Identification and Degradation of Toxic Composites during the Composting of Terrestrial Weeds

A thesis submitted in partial fulfilment of the requirements
for the award of the degree of

Doctor of Philosophy

in

Environmental Engineering

by

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January, 2024

IN EXPRESSION OF MY THANKFULNESS TO MY

Mummy and **Daddy**

For their unconditional love and support even during my hardest times.

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Declaration of Originality

I, M. Krishna Chaitanya, declare that this thesis titled, "**Identification and Degradation of Toxic Composites during the Composting of Terrestrial Weeds**" and the work presented in it are my own. I confirm that:

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Certificate

This is to certify that the thesis entitled "**Identification and Degradation of Toxic Composites during the Composting of Terrestrial Weeds**", submitted by M. Krishna Chaitanya (196104013), 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

Acknowledgements

The pursuit of my doctoral degree at the Indian Institute of Technology Guwahati has provided me with an unparalleled and transformative academic experience. This Doctoral dissertation would not have been feasible without the invaluable assistance provided by several persons during the course of the previous four years. I would like to express my utmost appreciation to my supervisor, **Prof. Ajay Kalamdhad**, for his invaluable guidance and mentorship during my academic journey. This thesis is an outcome of his motivation and direction provided over the course of my research. His unwavering confidence in my aptitude has consistently served as a source of motivation, prompting me to maintain a steadfast commitment and concentration. I will make a concerted effort to uphold the ideals and work culture that he has imparted to me over this period, to the best of my capabilities.

I would like to express my gratitude to the members of my Doctoral Committee, namely **Prof. Sreedeep S. (Chairman)**, **Dr. Ravi K.**, and **Dr. Lalit Mohan Pandey**, for their invaluable contributions to my study. The recommendations and feedback provided by them have significantly enhanced the quality of my research. I am very thankful to **Prof. Ajay Kalamdhad** for his invaluable support and guidance, which played a pivotal role in my selection for the esteemed Research Intern position at **Polytechnique Montreal University, Canada** for the academic year 2022-2023.

I would like to express my gratitude to the **Director** of IIT Guwahati for their provision of essential resources and the establishment of a favourable academic atmosphere. I would like to express my gratitude to the Ministry of Education, India, for their invaluable financial assistance. I express my gratitude to the Central Instruments Facility (CIF) and Central Library at IIT Guwahati for providing me with the superior research infrastructure.

My greatest gratitude goes to the Head of the Civil Engineering Department for providing me with the required laboratory and research facilities. My heartfelt thanks to the Civil Department's technical personnel for their assistance with administrative tasks. I am grateful to **Mr. Chitaranjan Medhi**, **Mrs. Jonali Saikia** and **Mr. Payodhar Pathak** for their assistance during all stages of my study.

I express my gratitude to the dedicated individuals of the **Waste Management Research Group (WMRG)** for their indispensable support and stimulating dialogues. I experienced great pleasure in becoming affiliated with the members of the WMRG.

I wish to put on record my sincere gratitude to all anonymous reviewers and respective editors of journals for their valuable insights into all my published manuscripts. Their highly scientific inputs have helped in upgrading my research studies.

I will be very grateful to **Dr. Izharul Haq**, an assistant professor from Jaipur National University (a former Post-doctoral Research Associate in Department of civil engineering, IIT Guwahati) for teaching me valuable experiments, and getting me my 1st Scopus indexed book chapter. I am very much privilege to get a mentor and a friend, closest to my heart **Dr. Nzanthung Ngullie** (Assistant Professor at NIT Nagaland), who was there in every situation of my life and guided me through various paths to build my career. Over the years, I am privilege and lucky to have a brother, closest friend **Dr. Venkatesh Reddy** (Post-doctoral fellow at Hangyang University), who was always there for me as a own brother and guided, supported me in every aspect of my PhD career and life.

Over the course of several years, I have developed a unique and special bonding with **Ms. Anwesha Banerjee**, a former master student in the Department of Civil Engineering at the Indian Institute of Technology Guwahati (Currently a PhD scholar from McGill University, Canada). I express profound gratitude for her invaluable contributions to my scientific endeavour.

My deepest thanks to my special and supportive circle of friends, **Dr. Viswanth** , **late Mr. Kranthi Kumar**, **Mr. Mukesh**, **Dr. Muniraja**, **Dr. Vinay Gadi**, **Dr. Suresh**, **Mr. Bhaskar Kethireddy**, **Mr. Boddepalli Uday**, **Dr. Rohith Sangineni**, **Mr. Satish Chandra**, **Mr. Venkat Naga Chandra**, **Mr. Sreekanth Yadav**, **Mr. Anil Mekali**, **Mr. Ravi Kiran** for their care, intellectual guidance, encouragement, and love for making me feel homely and joyful during my stay in the Campus. My heartful thanks to my closest **Ms. Aparna Gupta**, for her intellectual and emotional support, throughout my PhD journey.

In the due course of time, I had cherish memories with my close friends **Ms. Vaishnavi Jahagirdar**, **Ms. Ashmita Kundu**, **Mr. Suryateja Pottipati**, **Mr. Sumantra Chaudhury, and Ms. Sneha Sree** in various technical discussions, trips, tea ceremonies.

I would like to express my sincere gratitude to my seniors Dr. Mayur Jain, Dr. Jayeeta Hazarika, Dr. Kunwar Raghavendra Singh, Dr. Sachin Kumar Tomar, Dr. Siddhant Dash, Dr. Ankit Pratim Goswami, Dr. Shweta Singh, Dr. Heena Kauser, Mr. Arun Sathyan, Mr. Induchoodan, Mr. Anjishnu Biswas, Ms. Menon Devi, Ms. Priyanka Kotoky, and Dr. Shinjini Paul Choudhury, who have given valuable inputs and suggestions during my experimental and analytical works in the laboratory. I express my gratitude to my colleagues, namely Mr. Prakash Singh, Ms. Silvia Saikia, Mr. Ankit Kumar, and Ms. Sangamitra Patra; my beloved PhD juniors Ms. Lamyanbi, Ms. Jyoti, Ms. Sujata, Mr. Zaminson, Ms. Sumona Koley, Ms. Sanjana, Ms. Akhila, Mrs. Nidhi Chhabra, and Mr. Kiran Venkatesh for fostering a sense of comfort and happiness throughout my tenure at the Campus.

I would like to express my sincere gratitude to the former master students especially Mr. Harsh Prit, Mr. Sajan Kumar Dansena, Mr. Aman, Mr. Vivek Kumar Saw, and Mr. Prakash for being there for me and supporting me with their positive attitude. I would also like to thank current M. Tech students Mr. Gagandeep Singh, Mr. Arif Khan, Mr. Jashraj, Ms. Sonali, and Ms. Beni for their insightful questions and curiosity related to my thesis.

I am grateful to my siblings, **Miss. Gopika Purnima Maturi** and **Mr. Chiranjeevi Venkat Harsha Maturi**, for their nurturing and affectionate support, which has always served as a source of inspiration for me. I would like to extend my heartfelt appreciation to my esteemed parents, my mother, **Mrs. Ganga Bhavani Maturi**, and my father, **Mr. Gopi Krishna Rao Maturi**, for their unwavering support, consistent motivation, unconditional affection, and blessings throughout my academic journey, including the arduous process of conducting research and composing this thesis. This achievement would not have been attainable without their contribution.

Above all, I would like to thank the Almighty for giving me the wisdom, health, strength and perseverance to complete this research.

Date:

Krishna Chaitanya Maturi

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List of Abbreviations

AAS	Atomic Absorption Spectrometer
AIL	Acid Insoluble Lignin
ASL	Acid Soluble Lignin
BD	Bulk Density
BOM	Biodegradable Organic Matter
BR	Burnt Residue
CA	Chromosomal Abnormalities
CEC	Cation Exchange Capacity
CFCF	Cell Free Culture Filtrate
DO	Dissolved Oxygen
DRIFTS	Diffuse Reflectance Infrared Fourier Transform Spectroscopy
DSC	Differential Scanning Calorimetry
DTPA	Diethylenetriamine Penta acetic acid
EC	Electrical Conductivity
EF	Enrichment Factor
FTIR	Fourier Transform Infrared Spectroscopy
GC-FID	Gas Chromatography Flame Ionization Detector
GC-MS	Gas Chromatography Mass Spectroscopy
GI	Germination Index
GR	Germination Rate
HVC	*Hydrilla Verticillate* Compost
IWM	Integrated Weed Management
LD	Lethal Dose
MI	Mitotic Index
NA	Nuclear Abnormalities
OUR	Oxygen Uptake Rate
RDC	Rotary Drum Composting
RL	Root Length
sBOD	Soluble Biochemical Oxygen Demand
sCOD	Soluble Chemical Oxygen Demand

SEM	Scanning Electron Microscope
SL	Shoot Length
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TCLP	Toxicity Characteristics Leaching Procedure
TDA	Thermodifferential Analysis
TGA	Thermogravimetric Analysis
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
UA	Uric Acid
UR	Unburnt Residue
VOCs	Volatile Organic Compounds
VWC	Vegetable Waste Rotary Drum Compost
WHC	Water Hyacinth Compost

Abstract

The proliferation of terrestrial weeds on a worldwide scale, such as *Ageratum conyzoides*, *Parthenium hysterophorus*, and *Lantana camara*, presents noteworthy ecological and economic complexities. *A. conyzoides*, sometimes referred to as goatweed, is widely distributed over diverse tropical and subtropical climates, where it exhibits prolific growth and often leads to significant infestations. *P. hysterophorus*, also referred to as "famine weed," is a well-known invasive species that exerts a detrimental impact on pastures, crops, and ecosystems. Its ability to transfer seeds by wind plays a significant role in facilitating its widespread distribution around the globe. The shrub *L. camara* demonstrates a remarkable ability to thrive in a wide range of ecological settings, hence exhibiting a competitive advantage over indigenous plant species. Cumulatively, the presence of these undesirable plant species has resulted in significant reductions in agricultural output, elevated expenses for their control and mitigation, and adverse effects on the variety of living organisms. The proliferation of these undesirable plants requires the implementation of comprehensive solutions to address their negative impacts and maintain the integrity of the ecosystem.

This doctorate research thesis explores and analyses the management strategies employed in the conversion of terrestrial weeds into agricultural products with added value. During the initial phase of the investigation, an analysis was conducted to characterize terrestrial weeds in order to assess their feasibility for the biodegradation process. The analysis encompassed the examination of many characterisation parameters, including volatile solids (VS), moisture content, C/N ratio, Total kjeldahl nitrogen, Available phosphorus, and potassium. The variability of *A. conyzoides*, *P. hysterophorus*, and *L. camara* was observed to vary from 70.1 to 73.5% for volatile solids (VS), whereas the moisture content ranged from 72 to 74%. Following the completion of the feasibility assessment, the Rotary Drum Composting (RDC) method was employed to facilitate the bioconversion of terrestrial weeds.

Furthermore, these terrestrial weeds have the tendency to uptake heavy metals (HMs) from their roots. To understand the transformation and reduction mechanism of HMs during the composting process, an experimental based approach was implemented which includes bioavailability, leachability characterization and chemical speciation fractions of HMs. During the RDC process of terrestrial weeds, most of the HMs in bioavailable and leachable fractions have been reduced at the end of composting process. In chemical speciation studies, HMs were extracted

in five different fractionable forms such as exchangeable form (F1), carbonate form (F2), reducible form (F3), oxidizable form (F4), and residual form (F5). F1, and F2 are highly mobile fractions, F3, and F4 are potential mobile fractions and F5 is immobile fraction. At the end of the composting process, most of the HMs were transformed to F4 and F5 fractions, resembling HMs in the immobile state.

Nevertheless, these terrestrial weeds provide challenges in terms of germination and impede the nutrient absorption of adjacent plants in their vicinity. The primary constituents responsible for the toxicity of these plants are toxic organic compounds. In order to gain insight into the inhibitory effects of toxicity, various evaluations were performed on *Vigna radiata* and *Allium cepa* throughout different stages of the composting process. These assessments included phytotoxicity, cytotoxicity, and genotoxicity tests. In contrast, the findings of the phytotoxicity experiment conducted on *V. radiata* demonstrated germination percentages ranging from 80 to 100% after exposure to 20th day terrestrial weeds compost (TWC) extract. Conversely, the germination percentages ranged from 40 to 50% when exposed to the initial day mix extract. The mitotic index (MI) of meristematic root tips of *A. cepa* in extracts obtained on the initial day showed a range of 20 to 30%. However, in extracts obtained from 25% concentrated TWC on the 20th day, the mitotic index ranged from 65 to 75%. The genotoxicity experiment demonstrates that the use of a 25% concentrated extract of TWC compost on the 20th day is the most effective dosage when applied to soil.

The instrumental examination of the RDC process of terrestrial weeds provided valuable observations regarding the transformation and reduction of toxic organic compounds. The identification and quantification of toxic organic compounds were conducted using analytical instruments such as Gas Chromatography Mass Spectrometry (GC-MS) and Gas Chromatography Flame Ionisation Detector (GC-FID). The presence of toxic organic compounds, including ageratochromene, farnesene, diethyl phthalate, stigmasterol, and caryophyllene oxide, was identified by GC-MS analysis. Furthermore, their quantities were determined using gas GC-FID. During the RDC process of terrestrial weeds, there has been a reduction in the presence of toxic organic compounds. Additionally, a significant transformation has occurred, whereby a majority of the aliphatic compounds have been converted into aromatic compounds.

The final study examines the efficacy of TWC amendment on the nutrients, engineering and heavy metal properties of the soil and morphological properties of the plant. The TWC (*A. conyzoides* compost (ACC), *P. hysterophorus* compost (PHC), and *L. camara* compost (LCC))

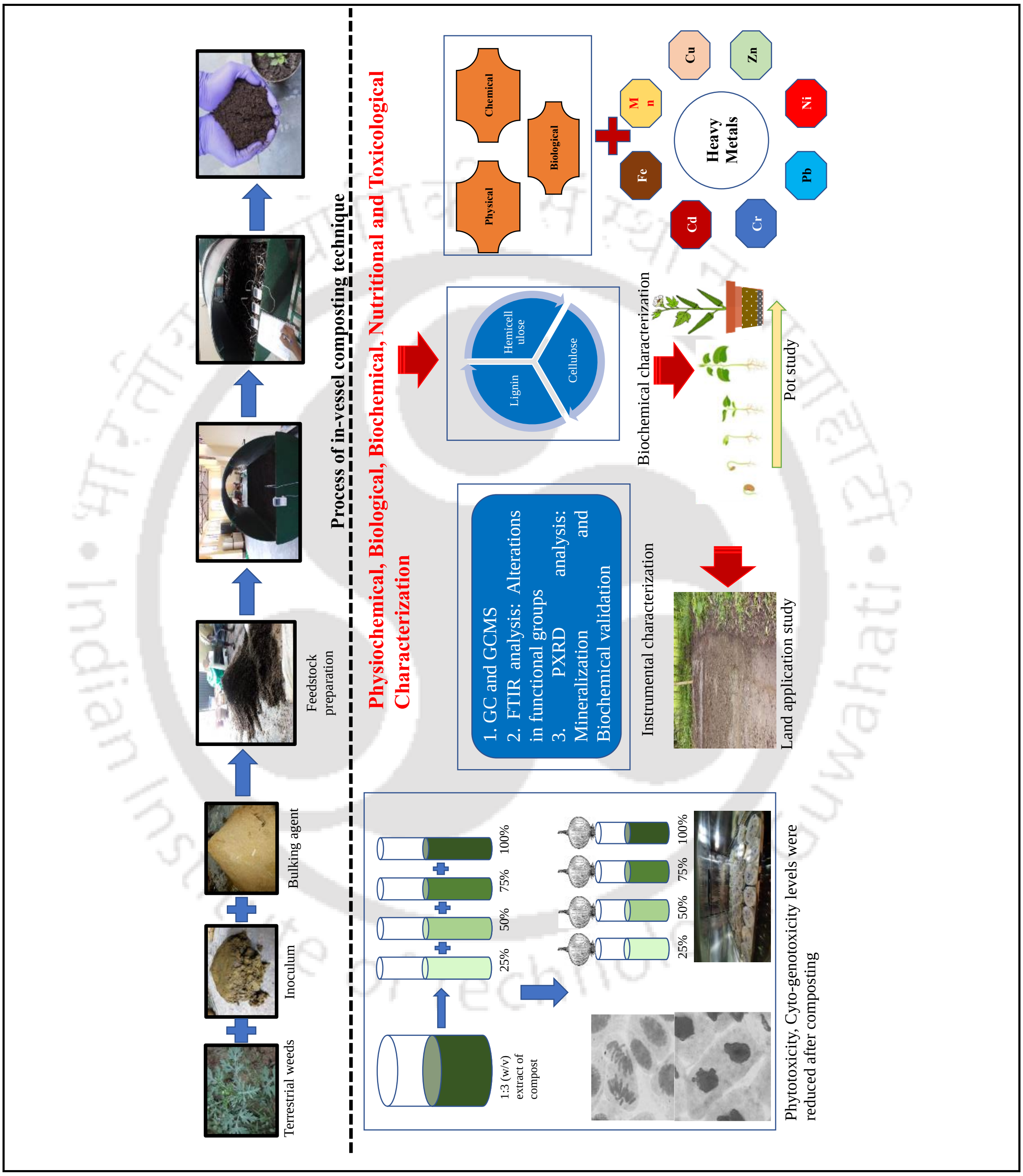
have significantly increased soil porosity at 120th day in 20% amendment by 10.56, 11.85, and 17.94%, respectively, compared to soil alone, and also significantly increased soil water holding capacity (WHC) at 120th day in 20% amendment by 68.42, 61.24, and 72.7%, respectively, compared to soil alone. The TWC has substantially decreased heavy metal transport into the plant system, and fruits of *Abelmoschus esculentus* grown in 20 to 25% amended composts had a 15 to 20% increase in nutrients compared to *A. esculentus* grown in soil alone. Based on central composite design Response surface methodology (CCD-RSM) optimizations, PHC and LLC has performed best by generating low bulk density (1.14 and 1.18 g/cc) and high WHC (48.3 and 59.9%) compared to ACC. The study's findings indicate that using TWC as a soil conditioner might be a feasible option in improving nutrient content and engineering properties for degraded soil.

The optimised ratio of pot study approach (20%) has been implemented onto the land for further investigation on application of compost. After amending TWC into the soil, the soil characteristics such as SOM and SOC has been increased in the range of 15 to 17% compared to the soil in its original form. The cation exchange capacity (CEC) of the soil has been increased in the range of 18 to 24% compared to the soil in its origin form. The soil's porosity has been increased by 11 to 16% after the application of TWC. Furthermore, compost application has also increased the yield and germination rates of *A. esculentus* and *Solanum lycopersicum*. The HMs concentration in the fruit has shown significantly low concentration. The toxic organic compounds have not detected in the fruits of *A. esculentus* and *S. lycopersicum*, and few essential compounds such as vitamin E, rhodopin, and oleic acid have been detected.

The study demonstrated that RDC process of terrestrial weeds yields a compost product that is both non-toxic and rich in nutrients. This compost product has the potential to serve as a soil conditioner in agricultural farmlands. The outcomes of the study also suggest that the use of TWC as a soil conditioner may be a viable approach for enhancing nutrient composition and engineering characteristics of depleted soil.

Keywords: Terrestrial weeds; Rotary drum composting; toxic organic compounds; Heavy metals; toxicity assessments

Graphical Abstract



> *For, usually and fitly, the presence of an introduction is held to imply that there is something of consequence and importance to be introduced.*
>
> Arthur Machen
> 1863-1947

1

Introduction

This chapter provides an analysis of the environmental consequences resulting from the presence of terrestrial weeds, with a specific focus on their impact on the agricultural sector. The terrestrial weeds under consideration include *Ageratum conyzoides*, *Parthenium hysterophorus*, and *Lantana camara*. This study aims to analyze the comprehensive influence exerted by the weeds in question, elucidate its introduction to the Indian context, and examine the prevalent methods employed for its management.

1.1. INTRODUCTION

The issue of invasive alien plant species has garnered significant attention from ecologists, biological conservationists, forestry planners, natural resource managers, and social development planners worldwide, primarily due to the detrimental effects they have on biodiversity and ecosystem functioning. The resultant consequences can be described as catastrophic, as they pose a significant threat to the ecological stability and, of utmost concern, the sustenance of the global population. An alien species refers to a unique or non-native species that originated in a different location and has been deliberately or inadvertently introduced beyond its original ecological range and capacity for dispersal (Saha et al., 2018). While it is true that not all plants introduced from other ecosystems are harmful, it is important to note that only a limited proportion of these plants possess a robust capacity for reproduction and proliferation, leading to their invasive behavior. The non-native species exhibit a significant increase in their growth rate, surpassing the native biota in terms of habitat occupancy and their ability to exploit water and nutritional resources. The phenomenon of plant invasions has garnered recognition as a highly significant global process that exerts profound impacts on the structure, composition, and function of natural and semi-natural ecosystems. The proliferation of invasive plant species often leads to the displacement of native plants, resulting in a significant impact on the availability of native forest products. Forest products play a significant role as inputs in the production activities of farm households within the village. The failure to supply timber and forest products of high quality can result in rural farmers altering their strategies for sustaining their livelihoods. The intricate connections between community livelihood and forest result in a situation where alterations in one variable are likely to have repercussions on other variables.

The investigation into weed management persisted over an extended period, yet the researchers encountered difficulties in identifying a definitive solution for the complete eradication of weeds. In recent times, notable strides and developments have been achieved in the realm of weed management through the utilization of cattle manure, thereby enhancing the soil's inherent characteristics. However, concurrently, it has resulted in a reduction in the importation of mineral fertilizers from foreign nations. According to a study conducted by Thomas (1956), numerous weed species can be found in the natural environment, although they are generally considered undesirable. In a separate investigation conducted by Tyag (1989), it was noted that there is a substantial abundance of weeds that have the potential to be transformed into a renewable resource. Subsequently, the implementation of biological treatment methods emerged as a viable approach for the management of weeds through the process of biological degradation (Verma and Prasad, 2005).

The terrestrial weeds used for this study, namely *Ageratum conyzoides*, *Parthenium hysterophorus*, and *Lantana camara*, were selected based on their prevalence and the significant issues they pose in the Assam and Meghalaya regions of India, as documented in the existing literature.

A. conyzoides is classified within the taxonomic family Asteraceae. This plant is widely recognised as Epimedium, often referred to as billy goat weed. The presence of this particular plant species is widespread in regions characterized by tropical and subtropical climates. According to Kong et al. (2002), *A. conyzoides* was documented in a total of thirty-six distinct agricultural fields distributed over forty-six nations. This particular weed species engages in competitive interactions with indigenous plant species, not just in cultivated areas but also in roadside and pasture land habitats. According to Kong et al. (2002), it has been assigned the 19th position among the most problematic weeds globally. *A. conyzoides*, an indigenous plant species originating from Central America and the Caribbean, has now achieved global distribution (Xuan et al., 2004). Fruits have evolved a specialized organ resembling a parachute that enhances the efficiency of long-range seed distribution.

P. hysterophorus, known by its vernacular name Congress Grass, is widely recognized as one of the most pernicious weed species globally. *P. hysterophorus* is a botanical species classified as a weed under the taxonomic family Asteraceae. The species in question is indigenous to the southern regions of the United States, Mexico, and Central and South America. However, due to unintentional introductions, it has spread to many nations and has emerged as a significant weed in agricultural and rangeland areas across Australia, Asia, Africa, and the Pacific Islands. This plant species is widely distributed over the geographical expanse of India. The weed's ability to withstand drought and exhibit broad adaptability enables its survival across diverse environmental circumstances. Several factors contribute to the rapid global invasion of *P. hysterophorus*, including morphological characteristics such as lightweight and small seeds that can be dispersed over long distances by various agents. Additionally, the seeds of this species have a high viability, allowing them to remain viable for extended periods of time. Furthermore, *P. hysterophorus* exhibits efficient seed production, enabling it to proliferate rapidly. Another factor that contributes to its successful invasion is its ability to outcompete other plants through allelopathy. These findings have been documented by Saini et al. (2014).

L. camara is a member of the Verbenaceae family and is widely recognized as one of the top 10 most problematic weeds globally. *L. camara*, an evergreen plant, originates from South and Central America. The introduction of this decorative plant in Calcutta's gardens in India occurred between 1809 and 1810 (Kohli et al., 2006). *L. camara* has encroached across approximately 13.2 million hectares of pasture and other land in India, establishing a widespread presence throughout the country (Kohli et al., 2006).

Various physical, chemical and biological technologies were implemented to eradicate these terrestrial weeds, since the residuals of weed exists in the soil, the weed regrows again, which ultimately led to failure. The only way to manage this plant is to transform it into a value-added product. Composting is an economical, biodegradable, and practical approach of dulcifying and recovering organic wastes. Traditional composting is a time-consuming procedure that, in some situations, leads to the partial degradation of substances. To overcome the limitations of conventional composting, investigations have suggested that composting with in-vessel composters may be used to remediate organic wastes (Ravindran et al., 2019). The rotary drum composter is easily mounted at the chosen location or the origin of biodegradable waste (Sharma et al., 2018). Numerous research has been performed on the use of in-vessel composting to manage organic waste such as flower waste (Sharma et al., 2018), *Azolla filiculoides* (W. R.Singh et al., 2016), *Hydrilla Verticillata* (Jain and Kalamdhad, 2018), municipal solid waste (Gikas et al., 2018), *Eichhornia crassipes* (Goswami et al., 2017), *A. conyzoides*, *Mikania micrantha* (Kauser et al., 2020) and paper mill sludge (Hazarika and

Khwairakpam, 2018). The rotary drum composting (RDC) process reduces the mass of the feeding substrate in the final compost product by 40 to 50 percent (Jain and Kalamdhad, 2019). Composting may also be impeded, owing to the substrate's refractory characteristics and the existence of a modest nitrogen level (2%), which slows microbial breakdown.

Furthermore, terrestrial weeds possess various toxic compounds due to the plant's secondary metabolites or decomposed substances of microbes (Cheng and Cheng, 2015; Nigatu et al., 2010). The toxicity potential of various waste and chemical extracts have been tested on various plants models and recommended *Vigna radiata* and *Allium cepa* as the plant models (OECD, 2006). Similarly, various studies have strongly suggested *V. radiata* and *A. cepa* because of their easy, rapid, sensitive, and adequately accurate plant models for evaluating the toxicity assessments of environmental pollutants present in natural ecosystem resources (Haq et al., 2017; Siles-Castellano et al., 2020; Yadav et al., 2019). Haq et al. (2018) conducted a study to evaluate the phytotoxicity assay using *V. radiata* and the genotoxicity assay using *A. cepa*. These plant models are frequently employed to evaluate the toxicity of environmental contaminants.

1.2. THESIS ORGANIZATION

The thesis has been organized in following chapters:

- **Chapter 1** presents an overview of the origins of terrestrial weeds, explores their effects, and examines the reasons behind their emergence.
- **Chapter 2** presents a comprehensive literature review. This review establishes clear definitions of terrestrial weeds, discusses diverse strategies for managing their proliferation, and explores potential conversion technologies to transform terrestrial weeds into valuable products.
- **Chapter 3** identifies gaps in existing knowledge drawn from the literature. It outlines the objectives of the study, emphasizing its significance, and delineates the scope within which the research operates.
- **Chapter 4** elaborates on the materials and methodologies employed to achieve the stipulated objectives of the study.
- **Chapter 5** delves into the bioconversion process of terrestrial weeds into compost, highlighting the outcomes of RDC. Additionally, it investigates the behavior of heavy metals, including their chemical forms, throughout this composting process.
- **Chapter 6** focuses on the assessment of toxic organic compounds within terrestrial weeds during RDC.

- **Chapter 7** Examines the applications of the compost in soil, it evaluates the impact of compost on soil health and plant morphology, while also exploring the uptake of toxic compounds by plants.
- **Chapter 8** presents crucial insights derived from the study's findings, serving as a conclusive summary. Additionally, it provides recommendations for future research directions based on the study's outcomes.

◆

What do researchers know? What do they not know? What has been researched and what has not been researched? Is the research reliable and trustworthy? Where are the gaps in the knowledge? When you compile all that together, you have yourself a literature review.

Jim Ollhoff
1959-present

2

Literature Review

This chapter provides the definitions and explanations of each subset and covers the diverse literature on composting process and its derivatives, different types of composting process, toxicity assessments, identification of toxic organic compounds, and compost applications.

2.1. WEED

A weed is a plant considered undesirable in a particular situation, "a plant in the wrong place". Examples commonly are plants unwanted in human-controlled settings, such as farm fields, gardens, lawns, and parks. Taxonomically, the term "weed" has no botanical significance, because a plant that is a weed in one context is not a weed when growing in a situation where it is in fact wanted, and where one species of plant is a valuable crop plant, another species in the same genus might be a serious weed, such as a wild bramble growing among cultivated loganberries. In the same way, volunteer crops (plants) are regarded as weeds in a subsequent crop. Many plants that people widely regard as weeds also are intentionally grown in gardens and other cultivated settings, in which case they are sometimes called beneficial weeds (Janick, 1986). Invasive plant species not only change the dynamics of species composition and biodiversity but also hamper the system productivity and efficiency in invaded regions (Bajwa et al., 2016). Besides rapidly colonizing areas replacing the native vegetation, it is also known to cause a number of human health problems, environmental degradation including threat to tourism activities (Kumar and Prasad, 2014). Terrestrial weeds which are evident in India and many other parts of the world causing economic and environmental threats are *L. camara*, *A. conyzoides*, *P. hysterophorus*, *Galinsoga purviflora*, *Saccharum spontaenium*, *Argemone mexicana*, *Mikania micrantha* kunth, is also known as billy goat weed or goat weed. It belongs to the plant family Asteraceae. It is widely distributed tropical and subtropical areas of the world. Commonly found in cultivated areas, pasture land, road sides interfering with native plants. *P. hysterophorus* is considered as one of the worst weed of the world. The plant belongs to the plant family Asteraceae, commonly known as Congress Grass. Native to southern United States, Mexico and Central and South America, it has been accidentally introduced into several countries and has become a serious agricultural and rangeland weed in parts of Australia, Asia, Africa and the Pacific Islands. *Saccharum spontaneum* is a perennial, herbaceous plant belonging to the family Poaceae. This is a close relative and one of the most important parents in the interspecific hybridization of sugarcane. It has adapted to live over a wide range of grassland climatic habitats: from oriental Asia to the southern region, in the warm-temperate areas of Africa and in Mediterranean regions (Saha et al., 2018).

2.2. TERRESTRIAL WEEDS

Terrestrial weeds, referred to as land weeds or simply weeds, are botanical entities that thrive in terrestrial or non-aquatic habitats and are deemed undesirable or problematic within specific contexts, such as agriculture, horticulture, landscape management, or natural ecosystems. These plants are frequently distinguished by their capacity to adapt, compete, and propagate in disrupted or advantageous environments, often surpassing desired plant species in competition. Weeds exhibit taxonomic diversity, encompassing a multitude of plant families and species, thereby resulting in variations in their characteristics and consequential effects (Devi and Khwairakpam, 2020a). Several typical instances of terrestrial weeds include:

Annual weeds are characterized by their ability to complete their life cycle in a year or less. The organisms exhibit a rapid germination process, followed by accelerated growth, reproduction, and seed production, culminating in their eventual demise. Some examples of annual weeds include *Portulaca oleracea*, also referred to as lamb's quarters, *P. hysterophorus*, *A. conyzoides*, and *Ambrosia artemisiifolia*, commonly known as common ragweed.

Perennial weeds are characterized by their ability to endure for multiple years, as they possess the capacity to regenerate from their root systems or underground structures on a recurring basis throughout various seasons. The plants possess a root system that is both extensive and resilient, thereby presenting difficulties in their management and control. Perennial weeds, such as dandelion (*Taraxacum officinale*), bindweed (*Convolvulus arvensis*), and Canada thistle (*Cirsium arvense*), *L. camara* serve as illustrative instances.

Grass weeds are classified within the Poaceae family and are distinguished by their slender leaves, hollow stems, and fibrous root structures. Grass weeds, such as crabgrass (*Digitaria* spp.), foxtail (*Setaria* spp.), and annual bluegrass (*Poa annua*), serve as prime illustrations.

Broadleaf weeds Broadleaf weeds, alternatively referred to as dicotyledonous weeds, exhibit distinctive features such as wide, flattened leaves and commonly possess taproots or fibrous root structures. Frequently, they engage in direct competition with desirable plants for essential resources such as nutrients, light, and space. Some examples of broadleaf weeds include Stellaria media, commonly known as common chickweed, various species of Amaranthus, commonly referred to as pigweed, and Plantago major, commonly known as broadleaf plantain.

2.2.1. *Parthenium hysterophorus*

P. hysterophorus is anciently considered as foremost worst weeds ever in the world. It belongs to the herbs family Asteraceae, prominently known to be Congress Grass (Saha et al., 2018). It is native to Southern United States, Mexico, and Central and South America. The seeds of the weed were transported to several countries by weathering, anthropogenic and natural activities. *P. hysterophorus* has urged so many threats against agricultural grasslands in different regions of the world. Few are the listed continents, namely Asia, Australia, Africa were mostly affected continents (Saha et al., 2018). The transportation of this weed proceeded through weathering, drought effect, animal traffic, wind directories to different parts of the world. The weed can produce a large number of seeds, tiny and light weighed seeds, broad flexibility, intense competition against harvested crops, phytotoxic, and various allelochemicals, which makes the weed cross the national boundaries and flexibility towards sustaining climatic barriers (Saha et al., 2018). A *P. hysterophorus* weed can process 10000 to 15000 viable seeds due to high abundance. However, these seeds can grow at large extents and can be scattered to the large nearest vicinities of the area. The allelochemicals present in the weed have reduced the crop production of agriculture excessively. The *P. hysterophorus* weed has threatened biodiversity with its hostile dominance in nature. The *P. hysterophorus* weed has reported a high level of threats to humans by creating problems in respiratory systems. It also affects humans with disease called contact dermatitis and effected livestock by imposing mutagenicity (Patel, 2011). *P. hysterophorus* has become evident as a noxious weed by the occurrence of allelochemicals through various experimental procedures carried out through particular intervals of time. It was apparent from a study that *P. hysterophorus* can inhibit the early growth of a neighboring harvested crop (Belgeri and Adkins, 2015). A study conducted by Demissie et al. (2013) has investigated the allelopathy of *P. hysterophorus* and illustrated that it contains allelochemicals.

The authors demonstrated an experiment and found that the extracts of *P. hysterophorus* could inhibit the seed germination and prolongation of root and shoot of Onion *(A. cepa)* and

Bean *(Phaseolus vulgaris)*. Wakjira et al. (2009) reported reducing allelochemicals in *P. hysterophorus* by managing the weed by composting process. They studied the impact of allelopathic effects on the plant model Lettuce *(Lactuca Sativa)*. The authors found composting an efficient biological technique to convert *P. hysterophorus* into a value-added product called compost. The authors suggested that *P. hysterophorus* composting could reduce the allelopathic inhibitory effect with the locally available plant. Rashid et al. (2008) have investigated the impact caused by the root extracts of *P. hysterophorus* against the Maize and Barley crops. The authors suggested that the increase in the concentration of root extract of *P. hysteophorus* in crops has decreased the growth trends of the crops with decreased seed growth and shoot growth of the crops. A study conducted by Kumar and Jagannath (2015) suggested a significant decrease in growth trends in beans when exposed to the flower, leaf, and stem extracts of *P. hysterophorus*. The authors found that shoot and root lengths, dry weight, and tolerance index were decreased in the bean with leaf extracts of *P. hysterophorus*. Masum et al. (2013) investigated a significant decrease in growth and bud percentage of soybean (Glycine max) when exposed to liquid extracts of *P. hysterophorus* root and shoot. The generated revenue has also gone down due to *P. hysterophorus* weed. Kanchan (1979) has investigated that the toxic in *P. hysterophorus* will penetrate soil through leaching and decaying purely dried *P. hysterophorus* weed. Muniyappa and Krishnamurthy (1981) found that pulses' growth was reduced compared to cereals when *P. hysterophorus* was introduced as a neighboring plant to soil. Furthermore, it is imperative to know the study on the biological management of *P. hysterophorus* weed. The global and Indian distribution of *P. hysterophorous* is presented in Fig. 2.1 and Fig. 2.2.

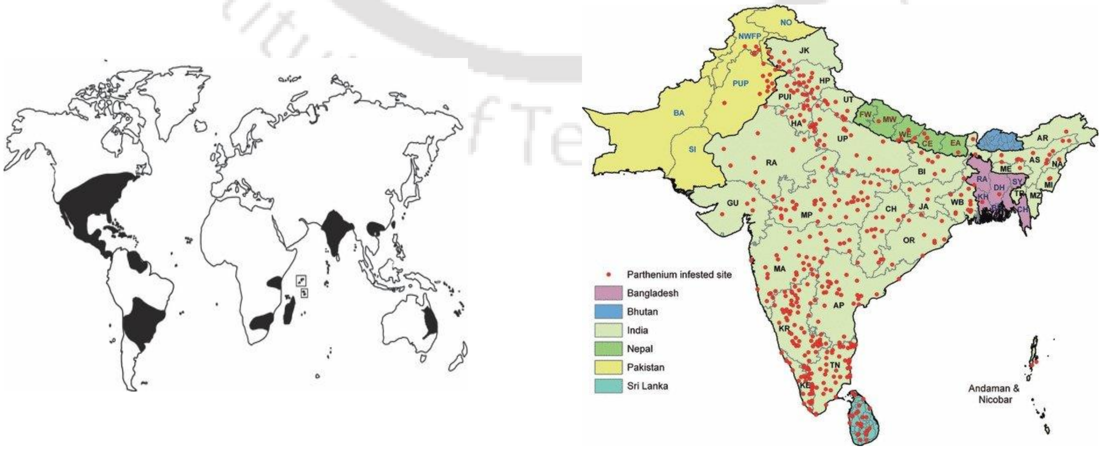


Fig. 2.1. Global and Indian distribution of *P. hysterophorus* weed (Adkins and Navie, 2006; Dhileepan and Wilmot Senartne, 2009)

Fig. 2.2. Species of *P. hysterophorus* (Adkins andNavie, 2006)

2.2.2. *Ageratum conyzoides*

The family of Asteraceae consists of 40 spp. Approximately in the genus *Ageratum*, which is native to Central America (Kong et al., 2000). In this family of herbs, two species were very familiar, and those are *A. conyzoides L.* and *Ageratum houstonianum* Mill (Kong et al., 2000). *A. conyzoides* have been spread to different parts of the world, mainly in West Africa, Southeast Asia, India, South America, India, and South China (Okunade, 2002). These weeds were invasive and have grown in waste and ruin sites, including agricultural fields. *A. conyzoides* is an annual straight, branched herb growing 0.15 m to 1 m tall. The plant stem is covered with fine white hairs, and its leaves are on the opposite side, pubescent with long petioles and include glandular trichrome. The root system of the plant is “tape”. The plants contain 35 to 60 purple or pink flowers organized in a corymb and self – incompatible (Jhansi and Ramanujam, 1987). The *A. conyzoides*, when intercropped with citrus orchards soil, have reduced the population of most splendid pathogenic fungi (Hu et al., 2002; Kong et al., 2001, 2004b). Kong et al. (2004b) have investigated the intercropping among the sudden transformation between ageratochromene and the two dimers of *A. conyzoides* with citrus orchard soil could be the crucial mechanism to maintain efficacious concentration for soil pathogenic fungi. The derivatives of ageratochromene, sesquiterpenes, and monoterpenes are the major volatile components in *A. conyzoides* (Kong et al., 2004b). Demethoxy-ageratochromene and ageratochromene of *A. conyzoides* are responsible for soil erosion at the soil stratum (Fei and Chuihua, 1997). Jianjun et al. (2002) have investigated the usage of *A. conyzoides* as an agricultural product in South China. The authors suggested *that A. conyzoides* could be utilized as green manure of farm fields for good crop yields and controlling other weeds. The authors found that the allelopathic effects of *A. conyzoides* depend on green waste and decaying intervals of soil. Roder et al. (2000) saw a decrease in germination and production of rice with the highest densities of *A. conyzoides*. A study conducted by Kohli et al. (2006) has reported that the seeds of *A.conyzoides* are photoelastic and can remain feasible for at least one year. Kamboj and Saluja (2008) have said that *A. conyzoides* could be a possible pharmaceutical appliance burns and wounds in humans, skin diseases in humans. Okunade (2002) has conducted a study to investigate the medicinal usage of *A. conyzoides* extracts. The authors reported a broad circle of chemical compounds like terpenoids, flavonoids, alkaloids, etc. A study conducted by Ming (1999) has reported that *A. conyzoides* is unfavourable for cultivated crops and has invaded many agricultural fields. Still, it can harm insects in fields and can be used as an insecticidal, antimicrobial agent. The world wide spread of *A. conyzoides* is shown in the Fig. 2.3 and Fig. 2.4.

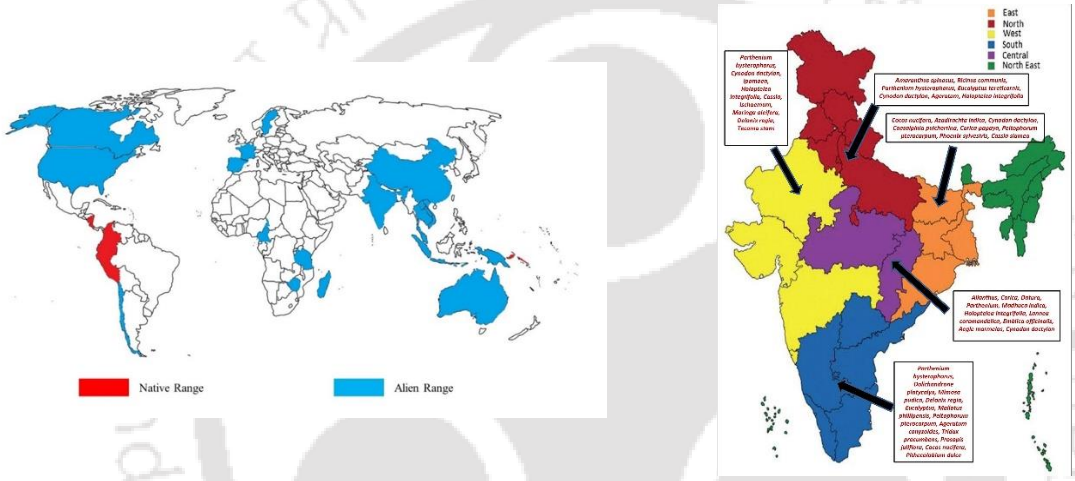


Fig. 2.3. Global and Indian distribution of *A. conyzoides* weed (Dhami, 2018; Laha et al., 2023)

Fig. 2.4. Species of *A. conyzoides* (Dhami, 2018)

2.2.3. *Lantana camara*

Linnaeus was the scientist who discovered the genus *Lantana* in 1753 and explained that *Lantana* was the genus with seven species; out of those, six were from South America and one from Ethiopia (Munir, 1996). The term *Lantana* was derived from the Latin *lento*, to blend. *Lantana* is a prominent native herb in tropical regions of America. Due to various transport activities, some of these seeds were, directly and indirectly, brought to a few low areas of Asia and Africa. The variation of *L. camara* species could be precisely and prominently ranged between 50 to 270 entities. *L. camara* is very difficult to classify taxonomically because of its stability problem. Widespread hybridization, inflorescence shape change with age, and the colour of the flowers vary with age and maturity (Munir, 1996).

L. camara is commonly known as the most widespread species of this genus, and it is also known as wild or red sage. It can grow luxuriantly at elevations up to 2000m in tropical sub-tropical and temperature regions (Sharma et al., 1988). The species name, *camara*, is probably adopted from the West Indian colloquial name for the common species (Parsons et al., 2001). The colour of the flowers is red, pink, white, yellow and violet. The stems and branches are armed with prickles. In India, it was introduced during the 18th century as a decorative plant in Calcutta's gardens (Kohli et al., 2006). From the 20th century, this weed was commonly found in India. *L. camara* has invaded nearly 13.2 million hectares of cultivated land and various regions in India. In India, *Quercus leucotrichphora* and *Pinus roxburghii* were replaced by *L. camara* at Kumaun hills (U.P) (Bhatt et al., 1994); it has occupied the plantation of Teak at Tamil Nadu (Muniappan and Viraktamath, 1993); it has also invaded the region in Indian western ghats (Muniappan and Viraktamath, 1993) and damaged the fluoresce of Garhwal (U.P) by damaging the heartwater region (Rajwar, 1998). The herb of *L. camara* has been used as a medicine for various disorders in the world (Ross, 2000). In some countries like Ghana, the *L. camara* was infused against stomach ache and fever (Irvine, 1961). The first toxic allelochemical reported from the extracts of *L. camara* weed was Lantadene (A) and Lantadene (B). The leaves of the *L. camara* weed consist of flavonoids, Lantadene A, and icterogenin (Wollenweber et al., 1997). The invasion of *L. camara* took place with the soil nutrients; a study conducted by Mandal and Joshi (2014) has investigated the soil's nutrients and dynamics and concluded that the physio-chemical properties of the soil got improved with the soil invasion of *L. camara* weed. *L. camara* invasion significantly increases the growth of abundant plants. The invasiveness of *L. camara* lies in morphological conditions, reproduction modes, and grains production (Sharma et al., 2005). In Indian forests, the density of *L. camara* got increased. Therefore, due to its allelopathic effects, the other plant species declined (Day et al., 2003). The allelopathic effects caused due to the presence of allelochemical in *L. camara* can destroy the production and growth of the native plants of that particular region (Sharma et al., 1988). A study conducted by Sharma et al. (1987) reported a difference in both purely lantadene A and partially lantadene A. The authors noted that the partially lantadene mixed with distilled water was toxic, whereas pure lantadene A was not toxic. There were many wildfire hazards due to *L. camara* in forests (Sharma et al., 2005). Quan et al. (2009) has reported that the leaching nature of the fruit and leaf of *L. camara* has inhibited the growth of other intrusive alien species. Mishra (2014) has scrutinized the aqueous extracts of leaf, root and stem of *L. camara* and reported a decline in the growth of crops from the germination stage. According to Suthar and Sharma (2013), management of *L. camara* has become a menace for policymakers and community because of its invasive nature and allelochemicals. The global and Indian distribution of *L. camara* is given in Fig. 2.5 and Fig. 2.6.

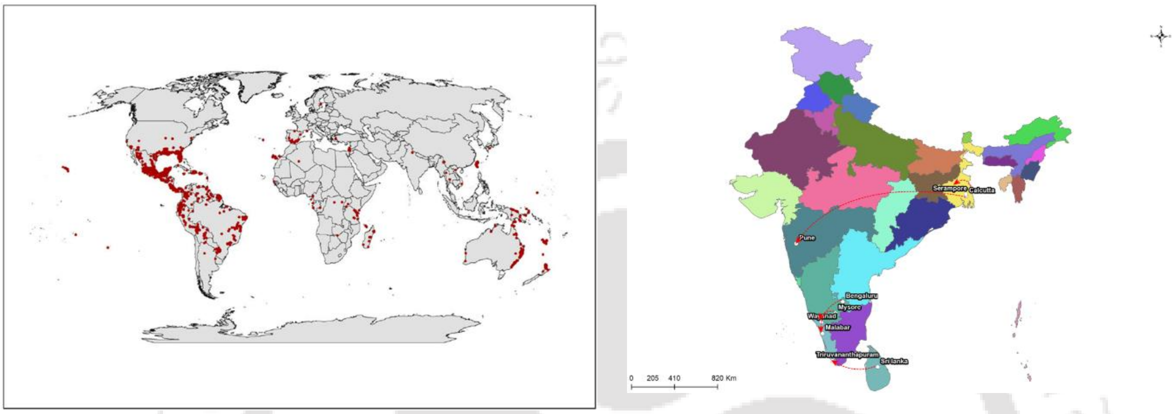


Fig. 2.5. Global and Indian distribution of *L. camara* weed (Soumya andSajeev, 2020; Taylor et al., 2012)

Fig. 2.6. Species of *L. camara* (Taylor et al., 2012)

2.2.4. Invasion and Effects on Ecosystem in Global Scenario

According to projections, the global population, which currently stands at 7.7 billion individuals, is expected to increase to nearly 9 billion by the year 2050. According to Chauhan (2020), there is a pressing need for a significant increase in global food production, ranging from 70% to 100%. Weeds are a prominent biotic constraint to agricultural production in both developing and developed nations. According to Oerke (2006), the presence of weeds, along with pathogens such as fungi and bacteria, as well as animal pests including insects, rodents, nematodes, mites, and birds, poses a significant risk to crop yield. Weeds engage in competition with crops for essential resources such as sunlight, water, nutrients, and space. Moreover, these ecosystems harbor various insect species and viral pathogens that inflict damage upon agricultural crops. Moreover, they inflict severe damage upon indigenous ecosystems, thereby posing a threat to the survival of native flora and fauna. Several factors, including weed emergence time, weed density, and weed type, have been identified as influential factors that contribute to crop yield losses. If weeds are not properly managed, they have the potential to result in a complete loss of yield, reaching up to 100 percent. According to a study conducted by Llewellyn et al. (2016), it has been estimated that Australian grain growers incur an annual cost of AUD 3.3 billion due to the presence of weeds. The agricultural industry in the United States experiences a significant loss of 2.7 million tonnes of grain due to the presence of weeds, resulting in reduced yields. The prices in India exhibited a significant increase. According to Gharde et al. (2018), the annual economic impact of weeds on India's agricultural productivity exceeds USD 11 billion. The potential exists for changes in the current distribution of species due to shifts in global climate factors, including precipitation patterns and environmental parameters such as carbon dioxide and ultraviolet exposure (Garrett et al., 2006). In recent years, there has been a growing recognition among environmentalists and other stakeholders regarding the significance of invasive species. This is evident from the surge in scholarly research dedicated to this topic (Culliney, 2005).

The encroachment of weeds poses a significant global challenge to both managed and unmanaged ecosystems (Panetta and Timmins, 2004). *Poncirus trifoliate* (Trifoliate orange), *Vinca minor* (Common Periwinkle), and *Mikania micrantha* (Bitter Vine) are among the numerous terrestrial invasive weeds. Similarly, *Egeria densa* (Brazilian waterweed), *Eichhornia crassipes* (common water hyacinth), and *Pistia stratiotes* (water lettuce) are examples of aquatic invasive weeds. In the context of India, certain invasive weed species, namely *P. hysterophorus* in urban regions, *L. camara* in forested areas, and *A. conyzoides* in agricultural land, have been found to contain a significant concentration of toxic pollutants (Saha et al., 2018). Recent research findings indicate that the introduction of non-native plant species has not only resulted in modifications to the physiochemical characteristics of the soil, but has also significantly impacted the composition, diversity, and functionality of soil microbiota (Callaway et al., 2004). Naturalization refers to the subsequent phase in the process of invasion, which occurs after a species has successfully overcome various obstacles to its survival and reproduction, and has established sufficiently large populations that the likelihood of extinction is diminished by environmental fluctuation (Richardson et al., 2000). According to Panetta and Timmins (2004), for a plant to exhibit invasive behavior, it must possess the ability to disperse rapidly and demonstrate adaptability to the prevailing environmental conditions. The introduction of non-native species and the subsequent increase in diversity and spread within an ecosystem is influenced by ecological changes. However, the extent of this change is contingent upon the type of disturbance, with particular emphasis on grassland and anthropogenic activities in terrestrial habitats (Kauser et al., 2020). The phenomenon of globalization has led to the emergence of novel pathways for the dispersal of invasive species, as highlighted by Shabani et al. (2020). This can be attributed to the amplified levels of trade and travel that have accompanied globalization. The population dynamics of weeds exhibit variability over time. The structure and infestation of weed flora are influenced by various factors. In the last twenty years, there have been significant transformations in agricultural practices, which have consequently led to alterations in weed populations (Krähmer et al., 2020). Weeds encounter intricate challenges in crop environments due to the lack of diversity and purity in the end product. The variability in latitudinal gradients can have a substantial impact on the invasiveness and ecological impact of invasive species. Certain exotic grass species have been discovered to inhibit the germination of native plant seedlings and pose a threat to species diversity, particularly in the western regions of the United States at higher latitudes. However, in lower latitudes, these same species have been observed to promote the growth of native plant populations (Drenovsky et al., 2012). The impact of nitrogen accumulation on the ecological invasion of weed species with varying advantages remains poorly understood, particularly in the context of invasive plants in aquatic and terrestrial ecosystems (Currie et al., 2014). It is widely recognized in the scientific community that genetic bottlenecks are often associated with biological invasions. This is due to the fact that a restricted number of individuals, which may have limited genetic diversity, are typically introduced into the populations (Dlugosch and Parker, 2008). The utilization of intra- and interspecific hybridization has been suggested as a means to promote invasive tendencies in plants. The occurrence of polyploidy or genome duplication played a crucial role in the evolutionary process of plants (Adams and Wendel, 2005). The field of invasion ecology encompasses an understanding of the speed and mechanisms by which organisms are transported and dispersed, the characteristics that enable a species to successfully establish itself in a new environment, and the attributes of habitats that render them susceptible to invasion by non-native species. Weeds pose a substantial constraint on agricultural production in India, significantly limiting plant productivity. Crop plants engage in resource competition with weeds, both for naturally available resources and those provided through human intervention. According to Varshney and Babu (2008), a separate study has indicated that the annual economic impact of weed-related agricultural production losses is estimated to be INR 1050 billion. Typically, the reduction in crop yield attributed to weeds is predominantly attributed to a diverse array of weed species, each possessing distinct competitive capacities (Milberg and Hallgren, 2004). Estimating the yield loss caused by a single weed species presents significant challenges in practice, leading to the adoption of a collective approach that considers the combined impact of all weed species. In a global context, it is evident that weeds pose the greatest potential for loss, accounting for 34% of overall losses. Conversely, animal pests and pathogens are comparatively less significant, contributing to losses of 18% and 16% respectively (Oerke, 2006).

2.3. INVASION AND EFFECTS ON ECOSYSTEM IN INDIAN SCENARIO

India receives indigenous species through multiple pathways, encompassing both deliberate introductions for agricultural, horticultural, and aqua cultural purposes, as well as inadvertent introductions resulting from trade, transportation, and human activities. Several examples of invasive species in India include *E. crassipes,* commonly known as water hyacinth, *L. camara*.

India possesses a diverse array of geographical features, including majestic mountain ranges, expansive river deltas, elevated forests at high altitudes, plateaus on its peninsular region, as well as a wide range of climatic conditions (Devi and Khwairakpam, 2020b). The nation exhibits a wide spectrum of temperatures, spanning from frigid Arctic conditions to scorching tropical climates. Similarly, the country experiences a diverse range of precipitation patterns, varying from extremely dry regions with less than 10 cm of rainfall annually to excessively humid areas. Notably, specific locations within the country receive the highest recorded levels of rainfall globally, reaching up to 1,120 cm. India exhibits a diverse topography, encompassing elevated plateaus, expansive valleys, undulating highlands, vast plains, marshy lowlands, and arid deserts. The nation is geographically classified into a total of 20 agro-ecological regions and 60 agro-ecological sub-regions, which are delineated based on factors such as soil composition, bio-climate conditions, and physiographic characteristics. Bhattacharyya et al. (2006) have proposed the subdivision of agro-eco-sub regions at the district level into agro-eco-units, with the aim of developing comprehensive land-use policies for the long term. The issue of weed management varies depending on the specific agro-ecological location and crop being considered (Kauser et al., 2020).

There have been between 50 and 270 distinct and various *L. camara* species identified; however, 150 species likely to be a more accurate forecast. The genus has been extremely useful throughout the majority of India. As a decorative shrub, the British created it in 1809 at the Calcutta Botanical Garden (Nanjappa et al., 2005). Apart from the Thar Desert and nearby areas, their existence and abundance are opportunistic in almost every part of India, including fields, grass, fallow land, and forests (Dobhal et al., 2011).

P. hysterophorus was erroneously introduced to Pune, India in 1956 from Central America, along with wheat (Saha et al., 2018). According to the herbarium record, it has been asserted that there was at least one previous adoption around the year 1800 (Devi and Khwairakpam, 2021). The rapid global expansion of the plant species under consideration can be attributed to several advantageous traits. These include broad resilience, insensitivity to both light and temperature variations, adaptability to drought conditions, strong competitiveness and allelopathic properties, high capacity for seed production, the ability of seeds to persist in soil seed banks, and the small and lightweight nature of the seeds, which facilitates long-distance dispersal through various means such as wind, water, birds, vehicles, farm machinery, and other animal traffic (Saha et al., 2018). Singh et al. (2005) conducted a study to examine the allelopathic impacts of unburnt residue (UR) and burnt residue (BR) of *P. hysterophorus* on the growth of winter crops, specifically radishes and chickpeas. The extract derived from both UR and BR has been found to exhibit toxicity towards the seedling length and dry weight of the test crops. In the field, the burning of *P. hysterophorus* has been observed to result in reduced germination, biomass formation, plumulus length, and Phaseolus mungo radicle length (Devi and Khwairakpam, 2021). Singh et al. (2005) estimated a significant portion of phenolics in residue extracts and residue-incorporated *P. hysterophorus* dust, which primarily consists of secondary metabolites that are commonly associated with allelopathy. *P. hysterophorus* exhibits potent allelopathic properties that significantly impact the germination process and growth of plant species cultivated alongside it. *P. hysterophorus* weeds have the ability to impact the natural biodiversity by influencing the regeneration processes of native plants and inhibiting the growth of neighboring plant species through the emission of volatile and non-volatile allelopathic compounds (Khaliq et al., 2015).

2.4. ALLELOCHEMICAL INTERACTIONS IN TERRESTRIAL WEEDS

Allelopathy is a botanical phenomenon characterized by the release of allelochemicals, which are biochemical compounds, by plants. These allelochemicals have the ability to impact the growth, development, and behavior of nearby plants. Terrestrial weeds, which are commonly regarded as invasive or undesirable flora, exhibit a diverse array of allelochemicals that contribute substantially to their competitive edge and ecological interactions. The primary objective of this article is to furnish comprehensive insights into allelochemical interactions occurring in terrestrial weeds, elucidating the underlying mechanisms, consequences, and ramifications associated with these chemical interactions (Muller, 1969).

It is well-documented that terrestrial weeds have the capacity to synthesize and emit a wide range of allelochemicals, which encompass phenolics, terpenoids, alkaloids, and volatile organic compounds (VOCs). These compounds have the potential to be synthesized within a range of plant organs, including leaves, roots, and flowers. The environmental dissemination of allelochemicals can transpire via various mechanisms, including root exudation, leaching, decomposition of plant residues, or volatilization (Adams et al., 2019). The release of allelochemicals can exhibit variability in both quantity and composition, which is contingent upon environmental factors and the specific plant species involved. The release of allelochemicals by terrestrial weeds can result in both stimulatory and inhibitory effects on adjacent plants. Various factors have the potential to exert an influence on multiple aspects of plant physiology and ecology (Vickers, 2017). These factors encompass seed germination, root development, shoot growth, nutrient uptake, photosynthesis, as well as the activity of both plant pathogens and beneficial soil microorganisms. Certain allelochemicals demonstrate phytotoxic properties, impeding the growth of alternative plant species, whereas others may elicit growth stimulation or display selective effects, targeting particular plant species or groups. The mechanisms that govern allelochemical interactions in terrestrial weeds are intricate and diverse. These interactions may encompass direct impacts via chemical inhibition or stimulation, as well as indirect impacts facilitated by alterations in soil microbial activity, nutrient availability, or plant-microbe interactions (Adams et al., 2019). Allelochemicals have the potential to impede plant hormonal regulation, enzyme activities, cell membrane functions, or disrupt specific physiological processes, thereby exerting an influence on the growth and performance of adjacent plants. The allelochemical interactions occurring among terrestrial weeds possess notable ecological implications. Alterations in competitive interactions among plants can have a significant impact on plant community structure, species composition, and biodiversity (Vickers, 2017). The phenomenon of allelopathy can play a significant role in enhancing the proliferation of invasive weeds, thereby granting them a competitive edge over indigenous plant species. In addition, the release of allelochemicals by terrestrial weeds has the potential to exert an influence on the composition and functionality of soil microbial communities, thereby affecting crucial processes such as nutrient cycling, soil fertility, and overall ecosystem dynamics (Kauser and Khwairakpam, 2022).

