product quality.

In **Phase I**, batch scale studies carried out for vegetable waste, sewage sludge, aquatic and terrestrial weeds validated effectiveness of the two-stage biodegradation process.

- Batch scale study of vegetable waste using the dual-stage biodegradation process, yielded nutrient-rich vermicompost from vegetable waste in the shortest feasible period (27 days). In-depth optimization of process parameters combined with the reduction in EC, CO_2 evolution, and increased SGI, validated the technique and product.
- Batch scale study of freshly dewatered sewage via the dual-stage biodegradation process evinced quick sludge stabilization post the 7-day thermophilic degradation in RDC, followed by 20-day mesophilic degradation using *E. fetida*-based vermicomposting. Though bioaccumulation of toxic heavy metals like Zn, Pb and Cr was observed throughout sewage sludge stabilization, overall plant growth was not adversely affected by the resultant vermicompost.
- In the batch scale study of aquatic weed waste the dual-stage biodegradation process, the RDC active thermophilic was achieved within the initial 24-hour period, which facilitated the growth of earthworms within the 20-day VC phase. Notable reduction in TOC was evinced as feedstock degradation progressed. Spectroscopy-based investigations validated the efficiency of the RV method in breaking down feedstock lignocellulose compounds.
- Batch scale study of terrestrial weed waste via the dual-stage biodegradation process led to an increased concentration of TN, TP, and TK and reduction of toxic pathogens in the weeds. The *E. Fetida* derived vermicompost exhibited significant and positive impact on plant growth in alluvial soils, validated by the SGI (%) value exceeding 80%. This methodology is distinct in managing lignocellulosic weeds that are toxic, and it yields a final product of superior quality and safety within a compressed timeline of 27 days.

In **Phase II** of the study, Phytotoxicity tests and safety assessments for soil conditioners validated the end products from Phase I batch scale studies derived from vegetable waste, sewage sludge, aquatic and terrestrial weeds.

- The seed germination index of the vermicompost produced through two-stage biodegradation was significantly > 95%, which categorically endorsed the conversion of the toxic terrestrial weed-based vermicompost into a nontoxic soil conditioner.
- Pot studies evidenced by the healthy growth of *Coriandrum sativum* provided additional support for the safety of the VC product.

In **Phase III**, large scale studies validated the proven biodegradation process, in pilot-scale rotary drum composter (5000 L), including the bag (3000 L) and stack vermicomposting (3000 L) techniques.

- The pilot scale study using vegetable waste supported the effectiveness of large-scale in-vessel RDC in conjunction with vermicomposting as a successful organic waste management strategy. The study's biodegradation process efficiently lowered the heavy metal content and seed inhibitory toxic nature (SGI-98%). Furthermore, the two-stage composting consistently produced a nutrient-rich soil conditioner from waste (4.2% total nitrogen).
- At 1.95 mg/gVS/day of OUR, the end-product displayed stable and mature enough to apply to the soil. Taxonomic hit distribution at the domain level indicated that bacteria accounted for 83.17% of the sequences in the vermicompost sample, while Archaea accounted for 16.7%.

  Increased microbial activity was attributed to the two-stage composting of vegetable waste, leading to greater waste stabilization within a 27-day time frame. The large-scale reactors validated in the study can be a potential tool (70% volume reduction) in urban waste management.
- Pilot-scale study using water hyacinth supported the effectiveness of large-scale in-vessel rotary drum composting in conjunction with vermicomposting as a successful organic waste management strategy. The process efficiently increased the SGI to 98%. Furthermore, the technique consistently produced a nutrient-rich soil conditioner from weed biomass.
- The end-product was stable and mature, and increased microbial activity was discovered (bacteria; 83.17% and archaea; 16.7% in vermicompost) due to the two-stage composting of biomass, leading to more excellent waste stabilization within a 27-day time frame. The large-scale reactors validated in the study can be a potential tool (71% volume reduction) in urban waste management.
- Pilot scale study through CC, vegetable waste and water hyacinth underscored the efficacy of large-scale rotary drum composting and stack vermicomposting techniques in generating vermicompost from vegetable waste and water hyacinth, utilizing mono and co-composting approaches.
- Upon conclusion of the study, it was observed that during CC, there was an 80% reduction in the carbon-to-nitrogen ratio compared to the WHC and VWC. Also,

the EC values of compost are less than 4ms/cm, which suggests that the compost can be utilized in agricultural fields without any adverse consequences.

In **Phase IV** evaluation of vermicompost amendment on soil in natural conditions, using three different plant models Okra *or Abelmoscus esculentus*, Radish or *Raphanus sativus*, and Cilantro or *Coriandrum sativum* was successfully conducted on the IITG campus.

- The real-time study using an application of 10 tons per hectare and conducted for 100 days demonstrated that the increase in soil organic carbon was due to the amendment. The growth of the plants was healthy compared to the control soil. The yield was also three times higher than that of soil without vermicompost. It is noteworthy, that the vermicompost generated through aquatic weed, *Water hyacinth* was remarkable in terms of all the vegetables growth and yield.
- The results indicated that CC could offer a superior alternative to conventional aerobic composting and landfill disposal methods for organic waste, as evidenced by the lower carbon-to-nitrogen ratio, electrical conductivity, the resulting pH and nutrient content products compared to WHC and VWC

9.2 MAJOR OUTCOMES OF THE STUDY

- The application of rotary drum in-vessel composting, followed by vermicomposting of the phyto-mass into organic fertilizers, have emerged as efficient alternatives to decompose, treat and recycle organic waste.
- The significant reduction in time from minimum of 45 day to 27 days for quality vermicompost production, demonstrated the success of the two-stage biodegradation process.
- *E. fitida* worm culture has proven to be most robust, wide ranging and successful among the earthworm species applied in the bioconversion process.
- The combined technique is capable of handling sizable quantities of vegetable waste in an environmentally compatible mode to deescalate wide scale contamination hazards and produce an excellent soil conditioner.
- In a biologically appropriate mode, the biodegradation process validated in this study, could help sludge management facilities stabilize sludge faster, and

produce a marketable end product, in an optimized time frame. The application of this process has successfully decomposed and recycled resistant and toxic biomass like water hyacinth into soil conditioners.

- The novel approach for weed management holds promise for restoring nutritional vigour to areas affected by weeds across the globe.
- The successful decomposition also extends to biodegradable organic waste available with the community both in urban and rural settings.
- The proposed novel rapid treatment and recycling method in the present study has been successful in combating deterrents of composition and degradation in biodegradable organic waste.
- The present study hold its own among the very few studies on pilot-scale biodegradation processes documented on safety evaluation of the two-stage biodegradation efficiently in managing diverse organic wastes.
- Further the increased soil fertility due to the amendment of vermicompost suggest that the use or organic soil stabilizers are not only useful to plants but also to the soil. The two-stage biodegradation process was quick in stabilizing the biomass.
- Real time waste scenarios tested in pilot-scale study using water hyacinth and vegetable waste demonstrated that the two-stage biodegradation technique can tackle the humongous waste in large scale much more efficiently than in batch process.
- The findings indicate that using two-stage biodegradation holds considerable promise as a viable strategy for managing organic waste within urban areas through CC.
- Water hyacinth based vermicompost displayed the most significant positive impact on the crop yield, thus signifying the possibility of lowering the amount of chemical fertilizers used agriculture, besides increasing soil fertility and quality.