The presence of various compounds in *P. hysterophorus* and *L. camara* weed has been confirmed by multiple previous studies. Notably, draconic acid ($C_8H_8O_3$), (2E)-3-(4-

Hydroxyphenyl)prop-2-enoic acid ($C_9H_8O_3$), sodium caffeate ($C_9H_8O_4$), ferulate ($C_4H_4O_4$), fumaric acid ($C_4H_4O_4$), 4-Hydroxybenzoic ($C_7H_6O_3$), neochlorogenic acid ($C_{16}H_{18}O_9$), 3,4-Dihydroxybenzoic acid ($C_7H_6O_4$), and 4-hydroxy-3-methoxybenzoic acid ($C_4H_4O_4$) are considered to be of utmost significance (Bashar et al., 2023; Dhawan and Gupta, 2016). These compounds play a significant role in the germination and growth of the *P. hysterophorus* weed, affecting various natural and agricultural farmlands by inhibiting the growth of the plant species. Several previous studies have confirmed the presence of various compounds in *A. conyzoides* weed, including Ageratochromene ($C_{26}H_{32}O_6$), Caryophyllene ($C_{15}H_{24}$), Bisabolene ($C_{15}H_{24}$), Farnesene, Stigmasterol ($C_{29}H_{48}O$), and Hydroxyflavone ($C_{15}H_{10}O_3$). These compounds exert a substantial influence on the germination and growth processes of *A. conyzoides* weed, thereby impeding the growth of diverse plant species found in natural environments, agricultural crops, and pasture species (Kong et al., 2004a, 2002). The transport of allelochemicals from terrestrial weeds to the neighboring plants is depicted in Fig. 2.7. Table 2.1 depicts the brief summary on allelochemicals in terrestrial weeds and their potential effects

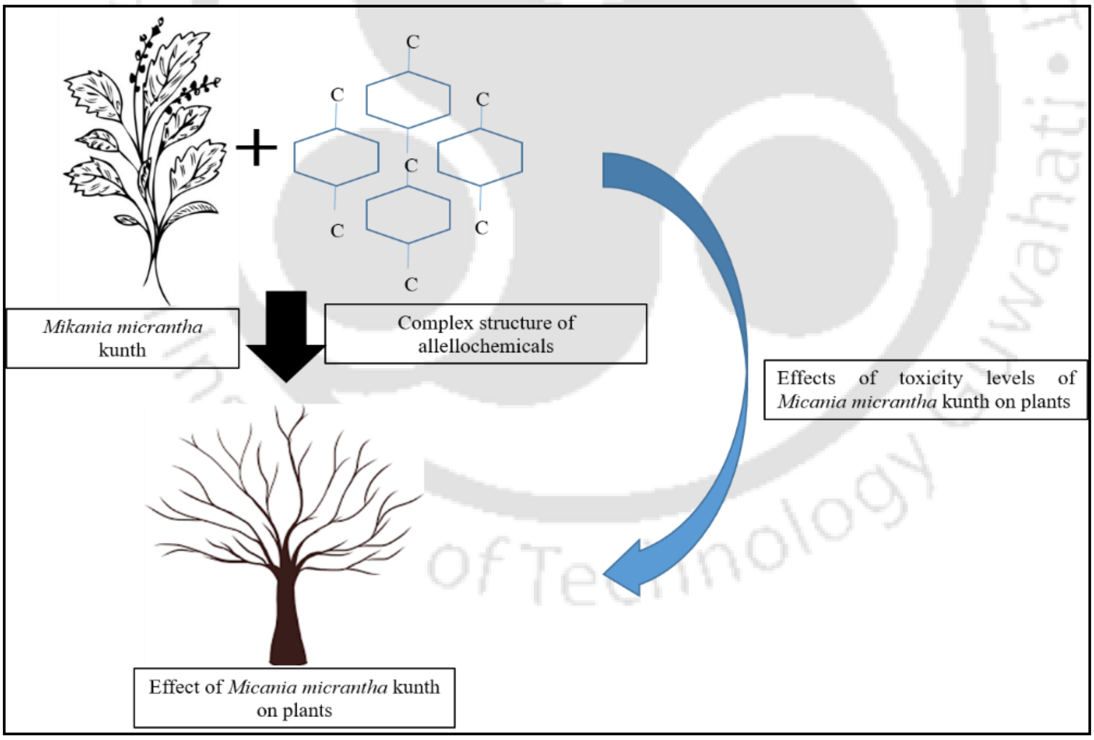


Fig. 2.7. Pathway of transportation of allelochemicals from a terrestrial plant to a neighboring plant

Table. 2.1. Brief summary on allelochemicals in terrestrial weeds and their potential effects

Type of allelochemicals	**Effects**	**Author year**
Caryophyllene ($C_{15}H_{24}$)	Caryophyllene has the potential to cause physiological stress in plants, impacting their growth and development. It has been noted for its allelopathic properties, impeding the germination and growth of nearby plants by disrupting their nutrient absorption and hormonal equilibrium.	(Kong, 2010)
Farnesene ($C_{15}H_{24}$)	Farnesene has the potential to disrupt the hormonal equilibrium in plants, leading to disturbances in critical processes like flowering and fruiting. Additionally, farnesene has been observed to trigger oxidative stress in plants, resulting in harm to cellular structures and compromising the overall vigor of the plant.	(Dobhal et al., 2011)
Stigmasterol ($C_{29}H_{48}O$)	Stigmasterol in plants may disrupt membrane fluidity, which can affect the plants' ability to transport nutrients and water, leading to reduced growth and development.	(Demissie et al., 2013)
Hydroxyflavone ($C_{15}H_{10}O_3$)	Hydroxyflavone has been discovered to hinder the growth and development of plants by disturbing a range of physiological processes. It can disrupt photosynthesis, the vital process through which plants convert sunlight into energy, resulting in diminished plant growth and productivity.	(Nasrin, 2013)
Ageratochromene ($C_{26}H_{32}O_6$),	Ageratochromene has the potential to trigger oxidative stress in plants, resulting in harm to cellular structures and diminished overall plant vigor. Moreover, it has been observed to disturb the natural defense mechanisms of plants,	(Joshi, 2014)

	rendering them more susceptible to pests, diseases, and environmental pressures.	
Parthenin ($C_{15}H_{18}O_4$)	It possesses anti-inflammatory properties, inhibiting the production of inflammatory mediators and reducing inflammation.	(Kumar and Jagannath, 2015)

2.5. TERRESTRIAL WEED MANAGEMENT STRATEGIES

Various mitigation and control strategies for managing terrestrial weeds, including physical, chemical, and biological methods, have the potential to decrease weed populations, albeit not entirely eradicate them. In addition to the inherent limitations associated with each of these methodologies.

Within the context of natural ecosystems, the presence of invasive weeds has the potential to surpass native plant species in terms of competitive advantage, thereby causing disturbances to the ecological equilibrium, diminishing biodiversity, and modifying habitats. Human activities have the potential to cause degradation of wildlife habitats, disrupt nutrient cycling processes, and hinder the natural regeneration of indigenous plant species. The ongoing challenge lies in the control and management of terrestrial weeds. Various strategies for managing weeds encompass cultural practices, mechanical methods, biological control, and chemical control. Cultural practices involve implementing techniques like crop rotation and mulching. Mechanical methods entail activities such as hand-pulling and mowing. Biological control involves the introduction of natural enemies to combat weeds. Lastly, chemical control involves the use of herbicides. The primary objective of Integrated Weed Management (IWM) approaches is to integrate various strategies in order to efficiently control weed populations while simultaneously reducing adverse effects on the environment. A comprehensive comprehension of the attributes, biology, and ecological interdependencies of terrestrial weeds is imperative in the formulation of efficacious weed control methodologies and the safeguarding of the integrity of both natural and agricultural systems.

Over the course of several decades, various methods and techniques have been employed to effectively manage terrestrial weeds. The functions encompass a range of practices, such as cultural practices, biological practices, mechanical practices, and others. Within the realm of cultural processes, a multitude of practices can be observed, including but not limited to mulching, tillage, rapid clean-ups, and drip irrigations. Through the implementation of these methodologies, the presence of undesirable vegetation can be effectively eliminated, albeit not completely eradicated. Another technique known as the stale seedbed technique involves the use of other weeds to suppress weed growth. However, this method still results in the presence of a residual amount of weed biomass. The utilization of rice crawfish rotation as a weed management strategy experienced a significant surge in south Louisiana, a region situated in the southeastern United States. Tillage is frequently employed in a restricted manner within this particular process. The primary factor contributing to the proliferation of weed varieties in fields is often the absence of proper land preparation for crop cultivation. This lack of preparation has led to the displacement of grass fields and broadleaf weeds by perennial aquatic weeds. In order to effectively manage certain aquatic weeds that are challenging to control, it is necessary to engage in tilling and fallowing of the affected area, or alternatively, rotating to a different crop such as soybean. This approach allows for the utilization of conventional tillage methods and the rotation of herbicides, as suggested by Webster and Levy (2014). Despite the implementation of diverse methodologies and the evolution of cultural norms, the complete eradication of weed biomass has proven to be unattainable. Following the establishment of cultural traditions, subsequent methods for weed management emerged, namely physical and mechanical approaches. These methods encompassed various techniques such as mourning, soil solarization, cultivation, flaming, and hand weeding. However, it was observed that these practices were not economically viable. Limited research has been conducted on alternative approaches to weed management, including the management of Red rice weed in Louisiana, a state located in the southeastern region of the United States. Table 2.2 presents a comprehensive overview of various weed management practices.

Table 2.2. Various techniques on weed management

Techniques	**Substrate**	**Applications**	**Author Year**
Manual picking	Terrestrial weeds	Effects of various weed management techniques on garlic crop yield	(Rahman et al., 2012)
Conventional Practices	Terrestrial weeds	Numerous weed management techniques on the production of wine grape in Albania	(Susaj et al., 2013)
Mechanical Screening and herbicidal techniques	Terrestrial weeds	Herbicidal techniques for managing weeds in transparent production systems	(Webster and Levy, 2014)

Various planting geometry	Terrestrial weeds	Different weed management practices in rice seeded crops	(Joshi et al., 2015)
Integrated approach	Terrestrial weeds	An integrated approach could be an efficient approach for managing terrestrial weeds	(Ghosh et al., 2017)

2.6. BIOLOGICAL TREATMENT OF TERRESTRIAL WEEDS

The first documented case of biological control of terrestrial weeds was introduced in Sri Lanka from India in 1860 by Rao et al. (1971). The authors have presented an insect (*Dactylopius ceylonicus* (Green)) to control the weed called cactus, *Opuntia Vulgaris* Miller. The various biological management techniques for managing terrestrial weeds are reported in Table 2.3.

Table 2.3. A brief summary on the various types of biological management techniques of terrestrial weeds

Type of biological management	**Substrate used**	**Applications**	**Author Year**
Vermicomposting	Multiple terrestrial weeds	According to the study, vermicomposting process increases the nutrient content and improves the soil properties.	(Ishii and Takii, 2003)
Biological agent (*Imperata cylindrical*)	*P. hysterophorus*	*P. hysterophorus* shoot extract has more inhibitory characteristics than root	(Anjum et al., 2005)
Grazing grasslands	*P. hysterophorus*	Grazing doesn't need any type of control agents	(Dhileepan, 2007)
Pile composting	*P. hysterophorus*	Composting of *P. hysterophorus* with natively available herbs is efficient in reducing the allelopathic effects of *P. hysterophorus* weed and also	(Wakjira et al., 2009)

		efficient in decreasing the inhibitory effects.	
Pest management	*A. conyzoides*	*A. conyzoides* could be managed efficiently by pests	(Kong, 2010)
herbicidal management by *Withania somnifera* (L.)	*P. hysterophorus*	*P. hysterophorus* could be managed by planting *Withania somnifera*	(Javaid et al., 2011)
Biological control agents	*P. hysterophorus*	Control agents could do management of *P. hysterophorus* weed	(Shabbir et al., 2013)
Anaerobic digestion	Terrestrial weeds	A feasible option as vermicomposting and anaerobic digestion for treating terrestrial weeds	(Saha et al., 2018)

According to Ishii and Takii (2003), terrestrial weeds can be good sources of substrate for vermicomposting in the presence of some additives. Microorganisms play a mutual relation with earthworms. Microorganisms (bacteria, fungi, and others) that populate substrates during composting reflect the evolution and performance of the composting process. The microbial study is still in its primitive stage, and more research needs to be focused on this area. Apart from this, compost and vermicompost application improve soil quality in terms of nutrients. In-vessel compost technology has shown significant efficiency in enhancing soil property, such as soil conductivity, stability, erosion resistance, soil fertility, and plant nutrition (Celik et al., 2004). But the implementation of vermicompost will be more helpful for soil improvement as vermicompost contains more effective nutrients than that other compost technology (Kauser and Khwairakpam, 2021).

Wakjira et al. (2009) have investigated to determine the effects caused by *P. hysterophorus* compost due to allelopathy on the *Lettuce* plant. For determining the impact, the authors have conducted growth experiments by taking *Lettuce* as a plant model. Fresh *P. hysterophorus* has reduced the *Lettuce* emergence percentage rate and radicle and plumule lengths by 93, 95, 97, and 93%, respectively. Furthermore, the author reported that *P. hysterophorus* compost had lessened the percentages of emergence, rate, and radicle and plumule lengths up to 45%, respectively. The study results distinctly showed that composting has prominently diminished allelopathic effects of *P. hysterophorus* compared to fresh *P. hysterophorus*. Moreover, the inhibition composition rates of emergence, speed, and radicle and plumule lengths of composting *P. hysterophorus* with other plants were low compared with different *P. hysterophorus* compost. Hence, the authors have suggested that composting of *P. hysterophorus* with natively available herbs is efficient in reducing the allelopathic effects of *P. hysterophorus* weed and also efficient in decreasing the inhibitory effects.

Khaket et al. (2012) conducted a study to examine the biochemical characteristics of consortium compost of toxic weeds *P. hysterophorus* and *Eichhornia crassipe*. In this study, the authors have prepared three types of compost, one with *P. hysterophorus, E. crassipe,* and combined compost to explore the compost quality from all the three various composts. To check the compost quality, they have performed a biological, physicochemical, and enzymatic analysis of the compost in addition to bioassay tests. The authors have found that phenolic compounds, organic carbon content, compost maturity ratios were decreased significantly, while the combined compost was increased dramatically with nutrients, nitrogen dynamics, and polyphenol oxidase. Moreover, blended compost has shown efficient and significant results against the bioassay test of *V. radiata* and *Triticum* seeds and revealed that the root length, shoot length, and germination index (GI) got increased with the application of combined compost. The authors have concluded that combined composting of *P. hysterophorus* with *E. crassipe* have reduced the allelopathic effect and increased the nutrient quality of the compost.

Suthar and Sharma (2013) have investigated the different ratios of *L. camara* with cow dung for the vermicomposting process using *Eisenia fetida*. The authors have set up an experiment with five other treatments of chemical and microbial analysis. The authors reported a significant decrease in pH, Total Organic Carbon (TOC), and C/N ratio. Furthermore, there was an increase in ash content, total N(Ntot), available phosphorous (Pavail), exchangeable potassium (Kexch), exchangeable calcium (Caexch) exchangeable calcium (Caexch). The mineralization rate of Vermibeds was shown better in Treatment 2 and treatment 3. The GI was reported to be between 47% and 83% in all the treatments through seed bioassay tests. According to the authors and their study, it was concluded that *L. camara* could be an efficient weed, which can produce vermicompost for sustainable organic agriculture.

In a review article by Kaur et al. (2014), the authors have mentioned methods for controlling *P. hysterophorus.* The methods include physical, chemical, and biological control of *P. hysterophorus*, but the primary focus of the authors was on biological control methods. According to the authors, there were three types of biological controls, they are: -

1) Classical biological control: - In this control method, there were two subclasses, Insects as classical biocontrol agents, another was Classical control by fungal plant pathogens.
2) Inundative biological control
3) Integrated weed management

These were the control systems that the authors have mentioned; according to the authors, the proper eradication of *P. hysterophorus* weed was still incomplete; even after so many control systems, there was no efficient system in eradicating the weed.

Rawat and Suthar (2014) have studied the composting process of invasive weed *L. camara* biomass and its applications in the agronomic sector. According to the authors, during the composting process, there was a significant decrease in pH, organic carbon, C/N ratio, K_{total}, C_{total} due to the moral degradation in the composting pile. The Total Kjeldahl Nitrogen (TKN) was increased from the initial stage; this was due to the subsequent Nitrogen enrichment through microbial activities results in enhanced TKN of the composted materials. The composting process has reduced the quantity of ammonium but has increased the nitrates due to the stimulation of nitrification processes. The total phosphorous was raised in composting time due to the mineralization of phosphorus and the release of nutrients by the microbial population. The C/N ratio specifies the putrefaction of organic waste. The authors found that the change in respiratory parameters of soil and C/N ratio has resulted that the biomass of *L. camara* could be putrefied within a span of 50 to 60 days thermal composting process.

Hussain et al. (2015) have conducted a study on vermicomposting of *L. camara*. It was an extension study of (Suthar and Sharma, 2013). In this study, the authors have procured vermicompost with good quality. Furthermore, to check the toxicity of the compost, a phytotoxicity study was done by taking green gram (*V. radiata*), lady finger (*Abelmoschus esculentus*), and cucumber (*Cucumis sativus*) as plant models. The compost was taken in different concentrations and mixed with soil (w/w); further, the plant models were planted. The vermicompost appeared plant-friendly at a concentration of 1.5% in soil (w/w). In this particular study, the authors have performed Fourier transform infrared spectrometry (FTIR) for the vermicompost to determine the degradation of phenols and the sesquiterpene lactones in the compost. The FTIR analysis has disclosed a significant degradation of allelopathic compounds in the vermicomposting process of *L. camara*. The study has also mentioned that there was a decrease in lignin content in the composting process. The authors have concluded that the invasive noxious *L. camara* weed can be used as an agricultural product for the crops.

Ameta et al. (2016) investigated carbon to nitrogen ratio for the combination of prepared feedstocks for the composting of *P. hysterophorus*. In this study, the authors have experimented with preparing good quality compost by fixing the C/N ratio. Furthermore, a feedstock was ready with the desired C/N ratio. Different combinations were established with varying proportions of organic waste stocks such as *P. hysterophorus*, cow dung, wheat straw, charcoal powder, sawdust, etc. The authors have prepared different C/N ratios varying between 11 to 105, depending on the initial raw material. They have concluded that a desired C/N ratio is required to make good quality compost.

Saha et al. (2018), in this study, the authors have done an in-depth review on the management of terrestrial weeds. According to the authors and previous studies, terrestrial weeds' management has become a severe concern in the scientific community. These weeds were tough to control because of their morphological advancements and adaptive capability. Various authors introduced various studies and control agents to eradicate these weeds, but all the methods have drawbacks. In this particular study, the authors have come up with two alternative techniques (Vermicomposting and Anaerobic Digestion), which were established a long way before for managing the weeds. However, these techniques were cost-effective and can also be used as revenue-generated models.

A study conducted by Devi and Khwairakpam (2020a) has reported the benefits of economy and environment through vermicomposting of an intrusive terrestrial weed *A. conyzoides*, which was registered as a feasible technique for managing terrestrial weeds. The authors have conducted an experimental study by taking distinct mix proportions of substrate and cow dung as amalgamate material for the vermicomposting process. The biological management of *A. conyzoides* was done by taking an earthworm species *Eisenia fetida*, to convert the weed into compost. The analysis was carried out by biological characterization with earthworm growth and cocoon production. The decomposition rate indicated a substantial decrease in TOC, C/N ratio, CO_2 evolution, and stabilization of the vermicompost.

In contrast, it was also rich in nutrients like TKN, total phosphorus, K, and Ca. The authors reported the compost maturity by conducting a CO_2 evolution rate experiment, which was an indicative measurement for compost maturity in the composting process (Kalamdhad et al., 2008). Furthermore, the authors have reported vermicomposting as an alternative eco-environmental technique for managing terrestrial weeds at the site. A study conducted by Devi and Khwairakpam (2020b) on the bioconversion of *L. camara* into an agricultural product by vermicomposting using two different earthworm species. In this study, the authors have set up ten reactors with five different prominent ratios by taking cow dung as a binding material. For the biological degradation, two earthworm species named *Eisena fetida and Eudrilus euginae* were taken. The authors have compared and evaluated the physicochemical parameters of the vermicompost produced by the two-earthworm species. The pH was in the range of 7.1 to 7.5 in all the reactors, whereas the TKN value was found for R_{ef4} with 2.78%, whereas it was lowest for R_{ee1} with 2.48%. There was a significant increase in TOC with 32.46% in R_{ef3}. Although as a result, the authors have reported that the C/N ratio of earthworms was between 11-14. In contrast, the gain in biomass was found in *E. fetida* in R_{ef3} with 37.5%. The authors have concluded that vermicomposting process is the best management practice for managing terrestrial weeds.

2.7. COMPOSTING

2.7.1. Definition

Composting is an inherent and environmentally responsible process that converts organic waste materials into compost, which is a valuable soil amendment enriched with nutrients. The process under consideration is a regulated decomposition mechanism that emulates the natural degradation of organic substances in the surrounding ecosystem, albeit at an expedited pace. The process of composting generates a highly valuable final product that possesses the capacity to enhance soil quality, promote optimal plant growth, and effectively divert organic waste away from landfills (Jain and Kalamdhad, 2018). The composting process entails the systematic gathering and stratification of organic substances, including but not limited to food remnants, garden refuse, agricultural byproducts, and other materials capable of undergoing biodegradation (Liu et al., 2017). The biological decomposition of these materials is facilitated by microorganisms, including bacteria, fungi, and actinomycetes. The microorganisms facilitate the decomposition of organic matter into more basic compounds, resulting in the release of heat and the production of metabolic byproducts (Kaudal and Weatherley, 2018).

In order to ensure the maintenance of an ideal composting environment, it is necessary to fulfill specific requirements. The essential factors for successful composting encompass a well-maintained carbon-to-nitrogen ratio (C/N ratio), appropriate moisture content, effective aeration, and an optimal temperature range. The carbon-to-nitrogen (C/N) ratio plays a crucial role in maintaining an optimal equilibrium between carbon-rich materials, commonly referred to as "browns," and nitrogen-rich materials, known as "greens" (Kalamdhad et al., 2008). This balance is essential for fostering microbial activity within a given system. Microbial growth and activity are dependent on the presence of moisture, while the provision of oxygen is ensured through aeration. The temperature range involved in the process of composting exhibits variation, typically spanning from mesophilic (moderate) to thermophilic (high) levels. This temperature range is instrumental in effectively eliminating weed seeds and pathogens (Pottipati et al., 2023b).

Over a period of time, the organic constituents within the compost pile undergo decomposition, resulting in the formation of a dark and granular substance commonly referred to as compost. The utilization of this compost, which is abundant in nutrients, yields advantageous outcomes such as the enhancement of soil structure, the augmentation of water retention capabilities, the facilitation of nutrient cycling, and the provision of essential elements necessary for the growth of plants (Hazarika and Khwairakpam, 2018). Composting provides a multitude of environmental advantages. The practice of reducing organic waste sent to landfills has the effect of diminishing methane emissions and thereby contributing to the mitigation of climate change. Composting additionally contributes to the process of carbon sequestration within the soil, thereby enhancing both soil health and biodiversity (Saini et al., 2014).

2.7.2. Types of composting process

(a) Backyard or Onsite Composting

Backyard or onsite composting refers to the practice of composting organic waste materials on a small scale, usually within the confines of a dwellings environment. The process entails the decomposition of organic matter, such as kitchen scraps, yard trimmings, and other relevant materials, within a specifically designated area located in the backyard or property. The process is dependent on the inherent biological activity of microorganisms, specifically bacteria and fungi, to decompose the materials into compost that is abundant in nutrients. The practice of backyard composting can be accomplished using uncomplicated methods such as compost piles or bins. These techniques involve layering organic waste and periodically agitating the materials to promote decomposition. This system offers individuals an ecologically sustainable method for managing organic waste, mitigating the amount of waste sent to landfills, and generating compost rich in nutrients that can be utilized in gardening and landscaping (Andersen et al., 2011; Ujj et al., 2021). Fig. 2.8 depicts the pictorial representation of backyard composting process.

Fig. 2.8. Backyard or onsite composting (https://www.fredericksburgva.gov/)

(b) Aerated (turned) windrow composting

The process of managing organic waste involves arranging it into elongated piles known as "windrows" and periodically aerating the piles through manual or mechanical turning. The optimal pile height, ranging from 4 to 8 feet, facilitates the creation of a sufficiently large pile that can generate ample heat and sustain desired temperatures, while simultaneously permitting the flow of oxygen to the core of the windrow. The optimal range for pile width is typically observed to be within the dimensions of 14 to 16 feet. The proposed approach has the capacity to effectively handle substantial quantities of various waste materials, encompassing yard trimmings, grease, liquids, and animal byproducts (such as fish and poultry wastes). However, it necessitates regular agitation and meticulous supervision to ensure optimal performance. This approach is well-suited for handling substantial quantities of waste, such as those produced by entire communities and managed by local governmental bodies, as well as large-scale food-processing establishments like restaurants, cafeterias, and packing plants. In regions characterized by high temperatures and low humidity, windrows are occasionally subjected to a covering or sheltering process in order to mitigate water evaporation. During periods of increased precipitation, it is possible to modify the configuration of the pile in order to facilitate the runoff of water from the top surface, thereby preventing its absorption into the pile. Moreover, windrow composting has been found to be effective even in regions with cold climates. Frequently, the exterior of the pile may undergo freezing, whereas the interior of a windrow has the potential to attain temperatures as high as 140° F. It is recommended that the compost samples undergo laboratory testing to assess their bacterial composition and heavy metal levels. The management of odors is also imperative. Windrow composting typically necessitates expansive land areas, robust machinery, a consistent labor force for facility maintenance and operation, and a willingness to engage in trial and error with different combinations of materials and frequencies of turning (Michel et al., 2022). Fig. 2.9 depicts the visual representation of windrow composting.

Fig. 2.9. Aerated Windrow composting (https://www.fredericksburgva.gov/)

(c) Aerated static pile composting

Aerated static pile composting involves the amalgamation of organic waste into a single, sizable pile, as opposed to arranging it in rows. In order to facilitate aeration, the pile is supplemented with layers of loosely arranged bulking agents, such as wood chips or shredded newspaper, which allow for the passage of air throughout the pile, from its base to its apex. Air blowers can be activated through the utilization of either a timer or a temperature sensor. Aerated static piles are a viable option for managing organic waste that is relatively uniform in composition. They are particularly effective for larger-scale generators of yard trimmings and compostable municipal solid waste, such as food scraps and paper products. Examples of entities that may benefit from this method include local governments, landscapers, and farms. However, this approach proves to be ineffective when it comes to composting animal byproducts or grease derived from food processing industries. The climatic conditions must exhibit similarity to those required for windrow composting. Given the absence of physical agitation, this approach necessitates meticulous supervision to guarantee that the exterior of the pile attains a comparable level of heat as its core. A potential method for mitigating unpleasant odors involves the application of a substantial layer of mature compost onto the pile, thereby facilitating the sustenance of elevated temperatures within the pile. An alternative approach to mitigating odor, contingent upon the air blower's extraction of air from the pile, involves the utilization of a bio filter composed of mature compost to filter said air. The implementation of this approach generally necessitates the utilization of various apparatus, including blowers, pipes, sensors, and fans, which may entail substantial financial investments and the need for technical expertise. The aforementioned technique yields compost at a relatively rapid rate, typically within a timeframe of 3 to 6 months. Fig. 2.10 displays the visual representation of aerated static pile composting.

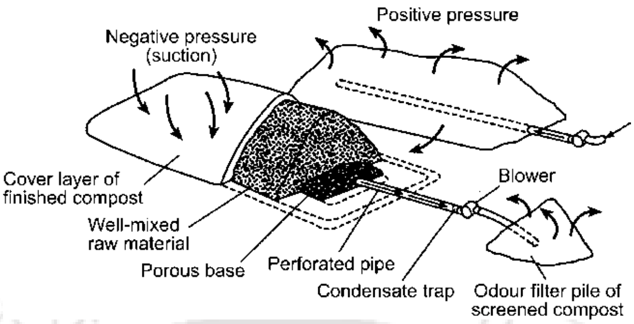


Fig. 2.10. Aerated static pile composting (Natural Resource, Agriculture, and Engineering Service (NRAES) -114, 1999)

(d) Vermicomposting

In this approach, red worms, specifically excluding night crawlers or field worms commonly found in gardens, are introduced into containers containing organic material with the purpose of decomposing it into a nutrient-rich compost known as castings. Worm bins can be easily constructed, and they are also commercially available (Kauser et al., 2022). Furthermore, these bins can be modified to effectively accommodate the volume of food scraps that are generated. Earthworms possess the ability to consume a wide range of organic matter commonly found in a conventional compost heap, including but not limited to food remnants, paper products, and plant materials (Soobhany, 2018). Vermicomposting may present an advantageous solution for individuals residing in apartments or small office spaces who seek to obtain certain advantages associated with composting while simultaneously mitigating solid waste. Worms exhibit sensitivity to fluctuations in climatic conditions. The adverse effects of high temperatures and direct sunlight on the well-being of worms should be taken into consideration. The optimal temperatures for vermicomposting range from 55° F to 77° F. In regions characterized by high temperatures and low humidity, it is advisable to position the bin in a shaded location. Vermicomposting necessitates a limited number of fundamental prerequisites, including the presence of worms, suitable worm bedding such as shredded newspaper or cardboard, and a designated container for housing the worms and organic materials (Hussain et al., 2015). Maintenance procedures encompass several tasks, namely the preparation of bedding material, the proper disposal of waste by burying it, and the segregation of worms from their castings. The production of harvestable castings by these worms usually requires a period of approximately three to four months, after which the resulting castings can be utilized as potting soil. Vermicomposting additionally yields compost or "worm" tea, which serves as a premium liquid fertilizer suitable for both indoor plants and gardens. Fig. 2.11 displays the visual representation of vermicomposting.

Fig. 2.11. Vermicomposting (http://aridagriculture.com/)

(e) In-vessel Composting

In-vessel composting refers to a regulated composting procedure that occurs within enclosed containers or vessels or circular drums. The process entails the breakdown of organic waste materials within a regulated setting, commonly employing mechanical agitation, forced aeration, and temperature supervision (Singh et al., 2013). The waste materials are introduced into the vessels, where they experience expedited decomposition within a regulated environment. This approach enables enhanced regulation of variables such as moisture, oxygen levels, and temperature, leading to accelerated composting and effective decomposition of organic substances. In-vessel composting is frequently employed in extensive operations, such as municipal or industrial composting facilities, wherein the procedure can be meticulously supervised and enhanced to achieve optimal efficiency and compost quality (Singh and Kalamdhad, 2012). Fig. 2.12 depicts the picture of in-vessel composter.

Fig. 2.12. In-vessel composter (Richentek, 2019)

The efficacy of pilot-scale in-vessel composting for the processing of food wastes was assessed by Kim et al. (2008). The composting facility was equipped with a composting bay volume of 324 m^3 and a composting material flow rate of 14,000 kg/day. The evaluations examined in this study encompassed the operational indices, compost maturity indices, and the quality of the final compost. The utilization of blowers in this system proved to be advantageous in sustaining optimal aerobic conditions, characterized by an off-gas oxygen concentration exceeding 6%, throughout the entirety of the compost bay. The assessed amounts of indices remained constant during the final phase of composting. The ultimate compost yielded satisfactory results in terms of its suitability for agricultural purposes.

The study conducted by An et al. (2012) examined the impact of coal ash (CA) and uric acid (UA) on the composting process of food waste within an in-vessel system. A comparative analysis was conducted to examine the patterns of composting food waste across different combinations. The findings indicated that the temperature level experienced a significant increase in the presence of CA and UA within the initial 8-day period. A notable decrease in pH was observed in the treatment lacking any amendment. However, the presence of calcium (CA) has the potential to mitigate the decrease in pH. The treatments with amended CA and UA exhibited a greater degree of organic mass reduction during the initial phase of the process. In the initial phase, the reactor containing both CA and UA exhibited a higher rate of O_2 uptake compared to the reactor with only CA. Throughout the composting period, microorganisms that thrive in high temperatures (thermophilic) as well as those that prefer moderate temperatures (mesophilic) were observed. The populations of thermophilic and mesophilic microorganisms were observed to be affected upon the addition of CA and UA.

The assessment of the efficiency of an in-vessel composting mechanism was investigated by Malamis et al. (2016). The study focused on exploring the kinetic responses of the methods employed for treating sewage sludge and agricultural waste. The composting process modeling takes into account a first-order kinetic model that incorporates environmental variables, which affect the oxidation of the biodegradable organic matter (BOM) in the substrate. The findings indicate that the system functions effectively, as it demonstrates elevated levels of BOM losses and rapid breakdown rates. However, any drawbacks in composting, resulting from environmental conditions, do not have a significant impact during the initial phases of the operations.

The study conducted by Hazarika et al. (2017) showcased the conversion of elemental hazardous heavy metals into inert fractions of waste generated by paper mills through the utilization of a rotating drum composter. The objective of this study was to assess the variability in the bioavailable and leachable portions of heavy metals (specifically Cd, Cu, Fe, Ni, Pb, Cr, Zn, Hg, and Mn) and investigate the influence of temperature, pH, degradation of organic matter, and humification processes over a 20-day period of RDC of paper mill sludge. According to the author, the bioavailability and leachability of heavy metals are affected by the decomposition of organic matter and the process of humification during composting. To optimize these processes in the composting of paper mill waste using a rotary drum, the author suggests incorporating an appropriate quantity of cow dung.

In their study, Jain and Kalamdhad (2018) examined the physical parameters associated with the composting of solid pulp and paper mill sludge. This investigation was conducted using a 550 L rotational drum composter. Throughout the process of composting, several physical factors exhibited variations, including bulk density, volatile solids, moisture content, free air space, void ratio, ash content, and particle density. The study observed an increase in bulk density, accompanied by a decrease in free air space, which was determined to be 52% in the final compost sample. The observed data indicated a rise in particle density from 610 to 680 kg/m^3. Nutritional characteristics of mature compost were evaluated and observed to exhibit progressive enhancement over the composting process.

The remedy and management of *M. micrantha* were assessed by Kauser et al. (2020) through the utilization of a 550 L rotary drum composter for in-vessel composting. The study employed six distinct mix proportions comprising biomass, cow dung, and sawdust. The Rotary Drum (RD2) with a concentration of 2.71% exhibits the highest level of TKN. The concentration of TOC exhibited a decrease to 19.7% by the conclusion of the 20th day. The final carbon-to-nitrogen (C/N) ratio ranges from 7 to 14 in all of the reactors. The phytotoxicity assessment of *M. micrantha* was conducted utilizing *V. radiata* and *A. cepa* as test organisms. The study's findings indicate that *M. micrantha* has the potential to be effectively utilized for the production of mature and stable compost, which could be suitable for field application due to its ability to reduce toxicity.

The study conducted by Pottipati et al. (2021) examined the efficacy of integrating in-vessel RDC with vermicomposting as a means of converting vegetable waste into vermicompost. Following a 7-day period of thermophilic exposure, during which the temperature reached a maximum of 51.5 °C within a 24-hour period, the partially degraded RDC waste was partitioned into four groups: R1 (without vermiculture), R2, R3, and R4 (with *E. eugeniae*, *E. fetida*, and *Perionyx excavates* monocultures, respectively). The vermicompost produced by R3 exhibited the highest levels of optimal process parameters and desirable compost qualities. In contrast to the consistent nitrogen content of 2.2% in R1, R3 exhibited a notable increase from 1.4% to 4.15%. Additionally, R3 demonstrated a substantial reduction of 52.5% in TOC. The unique integration of RDC thermophilic biodegradation and *E. fetida* based vermicomposting provides compelling evidence of its superior nutritional quality. Table 2.4 illustrates the comprehensive review on composting studies on terrestrial weeds

Table 2.4. Various reported composting studies on weeds

Type of composting	**Substrate used**	**Applications**	**Author year**
Composting pits	*Parthenium hysterophorus*	Effects of *P. hysterophorus* compost on *Lettuce* plant	(Wakjira et al., 2009)
Vermicomposting	*Parthenium hysterophorus*	Vermicomposting of *P. hysterophorus* is the well-suited technique for getting valuable soil product	(Anbalagan and Manivannan, 2012)
Windrow composting	*Parthenium hysterophorus* and *Eichhornia crassipe*	Consortium compost of toxic weeds *P. hysterophorus* and *Eichhornia crassipe* could be utilized as a soil conditioner	(Khaket et al., 2012)
In-vessel Composting (Rotary Drum Composting)	*Eichhornia crassipes*	Water hyacinth compost could be used as a conditioner in eroded soils	(Singh et al., 2012)

Vermicomposting	*Lantana camara*	Analyzing the different ratios of *L. camara* with cow dung for the vermicomposting process using *Eisenia fetida*	(Suthar and Sharma, 2013)
In-vessel Composting (Rotary Drum Composting)	*Eichhornia crassipes*	The natural zeolite has worked efficiently in reducing heavy metals during rotary drum composting of *Eichhornia crassipes* (water hyacinth)	(J.Singh et al., 2016)
Pile composting	*Lantana camara*	Composting could be a viable technique for *L. camara*, which could solve the land fertility issues.	(Rawat and Suthar, 2014)
Vermicomposting	*Lantana camara*	Vermicomposting of *L. camara* could be a potential source to be utilized against eroded soils.	(Hussain et al., 2015)
In-vessel composting	*Parthenium hysterophorus*	Analysis of carbon to nitrogen ratio for the combination of prepared feedstocks for the composting of *P. hysterophorus*	(Ameta et al., 2016)
Vermicomposting	All terrestrial weeds	A feasible option as vermicomposting and anaerobic digestion for treating terrestrial weeds	(Saha et al., 2018)
In-vessel Composting (Rotary Drum Composting)	*Hydrilla verticillata*	The rotary drum composting could be a possible viable option for high reduction towards biological parameters such as BOD, COD, CO_2 evolution, and OUR	(Jain and Kalamdhad, 2018)
Vermicomposting	*Ageratum conyzoides*	The authors demonstrated the benefits of economy and environment by	(Devi and Khwairakpam, 2020a)

		vermicomposting invasive terrestrial weed, *A. conyzoides*.	
Vermicomposting	*Lantana camara*	Bioconversion of *L. camara* into an agricultural product by vermicomposting using two different earthworm species	(Devi and Khwairakpam, 2020b)
In-vessel Composting (Rotary Drum Composting)	*Mikania micrantha* Kunth	*M. micrantha* could be a possible source of producing stable compost and could be applied in various agricultural aspects	(Kauser et al., 2020)
Vermicomposting	*Saccharum spontaenum*	*Saccharum spontaenum* could be used as a biofertilizer when it is composted with cow dung	(Devi and Khwairakpam, 2020c)
Rotary drum composting	Water hyacinth, *Hydrilla verticillata* and Vegetable waste	Vegetable waste compost would be the most efficient compost for degraded soil	(Mazumder et al., 2021)

2.8. SAFETY EVALUATION OF COMPOST PRODUCED

The evaluation of toxicity is a crucial factor in determining the suitability of compost for agricultural purposes and mitigating potential environmental issues prior to its reintroduction into agricultural areas. The potential for phytotoxicity from immature compost has been demonstrated by Tam and Tiquia (1994), particularly in relation to the presence of heavy metals. Phytotoxicity refers to the manifestation of various adverse effects on plants, such as delayed germination, inhibited growth, or other detrimental outcomes. These effects can be caused by specific compounds known as phytotoxins or by unfavorable growth conditions in the presence of a plant. Tam and Tiquia (1994) identified several chemical substances that have the potential to impede seed germination and hinder plant growth. These substances include excess salt accumulation, phenolic compounds, ethylene and ammonia, as well as organic acids.

A study conducted by Mersie and Singh (1987) to investigate the Allelopathic effects of *P. hysterophorous*. In this study, the authors have taken the entire shoot extract, plant part extracts, and shoot residue of *P. hysterophorous* on corn *(Zea mays)*, ryegrass *(Lolium multiflorum)*, wheat *(Triticum aestivum)*, velvetleaf *(Abutilon theophrasti)*, and soybean [*Glycine max* (L)

Merr.] and growth of each crop were examined. According to authors, *P. hysterophorous* contains water soluble materials in its shoot, which were toxic to root growth of velvetleaf and wheat. The root growth of wheat and velvetleaf were reduced by 60 and 75 %, respectively at 4% (w/v) concentrations. The increasing order of sensitivity for *P. hysterophorous* was ryegrass, corn, wheat, and velvetleaf. The correlation between extract concentration and toxicity was strong enough to test the species. Although, the toxicity of plant extracts was dependent on concentration. At 1 and 2% (w/v), the inflorescence and leaves caused more root inhibition than stem extract. The shoot of *P. hysterophorous*, when incorporated with soil at 1% (w/w) caused a significant increase in root inhibition of wheat as compared with soybean, corn, and ryegrass. With comparison to control, the root growth of all the test species was inhibited at 4 % (w/w). The toxicity of *P. hysterophorous* residue got reduced in wheat with increasing periods of decomposition. According to the authors, the decomposed residue was less toxic than the undecomposed residue.

Batish et al. (2002) has conducted a study to investigate the effect of residues of noxious weed *P. hysterohorous* on soil and laboratory conditions. The soil was mixed with different quantity of *P. hysterophorous* and was analyzed to determine the changes in soil characteristics and the phytotoxic effects of Chicpea (*Cicer arietinum*) and Radish (*Raphanus sativus*). The modified and unmodified (control) soils were taken and were analyzed for pH, conductivity, organic carbon, organic matter, available nitrogen, phosphorus, potassium and micronutrients such as sodium, iron, manganese and zinc. The pH of modified soils got decreased whereas organic matter, organic carbon and conductivity got increased. The available nitrogen and zinc were decreased in soil mixed with 4g of *P. hysterophorous*. The phenolic compounds were higher in *P. hysterophorous*, which indicates the change in chemical properties of the soil. Furthermore, the authors have done a phototoxic study of Chicpea and Radish on both modified and unmodified soils, which indicated a prominent growth in unmodified soil rather than in modified soil. The authors have concluded that, *P. hysterophorous* contains a huge amount phenolic which has decreased the growth of both the plant model, it was also observed that, soils infested with *P. hysterophorous* extracts were found to be more toxic than unmodified soils.

Tefera (2002) has investigated about the allelopathic effects of *P. hysterophorous* on seed germination and seedling growth of *Eragrostis tef*. In this study, authors have collected the *P. hysterophorous* plan from Alemaya University campus, the plants were uprooted and collected during their flowering stage. Root, flower, leaf and stem aqueous extracts of *P. hysterophorous* at 0, 1, 5, and 10 % concentrations were applied to determine their effect on tef seed germination and seedling growth under laboratory conditions. It was observed that, the increased concentrations of leaf and flower aqueous extracts of *P. hysterophorous* has inhibited seed germination and growth of seed at 10% and was recorded. Although, the aqueous extracts of stem and root of *P. hysterophorous* has doesn't shown any effect on tef seed germination. The roots were appeared to be more sensitive to allelopathic effect than shoots. According to the study, the shoot length of tef was prominently good with the extracts of flower, root and stem of *P. hysterophorous*. The authors have concluded that the leaf extracts of *P. hysterophorous* contains more toxicity towards the growth of tef. The root length of tef had a virtuous growth with root extracts at low concentration (1 %).

Singh et al. (2003) has conducted study, which was the continuation study of Batish et al. (2002). In this particular study, authors have explored the allelopathic properties of burnt and unburnt residues of *P. hysterophorous* on two winter crops radish and chickpea. They have conducted experiment by taking the extract of both burnt and unburnt *P. hysterophorous*. The extracts were infested with soil and growth of radish and chickpea was observed. The authors have found that, the growth was good in unburnt residue as compared to burnt residue, although the pH of unburnt residue was low as compared to burnt residue and the phenolics were more in burnt residue as compared to unburnt, but the authors found that the growth was good in unburnt residue. The authors have concluded that, burning *P. hysterophorous* is not a viable or recommended solution for managing the weed.

Chuihua Kong et al. (2004b) have conducted a study to investigate the effect of allelochemicals present in *A. conyzoides* intercropped with citrus orchards. The three flavones, ageratochromene and two dimers were identified and isolated from *A. conyzoides* intercropped with citrus orchards soil. It was been revealed that the flavones and ageratochromene could significantly inhibit the growth of weeds *Bidens pilosa*, *Digitaria sanguinalis* and *Cyperus difformis*, and spores germination of soil pathogenic fungi *Phytophthora citrophthora*, *Pythium aphanidermatum* and *Fusarium solani*. The study states that the presence of these allellochemical can prohibit the growth of other harmful weeds in citrus orchards. The authors have concluded that although, the dynamic transformation of ageratochromene to dimers and the dimer can monomerizes easily into the soil. But, the ageratochromene transformation intercropped with citrus orchards can be an important implementation to maintain the bioactive allelochemical at an optimum concentration.

Emino and Warman (2004) have conducted a study to assess the bioassay tests for compost quality. However, there was no universal acceptance or evident for compost quality. In this particular study authors have set up a plant bioassay study to report compost quality.

Furthermore, fourteen seed propagated species were surveyed to see if one or more would be useful as a bioassay for compost quality. The study confirmed that cress is a less sensitive indicator than several species, for example, lettuce, carrot or Chinese cabbage. *Amaranthus tricolor* was identified as a potential sensitive indicator species since it did not germinate in an immature compost extract. When the compost extract was diluted, the GI was linear with extract concentration. The authors concluded that most of the species, including the commonly used cress, are not sensitive enough to detect differences between mature and immature composts. However, Chinese cabbage appears to be the best of the commonly used assay plants. Amaranthus' potential as a sensitive compost maturity indicator was discovered and more studies are needed to confirm this finding.

Singh et al. (2005) has conducted a study to evaluate the phytotoxic properties *P. hysterophorous* residues towards the growth of three Brassica species (*Brassica oleracea, Brassica rapa and Brassica campestris*). In this study, they have taken the residues of *P. hysterophorous* and made 1%, 2%, 3%, 4% (v/v) *P. hysterophorous* extracts. The phenolic compounds were determined by Swain and Hillis (1959) using Folinciocalteu reagent. In the laboratory bioassays, they found that, the extracts of *P. hysterophorous* has reduced the earth growth of seedling. The early growth of seeds started reducing by increasing the concentrations of *P. hysterophorous* extracts, this was because as the concentrations increases phenolic content increases. By this, the authors have concluded that as the phenols increases early growth of seeds starts reducing.

A study conducted by Belz et al. (2007) to investigate whether the toxin parthenin present in *P. hysterophorous* plays a leading role. In their investigation they found that, parthenin a vital role in infecting neighbor crop by spraying through different parts of the plant system. But, according to the authors, major part of parthenin present in leaves of the *P. hysterophorous*. The authors have conducted an experiment by taking the leaf extracts of *P. hysterophorous* and imposed on five sensitive weeds *A. conyzoides, Echinochloa crus-galli* (L) P. Beauv, *Eragrostis curvula* (Schrad) Nees, *Eragrostis tef* (Zucc.) Trotter, *Lactuca sativa* L. to evaluate the parthenin effect on the plants by imposing dose response bioassay process. Among all the plant species, *A. conyzoides* was the most sensitive plant species. The quantities of parthenin in leaf extracts was analyzed through High performance liquid chromatography. The phytotoxicity of quantified extract concentrations was assessed in pure compound dose response bioassays. From the results, it was revealed that the concentrations of parthenin was increased with the increase in concentrations of the extracts. It was concluded that, parthenin was mostly found high in *P. hysterophorous* leaf extracts and it inhibits the neighboring crops a lot.

Igboasoiyi et al. (2007) have conducted a study to evaluate the leathal dose of ethanolic extracts of *A. conyzoides*. The wister rats were used to evaluate the toxicity of *A. conyzoides*. The serum levels of enzymes and biomolecules were assessed daily after imposing 500 mg/kg and 1000 mg/kg of extract in rats for 28 days. The results showed that the LD_{50} of *A. conyzoides* was 10,100mg/kg. The extracts with dosage 500 mg/kg and 1000mg/kg did not significantly affected the serum levels of alanine and asparate transaminases, alkaline phosphate amylase, glucose and total proteins. But, the extract doses have affected the serum levels of cholesterol and high-density lipoproteins. The authors have concluded that the extracts of *A. conyzoides* at dosage up to 1000 mg/kg has no toxic effects on wister rats. The *A. conyzoides* can be used in ethnomedicines as it has no toxic effects.

A study conducted by Selim et al. (2012) to evaluate the phytotoxicity of compost during composting process. Agricultural waste and cattle manure waste used to prepare compost and Cress seeds (*lepidum sativum*) were used for evaluating the phytotoxicity of the compost. The results shown that the NH_4^+-N and heavy metals in the organic wastes were major compounds inhibiting seed germination and root elongation. A positive correlation was established between GI and NO_3^--N, P and K content and a negative correlation was established between GI and heavy metals, Ammonia nitrogen. From the results, authors concluded that seed inhibition in cress seeds was due to the heavy metals in the compost, which makes the compost phytotoxic in nature.

Nasrin (2013) has conducted a study to evaluate the antioxidant and cytotoxic effects of *A. conyzoides* for the medicinal purpose. The author has taken methanol to extract the residues of *A. conyzoides* stem. The *A. conyzoides* extract was analyzed for 1, 1- diphenyl-2- picrylhydrazyl (DPPH) scavenging activity, reducing ability, and total antioxidant capacity as well as total phenolic contents. The composite phenols and antioxidants were found to be equivalent to gallic acid with 38.125 ± 2.01 mg/g and 333.37 ± 4.22 mg/g equivalent of ascorbic acid.

Dastan et al. (2014) has conducted a study on finding out the relative toxicity of a new disesquiterpene and five sesquiterpene from the roots of *Ferula pseudalliacea* on tobacco cells. The cyctotoxic effects of these compounds were evaluated on human cancer cell line (Sanandajin, methyl galbanate, ethy; galbanate, fekryonl acetate, farnesiferol B, kamonolol acetate). The phytotoxicity of Sanandajin on tobacco cells and seed germination on eggplant, common purslane and pepper was much better than the other compounds. Moreover, Sanandajin compound has a greater persistent towards the HeLa cells. From the study it was revealed that Sanandajin was the first disesquiterpene compound, which has common coumarin group. It was concluded that disesquiterpene and sesquiterpene coumarin can be a lead candidate and vigorous cancer therapy.

Fagodia et al. (2017) have conducted a study to investigate the phytotoxicity and cytotoxicity of *Citrus aurantiifolia* oil, and its major constituents-citral and limonene. *Citrus aurantiifolia* oil was selected due to its extreme commercialization and safe nature. GC–MS analysis has identified monoterpenes (83.93%), with limonene (40.92%) and citral (27.46%) as the major compounds *in Citrus aurantiifolia* oil. Phytotoxicity was assessed against three agricultural weeds, Avena fatua, *Echinochloa crus-galli* and *Phalaris minor*, at concentration ranging from 0.10–1.50 mg/ml. Percent germination, IC_{50} value and seedling growth (root and coleoptile length) were significantly reduced in a dose-response manner. *Citrus aurantiifolia* oil, citral and limonene caused alteration in the cell cycle of *A. cepa* root meristematic cells as evidenced by decrease in mitotic index (MI) and increase in chromosomal aberrations at progressive concentrations (0.01–0.10 mg/ml) and time periods (3 and 24 hours). Cytotoxic evaluation confirmed mitodepressive effect of the tested volatiles though the intensity was variable. The authors have concluded that, citral was the most toxic followed by *C. aurantiifolia* oil and limonene. The significant phytotoxic activity of *C. aurantiifolia* oil and citral suggests the possibility of being developed into eco-friendly and acceptable products for weed management in agriculture system.

A study conducted by Luo et al. (2018) to evaluate the phytotoxicity procedures for finding out the quality of the compost. According to the authors, the GI tests, which was used to determine phytotoxicity was not delivering the accurate results in plant bioassays. In this study, authors have studied about the roles, objectives, problems, prospects of GI from earlier studies. They found so many challenges and problems associated with the tests. Although, later studies proved that cress seeds were not efficient enough for germination tests. Then in recent studies, authors found that Chinese cabbage has its own advantageous in terms of toxicity, so authors suggested that Chinese cabbage may be the possible outcome for germination test assessments.

The study conducted by Khadra et al. (2019) assessed the chemical, biological, and eco-toxicological characteristics of compost derived from the co-composting of dewatered primary sludge and date palm trash. The present study aimed to investigate the individual and combined toxic effects of antibiotics, namely ciprofloxacin, enrofloxacin, nalidixic acid, roxithromycin, and sulfapyridine, in conjunction with chromium. Despite the considerable reduction in genotoxicity observed in the final compost product, approximately 50% of the micronucleus frequency persisted. This phenomenon could potentially be attributed to the presence of persistent refractory chemicals, such as chromium and various antibiotics. In general, the coexistence of antibiotics and chromium indicates that specific mixtures of pollutants can potentially pose an ecological hazard to soil health and ecosystems, even when present at concentrations that are considered environmentally insignificant. Table 2.5 depicts the comprehensive summary on the reported studies on safety evaluation of compost.

Table 2.5. Various reported safety evaluation studies in composting process

Type of safety parameter	**Type of plant model**	**Remarks from the study**	**Author year**
Phytotoxicity	*Lactuca sativa*	In this study, the effectiveness of co-composting in reducing phytotoxicity was verified through the germination index, which exceeded 90%, and by examining the concentration of metallic trace elements.	(ElFels et al., 2014)
Phytotoxicity	*Lactuca sativa*	The study recommends phytotoxicity as the essential parameter to assess the phytotoxin property of the compost.	(Cui et al., 2017)
Phytotoxicity	*Lepidium sativum*	The phytotoxicity was linked to the elevated electrical conductivity and pH levels of the food waste Residues compost, as well as the presence of heavy metals in the Municipal Solid Waste compost. To assess the effectiveness of industrial composting, it is recommended to monitor the germination index.	(Siles-Castellano et al., 2020)
Phytotoxicity	*Vigna radiata*	According to the study, RDC has the capability in reducing the toxic components in terrestrial weeds. Which was confirmed through phytotoxicity test.	(Pottipati et al., 2022)

Phytotoxicity, cytotoxicity, and genotoxicity	*Vigna radiata, and Allium cepa*	The dosage optimization of compost to soil has been optimized through the alterations in mitotic index, and chromosomal aberrations	(Kauser et al., 2022)
Phytotoxicity	*Cucumis sativus, Lactuca sativa*	The study reveals that the alterations in germination index depends on volatile fatty acids and ammonical nitrogen in the composting process.	(Kong et al., 2022)

2.9. IDENTIFICATION TECHNIQUES OF TOXIC COMPOUNDS

It is very important to find out the toxic compounds in a material from its initial growth stage for maintain clean and healthy environment.

A study conducted by Blanco and Almendros (1994) to assess the maturity of compost formed by wheat straw. Thermogravimetric analysis (TGA) in oxidizing atmosphere has been used to characterize composts prepared from wheat straw with different organic and mineral additives. After a wide range of classical parameters were determined for the chemical maturity of composts, plant yield improvement was studied in a greenhouse experiment. It was found that most chemical and agro biological maturity indices paralleled peak area values in the differential thermogravimetric curves. In particular, the weight loss corresponding to compost material destroyed between 360°C and 540°C, which has showed a very significant correlation with the GI and the plant yield of the soils amended with compost. As expected, the extent of such thermal effects reflected also the H/C, O/C, and C/N ratios and the lignin content of the composts. Experiments during the methodological optimization of TGA have shown the importance of removing the compost water-soluble fraction to prevent spurious results, probably due to the effect of salts on thermal decomposition in the lignocellulosic substrate.

Kong et al. (1999) have conducted a study to evaluate the allelochemicals and volatile oils produced by invasive weed *A. conyzoides.* The leaves of Ageratum were collected, grinded well and samples were prepared. Uniform seeds of cucumber *(Cucumis sativus* L.), ryegrass *(Lolium ultiforum* L.), radish *(Raphanus sativus* L.), mungbean *(Phaseolusaureus)*, wheat *(Triticum aestivum* L.), and tomato *(Lycapesicon)* were used as a plant models. The Gas chromatography mass spectroscopy (GC-MS) analysis was conducted for *A. conyzoides* leaves and volatile oils extracted from the leaves. A total of Eleven compounds were identified and six main compounds namely, precocene I, precocene II, 3,3-dimethyl-5-tert-butylindone, B-caryophyllene, G-bisabolene, and fenchyl acetate were identified in column chromatography.

Precocene I, precocene II, B-caryophyllene, and 3,3-dimethyl-5-tert-butylindone have inhibited the seedling growth of acceptor plants. Fenchyl acetate and G-bisabolene have no inhibitory activity, but when mixed with precocene II, they increased the inhibitory activity towards the growth of acceptor seedling plants. The authors concluded that the allelopathic symbiosis lies in allelochemicals of *A. conyzoides*.

Vikrant et al. (2006) have conducted a study to investigate the effect of phytotoxin obtained from *Phoma herbarum* towards the management of *P. hysterophorous*. The results of shoot cut, detached leaf and seedling bioassays revealed the presence of a toxic metabolite in the cell-free culture filtrate (CFCF) that was responsible for the toxicity against the target weed. Furthermore, the toxic metabolite was characterized after the CFCF extraction with butanol, hexane, chloroform acetone and ethyl acetate. The residues left after solvent evaporation were evaluated separately for their toxicity against the weed. Residue obtained from ethyl acetate fraction showed very distinct toxicity when compared with others. On the basis of analysis by GC-MS, the tentative structure of the phytotoxic compound was deduced to be 3-nitro-1, 2-benzenedicarboxylic acid (3-nitrophthalic acid). The authors have concluded that the particular toxic isolated from *Phoma herbarum* can be used as a phytotoxin against the invasive terrestrial weed *P. hysterophorous.*

A study conducted by Som et al. (2009) to investigate about the maturity of the compost made of green waste and bio-waste. The organic matter of the compost was evaluated for 8 months by correlating with the physiochemical parameters. For monitoring the bacterial activity, Thermochemolysis and thermodesorption were used, whereas diffuse reflectance infrared fourier transform spectroscopy (DRIFTS) and thermodifferential analysis (TDA) permitted to determine the degree of OM humification (maturity). The headspace GC-MS was used to determine the compounds present in VOCs. But the authors found that, VOCs got volatized in the initial stages of composting. The samples which were collected in the maturation stage has shown absence of VOCs. The VOCs observed in the initial stages has terpenes, low molecular weight acids (C_4, C_5 and C_6), aldehydes (C_{12} and C_{15}) and pentadecanone. The authors have concluded that, to determine the maturity index of a compost all the thermal analysis has to be conducted.

Zoubiri and Baaliouamer (2012) have conducted a study to investigate the effect of volatile oils present in *L. camara* against *Sitophilus granaries*. Three major compounds β-caryophyllene (35.70%), caryophyllene oxide (10.04%) and β-elemene (6.41%) were identified in GC-MS analyses of *L. camara* leaves. For doing fumigant activity *Sitophilus granaries* was taken and exposed to *L. camara* essential oils at three set of temperatures. The mortality rate got increased with increase in concentration of essential oil. The study was conducted for 2 weeks and the mortality rate of *Sitophilus granaries* was 70% to 100% of the initially introduced population. According to authors, for the tested oil, the main components e.g. β-caryophyllene and caryophyllene oxide, might be responsible for the observed insecticidal activity. However, it was very difficult to ascribe the insecticidal effect of a total essential oil to one or few active compounds.