9.3 FUTURE RECOMMENDATIONS

Based on the experience gained throughout the study certain future recommendations maybe possible as follows

- Identification and isolation of the bacterial culture dominant during the active thermophilic phase and study bio-augmentation and further its large-scale application.

- Exploring the fully automated facility to overcome the human resource requirement.
- Field application in the contaminated soil and study on the dynamics of plant growth and soil reclamation.
- Application of char/biochar to enhance the process and product quality on batch and large scale.
- Generation of mathematical models of the process to in better guiding the waste management sector.

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Annexure I

PUBLICATIONS

Published article

1. **Pottipati, S**., Kundu, A., & Kalamdhad, A. S. (2022). Process optimization by combining in-vessel composting and vermicomposting of vegetable waste. Bioresource Technology, 346, 126357. https://doi.org/10.1016/J.BIORTECH.2021.126357
2. **Pottipati, S**., Kundu, A., & Kalamdhad, A. S. (2022). Performance evaluation of a novel two-stage biodegradation technique through management of toxic lignocellulosic terrestrial weeds. Waste Management, 144, 191–202. https://doi.org/10.1016/J.WASMAN.2022.03.026
3. **Pottipati, S**., & Kalamdhad, A. S. (2022). Thermophilic-mesophilic biodegradation: An optimized dual-stage biodegradation technique for expeditious stabilization of sewage sludge. Journal of Environmental Management, 323, 116189. https://doi.org/10.1016/J.JENVMAN.2022.116189
4. **Pottipati, S**., Jat, N., & Kalamdhad, A. S. (2023). Bioconversion of Eichhornia crassipes into vermicompost on a large scale through improving operational aspects of in-vessel biodegradation process: Microbial dynamics. Bioresource Technology, 374, 128767. https://doi.org/10.1016/J.BIORTECH.2023.128767
5. **Pottipati, S**., Hazarika, J., & Kalamdhad, A. S. (2023). Bioconversion of phytotoxic terrestrial weeds into soil conditioning bioproduct through two-stage biodegradation process. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-023-04201-0 (**Online**)
6. **Pottipati, S**., Haq, I., & Kalamdhad, A. S. (2023). Large-scale production of a nutrient-rich soil conditioner by optimized biodegradation of vegetable waste: biodiversity and toxicity assessments. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-023-04050-x (**Online**)

Articles (Submitted)

1. **Pottipati, S**., Prit, H., Haq, I., & Kalamdhad, A. S. (2023). Large-scale vermicompost production improvement through two-stage co-composting of water hyacinth and vegetable waste (**Under review).**

2. **Pottipati, S.**, & Kalamdhad, A. S. (2022). Improved biodegradation technique for managing aquatic weeds Water hyacinth and Hydrilla verticillata by bio-converting in to nutrient-rich vermicompost: A two-step process (**Under review).**

International Conferences

1. **Pottipati, S.**, Kundu, A., & Kalamdhad, A. S. (2021). "Thermophilic degradation of vegetable waste using Rotary drum com- poster and efficacy of rotary drum followed by vermicomposting" Poster presentation at International Conference on **Sustainable Biowaste Management 2021 (SBM 2021)** Held at Hong Kong by Hong Kong Baptist University. **(Outstanding poster award)**
2. **Pottipati, S.**, & Kalamdhad, A. S. (2022). "Bioconversion of Eichhornia crassipes into vermicompost on large-scale through improving operational aspects of in-vessel biodegradation process: biodiversity and toxicity analysis" Flash talk and Poster presentation at International Conference on **Biotechnology, Sustainable Bioresources and Bioeconomy (BSBB- 2022)** held at **Indian Institute of Technology, Guwahati. (Best flash talk and poster award)**
3. **Pottipati, S.**, & Kalamdhad, A. S. (2023). "Effect of Reactor Scale on The Co-Composting of Vegetable Waste with Water Hyacinth Through Two-Stage Biodegradation" Oral presentation at the 4th International Conference on Waste Management (**Recycle 2023**) held at **Indian Institute of Technology, Guwahati.**

National Conferences

1. **Pottipati, S.**, & Kalamdhad, A. S. (2022). "Bioconversion of lignocellulosic weeds into nutrient-rich compost" Poster presentation at **North - East Research conclave 2022 (NERC-22)** an International Conference held at **Indian Institute of Technology, Guwahati.**
2. **Pottipati, S.**, & Kalamdhad, A. S. (2023). "An investigation into the scale shift of an efficient biodegradation practice from batch to pilot scale continuous feeding mode" oral presentation at National Conference on **Sustainable Waste Management Practices SWMP 2023**) held at **Sardar Vallabhbhai National Institute of Technology Surat.**

Book Chapters

1. Pottipati, S., Chakma, R., Haq, I., & Kalamdhad, A. S. (2022). Composting and vermicomposting: Process optimization for the management of organic waste. Advanced Organic Waste Management: Sustainable Practices and Approaches, 33–43. https://doi.org/10.1016/B978-0-323-85792-5.00015-0
2. Pottipati, S., Kalamdhad, A.S., 2023. Large-scale rotary drum composting for urban organic waste management. Waste Management: Climate change and Sustainability. Taylor & Francis (in press).
3. Pottipati, S., Kalamdhad, A.S., 2023.An Investigation into the Scale Shift of an Efficient Biodegradation Practice from Batch to Pilot Scale Continuous Feeding Mode. Sustainable waste management practices. Springer Nature (in press).

Annexure II

Table A1. Correlation between the parameters (R3).

	DC	**RT**	**AT**	**MC**	**VS**	**AC**	**TOC**	**pH**	**EC**	**TN**	**TP**	**AP**	**TK**	**Na**	**Ca**	**OUR**	**CE**	**AN**	**C/N**
DC	1.00																		
RT	-0.88	1.00																	
AT	-0.45	0.65	1.00																
MC	-0.58	0.89	0.69	1.00															
VS	-0.99	0.88	0.36	0.59	1.00														
AC	0.99	-0.88	-0.36	-0.59	-1.00	1.00													
TOC	-0.99	0.88	0.36	0.59	1.00	-1.00	1.00												
pH	0.86	-1.00	-0.68	-0.91	-0.86	0.86	-0.86	1.00											
EC	-0.95	0.85	0.22	0.58	0.98	-0.98	0.98	-0.83	1.00										
TN	0.98	-0.85	-0.27	-0.55	-1.00	1.00	-1.00	0.83	-0.99	1.00									
TP	0.99	-0.80	-0.30	-0.46	-0.99	0.99	-0.99	0.78	-0.96	0.99	1.00								
AP	1.00	-0.88	-0.47	-0.57	-0.99	0.99	-0.99	0.86	-0.94	0.97	0.98	1.00							
TK	1.00	-0.90	-0.42	-0.61	-1.00	1.00	-1.00	0.88	-0.97	0.99	0.98	0.99	1.00						
Na	0.98	-0.79	-0.30	-0.44	-0.98	0.98	-0.98	0.76	-0.95	0.98	1.00	0.98	0.98	1.00					
Ca	1.00	-0.87	-0.37	-0.57	-1.00	1.00	-1.00	0.85	-0.97	0.99	0.99	0.99	1.00	0.99	1.00				
OUR	-0.98	0.96	0.56	0.73	0.97	-0.97	0.97	-0.95	0.93	-0.95	-0.93	-0.98	-0.98	-0.93	-0.97	1.00			
CE	-0.97	0.97	0.55	0.76	0.97	-0.97	0.97	-0.96	0.93	-0.95	-0.92	-0.96	-0.98	-0.91	-0.96	1.00	1.00		
AN	-0.89	1.00	0.66	0.89	0.89	-0.89	0.89	-1.00	0.85	-0.85	-0.81	-0.88	-0.90	-0.80	-0.88	0.96	0.97	1.00	
C/N	-0.97	0.93	0.38	0.69	0.99	-0.99	0.99	-0.91	0.98	-0.98	-0.95	-0.96	-0.99	-0.94	-0.98	0.98	0.98	0.93	1.00