A study conducted by Arrieta et al. (2014) to investigate the disintegration of poly(lactic acid)–poly(hydroxybutyrate) (PLA–PHB) blends and intended for food packaging under composting conditions. Two different plasticizers, poly (ethylene glycol) (PEG) and acetyl-tri-n-butyl citrate (ATBC), were used to limit the inherent brittleness of both biopolymers. Neat PLA, plasticized PLA and PLA–PHB films were processed by melt-blending and compression molding and they were further treated under composting conditions in a laboratory-scale test at 58 ± 2°C. Disintegration levels were evaluated by monitoring their weight loss at different times: 0, 7, 14, 21 and 28 days. Morphological changes in all formulations were followed by optical and scanning electron microscopy (SEM). The influence of plasticizers on the disintegration of PLA and PLA–PHB blends was studied by evaluating their thermal and Nano mechanical properties by TGA and the Nano indentation technique, respectively. Meanwhile, structural changes were followed by FTIR. The ability of PHB to act as nucleating agent in PLA–PHB blends slowed down the PLA disintegration, while plasticizers speeded it up. The relationship between the meso-lactide to lactide forms of PLA was calculated with a Pyrolysis–GC-MS. The authors revealed that the meso-lactide form was increased during composting.

Zahra El Ouaqoudi et al. (2015) has studied about the evaluation of two types of compost based on lignocellulose content. The physio-chemical parameters (TOC, TKN, C/N) over 14 months were analyzed, the result showed a more rapid composting and greater maturation for the DPGC compost (containing a mixture of date palm waste with couch-grass clippings waste) as compared with DPW compost (containing date palm waste alone). However, parallelly nitrification and mineralization trends were recorded throughout both composting, which indicated that date palm waste has more degradation. The final values of $N\text{-}NH_4^+$ in both composting showed that maturity was not achieved yet. FTIR analysis disclosed that both composts were rich in aromatic, phenolic, aliphatic and polysaccharidic structures. The characteristic ratios of IR bands (R_{IR}) showed aromatization of the organic matter in the two composts, but higher in the DPGC compost parallel with an advanced maturation. The TGA/DTA analysis showed that the organic matter (OM) transformations were less extensive in DPW compost. The values of the R_{TGA} ratios gave the same end value for both composts, indicating that this index does not correctly assess biological stability. The variations recorded in mass losses associated to exothermal peaks showed that the transformations of OM did not lead to aromatization but rather to functionalization of the OM.

Hussain et al. (2016) has conducted a study to assess the transformations of allellochemicals present in *L. camara* into organic fertilizer product through vermicomposting. In this study, UV–visible and FTIR spectroscopy, TGA and differential scanning calorimetry (DSC), GC-MS, and SEM were used to analyze the toxic, volatile, degradation, mineralization properties through the composting process. The study reveals that a sharp reduction in humification index, substantial mineralization of organic matter and degradation of complex aromatics such as lignin and polyphenols into simpler carbohydrates and lipids occur in the course of vermicomposting. The significant fragmentation, bio-oxidation and molecular rearrangements of chemical compounds was found in vermicompost than in lantana through GC/MS analysis. SEM micrographs of vermicompost reflect strong disaggregation of material compared to the much better formed lantana matrices. The phenols and sesquiterpene lactones which are specifically responsible for the toxicity and allelopathy of lantana are seen to get significantly degraded in the course of vermicomposting —turning it into a plant-friendly organic fertilizer. The authors concluded that the million tons of phytomass of Lantana could be used as an organic fertilizer, when it is composted through vermicomposting.

Bhushan and Kumari (2017) have conducted a study to investigate the isolation of *Nigrospora sphaerica*, as an endophytic fungus from *P. hysterophorus*. The isolate has been identified on the basis of morphological and molecular characteristics. The isolated fungus was known for producing various secondary metabolites in its culture filtrate. The FT-IR spectrum of *Nigrospora sphaerica* extract in ethyl acetate reveals the dispensation of various functional groups whereas GC-MS analysis of fungal extract revealed that the polysiloxane compounds were predominant constituents. The major compounds reported in ethylacetate extract of *N. sphaerica* include Cyclohexasiloxane, dodecamethyl (21.86%), Decamethyl Cyclopentasiloxane (19.05%), 13-Docosenamide, (Z) (15.40%), Cycloheptasiloxane, and tetradecamethyl (8.55). The authors have concluded that *Nigrospora sphaerica* produces different secondary metabolites in its culture filtrate, which can be used for antimicrobial activities. Table 2.6 depicts the summarized literature on identification techniques of toxic organic compounds.

Table 2.6. A summarized literature on identification techniques of toxic organic compounds

Type of instrument	Remarks from the study	Author year
GC-MS	The shoot cut, detached leaf, and seedling bioassays demonstrated the existence of a harmful metabolite in the CFCF, which was accountable for its toxicity against the specific weed. Additionally, the toxic metabolite was identified and characterized through the extraction of CFCF using butanol, hexane, chloroform, acetone, and ethyl acetate.	(Vikrant et al., 2006)
FT-IR	The samples collected during the maturation stage did not exhibit any presence of VOCs. In the initial stages, VOCs such as terpenes, low molecular weight acids (C4, C5, and C6), aldehydes (C12 and C15), and pentadecanone were observed. The authors have reached the conclusion that in order to determine the maturity index of a compost, it is necessary to perform all thermal analyses.	(Som et al., 2009)
GC-MS	In the GC-MS analyses of *L. camara* leaves, three main compounds were identified: β-caryophyllene (35.70%), caryophyllene oxide (10.04%), and β-elemene (6.41%). To assess the fumigant activity, Sitophilus granaries was chosen as the test subject and exposed to *L. camara* essential oils at different temperatures. The mortality rate of the insects increased as the concentration of essential oil was raised.	(Zoubiri and Baaliouamer, 2012)
GC-MS	GC/MS analysis revealed that vermicompost exhibited noteworthy fragmentation, bio-oxidation, and molecular rearrangements of chemical compounds, surpassing those observed in lantana. SEM micrographs of vermicompost	(Hussain et al., 2016)

	displayed a distinct disaggregation of material, whereas the lantana matrices appeared to be more structurally intact.	
FT-IR, GC-MS	The ethyl acetate extract of *Nigrospora sphaerica* was analyzed using FT-IR spectroscopy, which demonstrated the presence of multiple functional groups. On the other hand, the GC-MS analysis of the fungal extract indicated that the polysiloxane compounds were the main constituents.	(Bhushan and Kumari, 2017)

2.10. EFFECTS OF COMPOST AMENDMENT ON SOIL AND PLANTS

Celik et al. (2004) has explored the role of mycorrhizal inoculation and organic fertilizers on the alteration of physical properties of a semi-arid Mediterranean soil (Entic Chromoxerert, Arik clay-loam soil). From 1995 to 1999, pepper (*Capsicum annuum* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.) were sequentially planted with one of five fertilizers: (1) inorganic (160–26–83 kg N–P–K ha−1) control, (2) control, (3) farm manure at 25 t ha−1, (4) compost at 25 t ha^{-1} and (5) mycorrhiza-inoculated compost at 10 t ha−1. Soil physical properties were significantly affected by organic fertilizers. For soil depths of 0–15 and 15–30 cm, mean weight diameter was highest under the manure treatment while total porosity and saturated hydraulic conductivity were highest under the compost treatment. For a soil depth of 0–15 cm, the compost and manure-treated plots significantly decreased soil bulk density and increased soil organic matter concentration compared with other treatments. Compost and manure treatments increased available water content of soils by 86 and 56%, respectively. The effect of inorganic fertilizer treatment on most soil physical properties was insignificant ($P > 0.05$) compared with the control. Mycorrhizal inoculation + compost was more effective in improving soil physical properties than the inorganic treatment. Organic fertilizer sources were shown to have major positive effects on soil physical properties.

Annabi et al. (2007) has conducted a study to assess the soil aggregate stability by addition of mature and immature composts. The authors have studied the improvement in soil stability of silt loam soil with three types of composts (a municipal solid waste compost, a co-compost of sewage sludge and green waste, and a biowaste compost). The composts were sampled at two different stages of composts and were studied during laboratory incubations. The results were related to (i) compost organic matter biodegradability, biochemical fractions, and humic substance content, (ii) microbial activity evaluated through organic C mineralization and microbial and fungal biomass evolution, (iii) hot-water-extractable polysaccharides, and (iv) aggregate hydrophobicity as revealed by the water drop penetration time test. Both the composts (immature and mature) has increased aggregate stability in different mechanisms. In addition, with immature compost, aggregate stability got increased by the intensify microbial activity with the increase in water repellency. It was also revealed that, the aggregate stability depends on fungal biomass. The municipal solid waste compost was very effective at improving resistance to slaking, probably because of its larger labile organic pool that enhanced microbial activity. The addition of mature composts immediately improved aggregate stability with similar efficiency for all composts as compared to immature composts. The observed increase of inter-particular cohesion could be due to the inward diffusion of binding organic substances within the aggregates.

Paul and Bhattacharya (2012) conducted a study to examine the performance of soil with the incorporation of water hyacinth compost in the region of Assam. The utilization of African marigold (*Tagetes erecta*) as a botanical specimen was employed to evaluate the efficacy of vermicomposted water hyacinth. This investigation involved a comparative analysis with alternative sources of organic and inorganic fertilizers, focusing on various aspects of soil quality, plant growth, and yield characteristics. The results indicated that the application of vermicomposted water hyacinth at a rate of 5 t ha^{-1} effectively outperformed conventional organic and inorganic fertilizers in enhancing the flower yield of marigold. In addition, a blend of vermicompost with horse manure and conventional cow dung manure was combined in a 1:1 ratio to evaluate the impact on soil characteristics when applied at a rate of 2.5 metric tons per hectare. The investigation also revealed a noticeable improvement in both soil quality and nutrient uptake efficiency of the crop as a result of applying vermicomposted water hyacinth at a rate of 5 tons per hectare. The authors propose that the control of the invasive weed water hyacinth can be achieved through the utilization of vermicomposting techniques. The resulting product of this process can then be utilized as an organic fertilizer in the region of Assam, as well as in the neighboring states of North Eastern India.

Balasubramanian et al. (2013) conducted research aimed at examining the impact of water hyacinth (*E. crassipes*) on soil properties within a lowland rain-fed rice farming system located in north-east India. The carbon and nutrient contents of the green mulch were found to be higher in comparison to its compost and vermicompost forms. Similarly, the hemicellulose, cellulose, and lignin contents were also observed to be higher in the green mulch. The composting process of fresh water hyacinth resulted in a noteworthy decrease in the carbon-to-nitrogen (C/N) ratio, suggesting a rapid release of nitrogen (N). In general, the application of mulch resulted in a statistically significant increase ($p < 0.05$) in soil carbon (C), total nitrogen (N), available phosphorus (P), and potassium (K) when compared to the plots without mulch. However, it was observed that the soil carbon content was higher in the plots with green mulch, followed by the plots with compost and vermicompost. The rate of net nitrogen mineralization exhibited comparable trends. In contrast to other aquatic weedy species, *E. crassipes* exhibited a higher content of carbon (ranging from 20% to 50% more) and nitrogen (ranging from 10% to 40% more) in its residues. Similarly, the compost derived from E. crassipes exhibits favorable quality characteristics, as evidenced by its C/N ratio being less than 25. This is in contrast to compost derived from Hydrilla spp., Najas spp., Ottelia spp., and Pistia stratiotes. Hence, it is recommended that the recycling of water hyacinth can serve as an environmentally conscious approach to managing aquatic weeds, thereby enhancing soil health and facilitating the redistribution of nutrients. This process, characterized by a rapid turnover, has the potential to foster sustainable agricultural production in tropical soil environments.

A study conducted by Zucco et al. (2015) aimed to examine the optimal application rate of vermicomposting and its impact on tomato growth responses across three different textural classes of soils, namely loamy sand, silt loam, and silt clay. The results of the study indicate that soils characterized by high vermicomposting rates (0.4 and 0.8 g/g) exhibited a significant positive impact on plant growth and development. Specifically, plants grown in these soils demonstrated increased height, greater numbers of leaves and flowers, higher levels of leaf chlorophyll content, greater overall plant biomass, and a larger total leaf area. In contrast, soils with low vermicomposting rates (0.05, 0.1, and 0.2 g/g) did not yield comparable results in terms of these growth parameters. In comparison to the untreated control group, it was also noted that tomato growth exhibited an increase when subjected to lower rates of vermicomposting soil amendment. The tomatoes cultivated in the sandy soil supplemented with vermicomposting exhibited the most significant growth responses in terms of plant height, leaf and flower quantity, and leaf chlorophyll content, in comparison to the clay or silt loam soils. Notably, the silt loam soil demonstrated the least pronounced response. The findings of the study indicate that vermicomposting can be considered as a viable substitute for traditional fertilizers in tomato cultivation. The optimal growth of tomato plants was observed when approximately 0.5-0.6 g/g of vermicomposting was incorporated into the soil. Additionally, the study indicated that the optimal combination for cultivating tomatoes is sandy soils with a high content of organic matter, specifically, vegetable compost, when compared to loamy soils.

In a study conducted by Taiwo et al. (2016), the focus was on investigating the application of compost and plant technology for the purpose of bio-remediating soil that has been contaminated by industrial pollutants. The findings indicated a notable alteration in the chemical composition of soil when combined with compost. During the one-month remediation experiment, the treatments involving the use of 'compost-only' were found to remove approximately 49 ± 8% of manganese (Mn), 32 ± 7% of iron (Fe), 29 ± 11% of zinc (Zn), 27 ± 6% of copper (Cu), and 11 ± 5% of chromium (Cr) from the soil contaminated with these metals. The treatments involving the application of compost combined with plants resulted in the remediation of approximately 71 ± 8% of manganese (Mn), 63 ± 3% of iron (Fe), 59 ± 11% of zinc (Zn), 40 ± 6% of copper (Cu), and 5 ± 4% of chromium (Cr). The enrichment factor (EF) of metals in the compost exhibited a low value, whereas the EF values for Cu (EF = 7.3) and Zn (EF = 8.6) were found to be high in the soils that were contaminated. The results of the study indicate that the bioaccumulation factor (BF) of the Kenaf plant exhibited a low level of metal uptake. The growth parameters of the Kenaf plant exhibited consistent increases from the first week to the fourth week of planting.

Goswami et al. (2017) conducted a study to assess the effects of water hyacinth drum compost and traditional vermicompost on soil quality and crop growth in an agro-ecosystem that was intensively cultivated with tomato and cabbage as the experimental crops. The application of drum compost and traditional vermicompost resulted in a notable enhancement in soil health in terms of nutrient availability, physical stability, and microbial diversity. The enhancement of soil organic carbon was observed as a result of increased levels of humic and fulvic acid carbon. Furthermore, the presence of heavy metal contamination was found to be less pronounced in soils treated with vermicompost compared to soils treated with other methods. The authors reached the conclusion that the utilization of vermicompost and drum compost, in conjunction with the application of recommended fertilizer, can significantly enhance the growth, yield, quality, and storage durability of tomato and cabbage crops.

Mazumder et al. (2020) has conducted an assessment of the seed emergence rate index, germination velocity coefficient, and rate of germination for *Lycopersicon esculentum* and *Brassica oleracea*. The assessment was carried out using different concentrations of water hyacinth compost. The germination percentage of *L. esculentum* reached a maximum of 95% when exposed to a concentration of 100 g/L, while *B. oleracea* exhibited a 100% germination rate when subjected to a concentration of 32 g/L of the compost extract. The probability of unintentional cessation of germination was determined to be less than 0.0001 for each of the species being tested. As a result, the utilization of water hyacinth compost has been found to facilitate plant growth and is highly recommended for effectively improving deteriorating ecological conditions.

Mazumder et al. (2023b) employed Water hyacinth compost WHC, water thyme compost, and vegetable waste rotary drum compost as strategies to mitigate the potential environmental risks associated with metal loids in agricultural soil. The addition of compost resulted in a notable decrease in the bioavailability and leachability of multiple metals. The present study involved the analysis of morphological parameters of the *Solanum lycopersicum* plant, including plant characteristics, fruit properties, crop yield, and crop quality. Additionally, the investigation examined the uptake and translocation of metals by the plant, while also determining the optimal compost addition for these purposes. The application of Vermicompost followed by WHC and compost derived from *Hydrilla verticillata* demonstrated enhanced outcomes in terms of soil metal immobilization and plant metal uptake. Table 2.7 depicts summarized literature on the application of compost as a soil conditioner.

Table 2.7. A summarized literature on the application of compost as a soil conditioner

Type of composting	**Type of plants used**	**Remarks from the study**	**Author year**
In-vessel followed by vermicomposting	*Oryza sativa*	It can be stated that incorporating various types of aquatic weed compost into agricultural soil through mulching has the potential to replenish the soil's capacity for carbon sequestration and mitigate greenhouse gas (GHG) emissions.	(Balasubramanian et al., 2013)
Vermicomposting	*Solanum lycopersicum*	Vermicomposting, with an approximate addition of 0.5-0.6 g/g to the soil, is a viable substitute fertilizer for tomatoes that promotes optimal growth of tomato plants.	(Zucco et al., 2015)
In-vessel composting	*Hibiscus cannabinus*	The contaminated soil filled with Heavy metals were reduced with 50 to 70% through up taking of heavy metals in the plants.	(Taiwo et al., 2016)

RDC and vermicomposting	*Solanum lycopersicum*	Combination of RDC and vermicomposting has increased dissolved organic carbon in soil. Intake of Bioavailable fractions of heavy metals were found to be reduced in both the plants.	(Goswami et al., 2017)
Bokashi composting	*Alpinia purpurata*	The application of Bokashi, a cow manure treatment at a rate of 3 t ha^{-1}, along with the addition of NPK inorganic fertilizer at a rate of 200 kg ha^{-1}, can lead to decreased evaporation rate, reduced soil temperature fluctuations, and increased shallot yield.	(Lasmini et al., 2018)
RDC	*Brassica oleracea*, *Solanum lycopersicum*	For water hyacinth compost, the most favorable germination rates were observed at 100 g/L for *S. lycopersicum* (95% germination) and at 32 g/L for *B. oleracea* (100% germination). Likewise, the germination index was highest at 100 g/L (183.74) for *S. lycopersicum* and at 32 g/L (135.47) for *B. oleracea*.	(Mazumder et al., 2020)
RDC	*Solanum lycopersicum*	The study has revealed the health impacts and various heavy metal indices of the tomato grown in various amendments of compost prepared from water hyacinth, vegetable, and *Hydrilla verticillate* to the soil.	(Mazumder et al., 2023a)

2.11. INFERENCES FROM THE LITERATURE

The management of weed poses a significant challenge within the realm of solid waste management. For several decades, the proliferation of invasive terrestrial weed has posed a significant global challenge. Conventional methods, such as cutting and uprooting, have exacerbated the issue of organic solid waste. The conventional methods employed were insufficient in effectively addressing the issue of weed proliferation, as the seeds possess sufficient potency to be dispersed through wind or water, even subsequent to cutting or uprooting. *A. conyzoides*, *P. hysterophorus*, and *L. camara* are widely recognized as significant terrestrial weed species with global implications. Terrestrial weeds, known for their highly destructive nature, are currently posing significant challenges in various agricultural sectors, including banana plantations, coffee plantations, tea plantations, and rubber plantations, on a global scale. The presence of allelopathic potential has been reported, leading to a significant focus on the study of controlling and treating these terrestrial weeds. In the interim, it is imperative to conduct a comprehensive examination of the extensive implementation of these botanical specimens in order to effectively address their ramifications within the field of agriculture. The substance in question consists of numerous toxic compounds that pose a significant threat to the integrity of the natural ecosystem, thereby exerting adverse effects on both economic and esthetic environmental dimensions. The implementation of remediation techniques that suggest the utilization of composting methods may present a novel approach in addressing the issue posed by this pernicious weed.

It is imperative to acknowledge and tackle these concerns, a task that can only be achieved by employing carbon-rich agents. To date, there have been limited investigations conducted on the composting of terrestrial weeds. In recent years, there has been a lack of emphasis on examining the phytotoxicity, cytotoxicity, and genotoxicity of the final compost product. Little consideration has been given in prior studies to the investigation of the utilization of compost derived from terrestrial weeds for the purpose of conducting plant growth experiments. The identification of allelochemicals is of utmost importance when considering the safe application of compost in soil. The investigation of allelochemical transformations during the composting process represents a relatively novel area of study, with limited existing research conducted thus far.

> *Objectives are not fate; they are direction. They are commands; they are commitments. They do not determine the future; they are a means to mobilize resources and energies of the business for the making of the future.*

Peter Drucker
1909-2005

3

Research Gaps and Objectives

This chapter covers the gap of knowledge, research objectives of the thesis, scope and need of the study.

3.1. GAP OF KNOWLEDGE

The existing literature highlights the significance of degrading terrestrial weeds and transforming them into valuable agricultural products through the composting process. However, it is crucial to acknowledge that terrestrial weeds differ from other organic wastes due to their possession of toxic organic compounds and a heightened capacity for absorbing heavy metals from the soil, both of which impede the growth of neighboring plants. Consequently, it becomes absolutely essential to focus on the transformation and reduction of these toxic compounds during the composting process. This aspect is of utmost importance as it not only provides valuable insights into the safety parameters of the compost but also confirms its suitability for application on soil and plants.

Furthermore, the application of compost derived from terrestrial weeds holds significant importance due to the rich content of nutrients such as nitrogen, phosphorus, and potassium, as well as the potential uptake of heavy metals and toxic organic compounds from the soil and plants. However, it is concerning that there is very limited literature on the degradation and transformation of toxic organic compounds into a stable and non-toxic compound through any biological systems.

This dearth of comprehensive research creates a significant knowledge gap in our understanding of the composting process for terrestrial weeds, particularly in terms of ensuring the safety and effectiveness of the produced compost. Moreover, the limited availability of literature on this subject restricts our ability to explore the potential benefits and applications of compost derived from terrestrial weeds in agricultural practices. To fully unlock the potential of composting terrestrial weeds and facilitate its safe and sustainable implementation, it is imperative to address this research gap through further investigations. Robust studies focusing on the degradation processes, reduction of toxic compounds, and evaluation of compost toxicity are critical for enhancing our understanding of terrestrial weed composting and its utilization for soil and plant enrichment, while also considering the potential risks associated with heavy metal and toxic compound uptake.

3.2. RESEARCH OBJECTIVES

The literature review identified a notable challenge in the eradication of terrestrial weeds (*A. conyzoides*, *P. hysterophorus*, and *L. camara*). The proliferation and detrimental effects of these invasive plants have rendered them a significant concern, given their extensive distribution and adverse consequences on ecological systems. Furthermore, the issue is compounded by the existence of toxic organic compounds. In order to tackle these challenges, the primary aim of this study is to investigate the viability of utilizing in-vessel composting as a method for transforming these three terrestrial weeds into a substance resembling soil or serving as a soil conditioner. In-vessel composting is a regulated procedure that takes place within enclosed containers, facilitating the effective breakdown of organic waste. The application of this composting technique to the weeds is anticipated to result in the conversion of their biomass into a valuable resource, thereby improving soil fertility and structure. This methodology exhibits potential for alleviating the adverse impacts of these invasive plant species while concurrently offering a sustainable resolution to the issue of organic waste management. Therefore, the study is structured and encompasses into three specific objectives, which are outlined as follows:

- **Objective 1:** Bioconversion of terrestrial weeds into compost and the assessment of heavy metals and their transformation during the RDC process. This objective evaluates the effectiveness of RDC and the biodegradability, leachability, and chemical speciation of heavy metals during the composting process.
- **Objective 2:** Toxicity assessment of the feedstock material in various stages of composting process. This objective includes phytotoxicity, cytotoxicity and genotoxicity as the toxicity assessment parameters. Identification and Quantification of toxic organic compounds in feedstock material in distinct stages of composting process. This objective also evaluates

the causes of the toxicity of these terrestrial weeds and determines the changes in toxic organic compounds during the composting process using instrumental techniques.

- **Objective 3:** Application of terrestrial weeds compost on soil and plants. This objective evaluates the morphological, nutritional, heavy metal uptake and toxic compounds transformations of oleoculture plants in compost-soil amendments and also the impact of compost on soil properties.

3.3. NEED OF THE STUDY

India, being one of the world's largest and most populated countries, relies heavily on agriculture, with 61.5% of its population depending on it for their livelihood (Ministry of Health & Family Welfare, 2020). Any decline in agricultural output would have far-reaching consequences for numerous individuals. Terrestrial weeds pose a significant threat to agricultural fields as they contain harmful organic compounds that impede the growth of other crops. While several studies have explored various methods and management strategies to eradicate these weeds, only a few have mentioned the crucial aspect of compost toxicity. Therefore, there is a need for comprehensive research that not only addresses the management of terrestrial weeds through composting but also examines the toxicity of the resulting compost. The findings of this study will offer strategic solutions for managing terrestrial weeds while evaluating the effectiveness of composting in reducing and transforming toxic organic compounds. Additionally, the study will provide valuable insights into the application of terrestrial weed compost on soil and plants, assessing factors such as morphology, nutrient content, toxicology, and engineering properties.

3.4. SCOPE OF THE STUDY

The main objective of this research is to utilize terrestrial weed as a substrate for the production of sustainable compost, thereby transforming it into a useful product. The study encompasses several scopes. Firstly, the sampling process in composting involves the collection of grab samples followed by composite samples. To ensure accuracy, the samples were analyzed immediately to avoid any potential errors, considering the influence of biological parameters on the composting duration. Secondly, the study focuses on maintaining appropriate temperatures for toxicity analysis. Careful attention was given to the instrumental procedures, including the handling of samples for solvent extractions, where the use of GC-MS grade solvents was mandatory. Additionally, applying the compost to soil and conducting pot studies on plants required agricultural expertise to establish the experimental setup. Overall, this research is an interdisciplinary endeavor, involving fields such as ecology, environmental engineering, and others.

A method is more important than a discovery, since the right method will lead to new and even more important discoveries.

Lev Landau
1908-1968

4

Materials and Methodology

The present study employed several experimental procedures in order to achieve the specified aims. The study was carried out through a series of phases, encompassing the examination of various combinations of essential materials. This chapter presents an in-depth methodology for conducting the study.

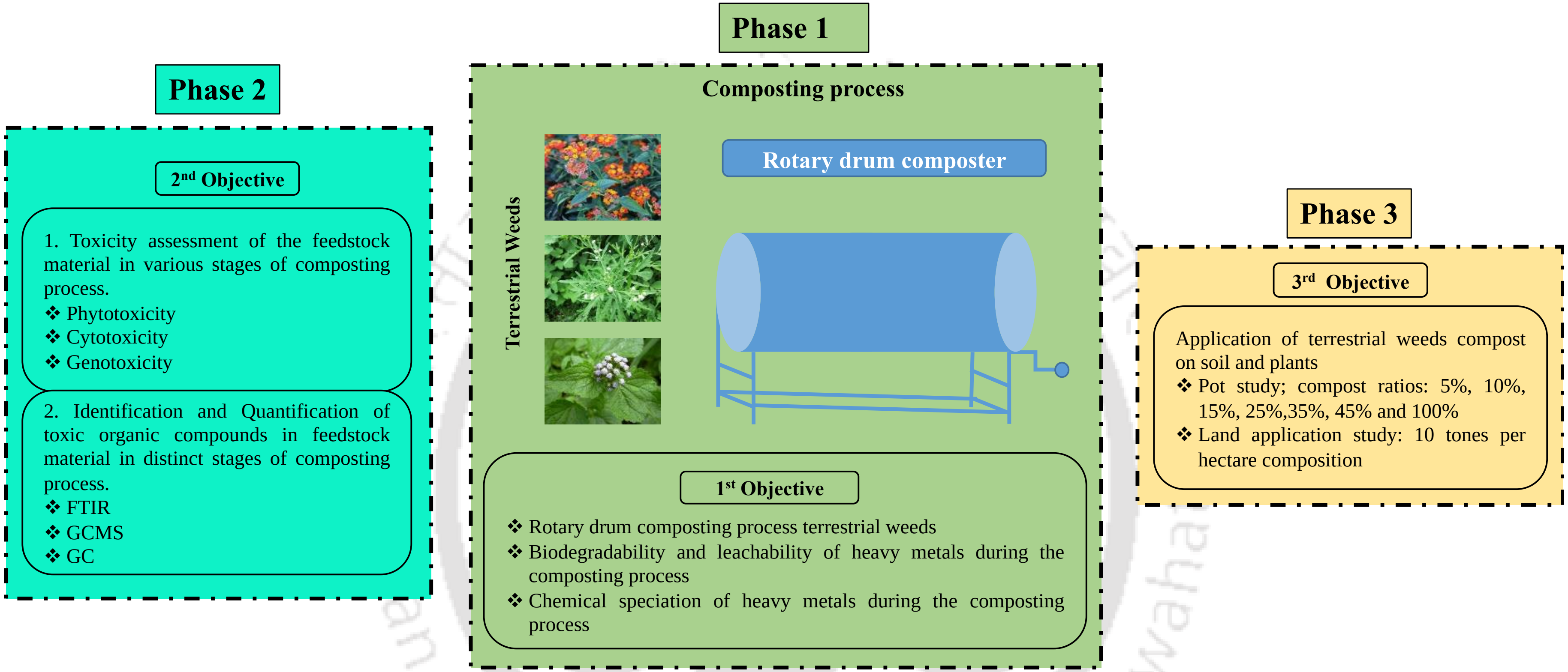
4.1. RESEARCH FRAMEWORK

In order to fully understand the toxic nature of terrestrial weeds and its treatment through composting process, the research was conducted in various stages, and a summary of these stages is provided below. The study required a methodical evaluation, thus necessitating the implementation of an experimental framework, as illustrated in Fig. 4.1. As depicted in the figure, the entirety of the investigation was conducted in three distinct phases.

During the initial phase, the collection of terrestrial weeds was conducted in the vicinity of IIT Guwahati, with a specific focus on agricultural fields. Subsequently, the weeds were subjected to composting using a rotary drum reactor, followed by the collection of samples for subsequent physiochemical, biological, biochemical and heavy metal analyses.

During the second phase, a series of toxicity assessments, including phytotoxicity, cytotoxicity, and genotoxicity tests, were performed subsequent to the composting process. Concurrently, the samples were subjected to analysis in order to identify and quantify toxic organic compounds.

During the third phase, the application of compost to both soil and plants was carried out. A study was conducted to investigate the optimal compost percentage in soil for agricultural purposes by varying the compost to soil ratios in pot experiments. Subsequently, the application of compost was conducted on the land in order to evaluate the soil's overall health, the absorption of heavy metals, and the potential changes in toxic organic compounds within plants.



1. Toxicity assessment of the feedstock material in various stages of composting process.
- ❖ Phytotoxicity
- ❖ Cytotoxicity
- ❖ Genotoxicity

2. Identification and Quantification of toxic organic compounds in feedstock material in distinct stages of composting process.
- ❖ FTIR
- ❖ GCMS
- ❖ GC

- ❖ Rotary drum composting process terrestrial weeds
- ❖ Biodegradability and leachability of heavy metals during the composting process
- ❖ Chemical speciation of heavy metals during the composting process

Application of terrestrial weeds compost on soil and plants
- ❖ Pot study; compost ratios: 5%, 10%, 15%, 25%,35%, 45% and 100%
- ❖ Land application study: 10 tones per hectare composition

Fig. 4.1. Research framework of the study in phase wise

4.2. PHASE-I: BIODEGRADATION OF AN INTRUSIVE TERRESTRIAL WEEDS THROUGH IN-VESSEL COMPOSTING TECHNIQUE: (OBJECTIVE 1)

4.2.1. Substrate Characterization

The substrates used in the study were *A. conyzoides*, *P. hysterophorus*, and *L. camara*. These were collected from the vicinities nearby the Indian Institute of Technology Guwahati (IITG) campus, Assam, India. Inoculum (Cow dung) and sawdust were acquired from the dairy farm and sawmill at an accessible distance of IITG campus. Fig. 4.2. depicts the pictorial representation of the terrestrial weeds, inoculum and saw dust used in the study.

A. conyzoides *P. hysterophorus*

L. camara

Inoculum Saw dust

Fig. 4.2. Substrates, inoculum and saw dust used in the study

To thoroughly understand the waste substrate, inoculum, and sawdust, it was crucial to analyze their physicochemical, biological, and biochemical parameters for characterization. This analysis aimed to provide valuable insights into the composition and suitability of these materials for the composting process. The initial characterization of the terrestrial weeds is presented in Table 4.1. The parameters examined included moisture content, volatile content, total nitrogen, and lignin. The results revealed that all three types of terrestrial weeds fell within a certain range for each parameter. The moisture content ranged from 71 to 74%, indicating a moderate level of moisture within the weeds. The volatile content also fell within a range of 70% to 74%, suggesting that the weeds contained a substantial proportion of organic matter that could potentially contribute to the composting process. Furthermore, the total nitrogen content ranged from 1.2% to 1.6%, indicating the presence of nitrogen, an essential nutrient for microbial activity and decomposition in the composting process. Lastly, the lignin content ranged from 15% to 24%, which is significant as lignin is a complex organic compound that can influence the decomposition rate of the compost. Based on these findings, it can be determined that the selected terrestrial weeds are feasible for the composting process, as their physicochemical, biological, and biochemical characteristics align with the desired parameters. Furthermore, the C/N ratio of the substrates were observed in the range of 20 to 35. According to Jain and Kalamdhad (2018), the C/N ratio of any substrate should be between 20 to 40 for an ideal composting process. In the study, cow manure was used as an inoculum. The inoculum contained a combination of microorganisms and organic matter, which played a crucial role as a primary consumer in the degradation process. The inoculum also helps in reducing the time for degradation process (Pottipati et al., 2023a). Saw acts as a bulking agent, creating air spaces within the compost heap and improving aeration. This is crucial for the growth and activity of aerobic microorganisms that rely on oxygen to effectively break down organic matter. Sawdust contributes to composting by enhancing aeration, balancing the C/N ratio, and regulating moisture levels, all of which support the efficient decomposition of organic materials into nutrient-rich compost. This initial characterization provides a foundation for further investigation and optimization of the composting process using these materials as substrates.

Table 4.1. Initial Characterization of Substrates, Inoculum and Saw dust

Initial Characterization	***A. conyzoides***	***L. camara***	***P. hysterophorus***	**Cow dung**	**Saw dust**
sBOD (g/kg)	6.01 ± 14	6.30 ± 17	7.86 ± 21	1.20 ± 20	2.80 ± 20
sCOD (g/kg)	26.74 ± 54	31.03 ± 42	31.13 ± 43	5.40 ± 16	5.00 ± 30
pH	6.09 ± 0.08	6.01 ± 0.04	5.98 ± 0.12	6.5 ± 0.01	6.1 ± 0.01
EC (dS/m)	4.89 ± 0.12	6.3 ± 0.01	6.5 ± 0.27	3.4 ± 0.02	0.6 ± 0.03
Moisture content (%)	73.2 ± 1.78	72.1 ± 3.1	71.3 ± 2.12	85.3 ± 1.9	13.2 ± 1.1
% Volatile Solids	72.4 ± 1.5	71.9 ± 4.2	74.2 ± 4.2	87.8 ± 1.4	86.6 ± 1.3

Ash Content	27.6 ± 0.9	28.1 ± 4.1	25.8 ± 1.21	12.6 ± 1.4	13.4 ± 1.3
% TOC	40.2 ± 1.05	39.9 ± 2.3	41.2 ± 4.76	48.7 ± 0.77	48.1 ± 0.72
TN %	1.61 ± 0.03	1.35 ± 0.07	1.22 ± 0.09	1.5 ± 0.1	0.35 ± 0.1
TP (g/kg)	4.1 ± 0.24	4.14 ± 1.2	4 ± 0.79	5.0 ± 0.3	2.2 ± 0.3
AP (g/kg)	1.9 ± 0.03	2.09 ± 0.3	1.3 ± 0.2	3.1 ± 0.1	1.2 ± 0.2
C:N Ratio	24.9 ± 1.2	29.5 ± 1.9	33.7 ± 2.03	32.51 ± 1.5	137.4 ± 12
Na$^+$ (g/kg)	2.4 ± 0.8	2 ± 0.1	2.1 ± 0.33	1.67 ± 0.52	2.48 ± 0.89
K$^+$ (g/kg)	26 ± 2.43	19.2 ± 1.67	23.2 ± 3.33	0.43 ± 0.01	0.83 ± 0.08
Ca^{2+} (g/kg)	4.1 ± 1.03	3.12 ± 1.1	4.67 ± 1.51	2.46 ± 0.77	8.12 ± 2.65
Lignin	20.2 ± 0.01	22.2 ± 0.12	11.2 ± 1.2		
Cellulose (%)	31.34 ± 1.9	28.1 ± 2.3	34.6 ± 2.1		
Hemicellulose (%)	36.23 ± 3.2	29.2 ± 2.1	30.3 ± 3.6		

4.2.2. Experimental Set Up

The schematic representation of a batch rotary drum composter was illustrated in Fig. 4.3, and 4.4, which was utilized in the study. The composter featured a drum with a capacity of 550 liters, mounted on four rubber rollers and attached to a sturdy metal stand. The mechanical rotation of the drum was facilitated by a revolving handle, allowing for batch mode operation. The central unit of the drum had a length of 1 meter and a diameter of 0.76 meters, providing sufficient space for the composting process. To ensure effective mixing, agitation, and aeration of the waste materials during rotation, a thick metal sheet measuring 4 mm in thickness was welded longitudinally at 40×40 mm angles inside the drum. This design element helped promote the proper blending of the waste substrate, inoculum, and sawdust, facilitating optimal decomposition and composting. Moreover, the interior of the drum was coated with an anti-corrosive material. This protective coating served to prevent any potential damage or degradation of the drum due to the corrosive nature of the composting process. By safeguarding the drum against corrosion, the longevity and durability of the composter were enhanced, allowing for repeated and efficient use over an extended period.

The utilization of this batch rotary drum composter provided a practical and controlled environment for the composting process. The design elements, including the drum capacity, mechanical rotation, internal metal sheet, and anti-corrosive coating, were carefully incorporated to optimize the mixing, agitation, and aeration of the waste materials, ensuring effective decomposition and the production of high-quality compost.

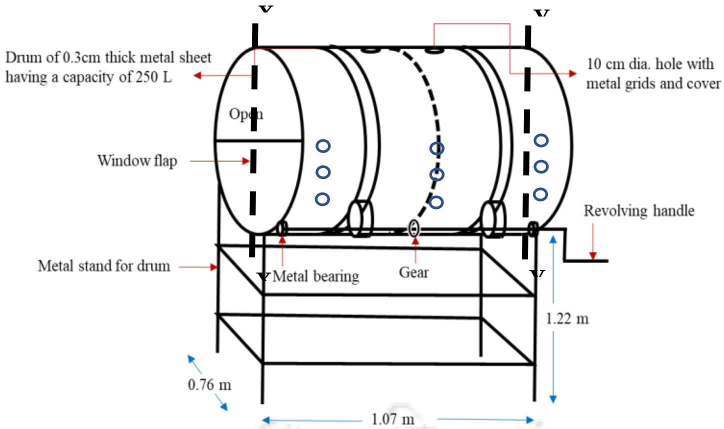


Fig. 4.3. Pictorial Depiction of Rotary Drum Reactor (Kalamdhad et al., 2009)

Blue colour circular shape indicates the sampling sites at 2 cm, 5 cm and 10 cm depth in front, middle and back side of the reactor

Fig. 4.4. Rotary Drum Composter

The terrestrial weeds material was transported to the solid waste laboratory located on the IITG campus. Upon arrival, the entire plant was carefully pulverized to achieve a particle size of approximately 1-2 cm. It is worth noting that achieving a finer particle size is crucial for ensuring adequate degradation of the plant material during the composting process, as highlighted by Ge et al. (2015) in their study. To initiate the composting process, the reactor was fed with a mixture of finely minced substrates in a specific ratio. The ratio used was 5:4:1, which corresponded to 65 kg of terrestrial weeds, 52 kg of inoculum, and 13 kg of bulking agent, as described by Kauser et al. (2020) in their research. This combined feed, totaling 130 kg, provided the necessary organic matter and microbial inoculum required for efficient decomposition. To ensure proper mixing and facilitate easy access of organic matter to microorganisms, the reactor was turned or rotated once a day. This regular turning or rotation of the reactor allowed for the homogenous distribution of organic materials throughout the composting matrix. It also aided in maximizing contact between microorganisms and the organic substrates, promoting the breakdown and degradation processes. By implementing these practices, such as finely mincing the plant material, using a specific ratio of substrates, and providing regular mixing, the composting process aimed to optimize the conditions for microbial activity and organic matter decomposition. Fig. 4.5 depicts the mechanism of the composting process performed in the study.

Fig. 4.5. Composting mechanism performed in the study

4.2.3. Sampling and Analysis

For the purpose of biological, physicochemical, and biochemical analysis, a total of 500 grams and 200 grams of samples were collected from the Rotary drum composter. The sampling process involved taking representative samples from 9 different points within the composter (Fig. 4.3). These points were strategically selected, focusing on the mid span and end terminals of the pilot-scale rotary drum composter. By sampling from various locations, it ensured that the collected samples were representative and provided a comprehensive understanding of the composting process. To maintain homogeneity in the samples, great care was taken during the collection process. The samples were obtained after the rotation of the drum to ensure thorough mixing and even distribution of the composting materials. This step was crucial in obtaining a homogenized sample that accurately represented the overall composition of the compost.

Throughout the drum composting period, samples were collected at two-day intervals. Each time, the grab samples were carefully mixed together to create a homogenized sample. This step helped to reduce any potential variations or inconsistencies within the composting process and ensured that the samples were representative of the entire duration. The collected samples were then subjected to different preparation procedures depending on the analysis required. For physicochemical and biochemical analysis, triplicate samples were collected and immediately air-dried. Afterward, they were ground to a size that could pass through a 0.2 mm sieve. These samples were then stored appropriately, ready for further analysis. In contrast, for biological analysis of the wet sample, sub-samples were taken from the collected samples and either used immediately or stored at 4°C. It was important to conduct the biological analysis within two days to ensure the integrity and reliability of the results. By following this systematic sampling and preparation protocol, the study aimed to obtain accurate and representative data regarding the biological, physicochemical, and biochemical parameters throughout the drum composting process. This approach allowed for a comprehensive analysis of the composting process and its effects on the waste materials.

4.2.4. Physicochemical analysis

To achieve the objectives of the study, various experimental methods were employed, with a particular focus on the physicochemical analysis of the waste substrate, cow dung, and sawdust. These analyses were conducted in the Environmental Engineering laboratory, which falls under the purview of the Civil Engineering Department at IIT Guwahati. The experimental procedures for the physicochemical analysis involved a systematic approach to gather relevant data. The procedures were designed to provide insights into the physical and chemical properties of the feedstock, which are vital in understanding their suitability for the composting process. The experiments entailed careful measurement and observation of various physicochemical parameters. These parameters included but were not limited to temperature, moisture content, organic matter content, pH, electrical conductivity, carbon-to-nitrogen ratio, and ash content. Each parameter played a significant role in evaluating the composition, stability, and nutrient content of the waste substrate and its constituents. To ensure accuracy and reliability, standardized experimental methods were followed. These methods adhered to established protocols and guidelines in the field of environmental engineering. Precise techniques and instruments were utilized to measure and analyze the physicochemical properties of the samples. The experimental procedures were conducted with meticulous attention to detail to minimize errors and ensure consistency. Samples were appropriately prepared, and measurements were taken under controlled conditions. Additionally, replicate samples were tested to validate the results and ensure the reproducibility of the findings.

Temperature

Temperature was monitored using a digital thermometer throughout the composting period in the interval of 6 hours.

Mositure content

To determine the moisture content of the compost, the gravimetric method was employed. This method involved weighing the compost before and after subjecting it to drying at a temperature of 105°C for a duration of 24 hours.

pH and Electrical Conductivity (EC)

To measure the pH and electrical conductivity of the compost, a sub-sample of the compost was mixed with distilled water and stirred. The pH of the mixture was then determined using a calibrated pH meter. Subsequently, the electrical conductivity was measured by passing the mixture through Whatman filter paper No. 42 to filter out any impurities, and the EC measurement was carried out on the filtered supernatant (APHA, 2012).

Volatile Solids and Ash Content

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

$$\frac{\text{W2-W3}}{\text{W2-W1}} = \text{Volatile solids (\%)} \tag{4.1}$$

Total Nitrogen (TN) and Ammonical Nitrogen (NH_4-N)

TN was analyzed using the Kjeldahl method and NH_4-N using KCl extraction (Tiquia and Tam, 2000). For TN analysis 0.2 grams of sample (passed through 0.22 mm sieve) was taken and catalyst mixture (potassium sulphate and cupric sulphate, 5:1) of 3 grams was added, and digested with 10 mL conc. H_2SO_4 using digestion equipment at 400°C for 4 hours (end color of digested sample was green). After digestion, make the digested sample 100 mL. 10 mL of diluted sample was distilled using distillation unit (Pelican Equipments Chennai, India) with 40% NaOH and distilled water, distillate was collected in 25 mL boric acid with mixed indicator. Collected distillate (clear green color) and titrate with 0.02 N H_2SO_4 at end point purple color.

The TN was calculated by the given equation 4.2:

$$\text{TN (\%)} = \frac{14 * (\text{S} - \text{B}) * \text{N}}{\text{W}} \tag{4.2}$$

Where, S = mL of standard sulphuric acid used for sample, B = mL of standard sulphuric acid used for blank, N = Normality of standard sulphuric acid, W = Weight of compost sample in g.

For the analysis of NH_4-N, 5 grams sample (passed through 0.22 mm sieve) was taken in a reagent bottle and shaken with add 50 mL of 2M KCl in a horizontal shaker for 2 hours. After shaking, sample was filtered and supernatant was taken for NH_4-N analysis using Phenate method of Standard methods (APHA, 2012).

Nutrients and trace elements

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

4.2.5. Biological Analysis

Soluble Biochemical Oxygen Demand (sBOD)

About 10 ± 0.1grams 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 2 hours. Then it was filtered using Whattman filter paper (Standard filter paper). The supernatant of samples was taken and analyzed for BOD test using BOD_5 test and equation as given in equation 4.3, and 4.4.

Procedure for measurement of BOD_5

- To create dilutions for the Biochemical Oxygen Demand (BOD) analysis, the dilution water, which contains specific nutrients as outlined in Standard Methods, was utilized. The dilutions were prepared as follows: a blank dilution consisting solely of dilution water, and a dilution where 10 mL of the sample was combined with 300 mL of a BOD bottle, which was then filled up with dilution water.
- Obtain a 300 mL sample and divide it into two sets. The first set will be used for Dissolved Oxygen (DO) analysis immediately (referred to as the "Sample 0th day"). The second set will be placed in a BOD incubator at a temperature of 20°C for a duration of 5 days (referred to as the "Sample 5th day").
- Measure DO in different samples at t=0.
- Incubate samples at 20°C for 5 days, after 5 days, and record dissolved oxygen.

Calculation:

$$BOD_5\text{, mg/L}=\frac{D1\text{-}D2}{P} \tag{4.3}$$

$$BOD_5\text{, g/kg}=\frac{BOD_5\text{, mg/L}}{100} \tag{4.4}$$

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)

About 10 ± 0.1 grams 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 2 hours. Then it was filtered using Whattman filter paper (Slandered filter paper). The supernatant of samples was taken and analysed for COD using closed reflux method, where 1.5 mL $K_2Cr_2O_7$, 2.5 mL of sample and 3.5 mL of COD acid are added to the COD vials and shaken well. The mixture is digested in a COD digester at 150^{o}C for 2 hours and then left to cool down to room temperature. The mixture is then titrated against Ferrous Ammonium Sulphate (FAS) using Ferroin indicator. The color change is from yellow to wine red. The sCOD can be calculated using the following equation 4.5, and 4.6.

$$\text{sCOD (mg/L)}=\frac{\text{(A-B)*Molarity of FAS*D.F*8000}}{\text{Volume of sample}} \tag{4.5}$$

$$\text{sCOD, g/kg}=\frac{\text{COD, mg/L}}{100} \tag{4.6}$$

CO_2 evaluation by Soda-Lime method

About (25 ± 0.1 grams) of fresh compost sample was taken in 1liter airtight container. About 10 grams of Oven dried (105°C) soda lime (1.5 - 2.0 mesh) soda-lime was taken in a 100 mL beaker and it was placed in the above container. The initial weight of the soda-lime taken as (W_1) grams. The container with soda-lime beaker was kept in an incubator, setted at temperature of 25°C. After 20 - 24 hours the soda-lime was taken out and oven dried it again, then the final weight is noted down as (W_2) grams. The difference in mass of soda lime will give the amount of CO_2 absorbed. The calculation can be done using equation 4.7.

Calculation:

$$\text{CO}_2\text{evolution rate}(\text{mg/g VS/day}) = \frac{(W_2 - W_1)}{W \times T} \times 1000 \tag{4.7}$$

Where, W_1 = 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)

OUR was performed according to the method described by (Lasaridi andStentiford, 1996). The OUR was measured in a liquid suspension of compost (5-8 grams of compost in 500 mL of distilled water added with $CaCl_2$, $MgSO_4$, $FeCl_3$ and phosphate buffer at pH 7.2) the solution was kept in suspension by placing it on the magnetic stirrer at constant temperature by keeping the whole assembly into the water bath held at 30°C. During this time, the dissolved O_2 of the suspension was continuously observed through the digital DO meter attached to it. The oxygen consumption rate was calculated by taking the difference of DO with respect to the time intervals and this value was quoted as the OUR in mg O_2/g VS/hour.

4.2.6. Biochemical Analysis

Lignin measurement was conducted by taking 0.3 grams of powdered sample and recorded as W_1 and digested using 72% H_2SO_4 and the extract was filtered. Acid soluble lignin was measured from the filtered sample by measuring the absorbance at 205 nm and calculated using equation 4.8.

$$\%\text{Acid Soluble Lignin} = \frac{\left(\frac{A}{a*b}\right)*\mathrm{df}*V*\left(\frac{L}{1000\mathrm{ml}}\right)*100}{\left(W*\frac{T105}{100}\right)} \qquad (4.8)$$

Where, A = absorbance at 205 nm

df = dilution factor

b = cell path length

a = absorptivity, equal to 110 L/g-cm

V = Filtrate volume

W = Initial biomass sample weight (g)

T_{105} = %total solids content of biomass determined at 105°C dry weight basis

Acid insoluble lignin was calculated by drying the filtrate at 105 ± 3°C for 2 hours in drying oven and recorded weight as W_2, then after the sample was placed in muffle furnace and ignited at 575 ± 25°C for 3 hours and recorded weight as W_3. The acid insoluble lignin was calculated using the equation 4.9.

$$\%\text{Acid Insoluble Lignin} = \frac{W2 - W3}{W1 * T105} * 100 \qquad (4.9)$$

Where, W1 = Initial weight of sample

W2 = Weight of sample after oven drying at 105°C

W3 = Weight of sample after igniting at 575°C

The difference of acid soluble lignin and acid insoluble lignin was taken as lignin according to National Renewable Energy Laboratory procedure (Ehrman, 1996; Templeton, 1995). Cellulose was determined by the acetic/nitric reagent extraction method, as reported by (Varma et al., 2017a) and hemicellulose was determined by the protocol explained by (Varma et al., 2017a).

4.2.7. Humification Characterization

Humification was determined by measuring the quantity of Humic substances (HS) and their spectral characteristics, as described by Xu et al. (2021). In brief, HS was obtained from air-dried compost samples using a w/v ratio of 1:20 of a composite mixture of 0.1 M sodium hydroxide (NaOH) and 0.1 M sodium pyrophosphate (1:1, v/v). To extract the HS supernatant, the solution was agitated at 200 rpm for 1 day and then centrifuged at 4000 rpm for 20 minutes. The pH of the HS effluent was lowered to 1 employing 6M HCL and then left to extract humic acid in the precipitate and fulvic acid in the solution for 12 hours. 0.1 M NaOH was used to re-dissolve the humic acid in the residue. The extracts of HS, humic acid and fulvic acid were filtered using 0.4 μm and then analyzed by a TOC analyzer. The humic acid/fulvic acid ratio was used to determine the Degree of polymerization (DP) and the ratio of humic acid/TOC was used to determine the Humification Index (HI) (Gao et al., 2022).

4.2.8. Heavy Metal Analysis

Total Metals (EPA, 1996)

After digestion using 0.2 grams sample in block digestion system (Pelican Equipments Chennai-India) for 2 h at 300°C with 10 mL H_2SO_4 and $HClO_4$ (5:1) mixture, Mg, Zn, Cu, Mn, Fe, Ni, Pb, Cd, and Cr concentrations were measured using an atomic absorption spectrometer (Varian Spectra 55B).

Water soluble *(Singh et al., 2013)*

After extracting 2.5 grams of sample with 50 mL of distilled water (sample: solution ratio = 1:20) at room temperature for 2 hours in a shaker at 100 rpm, water-soluble heavy metals were determined by Singh et al. (2013). Samples collected were preserved in sampling tube in laboratory refrigerator at 4°C till the analyses are carried out.

Diethylenetriamine Pentaacetic Acidic Fraction (DTPA) *(Singh et al., 2013)*

At 100 rpm, 4 g of ground sample (screened through 0.22 mm sieve) was mechanically shaken with 40 mL of 0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M (triethanolamine) buffered to pH 7.3 (Guan et al., 2011). Samples were collected after centrifuging at 5000 rpm for 5 minutes and preserved in sampling tube in laboratory refrigerator at 4°C till the analyses are carried out.

Toxicity Characteristics Leachable Procedure (TCLP) *(Singh et al., 2013)*

To assess the possible leachability of heavy metals, the solid samples were subjected to the standard TCLP process according to EPA Method 1311. 5 grams solid sample (size less than 9.5 mm) was mixed with 100 mL acetic acid at pH 4.93 ± 0.05 (pH was changed with 1 N NaOH) (sample: solution ratio = 1:20) in a 125 mL reagent bottle and held at room temperature for 18 hours in a shaker at 30 rpm. The suspensions were centrifuged for five minutes at 10,000 rpm, then filtered through Whatman no. 42 filter paper and processed at 4°C for heavy metal analysis.

***Metal Speciation** (Tessier et al., 1979)*

Tessier et al. (1979) has designed and created the traditional heavy metal speciation process. It was named into five fractions: exchangeable (F1), carbonate (F2), reducible (F3), oxidizable (F4), and residual (F5). The steps show the sequential extraction technique in detail. Metals in the extracted samples from the various steps were analyzed using AAS. The sum of the total concentration of F1, F2, F3, and F4 for one heavy metal represents its total concentration of mobile fractions (FA) in mg/kg (dry matter). Furthermore, the total concentration of the heavy metal's F1, F2, F3, F4, and F5 fractions in mg/kg (dry matter) is classified as FT. The ratio of FA to FT is used to calculate the bioavailability factors (BF) of a heavy metal.

Step I:

1 gram of sediment sample is treated with 1 M magnesium chloride at pH 7 and agitated using shaker for 24 hours at room temperature; suspension is then centrifuged at 5000 rpm for 10 minutes , diluted to with deionized water and analyzed.

Step II:

The residual solid of the previous step is treated with Sodium Acetate. After 5 hours of shaking in the shaker at room temperature a solid-liquid separation is performed by centrifugation as before and the metal-bearing solution is diluted and analyzed.

Step III:

The residual solid of the previous step is treated with Hydroxyl ammonium chloride agitated using water bath shaker for 6 hours at 85°C temperature. As in previous steps, a solid–liquid separation is performed and the solution is diluted and analyzed.

Step IV:

The residual solid of the previous step is treated with Nitric Acid and Hydrogen peroxide and agitated for 2 hours at 85°C in hot water bath shaker. After 2 hours, 3 mL H_2O_2 is added and continued for heating with further adding of NH_4OAc and HNO_3 diluted to 20 mL solution. Agitate it for 30 minutes and remove the supernatant.

Step V:

The residual solid of the previous step is added to mixed acid of H_2SO_4 and $HClO_4$ (in 5:1 ratio) and digested for 2 hours at 300°C and filtered using Whatman filter paper.

4.3. PHASE II: TOXICITY ASSESSMENT OF FEEDSTOCK MATERIAL IN VARIOUS PHASES OF COMPOSTING PROCESS (OBJECTIVE 2)

4.3.1. Phytotoxicity Test Using *V. radiata*

The procedure for acquiring the aqueous extracts of the raw substrate and different staged compost samples was conducted using a modified approach derived from the methodology outlined by Matthews and Hastings (1987). During the experimental procedure, a specimen with a mass of 100 grams was combined with 300 mL of distilled water, leading to the establishment of a weight-to-volume (w/v) ratio. Subsequently, the mixture underwent mechanical agitation for a period of 12 hours. Following the period of agitation, the mixture was permitted to undergo a settling process, during which the supernatant, referring to the clear liquid portion situated above the sediment, was gathered for utilization in the subsequent toxicity assessments.

In order to evaluate the phytotoxicity of the samples, a laboratory experiment was conducted utilizing mung bean (*V. radiata*) seeds. The mung bean seeds were procured from a certified vendor located in Guwahati, India. Prior to the commencement of the experiment, the seeds were subjected to surface sterilization through immersion in a solution containing mercuric chloride at a concentration of 0.1% w/v for a duration of 5 to 10 minutes. Subsequently, a comprehensive rinsing procedure was conducted using distilled water in order to eliminate any residual presence of the sterilizing agent, namely $HgCl_2$.

The seeds that exhibited good health were meticulously chosen and subsequently placed into petri dishes. These dishes were utilized to assess the germination of the seeds as well as to measure any potential inhibition in the length of the resulting sprouts. The test solutions were generated through the process of dilution, where the terrestrial weed and compost extracts were mixed with distilled water at different concentrations ranging from 25 to 100% (v/v).

The petri dishes were moistened with various test solutions, in addition to a control solution comprising tap water. Subsequently, the dishes housing the mung bean seeds were introduced into a controlled environment characterized by a temperature of 28 ± 1°C, while being maintained in a state of darkness. The number of germinated seeds was recorded after a 48 hours period. To conduct a more comprehensive examination, growth and biomass of the seedlings following a 5 days incubation period in a controlled environment featuring a light facility was performed. This facility provided the seedlings with an 8 hours light period followed by a 16 hours dark period. This facilitated the examination of potential impacts on the growth and total biomass of the mung bean seedlings.

The experiments were conducted to evaluate the germination rate, seedling growth, and biomass to assess the potential phytotoxic effects of aqueous extracts derived from the raw substrate and different staged compost samples. The provided information would enhance comprehension regarding the influence of these samples on the growth and development of mung bean plants, thereby offering valuable insights into their overall toxicity. The Germination rate (GR), Root length (RL), Shoot length (SL), GI and dry biomass of *V. radiata* was calculated by equations 4.10, 4.11, 4.12 and 4.13. Fig. 4.6 depicts the pictorial representation of phytotoxicity tests on *V. radiata*.

$$\text{GR (\%)} = \frac{\text{No. of germinated seeds in the extract}}{\text{No. of germinated seeds in the control}} * 100 \quad (4.10)$$

$$\text{RL (\%)} = \frac{\text{Root length of all seeds in the extract}}{\text{Root length of all seeds in the control}} * 100 \quad (4.11)$$

$$\text{SL (\%)} = \frac{\text{Shoot length of all seeds in the extract}}{\text{Shoot length of all seeds in the control}} * 100 \quad (4.12)$$

$$\text{GI (\%)} = \frac{\text{GR*(RL+SL)}}{100} \quad (4.13)$$

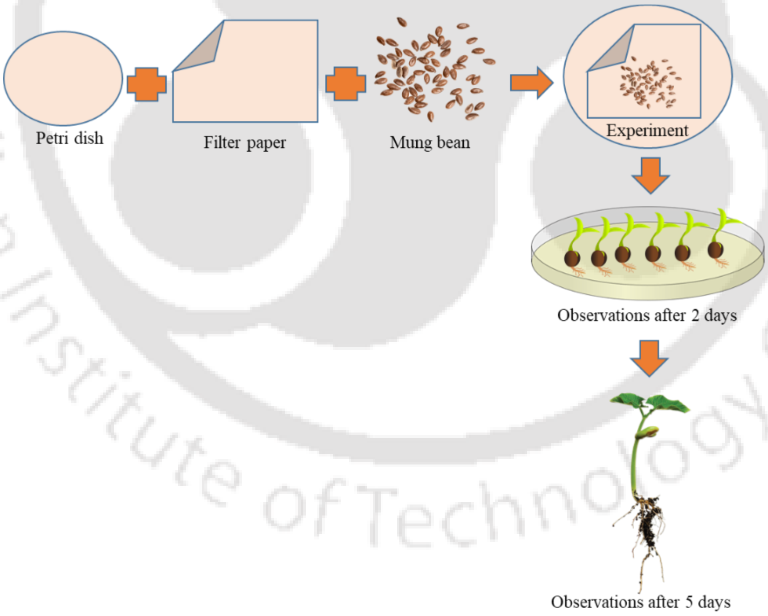


Fig. 4.6. Phytotoxicity test procedures on *V. radiata*

4.3.2. Phytotoxicity Test using *A. cepa*

The phytotoxicity test on *A. cepa* (onion bulbs) was conducted according to the established protocol outlined by Haq and Kalamdhad (2021). The bulbs of *A. cepa* were procured from a local market in Guwahati. In order to maintain uniformity, onion bulbs of equivalent dimensions were chosen for the experiment. The onion bulbs that were chosen for the experiment underwent irrigation using various dilutions of the raw substrate and different compost samples at different stages. Various dilutions were prepared using solutions with concentrations of 25, 50, 75, and 100% (v/v), respectively. Furthermore, a solution of tap water that had been dechlorinated was employed as a control. The irrigation process involved the placement of onion bulbs onto specimen tubes with a volumetric capacity of 50 mL. The specimen tubes were filled with various solutions, which encompassed the diluted samples as well as the control solution. The tubes were subsequently placed in an incubator for a period of five days, maintaining a controlled temperature of 23 ± 1°C. During the incubation period, regular monitoring was conducted on the test solutions in the specimen tubes to ensure consistent contact between the onion bulbs and their respective samples.

Following a 5 days incubation period, the onion bulbs were cautiously extracted from the tubes housing the test solutions. The measurements and recordings of root length and dry biomass of the onion bulbs were conducted in order to facilitate subsequent analysis. The quantification of growth inhibition in the roots of *A. cepa* was performed, and a correlation was established between this inhibition and an index that represents the level of toxicity. The objective of this study was to evaluate the potential phytotoxic impacts of the raw substrate and different staged compost samples on the *A. cepa* bulbs by conducting an experiment and analyzing the root length and dry biomass. The inclusion of this data would enhance comprehension regarding the degree of toxicity linked to these specimens and offer valuable perspectives on their influence on the growth and development of plants. Fig. 4.7 depicts the pictorial representation of phytotoxicity tests on *A. cepa*.

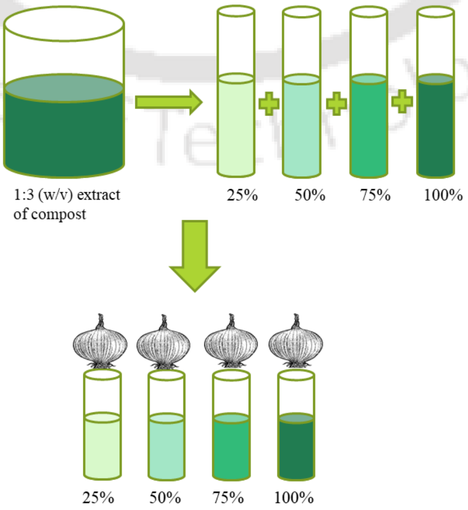


Fig. 4.7. Phytotoxicity test procedures on *A. cepa*

4.3.3. Cyto-genotoxicity tests using *A. cepa*

In the study, cytotoxicity and genotoxicity tests were performed using *A. cepa* as the plant model to assess the effects of waste substrate and various staged compost samples. The onion bulbs used in the experiment were purchased from a local market near IIT Guwahati, and bulbs of uniform size were selected to ensure consistency. To prepare the test samples, the onion bulbs were peeled, and the rooted part was carefully washed with distilled water. After washing, the bulbs were placed in incubation for 48 hours in dechlorinated water (tap water) at a controlled temperature of 23 ± 1°C. This incubation period allowed the bulbs to germinate and establish root growth. The well-germinated onion bulbs were then transferred to the prepared test solutions containing the waste substrate and various staged compost samples. The bulbs remained in contact with the test solutions for 24 hours while being maintained at a temperature of 23 ± 1°C. After the exposure period, the samples were processed to evaluate cytotoxicity and genotoxicity based on two parameters: MI and chromosomal abnormalities (CA). For this purpose, root tips were carefully removed from the onion bulbs and fixed in a mixture of absolute alcohol and glacial acetic acid (in a ratio of 3:1) for 12 hours at 4°C. The fixed root tips were then washed with distilled water to remove the fixative. To prepare the root tips for further analysis, they were hydrolyzed using 1 N HCl at a temperature of 60°C to 70°C for 5 minutes and then washed with distilled water. Following hydrolysis, approximately 1 mm to 2 mm sections of the root tips were cut and processed for staining with hematoxylin, according to the method described by LKS and Sundararaman (1990). All prepared slides were examined under a microscope to calculate the MI and score any chromosomal aberrations (CA) observed. The MI represents the ratio of dividing cells to the total number of cells observed and provides an indication of cell proliferation. CA are evaluated and recorded based on established procedures. Fig. 4.8 depicts the procedures of cyto-genotoxicity experiments.

By analyzing the MI and CA, the researchers aimed to assess the cytotoxic and genotoxic effects of the waste substrate and various staged compost samples on the onion bulbs. This information helps determine the potential harm caused by the samples at the cellular level, providing insights into their toxicological impact on plant cells. MI was determined by scoring approximately 5000 cells (500 to 1000 cells per slide). MI was calculated as:

$$\mathrm{MI} = \frac{\mathrm{A}}{\mathrm{B}} * 100 \tag{4.14}$$

where A: total dividing cells and B: total counted cells per concentration. The number of CA was analyzed by 2500 cells per treatment (100 to 500 cells per slide). CA was calculated as:

$$CA=\frac{C}{B}*100 \tag{4.15}$$

Equations 4.14, and 4.15 were used to calculate MI and CA, which resembles the cyto-genotoxicity assay results.

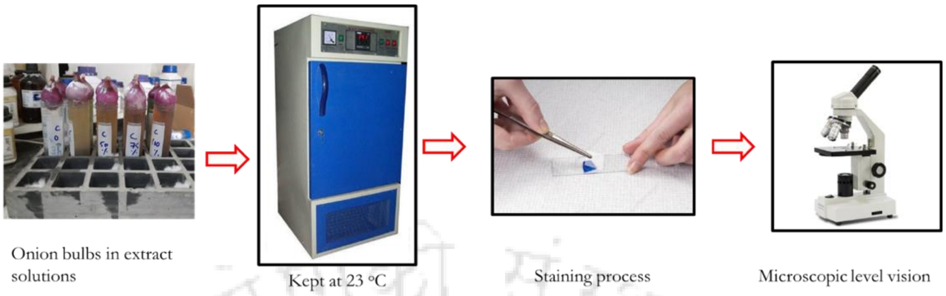


Fig. 4.8. Cyto-genotoxicity assessment procedures of terrestrial weeds and its compost extracts using *A. cepa* as the plant model

4.4. PHASE II: IDENTIFICATION AND QUANTIFICATION OF TOXIC ORGANIC COMPOUNDS IN FEEDSTOCK MATERIAL IN DISTINCT STAGES OF COMPOSTING PROCESS (OBJECTIVE 2)

4.4.1. Identification of functional groups by FTIR

In the study, FTIR was employed to investigate the biochemical differences in compost blends at various stages. FTIR is a powerful analytical technique that can provide information about the molecular composition and functional groups present in a sample. To prepare the samples for FTIR analysis, a mixture was created by combining 300 milligrams of desiccated Potassium bromide (KBr) powder with 1 milligram of pre-dehydrated content from the compost blends. KBr is commonly used in FTIR analysis as a transparent medium to create solid pellets for spectroscopic measurements. The KBr powder and pre-dehydrated content were carefully mixed to ensure homogeneity of the sample. Then, the mixture was compacted using a hydraulic press at a pressure of 10 MPa for a duration of 180 seconds. This compaction process allowed the formation of uniform and flat disc-shaped specimens made of the KBr and compost blend mixture.

The compacted specimens were created to be immaculate and crisp, ensuring a consistent and reproducible sample for capturing FTIR spectra. These specimens provide a medium through which the infrared radiation can pass, allowing the measurement of the sample's interaction with the infrared light. The FTIR spectra were captured using an FTIR spectrometer with a resolution of 0.04 m^{-1} (reciprocal centimeters) and 16 scans. The wavenumber range for the spectra acquisition was set from 4 to 40 m-1. The wavenumber represents the number of waves (cycles) per unit distance, and in this case, it refers to the infrared light's frequency or energy level. By analyzing the captured FTIR spectra, the researchers aimed to examine the biochemical differences in the compost blends at various stages. The FTIR technique provides information about the functional groups present in the sample, such as bonds and molecular structures. These differences in the compost blends' chemical composition can help understand the biological processes occurring during composting and evaluate the quality and maturity of the compost at different stages. Overall, FTIR analysis allows for a detailed characterization of the biochemical properties of the compost blends by examining their molecular composition and functional group contributions, providing valuable insights into the composting process and its potential applications in various fields.

4.4.2. Novel method for the identification of toxic compounds by GC-MS

The present investigation involved the utilization of GC-MS with the Agilent GC-MS instrument (Model: 8860 GC connected with 5977B MSD) for the purpose of identifying toxic organic compounds and organic pollutants. The analysis of the samples involved the utilization of an HP-5MS capillary column (30mm×0.32mm×0.25µm) within the GC-MS system. The specimens utilized in the analysis underwent initial drying in an oven. Subsequently, the substances were combined with a n-hexane (GC-HS grade) solvent in a weight-to-volume ratio of 1:10. The mixture was introduced into a horizontally oriented shaker and subjected to agitation at a rotational speed of 120 revolutions per minute for a duration of 24 hours. The aforementioned procedure facilitated the effective retrieval of the desired compounds into the methanol solvent. Following the period of agitation, the solutions underwent filtration utilizing a Grade 41 Whatman filter paper. The supernatant obtained from the filtration process was stored at a temperature of 4°C. Meanwhile, the matter that remained after filtration underwent two additional extraction cycles using the identical procedure. The aforementioned procedure was conducted in order to achieve a comprehensive extraction of the organic compounds present in the samples. The supernatant that had undergone filtration was subsequently subjected to additional processing in order to facilitate analysis. The sample underwent an additional filtration step using a syringe filter with a pore size of 0.2 micrometer to eliminate any residual impurities or particulate matter prior to its introduction into the GC-MS system.

In the context of GC-MS analysis, a 1 microlitre aliquot of the processed supernatant was introduced into the system via a 10 microlitre syringe. The temperature of the injector was maintained at 280°C. The temperature of the oven was ramped from 70°C for a hold time of 3 minutes, and increased to 300°C at a rate of 10°C/minute for a hold time of 9 minutes, whereas the ion source and interface temperatures were set to 230°C and 250°C, respectively. The compounds under investigation were subjected to mass spectrometry analysis using a full scanning mode. The mass spectra were obtained within the mass-to-charge ratio range of 32 – 500 m/z, with an electron energy of 70 eV. The utilization of scanning techniques facilitated the detection and identification of diverse compounds that were found within the samples. In order to ascertain the presence of toxic organic compounds, a comparative analysis was conducted between their mass spectra and the mass spectra accessible in the National Institute of Standards and Technology (NIST) database. This database was made available in conjunction with the GC-MS device. The database comprises reference mass spectra of established compounds, facilitating the identification of detected compounds through spectral similarity analysis. The comprehensive GC-MS analysis encompassed a duration of 35 minutes, wherein the instrument successfully detected and identified the toxic organic compounds within the samples by analyzing their mass spectra and comparing them to a database. The primary objective of this analysis was to identify and characterize the precise toxic organic compounds and organic pollutants found in the samples. This would yield significant insights into the nature and composition of the substances under investigation, thereby offering valuable information.

4.4.3. Quantification of targeted toxic organic compounds by Gas Chromatography-Flame Ionization Detector (GC-FID)

The methods for quantification of targeted toxic organic compounds such as ageratochromene, diethyl phthalate, stigmasterol, caryophyllene oxide, and farnesene were optimized from various literatures. The methods were illustrated in Table 4.2.

Table 4.2. Methods for quantifying the targeted toxic organic compounds

Toxic organic compound	**Injector temperature (°C)**	**Detector temperature (°C)**	**Oven temperature (°C)**		
			temperature	**Rate of increase (°C/min)**	**Hold time (mins)**
Ageratochromene	230	250	60		0
			220	3	0
Farnesene	200	250	50		0
			170	30	1.5
			300	15	3
Diethyl Phthalate	300	300	260		10

Caryophyllene oxide	250	300	60		10
			220	4	10
			240	1	10
Stigmasterol	290	320	70	0	0
			230	15	0
			250	5	0
			320	25	13

The sample injection was maintained at 1µL, and spitless mode was operated in all the methods. The column used in the instrument was DB-5 packed column. The carrier gas used in the instrument was Argon.

4.5. PHASE III: APPLICATION OF TERRESTRIAL WEEDS COMPOST ON SOIL AND PLANTS (OBJECTIVES 3)

Pot studies, also known as pot experiments, hold significant importance in the process of optimizing compost ratio prior to its application on land. This is attributed to various reasons:

- Controlled environments are utilized in pot studies to facilitate the conduction of experiments involving compost. This experimental setup enables researchers to systematically manipulate and closely observe targeted variables, such as compost ratios, within a controlled environment. The utilization of this control aids in the evaluation of the effects of various compost ratios on both plant growth and soil properties, while minimizing the influence of extraneous factors.
- Reproducibility is a key characteristic of pot studies, as these experiments can be replicated under identical conditions, employing consistent compost ratios. This practice guarantees uniformity and facilitates the evaluation of outcomes across various experiments. The establishment of reliable conclusions regarding the optimal compost ratio for desired outcomes is facilitated by this practice.
- The utilization of pot studies enables researchers to explore various compost ratios in a flexible manner. In order to ascertain the optimal combination for desired outcomes, researchers have the ability to manipulate the ratios of compost to soil or compost to other amendments. In addition, individuals have the capacity to assess various compost sources or types in order to identify the most appropriate alternative.

- The utilization of pot studies enables the meticulous observation of various plant growth parameters, including shoot height, biomass accumulation, leaf area, and root development. Through the process of comparing the growth performance of plants in various pot configurations with differing compost ratios, researchers are able to evaluate the influence of compost on the overall well-being and vitality of the plants.
- The assessment of soil properties and nutrient availability can be effectively conducted through the use of pot studies. Through the examination of soil samples obtained from the containers upon the culmination of the experiment, investigators are able to evaluate alterations in soil fertility, nutrient concentrations, and soil composition that arise as a consequence of the utilization of various compost ratios. This data contributes to the comprehension of the influence of compost on the overall well-being of soil and the dynamics of nutrient availability.
- In comparison to large-scale field trials, pot studies generally incur lower costs and demand fewer resources. Prior to implementing large-scale field applications, they offer an initial evaluation of compost ratios. This economically efficient methodology enables researchers to maximize the efficiency of compost ratios prior to expanding operations to larger land areas.

The temporal efficiency of pot studies is frequently higher in comparison to field trials, as they tend to have shorter experimental durations. This facilitates the acquisition of expedited outcomes and enabled to make prompt decisions pertaining to compost ratios. The reduced duration allows for more rapid optimization and adjustments to be made prior to the widespread implementation of the compost application.

4.5.1. Set up of Small-Scale Pot Experiment

In order to assess the viability of utilizing the Terrestrial weeds compost (TWC) products, alluvial soil was collected from the Brahmaputra riverbanks at a specific latitude and longitude of 26°10'55.3"N 91°41'36.7"E. This specific location was chosen to ensure consistency and accuracy in the study. The collected alluvial soil underwent a series of physiochemical and geotechnical characterizations to evaluate its suitability for use in the study. These characterizations involved analyzing various properties such as nutrient content, pH level, texture, organic matter content, and particle size distribution. The results of these characterizations helped determine the soil's quality and its appropriateness for the research. For the experimental setup, pots with a volume of 10 litres and approximate dimensions of 50 cm in height and 30 cm in upper diameter were selected. These pots were carefully constructed using high-quality and long-lasting hard plastic materials, ensuring their durability and reliability throughout the study.

To ensure proper drainage and prevent waterlogging, small holes were skillfully drilled into the bottom of each pot. These perforations allow excess water to drain efficiently, promoting healthy root development by preventing the roots from being submerged in stagnant water.

To create an optimal growing environment for the plants, a layer of gravel, approximately 2-3 cm thick, was added to the bottom of each pot. This gravel layer serves multiple purposes: it prevents soil compaction, improves aeration in the root zone, and facilitates appropriate drainage. These factors contribute to optimal plant growth and development by creating favorable conditions for the roots to access oxygen and nutrients.

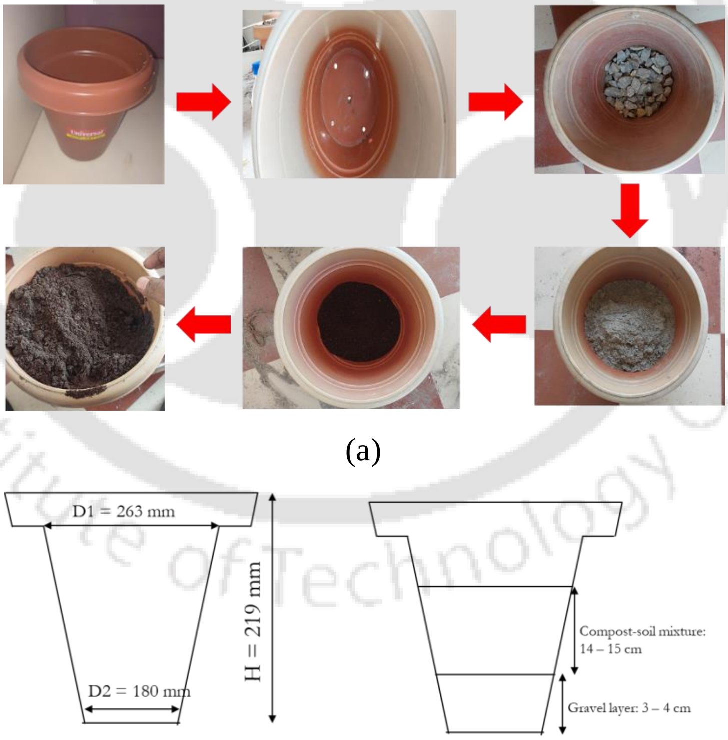
In the experiment, different ratios of compost to soil amendments were used. The specific ratios are provided in Table 4.2, which likely outlines the proportions or percentages of compost and other soil amendments used in the study. These ratios were carefully chosen based on the objectives of the research, aiming to optimize the composition of the soil mixture and assess the effects of different compost-to-amendment ratios on plant growth and development. By providing a detailed description of the experimental setup, including the collection and characterization of alluvial soil, the selection and construction of pots, the inclusion of drainage holes, the addition of a gravel layer, and the use of specific compost-to-amendment ratios, the researchers aimed to establish a controlled and optimal growing environment for the plants. This standardized setup ensures consistency in the study and facilitates the evaluation of the effects of TWC products on plant performance and soil quality. Fig. 4.9 depicts the pot study approach and dimensions of the pot used in the study.

Table 4.3. Mix proportions of TWC amended trails

Treatment name	**Total volume of pot (kg)**	**Percentage of compost**	**Compost composition (kg)**	**Alluvial soil composition (kg)**
Control	4	0	0	4
A5	4	5	0.2	4.2
A10	4	10	0.4	4.4
A15	4	15	0.6	4.6
A20	4	20	0.8	4.8
A25	4	25	1	5
A35	4	35	1.4	5.4
A45	4	45	1.8	5.8
A100	4	100	4	0

P5	4	5	0.2	4.2
P10	4	10	0.4	4.4
P15	4	15	0.6	4.6
P20	4	20	0.8	4.8
P25	4	25	1	5
P35	4	35	1.4	5.4
P45	4	45	1.8	5.8
P100	4	100	4	0
L5	4	5	0.2	4.2
L10	4	10	0.4	4.4
L15	4	15	0.6	4.6
L20	4	20	0.8	4.8
L25	4	25	1	5
L35	4	35	1.4	5.4
L45	4	45	1.8	5.8
L100	4	100	4	0

A: *A. conyzoides* compost; P: *Parthenium hysterophrus* compost; L: *L. camara* compost



(a)

(b)

Fig. 4.9. (a) Mechanism of pot study approach; (b) Dimensions of the pot used in the study

4.5.2. Set up of large-scale field approach

The study area was situated within the campus of the IITG, which is located in the state of Assam, India. The landform observed in the Brahmaputra river basin, situated between 24°39′N–28°15′N and 89°42′E–97°25′E, is known as the plain-river-valley. The area of cultivated land has witnessed a growth from 5.78 million hectares in the fiscal year 2000-01 to 6.05 million hectares in the fiscal year 2018-19. Assam exhibits a humid subtropical climate, characterized by substantial precipitation during the monsoon period and moderate temperatures throughout the year. The average annual temperature in the region varies between 19°C and 27°C, contingent upon the specific geographical location. The average annual precipitation is approximately 2300 mm, exhibiting notable regional disparities. Conversely, the average annual evaporation ranges from 1500 to 2000 mm. The Brahmaputra River and its tributaries are primarily responsible for an average annual surface water runoff of 620 billion cubic meters. The annual fluctuations in surface water exhibit significant variability, primarily attributed to the influence of the monsoon season, which contributes approximately 70-80% of the overall runoff. Conversely, the dry season is responsible for a comparatively smaller proportion, approximately 20-30%, of the total surface water runoff.

In the IITG campus premises, three plots with dimensions of 1.5×1.5 m^2 were prepared for the study. These plots were carefully constructed to have an elevation of 0.15 m, ensuring a consistent level across all three plots. The process involved ploughing the soil multiple times to create the desired elevation and maintain it throughout the experiment, as described by Duong et al. (2012). Based on the results obtained from the previous pot study, an application of 20% (w/w) of TWC was applied to the entire area of each plot. This application ratio was determined based on the findings from the pot study, which likely showed positive effects or optimal results when using this particular percentage of TWC. The decision to apply 20% (w/w) of TWC to the plot area may have been influenced by similar studies conducted by Al-Sayed et al. (2023) and Wang et al. (2021). These studies might have reported successful outcomes or recommended the same application ratio in their respective research. By referring to these studies, the researchers ensured that their approach was aligned with existing knowledge and findings in the field. Fig. 4.10 depicts the field used for the compost application. The 20% (w/w) of compost has turned out to an application rate of 1kg/m^2, which turns about 10 tonnes/hectare.

15th Dec, 2022 (Ploughing)

1st Jan, 2023 (compost amendment)

2nd Jan, 2023 (after compost application

14th April, 2023

14th April, 2023

24th Feb, 2023

12th Feb, 2023 (growth)

Fig. 4.10. Field preparation and mechanism of applying compost to the soil

4.5.3. Physiochemical characterization

The soil's moisture content was determined by subjecting it to a 24-hour period of drying in a hot air oven at 105 ± 5°C. Determining the soil organic matter (SOM) composition is an important aspect of soil analysis as it provides information on the organic matter content. The muffle furnace method is commonly used to determine soil organic composition and involves igniting a representative soil sample in a furnace at a temperature of 550°C for a specified period of time, typically around 2 hours. During this process, the organic matter in the sample is converted into ash and volatile gases, and the mass reduction due to this conversion is measured. The percentage of SOM is then calculated as the mass loss during ignition divided by the initial dry mass of the sample. To determine the amount of soil organic carbon (SOC) present, the Walkley and Black oxidation method was employed. First, a 0.5-1-gram sample of dried soil (which has been sieved through a 0.22 mm sieve) was placed in a 500 ml conical flask. Then, 10 mL of $K_2Cr_2O_7$ was pipetted into the flask and swirled. Next, 20 mL of concentrated sulfuric acid was added to the flask and swirled until the soil and reagent were thoroughly mixed. The flask was then left to cool for 30 minutes. Once cooled, the mixture was diluted to a final volume of 200 mL, and 10 mL of 85% H_3PO_4, 0.2 grams of NaF, and 15 drops of diphenylamine indicator were added. The solution was then titrated with 0.5 N FAS. Finally, SOC was calculated using the formula (equation 4.16).

$$\text{SOC (\%)} = (B - S) \times N \times 0.003 \times \frac{100}{\text{weight of dry soil}} \qquad (4.16)$$

B = ml of standard 0.5 N ferrous ammonium sulphate required for the blank.

S= ml of standard 0.5 N ferrous ammonium sulphate required for blank sample

N = Normality of std. ferrous ammonium sulphate (0.5N)

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

In order to obtain the pH and EC values of the soil samples, a mechanical shaking procedure was employed. The samples were shaken for a period of 2 hours in a 1:2.5 (w/v) ratio of distilled water. This process was used to extract the relevant parameters and obtain a comprehensive understanding of the soil's potential for hydrogen and conductivity. Cation exchange capacity (CEC) was evaluated using Ammonium acetate extraction technique under a maintained pH of 7 for half an hour and analysed through Atomic absorption spectroscopy (AAS) (Jain and Kalamdhad, 2020). A KCl extraction technique was used to ascertain the potency of ammonium nitrogen (NH_4-N). For the analysis of TKN, the Kjeldalh method was employed. Firstly, 0.2 grams of the sample, which has been dried and passed through a 0.22 mm sieve, is combined with a catalyst mixture consisting of Potassium Sulphate and Cupric Sulphate in a 5:1 ratio. The resulting mixture is then subjected to digestion using 10 mL of concentrated H_2SO_4 and a digestion apparatus at a temperature of 400°C for a duration of 4 hours. Following digestion, the volume of the sample is increased to a total of 100 mL. A 10 ml aliquot of the diluted sample is distilled using the Kelplus distillation unit. The distillate obtained from this process is then titrated using either 0.02 N H_2SO_4. Finally, TKN was determined using the formula (equation 4.17).

$$\text{TKN (\%)} = \frac{14 \text{ x } (S - B) \text{ x } N}{\text{Weight of sample}} \tag{4.17}$$

Where, S = mL of standard sulphuric acid used for sample, B = mL of standard sulphuric acid used for blank, N = Normality of standard sulphuric acid, W = Weight of compost sample in grams.

4.5.4. Soil characterization

Bulk density is a key parameter used to assess the physical characteristics of compost, representing the amount of material contained within a particular volume. This value is calculated using a specific formula, which takes into account both the mass and volume of the compost sample. By determining the bulk density, it is possible to better understand the overall density and weight distribution of the compost, which can have important implications for its handling, transport, and use in various applications (equation 4.18).

$$\text{Bulk density, BD (kg/m}^3) = \frac{\text{Mass}}{\text{Volume}} \tag{4.18}$$

The Specific Gravity (SG) of soil was determined in accordance with IS: 2720:1980 using a pycnometer. A clean and dry pycnometer with stopper was weighed (W1) using a balance accurate to 0.001 grams. A portion of air-dried soil sample, passed through a 0.22 mm sieve, was added to fill about 1/3 of the pycnometer. The pycnometer with soil and stopper was then weighed (W2). After filling the pycnometer with distilled water to completely soak the soil, the outside was wiped clean and the weight of the pycnometer was determined (W3). Finally, the pycnometer was emptied, cleaned, and filled with distilled water before being weighed again (W4) (equation 4.19).

$$SG = \frac{(W2\text{-}W1)}{(W2\text{-}W1)\text{-}(W3\text{-}W4)} \tag{4.19}$$

$$\text{Total Porosity} = \left(1 - \frac{\text{Bulk density}}{\text{particle density}}\right) \tag{4.20}$$

The soil's water holding capacity (WHC) was determined using the method described by Jain and Kalamdhad (2019), wherein a soil sample was soaked in water for a duration of 2 h and then allowed to drain for 2 h before measuring the amount of water retained by the soil.

Look deep into nature, and then you will understand everything better.

Albert Einstein
1879-1955

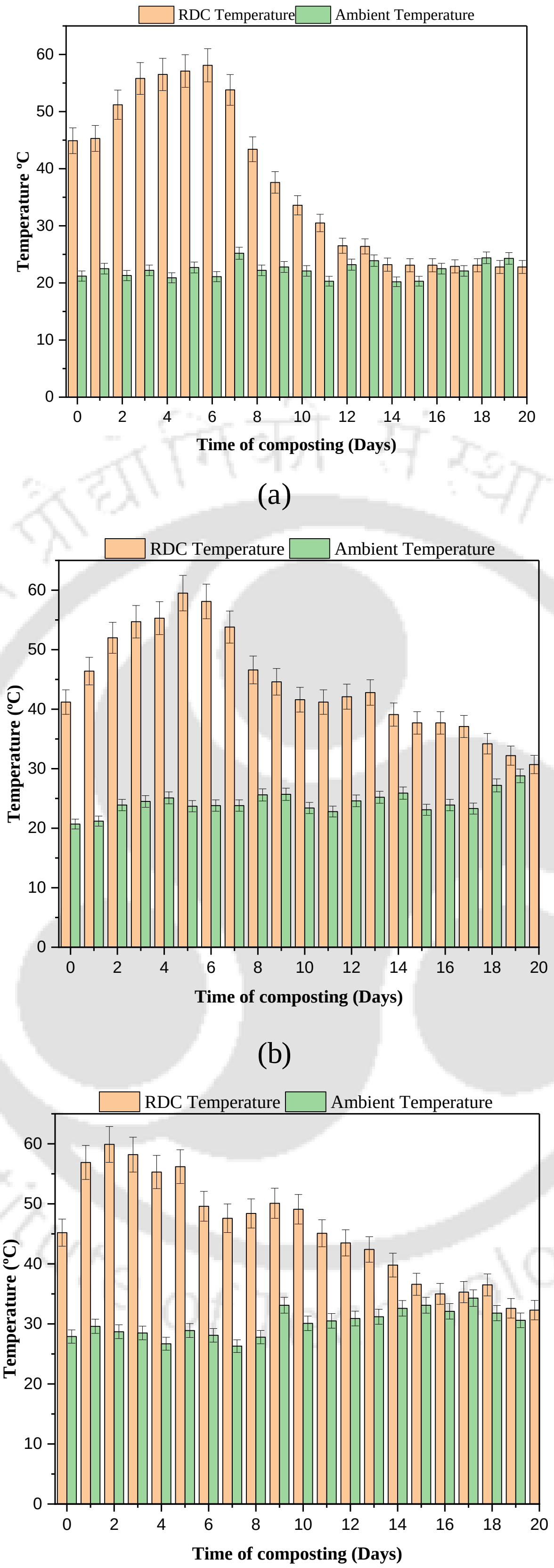
5

Biodegradation of an intrusive terrestrial weeds through in-vessel composting technique

5.1. PHYSIO-CHEMICAL ANALYSIS

5.1.1. Temperature Profile

Temperature profiles play a critical role in the RDC process. Temperature profiles and measurements are essential for comprehending the dynamics of composting processes (Smith et al., 2020). During the breakdown of substrates, the bioheat created will raise the temperature profile of the composting process, and these temperature variations may be used to measure the pace of the fermentation. Fig. 5.1 illustrates the temperature variation that occurs during the composting process. On the sixth, fifth, and second days of the composting process of *A. conyzoides*, *P. hysterophorus* and *L. camara*, the temperature in the reactor reached a high of 58.1, 59.5 and 59.9°C, signifying the organic waste's most vigorous decomposition activity (Kauser et al., 2020). The thermophilic phase was observed on 1st day and lasted until the 8th day in all the reactors. The phase transition from thermophilic to secondary mesophilic might be attributed to the reactor's rotational frequency (Jain and Kalamdhad, 2018). The heat release in the composting process is due to the substrates' thermal heat coefficients and convection and conduction losses. Composting progresses by changing the color of the feed material from light green to brown, which may be attributed to the rapid decay rate and exothermic activity (Hazarika and Khwairakpam, 2018).



(a)

(b)

(c)

Fig. 5.1. Temperature profiling of the feedstock during the composting process of (a) *A. conyzoides*, (b) *P. hysterophorus*, and *L. camara*

5.1.2. Moisture Content

As the composting materials warm, evaporation occurs, resulting in moisture loss. On the other hand, the increased metabolic activity of bacteria during the degradation process creates heat and moisture (Hazarika and Khwairakpam, 2018). As indicated in Fig. 5.2, the final compost's moisture content decreased by 28.4% (*A. conyzoides*), 27.4% (*P. hysterophorus*) and 26.7% (*L. camara*) from the initial day, suggesting efficient degradation activity, which depends on the inceptive moisture content of the substrate (Jain et al., 2020). Composting produces a product with a reasonable moisture level. According to Biotreat (2003), the optimal moisture level of the compost product should be between 42 – 60%. Low moisture content can hinder the operation of microorganisms, whereas a high moisture content can hamper the microorganisms' oxygen supply (H.Wang et al., 2021). This decrease in moisture content was due to the depletion of water vapour due to biologically generated heat. Various studies have reported 50 – 70% moisture during composting of vegetable waste (Kalamdhad and Kazmi, 2009), water hyacinth (Singh and Kalamdhad, 2012), *Mikania micrantha* (Kauser et al., 2020). The inclusion of sawdust in composting mixes aids in reducing moisture content (Eftoda and McCartney, 2004). It was apparent that moisture content was a fundamental result of organic matter breakdown throughout the composting process. RDC have demonstrated their adoption in processing various organic wastes with a substantial reduction in moisture content within 20 days.

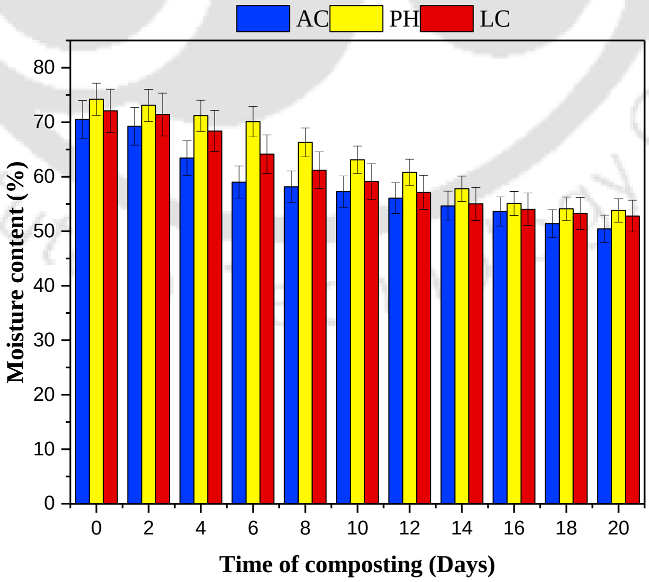
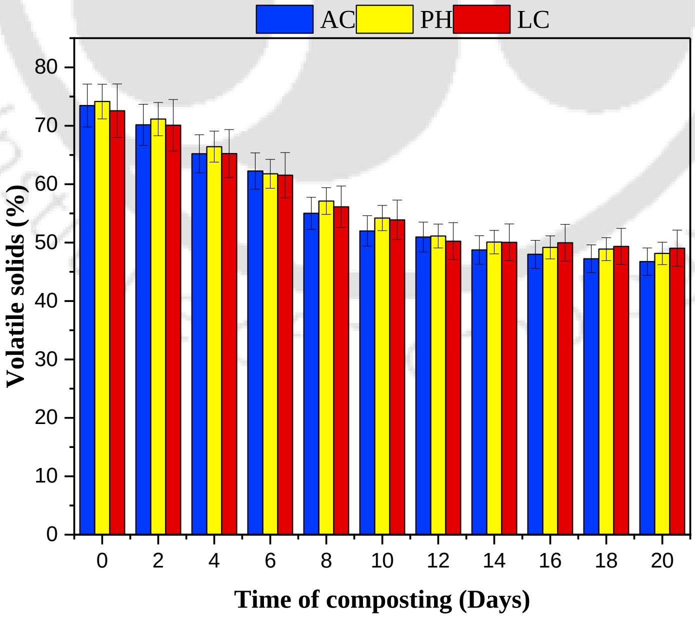


Fig. 5.2. Alteration in Moisture content during the composting process of terrestrial plants

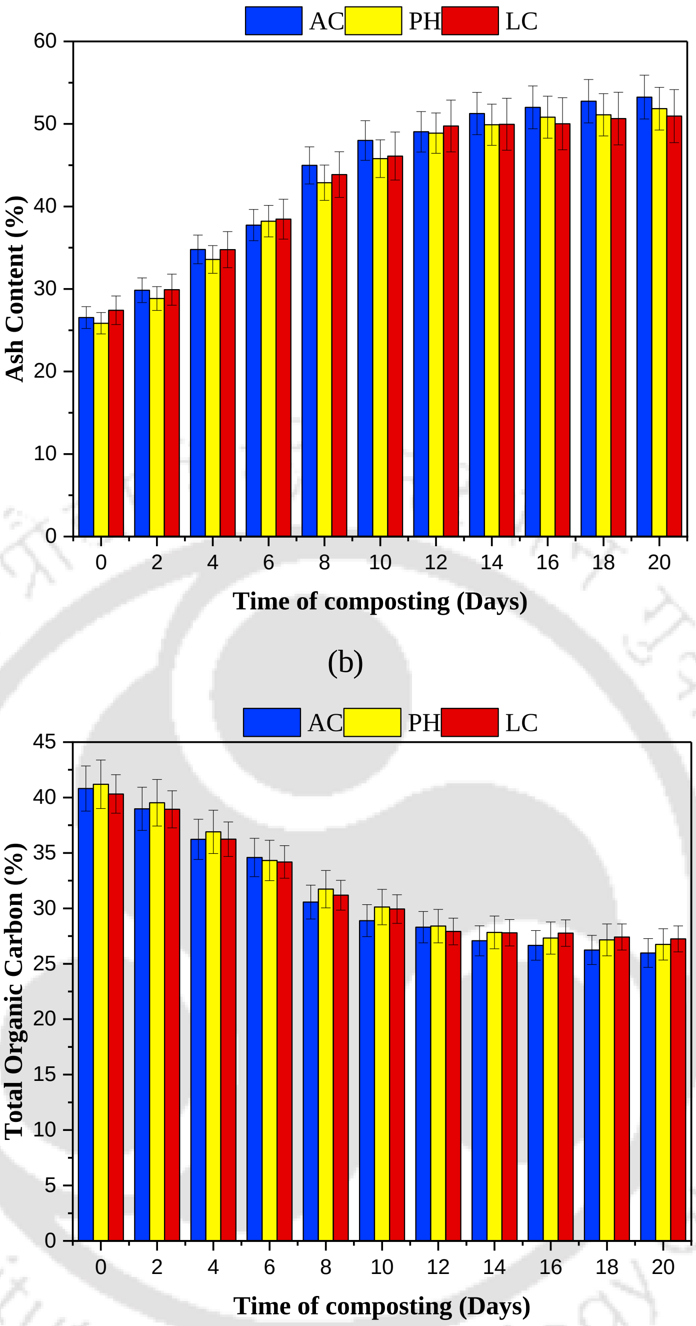
*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

5.1.3. Organic Matter Transformation

During composting, the organic matter is mineralized and degraded into CO_2. In the investigation, organic matter was described by finding out the compositions of volatile solids, TOC and ash content. Fig. 5.3 illustrates the alterations in organic matter breakdown. The characteristics of organic matter in final compost were reduced by 36.3, 35 and 32.4% (volatile solids, TOC) and raised by 50.2, 50.1 and 46.1% (ash content) in composting of *A. conyzoides*, *P. hysterophorus* and *L. camara* compared to the initial day mix. The maximum reduction in organic matter was observed in the thermophilic phase by 25, 23, and 28% (volatile solids, TOC) in composting of *A. conyzoides*, *P. hysterophorus* and *L. camara* compared to initial day blend. The organic matter content was decreased during the composting process, similar patterns were observed by Li et al. (2021) in composting of green waste. The organic matter concentration steadily degraded as the composting process progressed, owing to the microbes' use of organic matter as an energy source and the CO_2 produced by the bacteria. According to Varma et al. (2017a), the breakdown of carbon-rich agents in the composting process is caused by the inclusion of bulking agent, which may improve volatile solids and TOC reduction. The physical parameters of compost samples at initial and final day shown a significant difference at $p<0.05$ and $p<0.01$ using student *t*-test.



(a)



(b)

(c)

Fig. 5.3. Organic matter transformation during the composting process of terrestrial plants (a) Volatile solids, (b) Ash content, and (c) TOC

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

5.1.4. Chemical characterization of the composting process

The pH of the composting process determines its acidic or alkaline nature. The pH changes in the *A. conyzoides*, *P. hysterophorus* and *L. camara* compost were depicted in Fig. 5.4. The pH of the process was marginally elevated in the early days, but as the process progressed, the pH increased moderately. In the final compost *A. conyzoides*, *P. hysterophorus* and *L. camara*, the pH of the process increased from 6.2 to 7.9, 7.01 to 8.19 and 6.4 to 8.3. The increment in pH is due to the breakdown of organic substances such as peptides, amino acids and the subsequent emission of ammonium ions (Lopez et al., 2021). The carbonaceous matter is also regarded as an acid-neutralizing material that aids in composting by raising the pH (Jain et al., 2020). Furthermore, the mineral cation concentration increased as organic material degraded (Francou et al., 2005).

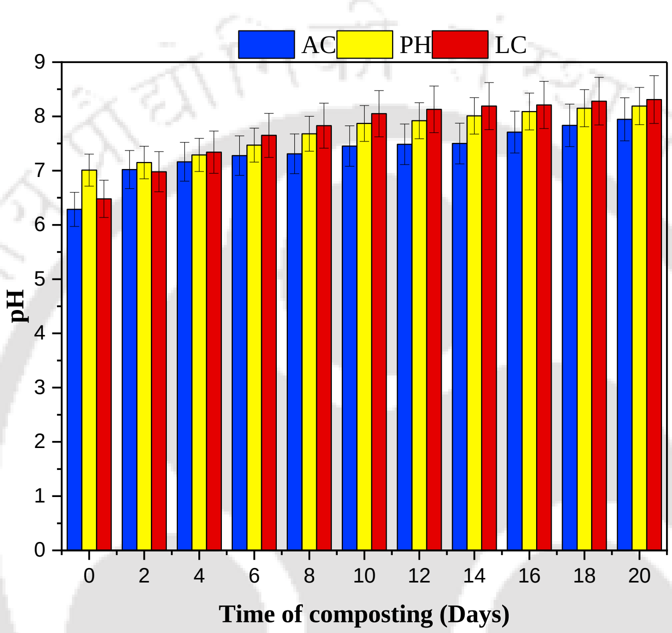


Fig. 5.4. pH alteration during the composting process of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

EC was used as a salinity and phytotoxin predictor to assess any possible issues associated with utilizing compost as a soil supplement. The EC values decreased from 3.75 to 1.45, 5.98 to 3.77 and 6.13 to 3.84 dS/m in final compost product of *A. conyzoides*, *P. hysterophorus* and *L. camara* compared to initial mix, as illustrated in Fig. 5.5. This reduction may be attributed to organic material loss, which resulted in an increase in mineral cation concentrations not mitigated by salt dissolution or adherence to stable organic complexes. Phytotoxicity could be related to EC greater than or nearer to 4 dS/m (Francou et al., 2005). Phytotoxicity experiments were carried out to determine the optimal dosage of compost that may be used in agricultural farmlands. The chemical parameters of compost samples at initial and final days shown a significant difference at $p<0.05$ and $p<0.01$ using student *t*-test.

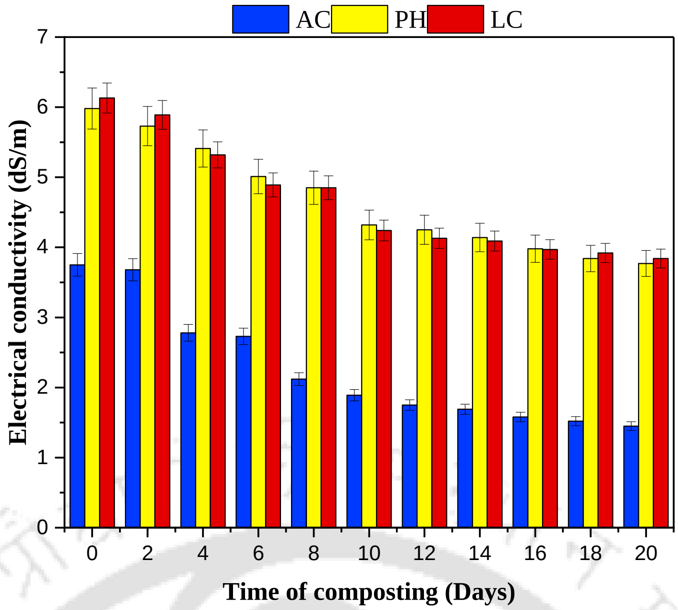


Fig. 5.5. Electrical conductivity during the composting of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

5.2. BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF THE COMPOSTING PROCESS

5.2.1. sBOD and sCOD

The assessment of readily accessible carbonaceous matter determines the quality of compost in terms of sBOD and sCOD. The higher reduction rate of sBOD (91.1, 71.8, and 75.2%) and sCOD (83.9, 80, and 81%) was achieved in final compost of *A. conyzoides*, *P. hysterophorus* and *L. camara* compared to the initial day blend with adequate blending and circulation during the process as depicted in Fig. 5.6 and 5.7, leading in a significant decline in biologic parameters, culminating in a reduction in CO_2 emission, eventually showing compost stability. In brief, composting entails the microbial process of decomposing organic matter, resulting in the degradation of intricate organic compounds into more simplistic and readily biodegradable forms. The concentration of sBOD and sCOD is decreased through this process, wherein the organic matter undergoes transformation into carbon dioxide, water, and stable organic matter. Consequently, a compost product with enhanced stability and reduced environmental pollution is produced. The practice of appropriate composting contributes to the recycling of nutrients, the reduction of waste volume, and the production of a valuable soil amendment that is beneficial for both agricultural and gardening purposes (Kauser and Khwairakpam, 2022).

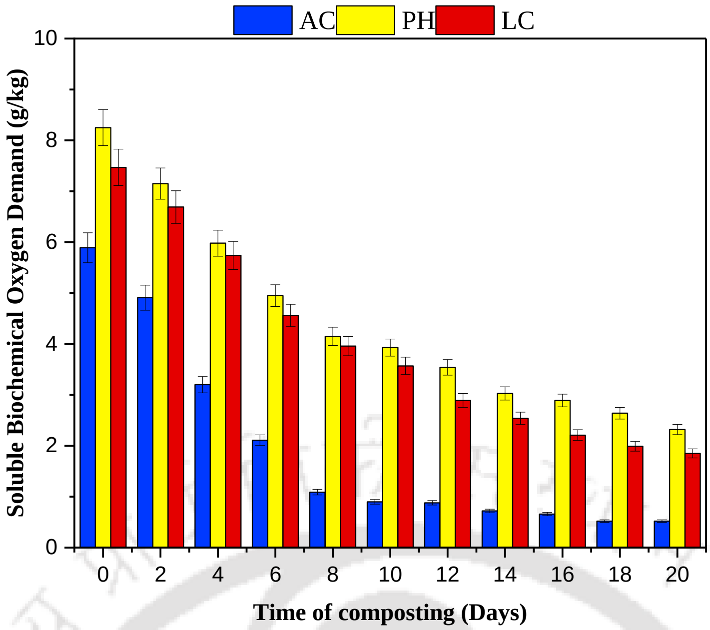


Fig. 5.6. Reduction in sBOD during the composting process of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

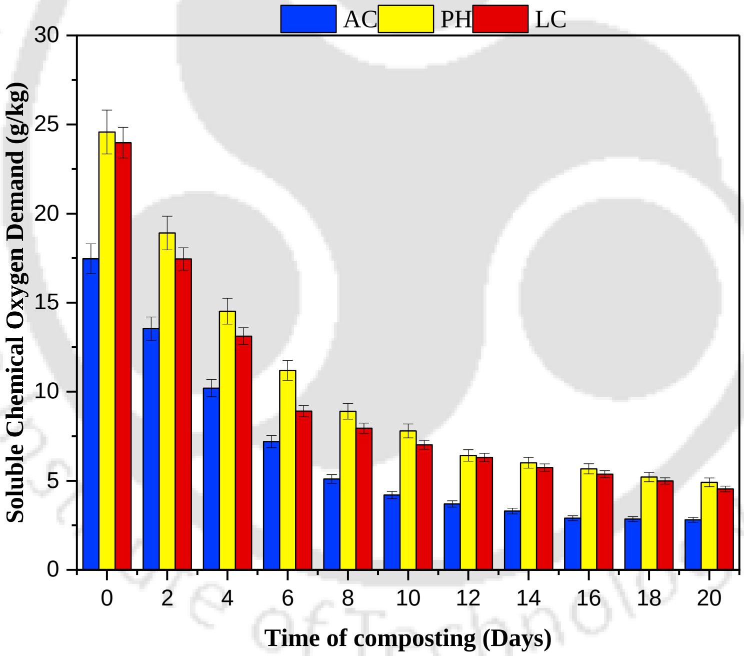


Fig. 5.7. Reduction in sCOD during the composting process of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

5.2.2. Coliform Precedence

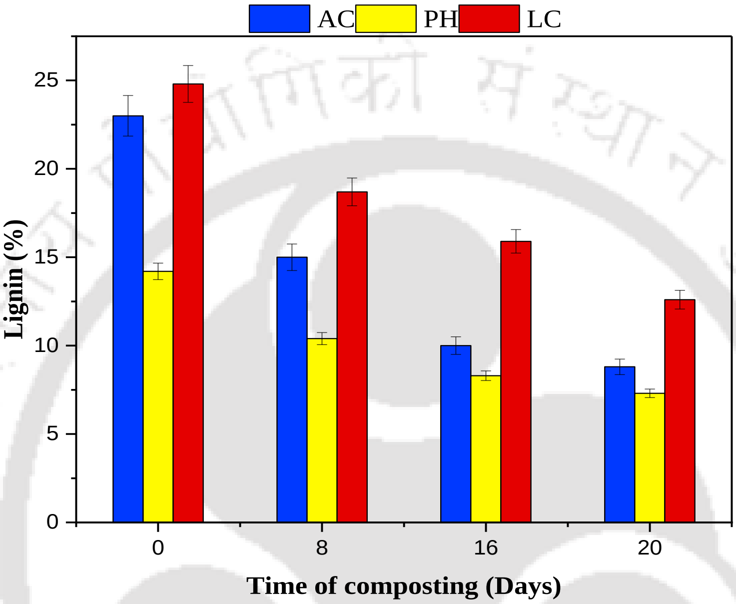
The MPN was performed to assess the alterations in total and fecal coliforms and determine the compost's sanitary quality. The total and fecal coliforms were decreased drastically during the thermophilic phase; this may be due to the elevated temperatures and adverse circumstances. The decreasing pattern of total coliforms was found from 2.4 × 10^6 to 1.7 × 10^2, 2.5 × 10^7 to 2.3 × 10^2, and 2.6 × 10^6 to 2.4 × 10^2, whereas in fecal coliforms from 4.2 × 10^5 to 1.3 × 10^2, 4.6 × 10^5 to 1.6 × 10^2 and 3.9 × 10^5 to 90 MPN g^{-1} dry weight from initial to final days compost blend of *A. conyzoides*, *P. hysterophorus* and *L. camara* is presented in Table 5.1. Similar patterns were reported by Jain and Kalamdhad (2018); Kauser et al. (2020) in RDC of *H. verticillata* and *M. micrantha*. During the process of composting, microorganisms generate organic acids and other substances as byproducts of their metabolic processes. Certain compounds exhibit toxicity towards coliform bacteria, thereby impeding their growth and viability (Kauser and Khwairakpam, 2022). As the process of composting advances and the organic material undergoes decomposition, the feedstock in the composter reaches a state of maturity and stability. The gradual decline of coliform populations can be attributed to prolonged exposure to hostile environmental conditions, microbial competition, and the decomposition of organic matter.

Table 5.1. Reduction in (a) Total coliform (b) Fecal coliform during the composting process of terrestrial plants

		(a)	
Days	***A. conyzoides***	***P. hysterophorus***	***L. camara***
0	2.40E+06	3.20E+06	2.60E+06
10	1.60E+03	1.80E+03	1.80E+03
20	1.70E+02	2.60E+02	2.40E+02
		(b)	
0	4.20E+05	4.60E+05	3.90E+05
10	2.40E+04	2.80E+04	1.10E+04
20	1.30E+02	1.60E+02	9.00E+01

5.2.3. Biochemical Characterization

Due to the resistive nature and role as an essential cell wall element, lignin is regarded as the most complex component to breakdown during composting. The reduction in lignin (55.5, 43.5, and 49.19%), hemicellulose (37.5, 50.7 and 49.8%) and cellulose (45.4, 57.3, and 48.5%) was observed in the final compost of *A. conyzoides*, *P. hysterophorus* and *L. camara* compared to the initial day bend. The maximum reduction in lignin (24%), hemicellulose (27.7%) and cellulose (27.3%) was observed in the thermophilic phase; as per Varma et al. (2017), the bond breakages between lignocellulose will occur in elevated temperature. Adequate rotation and aeration were also crucial in degrading these compounds. Hemicellulose is the foremost component used by microbes as a carbon and energy source after cellulose (Pottipati et al., 2022). Furthermore, the presence of increased populations of microorganisms, including fungi and thermophilic bacteria, during drum composting led to significant reductions in hemicellulose and cellulose. These reductions were more pronounced in the study due to the appropriate combinations of waste materials utilized (Varma et al., 2017a). Fig. 5.8 depicts the degradation of biochemical parameters during the composting of *P. hysterophorus*. The biological and biochemical parameters of compost samples at initial and final days shown a significant difference at $p<0.05$ and $p<0.01$ using student *t*-test.



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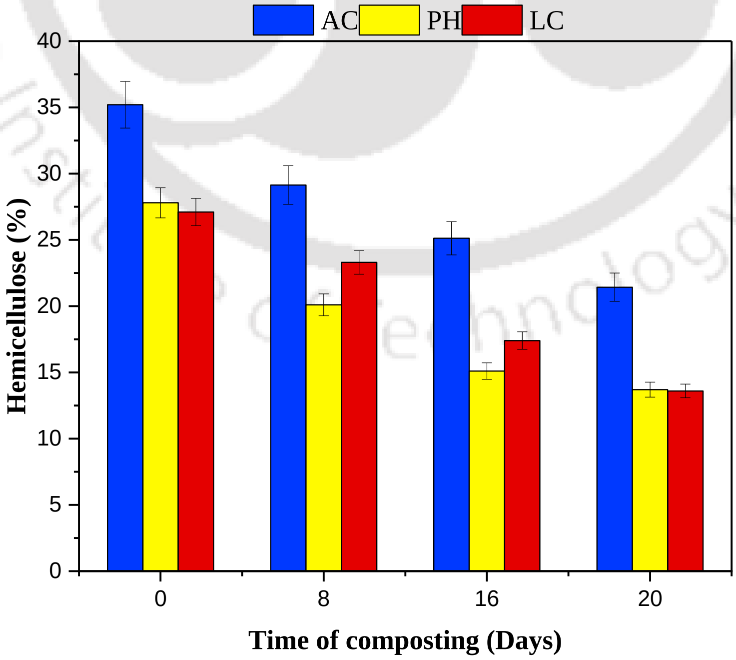

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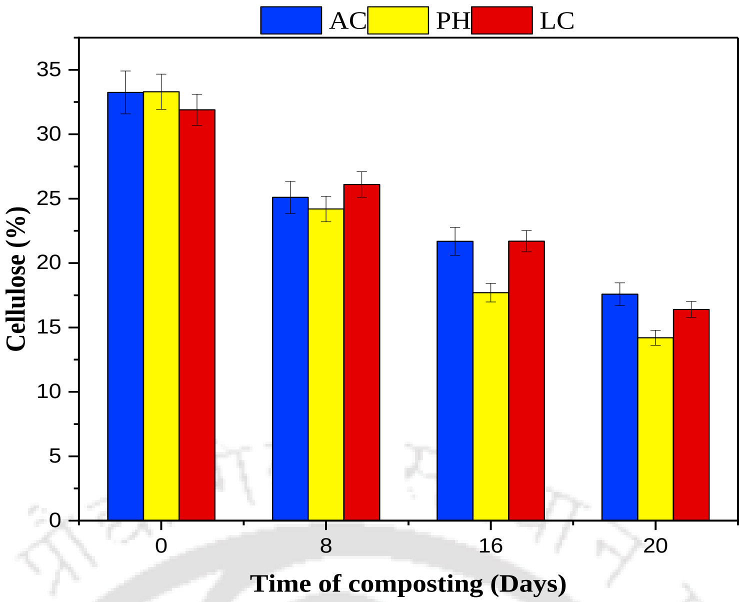


(c)

Fig. 5.8. Reduction in (a) Lignin, (b) Hemicellulose, and (c) Cellulose during the composting process of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

5.3. ASSESSMENT OF COMPOST STABILIZATION

There is a correlation between aerobic respiratory throughout composting and the production of CO_2 from microbial action and this approach is used to determine the rate of compost stabilization (Varma and Kalamdhad, 2015). Fig. 5.9 depicts the decreasing pattern of CO_2 evolution throughout the composting process. The high amount of CO_2 released in the initial days was due to the high content of organic matter degradation by microbial activity in the substrates. The reduction in CO_2 emissions in the final compost of *A. conyzoides*, *P. hysterophorus* and *L. camara* was 68, 63.5, and 61.6% compared to the initial day blend. The reduction in CO_2 emissions was seen near the ending of the composting period, clearly indicating the depreciation of organic matter and subsequently increased breakdown. Due to the increased biodegradable content in the terrestrial plants and the proliferation of microbes throughout the composting process, OUR was elevated in the initial days of composting. Fig. 5.10 illustrates the OUR reduction from 9.52 to 2.84, 9.61 to 2.94 and 9.40 to 3 mg/gVS/day in the process of RDC of *A. conyzoides*, *P. hysterophorus* and *L. camara*. The decreased OUR readings in the final day compost indicate that the terrestrial plants and the other feedstocks have been decomposed by microbes and transformed into a stable compost (Kalamdhad et al., 2009). The stability parameters of compost samples at initial and final days shown a significant difference at $p<0.05$ and $p<0.01$ using student *t*-test.