DC: Duration of composting, RT: Reactor temperature, AT: Ambient temperature, CE: CO_2 Evolution rate AN: NH_4-N

Table A2: Correlation between the parameters (R1)

	DAY	RT	AT	MC	VS	AC	TOC	pH	EC	TN	TP	AP	TK	Na	Ca	OU	CE	AN	C/N
DAY	1.00																		
RT	-0.86	1.00																	
AT	-0.45	0.72	1.00																
MC	-0.90	0.90	0.35	1.00															
VS	-0.99	0.88	0.41	0.95	1.00														
AC	0.99	-0.88	-0.41	-0.95	-1.00	1.00													
TOC	-0.99	0.88	0.41	0.95	1.00	-1.00	1.00												
pH	0.86	-1.00	-0.73	-0.90	-0.89	0.89	-0.89	1.00											
EC	-0.93	0.80	0.21	0.97	0.97	-0.97	0.97	-0.80	1.00										
TN	0.96	-0.96	-0.62	-0.92	-0.97	0.97	-0.97	0.96	-0.89	1.00									
TP	0.99	-0.86	-0.54	-0.85	-0.96	0.96	-0.96	0.87	-0.88	0.97	1.00								
AP	0.92	-0.98	-0.73	-0.87	-0.92	0.92	-0.92	0.98	-0.82	0.99	0.94	1.00							
TK	0.99	-0.91	-0.49	-0.94	-1.00	1.00	-1.00	0.91	-0.94	0.99	0.98	0.95	1.00						
Na	0.99	-0.79	-0.33	-0.87	-0.98	0.98	-0.98	0.79	-0.94	0.92	0.97	0.86	0.97	1.00					
Ca	0.99	-0.91	-0.51	-0.93	-0.99	0.99	-0.99	0.91	-0.93	0.99	0.98	0.95	1.00	0.97	1.00				
OU	-0.97	0.96	0.64	0.90	0.96	-0.96	0.96	-0.96	0.88	-1.00	-0.97	-0.99	-0.98	-0.92	-0.99	1.00			
CE	-0.95	0.97	0.57	0.96	0.97	-0.97	0.97	-0.97	0.92	-0.99	-0.93	-0.97	-0.98	-0.90	-0.98	0.98	1.00		
AN	-0.83	1.00	0.77	0.87	0.85	-0.85	0.85	-1.00	0.76	-0.95	-0.84	-0.98	-0.89	-0.76	-0.89	0.95	0.95	1.00	
C/N	-0.97	0.95	0.53	0.96	0.99	-0.99	0.99	-0.95	0.94	-0.99	-0.96	-0.97	-0.99	-0.94	-0.99	0.99	1.00	0.93	1.00

Table A3: Correlation between the parameters (R2)

	DAY	RT	AT	MC	VS	AC	TOC	pH	EC	TN	TP	AP	TK	Na	Ca	OU	CE	AN	C/N
DAY	1.00																		
RT	-0.88	1.00																	
AT	-0.45	0.65	1.00																
MC	-0.30	0.71	0.65	1.00															
VS	-0.99	0.89	0.36	0.34	1.00														
AC	0.99	-0.89	-0.36	-0.34	-1.00	1.00													
TOC	-0.99	0.89	0.36	0.34	1.00	-1.00	1.00												
pH	0.85	-1.00	-0.69	-0.75	-0.86	0.86	-0.86	1.00											
EC	-0.95	0.86	0.23	0.32	0.99	-0.99	0.99	-0.82	1.00										
TN	0.99	-0.86	-0.32	-0.28	-1.00	1.00	-1.00	0.83	-0.99	1.00									
TP	0.99	-0.80	-0.32	-0.15	-0.98	0.98	-0.98	0.76	-0.95	0.99	1.00								
AP	0.99	-0.93	-0.54	-0.41	-0.98	0.98	-0.98	0.91	-0.94	0.97	0.96	1.00							
TK	1.00	-0.88	-0.46	-0.30	-0.99	0.99	-0.99	0.85	-0.95	0.98	0.99	0.99	1.00						
Na	0.98	-0.76	-0.34	-0.09	-0.95	0.95	-0.95	0.72	-0.91	0.96	0.99	0.94	0.98	1.00					
Ca	0.99	-0.91	-0.42	-0.37	-1.00	1.00	-1.00	0.88	-0.97	0.99	0.98	0.99	0.99	0.95	1.00				
OU	-0.97	0.97	0.60	0.52	0.96	-0.96	0.96	-0.95	0.91	-0.95	-0.92	-0.99	-0.97	-0.90	-0.98	1.00			
CE	-0.95	0.98	0.57	0.58	0.96	-0.96	0.96	-0.97	0.93	-0.94	-0.89	-0.98	-0.95	-0.86	-0.97	0.99	1.00		
AN	-0.88	0.97	0.78	0.66	0.86	-0.86	0.86	-0.98	0.79	-0.83	-0.80	-0.94	-0.89	-0.78	-0.89	0.97	0.96	1.00	
C/N	-0.97	0.93	0.41	0.44	0.99	-0.99	0.99	-0.91	0.98	-0.98	-0.95	-0.98	-0.97	-0.91	-0.99	0.97	0.98	0.89	1.00

Table A4: Correlation between the parameters (R4)

	DAY	RT	AT	MC	VS	AC	TOC	pH	EC	TN	TP	AP	TK	Na	Ca	OU	CE	AN	C/N
DAY	1.00																		
RT	-0.88	1.00																	
AT	-0.45	0.65	1.00																
MC	-0.59	0.88	0.84	1.00															
VS	-0.98	0.91	0.36	0.61	1.00														
AC	0.98	-0.91	-0.36	-0.61	-1.00	1.00													
TOC	-0.98	0.91	0.36	0.61	1.00	-1.00	1.00												
pH	0.85	-0.99	-0.73	-0.92	-0.86	0.86	-0.86	1.00											
EC	-0.95	0.86	0.22	0.52	0.99	-0.99	0.99	-0.80	1.00										
TN	0.99	-0.83	-0.35	-0.50	-0.98	0.98	-0.98	0.79	-0.95	1.00									
TP	0.99	-0.82	-0.31	-0.47	-0.98	0.98	-0.98	0.77	-0.96	1.00	1.00								
AP	0.99	-0.86	-0.55	-0.60	-0.94	0.94	-0.94	0.84	-0.88	0.97	0.96	1.00							
TK	0.99	-0.93	-0.44	-0.65	-1.00	1.00	-1.00	0.89	-0.97	0.98	0.98	0.96	1.00						
Na	0.99	-0.81	-0.32	-0.46	-0.97	0.97	-0.97	0.76	-0.95	1.00	1.00	0.97	0.97	1.00					
Ca	1.00	-0.86	-0.43	-0.55	-0.97	0.97	-0.97	0.82	-0.94	1.00	0.99	0.99	0.98	0.99	1.00				
OU	-0.97	0.96	0.62	0.76	0.96	-0.96	0.96	-0.95	0.90	-0.94	-0.93	-0.97	-0.98	-0.92	-0.96	1.00			
CE	-0.95	0.98	0.53	0.78	0.97	-0.97	0.97	-0.96	0.94	-0.92	-0.91	-0.92	-0.98	-0.90	-0.93	0.98	1.00		
AN	-0.88	0.99	0.73	0.90	0.88	-0.88	0.88	-1.00	0.81	-0.82	-0.81	-0.88	-0.91	-0.80	-0.86	0.97	0.96	1.00	
C/N	-0.98	0.94	0.42	0.66	1.00	-1.00	1.00	-0.90	0.98	-0.96	-0.97	-0.94	-1.00	-0.95	-0.97	0.97	0.99	0.91	1.00