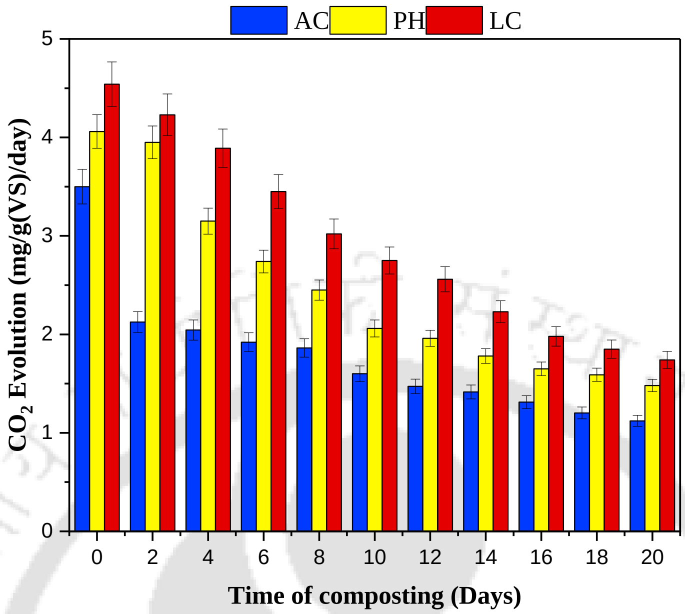


Fig. 5.9. CO_2 evolution during the composting process of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

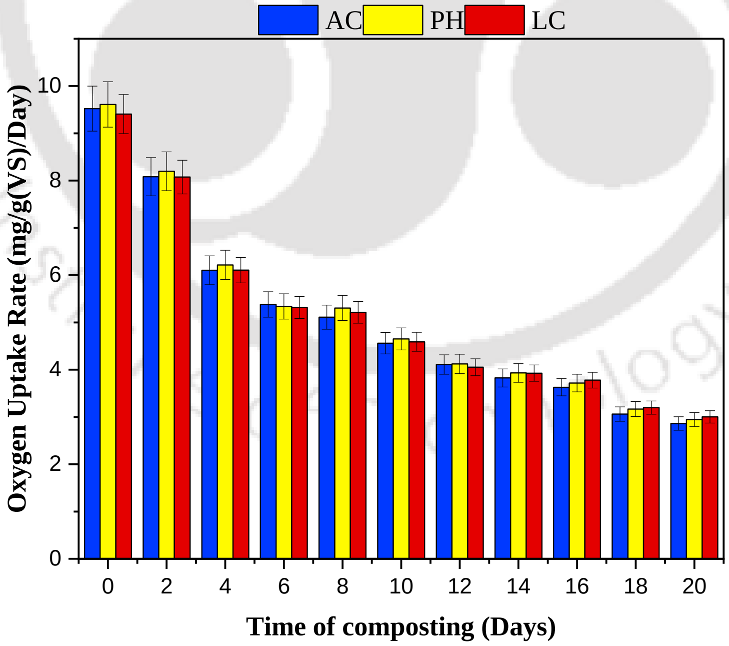
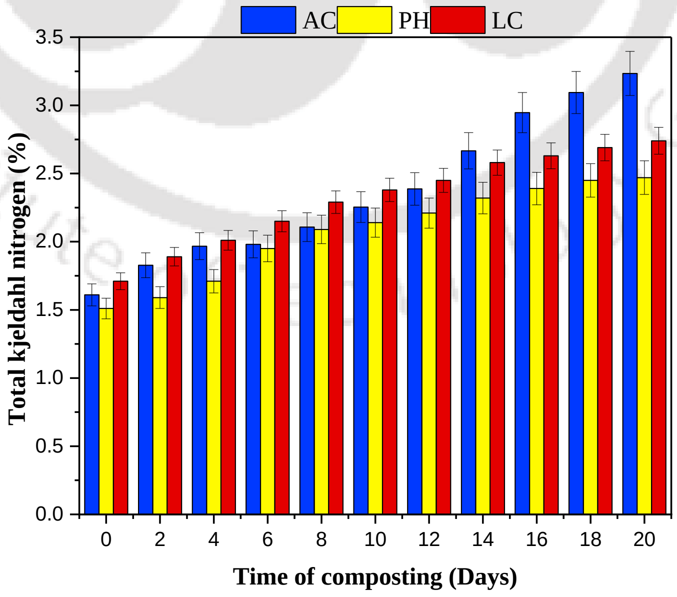


Fig. 5.10. OUR during the composting process of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

5.4. NUTRIENT DYNAMICS IN COMPOSTING PROCESS

The mineralization of nutrients is a complicated biologic mechanism. The increase in nutrients quantity is determined by various factors, including the elemental content of the organic material. Nitrogen is the most crucial nutrient in the composting process, which plays a vital role in metabolic and physiologic functions (Tabrika et al., 2021); the change in multiple forms of nitrogen are seen in Fig. 5.11. The increase in TKN was observed to be 50.1, 38.8 and 37.5% in the final compost of *A. conyzoides*, *P. hysterophorus* and *L. camara* compared to the initial day blend, whereas NH_4^+-N was reduced by 55.1, 83.9 and 88.1% compared to the initial day blend. The proportion of NH_4^+-N rose initially, then dropped, and eventually stabilized. The early composting phase would have seen high levels of microbial activity, significant decomposition and mineralization of organic waste, and an increase in the NH_4^+-N proportion (Xiong et al., 2021). The NH_4^+-N content would have decreased and eventually stabilized due to volatilization by nitrobacteria and nitrification bacteria. The mass decrease has a proportional influence on the increase in nitrogen concentration of the compostable material during the composting process. Nitrogen is immobilized and converted to humus during composting; it may also be used as an organic substance with a slow nutrient discharge. TKN increases during composting due to the total loss of biomass in terms of carbon dioxide and water loss attributable to evaporation. Proteolytic bacteria affect nitrogen during the initial stage of composting (Kauser et al., 2020).



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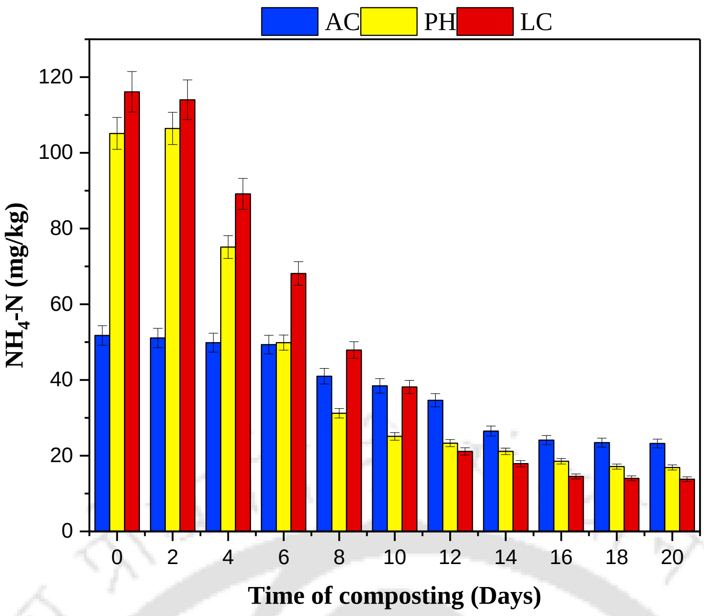


(b)

Fig. 5.11. Increase in (a) TKN and Reduction in (b) NH_4^+-N during the composting process of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

The kinematics of phosphorus could be described by the loss of dry matter, which increases the overall amount, and also by microbes degradation, which increases the amount of accessible phosphorus. Fig. 5.12 depicts the determination of phosphorus content in Total phosphorus and Available phosphorous. In the composting of *A. conyzoides*, *P. hysterophorus*, and *L. camara*, the patterns of Total phosphorus levels rose from 5.94 to 7.28, 5.12 to 8.41, and 4.69 to 8.71 g/kg and Available phosphorous levels rose from 2.34 to 3.62, 2.57 to 4.34, and 2.39 to 4.42 g/kg, respectively. Jain and Kalamdhad (2018); Varma and Kalamdhad (2015) discovered similar trends in composting *Hydrilla verticillata* and vegetable waste. Increase in phosphorus levels owing to microbial degradation and the reduction of organic matter throughout the composting process. However, various species of microbes will work throughout the composting process to solubilize phosphorus at a certain pH (Hameeda et al., 2008).

An optimal rate of decomposition is ensured by maintaining a balanced carbon-to-nitrogen (C/N) ratio within the initial composting materials, typically ranging from 25:1 to 30:1. In the event that the carbon-to-nitrogen (C/N) ratio is excessively high, the composting process may experience sluggishness due to an abundance of carbon (Hazarika and Khwairakpam, 2018). This is attributed to the fact that microorganisms primarily rely on nitrogen as their principal nutrient for growth and metabolic functions. Conversely, in the event that the carbon-to-nitrogen (C/N) ratio is insufficiently high, an excess of nitrogen may lead to the anaerobic decomposition of organic matter during the composting process, resulting in the generation of malodorous compost (Pottipati et al., 2023b). An optimal C/N ratio signifies the presence of nitrogen and carbon in quantities that can be effectively utilized by microorganisms in the process of decomposition. The C/N ratio on the initial day of the feedstock of *A. conyzoides*, *P. hysterophorus* and *L. camara* were 25.3, 27.2, and 23.5, which was decreased to 8, 11, and 9.94 in the final compost.

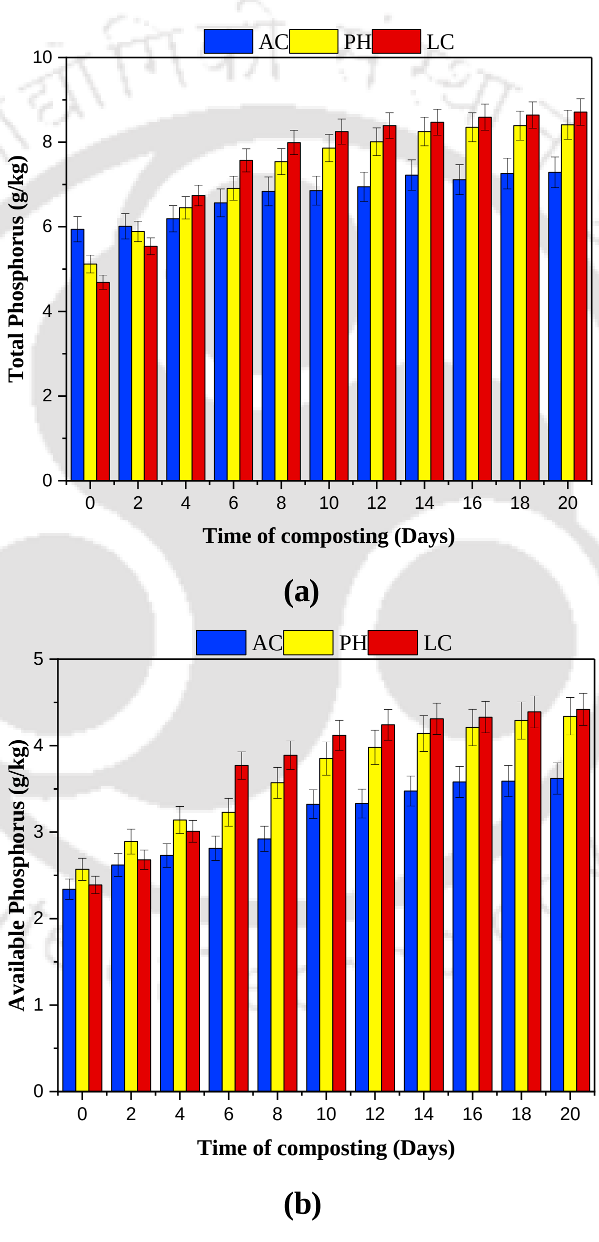
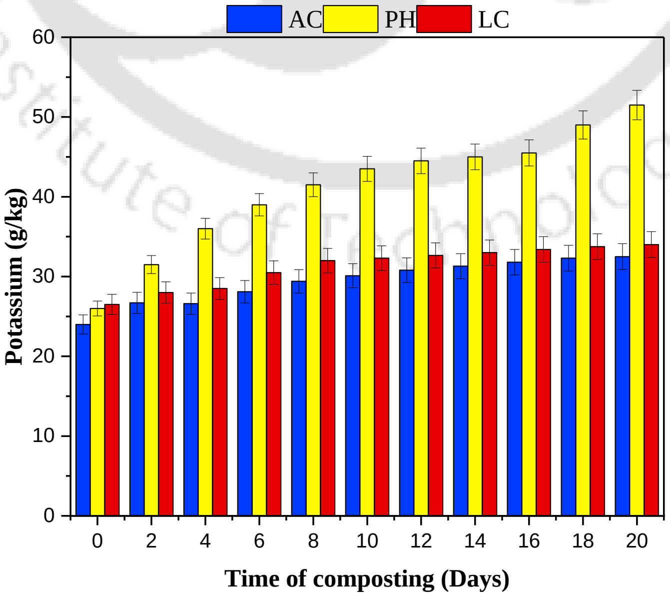


Fig. 5.12. Increament in (a) Total Phosphorus, and (b) Available Phosphorus during the composting of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

According to CPHEEO (2000), the compost's C/N ratio should be less than 20. A C/N ratio of less than 12 implies a high level of compost maturity (Kebibeche et al., 2019). In 20 days, the RDC of terrestrial plants produced high-quality compost. Apart from phosphorus and TKN, the other types of nutrients necessary for plant development are K, Ca, and Na. Fig. 5.13 showed the increasing patterns of these elements throughout the composting process. Artificial fertilizers have grown popular due to their quick accessibility of nutrients, which may, however, be supplemented in a more appropriate approach by compost. The progressive increment of potassium (K) was 26.1, 49.5, and 22%, Calcium (Ca) was 37.6, 37.1 and 39.8% and Sodium (Na) was 54.6, 54.5 and 70.3% in the final compost *A. conyzoides*, *P. hysterophorus* and *L. camara* compared to the initial day blend. Hazarika and Khwairakpam (2018) discovered similar trends in the RDC of paper mill waste. The observed rise in macronutrient levels may be attributed to the elevated macronutrient content present in the substrates (Jain et al., 2018). The RDC feedstock consists of organic matter that contains nutrients in organic forms that are chemically bound. During the process of composting, microorganisms facilitate the mineralization of nutrients, thereby transforming them into their inorganic states that can be readily absorbed by plants. The aforementioned procedure facilitates the liberation of potassium, calcium, and sodium ions from the organic matter, thereby enhancing their accessibility for absorption by plants (Mazumder et al., 2021). The nutritional parameters of compost samples on initial and final days shown a significant difference at $p<0.05$ and $p<0.01$ using student *t*-test.



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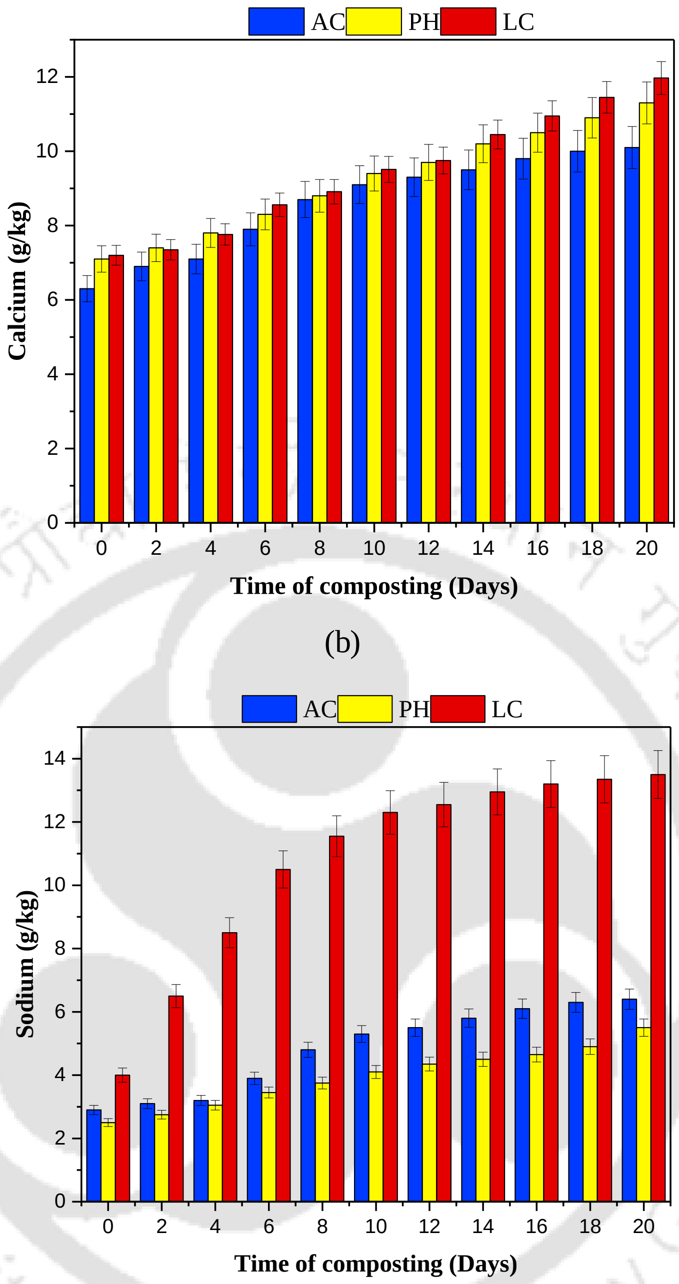


(b)

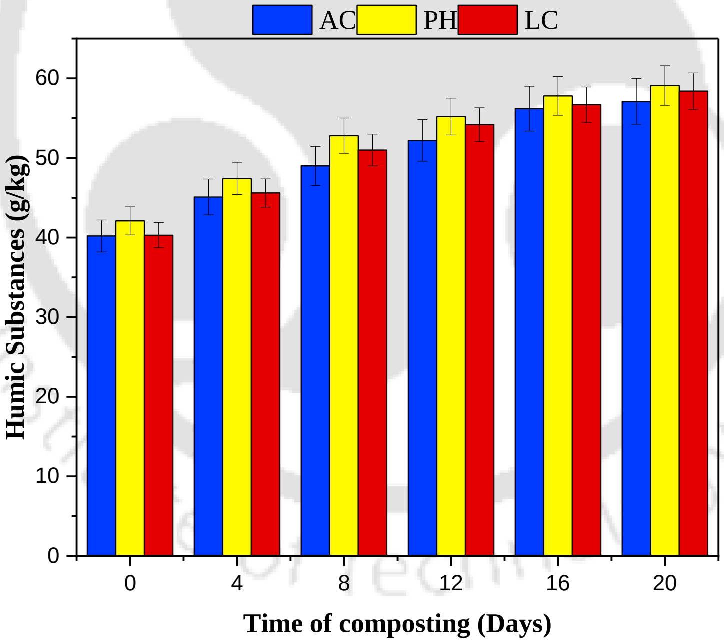
(c)

Fig. 5.13. Increment in macronutrients such (a) Potassium (b) Calcium (c) Sodium during the composting of terrestrial plants

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

5.5. HUMIFICATION CHARACTERIZATION

The key constituents of HS are humic acid and fulvic acid, which indicate the humification activity during the composting process (Zhou et al., 2022). HS fractions increased during the composting of *A. conyzoides*, *P. hysterophorus* and *L. camara*, indicating a continuous humification of feedstock biomass in the in-vessel composting. Fig. 5.14 illustrates the changes in HS, humic acid, and fulvic acid concentrations during the composting of terrestrial plants. When the final compost of *A. conyzoides*, *P. hysterophorus* and *L. camara* was compared to the initial feed, there was a significant increase in HS of 29.5, 28.7, and 30.9%. Similarly, the final compost had a 69.4, 65.2, and 65.7% increase in humic acid and a 37.7, 38, and 32.5% decrease in fulvic acid when compared to the initial feed. It is generally known that organic carbon is degraded by microbial activity and converted to fulvic acid and subsequently synthesized to persistent humic acid throughout composting to enhance the HS concentration (Wu et al., 2017). The highest drop in fulvic acid was seen in the thermophilic phase of the composting process, with a reduction of 22, 26.8, and 25.3% compared to the initial feed, which could be attributed to a significant spike in microbial metabolic rate and therefore compost temperature (Z.Xu et al., 2022). Fulvic acid has a lower molecular weight than humic acid, and microbes can utilize it as an energy source while converting it to a more rigid structure like humic acid. Similar trends were observed by various researchers in the composting of cattle manure (Z.Xu et al., 2022) and kitchen waste (Gao et al., 2022).



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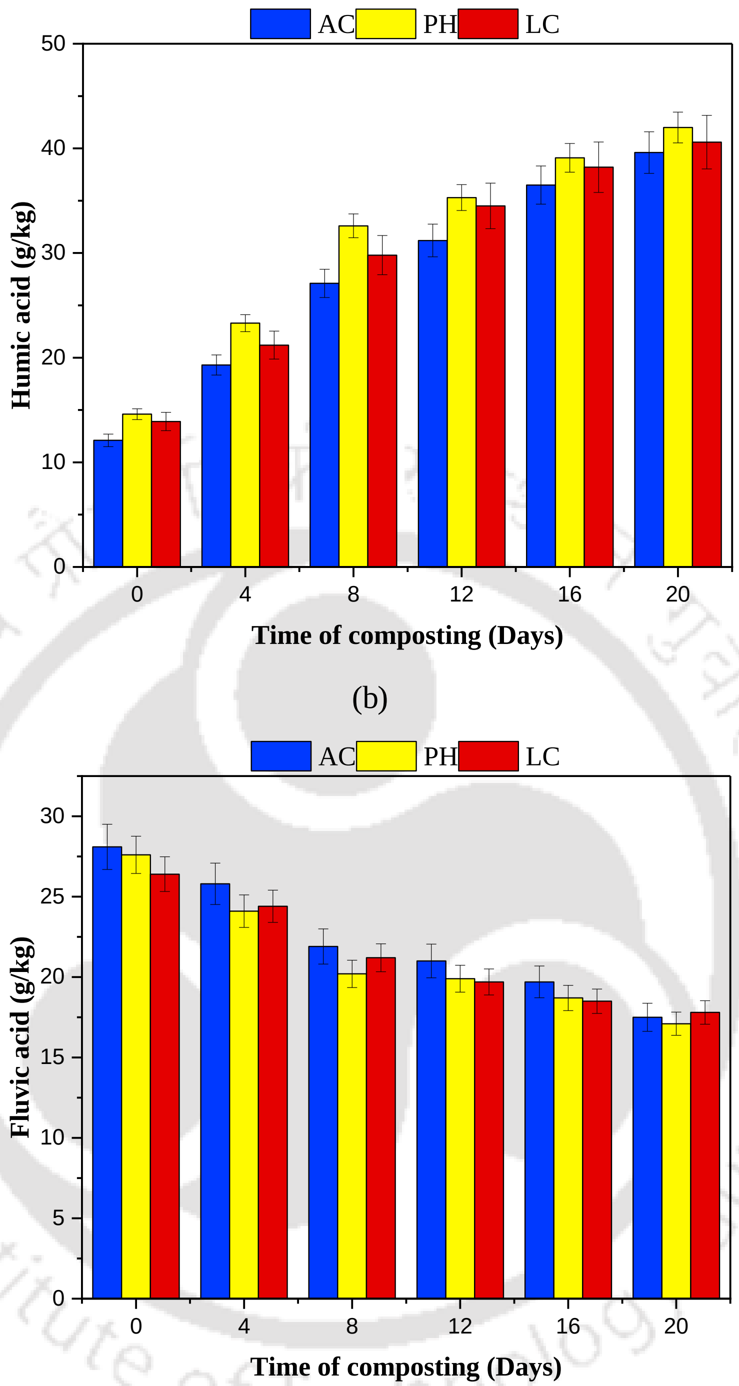


(b)

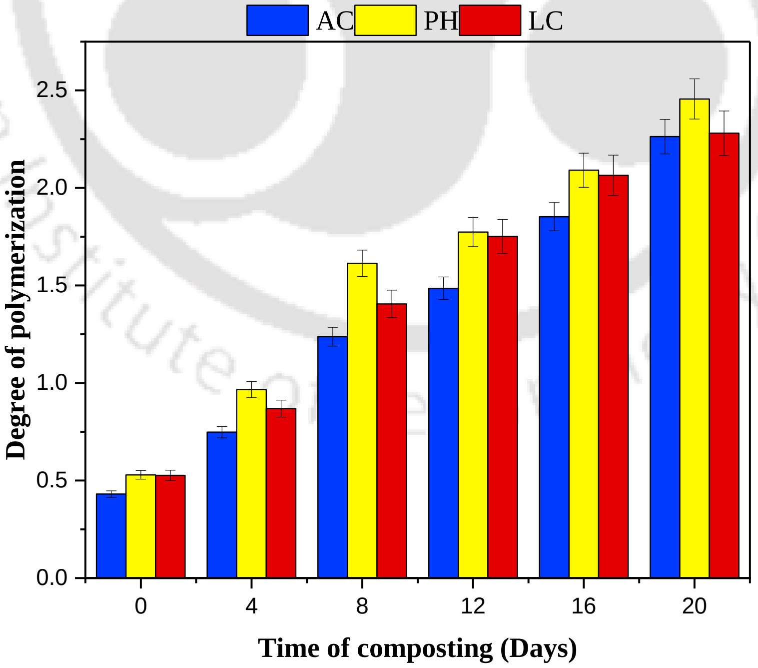
(c)

Fig. 5.14. Humification characterization during the composting process. (a) Humic Substances (b) Humic Acid (c) Fulvic acid

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

DP is regarded as an effective indicator for assessing the humification and relative immobilization rates during the composting process (Zhang et al., 2019). DP increased rapidly during the thermophilic phase (0–8 d) of composting from 0.4 to 1.23 (*A. conyzoides*), 0.5 to

1.6 (*P. hysterophorus*), and 0.52 to 1.4 (*L. camara*) then increased slightly from the 8th day to the end of the 20-day composting period from 1.23 to 2.26 (*A. conyzoides*), 1.6 to 2.4 (*P. hysterophorus*), and 1.4 to 2.28 (*L. camara*) indicating that the production of humic compounds happened mostly during the thermophilic phase as contrasted to the secondary maturation phase of composting. The constant rise in DP value was correlated with an increment in humic acid and a drop in fulvic acid across the composting process, which is illustrated in Fig. 5.15. The DP of the final compost product in the current study were 2.26, 2.4, and 2.28; according to Zhu et al. (2021), compost is regarded as mature when the DP surpasses 1.9. During the process of composting, condensation reactions take place among organic molecules, resulting in the production of humic substances. The organic compounds undergo reactions wherein the functional groups react, leading to the formation of covalent bonds that contribute to the stabilization of the resulting humic substances (Zhu et al., 2021). The composting process revealed an increase in HI in the final compost from 2.96 to 15.24 (*A. conyzoides*), 3.54 to 15.7 (*P. hysterophorus*), and 3.44 to 14.89 (*L. camara*), respectively. A greater HI signifies the successful conversion of organic matter into valuable humic substances during the composting process, rendering the resulting compost a valuable soil amendment for application in gardens, farms, and landscapes. Fig. 5.15 depicts the indices of humification parameters.



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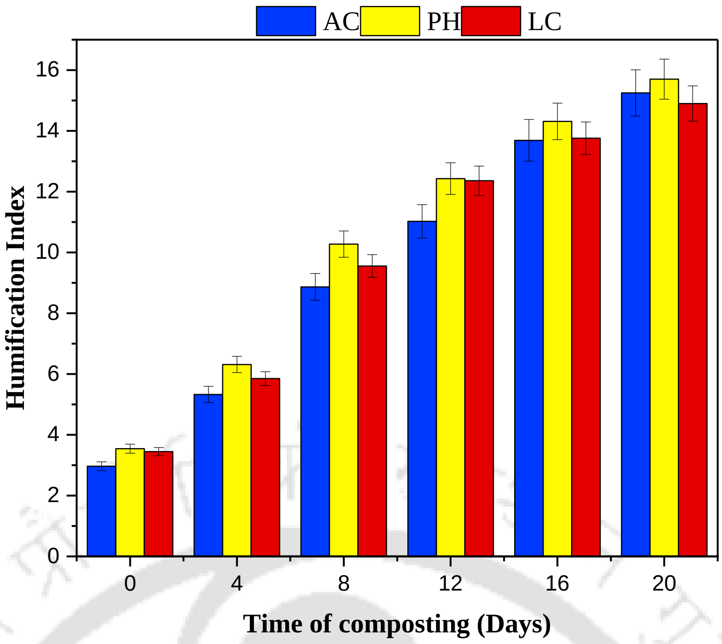


(b)

Fig. 5.15. (a) DP (b) HI are the indices of humification parameters

*AC: *A. conyzoides*, PH: *P. hysterophorus*, and LC: *L. camara*

5.6. HEAVY METALS ANALYSIS

5.6.1. Heavy metals characterization during the composting of *A. conyzoides*

Total Metal Analysis

The total heavy metal in the composting process includes the overall heavy metal concentration present in the sample. The rise in metal content is related to mass loss during composting due to degradation of organic matter, carbon dioxide and water release, and calcification processes (Amir et al., 2005). Table 5.2. illustrates the trends of total concentrations of heavy metals in the course of the composting process. The sequence of HM concentration during the composting process in descending order was Fe > Mn > Ni > Pb > Cr > Zn > Cd > Cu. The highest concentration was found in Fe with 13262.5 mg/kg of dry mass on 0th day and was increased to 16312.5 mg/kg of dry mass; similarly, Mn with 1469 mg/kg dry mass to 1855.3 mg/kg dry mass, Ni with 1470 mg/kg dry mass to 1591.9 mg/kg dry mass Pb, Cr with 952 mg/kg dry mass to 983.1 mg/kg dry mass, 241 mg/kg dry mass to 394 mg/kg dry mass, Zn with 138.5 mg/kg dry mass to 175.4 mg/kg dry mass, Cd with 125.81 mg/kg dry mass to 143.5 mg/kg dry mass, Cu with 77.7 mg/kg dry mass to 89.5 mg/kg dry mass. The variation in total heavy metals may be due to the organic compound biodegradation and metal leachability. Long-term increases in metal levels in compost-modified soil have been linked to higher heavy metal concentrations in the cells of soil-dwelling plants (Singh et al., 2013). Heavy metals' absolute content is usually assessed by their potential toxicity. On the other hand, the current study shows that heavy metal toxic effects on the ecosystem are influenced not only by the metal's overall amount in *A. conyzoides* compost but also by its bioavailable and leachable fraction.

Table 5.2. Alterations in total heavy metal levels during composting process of *A. conyzoides*

Time of composting (days)	**Cu**	**Zn**	**Fe**	**Mn**	**Pb**	**Cd**	**Cr**	**Ni**
0	77.7 ± 2.12	77.7 ± 1.14	13262.5 ± 51.21	1469 ± 11.25	952 ± 12.35	125.81 ± 10.23	241 ± 12.56	1470 ± 11.25
4	79.8 ± 2.01	79.8 ± 1.78	13838.75 ± 49.12	1650 ± 10.35	956.32 ± 21.24	129.12 ± 9.65	329.5 ± 13.32	1464.5 ± 51.32
8	83.3 ± 1.12	83.3 ± 1.47	13550 ± 43.90	1700 ± 15.12	963.1 ± 25.14	135.69 ± 9.23	291 ± 12.54	1555 ± 41.35
12	87.25 ± 1.15	87.25 ± 0.56	13987.5 ± 52.63	1775 ± 18.14	972.1 ± 26.36	137.21 ± 8.56	363 ± 13.58	1562 ± 40.26
16	88 ± 2.01	88 ± 2.23	15862.5 ± 41.23	1832.5 ± 10.47	975.23 ± 25.45	144.5 ± 11.24	385 ± 14.58	1585 ± 39.15
20	89.5 ± 1.58	89.5 ± 0.54	16312.5 ± 42.55	1855.33 ± 13.22	983.1 ± 21.52	143.5 ± 12.32	394 ± 10.32	1591.9 ± 33.12

All the values are in mg/kg within mean ± standard deviation

Water-Soluble Fraction

The most physiologically bound portion is the water-soluble fraction. The water-soluble fraction is the one that is readily available to any flora, potentially inflicting hazardous effects on plant and human health via the food chain, as well as affecting groundwater and surface water. Furthermore, the water-soluble proportion of metal could rapidly percolate and be available during the thermophilic phase due to the breakdown of organic matter but diminished in the final compost leading to variations in further oxidized and anionic conditions milieu (Singh et al., 2013).

Fig. 5.16 depicts the alterations in water-soluble Mn, Cu, Zn, Fe, Ni, Cd, Pb, and Cr concentrations throughout the course of 20 days of composting. The Water-Soluble fraction of Zn was reduced from 16.7% to 5.58% of Total metal. Similarly, for Cu, it was reduced from 4.20% to 0.36% of Total metal. The base-pair Hydroxyl and solitary bond Carboxylic groups found in cow manure boosted adhesion and adhered to Cu to form insoluble and stationary compounds, diminishing free Cu^{2+} levels and minimising the substantial threat to the environment (Guan et al., 2011). For Pb, it was reduced from 0.51 to 0.19% of Total metal, and for Cd, the initial 0th-day compost had a little of 0.51% of Total metal, which became zero by the end of the composting period. Hargreaves et al. (2008) reported that the fractions of water-soluble metals (such as Zn, Pb, Cu and Cd) were decreased and immobilised in the thermophilic stage of composting. In the initial phase of degradation, the pH of composting mixture gets acidic due to the degradation of organic matter, which triggered the availability of Pb and Zn. As a result, these metals had the highest water-extractable concentrations in acidic conditions (Hargreaves et al., 2008).

Mn concentrations decreased throughout composting, with the highest decline occurring on day 12. (from 3.56 to 2% of total Mn). The water solubility of Mn depends on the solubility of organic carbon, which increases during the thermophilic phase and then decreases significantly. The concentration of Cr was 0.72% of total metal during the initial 0th day, finally reducing to 0.5% of total metal. The water solubility of Fe was found to be decreasing, which could be attributed to the production of insoluble organometallic interactions during the composting process. Hence, it resulted in reduced metal availability. The concentration of Fe was 1.94% of total metal during the 0th day, which drastically reduced to 0.82% of total metal. Water-soluble Ni, Pb and Cd concentrations in mature compost of *A. conyzoides* mainly were not observed. Water-soluble Ni and Pb were about 2.04 and 1.79 mg/kg respectively in the mature compost. Furthermore, the transition of water-soluble metals towards a more robust state throughout the composting process may reduce the percentage of the water-soluble metals (Singh et al., 2013).

In composted *A. conyzoides,* the proportion of water-soluble metals was in the following order: Fe > Mn > Zn > Ni > Cr > Pb > Cu > Cd. Heavy metal distribution throughout the composting process was influenced by the discharge of heavy metals from organic matter mineralisation or metal solubilisation due to lower pH. Metals were refractory and tough to extract due to microbial biomass bio-sorption or metal chelation with freshly produced humic compounds.

Plant Accessibility Heavy Metals (DTPA Extraction)

The DTPA remedy, which is frequently recognised as a solubilising reagent, is utilised to assess the carbonate forms and biologically cavort heavy metal forms (Walter et al., 2006). Thus, the fate of toxic metals depends on their relations with organic and inorganic components of soil surfaces. Furthermore, the motility, bioavailability, and kindred ecotoxicity of trace metals to shrubs are highly dependent on their chemical compositions or binding mechanisms. During 20 days of composting, Fig. 5.16 shows the variants of DTPA extractable Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr levels. The sequence of metal concentration in DTPA extracts in the initial day of composted *A. conyzoides* was: Fe > Mn > Ni > Zn > Cu > Cr > Pb> Cd; whereas the sequence of metal contents in DTPA extracts at the end of 20 days composting was Fe > Mn > Cu > Cr > Zn > Pb > Cd > Ni. The study shows an initial concentration of Zn, Fe, Cu and Mn with total metal concentration to be 6.60, 3.8, 15.53,13.53%, respectively. During and after the end of the process, the same levels of Zn, Fe, Cu and Mn with total metal concentration decreased to 1.58, 2.64, 5.10, 10.67%, respectively. Also, it was observed that DTPA extractable Zn, Cu reduced drastically during the thermophilic stage of degradation during composting. In contrast, Fe, Mn, Pb, and Cr reduction was almost at a steady rate.

DTPA extractable Ni was not observed at the end of composting as its concentration got reduced to 0 mg/kg from 23.22 mg/kg initially. Also, the DTPA extractable concentrations of Cr, Cd decreased abruptly due to significantly less availability and mobility of these metals in their insoluble form in the final compost. The concentration of Cd was 0.71% of Total metal during the initial 0th day, finally reducing to 0.37% of Total metal; for Cr, it was 2.8% of Total metal initially to 1.09% of Total metal finally. Reduction in the Bioavailable fraction of Cd in the final compost is due to the binding of Cd to carboxylic and phenolic functional groups of the final compost. Cadmium ions are restrained in an inflexible inner sphere composite. Pb showed little or no change as it reduced from 0.24% to 0.22% of Total metal. The synthesis of more minor soluble metal carbonates and hydroxides is quick to attribute to decreased metal accessibility in plants (Wong andSelvam, 2006).

Valuation of leachable heavy metals

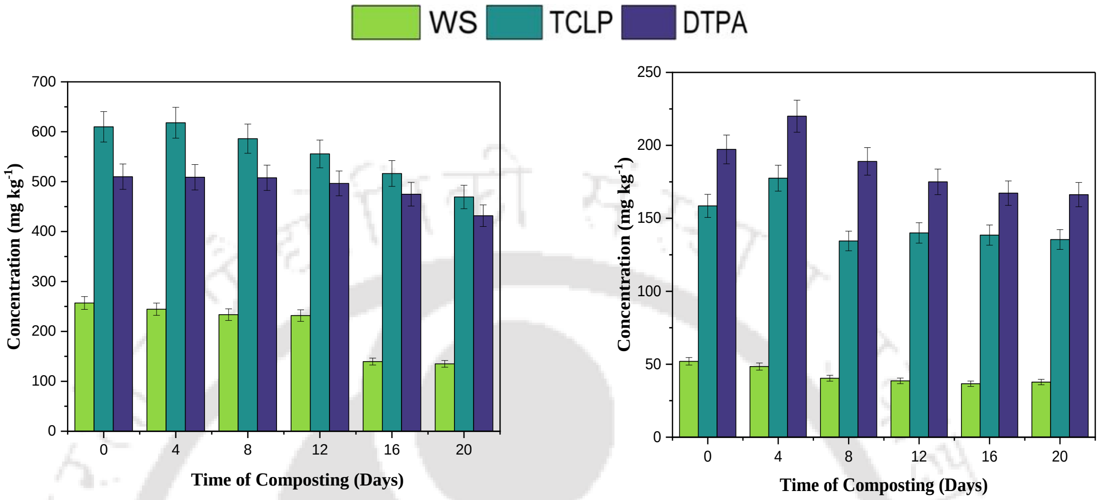
The toxicity characteristic leaching stratagem is designed to mimic the compost material's leaching ability when applied to the soil. The regulatory limits for leached toxic heavy metals are focused on preventing groundwater pollution, which constitutes a risk for humans and the environment. The presence of regulated heavy metals at such high concentrations in the TCLP extract means that the concentrations remain high after considering dilution through the various segments of the extract. If the waste exceeds the regulatory threshold for such metals, it is deemed to be hazardous.

During 20 days of composting, Fig. 5.16 shows the variants of TCLP extractable Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr levels. The sequence of metal concentration in TCLP extract in the initial day of composted *A. conyzoides* was: Fe > Pb > Mn > Ni > Cu > Zn > Cr > Cd; whereas the sequence of metal contents in DTPA extracts at the end of 20 days composting was Fe > Mn > Pb > Cr > Zn > Cu> Ni > Cd. It was observed that initially, Cd concentration after TCLP was 0.04 mg/kg, which reduced to 0 mg/kg at the end. The concentration of Cu was reduced from 18.46 to 3.37% of the total Cu during the composting process, but the maximum reduction was observed on day 16. The reduction could be attributed to Cu complexed with organic matter or humic substances during compost maturation. Copper is generally quite reactive and susceptible to an organic composting substance being washed off. With the fall in copper concentration, it's possible to presume that the best conditions for movement were achieved during the final stages of composting process.

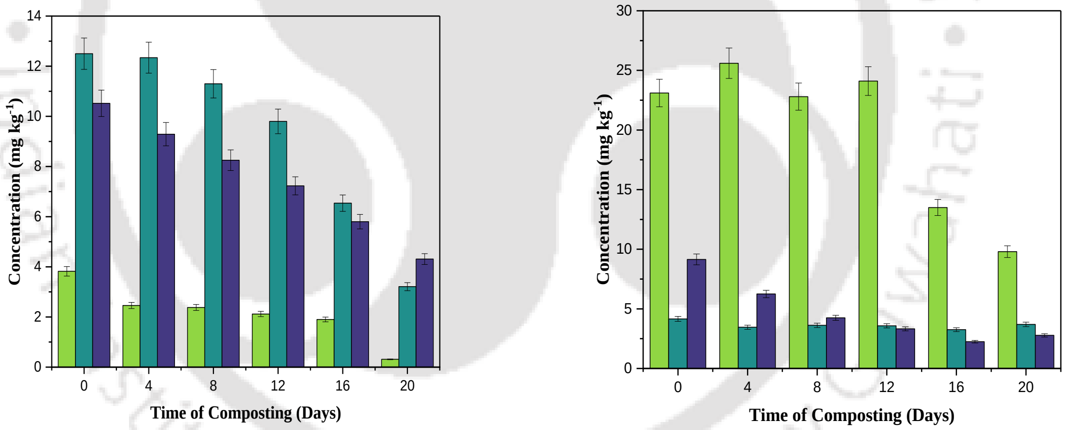
The leachable concentration of Zn, Fe, Pb, and Cr was reduced from 3.03 – 2.1%, 4.61 – 2.88%, 19.12 – 11.44%, and 4.14 – 2.47% of total Zn, Fe, Pb, and Cr metals, respectively, during the composting period. The increment in Fe concentration could be attributed to high organometallic complexation (Hazarika et al., 2017). Chromium has a considerable electrostatic attraction for humic substances, forming stable and stationary complexes (Singh et al., 2013). As a result, the clenching of all metals reduces with composting period; it may raise the pH and intricacy of the metal humic compounds. According to Maity et al. (2008), an increase in compost pH produces an increase in electrostatic interaction, boosting cationic adsorption, producing metal hydroxy ionic species with a more enormous affinity for adsorption surfaces than metal cations, and metal precipitation as metal hydroxides.

Although the *A. conyzoides* compost contains a high concentration of total heavy metals, the concentrations of water-soluble, DTPA, and leachable forms decreased from the 0th to the 20th day in Fe (47.55, 15.33 and 23.95%), Mn (27.34, 15.71 and 14.54%), Cu (91.85, 59.03 and 74.32%), Zn (57.57, 69.61 and 11.05%), Ni (92.21, 100 and 60.55%), and Pb (61.41, 24.48 and

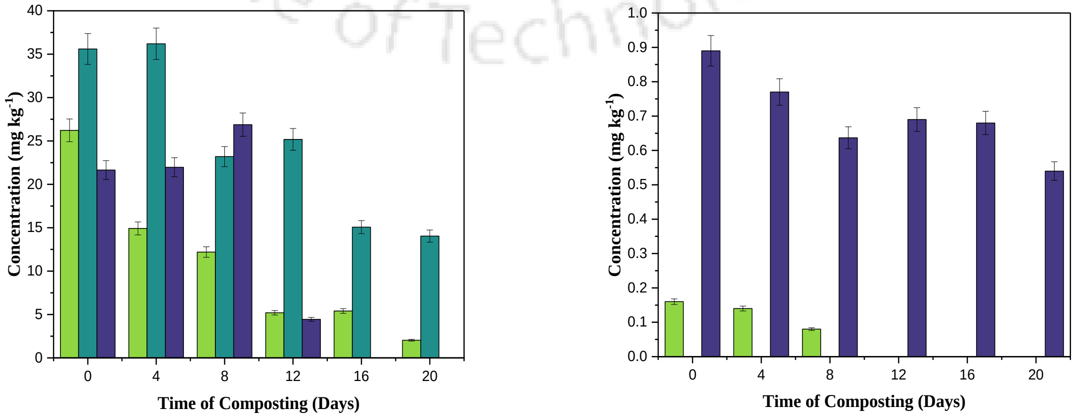
39.84%), respectively. These values of heavy metals strictly adhere to the permissible limits set by multiple global standards (CCME, 2005; CPCB, 2005; FCO, 1985). Hence, using *A. conyzoides* compost may prove to be of lower risk for soil application than using various chemical fertilisers.



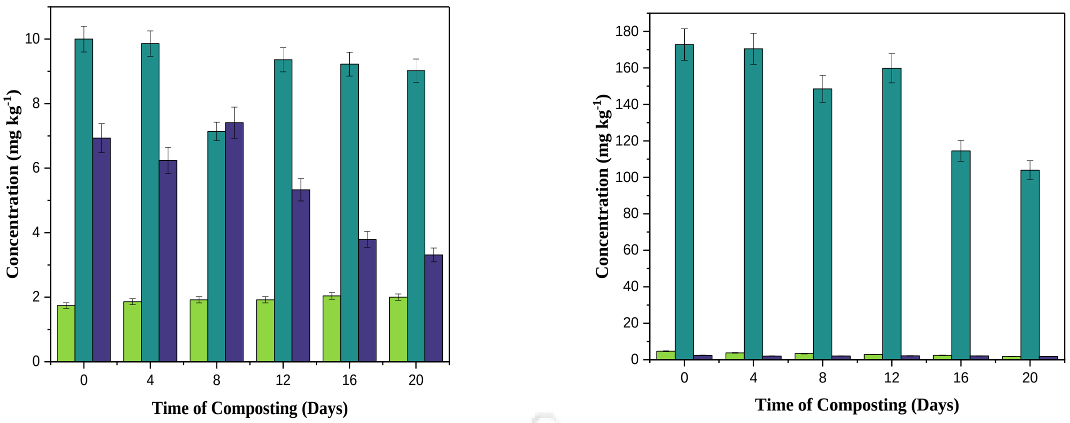
(a) Iron (Fe) (b) Manganese (Mn)



(c) Copper (Cu) (d) Zinc (Zn)



(e) Nickel (Ni) (f) Cadmium (Cd)



(g) Chromium (Cr) (h) Lead (Pb)

Fig. 5.16. Variation in bioavailable and leachable heavy metals throughout the composting process of *A. conyzoides*

Chemical Speciation of heavy metals

In investigating heavy metal speciation during composting, Amir et al. (2005) concluded that the conclusive prognosis of metal speciation through composting is affected by numerous parameters.

In the case of Cu, the F4 and F5 fractions were found to be prominent in all three raw materials. The order of different fractions of Cu in the initial day to final day composting process was: F5 (47.41%) > F4 (26.07%) > F2 (15.99%) > F3 (6.72%) > F1 (3.79%) to F5 (49.33%) > F4 (23.66%) > F2 (17.33%) > F3 (8.89%) > F1 (2.75%) as illustrated in Fig. 5.17 (a). At the end of the composting process, all moving fractions of Cu (F1, F2, F3, and F4 fractions) were converted into F5 fractions, possibly because of the formation, in a rigid internal-sphere compound, that of a Cu complex with multiple organic functional groups mainly R−COOH, C=O and OH, due to immobilisation of Cu at the end of composting (Singh et al., 2013). An organically bound fraction of Cu was found dominant next to the residual. This can be explained by the fact that Cu forms very stable complexes with organic ligands at the end of the composting.

In the case of Zn, the F5 fraction was found to be prominent in all three raw materials. The order of different fractions of Zn in the initial day to final day composting process was F5 (42.80%) > F3 (33.02%) > F4 (13.4%) > F1 (7.7%) > F2 (3.52%) to F5 (57.08%) > F3 (29.75%) > F4 (6.93%) > F2 (2.18%) > F1 (0.85%) as illustrated in Fig. 5.17 (b). The development of the Zn blend with humic components generated during the compost's mature stage may be responsible for the diminution of mobile forms. Humic compounds envelop various organic functional groups capable of absorbing metal ions via ionic force (Singh and Kalamdhad, 2013a). Because of its redox potential, Zn^{2+} is predicted to remain in an ionic state in a solution. The exchangeable Zn fraction was transformed into a reducible fraction during the composting process. The decrease in exchangeable and bioavailable Zn portions could increase pH and organic matter decomposition (Lu et al., 2014). Zn can build complexes with organic binders and dissolve with hydroxides, carbonates, phosphates, sulphides, and various additional anions. According to Kumpiene et al. (2008), the main Zn motility regulating mechanisms are organic ligands complexation and Cation exchange. The rise in pH during composting could be attributed to the reduction in exchangeable Zn portion and enhancement in reducible Zn fraction because Zn has a substantially higher propensity for sorption on the interfaces of Fe and Mn oxides.

The order of different fractions of Fe in the initial day to final day composting process was F5 (41.9%) > F4 (24.34%) > F3 (24.11%) > F1 (6.17%) > F1 (3.46%) to F5 (44.32%) > F4 (39.09%) > F3 (20.78%) > F2 (4.14%) > F1 (2.36%) as illustrated in Fig. 5.17 (c). The lowered pH long with reduction of Fe^{3+} to Fe^{2+} at the root surface enables Fe^{2+} to be taken up primarily through the young lateral roots. The root-absorbed Fe^{2+} is oxidised to Fe^{3+} and then transported to the plant top. The presence of higher carboxyl content accelerated soluble Fe^{2+} production. The easily bioavailable fractions are limited, but F3 and F4 fractions that can be made available based on special environmental conditions are found in considerable amounts. Although the total metal content obtained from a compost sample after intense acid digestion could be used as a pollution criterion.

The order of different fractions of Mn in the initial day to final day composting process was: F5 (55.65%) > F3 (34.52%) > F2 (8.38%) > F1 (0.88%) > F4 (0.54%) to F5 (60.01%) > F2 (20.37%) > F3 (18.40%) > F1 (0.69%) > F4 (0.51%) as illustrated in Fig. 5.17 (d). Manganese tends to form weak coordination complexes with organic matter. This means that Mn^{2+} cannot compete effectively with Cu^{2+}, Zn^{2+} and other more prevalent cations. Mn^{2+} is the most soluble form of Mn, and so under reducing conditions, higher concentrations of Mn^{2+} will be present in the soil solution. Under more oxidising conditions, soil solution concentrations of Mn decrease because the equilibrium shifts in favour of Mn^{4+}, which mainly exist as insoluble hydroxides and oxides. The production of precipitates can reduce Mn mobility by enhancing the frequency of adsorption sites and reducing H^+ competition for adsorption, leading to increased metal stability with humic compounds (Singh and Kalamdhad, 2013a).

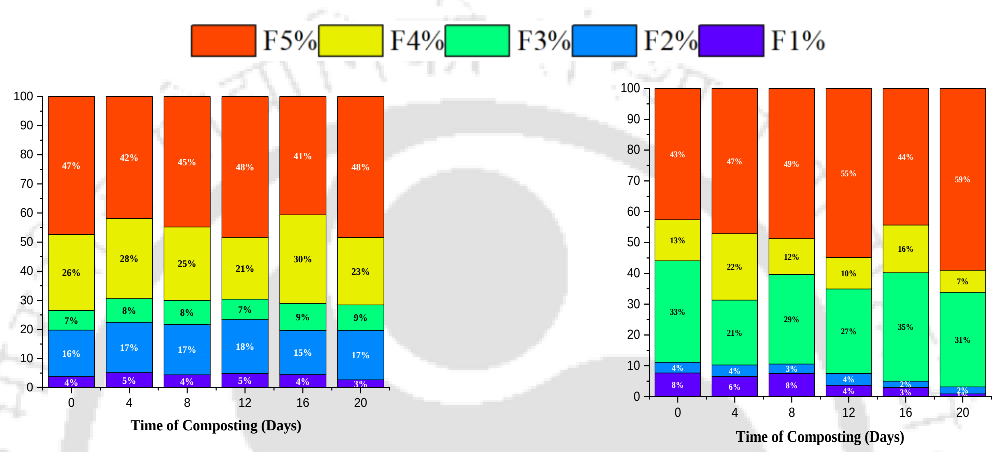
The order of different fractions of Pb in the initial day to final day composting process was: F5 (40.88%) > F3 (20.62%) > F1 (16.41%) > F2 (11.78%) > F4 (10.29%) to F5 (59.37%) > F3

(17.73%) > F4 (7.74%) > F1 (7.72%) > F2 (7.42%) as illustrated in Fig. 5.17 (e). Lead is generally found in carbonate form, which is a fairly mobile form; nevertheless, biologically bonded form, which is a less dynamic form, may be available for plants. Complexation by organic matter leads to a stable fixation state, leading to low mobility of Pb. The retention of Pb can occur not only on the organic matter but also on metallic hydroxides, either by cation exchange or by the fixation on carbonates (Koledzi et al., 2013). The mobile fractions of lead decreased throughout the composting process; the same trend was reported by Singh and Kalamdhad (2012); Wong and Selvam (2006). A slightly alkaline media can reduce Pb motility by creating a Pb-organic matter mixture due to the amphoteric properties of Pb. More alkaline circumstances, on the other hand, may have the inverse effect on Pb persistence. However, it may form soluble hydroxide complexes at high basic conditions that improve Pb motility (Kumpiene et al., 2008).

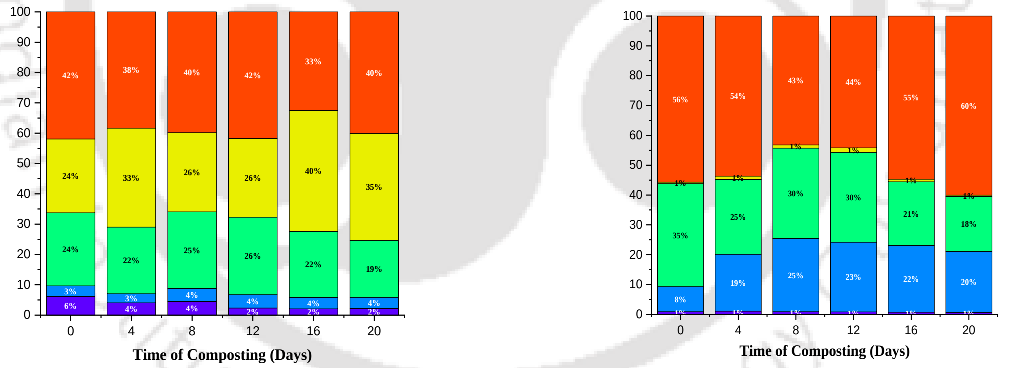
The order of different fractions of Cd in the initial day to final day composting process was: F5 (98.36%) > F1 (0.80%) > F2 (0.72%) > F3 (0.09%) > F3 (0) to F5 (99.54%) > F1 (0.45%) > F4 (0) = F2 (0) = F3 (0) as illustrated in Fig. 5.17 (f). In Cd's case, the metal's mobile fractions were zero on the final day of composting process, determining that the Cd became inert and stable during the composting process. One reason for the decrease in F1 and F2 fractions and elevation in F5 fractions could be Cd's propensity to chemically bind efficiently with organic molecules (Haroun et al., 2007). Moreover, the Cd ion is typically attached to multiple organic functional groups, the most common of which are COOH, C=O, and OH, resulting in the ion being immobilised in a stiff intrinsic complex (Singh and Kalamdhad, 2012).

The order of different fractions of Cr in the initial day to final day composting process was: F5 (61.74%) > F1 (19.98%) > F4 (10.65%) > F2 (6.05%) > F3 (1.56%) to F5 (70.25%) > F1 (11.83%) > F4 (8.7%) > F2 (5%) > F3 (4.17%) as illustrated in Fig. 5.17 (g). In a naturalistic environment, chromium can exist in various chemical valences ranging from +4 to +6, with trivalent chromium (Cr^{3+}) and hexavalent chromium (Cr^{6+}) being the most common. The toxicities and mobility of Cr^{3+} and Cr^{6+} are substantially different. In aqueous systems, Cr^{3+} is less soluble and has lower toxicity. Cr^{6+}, on the other hand, is a highly poisonous compound with high solubility. The findings reveal that compost biomass may easily reduce Cr^{6+} to Cr^{3+} (Yuan et al., 2010).

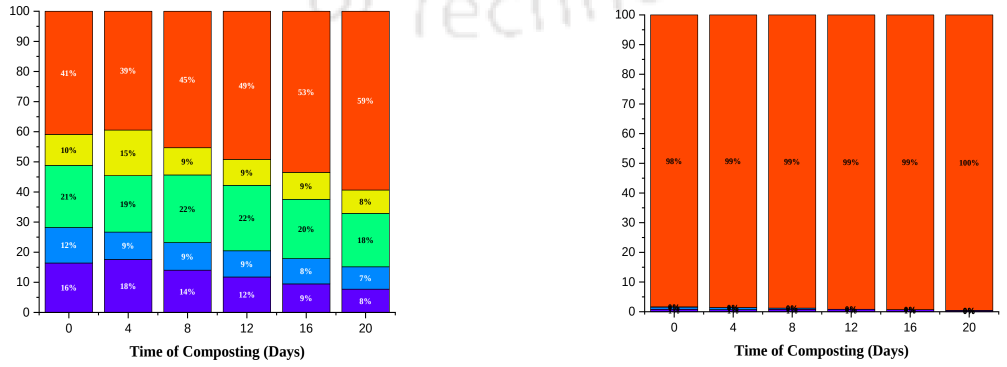
The order of different fractions of Ni in the initial day to final day composting process was F5 (99.6%) > F1 (0.12%) > F4 (0.10%) > F2 (0.08%) > F3 (0) to F5 (99.71%) > F1 (0.10%) > F4 (0.09%) > F2 (0.08%) > F3 (0) as illustrated in Fig. 5.17 (h). The F5 fraction remained the most prominent after composting process. At the beginning of composting, raw organic materials contain many non-specific compounds such as amino acids, polysaccharides, peptides that may form highly soluble complexes with heavy metals. This type of metal bonds are sufficiently easily extracted to allow their quantitative increase during the initial stages of composting, but further transformations (mineralization, humification) that compost humus compounds undergo result in the development of metal complexes that are either poorly or non-extractable. Humic acids are characterized by binding metal, oxide, and hydroxide ions and forming insoluble complexes of varying chemical stability.



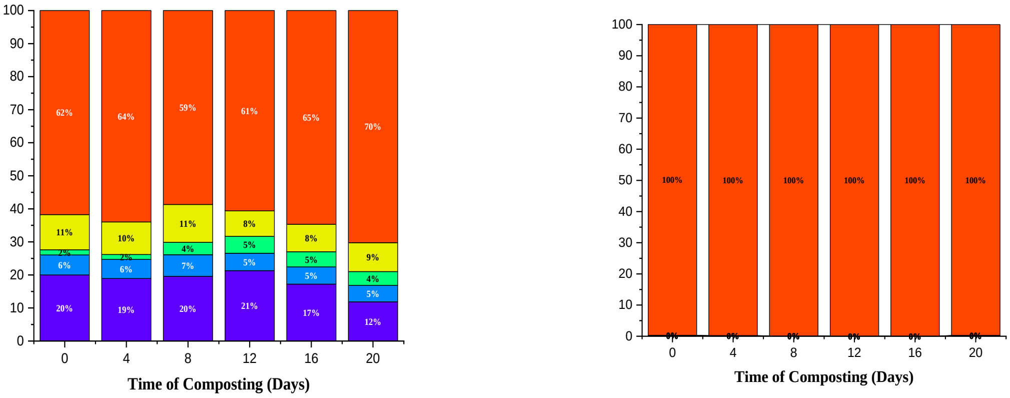
(a) Copper (Cu) (b) Zinc (Zn)



(c) Iron (Fe) (d) Manganese (Mn)



(e) Lead (Pb) (f) Cadmium (Cd)



(g) Chromium (Cr) (h) Nickel (Ni)

Fig. 5.17. Variation in chemical speciation of heavy metals throughout the composting process of *A. conyzoides*

5.6.2. Heavy metals characterization during the composting of *P. hysterophorus*

Total Metal Analysis

The boost in total heavy metal concentration is attributed to mass attrition during composting owing to organic matter breakdown, CO_2 and moisture emission, and mineralization processes (Singh and Kalamdhad, 2012). The total heavy metal concentration in *P. hysterophorus* was relatively high, which could be attributable to metals in the soil. The high metal content in the soil could be attributed to anthropogenic activities occurring at the collection sites, which were surrounded by diverse industries. Terrestrial plants have a high capacity for heavy metal accumulation during rhizofiltration (Sorokina and Zarubina, 2013). Table 5.3 depicts the variations in total heavy metal content during the composting of *P. hysterophorus*. The total heavy metal concentrations during the composting were increased due to the relative mass reduction of the feedstock biomass. The total heavy metal concentrations were found in the following order: Cu < Cd < Zn < Cr < Ni < Pb < Mn < Fe during the composting of *P. hysterophorus*. The highest total heavy metal concentrations were found in Fe, with an 18.9% increase on the 20th day compared to the initial feed; followed by Mn with 10.4%, Pb with 17.8%, Ni with 15.7%, Cr with 17.8%, Zn with 16.1%, Cd with 15.7% and Cu with a 50.2% increase in the final compost product compared to the initial feed. The total heavy metal content is often evaluated based on its potential toxicity. The present investigation, on the other hand, reveals that the detrimental effects of heavy metals on the environment are impacted not only by the total heavy metal concentrations in *P. hysterophorus* compost but also by the mobility potential of the metals.

Table 5.3. Alterations in total heavy metal levels during composting process of *P. hysterophorus*

Time of composting (Days)	**Mn**	**Fe**	**Cu**	**Zn**	**Ni**	**Cd**	**Cr**	**Pb**
0	1930 ± 12	8590 ± 51	47 ± 5	195 ± 21	482.5 ± 41	96.5 ± 13	299.5 ± 15.1	898.6 ± 21.3
4	1960 ± 15	9900.2 ± 63	60.5 ± 11	199.7 ± 10.2	492.5 ± 38	98.5 ± 21	312 ± 20.1	936 ± 23.1
8	2034.2 ± 33	10154.3 ± 48	76.5 ± 9.2	208 ± 27	525 ± 19.1	105 ± 11.1	315 ± 13	945 ± 37.8
12	2040.1 ± 21	10235.3 ± 62	89 ± 7.2	192.2 ± 22	556 ± 20.6	111.2 ± 26.2	351 ± 32.1	1053 ± 51.1
16	2115.2 ± 31	10408.2 ± 53	92 ± 5.8	202.7 ± 31	569.2 ± 31.4	113.8 ± 10.2	340.5 ± 22	1021.5 ± 36
20	2155.3 ± 26	10598.2 ± 39	94.5 ± 9.2	232.5 ± 17.1	573 ± 28.1	114.6 ± 11	364.5 ± 37.1	1093.5 ± 32

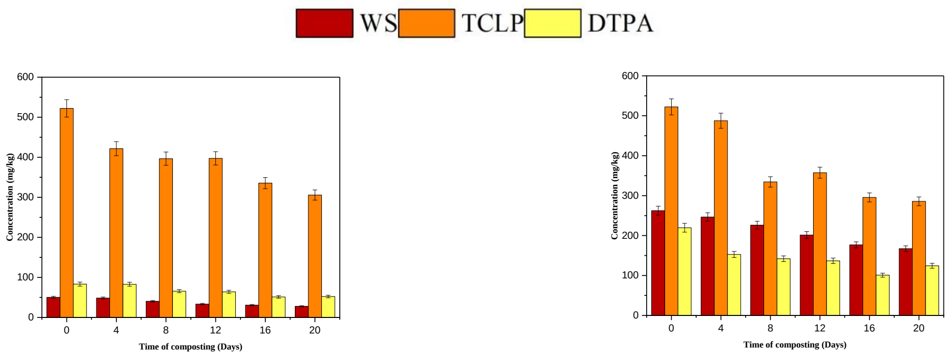
All the values are in mg/kg within mean ± standard deviation

Bioavailability and Leachability Fractions of Heavy Metals

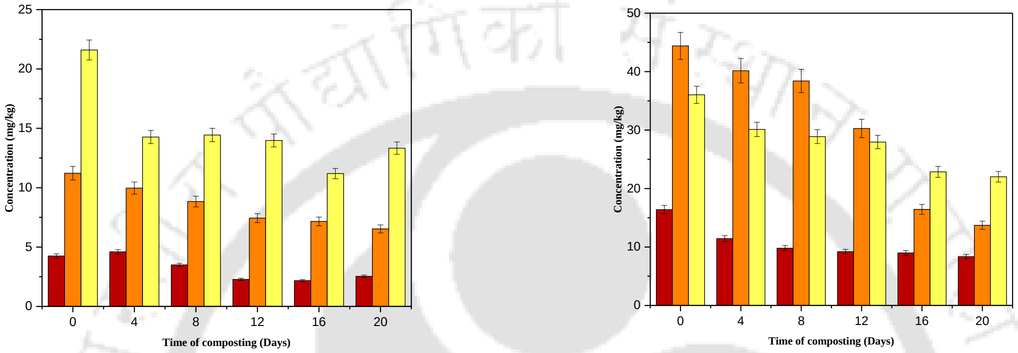
The heavy metal mobilization during *P. hysterophorus* composting was evaluated using bioavailability, leachability, and F_M. The bioavailable fraction is composed of water-soluble metals and plant accessible metals; F_M are mobile in nature and determine the various chemical speciated forms of the bioavailable and leachable fractionated metals. The heavy metals that are soluble in water have been discovered to be the most biologically bound fraction. The water-soluble portion is easily accessible to any flora, therefore causing harmful impacts on plant and human health, as well as damaging water bodies. Fig. 5.18 illustrates the bioavailable and leachable fractions of heavy metals. In the current study, the bioavailable and leachable fraction of *P. hysterophorus* was observed to decrease during composting. The reduction in bioavailable and leachable heavy metal content ranged from 21.5 to 69.1% during the composting process. The ascending sequence of bioavailable fractions in water soluble, DTPA and TCLP portions was Fe (36.2%) < Cu (40.2) < Pb (43.3%) < Mn (44.6) < Zn (49%) < Ni (49%) < Cr (49.4%) < Cd (54.1%); Pb (33%) < Mn (37.2%) < Cr (37.8%) < Cu (38.2%) < Zn (38.8%) < Ni (38.9%) < Cd (42.7%) < Fe (43.3%); and Pb (21.5%) < Cr (21.9%) < Mn (41.4%) < Cu (41.8%) < Fe (45.2%) < Cd (69%) < Zn (69.1%) < Ni (69.1%). The total metal concentration of Fe was high among all the metals in the composting process, but the reduction in bioavailable fractions of Fe in water soluble, DTPA, and TCLP portions was 36.2, 43.3, and 45.2%, it was attributed to the biodegradation of exchangeable and carbonate fractions of Fe during the composting process and also could be due to the production of intractable organometallic compounds of Fe during the composting process, resulting in decreased Fe bioavailability (Singh et al., 2013). The reduction in concentrations of Cu in bioavailable and leachable was in the range of 38.2 to 41.8%, it could be attributed to Cu getting complexed with organic materials or humic compounds during compost maturation could account for the decline. Cu is often extremely reactive and prone to getting swept away by organic composting elements. Additionally, it could be a result of the covalent hydroxyl bond and a single bond. Carboxylic groups discovered in cattle manure increased adhesion and covalently linked to Cu to produce hydrophobic and immobile compounds, lowering free Cu^{2+} levels and reducing the significant hazard to the ecology (Hazarika et al., 2017). The electrolyte content also has an effect on the absorption of Cu by humic compounds, as it raises the ionic intensity of the functional groups by increasing ionisation. With increasing salinity, humic acid's potential for complexation decreases substantially (Singh et al., 2013). Pb concentrations in bioavailable and leachable portions were reduced in the range of 21.5 to 43.3%. The reduction in Zn concentration in the TCLP forms was also observed by Hazarika et al. (2017) during the composting of paper mill sludge, it could be attributable to the increase in humic substances.

Water soluble concentrations of Cu and Zn have declined due to the humification that occurs during composting (Yousif Abdellah et al., 2022). During the initial phase of composting, the evolution of basic pH induced a mild binding of Cu and Zn onto organic materials, which likely leached out (Hazarika et al., 2017). The reduction of bioavailable fractions of Mn and Zn could be attributable to the elements (Mn and Zn) complexing with humic compounds during compost stabilization. The bioavailable and leachable concentrations of Ni, Cd, and Cr in the initial feed were low compared to the remaining heavy metals. The reduction in concentrations of Ni and Cd in bioavailable and leachable fractions could be due to the metals leaching at higher pH (pH > 8) due to the dissociation of metal complexes with organic materials. The bioavailable and leachable fractions of Cr in the composting process were decreased substantially in the range of 21.9 to 49.4% in the final compost product compared to the initial feed. The Cr^{3+} has the electron geometry of a noble gas, with great geometric stability and minimal polarisation potential. Because it has a dipole moment of three, it has a larger electrostatic attraction for the sorption sites than divalent cations. As a result, it forms the strongest persistent combination with humic compounds (Singh and Kalamdhad, 2016).

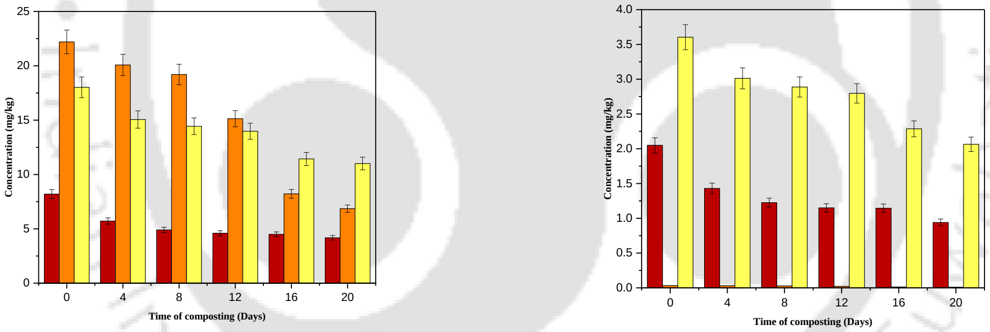
The effect of pH on heavy metal solubility is well established globally. In the current study, the alkalinisation was observed during the composting of *P. hysterophorus*. Raising pH over an average range affects the volatility and bioavailability of divalent trace metals, either through soluble equalization by accessible and interfacial ligands. The alkalinisation in the composting process drives the process into quick hydrolysis (Singh et al., 2013). The development of complexes with humic compounds is the cause of the decrease in leachable forms during the composting process. The composting process aids in the formation of persistent metal complexes, which can minimise the proportion of water soluble heavy metals (Hazarika et al., 2017). Bioaccumulation of metals by microbes, intercalation of metals with humic substances, and conversion of organic matter during composting alters the heavy metal bioavailability by converting the metals into persistent and immobilized forms.



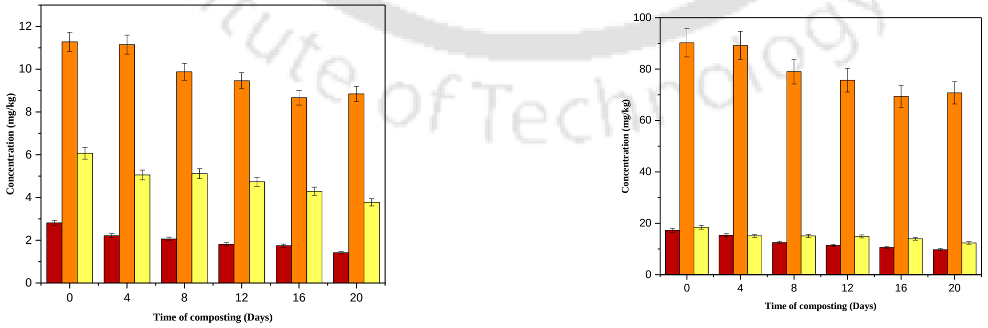
(a) Manganese (Mn) (b) Iron (Fe)



(c) Copper (Cu) (d) Zinc (Zn)



(e) Nickel (Ni) (f) Cadmium (Cd)



(g) Chromium (Cr) (h) Lead (Pb)

Fig. 5.18. Monotonic reductions in bioavailable and leachable fractions of Heavy metals during the composting process of *P. hysterophorus*.

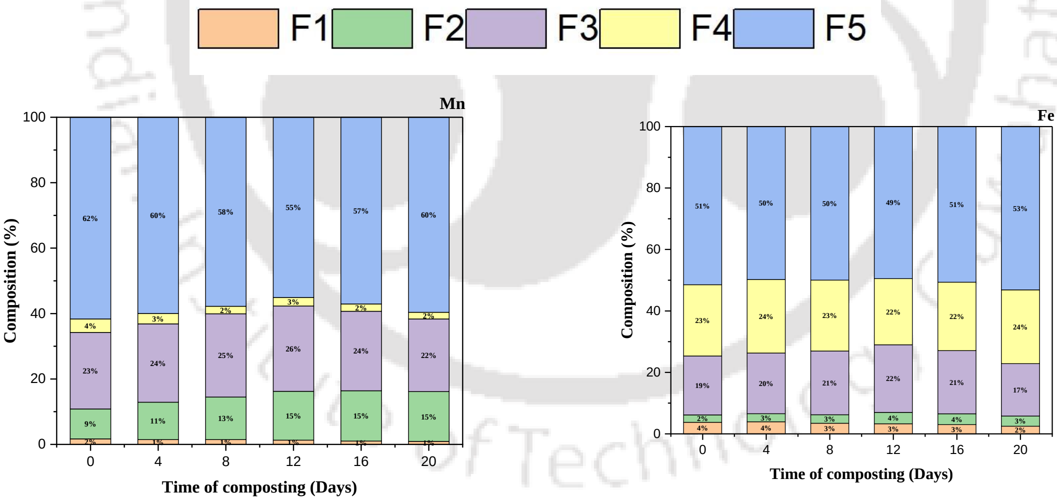
Chemical Speciation Fractions of heavy metals

The majority of the heavy metal mobile fractions will be exchangeable (F1) and carbonate fractions (F2), with potentially accessible mobile fractions being reducible (F3) and oxidizable fractions (F4). Fig. 5.19 illustrates the mobile and immobile fractions of heavy metals during the composting process. In the current study, most of the heavy metals were transformed to immobile fractions during the composting process. The composting process has the capability to transform the mobile fractions into immobile fractions (Wang et al., 2020).

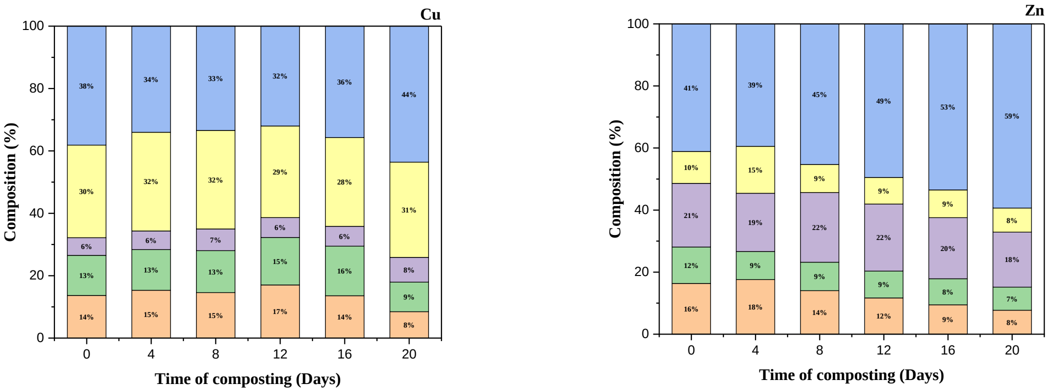
The total heavy metal concentrations of Fe, Mn, and Pb were the highest among all metals, although concentrations of these metals in F1, F2, F3, and F4 fractions were very low and altered throughout the composting process. When comparing the final compost product to the initial feed, the reduction in mobile fractions of Fe in F1 and F3 was 30.6 and 6.8%, respectively, and the increase in F2 and F4 was 30.3 and 7.9%. The existence of a greater carboxyl content increased the synthesis of soluble Fe^{2+}. The readily accessible fractions are minimal, however there are significant proportions of F3 and F4 fractions that can be made bioavailable dependent on particular environmental factors. Despite the fact that the total metal content of a compost sample after intensive acid digestion could be used as a pollution indicator. In the final compost product, the mobile fractions of Mn in F1, F3, and F4 were reduced by 44, 5.1, and 50.1%, respectively, while F2 increased by 39.8% compared to the initial feed. The Mn in the current study was observed to be extractable in F3 and F2, which determines the potential mobility of the Mn under constructed environmental conditions. Precipitation can decrease Mn movement by increasing the incidence of adsorption sites and decreasing H^+ competition for adsorption, resulting in greater metal stability when combined with HS. In the final compost product, the mobile fractions of Zn in F1 and F2 were zero, whereas F3 and F4 were reduced by 36.5 and 11.2% respectively, compared to the initial feed. The accessible mobile fractions (F1 and F2) of Zn were transformed into potential mobile fractions (F3 and F4) during the composting process. The decrease in F1 and F2 portions of Zn and rise in F3 and F4 portions of Zn could be attributable to the elevated pH during the composting process (S.Xu et al., 2022). Meanwhile, Zn has a significantly greater proclivity for sorption at the Fe and Mn oxide contacts. In the final compost product, the mobile fractions of Cu in F1 and F2 were reduced by 37.9 and 25.9%, whereas F3 and F4 were increased by 28.8 and 2.9% respectively, compared to the initial feed. In comparison to the residual portion (F5), an oxidizable bound content (F4) of Cu was observed to be dominating next to it. This is because copper could be made potentially motile at extreme environmental conditions, when pH is regulated between 4 and 5. In the final compost product, the mobile fractions of Cr in F1 and F3 were reduced by

19.7 and 54%, whereas F2 was zero and F4 was increased by 12.4% respectively, compared to the initial feed. Cr^{3+} is less mobile and hazardous in alkaline solutions. In contrast, Cr^{6+} is an extremely toxic molecule with high mobility. The results show that the composting process may easily decrease Cr^{6+} to Cr^{3+}. The most mobile fractions (F1 and F2) and potentially mobile fractions (F3 and F4) of Ni and Cd were almost tends less than 4% during the composting.

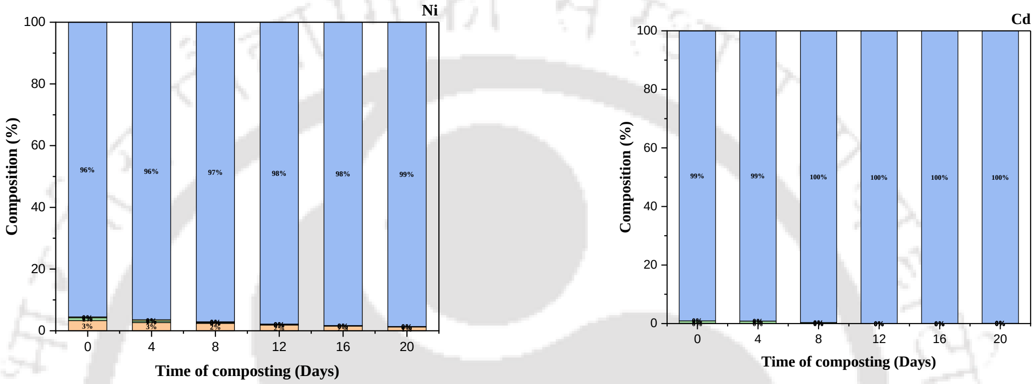
In the current study, the composting of *P. hysterophorus* has transformed the mobile and potentially mobile fractions into immobile fractions. In the heavy metals such as Ni and Cd having the higher concentration of F5 fractions with more than 98% in the final compost product. The Cd ion is effectively attached to multiple organic functional groups, primarily carboxylic, carbonyl, and phenolic, resulting in immobilisation in a hard internal-sphere complex. In the initial feed, the feed biomass contains numerous aliphatic compounds, which attach to the heavy metals in the most soluble form. The immobile fractions (F5) of heavy metals were observed to be increased during the composting process; the rise may be attributable to the transformation of mobile and unstable fractions of heavy metals into immobile fractions. The humification and increase in HS result in the formation of metal combinations that are possibly poorly or non-extractable.



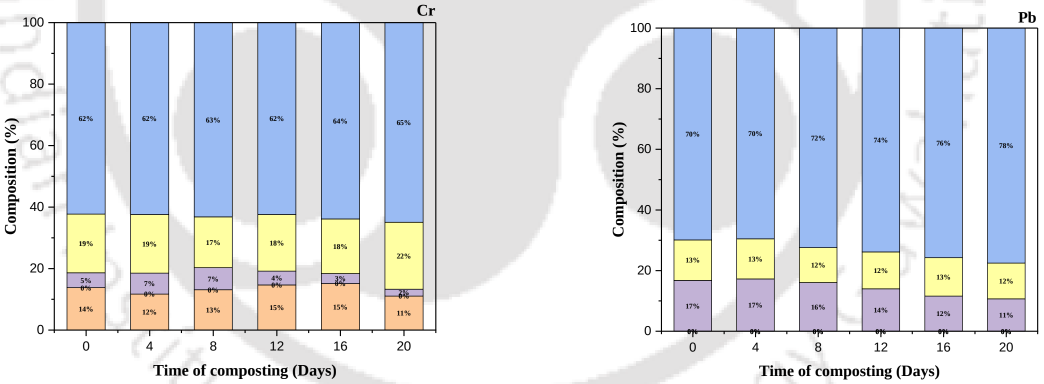
Manganese (Mn) (b) Iron (Fe)



(c) Copper (Cu) (d) Zinc (Zn)



(e) Nickel (Ni) (f) Cadmium (Cd)



(g) Chromium (Cr) (h) Lead (Pb)

Fig. 5.19. Compositions of mobile and immobile heavy metal fractions during the composting process

5.6.3. Heavy metals characterization during the composting of *L. camara*

Total Metal Analysis

The increase in metal concentration is associated with the reduction in mass that occurs during the composting process as a result of the breakdown of biodegradable matter, the emission of various components of carbon element and water (Hazarika et al., 2017). Table 5.4

presents an overview of the variations in the overall levels of heavy metals during the RDC of *L. camara*.

The descending order of heavy metal (HM) concentrations observed during the composting process was as follows: Fe > Mn > Pb > Ni > Cr > Zn > Cu > Cd. The initial concentration of Fe in the dry mass was 4954 mg/kg on day 0, which increased to 8989.5 mg/kg. Similarly, the concentration of Mn increased from 1126 mg/kg to 1805.1 mg/kg, Pb increased from 646.9 mg/kg to 835.4 mg/kg, Ni increased from 235.8 mg/kg to 319.8 mg/kg, Cr increased from 131 mg/kg to 196.3 mg/kg, Zn increased from 116.2 mg/kg to 177.4 mg/kg, Cu increased from 60.1 mg/kg to 77.5 mg/kg, and Cd increased from 45.8 mg/kg to 67.5 mg/kg in the dry mass. The observed fluctuations in the overall concentration of heavy metals could potentially be attributed to the process of organic compound biodegradation and the subsequent leaching of metals. Singh et al. (2016) established a correlation between elevated heavy metal concentrations in the cells of soil-dwelling plants and the persistent accumulation of metal levels in compost-amended soil over an extended period. The quantification of heavy metals is typically determined based on their inherent toxicity. In contrast, the present study demonstrates that the impact of heavy metal toxicity on the ecosystem is determined not solely by the total quantity of heavy metals present in *L. camara* compost, but also by the bioavailable and leachable fraction of these metals.

Table 5.4. Variation of Total metals in composting of *L. camara*

Time of composting (Days)	**Zn**	**Mn**	**Fe**	**Cu**	**Cr**	**Cd**	**Ni**	**Pb**
0	116.23 ± 5	1126.933 ± 23	4954 ± 36.2	60.11 ± 1.03	131 ± 2.6	45.8 ± 2.4	235.8 ± 8.7	646.9 ± 10.7
4	136.5 ± 4.9	1583.6 ± 13.2	5954.1 ± 23.5	63.2 ± 2.5	154 ± 2.9	51.2 ± 1.9	266 ± 10.6	700.2 ± 5.8
8	139.7 ± 4.8	1664.2 ± 14.3	6687.5 ± 25.8	64.2 ± 2.5	187.2 ± 1.5	55 ± 2.5	271.5 ± 10.7	714 ± 13.4
12	152 ± 5.2	1711.2 ± 10.3	7112 ± 21.5	67.85 ± 1.05	191.2 ± 3.8	59.1 ± 5.6	298.4 ± 11.9	763.3 ± 14.2
16	156.8 ± 5.8	1799.6 ± 20.2	7576 ± 32.2	72.3 ± 0.9	193 ± 3.1	64 ± 1.8	311 ± 13.4	802 ± 10.9
20	177.41 ± 5.6	1805.1 ± 13.9	8989.5 ± 26.8	77.5 ± 1.5	196.3 ± 2.5	67.5 ± 1.7	319.8 ± 5.9	835.4 ± 11.8

All the values are in mg/kg within mean ± standard deviation

Water Soluble fractions

Fig. 5.20 illustrates the changes in the level of water-soluble Fe, Mn, Zn, Cu, Pb, Ni, Cd, and Cr over a period of 20 days during the composting process. On the initial day, the concentration of iron (Fe) accounted for 4.48% of the total metal content. However, this concentration experienced a significant decrease, reaching 1.71% of the total metal content. The decrease in water solubility of Fe observed in this study may be connected to the formation of inaccessible organometallic compounds that occur during the composting process. The levels of Mn exhibited a steady reduction over the course of composting, with the most significant decline observed on day 10, where the Mn concentration decreased from 3.31 to 1.24 % of the total Mn content. The dissolution of Mn in water is influenced by the dissolution of organic carbon, which exhibits an initial rise during the thermophilic phase followed by a subsequent substantial drop. The percentage of the water-soluble fraction of Zn decreased from 9.99 to 2.82 % of the total metal in the 20th day compost compared to initial mix. In a similar vein, the percentage of Cu encountered a reduction from 9.35 to 4.67 % of the total metal in the 20th day compost compared to initial mix. According to Hazarika et al. (2017), the presence of –OH and –COOH groups in cow manure resulted in increased adhesion and binding to copper (Cu), forming insoluble and immobile compounds. This process effectively reduced the concentration of free Cu^{2+} ions, thereby mitigating the significant environmental risk associated with their presence. The concentration of Pb decreased from 1.36 to 0.51% of the total metal content in the 20th day compost compared to initial mix. In the case of Cd, the initial compost sample on day 0 contained a small amount of 0.12% of the total metal content, which was completely eliminated by the end of the composting period. According to Hargreaves et al. (2008) , the thermophilic stage of composting resulted in a reduction and immobilization of water-soluble metals, including Zn, Pb, Cu, and Cd. During the initial stage of breakdown, the composting blend undergoes a decrease in pH as a result of the breakdown of organic matter. This decrease in pH subsequently leads to the release of Pb and Zn. The initial level of chromium (Cr) accounted for 0.95% of the total metal content on the 0th day, gradually decreasing to a final concentration of 0.23% of the total metal. Consequently, this led to a decrease in the availability of metals. The concentrations of water-soluble Ni, Pb, and Cd in mature compost of *L. camara* were predominantly absent. The levels of Ni on 20th day compost was reduced from 2.21 to 0.05 mg/kg compared to initial day mix.

Moreover, it has been observed that the composting process can lead to the transformation of water-soluble metals into a more steady form, resulting in a decrease in the proportion of water-soluble metals (Hazarika et al., 2017). The order of water-soluble metals in composted

L. camara was found to be as follows: Fe > Mn > Zn > Pb > Cu > Cr > Ni > Cd. The distribution of heavy metals during the composting process was impacted by the release of heavy metals resulting from the mineralization of biodegradable content or the solubilization of metals due to a decrease in pH. Metals posed challenges in terms of their refractory nature and the difficulty in their extraction, primarily attributed to the processes of microbial biomass biosorption or the chelation of metals with recently synthesized humic compounds.

DTPA Extraction fractions

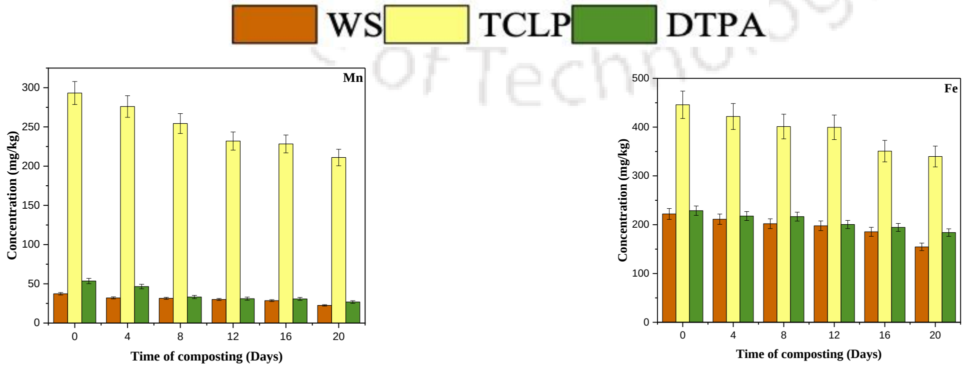
Over a period of 20 days, the levels of DTPA extractable Fe, Mn, Zn, Cu, Pb, Ni, Cd, and Cr were observed and are presented in Fig. 5.20. The metal concentration sequence observed in the DTPA extracts of composted *L. camara* on initial and 20th day was Fe > Mn > Zn > Pb > Cu > Ni> Cr > Cd. The study demonstrates that the initial concentrations of Zn, Fe, Cu, and Mn, along with their respective total metal concentrations, are 34.69, 4.49, 25.06, and 4.76%. Following the conclusion of the process, it was observed that the levels of Zn, Fe, Cu, and Mn, along with the total metal concentration, experienced a decrease to 9.8, 3.6, 12.5, 2.38%, respectively. Furthermore, a significant reduction in the levels of DTPA extractable zinc (Zn) and copper (Cu) was observed during the thermophilic stage of degradation in the composting process. On the other hand, the reduction of Fe, Mn, Pb, and Cr exhibited a relatively consistent rate.

The DTPA extractability of Ni in the 20th day compost product was reduced to 3.7 from 11 mg/kg compared to initial mix. Furthermore, the levels of Cr and Cd that can be extracted using the DTPA method exhibited a sharp decline. This is attributable to the diminished accessibility and movement of these metallic substances in their inert state within the compost's final substance. The initial level of Cd accounted for 5% of the total metal content, which subsequently decreased to 1.01% of the total metal content. Similarly, the initial level of Cr constituted 3.6% of the total metal content, ultimately decreasing to 1.06% of the total metal content. The decrease in the accessible portion of Cd within the final product of compost could be attributed to the interaction between Cd and carboxylic and phenolic functional groups present in the compost. The cadmium ions are confined within a rigid inner sphere composite. The element Pb exhibited minimal or negligible alteration as it decreased from 4.3% to 1.3% of the overall metal content. The rapid generation of easily dissolved metal carbonates and hydroxides can be attributed to the reduced availability of metals in plants (Wong and Selvam, 2006).

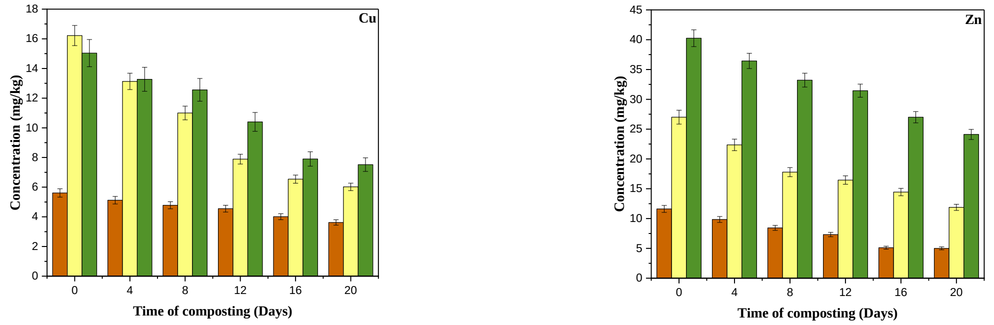
TCLP Extractable Heavy Metals

Over a period of 20 days, the levels of TCLP extractable Fe, Mn, Zn, Cu, Pb, Ni, Cd, and

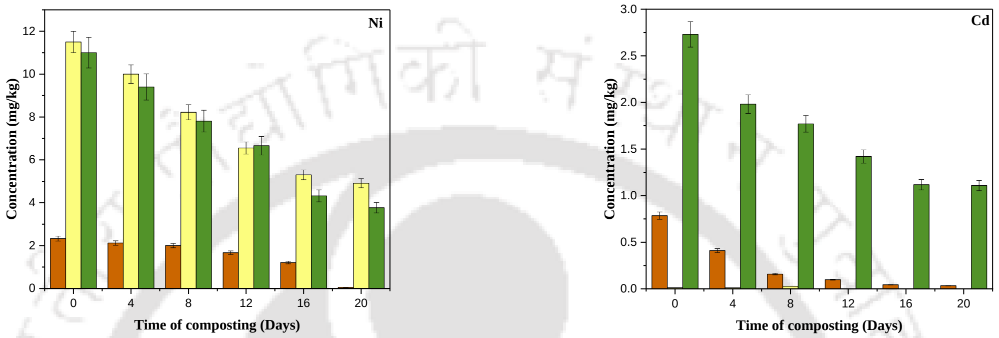
Cr were observed and are presented in Fig. 5.20. The sequence of metal concentrations in TCLP extract in the initial day and at the end of 20 days composting was Fe > Mn > Pb > Zn > Cu > Ni> Cr > Cd. On the initial day mix, the concentration of Cd was 0.03 mg/kg and has been not detected after the composting of *L. camara*. During the composting process, the concentration of Cu experienced a decrease from an initial value of 26.6 to a final value of 8.7% of the total Cu. Notably, the most significant reduction in Cu concentration was observed on day 12. The decrease in concentration can be ascribed to the formation of Cu complexes with organic matter or HS during the process of compost maturation. Copper exhibits a tendency towards reactivity and vulnerability to the removal of an organic composting substance through washing. Based on the observed decrease in copper concentration, it can be inferred that the optimal circumstances for movement were likely attained in the later phases of the composting process. The levels of Zn, Fe, Pb and Cr experienced a decrease from an initial value of 22.3, 9.08, 7.5 and 6.1 to a final value of 6.2, 3.8, 2.5 and 2.5% of the total Zn, Fe, Pb and Cr. The observed increase in iron content can be related to the strong association of organometallic substances, as suggested by Singh and Kalamdhad (2016). Chromium exhibits a significant electrostatic affinity towards HS, resulting in the formation of steady and immobile complexes (Hazarika et al., 2017). Consequently, the process of composting leads to a decrease in the holding of metals across all types, potentially resulting in an elevation of both pH levels and the complexity of metal humic compounds. Maity et al. (2008) reported that an elevation in compost pH results in the augmentation of electrostatic interaction, thereby enhancing cationic adsorption. This leads to the formation of metal hydroxy ionic species, which exhibit a greater resemblance for adsorption surfaces compared to metal cations. Additionally, the increased compost pH facilitates the precipitation of metals as metal hydroxides.



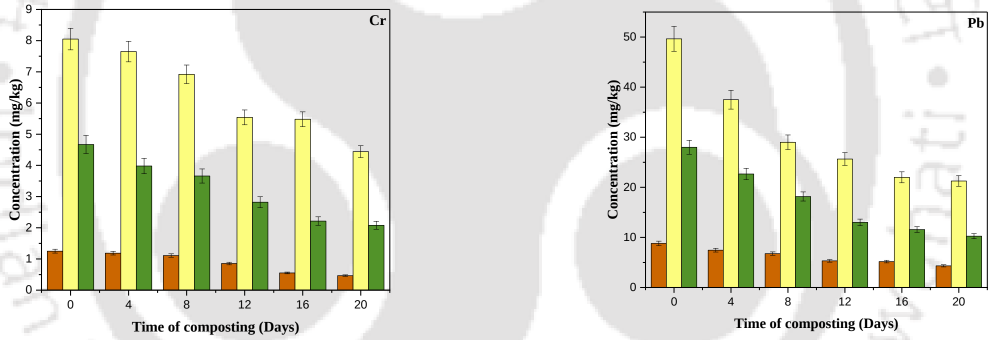
(a) Manganese (Mn) (b) Iron (Fe)



(c) Copper (Cu) (d) Zinc (Zn)



(e) Nickel (Ni) (f) Cadmium (Cd)



(g) Chromium (Cr) (h) Lead (Pb)

Fig. 5.20. Monotonic reductions in bioavailable and leachable fractions of Heavy metals in *L. camara* during the composting process

Chemical Speciation Fractions of heavy metals

The order of different fractions of Mn in the initial day to final day composting activity of *L. camara* was F5 (54.23%) > F3 (30.04%) > F2 (5.65%) > F1 (3.95%) > F4 (1.94%) to F5 (73.14%) > F3 (18.50%) > F2 (5.65%) > F4 (1.39%) > F1 (1.28%). Manganese tends to form weak coordination complexes with organic matter. This means that Mn^{2+} cannot compete effectively with Cu^{2+}, Zn^{2+} and other more prevalent cations, such as Ca^{2+} and Mg^{2+} for sites on organic matter and, hence, less Mn are generally found bound to organic matter. Manganese (II) is the most soluble form of Mn, and so under reducing conditions, higher concentrations of

Mn^{2+} will be present in the soil solution. Under more oxidising conditions, soil solution concentrations of Mn decrease because the equilibrium shifts in favour of Mn (IV), which tend to exist mainly as insoluble hydroxides and oxides (McGrath et al., 1988). The production of precipitates can reduce Mn mobility by enhancing the frequency of adsorption sites and reducing H^+ competition for adsorption, leading to increased metal stability with humic compounds (Singh and Kalamdhad, 2013b).

The order of different fractions of Fe in the initial day to final day composting activity of *L. camara* was F5 (54.94%) > F4 (22.53%) > F3 (18.07%) > F2 (2.45%) > F1 (2.00%) to F5 (61.42%) > F4 (24.06%) > F3 (11.69%) > F2 (1.48%) > F1 (1.33%). The lowered pH long with reduction of Fe^{3+} to Fe^{2+} at the root surface enables Fe^{2+} to be taken up primarily through the young lateral roots. The root-absorbed Fe^{2+} is oxidised to Fe^{3+} and then transported to the plant top. The presence of higher carboxyl content accelerated soluble Fe (II) production. The easily bioavailable fractions are limited, but F3 and F4 fractions that can be made available based on special environmental conditions are found in considerable amount. Although the total metal content obtained from compost sample after intense acid digestion could be used as a pollution criterion. Nonetheless, it provides little relevant information on the bioavailability menace.

In the case of Cu, the F4 and F5 fractions were found to be prominent in all three raw materials. The order of different fractions of Cu in the initial day to final day composting activity of *L. camara* was F5 (43.15%) > F4 (22.3%) > F2 (14.57%) > F1 (13.17%) > F3 (6.72%) to F5 (67.22%) > F4 (12.31%) > F1 (8.64%) > F3 (6.93%) > F2 (4.87%) as illustrated in Fig. 5.15..At the end of the composting activity, all moving fractions of Cu (F1, F2, F3, and F4 fractions) were converted into F5 fractions, possibly because of the formation, in a rigid internal-sphere compound, that of a Cu complex with multiple organic functional groups mainly R−COOH, C=O and OH, due to immobilisation of Cu at the end of composting (Singh and Kalamdhad, 2012). An organically bound fraction of Cu was found dominant next to the residual. This can be explained by the fact that Cu forms very stable complexes with organic ligands at the end of the composting.

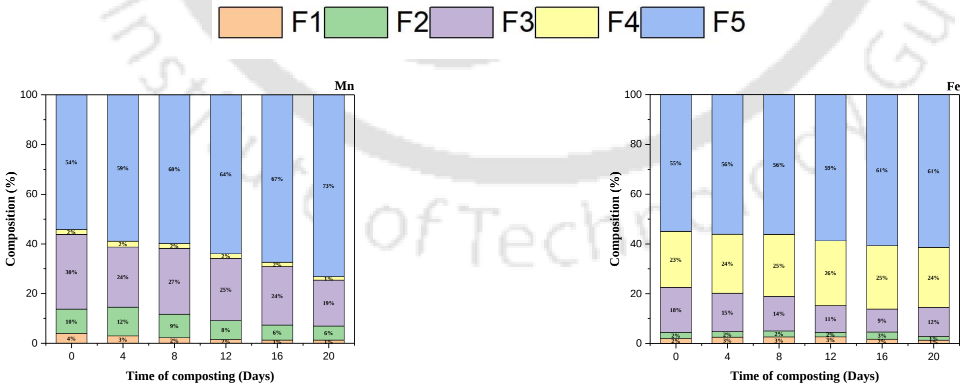
In the case of Zn, the F5 fraction was found to be prominent in all three raw materials. The order of different fractions of Zn in the initial day to final day composting activity of *L. camara* was F5 (48.76%) > F3 (17.14%) > F4 (15.50%) > F1 (9.48%) > F2 (9.09%) to F5 (59.20%) > F3 (25.94%) > F4 (7.31%) > F1 (3.99%) > F2 (3.54%). The development of the Zn blend with humic components generated during the compost's mature stage may be responsible for the diminution of mobile forms. Humic compounds envelop various organic functional groups capable of absorbing metal ions via ionic force (Singh and Kalamdhad, 2013b). Because of its redox potential, a Zn^{2+} is predicted to remain in an ionic state in a solution. The exchangeable Zn fraction was transformed into a reducible fraction during the composting activity. The decrease in exchangeable and bioavailable Zn portions could be due to an increase in pH and organic matter decomposition (Maity et al., 2008). Zn can build complexes with organic binders and dissolve with hydroxides, carbonates, phosphates, sulphides, and various additional anions. According to Kumpiene et al. (2008), the main Zn motility regulating mechanisms are organic ligands complexation and cation exchange. The rise in pH during composting could be attributed to the reduction in exchangeable Zn portion and enhancement in reducible Zn fraction because Zn has a substantially higher propensity for sorption on the interfaces of Fe and Mn oxides. The high Zn bioavailability factor could cause phytotoxicity (Fuentes et al., 2004). The reduction of movable forms might be due to the formation of the Zn complex with HS formed at the maturity stage of the compost. HS surround various organic functional groups that can absorb metal ions through ionic force.

The order of different fractions of Ni in the initial day to final day composting activity of *L. camara* was F5 (98.29%) > F2 (0.72%) > F4 (0.49%) > F1 (0.47%) > F3 (0%) to F5 (99.99%) > F4 (0.009%) > F3 = F2 = F1 = 0. The F5 fraction remained the most prominent after composting activity. At the beginning of composting, raw organic materials contain large amounts of non-specific compounds such as amino acids, polysaccharides, peptides that may form highly soluble complexes with heavy metals. This type of metal bonds are sufficiently easily extracted to allow their quantitative increase during the initial stages of composting, but further transformations (mineralization, humification) that compost humus compounds undergo result in the development of metal complexes that are either poorly or non-extractable (Jakubus, 2016). Humic acids are characterized by the ability to bind metal, oxide and hydroxide ions and form insoluble complexes of varying chemical stability.

The order of different fractions of Cd in the initial day to final day composting activity of *L. camara* was F5 (99.27%) > F2 (0.55%) > F1 (0.17%) > F3 = F2 (0%) to F5 (100%) > F4 = F3 = F2 = F1 = 0. In Cd's case, the metal's mobile fractions were zero on the final day of composting activity, determining that the Cd became inert and stable during the composting activity. One reason for the decrease in F1 and F2 fractions and elevation in F5 fraction could be Cd's propensity to chemically bind efficiently with organic molecules (Haroun et al., 2007). Moreover, the Cd ion is typically attached to multiple organic functional groups, the most common of which are COOH, C=O, and OH, resulting in the ion being immobilised in a stiff intrinsic complex (Singh and Kalamdhad, 2014).

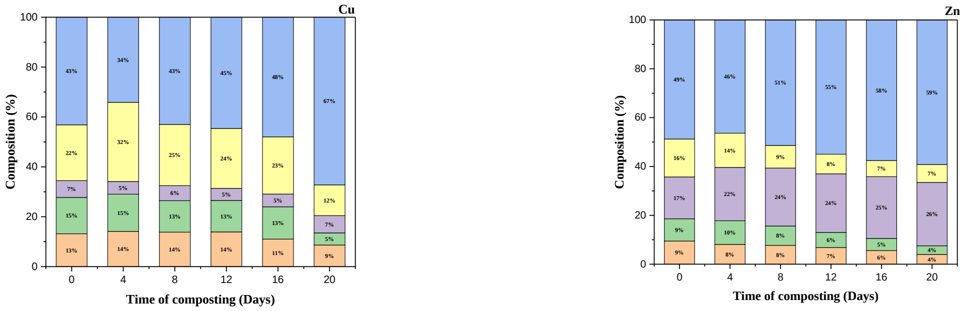
The order of different fractions of Cr in the initial day to final day composting activity of *L. camara* was F5 (64.75%) > F4 (19.34%) > F3 (9.24%) > F1 (6.366%) > F2 (0%) to F5 (74.26%) > F4 (24.28%) > F3 (1.44%) > F2 = F1 = 0. In a naturalistic environment, chromium can exist in a variety of chemical valences ranging from +4 to +6, with trivalent chromium (Cr (III)) and hexavalent chromium (Cr (VI)) being the most common. The toxicities and mobility of Cr (III) and Cr (VI) are substantially different. In aqueous systems, Cr (III) is less soluble and has lower toxicity. Cr (VI), on the other hand, is a highly poisonous compound with high solubility. The findings reveal that compost biomass may easily reduce Cr (VI) to Cr (III) (Yuan et al., 2010).

The order of different fractions of Pb in the initial day to final day composting activity *L. camara* was F5 (66.42%) > F4 (18.81%) > F3 (14.76%) > F2 = F1 = 0% to F5 (85.79%) > F3 (7.34%) > F4 (6.85%) > F2 = F1 = 0% . Lead is generally found in carbonate form, which is a fairly mobile form; nevertheless, biologically bonded form, which is a less dynamic form, may be available for plants. Complexation by organic matter leads to a stable fixation state, leading to low mobility of Pb. The mobile fractions of lead got decreased throughout the composting activity; the same trend was reported by (Wong and Selvam, 2006). A slightly alkaline media can reduce Pb motility by creating a Pb-organic matter mixture due to the amphoteric properties of Pb. More alkaline circumstances, on the other hand, may have the inverse effect on Pb persistence. However, it may form soluble hydroxide complexes at high basic conditions that improve Pb motility (Kumpiene et al., 2008; Whittle and Dyson, 2002). Fig. 5.21 illustrates the chemical speciation fractions during the composting of *L. camara*.

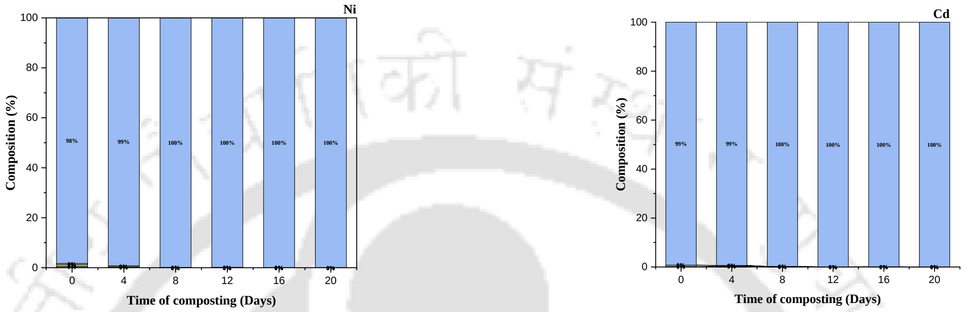


(a) Manganese (Mn)

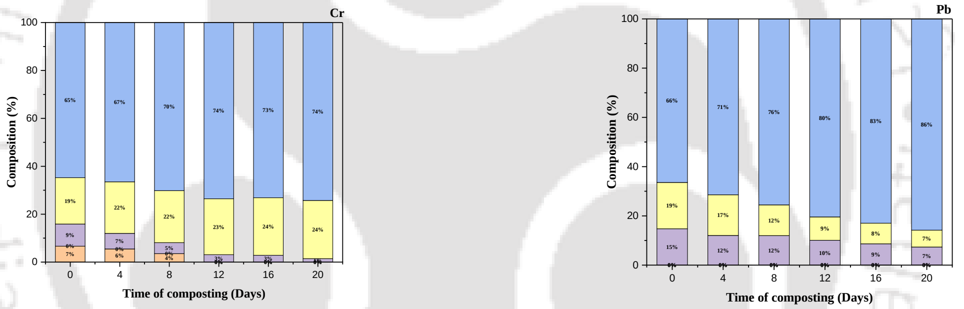
(b) Iron (Fe)



(c) Copper (Cu) (d) Zinc (Zn)



(e) Nickel (Ni) (f) Cadmium (Cd)



(g) Chromium (Cr) (h) Lead (Pb)

Fig. 5.21. Variations in F1, F2, F3, F4 and F5 fractions during composting of *L. camara*

5.6.4. Comparison of Heavy metal concentrations between the terrestrial weeds and its composts

The heavy metal concentrations of any biomass depend on the sampling sites and the capability of up taking the heavy metal. In the current study, all the three terrestrial weeds were taken from the vicinities of IITG campus. The concentrations of bioavailable and leachable fractions of heavy metals depends on the total heavy metal concentrations. According to FSSAI (2011), the permissible limits for Cd, Cr, Pb, and Ni in food have been established due to the severe carcinogenic health effects associated with these metals. These limits are put in place to ensure that the levels of these heavy metals in food do not exceed safe thresholds, reducing the risk of cancer and other adverse health effects. In the current study, it was observed that the total heavy metal concentrations of Ni (1470 – 1600 mg/kg), Cd (125 – 143 mg/kg), Cr (241 – 394 mg/kg), and Pb (950 – 1000 mg/kg) were found to be high in *A. conyzoides* composting compared to other two terrestrial weeds composting. The high concentrations in *A. conyzoides* could be attributable to the metal intake through Hyperaccumulation, and Metal tolerance in their root system (Nasrin, 2013). Compared to *A. conyzoides,* and *P. hysterophorus, L. camara* has low concentrations of total heavy metals. During the composting of terrestrial weeds, it was observed that the water-soluble and leachable fractions of Cd, Cr, Pb, and Ni decreased in the final day compost samples. Specifically, the water-soluble and DTPA fractions of Pb showed a reduction of around 40 to 44% throughout the composting process. Moreover, the bioavailable and leachable fractions of Cr and Ni were found to be in negligible values in the final day compost samples. These findings indicate that the composting process of terrestrial weeds effectively transformed the mobile fractions of these heavy metals into immobile fractions (F5), thereby reducing their mobility and potential environmental impact.

The waste products in Earth's crust are also the human in this expanded, spectral sense. One's garbage doesn't go away, it just goes somewhere else.

Timothy Morton
1968-present

6

Toxicity Assessments and Identification of Toxic Organic Compounds in distinct phases of composting process

6.1. TOXICITY ASSESSMENTS IN DISTINCT PHASES OF COMPOSTING *A. CONYZOIDES*

6.1.1. Phytotoxicity

The seed germination test is susceptible, and it can be effectively blocked by any hazardous chemical present in the environment (Haq and Kalamdhad, 2021). The phytotoxicity of *A. conyzoides* was determined before and after composting process using 48 h mung bean seed germination assays. Table 6.1 (a), (b) and Fig. 6.1 illustrates both scenarios' outcomes and inhibition results of *V. radiata* when exposed to *A. conyzoides* and compost extracts. The seed germination of *V. radiata* was reported only up to 50% dosage in *A. conyzoides* sample and a drastic decrease in GP, root length, shoot length and biomass index from 25 to 100% concentrations of *A. conyzoides* sample. In contrast, the rate of GP and other characteristics of *V. radiata* in compost sample was significantly increased.

The growth of the seeds is dependent on the concentrations of the sample. As the concentration of the *A. conyzoides* sample increases, a reduction in growth was observed in seeds. The maximum root and shoot lengths of *V. radiata* was observed in 25% of the *A. conyzoides* sample (1 ± 0.35 and 1.96 ± 0.36). Whereas, in the same concentration of compost sample, a significant increase in root and shoot lengths was observed (3.66 ± 1.37 and 4.24 ± 0.75). The root and shoot lengths of 100% *A. conyzoides* sample almost tended to zero, whereas the 100% compost sample shown significant growth in the root and shoot part of *V. radiata*

(2.36 ± 0.19 and 2.89 ± 0.63). The reduction in biomass of *V. radiata* was observed from 33 to 84.2% as compared to control in *A. conyzoides* samples when the concentrations were increased from 25 to 100%. Whereas in compost samples, the biomass quantity was increased significantly in 25 % concentration with 12.5% compared to control. The substantial decrease in seed germination, root length, shoot length, and biomass of *V. radiata* cultivated in *A. conyzoides* might result from the cumulative effect of EC, sBOD, sCOD, TOC and volatile content. When *V. radiata* induced with both *A. conyzoides* and compost samples demonstrate substantial variance compared to controls ($p < 0.05$).

The effects of *A. conyzoides* and compost sample concentrations on the root development and length of *A. cepa* is demonstrated in Table 6.1 (c) and (d). As the concentrations of the substrate sample increased from 25 to 100%, there was a reduction in growth and lengths of roots from 69.2 to 96.9% compared with the control. Whereas, in the compost sample at 25% concentration, the length of the roots increased by 3.39% compared with the control. The biomass reduction was observed from 45.4 to 88.6% in the *A. conyzoides* sample when the concentration was increased from 25 to 100%, but, in the compost sample, at 25 % concentration, the biomass quantity was increased to 2.12% as compared with the control. At 100 % concentrations of both the samples, a substantial decrease in biomass was found in contrast to the control. The inability of *A. cepa* cultivated in organic substrates to develop roots and biomass suggests the presence of harmful chemicals in *A. conyzoides*. When *A. cepa* induced with both *A. conyzoides* samples demonstrate substantial variance compared to controls ($p < 0.05$).

Table 6.1. Phytotoxicity test results of *V. radiata* and *A. cepa* in *A. conyzoides* and final compost samples

(a) *V. radiata* in *A. conyzoides* sample					**(c) *A. cepa* in *A. conyzoides* sample**	
Conc (v/v)	**Germination (%)**	**Root Length (cm)**	**Shoot Length (cm)**	**Biomass (g)**	**Root length (cm)**	**Biomass (g)**
Control	100 ± 0	2.4 ± 0.8	4.3 ± 0.67	1.27 ± 0.07	6.5 ± 1.23	1.85 ± 0.23
25%	53.3 ± 0.86*	1± 0.35*	1.96 ± 0.36*	0.85 ± 0.02	2 ± 0.22*	1.01 ± 0.17
50%	50 ± 1*	0.3 ± 0.09*	0.7 ± 0.27*	0.62 ± 0.06*	1.1 ± 0.15*	0.78 ± 0.11*
75%	23.3 ± 1.52*	0.08 ± 0.0*	0.32 ± 0.01*	0.3 ± 0.01*	0.5 ± 0.09*	0.45 ± 0.13*
100%	16.6 ± 0.92*	0.04 ± 0.0*	0.04 ± 0.0*	0.2 ± 0.04*	0.2 ± 0.02*	0.21 ± 0.09*
(b) *V. radiata* in final compost sample					**(d) *A. cepa* in final compost sample**	
Conc (v/v)	**Germination (%)**	**Root Length (cm)**	**Shoot Length (cm)**	**Biomass (g)**	**Root length (cm)**	**Biomass (g)**
Control	100 ± 0	3.92 ± 0.23	4.1 ± 0.65	1.44 ± 0.09	5.9 ± 1.13	1.88 ± 0.18
25%	90 ± 0.9	3.66 ± 1.37	4.24 ± 0.75	1.62 ± 0.16	6.1 ± 1.25	1.92 ± 0.52
50%	86.6 ± 0.57	3 ± 0.61	3.7 ± 0.44	1.38 ± 0.17	6 ± 1.32	1.84 ± 0.45
75%	83.3 ± 1.86	2.89 ± 0.77	3.1 ± 0.84	1.28 ± 0.09	5.7 ± 0.92	1.68 ± 0.49
100%	86.6 ± 0.56	2.36 ± 0.19*	2.89 ± 0.63*	1.14 ± 0.08	5.1 ± 0.98	1.66 ± 0.19

All the parameters are expressed in mean ± SD of triplicates

* Significant difference observed at $p < 0.05$, when compared to control

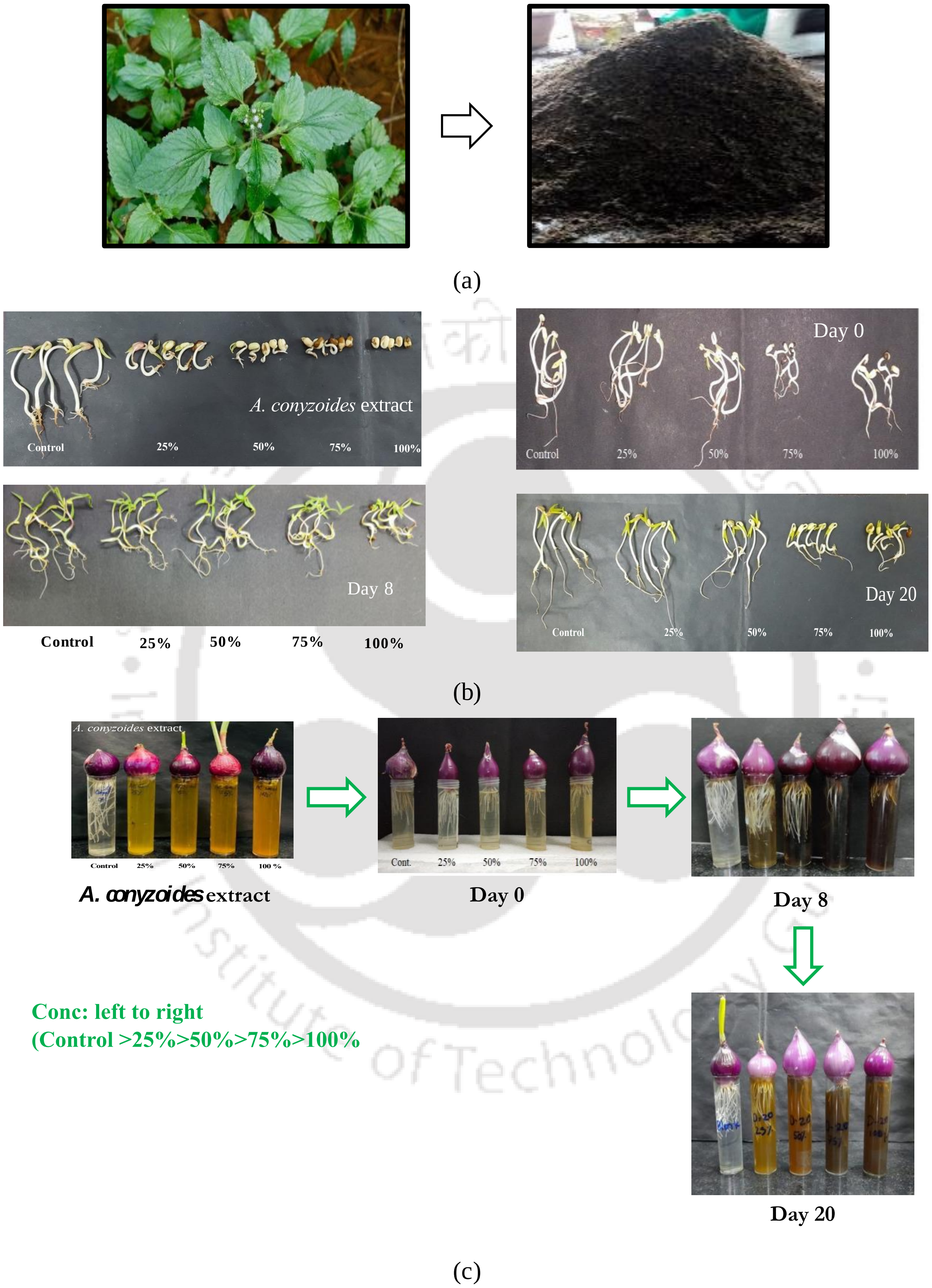


Fig. 6.1. (a) Initial and final day compost samples; Phytotoxicity assay during the composting process of *A. conyzoides* (b) *V. radiata*, (c) *A. cepa*

6.1.2. Cytotoxicity test

The cytotoxicity of environmentally toxic substances and compost samples can be determined by evaluating the MI (Haq et al., 2017). MI is an effective bioindicator technique for determining the impact of contaminants on cell division (Haq et al., 2017). Root growth inhibition of *A. cepa* was interpreted as a sign of toxicity resulting from cellular damage or a block in cell division. It was evident that the reduction in MI of *A. cepa* in *A. conyzoides* samples was due to an increase in concentrations. The MI of the negative control with dechlorinated tap water was 75.3 ± 0.8, whereas the MI of the positive control with 4 mM EMS was recorded as 5.4 ± 0.7. Table 6.2 depicts the alterations of MI in *A. cepa* root tips, when exposed *A. conyzoides* and compost extracts. The reductions in MI was observed from 50.59 to 76.75% in *A. conyzoides* sample from 25 to 100% concentrations compared to negative control. The drops was attributed to the cytotoxic impacts of the *A. conyzoides* sample, which may induce indeterminate prophase, resulting in an erratic mitotic phase and therefore suppressing division during interphase (ElFels et al., 2016). Whereas, there was a significant increase in MI of *A. cepa* roots with compost sample compared to *A. conyzoides* sample. The maximum MI in the compost sample was observed in 25% concentration with 70.8%. The increasing MI trends in compost samples suggest that the cytotoxic impacts of *A. conyzoides* were efficiently reduced due to composting activity. When *A. cepa* induced with *A. conyzoides* sample demonstrate substantial variance in MI compared to compost sample and control ($p < 0.05$).