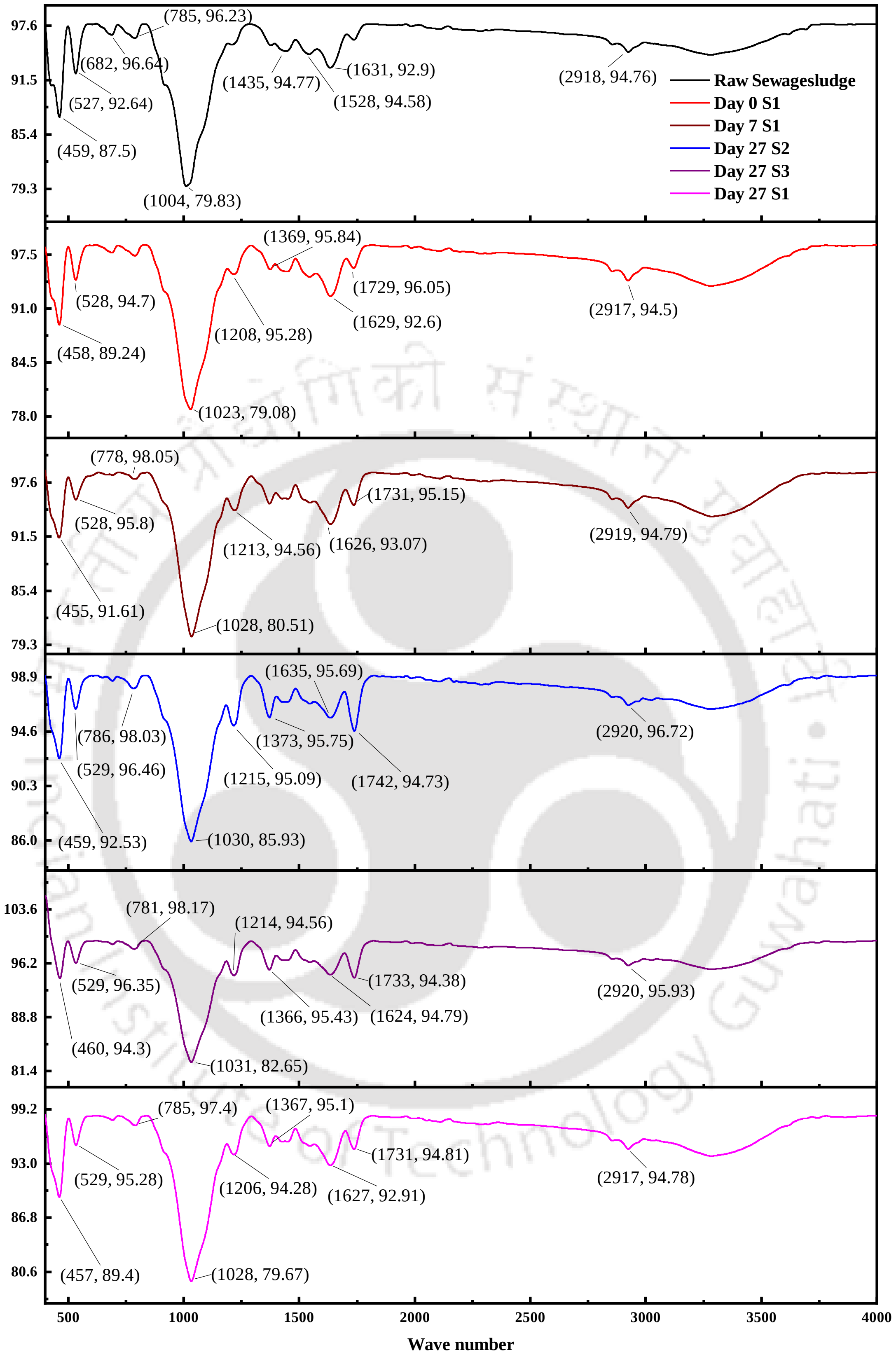
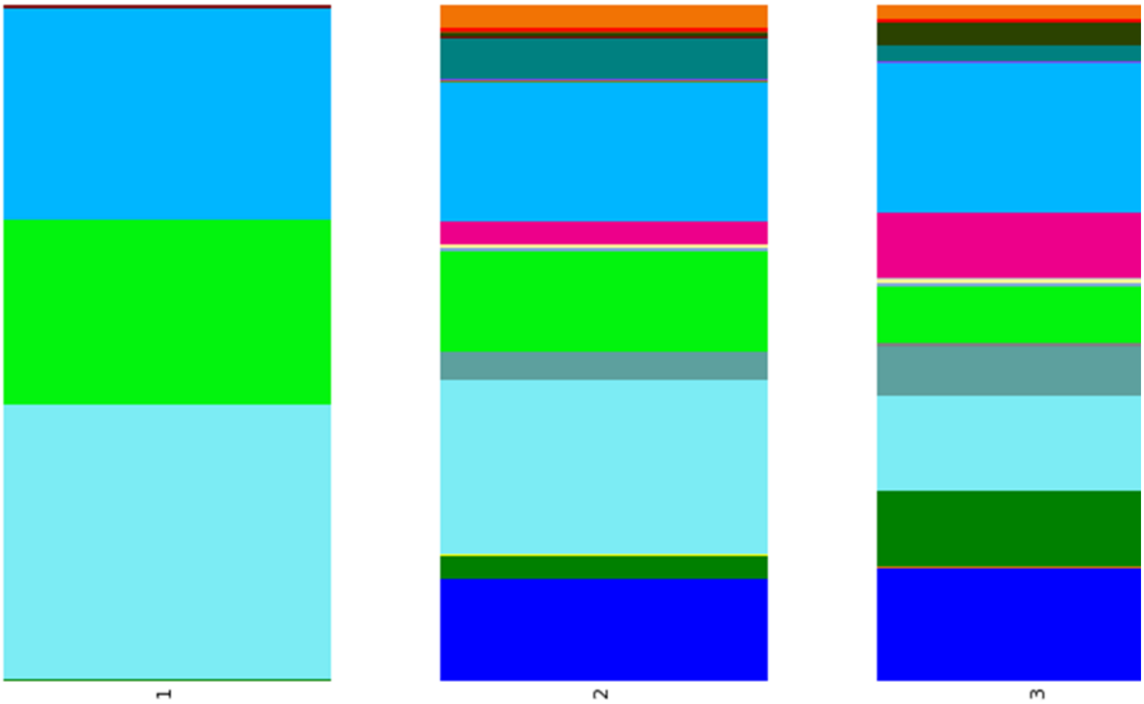
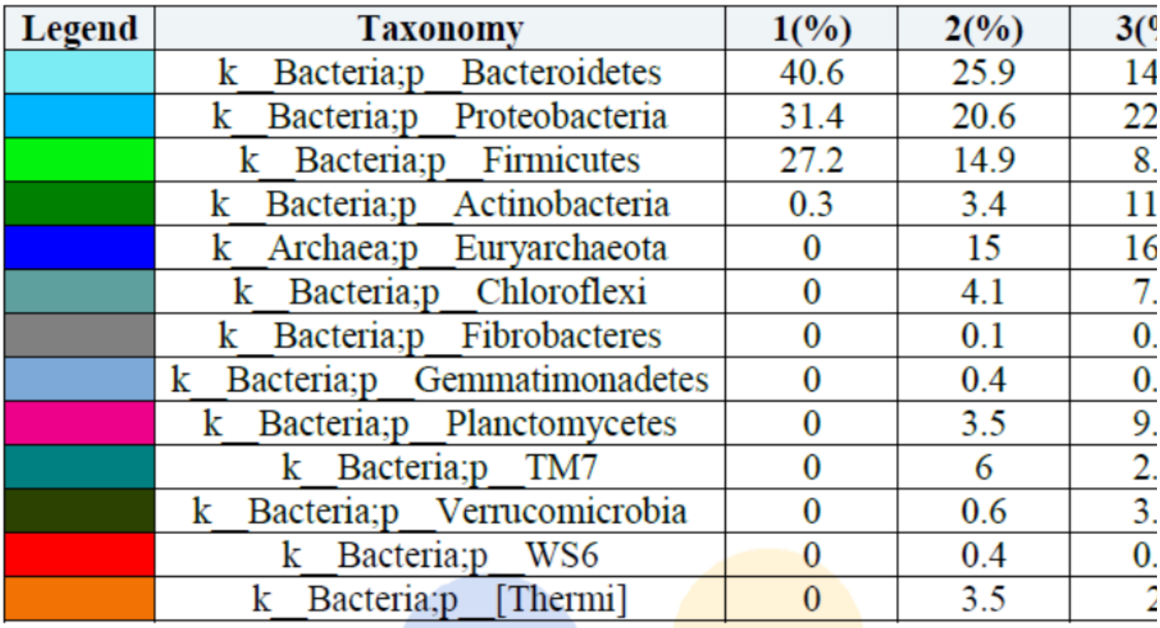