Table 6.2. MI (%) of root tip cells of *A. cepa* following with *A. conyzoides* and final compost samples

Exposure (24 h)	**Concentrations**	**Total cells**	**Dividing cells**	**MI %**
Tap water		1201	905	75.3 ± 0.8
EMS		895	49	$5.4 \pm 0.7^{*}$
A. conyzoides	25%	1274	475	$37.2 \pm 0.6^{*}$
compost		1369	970	$70.8 \pm 0.9^{\#}$
A. conyzoides	50%	1235	393	$31.8 \pm 0.4^{*}$
compost		1327	773	$58.2 \pm 0.8^{\#}$
A. conyzoides	75%	1223	310	$25.3 \pm 0.9^{*}$
compost		1354	544	$40.1 \pm 0.3^{\#}$

A. conyzoides	100%	1314	230	17.5 ± 0.1*
compost		1287	367	28.5 ± 0.6$^{\#}$

All the parameters are expressed in mean ± SD of triplicates

*. Significant difference observed at $p < 0.05$ compared to tap water (negative control)

$^{\#}$. Significant difference observed at $p < 0.05$, when compared to *A. conyzoides* sample at same concentration

6.1.3. Genotoxicity test

A. cepa's developing root tips provide easily attainable plant material for investigating the deleterious effects of toxins on chromosomes. Numerous forms of CAs are examined while considering chromosomal aberrations during cell division's mitotic phase (Haq and Kalamdhad, 2021). CA is defined by alterations in the integrity of one or more chromosomes, which can arise both dynamically and as a consequence of interaction to physical or chemical stimuli (Yadav et al., 2019). The CA and NA were not identified in the root tips of *A. cepa* exposed to dechlorinated water. However, CA was observed less in the root tips of *A. cepa* exposed to 4mM EMS due to limited cell division occurred in root tips. The aberrant cell percentages of *A. cepa* root tips exposed to various concentrations of *A. conyzoides* and compost extracts were illustrated in Table 6.3 and Fig. 6.2. The observed CAs were vagrant chromosome, chromosome loss, C mitosis (Colchicine mitosis), and NA was Binucleated cells. The C-mitosis abnormality was highest in 100 % concentrations of *A. conyzoides* sample with 16.4. Whereas in the compost sample, the range of C-mitosis was < 2.4. The occurrence of c-mitosis indicated the loss of poles, preceded by arbitrary chromosome dispersal. The anaphase bridge in *A. conyzoides* sample ranged from 1.8 to 3.6, but it was reduced to 1 in compost sample. Bridges in chromosomes may develop due to chromosomal adhesion, dividing and collecting damaged ends, which hinder pole detachment in the anaphase period of the cell cycle (Haq and Kalamdhad, 2021). The existence of chromosomal loss and vagrant chromosomes shows that the spindle is deteriorating (Yadav et al., 2019). NAs such as binucleated cells were seen in the root tips of *A. cepa* subjected to *A. conyzoides* samples ranging from 2.4 to 2.8, but were reduced when exposed to compost samples. The NAs in the *A. conyzoides* sample were attributed to the initial stage of the telophase chromosome, preventing cell plate expansion among cells and possibly resulting in the binucleated cell (Haq et al., 2017). There was a significant decrease in aberrant cells in the compost sample compared to the *A. conyzoides* sample; this could be attributable to the proper degradation of organic matter during the composting process. When *A. cepa* induced with *A. conyzoides* sample, demonstrate substantial variance in CA and NA compared to compost sample and control ($p < 0.05$).

Table 6.3. Variation in chromosomal aberrations and nuclear abnormalities in root tip cells of *A. cepa* in *A. conyzoides* and compost sample

Concentrations	**Extracts**	**Anaphase Bridge**	**Vagrant chromosome**	**Chromosomal loss**	**C mitosis**	**Binucleated cells**	**Aberrant cells (%)**
Tap water		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EMF		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 0.4	1.2 ± 0.3
25%	*A. conyzoides*	1.8 ± 0.1	2.2 ± 0.22	1.4 ± 0.64	6.2 ± 1.2	2.6 ± 0.98	2.5 ± 0.46
	compost	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.12*	0.0 ± 0.0	0.1 ± 0.01
50%	*A. conyzoides*	2 ± 0.3	2.4 ± 0.67	3.2 ± 0.98	15.2 ± 2.4	2.4 ± 0.76	4.9 ± 0.4
	compost	0.0 ± 0.0	1.7 ± 0.1	0.0 ± 0.0*	0.8 ± 0.6*	0.0 ± 0.0	0.5 ± 0.09*
75%	*A. conyzoides*	3.2 ± 0.65	2.98 ± 0.98	3.62 ± 1.1	15.8 ± 1.4	2.8 ± 0.12	5.62 ± 0.88
	compost	0.0 ± 0.0*	1.9 ± 0.23	1.5 ± 0.2	2.1 ± 0.24*	1.0 ± 0.06	1.2 ± 0.2*
100%	*A. conyzoides*	3.62 ± 0.2	3.3 ± 0.24	4.3 ± 0.56	16.4 ± 2.3	2.4 ± 0.2	5.92 ± 0.45
	compost	1.0 ± 0.0	2.3 ± 0.06	1.8 ± 0.8	2.4 ± 0.13*	1.2 ± 0.03	1.72 ± 0.08*

All the parameters are expressed in mean ± SD of triplicates

* Significant difference observed at $p < 0.05$, when compared to *A. conyzoides* sample at same concentration

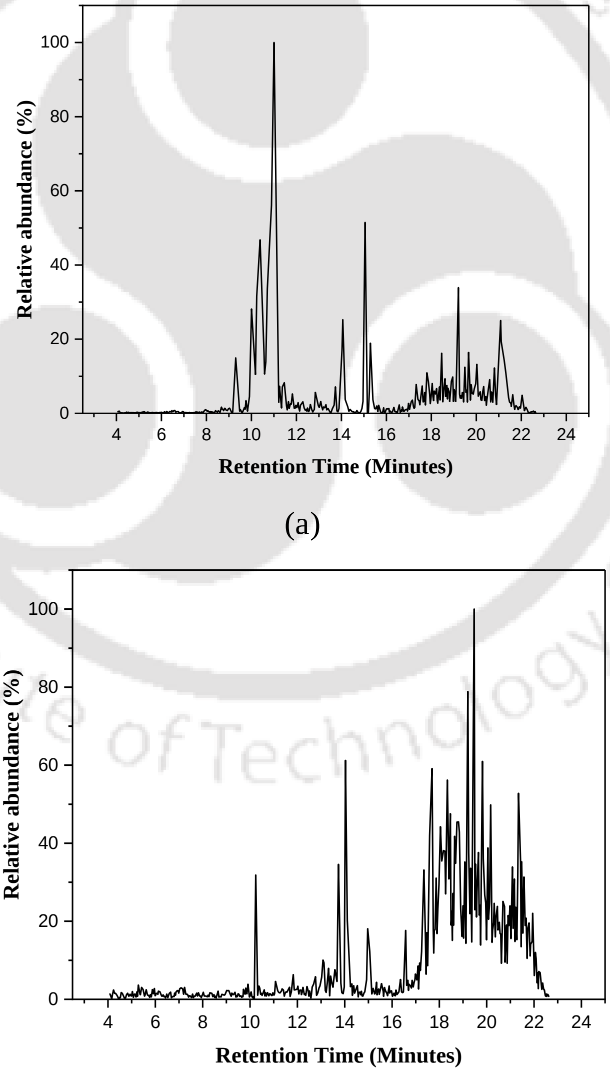
6.2. TRANSFORMATION OF TOXIC ORGANIC COMPOUNDS DURING THE COMPOSTING PROCESS

Terrestrial weeds, despite being commonly perceived as nuisances in agricultural and natural ecosystems, exhibit distinct characteristics that render them noteworthy contributors to the ecological dynamics of their respective environments. A noteworthy characteristic exhibited by numerous weed species is their allelopathic behavior, denoting their capacity to emit allelochemicals, chemical compounds that have an impact on the growth and development of adjacent plants. Allelochemicals, which are commonly of organic origin, possess the ability to exert toxic effects on neighboring vegetation, thereby impacting resource competition and influencing the composition of plant communities. The study of allelopathic interactions among plants has garnered significant interest due to its potential implications for the structure and functioning of ecosystems. The primary constituents of allelochemicals synthesized by weeds consist mainly of aliphatic and aromatic hydrocarbons. These organic compounds are characterized by their composition of carbon and hydrogen atoms, organized in either linear or cyclic configurations. The aforementioned compounds are produced and emitted by weeds into the surrounding environment, encompassing the soil, water, and air, via diverse mechanisms such as root exudation and the breakdown of plant matter (Tafese Bezuneh, 2015). The adverse impacts of allelochemicals on adjacent plants can manifest as growth suppression, diminished seed germination, modified root growth, and even mortality of the plants. Hence, it is imperative to comprehend the allelopathic capacity of weeds and their chemical constituents in order to effectively control weed populations and enhance agricultural yield (Kapeua Ndacnou et al., 2020).

The current study employed an innovative methodology to address the allelopathic impacts of specific terrestrial weeds by utilizing composting technology. Composting, an extensively employed method for waste management and soil enhancement, involves the controlled decomposition of organic matter. The objective of the study was to investigate the use of composting as a method for the degradation of toxic allelochemicals commonly found in weeds. The intention was to mitigate the phytotoxic effects of these chemicals and minimize the potential harm they pose to nearby plants.

The study utilized a two-step methodology: initially, the identification of the precise allelochemicals generated by the chosen weed species, followed by the quantification and evaluation of the degradation of these allelochemicals throughout the composting procedure. The analytical method utilized for the identification and characterization of allelochemicals in the weed species *A. conyzoides* was GC-MS. The conducted analysis unveiled a diverse array of chemical compounds, such as phenols, chromene, flavone, sterols, terpenes, and fatty acids. It was observed that several of these compounds were accountable for the observed toxic effects in adjacent plants.

The study revealed that the composting process resulted in substantial alterations in the composition of allelochemicals. The aliphatic hydrocarbons present in the original weed samples exhibited stability at the outset, but underwent changes during the composting process, leading to the generation of aromatic hydrocarbons. The observed alteration demonstrated the possibility of disintegration and deterioration of hazardous substances, thereby facilitating the mitigation of phytotoxic effects. Aromatic hydrocarbons, known for their increased stability and reduced phytotoxicity compared to aliphatic hydrocarbons, exhibited a notable presence in the ultimate compost samples. This observation implies that composting is an effective process for modifying the chemical properties of allelochemicals. The GC-MS analysis of methanol extracts of *A. conyzoides* has revealed toxic organic compounds such as caryophyllene oxide (RT: 19.20), stigmasterol (15.01), ageratochromene (RT: 15.29), Dibutyl phthalate (RT: 13.01). The presence of toxic organic compounds in extracts of *A. conyzoides* primarily consists of secondary metabolites that are synthesized by the plant to serve diverse ecological and physiological purposes (Hu et al., 2002). These compounds have the potential to display antimicrobial or allelopathic characteristics; however, their prolonged presence in the environment can have detrimental effects. Microorganisms play a crucial role in composting by breaking down complex organic molecules into simpler forms. The presence of microorganisms capable of utilizing or metabolizing the toxic compounds could lead to their degradation. This microbial activity transforms the toxic compounds into less harmful substances. The process of composting involves a wide array of biochemical reactions, including hydrolysis, oxidation, and reduction. These reactions facilitate the degradation of chemical bonds within the toxic compounds, resulting in reduced stability and eventual elimination. For example, the aromatic rings found in compounds such as caryophyllene oxide and Dibutyl phthalate have the ability to undergo ring-opening reactions when exposed to enzymes and microbial activity. Certain toxic compounds have the potential to undergo adsorption onto the surfaces of compost particles, whereas others may be absorbed by microbes as a carbon source (T.C. and G., 2012). This process has the ability to isolate the harmful substances from the surrounding environment and facilitate their subsequent conversion or degradation. Microorganisms possess the ability to decompose intricate organic substances and subsequently secrete enzymes capable of catalyzing diverse reactions. Oleic acid, a type of fatty acid, may be generated as a result of lipid degradation facilitated by lipolytic enzymes. Likewise, the synthesis of 7-Propylidene-bicyclo[4.1.0]heptane may encompass microbial processes such as isomerization or cyclization reactions (Haq et al., 2018). The elevated temperature and dynamic conditions present in the RDC system can promote the occurrence of rearrangement and cyclization reactions. The generation of 7-Propylidene-bicyclo[4.1.0]heptane may entail carbon-carbon bond rearrangements, resulting in the formation of unique cyclic architectures. Fig. 6.2 and Table 6.4 illustrates the presence and absence of toxic organic compounds in *A. conyzoides* feedstock before and after composting process.



(a)

(b)

Fig. 6.2. GC-MS chromatograms of (a) *A. conyzoides* extract (b) final day compost extract

Table 6.4. GCMS analysis of *A. conyzoides* and its compost

Retention time	Compounds	*A. conyzoides* extract	Compost extract
10.23	Phenol, 2,4-bis(1,1-dimethylethyl)-	+	+
15.29	Ageratochromene	+	+
19.20	Caryophyllene oxide	+	+
13.3	Phthalic acid, 7-methyloct-3-yn-5-yl propyl ester	+	-
13.01	Dibutyl phthalate	+	-
15.01	Stigmasterol trimethylsilyl ether	+	+
16.8	6-Methyl-4-prop-2-en-1-yl-3-propyl-2,6-dioxo-4,5,6,7-tetrahydro-1,2,3-triazolo[4,5-d]pyrimidine	+	-
14.10	Oleic acid	-	+
14.97	7-Propylidene-bicyclo[4.1.0]heptane	-	+

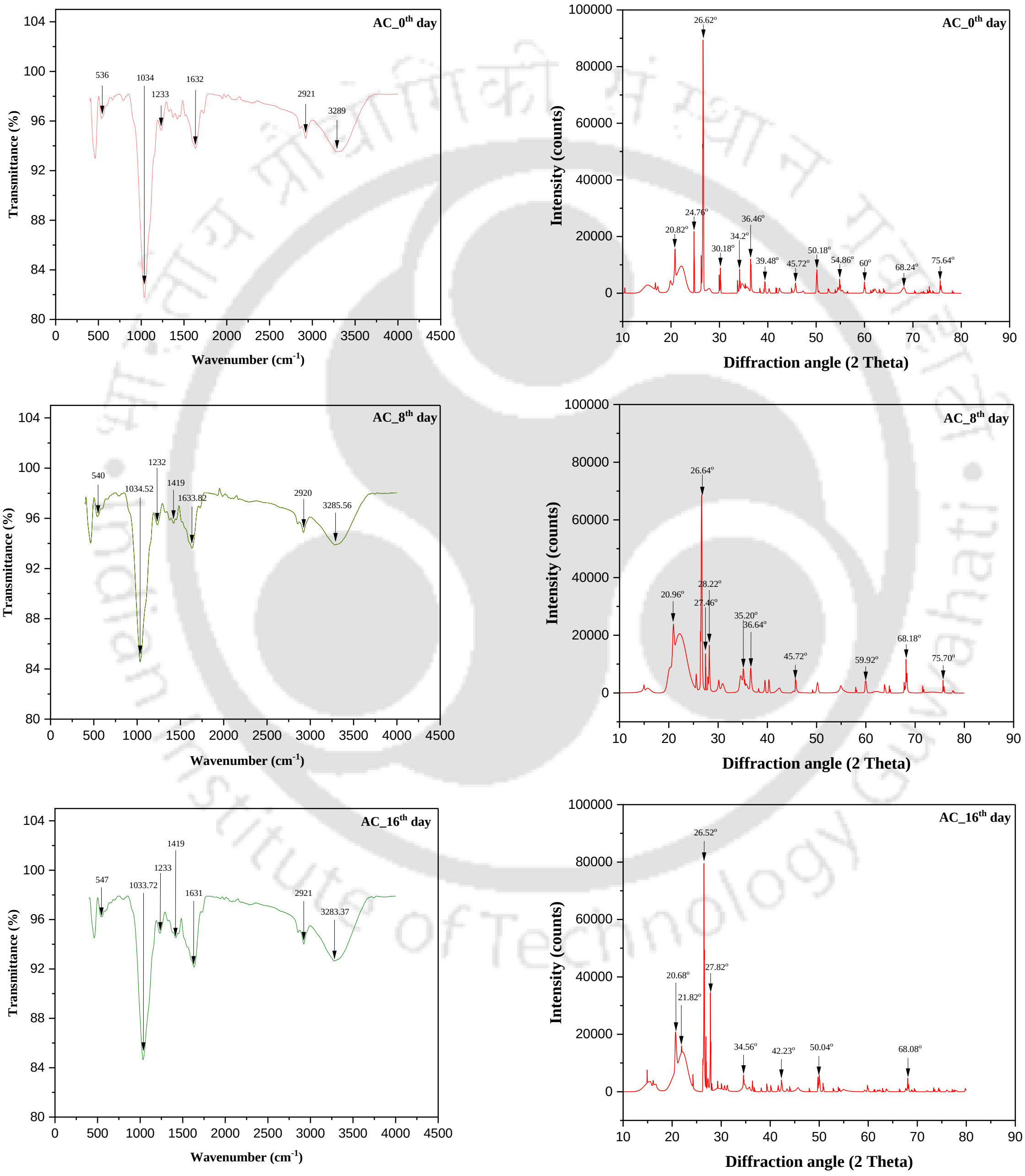
6.3. INSTRUMENTAL CHARACTERIZATION

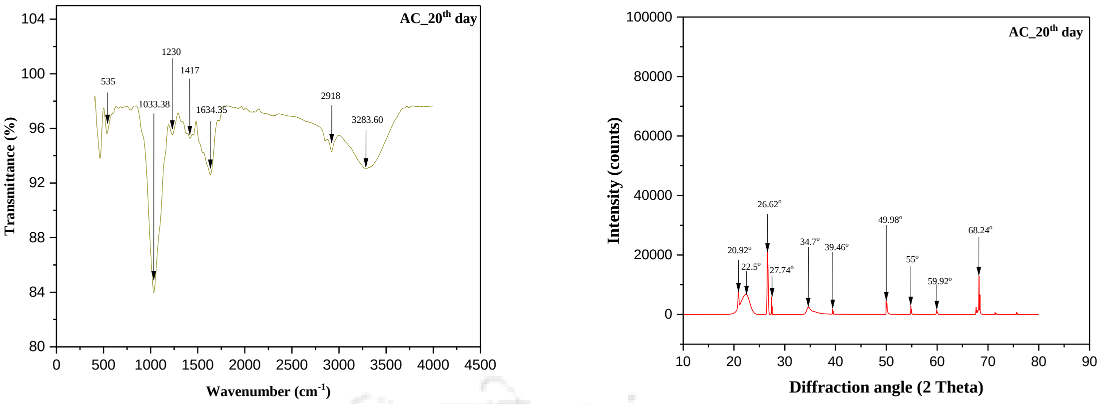
6.3.1. FTIR

FTIR spectra were collected based on the strengths of specific distinctive absorbance peaks to analyse the evolution of the functional groups and related carbon structures of distinct compounds during composting (Biyada et al., 2020). The same transmittance peaks were observed during the composting activity with a difference in amplitude at distinct phases Fig. 6.3 (a). The spectra ranging from 1031 to 1035 cm^{-1} is common in all the phases of composting activity, which was due to the presence of S=O stretching in the form of sulfoxide compound, strong C-O stretching in the form of vinyl ether compound, medium C-N stretching in the form amine compounds and silicate impurity with an asymmetric Si-O stretch (Paul et al., 2020). Furthermore, It also corresponds to the aromatic C-H in-plain distortion of syringyl and guaiacyl alcohols, two structural constituents of lignin (Varma et al., 2017b). The spectra stretch ranging between 3283 to 3289 cm^{-1} showing the crystalline structures of cellulose, hemicellulose and lignin resembling the profusion aromatic hydrocarbons and phenolic compounds (C-H stretching) (Zahra et al., 2015). The ratio of aromatic C/aliphatic C from initial to final days (1632/2921 to 1634.35/2918) was increased from 0.55 to 0.56; This occurred as a result of the humic polymer's synthesis (Rich et al., 2018). The Aromatic C/polysaccharide ratio increased from the initial day to the final day of composting activity (1632/1034 to 1634.35/1033.38) from 1.57 to 1.58; This showed the protein's and polysaccharide's biodegradation efficiency (Rich et al., 2018). The intensity of spectra ranging between 535 to 547 cm^{-1} represents the elongation of phosphate clusters asymmetrically in the process of composting (Zhang et al., 2021).

6.3.2. PXRD characterization

X-ray diffraction technique is advantageous for determining phasing, crystalline structure, and other structural properties (Hajji et al., 2015). The compost samples in various degradation phases were analysed using X-ray diffraction to assess the possible changes that might emerge during the composting activity. Fig. 6.3 (b) illustrates the alterations in X-ray spectra during the composting activity. The spectra of compost samples were revealed to be reduced during the composting activity; these outcomes are consistent with organic material breakdown, resulting in a drop in the C/N ratios. The cellulose found in fibre-rich substance's cell wall has an intricate crystal lattice that defies degradation. Chains are linked together in crystalline cellulose by shared hydrogen bonds, whereas in amorphous cellulose, hydrogen bonds do not form (Paul et al., 2020). There were numerous peaks spread in the range 2θ from 20° to 40°. As seen on the initial day, the cellulose is polycrystalline with peaks placed at Bragg's angle 2θ in Fig. 6.3 (b), as evident from other studies (Paul et al., 2020). According to Biyada et al. (2020), the range between 20° to 50° 2θ is more suitable for identifying all relevant cellulose crystalline composition sensitivities. It was evident from the few studies on green waste, which demonstrates a high concentration of cellulose fibre and lignocellulosic chemicals (Ho et al., 2010). Furthermore, It would be highlighted that the range 2θ of 22.55° to 22.65° denotes the crystalline plane of cellulose I, while the interval between 34.5° and 35.2° denotes the crystalline plane of cellulose II (Biyada et al., 2020). Fig. 6.3 (b) illustrates the increase in cellulose I content between 22.24° to 22.98° compared to the *A. conyzoides* sample. There was a significant decrease in cellulose II from the initial to the final day compost. Nonetheless, a substantial reduction in the amplitude of these peaks was evidenced in the final compost, which could be ascribed to microorganisms degrading the cellulose molecular chains, explaining the decrease in the C/N ratios indicating the breakdown of organic material and thus the cellulose compounds. Additionally, XRD analysis established the mineral proportion of the compost samples. $CaCO_3$ and P_2O_5 is the most readily visible mineral component in composting stages in the range of 22.64°, 29.52°, 68.24°, 26.85°, 40.04°, 21.55° and 20.87°, 40.86°, 31.07°, 34.21°, 50.24° respectively, as evident from the study Hajji et al. (2016).





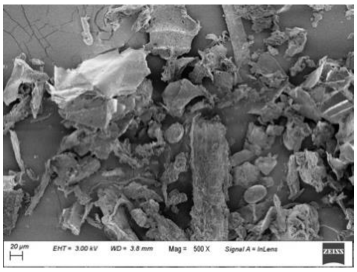
(a) FTIR spectra (b) PXRD spectra

Fig. 6.3. Spectroscopic characterization during the composting process of *A. conyzoides*

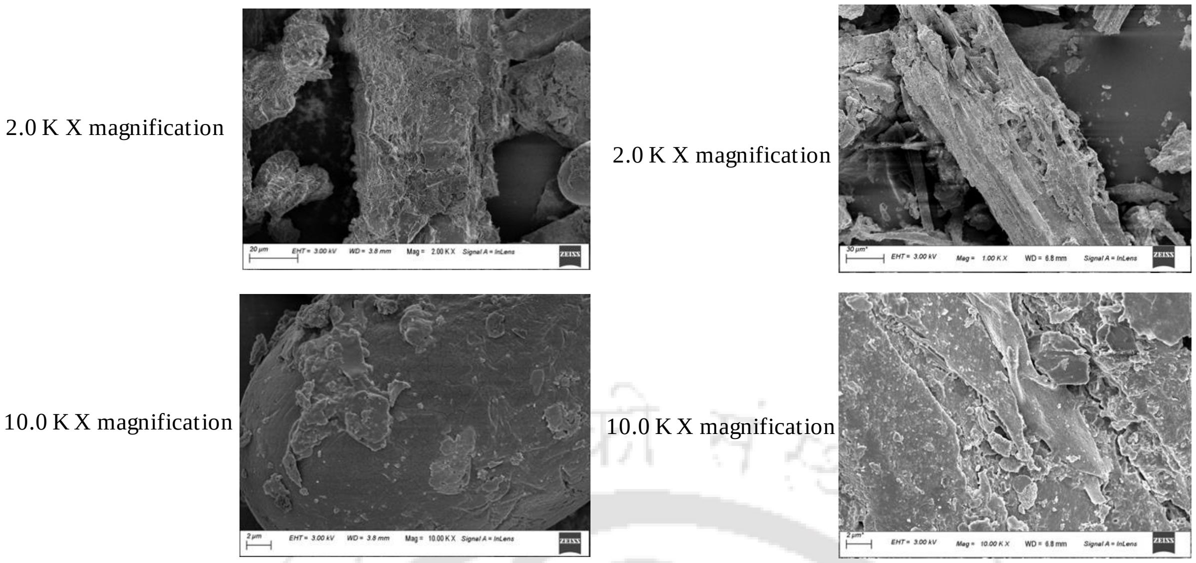
6.3.3. FESEM characterization

The FESEM images illustrate the morphological changes that occur throughout distinct phases of *A. conyzoides* compost. The morphology of the initial day of the composting process revealed a broader solid substance with lesser pores developed on the surface. With the progression of composting time and more significant deterioration, the particle size began to decrease. On the final day compost, the particles had been transformed into smaller-sized particles with larger gaps on the surface, suggesting the development of mature compost (Sharma et al., 2019). The *A. conyzoides* include modest amounts of lignin and fibrous materials; a lengthy verticle structure was discovered in the initial day mix, representing fibrous material. However, as the magnification was raised in a specific location of the vertical structure, the void spaces became very small, indicating a stiff lignin structure. The initial day mix had several fibrous elements. However, in the case of final day compost, the pore space between each particle was minimal, indicating that microorganisms efficiently degraded organic matter during the composting process. The FESEM study demonstrates lignin biodegradation during the in-vessel composting process. The FESEM images are depicted in Fig. 6.4 (c) and (d).

500 X magnification



500 X magnification



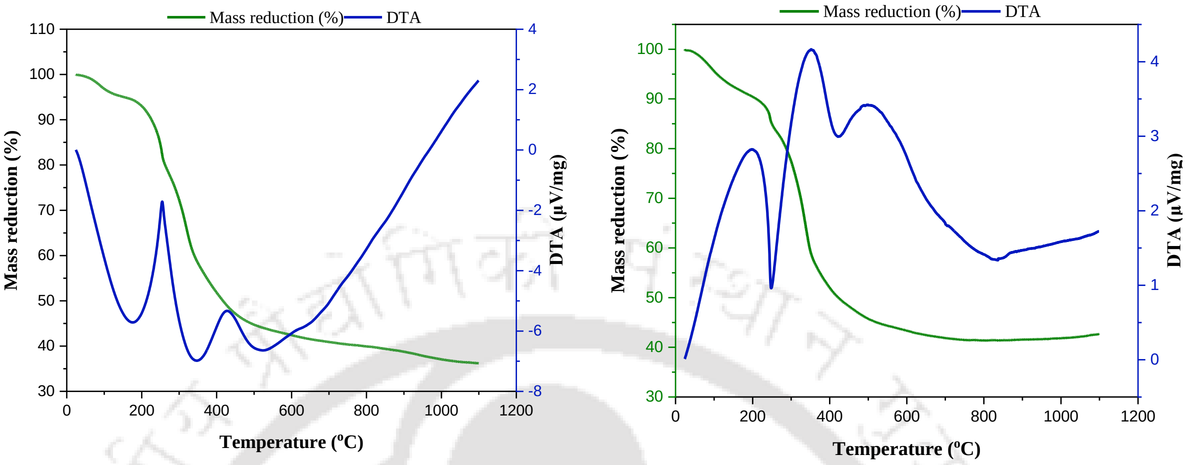
(a) FESEM image of initial day compost (b) FESEM image of final day compost

Fig. 6.4. Micrographic analysis of initial and final day compost. (a) and (b) FESEM images of initial and final day compost made of *A. conyzoides* at various magnifications

6.3.4. TGA/DTA characterization

Thermal studies of the initial and final day composts reveal two exothermic peaks in the range of 200–600°C, as depicted in Fig. 6.5 (a) and (b). At the initial and final days of the composting process, a gradual decrease in the mass reduction related to the temperature ranging 200–350°C was observed. This indicates that carbohydrates, aliphatic molecules, and some potentially biodegradable aromatic compounds gradually degrade (Fernández et al., 2012). The mass reduction in this phase was higher on the initial day (37.2%) compared to final day (32.1%) compost; this low reduction in final day compost could be attributable to the loss of carbohydrates and aromatic compounds in the composting process. The magnitude of mass reduction attributed with the temperature ranging 400–500°C was associated with more complex organic compounds, such as lignin, hemicellulose and cellulose (Zahra et al., 2015). The decrease in the peak intensity could be attributed to the breakdown of complex structured compounds such as lignin, hemicellulose, whereas the increase in intensity is most likely due to the discharge of aromatic structures following lignocellulose complex breakdown, resulting in the convection of these structures. The discharge of core components and the degradation of hemicellulose, cellulose, and lignin occur during this phase (Pardo et al., 2021). The mass reduction in this phase was higher on the initial day (13.6%) compared to the final day (9.8%)

compost; the higher reduction on the initial day was due to the presence of higher amounts of lignocellulose matter.



(a) TGA/DTA curves of initial day

(b) TGA/DTA curves of final day

Fig. 6.5. Thermal analysis of initial and final day compost of *A. conyzoides*. (a) and (b) TGA/DTA curves of initial and final day compost

6.4. TOXICITY ASSESSMENTS IN DISTINCT PHASES OF COMPOSTING *P. HYSTEROPHORUS*

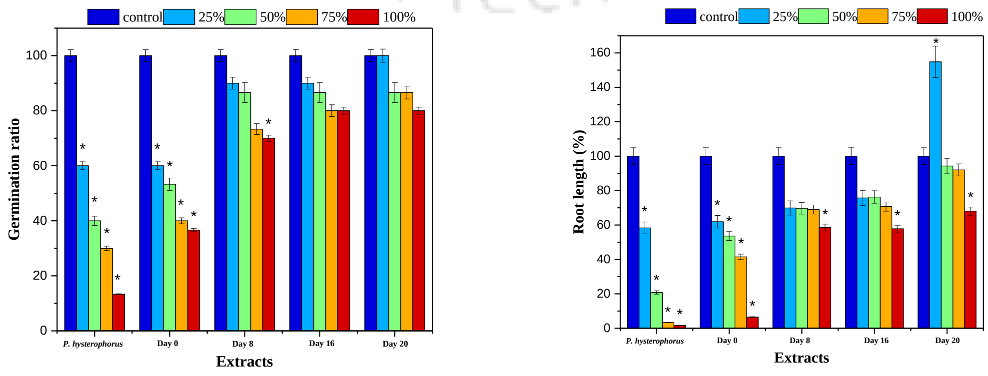
6.4.1. Phytotoxicity test

Phytotoxicity assay is crucial for assessing compost quality, and indices such as GR, RL, SL and GI are valid markers of phytotoxicity; inadequately digested compost can impede seed sprouting (Luo et al., 2018). Fig. 6.6 (I) illustrates the pattern alterations in the GR, RL, SL, GI and Biomass weight during the composting process. The GR of the *V. radiata* in *P. hysterophorus* extract was reduced by 86.7, 63.6 and 83.3% compared to control, 100% concentration of initial and final day, which resembles the reduction in toxic composites during the composting process. The RL, SL and GI of the *V. radiata* in 25% concentration of 20th day were increased by 54.9, 17.8 and 72.7% and 150.2, 123.5 and 296.3% compared to control and 25% concentration of initial day compost extract. Seedling growth is hindered by phytotoxic compounds found in the compost extracts, such as phenolic compounds, organic acids, and inorganic nitrogen (Chen et al., 2021). Composting could help reduce phytotoxicity by degrading, transforming, and aggregating toxic compounds and lowering the bioavailability of noxious compounds (Kebibeche et al., 2019). The GI of *V. radiata* in 25 to 100% concentrations of *P. hysterophorus* extract is reduced by 67.4 to 99.9% compared to the control at $p<0.05$.

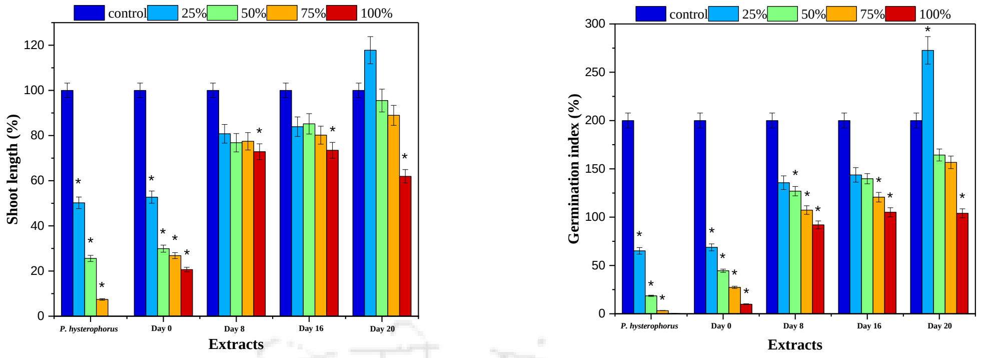
Furthermore, it was observed that the biomass reduction of *V. radiata* in 100% concentrated *P. hysterophorus* extract was 86.3% significant at $p<0.05$ compared to control. During the composting process, a substantial increase in biomass was observed, and similar patterns were reported by Kauser et al. (2020) in their study. The biomass percentage was reduced in the range of 18.3 to 86.3% from 25 to 100% concentrations of *P. hysterophorus* extract compared to control. There was a significant increase in biomass percentage in the 25% concentration of 20th day compost extract by 20.1% and a decrease of 24.20% in the 100% concentration of 20th day compost extract compared to control. Additionally, it was highlighted that direct usage of *P. hysterophorus* and 100% concentrated 20th day compost extracts resulted in a loss of benefits owing to increased toxicity, which also prohibits seedlings from utilizing nutrients, leading to a reduction in the biomass content, root and shoot lengths of *V. radiata*.

The plant bioassay of *A. cepa* is regarded as an indicator of the phytotoxicity experiment. The root growth of the *A. cepa* in the 25% concentrated compost extracts increased in the range of 52 to 60% compared to the initial day compost and *P. hysterophorus* extracts. Fig. 6.6 (II) depicts the alterations in the growth of *A. cepa* in various extracts. The maximum root length was observed at a 25% concentration of 20th day compost extract (6.1 cm). But as the concentration in the 20th day compost extract increased, the length of the roots decreased. This might be due to the high dosage of nutrient intake by the onion bulb. The increase in biomass and root length percentages on the 8th and 16th days of the composting process might be attributed to the biodegradation of soluble organic matter, and PXRD demonstrates the transformation of insoluble lignocellulose material into soluble material. The recorded findings indicated that composting may be the most environmentally friendly strategy for managing and reducing the damaging impacts of weeds (Kauser et al., 2020). Fig. 6.7 depicts the growth of *V. radiata* and *A. cepa* in various extracts.

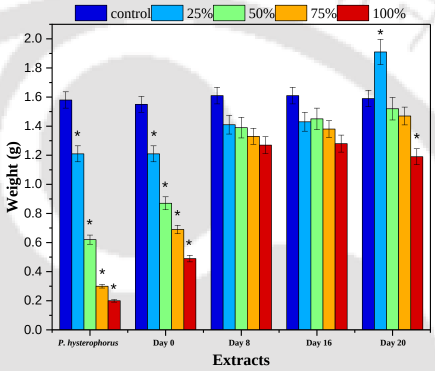
I



(a) Germination ratio (b) Root length (%)

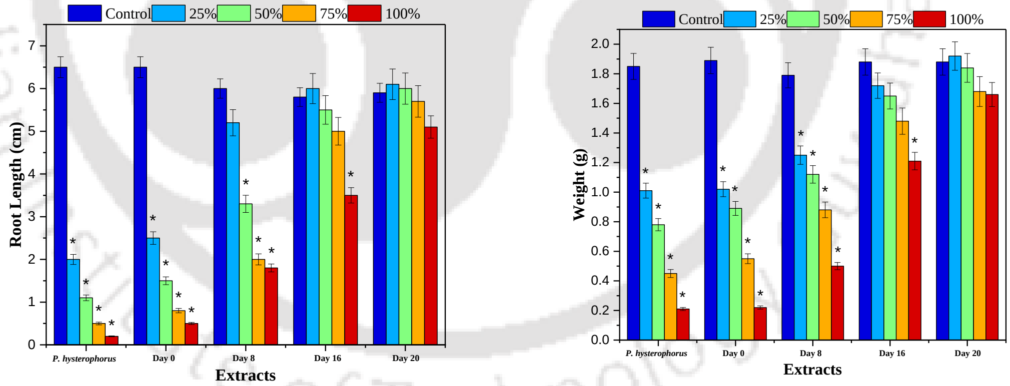


(c) Shoot length (%) (d) Germination index (%)



(e) Biomass (g)

II



(a) Root length (cm) (b) Biomass (g)

Fig. 6.6. (I). Graphical depiction of phytotoxicity parameters of *V. radiata* in *P. hysterophorus* and various staged compost extracts; (II) Graphical depiction of phytotoxicity parameters of *A. cepa* in *P. hysterophorus* and various staged compost extracts.

*. Significant difference observed at $p < 0.05$ compared to tap water (negative control)

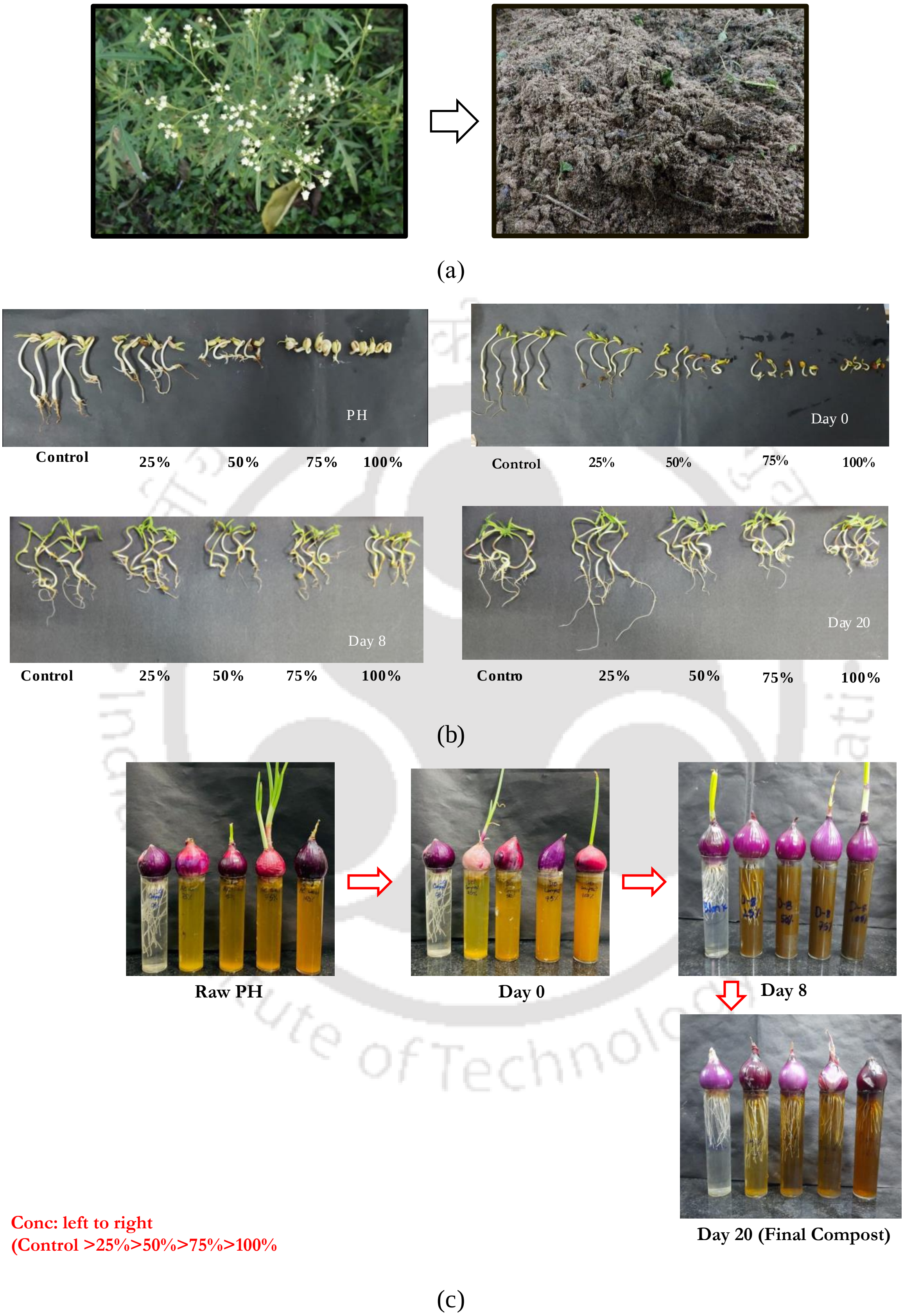


Fig. 6.7. (a) Initial and final day compost samples; Phytotoxicity assay during the composting process of *P. hysterophorus* (b) *V. radiata*, (c) *A. cepa*

6.4.2. Cytotoxicity test

The cytotoxic characteristics of *P. hysterophorus* and its compost was determined by calculating MI of *A. cepa* meristematic roots. The MI is an effective experimental approach for determining the cytotoxicity of a variety of toxic composites during cell division (Yadav et al., 2019). The MI of *A. cepa* roots in various extracts is depicted in Table 6.5. The percentage reduction in MI of *A. cepa* roots in *P. hysterophorus* extract was decreased from 54.8 to 73.91% in 25 to 100% compared to control, which clearly determines the presence of highly toxic composites in *P. hysterophorus* extract (Adkins and Shabbir, 2014). The results of compost extracts from initial to final days revealed an increase in MI of *A. cepa* roots in all concentrations from 9.5 to 55.3% compared to *P. hysterophorus* extracts. The maximum MI percentage of *A. cepa* roots was observed in 25% concentration of 20th day compost extract with 1.1% compared to control. But as the concentration was further increased to 50, 75 and 100% in 20th day compost, the MI was decreased by 15.5, 22.1 and 31.1% compared to control, revealing the existence of a variety of cytotoxic persistent organic contaminants. These contaminants may disrupt the mitosis process by cessation the high amount of cells from undertaking prophase and averting the mitosis cycle from continuing throughout interphase (Haq et al., 2017). The percentage MI of *A. cepa* roots in all the compost extracts were varied significantly at $p<0.05$ compared to control and *P. hysterophorus* extract.

Table 6.5. MI (%) of root tip cells of *A. cepa* following with *P. hysterophorus* and various staged compost extracts

Concentrations	**Extracts**	**Total cells**	**Dividing cells**	**MI %**
Tap water		702	501	71.3 ± 1.8
EMS		713	41	5.75 ± 1.01^{a}
25%	PH	860	268	32.2 ± 0.6^{a}
	Initial mix	832	297	35.6 ± 0.5^{a}
	8th day	836	374	44.73 ± 0.8^{a}
	16th day	809	407	50.30 ± 1.2$^{a, b}$
	20th day	834	602	72.1 ± 0.9^{b}
50%	PH	796	213	26.7 ± 0.4^{a}
	Initial mix	823	254	30.8 ± 1.1^{a}
	8th day	849	354	41.69 ± 0.5^{a}

	16th day	856	421	49.18 ± 1.5$^{a, b}$
	20th day	844	509	60.3 ± 1.2^{b}
75%	PH	801	177	22.09 ± 0.9^{a}
	Initial mix	812	203	25 ± 0.6^{a}
	8th day	824	348	42.23 ± 1.6^{a}
	16th day	792	410	51.76 ± 1.5^{b}
	20th day	798	439	55.5 ± 1.3^{b}
100%	PH	805	150	18.6 ± 0.1^{a}
	Initial mix	813	174	21.4 ± 0.7^{a}
	8th day	833	313	37.57 ± 1.1^{a}
	16th day	806	353	43.79 ± 0.8^{a}
	20th day	855	420	49.12 ± 1.6$^{a, b}$

All the parameters are expressed in mean ± SD of triplicates

^{a}. Significant difference observed at $p < 0.05$ compared to tap water (negative control)

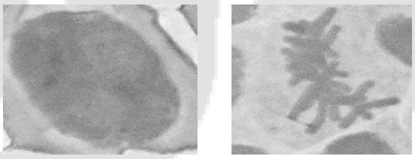
^{b}. Significant difference observed at $p < 0.05$, when compared to *P. hysterophorus* extract at same concentration

6.4.3. Genotoxicity test

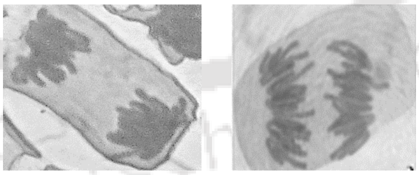
The sprouting root hairs of *A. cepa* provide widely available plant material for research into the deleterious impact of toxins on chromosomes. CA and NA study of *A. cepa* root tip cells is regarded as an effective method for determining the genotoxic potential of various environmental applications such as compost. CA is defined by alterations in the shape of one or more chromosomes, which could affect the cell division process in the plant. Various forms of CAs are used to assess chromosomal aberrations throughout cell division's mitotic phase (Leme and Marin-Morales, 2009). The induced genotoxic effects in the *A. cepa* root tips are depicted in Table 6.6 and Fig. 6.8 as CAs and NAs. CAs and NAs were not observed in 4mM EMS and tap water. The root tips of *A. cepa* revealed the initiation of various kinds of CAs and NAs when subjected to *P. hysterophorus* and compost extracts. The CAs identified in the genotoxic investigation of *A. cepa* root tips were chromosomal bridge, sticky chromosome, chromosomal loss, C-mitosis, vagrant chromosome and, whereas NAs identified were Micro-nucleated cell, binucleated cell, nuclear enlargement (Fig. 6.8). The aberrant cell percentage observed in the genotoxic potential of *A. cepa* root tips in *P. hysterophorus* extracts was most significantly varied from 16th and 20th day compost extracts in the same concentrations at $p<0.05$.

The substantial observed CAs were C-mitosis, sticky chromosome and vagrant chromosome in the investigation. The C-mitosis in 25 to 100% concentrated *P. hysterophorus* extract was 6.2, 15.2, 15.8 and 16.4 and was reduced during the composting process. The maximum reduction was observed in 25% dilution on 20th day compost extract with 80.6% compared to same concentration of *P. hysterophorus* extract. C-mitosis is described as pole deactivation accompanied by spontaneous chromosomal dispersion across the cells. The *P. hysterophorus* extract produced a high prevalence of c-mitosis, as evidenced by earlier research demonstrating that wastewater is poisonous to colchicine and therefore capable of inducing C-mitosis. The maximum numbers of sticky chromosome were observed at 100% diluted *P. hysterophorus* extract with 3.3, whereas during the composting process, sticky chromosome was not observed in 16th and 20th day compost extracts. Sticky chromosome is regarded as prevalent indicator of toxic effects on chromosomes, which may result in cell damage (Rojas et al., 1993). Sticky chromosome can also develop as a result of enhanced chromosomal shrinkage and consolidation, or as a result of solubilization of Deoxyribonucleic acid (DNA) and partial dissociation of nucleoproteins (Yadav et al., 2019).

I

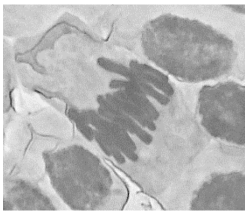


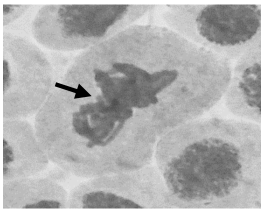
(a) Prophase (b) Metaphase



(d) Telophase (c) Anaphase

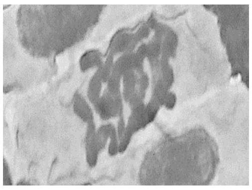
II

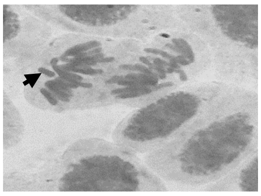




(a) Sticky chromosome

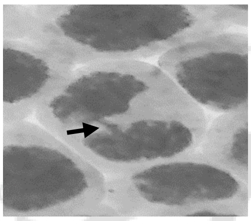
(b) Non-structured sticky chromosome

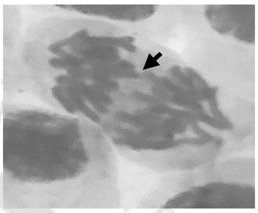




(c) C-mitosis

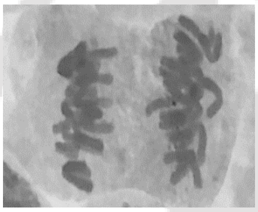
(d) Chromosomal loss

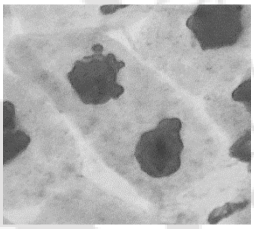




(e) Bridge at telophase

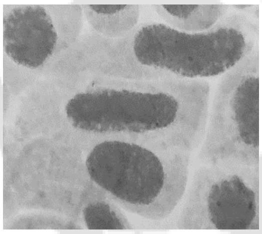
(f) Anaphase bridge

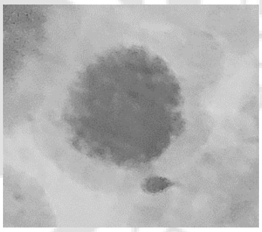




(g) Vagrant chromosome

(h) Binucleated cell





(i) Nuclear enlargement

(j) Micro nuclei

Fig. 6.8. I-Photographs: Normal mitosis of *A. cepa* root tips treated with tap water (control). II-Photographs: Induced Chromosomal aberrations and Nuclear abnormalities by various concentrations *P. hysterophorus* and compost extracts in *A. cepa* root tip cells

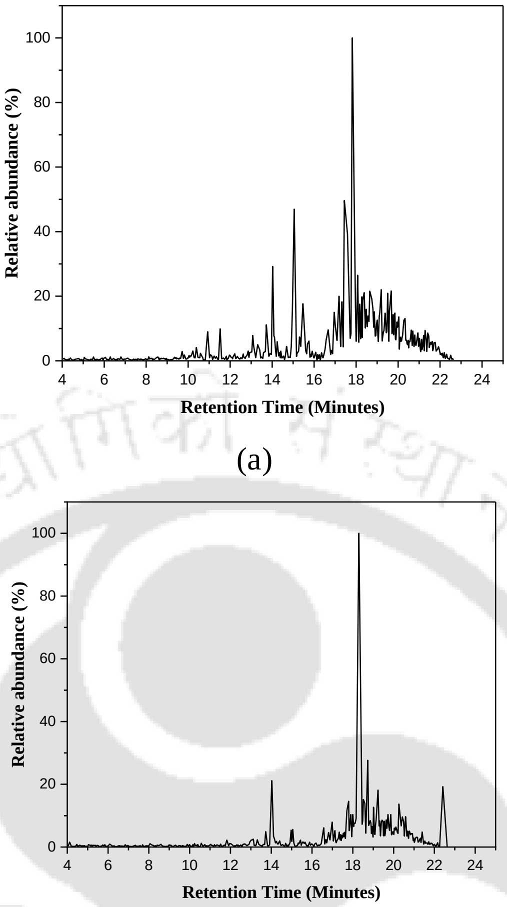
Table 6.6. Various Chromosomal and nuclear abnormalities found in root tip of *A. cepa* following with *P. hysterophorus* (PH) and various staged compost extracts

Concentrations	**Extracts**	**Chromosomal bridge**	**Sticky chromosome**	**Chromosomal loss**	**C mitosis**	**Vagrant chromosome**	**Micro nucleated cells**	**Nuclear enlargement**	**Binucleated cells**	**Aberrant cells (%)**
Tap water		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
25%	PH	1.8 ± 0.1	2.2 ± 0.22	1.4 ± 0.64	6.2 ± 1.2	5.2 ± 1.1	1.8 ± 0.88	2.4 ± 0.1	2.6 ± 0.98	2.95 ± 0.46
	Initial mix	1.6 ± 0.1	1.8 ± 0.45	1.4 ± 0.88	5.8 ± 1.4	4.9 ± 0.8	1.6 ± 0.68	1.3 ± 0.09	2.2 ± 0.86	2.57 ± 0.24
	8th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	5.2 ± 0.9	3.2 ± 0.34	1.2 ± 0.88	1.1 ± 0.06	1.6 ± 0.66	1.53 ± 0.17
	16th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	1.2 ± 0.43*	0 ± 0*	1 ± 0.1	0.0 ± 0.0*	1.2 ± 0.1	0.42 ± 0.1*
	20th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	1.2 ± 0.12*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.15 ± 0.05*
50%	PH	2 ± 0.3	2.4 ± 0.67	3.2 ± 0.98	15.2 ± 2.4	13.1 ± 1.4	2.2 ± 0.68	2 ± 0.13	2.4 ± 0.76	5.31 ± 1.4
	Initial mix	1.8 ± 0.88	2.2 ± 0.78	2.4 ± 0.88	13.2 ± 1.8	11.4 ± 0.4	1.8 ± 1.2	1.2 ± 0.05	2.4 ± 0.68	4.55 ± 1.44
	8th day	0.0 ± 0.0*	1.2 ± 0.88	1.2 ± 0.76*	5.4 ± 0.45*	1.2 ± 0.31*	0.0 ± 0.0*	0.0 ± 0.0*	1.2 ± 0.1	1.27 ± 0.24*
	16th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	3.2 ± 1.2*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	1.2 ± 0.1	0.62 ± 0.23*
	20th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	2.9 ± 0.66*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	1.2 ± 0.42	0.45 ± 0.54*
75%	PH	3.2 ± 0.65	3.5 ± 0.98	3.7± 1.1	15.8 ± 1.4	14.1 ± 0.12	2.2 ± 0.88	2.5 ± 1.1	2.8 ± 0.12	5.97 ± 0.88
	Initial mix	2.4 ± 0.43	2.4 ± 0.44	2.8 ± 0.76	12.8 ± 0.88	13.1 ± 0.23	1.6 ± 0.12	1.7 ± 0.98	2.2 ± 0.56	4.88 ± 0.4
	8th day	1.2 ± 0.21*	0.0 ± 0.0*	1.9 ± 0.0*	5.7 ± 0.12*	1.1 ± 0.09*	1.0 ± 0.0*	0.0 ± 0.0*	1.9 ± 0.0*	1.61 ± 0.24*
	16th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	4.1 ± 0.24*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	1.5 ± 0.0*	0.7 ± 0.2*
	20th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	3.6 ± 0.24*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	2.1 ± 0.0*	0.82 ± 0.2*
100%	PH	3.2 ± 0.2	3.6 ± 0.24	3.9 ± 0.56	16.4 ± 2.3	12.1 ± 1.5	2.8 ± 1.4	2.7 ± 0.67	3.1 ± 0.2	5.98 ± 1.2
	Initial mix	2.8 ± 0.34	2.9 ± 0.88	3.2 ± 0.0*	13.4 ± 1.24	11.1 ± 0.34	1.8 ± 1.62	2.1 ± 0.13	2.6 ± 0.12	4.97 ± 2.4
	8th day	1.2 ± 0.2*	0.0 ± 0.0*	1.6 ± 0.0*	5.4 ± 0.44*	1.3 ± 0.14*	0.2 ± 0.1*	0.0 ± 0.0*	2.3 ± 0.1*	1.5 ± 0.12*
	16th day	1.6 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	3.6 ± 0.67*	1.8 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.4 ± 0.1*	0.92 ± 0.43*
	20th day	1.9 ± 0.13	2.6 ± 0.0*	1.5 ± 0.0*	1.4 ± 0.76*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.9 ± 0.0*	1.03 ± 0.2*

*. Substantial variance observed at $p < 0.05$ compared to negative control (Tap water)

6.5. TRANSFORMATION OF TOXIC ORGANIC COMPOUNDS DURING THE COMPOSTING OF *P. HYSTEROPHORUS*

The identification of toxic organic compounds in the composting of *P. hysterophorus* was analyzed through GC-MS instrument. Fig. 6.9, and Table 6.7 depicts the chromatogram peaks and toxic organic compounds present in the *P. hysterophorus* substrate and compost sample. The compounds such as benzoic acid (RT: 14.02), caryophyllene oxide (RT: 15.04), Beta stigmasterol (RT: 17.44), Diethyl phthalate (RT: 17.81) were found in n-hexane extracts of *P. hysterophorus*. Diethyl phthalate is considered to be a toxic compound with respect to plants. The high concentration of this compound could lead to plant mortality (Carolina de Almeida et al., 2023). The sensitivity of the peak of this compound was less in the compost sample, resembles the degradation of the compound, the further degradation mechanism was performed through GC-FID. Toxic organic compounds such as beta sitosterol, caryophyllene oxide, benzoic acid was found in the extracts of *P. hysterophorus*, while not detected in compost sample. Caryophyllene oxide has the ability to undertake oxidative reactions during the RDC process, where aeration is provided by keeping valves open and proper turning frequency. These reactions have the potential to induce the splitting of the oxide ring, resulting in the generation of diverse oxygenated molecules such as aldehydes, ketones, and carboxylic acids. The aforementioned oxidative transformations can be facilitated by enzymes or take place via non-enzymatic processes. The transformation of caryophyllene could also due to the hydrolytic splitting of epoxide rings transforming into alcohols. Within an aerobic environment, the primary objective of degradation involves the process of mineralization, wherein benzoic acid is transformed into carbon dioxide (CO_2) and water (H_2O). Microorganisms exhibit variability in their use of metabolic pathways, while a common feature involves the degradation of the aromatic ring structure, afterwards using the resultant fragments as sources of carbon and energy. Microbial species have the capability to synthesize enzymes such as cytochrome P450 monooxygenases, which possess the ability to catalyze the hydroxylation of beta-sitosterol. This enzymatic process involves the addition of hydroxyl groups (-OH) at different places within the beta-sitosterol molecule. The process of hydroxylation can lead to the generation of many derivatives of hydroxylated beta-sitosterol (Yin et al., 2018). The composting process of *P. hysterophorus* has transformed and non-detected toxic organic compounds in its compost sample.