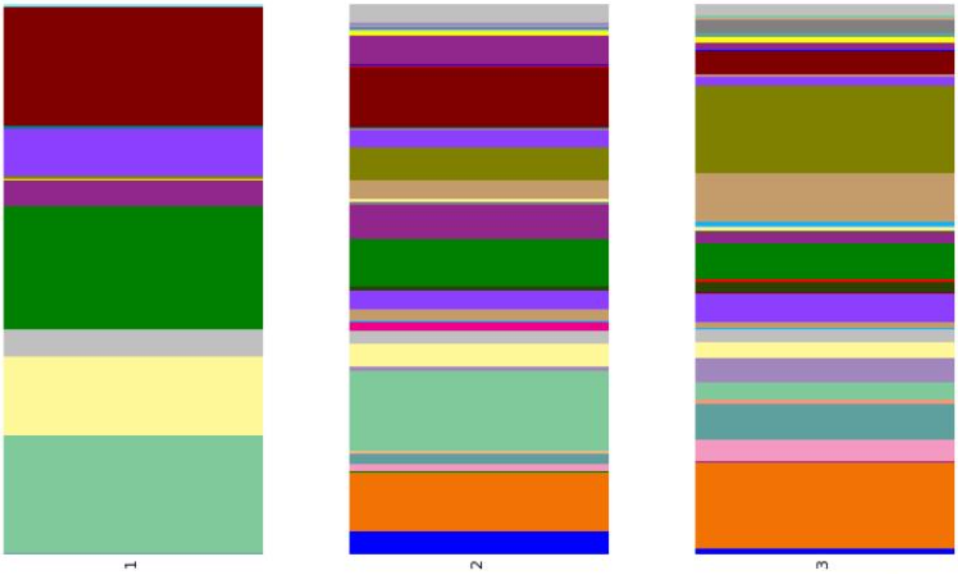
Figure A1. FTIR spectroscopic of the feedstock samples of S1, S2 and S3 experimented with trails during the stabilization process and raw sewage sludge.





Legend	Taxonomy	1(%)	2(%)	3(
🟦	k__Bacteria;p__Bacteroidetes	40.6	25.9	14
🟦	k__Bacteria;p__Proteobacteria	31.4	20.6	22
🟩	k__Bacteria;p__Firmicutes	27.2	14.9	8.
🟩	k__Bacteria;p__Actinobacteria	0.3	3.4	11
🟦	k__Archaea;p__Euryarchaeota	0	15	16
🟦	k__Bacteria;p__Chloroflexi	0	4.1	7.
⬜	k__Bacteria;p__Fibrobacteres	0	0.1	0.
🟦	k__Bacteria;p__Gemmatimonadetes	0	0.4	0.
🟪	k__Bacteria;p__Planctomycetes	0	3.5	9.
🟦	k__Bacteria;p__TM7	0	6	2.
⬛	k__Bacteria;p__Verrucomicrobia	0	0.6	3.
🟥	k__Bacteria;p__WS6	0	0.4	0.
🟧	k__Bacteria;p__[Thermi]	0	3.5	2

Fig. A2. Relative abundance of each phylum within each sample



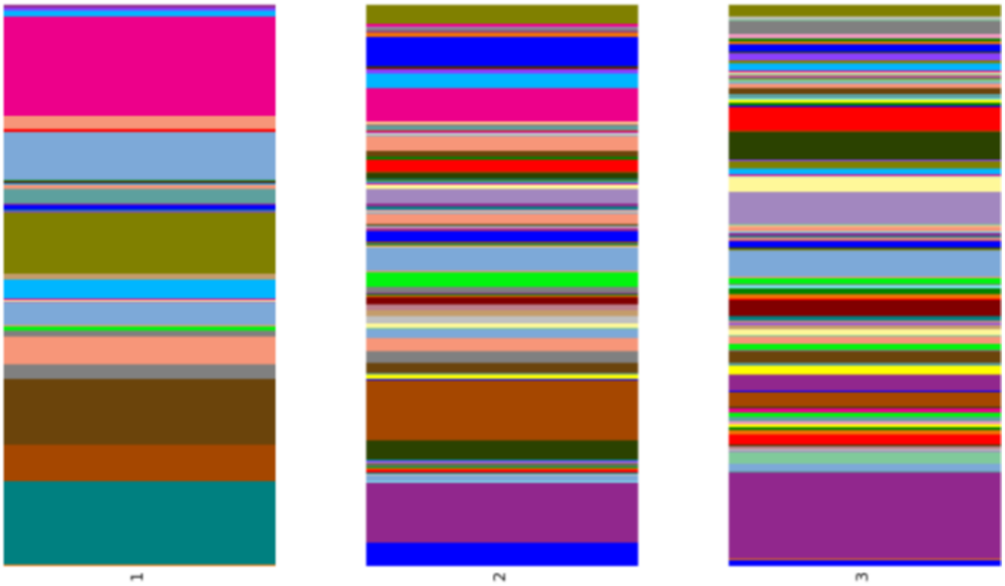
Legend	Taxonomy	1(%)	2(%)	3(%)
🟩	k__Bacteria;p__Firmicutes;c__Bacilli	22.4	8.6	6.6
🟥	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	21.8	11	4.2
🟩	k__Bacteria;p__Bacteroidetes;c__Bacteroidia	21.4	14.7	3
🟨	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia	14.3	4.2	2.9
🟪	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria	8.8	3	1.6
⬜	k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia	4.9	2.3	2.2
🟪	k__Bacteria;p__Firmicutes;c__Clostridia	4.8	6.3	1.9
🟫	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria	0.5	6	15.8
🟦	k__Bacteria;p__Actinobacteria;c__Actinobacteria	0.3	2	6.6
🟦	k__Archaea;p__Euryarchaeota;c__Methanobacteria	0	4	1.1
🟧	k__Archaea;p__Euryarchaeota;c__Methanomicrobia	0	10.7	15.5
🟪	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia	0	1.2	3.7
🟧	k__Bacteria;p__Actinobacteria;c__Thermoleophilia	0	0.2	0.7
🟪	k__Bacteria;p__Bacteroidetes;c__Cytophagia	0	0.8	4.6
🟥	k__Bacteria;p__Bacteroidetes;c__VC2_1_Bac22	0	1.4	0
🟫	k__Bacteria;p__Bacteroidetes;c__[Saprospirae]	0	2.1	1.2
🟪	k__Bacteria;p__Chloroflexi;c__Anaerolineae	0	3.3	5.2
⬛	k__Bacteria;p__Chloroflexi;c__Thermomicrobia	0	0.8	1.8
🟥	k__Bacteria;p__Fibrobacteres;c__Fibrobacteria	0	0.1	0.5
🟦	k__Bacteria;p__Planctomycetes;c__Phycisphaerae	0	0.2	1
🟫	k__Bacteria;p__Planctomycetes;c__Planctomycetia	0	3.3	8.8
🟥	k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria	0	0.5	0.5
🟪	k__Bacteria;p__TM7;c__TM7-1	0	5.2	1.3
🟨	k__Bacteria;p__TM7;c__TM7-3	0	0.8	1
🟦	k__Bacteria;p__Verrucomicrobia;c__Opitutae	0	0	0.8
⬛	k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae	0	0.4	2.3
🟩	k__Bacteria;p__WS6;c__B142	0	0	0.5
⬜	k__Bacteria;p__[Thermi];c__Deinococci	0	3.5	2

Fig. A3. Relative abundance of each class within each sample

Legend	Taxonomy	1(%)	2(%)	3(%)
🟥	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales	21.4	14.7	3
🟫	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales	18.9	8.7	1.6
🟨	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales	15.9	0.2	0.2
🟫	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales	14.3	4.2	2.9
🟪	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales	8.6	2.8	1.2
🟩	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales	6.1	8.3	6.4
🟫	k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales	4.9	2.3	2.2
🟫	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales	4.6	5.2	1.8
🟫	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales	2.3	0	0
🟥	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales	0.5	0.8	1.8
🟩	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales	0.3	2	6.6
⬛	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales	0.2	4	11

Legend	Taxonomy	1(%)	2(%)	3(%)
🟦	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales	0.1	0.2	1.2
🟫	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales	0.1	0.4	0.7
🟦	k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales	0	4	1.1
🟩	k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales	0	10.5	15.3
🟫	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales	0	1.2	3.7
🟪	k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales	0	0	0.6
🟦	k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales	0	0.8	4.6
🟪	k__Bacteria;p__Bacteroidetes;c__VC2_1_Bac22;o__	0	1.4	0
🟦	k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales]	0	2.1	1.2
🟩	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales	0	1	0.7
🟦	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__GCA004	0	0.9	0.7
🟧	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031	0	1.3	3.6
🟥	k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__AKYG1722	0	0.4	0.8
🟦	k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45	0	0.2	0.9
🟩	k__Bacteria;p__Fibrobacteres;c__Fibrobacteria;o__258ds10	0	0	0.5
⬜	k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__WD2101	0	0.1	0.8
🟫	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales	0	2.4	5.7
🟩	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales	0	0.9	2.8
🟥	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales	0	0	0.6
🟧	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales	0	1	1.9
🟪	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales	0	1.1	0.3
🟪	k__Bacteria;p__TM7;c__TM7-1;o__	0	5.2	1.3
🟨	k__Bacteria;p__TM7;c__TM7-3;o__	0	0.7	0.6
🟦	k__Bacteria;p__TM7;c__TM7-3;o__EW055	0	0.1	0.5
⬛	k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales	0	0	0.6
🟦	k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales	0	0.4	2.3
⬜	k__Bacteria;p__WS6;c__B142;o__	0	0	0.5
🟩	k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales	0	3.5	2

Fig. A4 Relative abundance of each order within each sample



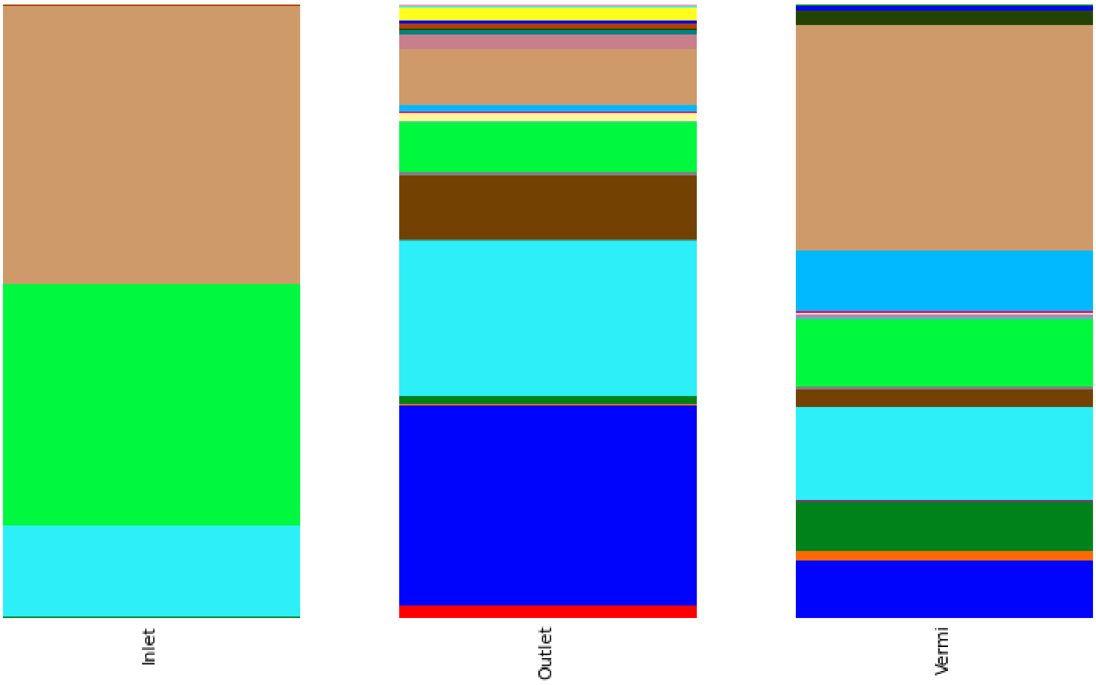
Legend	Taxonomy	1(%)	2(%)	3(%)
🟪	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae	17.8	6.1	0.3
🟦	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae	14.7	0	0
🟫	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae	11.5	1.8	2.2
🟩	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae	11.1	0.1	0.1
🟦	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	8.5	0.4	0.3
🟧	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae	6.7	10.7	2.5
🟧	k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae	4.9	2.3	1.3
🟦	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae	3.8	4.4	4.7
🟦	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae	3.4	0	0

Legend	Taxonomy	1(%)	2(%)	3(%)
⬛	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae]	2.7	2.1	0.3
🟦	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Symbiobacteriaceae	2.5	0.3	0
🟧	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae	2.3	0	0
🟦	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae	1.1	2.6	1.3
⬛	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__	1	1.1	0.3
🟫	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae	1	0	0.1
🟧	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae]	0.9	1.8	0.2
🟩	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae	0.8	2.5	1.2
🟦	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	0.6	1.7	1.1
🟪	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae	0.5	0.7	1.4
🟦	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae	0.1	0.2	1.2
🟩	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae	0.1	0.4	0.5
🟧	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae	0.1	2.4	0.8
🟦	k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae	0	4	1.1
🟪	k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales;f__Methanosarcinaceae	0	10.5	15.3
🟦	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__	0	0.9	1.1
🟩	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111	0	0.1	2.1
🟥	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae	0	0.3	1.9
🟧	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae	0	0.5	0.8
🟨	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae	0	0	0.7
🟪	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae	0	0	0.5
🟦	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae	0	0.2	0.7
🟩	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae	0	0.1	0.8
🟪	k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__	0	0	0.6

Legend	Taxonomy	1(%)	2(%)	3(%)
🟩	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Marinilabiaceae	0	3.4	0.2
🟪	k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cyclobacteriaceae	0	0	3
🟨	k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae	0	0.8	1.6
🟩	k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__	0	0	0.9
🟦	k__Bacteria;p__Bacteroidetes;c__VC2_1_Bac22;o__;f__	0	1.4	0
🟨	k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae	0	0.8	1.1
⬜	k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Saprospiraceae	0	1.2	0.1
🟫	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolinaceae	0	1	0.7
🟪	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__GCA004;f__	0	0.9	0.7
🟦	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__A4b	0	0.1	0.8
🟥	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__SHA-31	0	1.3	2.9
🟧	k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__AKYG1722;f__	0	0.4	0.8
🟩	k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45;f__	0	0.2	0.9
🟦	k__Bacteria;p__Fibrobacteres;c__Fibrobacteria;o__258ds10;f__	0	0	0.5
🟧	k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__WD2101;f__	0	0.1	0.8
🟪	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae	0	2.4	5.7
🟨	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae	0	0.9	2.8
🟩	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__	0	0.2	1.3
🟩	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae	0	1.3	5
🟥	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae	0	2.2	4.2
🟨	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae	0	0	0.5
🟦	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae	0	0	0.9
🟫	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae	0	1	0.9
🟦	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Idiomarinaceae	0	0.7	0
🟩	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae	0	0.1	0.5
🟦	k__Bacteria;p__TM7;c__TM7-1;o__;f__	0	5.2	1.3

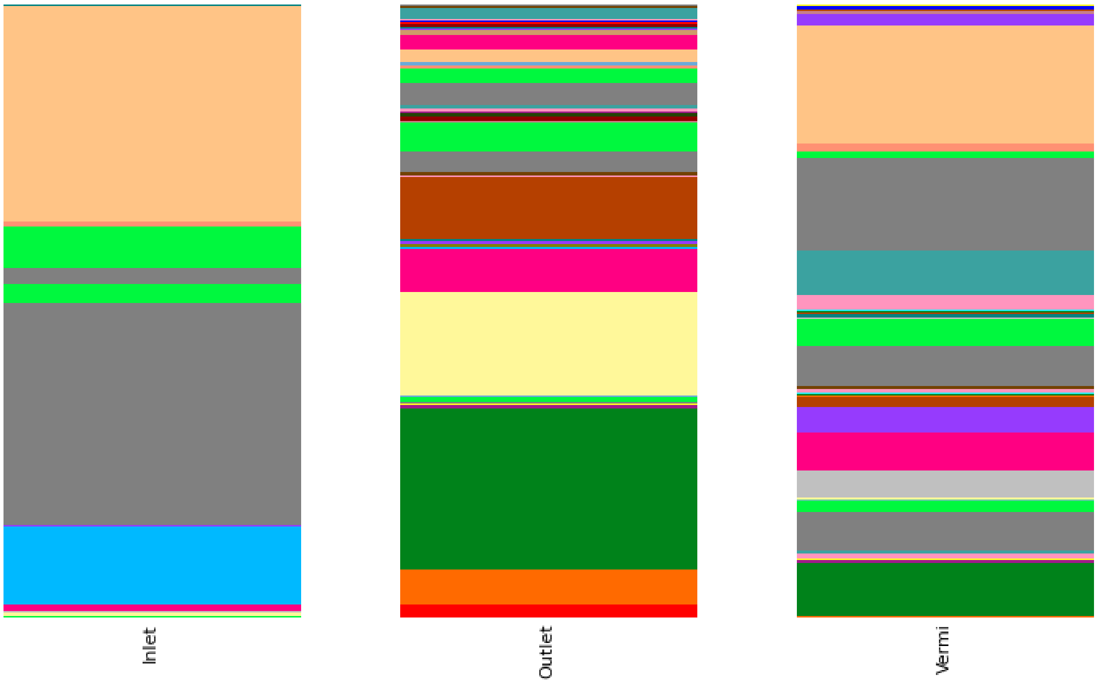
Legend	Taxonomy	1(%)	2(%)	3(%)
🟧	k__Bacteria;p__TM7;c__TM7-3;o__;f__	0	0.7	0.6
🟩	k__Bacteria;p__TM7;c__TM7-3;o__EW055;f__	0	0.1	0.5
🟪	k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae	0	0	0.6
⬛	k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae	0	0.4	2.3
⬜	k__Bacteria;p__WS6;c__B142;o__;f__	0	0	0.5
🟫	k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales;f__Trueperaceae	0	3.5	2

Fig A5 Relative abundance of each family within each sample



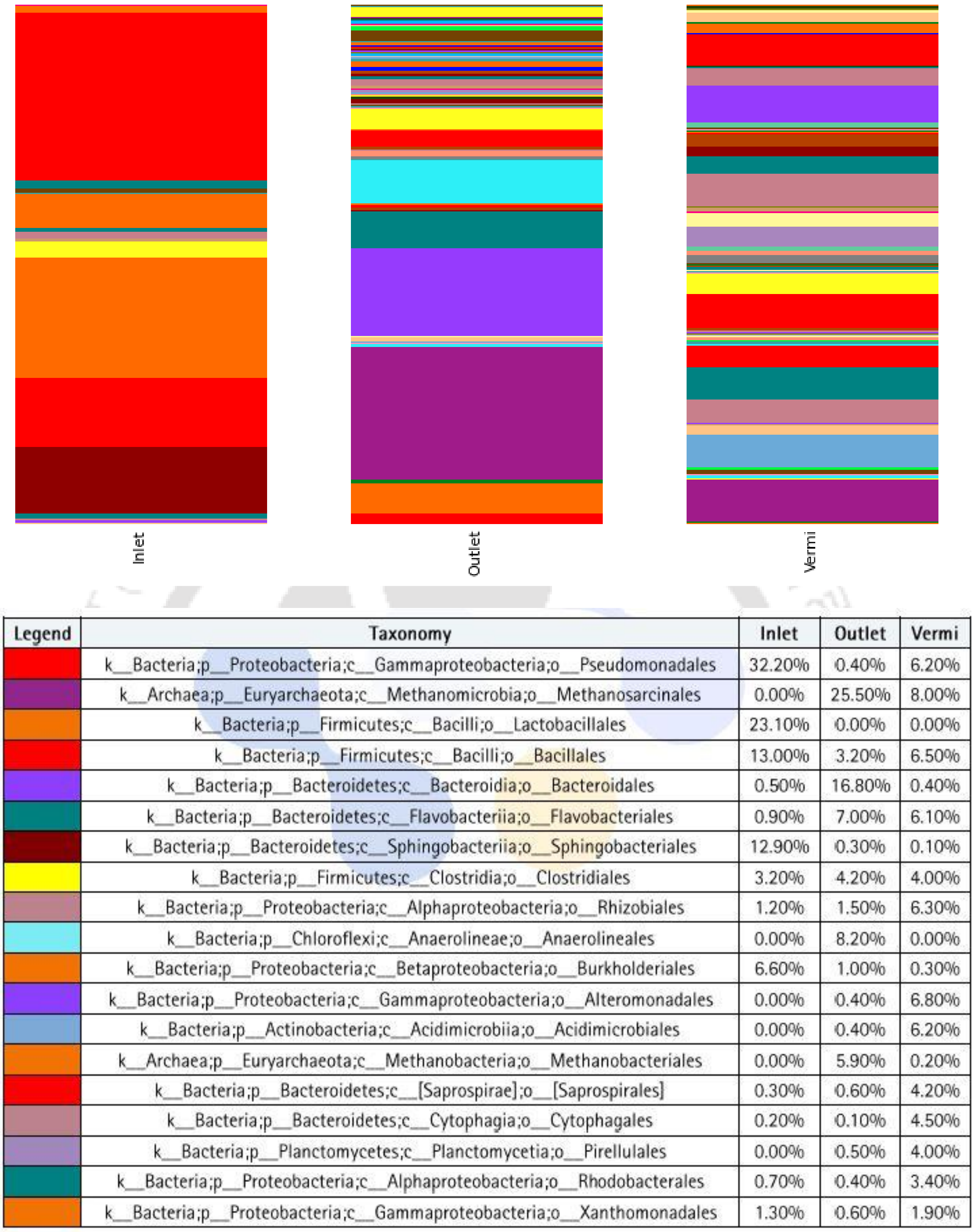
Legend	Taxonomy	Inlet	Outlet	Vermi
🟫	k__Bacteria;p__Proteobacteria	45.20%	9.00%	36.60%
🟩	k__Bacteria;p__Firmicutes	39.30%	7.90%	11.00%
🟦	k__Bacteria;p__Bacteroidetes	14.70%	25.30%	15.20%
🟦	k__Archaea;p__Euryarchaeota	0.00%	32.50%	9.20%
🟫	k__Bacteria;p__Chloroflexi	0.00%	10.50%	2.80%
🟦	k__Bacteria;p__Planctomycetes	0.00%	1.10%	9.80%
🟩	k__Bacteria;p__Actinobacteria	0.30%	1.30%	8.20%
🟩	k__Bacteria;p__TM7	0.10%	0.20%	2.30%
🟪	k__Bacteria;p__Spirochaetes	0.00%	2.30%	0.00%
🟥	k__Archaea;p__Crenarchaeota	0.00%	2.00%	0.10%
🟧	k__Bacteria;p__Acidobacteria	0.00%	0.30%	1.60%
🟨	k__Bacteria;p__WS6	0.00%	2.10%	0.00%
🟨	k__Bacteria;p__OD1	0.00%	1.20%	0.30%
🟦	k__Bacteria;p__Verrucomicrobia	0.10%	0.40%	0.80%
⬛	k__Bacteria;p__Fibrobacteres	0.00%	0.60%	0.40%
🟦	k__Bacteria;p__Synergistetes	0.00%	0.90%	0.00%

Fig. A6. Comparative analysis of samples at Phylum level



Legend	Taxonomy	Inlet	Outlet	Vermi
🟧	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	35.00%	2.00%	19.10%
⬛	k__Bacteria;p__Firmicutes;c__Bacilli	36.10%	3.20%	6.60%
🟩	k__Archaea;p__Euryarchaeota;c__Methanomicrobia	0.00%	26.30%	8.50%
⬛	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria	2.60%	3.60%	15.20%
🟨	k__Bacteria;p__Bacteroidetes;c__Bacteroidia	0.50%	16.80%	0.40%
🟥	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia	0.90%	7.00%	6.10%
🟦	k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia	12.90%	0.30%	0.10%
🟩	k__Bacteria;p__Firmicutes;c__Clostridia	3.20%	4.70%	4.40%
🟫	k__Bacteria;p__Chloroflexi;c__Anaerolineae	0.00%	10.30%	1.50%
🟩	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria	6.60%	2.20%	0.90%
🟦	k__Bacteria;p__Planctomycetes;c__Planctomycetia	0.00%	0.70%	7.40%
⬛	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia	0.00%	0.40%	6.20%
🟧	k__Archaea;p__Euryarchaeota;c__Methanobacteria	0.00%	5.90%	0.20%
🟪	k__Bacteria;p__Bacteroidetes;c__[Saprospirae]	0.30%	0.60%	4.20%
⬜	k__Bacteria;p__Bacteroidetes;c__Cytophagia	0.20%	0.10%	4.50%
🟧	k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria	0.90%	0.60%	1.40%
🟩	k__Bacteria;p__Actinobacteria;c__Actinobacteria	0.30%	0.70%	1.80%
🟪	k__Bacteria;p__Planctomycetes;c__Phycisphaerae	0.00%	0.40%	2.40%
🟥	k__Bacteria;p__Spirochaetes;c__Spirochaetes	0.00%	2.30%	0.00%

Fig. A7 Comparative analysis of samples at Class level



Legend	Taxonomy	Inlet	Outlet	Vermi
🟥	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales	32.20%	0.40%	6.20%
🟪	k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales	0.00%	25.50%	8.00%
🟧	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales	23.10%	0.00%	0.00%
🟥	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales	13.00%	3.20%	6.50%
🟪	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales	0.50%	16.80%	0.40%
🟦	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales	0.90%	7.00%	6.10%
🟫	k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales	12.90%	0.30%	0.10%
🟨	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales	3.20%	4.20%	4.00%
🟫	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales	1.20%	1.50%	6.30%
🟦	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales	0.00%	8.20%	0.00%
🟧	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales	6.60%	1.00%	0.30%
🟪	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales	0.00%	0.40%	6.80%
🟦	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales	0.00%	0.40%	6.20%
🟧	k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales	0.00%	5.90%	0.20%
🟥	k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales]	0.30%	0.60%	4.20%
🟫	k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales	0.20%	0.10%	4.50%
🟪	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales	0.00%	0.50%	4.00%
🟦	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales	0.70%	0.40%	3.40%
🟧	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales	1.30%	0.60%	1.90%

Fig. A8. Comparative analysis of samples at Order level