(a)

(b)

Fig. 6.9. GC-MS chromatograms of (a) *P. hysterophorus* extract (b) final day compost extract

Table 6.7. GCMS analysis of *P. hysterophorus* and its compost

Retention time	**Compounds**	***P. hysterophorus* extract**	**Compost extract**
10.22	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	+	-
11.46	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	+	
14.02	Benzoic acid, 2,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	+	-
14.05	Phthalic acid, butyl 2-chloropropyl ester	-	+
15.04	Caryophyllene oxide	+	-
17.44	Beta-sitosterol	+	-
17.81	Diethyl phthalate	+	+

18.73	Cyclodecasiloxane, eicosamethyl-	-	+
19.23	Stigmasterol	-	+

6.6. INSTRUMENTAL CHARACTERIZATION

6.6.1. FTIR

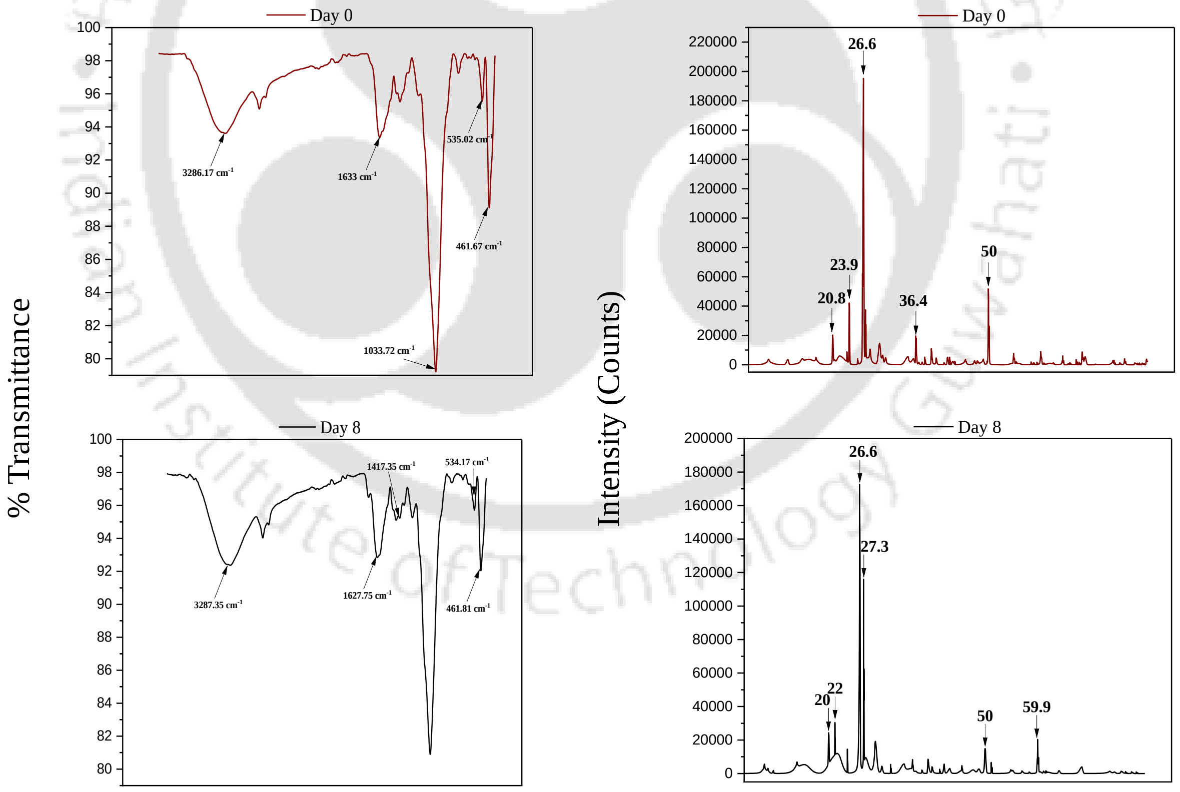
The FTIR spectroscopic analysis has been extensively employed to analyze the alterations in the structure and functional groups of composting feedstock. Similar absorbance spectra with changes in transmittance were detected during the composting process. Fig. 6.10 (a) depicts the alterations in FTIR spectra in the due course of composting process. The FTIR spectra were mostly absorbed at a wave numbers of 3286.17, 1633, 1033.72 and 535.02 cm^{-1} at different transmittance showing the abundance of phenolic, aliphatic, aromatic and polysaccharide structures in *P. hysterophorus*, inoculum and saw dust (Biyada et al., 2020; Zahra et al., 2015). The transmittance of spectra ranging 1032 to 1034 cm^{-1} (complicated C–O and C–C stretching, anti-symmetric bridge C–O–C, CCH and OCH vibrations), C-N stretching (amine group) was decreased and also attributes to the patterns of cellulose, hemicellulose, phenolic compounds and the presence of polysaccharide compounds during the composting process. The spectra at 535 cm^{-1} was observed to be diminished in the due course of composting process from 16th day, this band attributes to the C-I and C-Br stretching and belongs to halogen group. When halogens combine with hydrogen, a strong acidic chemical is formed. However, during the thermophilic stage of the composting process, the microbial activity has mineralized. Whereas, the band at 1633.22 cm^{-1} showed the transformation of carbonyl (C = O) compound in the feedstock during the composting process (Rich et al., 2018). The band at 3286.1 cm^{-1}, which corresponds to C-H stretching in the methylene and methyl forms, remained after composting process, resulting in a decrease in root and shoot lengths of *V. radiata* and *A. cepa* grown in 100% concentrated 20th day compost.

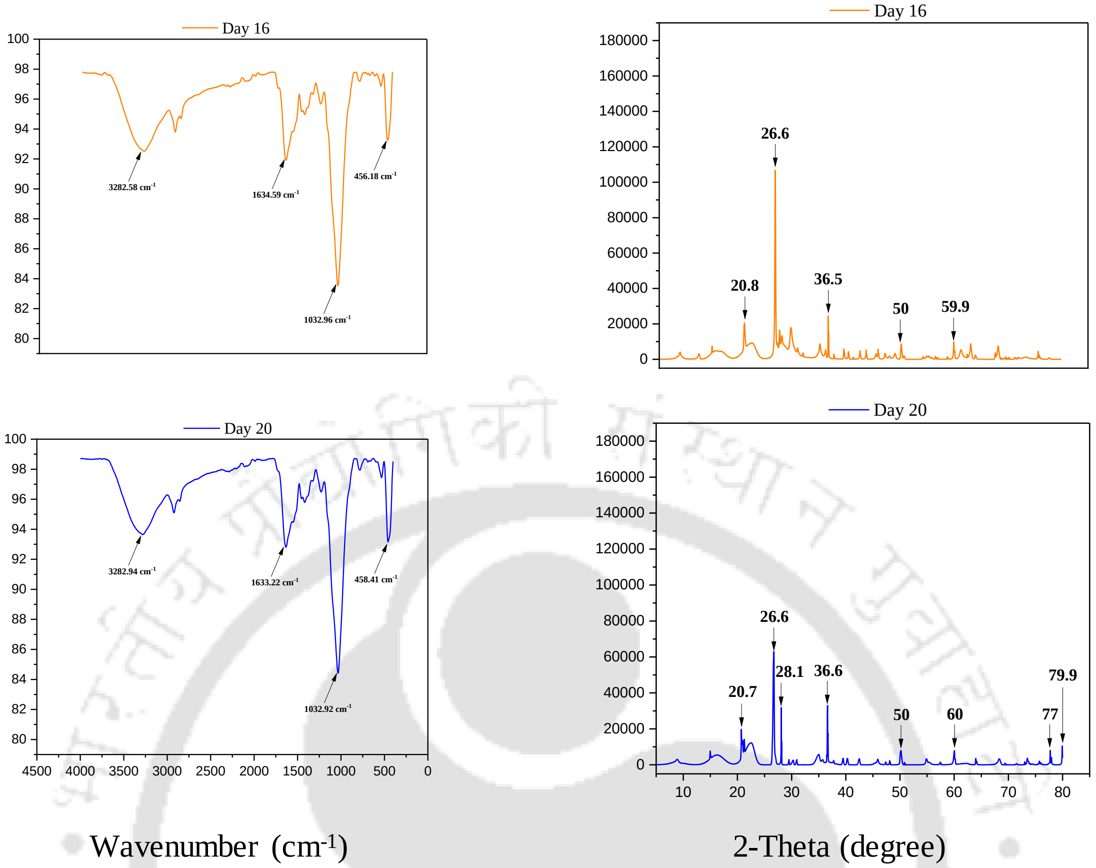
6.6.2. PXRD

Composting is the breakdown of organic waste and the creation of inorganic compounds on transformation of organic compounds. As a result, PXRD examination of composting feedstocks could immediately detect the decomposition and mineralization processes that occur during the composting process. Fig. 6.10 (b) depicts the variations in crystalline structures of the compost during the composting process. The spectra of the compost samples analyzed on the initial day was recorded the highest acute peaks with high intensity at 2θ on 26.6°, 23.9°, 36.4° and 50°. The cellulose and hemicellulose content may be attributed to spectra ranging from 20° to 30° on the initial day but the intensities of these were decreased during the composting process, similar patterns were reported by Li et al. (2020) in their study.

The intensities of spectra ranging from 30° to 40° at 2θ increased during the composting process, which was attributed to phosphorus and amino acids, which interacted during the composting process to make phosphonomethyl and peptide, which produced phosphate (Li et al., 2020). The spectra ranging between 25° to 35° at 2θ were attributed to the combination of Mg^{2+} and PO_4^{3-} during the composting process, which might enhance struvite crystallization and reduce nitrogen loss. Magnesium phosphate salts may have a clear suppressive impact on release of ammonia during composting process.

There were many developing peaks on the 16th and 20th days, including 50°, 59.9°, 77°, and 79.9°, which led to the conclusion that organic material was mineralized into inorganic materials as a result of the compost bacteria utilizing it as a source of energy (Biyada et al., 2020).



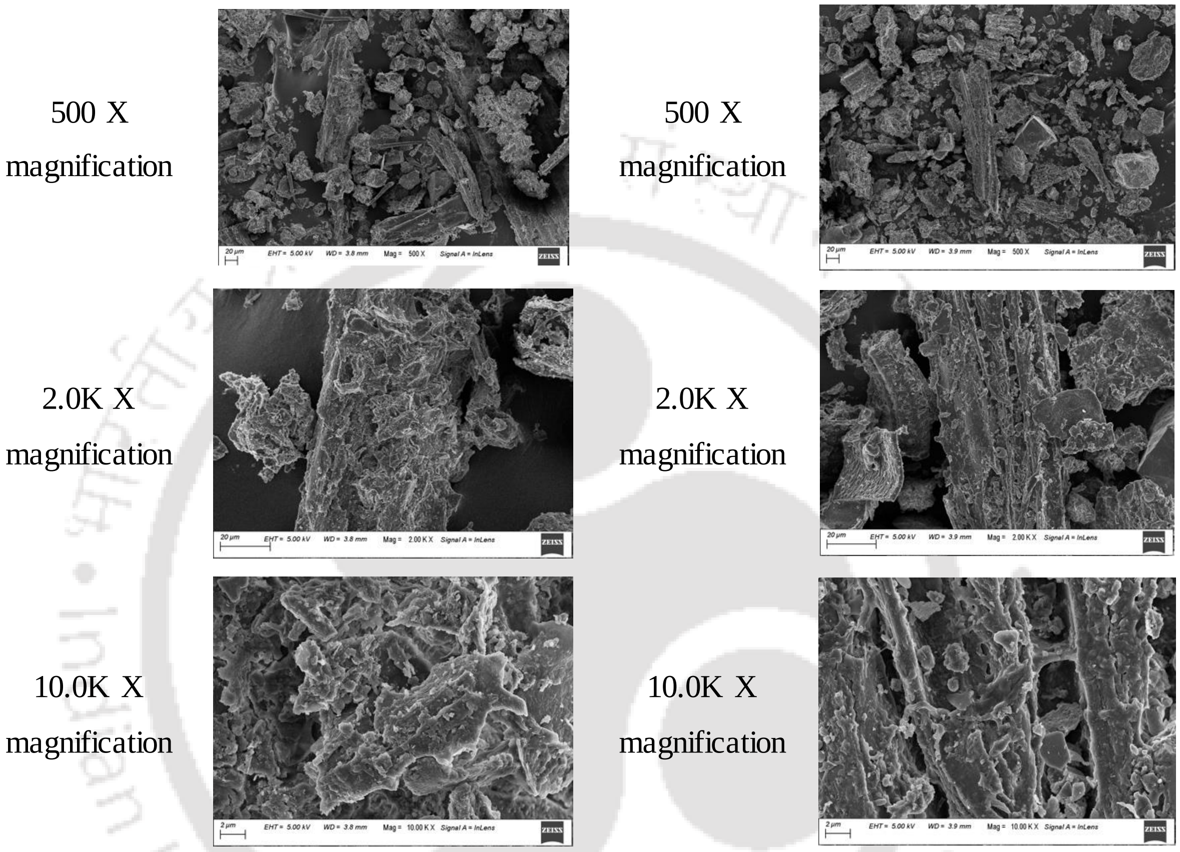


(a) FTIR spectra (b) PXRD spectra

Fig. 6.10. Spectroscopic characterization during the composting process of *P. hysterophorus*

6.6.3. FESEM

The FESEM images depict the structural modifications that occur during various phases of the *P. hysterophorus* composting process. Fig. 6.11 depicts the FESEM images of *P. hysterophorus* and its corresponding compost samples. The microstructural analysis conducted on the first day of composting revealed a solid mass with a relatively larger volume and a reduced number of surface openings. As the composting process advanced and degradation became more pronounced, there was a noticeable reduction in particle size. According to Pottipati et al. (2023b), the final day compost exhibits the transformation of larger fragments into smaller fragments characterized by significant voids on the surface, suggesting the development of a stable compost. The *P. hysterophorus*, characterized by its rigid architecture and substantial lignocellulosic biomass composition, underwent effective degradation during the in-vessel composting procedure. The initial composition of the mixture on the first day consisted of elongated fibrous structures. However, as the magnification increased for the compost sample taken on the 20th day, these fibrous structures underwent fragmentation into smaller particles. This observation serves as evidence that the microbial activity effectively decomposed the organic matter throughout the composting process. The analysis using FESEM demonstrated that the lignocellulosic biomass derived from *P. hysterophorus* underwent biodegradation throughout the composting process.



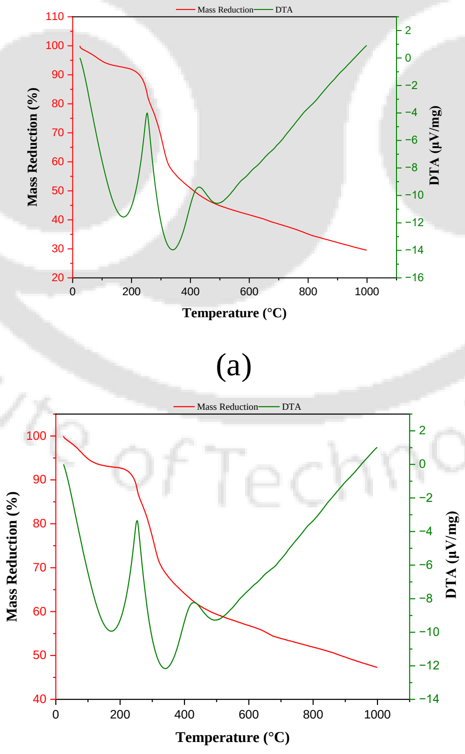
(a) FESEM images of 0th day compost (b) FESEM images of 20th day compost

Fig. 6.11. FESEM images of compost samples at 0th and 20th day of composting process of *P. hysterophorus*

6.6.4. TGA/DTA

The thermal analysis conducted on the compost samples during the first and 20th days of the study reveals the existence of two exothermic peaks within the temperature range of 150–650°C, as depicted in Fig. 6.12 (a) and (b). A gradual decrease in mass reduction was observed during the initial and 20th day of the composting process, which exhibited a correlation with temperatures ranging from 200 to 350°C. The aforementioned discovery indicates that the degradation mechanism of carbohydrates, aliphatic molecules, and specific aromatic compounds with potential for biodegradability takes place in a gradual manner in *P.*

hysterophorus plant (Fernández et al., 2012). The magnitude of mass decline during this phase was greater on the initial day (38%) compared to the final day (33%) of composting. The comparatively lesser decrease observed in the compost on the 20th day can be attributed to the degradation of carbonaceous material and aromatic compounds during the process of composting. Zahra et al. (2015) observed that the mass reduction was notably associated with the temperature range of 400-500°C, indicating the presence of intricate organic compounds such as lignocellulose. The decrease in maximum intensity observed can be attributed to the degradation of complex compounds, such as lignin and hemicellulose. On the other hand, it is probable that the observed rise in intensity is a result of the liberation of aromatic structures following the dissolution of the lignocellulose complex, which subsequently leads to the convective transport of said structures. In this stage, there is a release of fundamental constituents and a breakdown of lignocellulose. The magnitude of mass decline during this phase was greater on the initial day (14%) compared to the final day (13%) of compost. The variation in reduction can be ascribed to the elevated concentration of lignocellulosic material observed on the initial day.



(b)

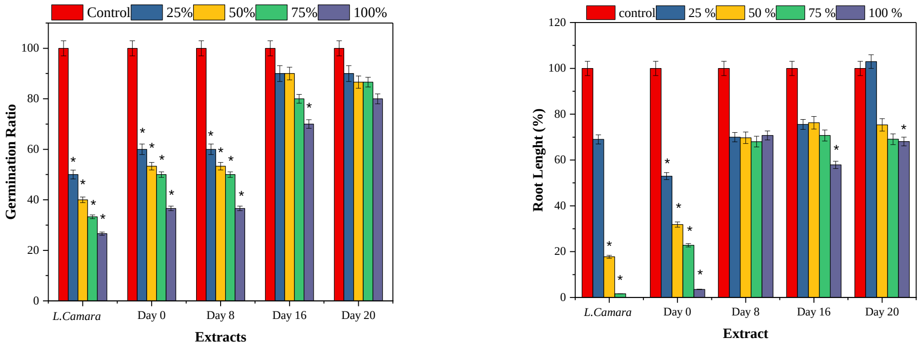
Fig. 6.12. Thermogravimetric analysis of *P. hysterophorus* feedstock (a) on initial day; (b) 20th day

6.7. TOXICITY ASSESSMENTS IN DISTINCT PHASES OF COMPOSTING *L. CAMARA*

6.7.1. Phytotoxicity test

Phytotoxicity analysis is essential for determining compost quality, and metrics such as GR, RL, SL, and GI are reliable indicators of phytotoxicity. Inadequately metabolized compost might stymie seedling growth (Haq et al., 2018). The seedlings' progress is sensitive to the sample concentrations. Seeds' germination was shown to slow down in response to increasing concentrations of the *L. camara* extracts. The root and shoot length percentages of *V. radiata* assessed in 25% *L. camara* extract were 69 and 42.9% respectively (Fig. 6.13 (I)). In contrast, a considerable increase in root and shoot lengths was reported in the same concentration of 20th day compost extract (102.9 and 101%). In 25 to 100% *L. camara* extracts, the biomass of *V. radiata* decreased from 30.5 to 82.4% compared to the control. In contrast, the biomass content of 25% of 20th day compost extract was found to be enhanced by 2% (Fig. 6.13 (I)). The 25% of 20th day compost extract has shown a significant increase in GI compared to the control, and for all the concentrations of *L. camara* extract, similar trends were evident from the study by Kauser et al. (2020). Composting could aid in the reduction of phytotoxicity by breaking, oxidizing, and clumping harmful chemicals and decreasing their solubility (Kebibeche et al., 2019). Furthermore, direct use of *L. camara* and 100% concentrated 20th day compost extracts culminated in a lack of advantages due to higher toxicity, which further prevents plants from soaking up nutrients, resulting in a decrease in biomass content, root, and shoot lengths of *V. radiata*.

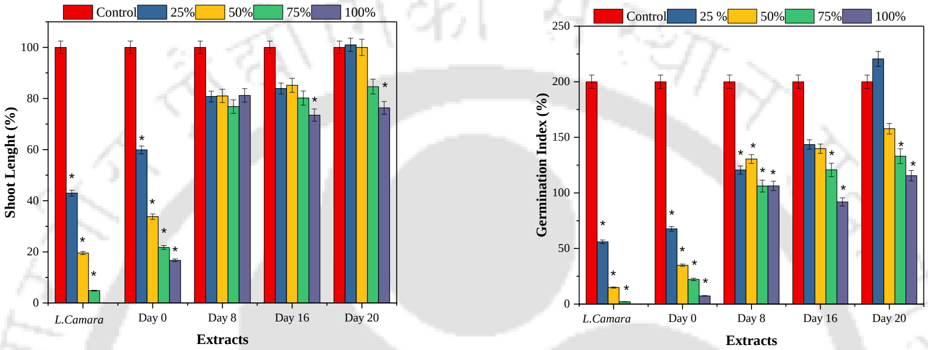
The *A. cepa* plant bioassay is considered as a phytotoxicity experiment indication. The root length and biomass of *A. cepa* cultivated in *L. camara* extract decreased when the quantities of the extracts were raised. This is likely due to the presence of phenolic compounds and DEPs in the plant system (Haq et al., 2022). The root length and biomass of *A. cepa* grown in 25% concentrated compost extracts grew by 1.1 and 2.5%, respectively, when compared to the control. The variations in the development of *A. cepa* in various extracts are depicted in Fig. 6.13 (II). At a concentration of 25% 20th-day compost, the longest roots were seen (7.19cm). However, when the content of the 20th-day compost extract rose, root length diminished. The root length and biomass of onion bulb roots were found to be reduced from 4.5 to 19.1% and 3.1 to 20.7% in 50 to 100% concentrations of 20th day compost extracts. The pictorial depiction of phytotoxicity results of *V. radiata* and *A. cepa* are shown in Fig. 6.14.



(a) Germination ratio

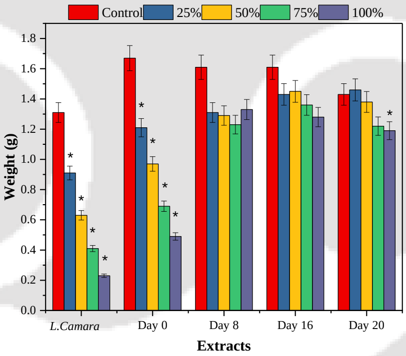
(b) Root Length

I



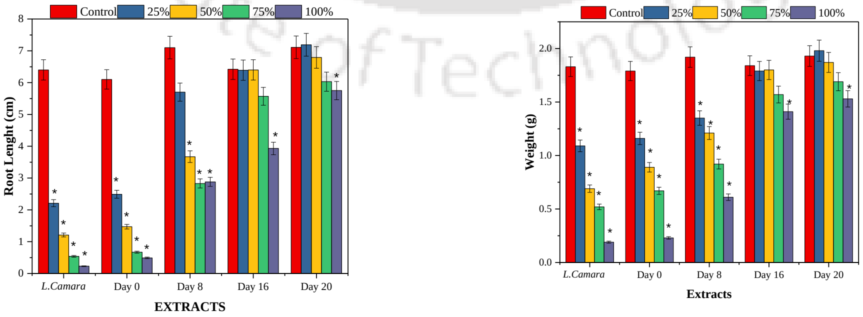
(c) Shoot Length

(d) Germination Index



(e) Biomass of *V. radiata*

II



(a) Root Length

(b) Biomass of *A. cepa*

*. Substantial variance observed at $p < 0.05$ compared to negative control (Tap water)

Fig. 6.13. Phytotoxicity parameters of (I) *V. radiata* and (II) *A. cepa*

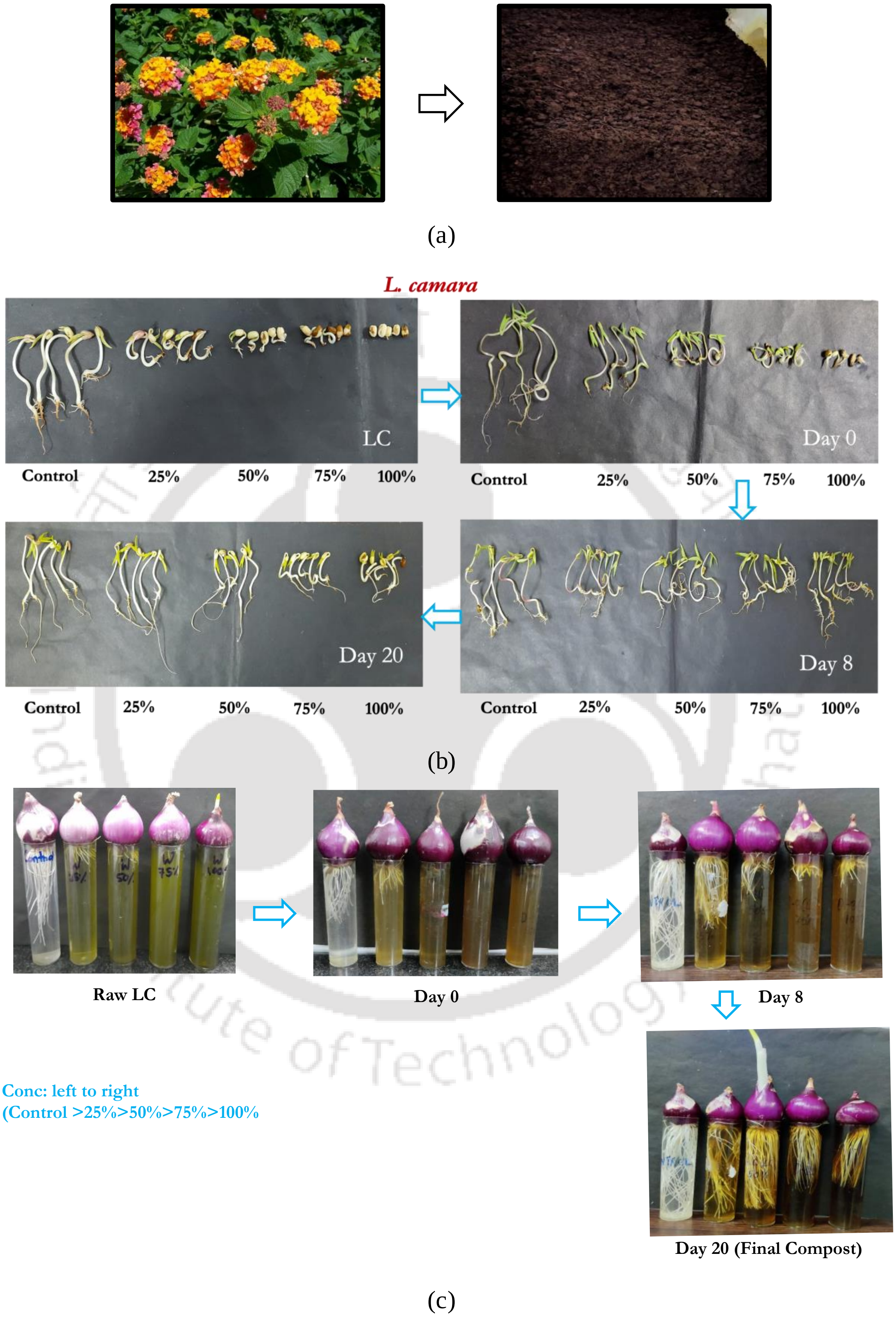


Fig. 6.14. (a) Initial and final day compost samples; Phytotoxicity assay during the composting process of *L. camara* (b) *V. radiata*, (c) *A. cepa*

6.7.2. Cytotoxicity test

The *A. cepa* chromosomal aberration assay has been authorized by the International Chemical Safety Program and the United Nations Environment Program as a standardized chemical assessment tool for environmental products (Haq et al., 2022). To investigate the cytotoxic properties of *L. camara* and its compost, the MI of *A. cepa* apical germinative roots was calculated. Table 6.8 depicts the MI of *A. cepa* roots in various extracts of *L. camara* and compost extracts. The existence of extremely toxic compounds in *L. camara* extracts was clearly determined by the fact that the MI of *A. cepa* roots was reduced from 51.4% to 68.5% in 25 to 100% compared to control and toxic compounds existence was also confirmed by the instrumental analysis. When *A. cepa* were exposed to different phases of compost extracts, the MI of roots increased. The maximal MI of *A. cepa* roots was found to be 67.3% in a 20th day compost extract at 25% concentration. The MI percentages of *A. cepa* in *L. camara* extracts has shown a significant difference compared to the compost extracts at $p < 0.05$.

Table 6.8. MI (%) of root tip cells of *A. cepa* following with *L. camara* and various staged compost extracts

Concentrations	**Extracts**	**Total cells**	**Dividing cells**	**MI %**
Tap water		1826	1289	70.59 ± 0.98
EMS		902	50	5.54 ± 0.67^{a}
25%	LC	1912	656	34.30 ± 1.31^{a}
	0th day	1803	690	38.20 ± 1.43^{a}
	8th day	1896	820	43.2 ± 0.65^{a}
	16th day	1567	853	54.4 ± 1.01$^{a, b}$
	20th day	2003	1350	67.39 ± 0.53^{b}
50%	LC	1956	570	29.14 ± 0.13^{a}
	0th day	1878	650	34.61 ± 0.21^{a}
	8th day	1909	757	39.13 ± 0.54^{a}
	16th day	1589	789	49.65 ± 0.43$^{a, b}$
	20th day	1765	1103	62.49 ± 0.86^{b}
75%	LC	1990	537	26.98 ± 0.98^{a}
	0th day	1875	532	28.37 ± 0.12^{a}
	8th day	1789	575	32.14 ± 0.67^{a}

	16th day	1998	888	44.44 ± 0.32[a]
	20th day	1890	1056	55.87 ± 0.87[b]
100%	LC	1932	430	22.22 ± 0.55[a]
	0th day	2123	502	23.64 ± 0.76[a]
	8th day	1679	489	29.12 ± 0.53[a]
	16th day	2004	815	40.66 ± 0.34[a]
	20th day	1789	912	50.97 ± 0.76[b]

All the parameters are expressed in mean ± SD of triplicate samples

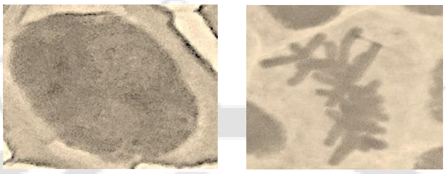
[a]. Substantial variance observed at $p < 0.05$ compared to negative control (Tap water)

[b]. Substantial variance observed at $p < 0.05$, when compared to *L. camara* extract at same concentration.

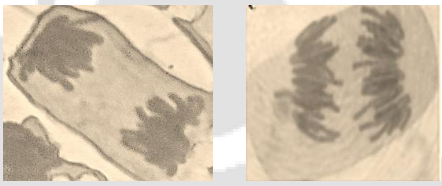
6.7.3. Genotoxicity test

The genotoxicity potential of any plant system could be assessed based on the CAs and NAs of their meristematic roots. The maturing root terminals of *A. cepa* offer conveniently accessible plant material for studying the detrimental implications of contaminants on chromosomes. Diverse CAs are investigated during the mitotic phase of cell division. CAs and NAs are the abnormalities that occurs during the mitosis. Table 6.9 and Fig. 6.15, show the elicited genotoxic impacts in *A. cepa* root tips as CAs and NAs. CAs and NAs were not found in the 4 mM EMS or tap water. When *A. cepa* root tips were exposed to *L. camara*, and compost extracts, they exhibited the commencement of distinct types of CAs and NAs. The CAs observed in the study were chromosomal bridge, sticky chromosome, chromosomal loss, C-mitosis, and vagrant chromosome, whereas the NAs found were Micro-nucleated cell, binucleated cell. The proportion of abnormal cells identified in the genotoxic activity of *A. cepa* root tips in *L. camara* extracts differed most substantially between 16th and 20th day compost extracts at the similar doses. In the research, the prominent CAs detected were C-mitosis, sticky chromosome, and vagrant chromosome. C-mitosis concentrations in *L. camara* extracts ranging from 25% to 100% concentration were 5.3, 8.4, 15.2, and 12.2 and diminished over the composting process. The highest decline was recorded in a 25% diluted 20th day compost extract with 75.5% drop compared to the similar level of *L. camara* extract. The breakdown of spindles and subsequent spontaneous dispersal of compacted chromosomes is indicated by the existence of c-mitosis (colchicine mitosis) and the vagrant chromosome. The highest proportion of sticky chromosomes was identified in 100% concentrated *L. camara* extract with 3.21, but sticky chromosomes were not identified in 16th and 20th day compost extracts. Sticky chromosomes are recognized as a common sign of toxic impacts on chromosomes, which can cause cell death. The amount of binucleated cells observed in 100% concentrated *L. camara* extracts were 8.9, whereas it was reduced by 88% in all the 100% concentrated compost extracts. Inhibiting cell plate growth across cells during the first phase of the telophase chromosomes could result in the generation of binucleated cells (Haq and Kalamdhad, 2021).

I



(a) Prophase (b) Metaphase



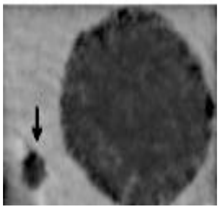
(d) Telophase (c) Anaphase

II

(a) Vagrant Chromosome (b) Sticky metaphase

(c) Chromosomal break (d) Chromosomal bridge

(e) c-mitosis (f) Binucleated cell



(g) Micro nuclei

Fig. 6.15. I-Photographs: Normal mitosis of *A. cepa* root tips treated with tap water (control). II-Photographs: Induced Chromosomal aberrations and Nuclear abnormalities by various concentrations *L. camara* and compost extracts in *A. cepa* root tip cells

Table 6.9. Various Chromosomal and nuclear abnormalities found in root tip of *A. cepa* following with *L. camara* and various staged compost extracts

Concentrations	**Extracts**	**Bridge**	**Sticky metaphase**	**Chromosomal loss**	**C mitosis**	**Vagrant chromosome**	**Micro nucleated cells**	**Nuclear enlargement**	**Aberrant cells (%)**
Tap water		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
25%	LC	1.9 ± 0.12	3.4 ± 0.12	1 ± 0.1	5.32 ± 0.12	6.1 ± 1.34	3.48 ± 1.03	1.2 ± 0.02	3.45± 0.89
	0th day	1.7 ± 0.24	2.89 ± 0.98	1 ± 0.23	4.98 ± 0.09	3.12 ± 0.12	3.12 ± 1.98	1.1 ± 0.13	3.09 ± 1.01
	8th day	1.0 ± 0.1	1.3 ± 0.0*	0.0 ± 0.0*	4.79 ± 0.78	2.01 ± 0.17*	2.13 ± 0.15	0.0 ± 0.0*	2.67 ± 0.13
	16th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	1.3 ± 0.13*	1.1 ± 0.34*	0.0 ± 0.0*	0.0 ± 0.0*	0.67 ± 0.09*
	20th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	1.3 ± 0.09*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	0.5 ± 0.09*
50%	LC	2.2 ± 0.24	3.51 ± 0.09	1.2 ± 0.01	8.2 ± 1.3	7.89 ± 1.02	4.01 ± 0.14	2.13 ± 0.15	4.14 ± 1.01
	0th day	1.9 ± 0.18	2.91 ± 0.14	1.1 ± 0.09	13.2 ± 1.8*	3.65 ± 0.29	3.67 ± 0.89	0.0 ± 0.0*	3.67 ± 0.78
	8th day	1.0 ± 0.13	1.67 ± 0.03*	1.2. ± 0.76	7.98 ± 0.24	1.78 ± 0.19*	1.89 ± 0.09*	0.0 ± 0.0*	2.79 ± 0.98
	16th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	1.67 ± 0.04*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	0.71 ± 0.09*
	20th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	1.43 ± 0.09*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	0.63 ± 0.02*
75%	LC	2.1 ± 0.98	3.12 ± 0.18	1.56 ± 0.07	15.23 ± 2.75	7.74 ± 1.25	3.98 ± 0.77	1.58 ± 0.89	4.21 ± 0.76
	0th day	1.8 ± 0.56	2.65 ± 0.89	0.0 ± 0.0*	10.89 ± 1.43	3.87 ± 1.02	2.45 ± 0.18	0.0 ± 0.0*	3.71 ± 0.25
	8th day	1.1 ± 0.25	0.0 ± 0.0*	0.0 ± 0.0*	6.87 ± 1.01*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	2.24 ± 0.11
	16th day	1.3 ± 0.1	0.0 ± 0.0*	0.0 ± 0.0*	1.87 ± 0.01*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	0.68 ± 0.01*

	20th day	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	1.32 ± 0.15*	0 ± 0*	0.0 ± 0.0*	0.0 ± 0.0*	0.61 ± 0.08*
100%	LC	3.4 ± 0.13	3.21 ± 0.13	2.3 ± 0.76	12.34 ± 2.56	7.35 ± 1.78	4.09 ± 1.14	2.56 ± 0.14	4.23 ± 1.1
	0th day	2.5 ± 0.21	2.87 ± 0.78	0.0 ± 0.0*	11.45 ± 1.25	3.32 ± 0.78	2.98 ± 0.78	0.0 ± 0.0*	3.72 ± 0.87
	8th day	1.4 ± 0.56*	0.0 ± 0.0*	0.0 ± 0.0*	5.45 ± 0.14*	0 ± 0*	1.1 ± 0.23*	0.0 ± 0.0*	2.23 ± 0.98
	16th day	1.4 ± 0.09*	0.0 ± 0.0*	0.0 ± 0.0*	1.82 ± 0.45*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.69 ± 0.12*
	20th day	1.1 ± 0.01*	0.0 ± 0.0*	0.0 ± 0.0*	1.31 ± 0.08*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.6 ± 0.0.04*

All the parameters are expressed in mean ± SD of triplicates

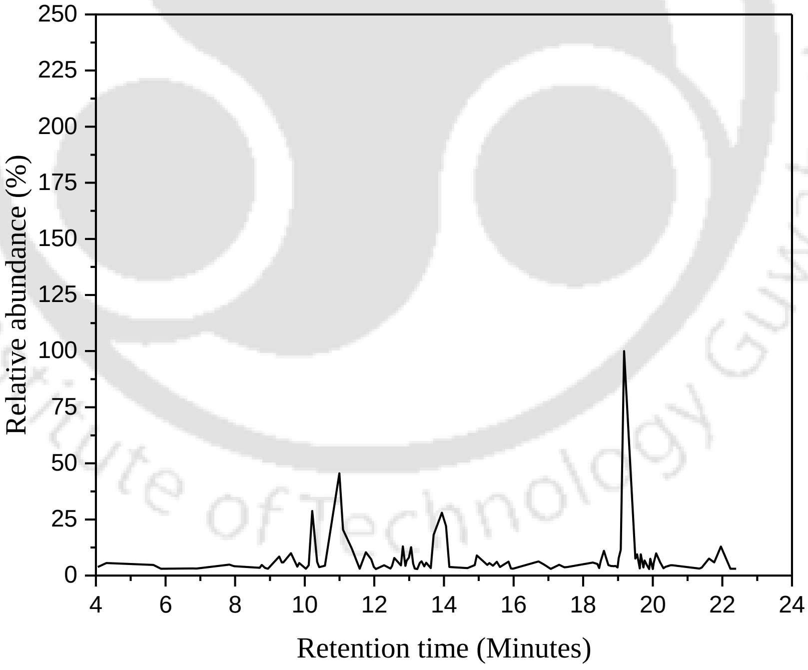
*. Substantial variance observed at $p < 0.05$ compared to *L. camara* extract at same concentration.

6.8. TRANSFORMATION OF TOXIC ORGANIC COMPOUNDS DURING THE COMPOSTING OF *L. CAMARA*

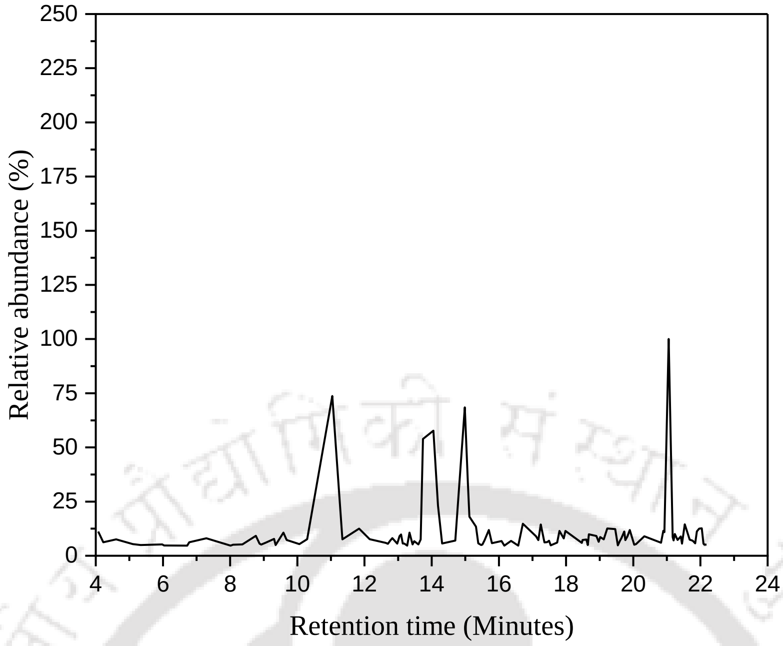
The identification of toxic organic compounds in the composting of L. *camara* was performed through GC-MS instrument and its graphical depiction is shown in Fig. 6.16 and the detail organic contaminants list is given in Table 6.10. The compounds identified in the methanol extracts of L. *camara* were phenol, 2, 4-bis(1,1-dimethylethyl)-, Farnesene (2-Butendioic acid (Z)-, monobutylester), Diethyl Phthalate (DEP), Oleanolic acid, Phthalic acid-butyl 2-chloropropyl ester, (4,8, 2, 16-Octadecatetraen-1-ol, 4, 9, 13, 17-tetramethyl-. Furthermore, the compositional areas of toxic organic compounds such as phenol, 2, 4-bis(1,1-dimethylethyl)- and Phthalic acid-butyl 2-chloropropyl ester have been found to be decreased in the final compost product. But, most of the compounds has been transformed to other stabilized compounds due to the course of degradation during the composting process. Phenol, 2, 4-bis(1,1-dimethylethyl)- has the tendency to reduce the seedling growth of the plant, which was termed as a toxic compound for plants (Chen et al., 2012). Oleanolic acids are secondary metabolites and pentacyclic compounds found in the majority of plants found in different climatic zones and geographies. These compounds are usually used to derive new pharmaceutical medications (Luchnikova et al., 2020). The biotransformation of pentacyclic compounds could be attributed to the microbial degradation during the composting process. According to Luchnikova et al. (2020), *Streptomyces* have the ability to transform pentacyclic compounds to secondary triterpenes during the composting process. The compounds such as Phenol, 2,4-bis(1,1-dimethylethyl)- (RT: 10.25), Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester (RT: 13.03) and Furan, 2,3-dihydro-4-(1-methylethyl)- (RT: 18.66) were identified in L. *camara* extract but not identified in compost extract. The transformation of phenolic compounds tends to increase the rate of humification during the composting process (Zhao et al., 2022). The majority of plants contain benzoic acid derivatives as the building blocks for the various metabolites, however excessive quantities of these acids may cause skin issues in humans (Widhalm and Dudareva, 2015).

The terrestrial plants also secrete few plasticizing characteristic compounds such as DEP, phthalic acids in their plant system, which will directly affect the germination of other neighboring plants. Most of the phthalic acids could be found numerously in soil due to the weathering effect (Li et al., 2005; Qiu et al., 2023). DEP and phthalic acids were identified both in methanol extracts of L. *camara* and its compost. But, the compost extract has shown less area and 44% reduction in DEP, when analyzed in GC-MS compared to L. *camara* extract. The biodegradation of DEPs and transformation of DEPs to phthalic acid could be attributed to aerobic bacterial strains such as *Bacillus* sp. and *Micrococcus* sp. functions during the composting process (Patil and Jena, 2019).

Furthermore, furan compounds were found in the *L. camara* extract at RT = 18.6 minutes but not in the compost sample. The biodegradation of furan compounds in the composting process could be attributed to fungal species such as *Paecilomyces* spp. at alkaline pH (Nakasaki et al., 2015). The RDC was effective in producing mesophilic temperatures during the early and maturation phases, as well as thermophilic temperatures during the composting process. The majority of the fungal species will be prominent in mesophilic temperatures, resulting in furan compounds degradation. The majority of the organic compounds found in *L. camara* methanol extracts are hazardous to nearby plants in a variety of ways, including germination, growth, and disease. The RDC of *L. camara* has been proven to be an effective mechanism for reducing hazardous chemical substances and converting the most complex bonds to simpler bound compounds.



(a)



(b)

Fig. 6.16. GC-MS chromatograms of (a) *L. camara* extract (b) final day compost extract

Table 6.10. Organic contaminants extracted from methanol extracts of *L. camara* and compost were analyzed by GC-MS

Retention time	Compounds	*L. camara* extract	Compost extract
9.305	Cyclohexene, 3-ethenyl-4-(1-methylethenyl)-	+	-
9.37	Propanenitrile, 3-hydroxy-	+	-
9.42	5-Benzotriazol-1-yl-pyrrolidin-2-one	+	-
9.72	Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	+	-
10.25	Phenol, 2,4-bis(1,1-dimethylethyl)-	+	-
11.042	Diethyl Phthalate	+	+
11.124	1,5,9,11-Tridecatetraene, 12-methyl-, (E,E)-	+	-
11.393	3-Cyclohexene-1-carboxaldehyde, 2,4,6-trimethyl-	+	-
11.839	Farnesene (2-Butendioic acid (Z)-, monobutylester)	+	-
12.629	Cyclononasiloxane, octadecamethyl-	+	-
12.85	1-(3-Isopropylidene-5,5-dimethyl-bicyclo[2.1.0]pent-2-yl)-ethanone	+	-

13.03	Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	+	-
13.09	3-Tetradecyne	+	-
13.743	Oleanolic acid	+	-
14.052	Phthalic acid, butyl 2-chloropropyl ester	+	+
14.094	Undecanoic acid, 10-bromo-	+	-
14.189	1-Butanol, 3-methyl-, formate	-	+
14.986	11,14,17-Eicosatrienoic acid, methyl ester	-	+
15.074	3-Tridecene	-	+
15.12	Undecanoic acid, 2-nonyl-, methyl ester	-	+
16.712	3-(n-Propylamino)-2,1-benzisothiazole	-	+
17.245	Octadecanoic acid, trimethylsilyl ester	-	+
18.66	Furan, 2,3-dihydro-4-(1-methylethyl)-	+	-
19.21	4,8,12,16-Octadecatetraen-1-ol, 4,9,13,17-tetramethyl-	+	-
21.052	Cyclodecacyclotetradecene, 14,15-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro-	-	+
21.535	8-(Dimethylamino)-7-(3-(4-ethylphenoxy)-2-hydroxypropyl)-3-methyl-3,7-dihydro-1H-purine-2,6-dione tms	-	+

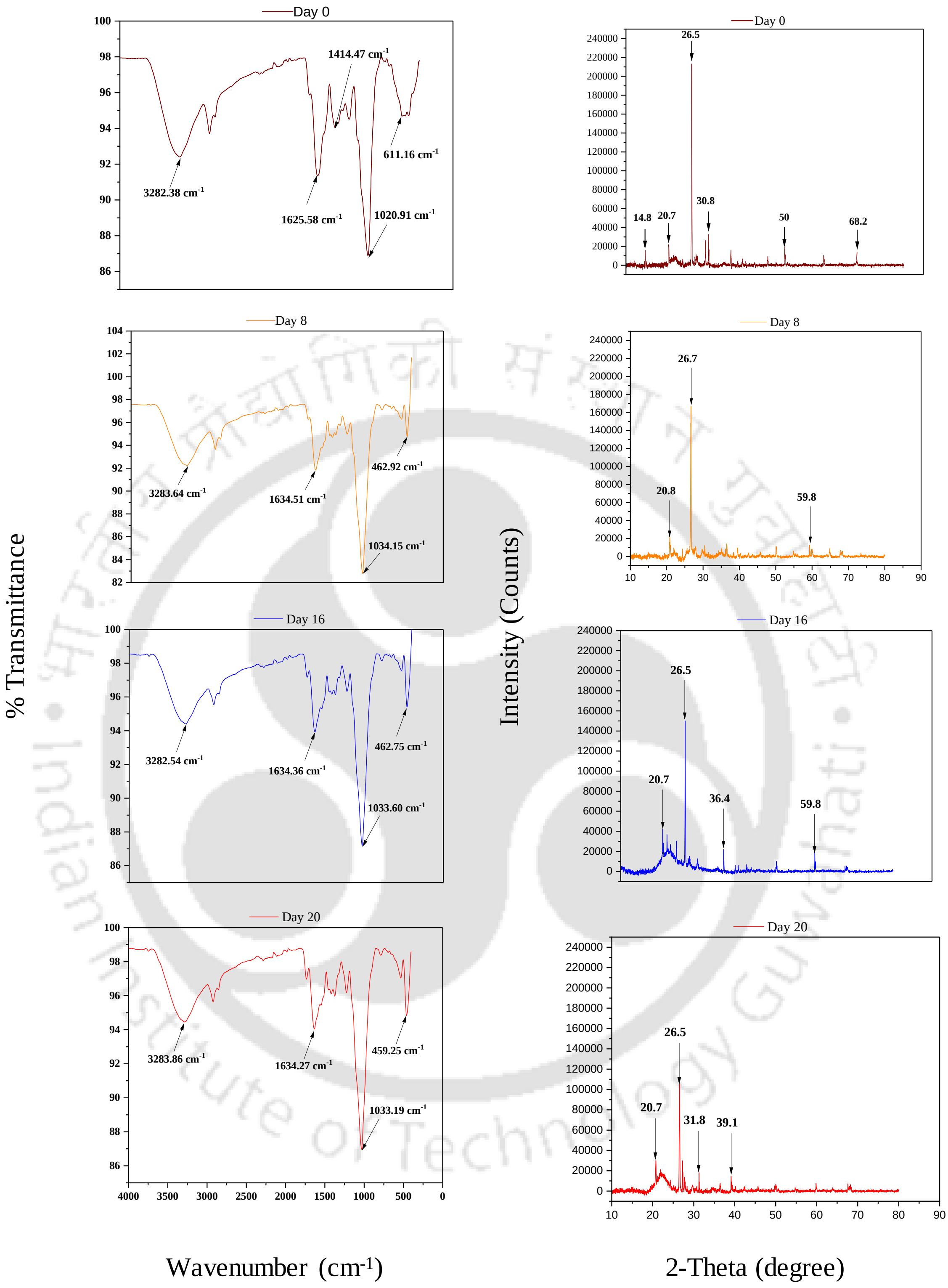
6.9. INSTRUMENTAL CHARACTERIZATION

6.9.1. FTIR

FTIR signals were obtained depending on the intensities of certain unique absorbance peaks in order to examine the progression of functional groups and associated carbon chains of different toxins during composting. Fig. 6.17 (a) illustrates the spectra of various samples during the composting process. Most of the spectra were absorbed at wave numbers such as 3282.3, 1625.8, 1020.9 and 462.9 cm^{-1}with reduction in transmittance during the composting process. The spectra such as 1414. 4 and 611.1, which belongs to carboxylic acid and halogen compounds were found in *L. camara* sample but diminished during the composting process. The disappearance of these spectra could be attributed to the mineralization phenomena during the composting process. The spectra 3282.3 belongs to C-H, O-H stretching and alkyne groups and have been shown reduction in transmittance during the composting process. The spectra ranging between 1625 and 1634 cm^{-1} belongs to C=C stretching alkene, amine and cyclic alkene groups, whose transmittance were reduced during the course of composting process. The spectra ranging between 1020 and 1033 cm^{-1} belongs to strong bonding of C-O stretching consisting of vinyl ether and alkyl aryl ether, whose transmittance were reduced during the course of composting process and the organic compounds of ether functional group (1-Monolinoleoylglycerol trimethylsilyl ether with RT=20.669) was identified in GC-MS. Furthermore, these spectra also validate the reduction patterns of lignin, hemicellulose and cellulose biomass of feedstock during the composting process.

6.9.2. PXRD

The process of composting involves the decomposition of organic waste and the production of mineral substances as a byproduct of the conversion of organic substances. As a consequence, the breakdown and mineralization activities that take place throughout the composting process may be promptly detected using PXRD analysis of the composting biomass. Fig. 6.17 (b) illustrates the spectra of samples taken in distinct phases of composting process of *L. camara*. The initial day compost's sample contained various spectra at 14.8°, 20.7°, 26.5°, 30.8°, 50° and 68° on 2θ angle. The spectra ranging between 20° and 30° could be attributed to the presence of lignocellulose biomass. In the initial day, it was found that the spectra 26.5° have high intensity and have reduced during the composting process. Furthermore, it could be noted that that the RDC was prominent in biodegrading lignocellulose biomass. However, the final compost displayed a significant decrement in the magnitude of these peaks, that might be attributed to microbes degrading the cellulose molecular bonds, thus outlining the decline in the C/N ratios denoting the degradation of organic substance and, by extension, the cellulose substances. The spectra between 30° and 40° at 2θ angle were observed to have persisted during the composting process; this was ascribed to PO_4 and R-CH(NH_2)-COOH, which reacted during the aerobic digestion to form phosphonomethyl and peptide (Li et al., 2020).

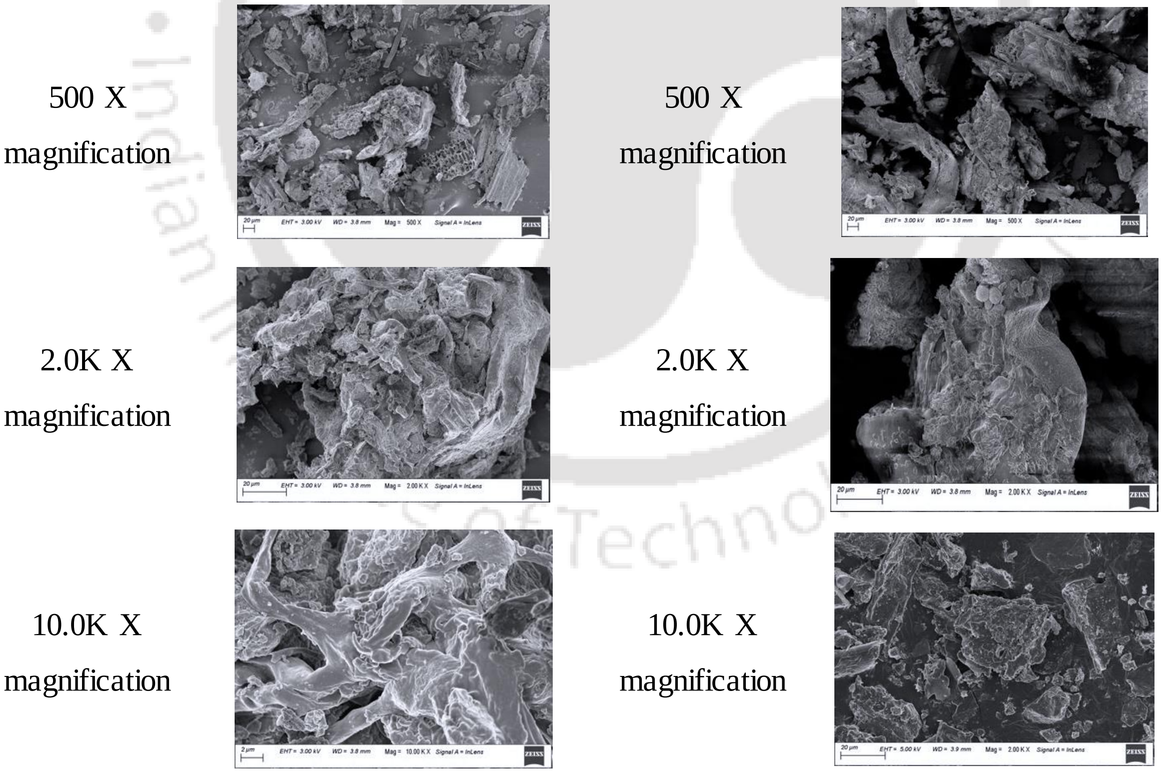


(a) FTIR Spectra (b) PXRD Spectra

Fig. 6.17. Mineralization and Functional group characterization during composting process

6.9.3. FESEM

The FESEM photographs show the structural alterations that arise throughout different stages of *L. camara* compost. Fig. 6.18 illustrates the FESEM images of *L. camara* and its compost samples. The microstructure of the initial day of composting indicated a wider solid mass with fewer openings on the surface. The particle size tended to shrink as composting time progressed and increasingly substantial degradation occurred. The larger fragments have been turned into tiny fragments with massive voids on the surface in the final day compost, indicating the formation of stable compost (Pottipati et al., 2023a). The *L. camara*, which has a very stiff structure and high lignocellulose biomass content, was finely degraded in the in-vessel composting process. The initial day mix featured long fibrous structures, however when the magnification for the 20th day compost sample rose, the fibrous structures fragmented into small particles, demonstrating that microbes decomposed organic substances effectively throughout the composting process. The FESEM analysis revealed that the lignocellulose biomass of *L. camara* biodegraded during the composting process.

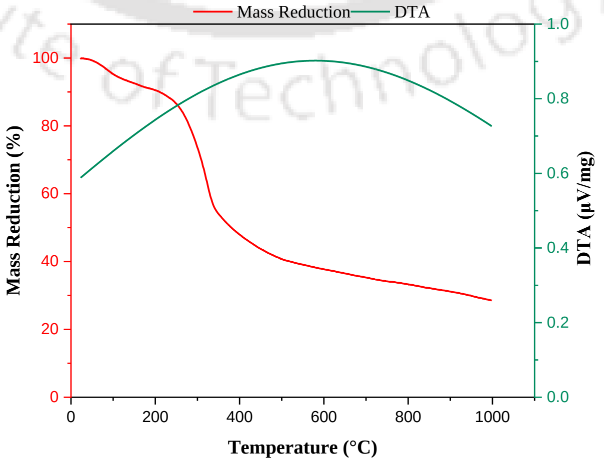


(a) FESEM images of 0th day compost (b) FESEM images of 20th day compost

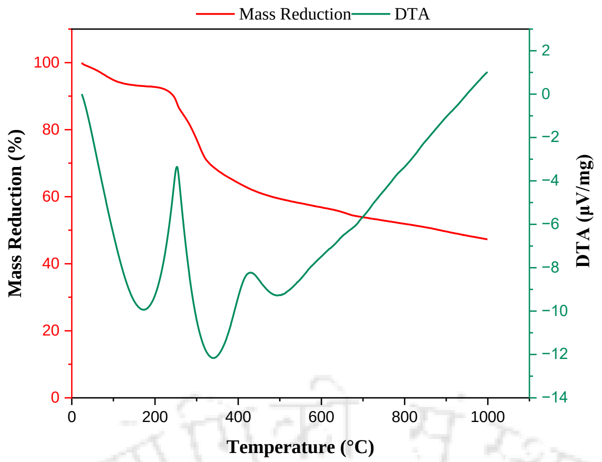
Fig. 6.18. FESEM images of compost samples at 0th and 20th day of composting process of *L. camara*

6.9.4. TGA/DTA

The thermal analysis of the compost samples on the initial and 20th days of the study indicates the presence of two exothermic spikes within the temperature between 150–650°C, as illustrated in Fig. 6.19 (a) and (b). During the initial and 20th day of the composting process, there was an observed gradual decline in the reduction of mass, which was correlated with temperatures ranging from 200 to 350°C. This finding suggests that the degradation process of carbohydrates, aliphatic molecules, and certain aromatic compounds with prospective biodegradability occurs progressively (Fernández et al., 2012). The decline in mass during this phase exhibited a greater magnitude on the initial day (35%) in comparison to the final day (30%) of composting. The relatively lower reduction observed in the 20th day compost may be attributed to the degradation of carbon material and aromatic compounds during the composting process. The temperature range of 400-500°C was found to be responsible for a significant decrease in mass, which can be attributed to the presence of complex organic compounds like lignocellulose (Zahra et al., 2015). The observed decline in peak intensity can be ascribed to the degradation of intricate compounds, such as lignin and hemicellulose. Conversely, the observed increase in intensity is likely a consequence of the release of aromatic structures subsequent to the dissolution of the lignocellulose complex, leading to the convective transport of these structures. During this phase, there is a discharge of core components and a degradation of lignocellulose (Pardo et al., 2021). The decline in mass during this phase exhibited a greater magnitude on the initial day (12%) in comparison to the final day (10%) of compost. This disparity in reduction can be attributed to the higher concentration of lignocellulosic material present on the initial day.



(a)

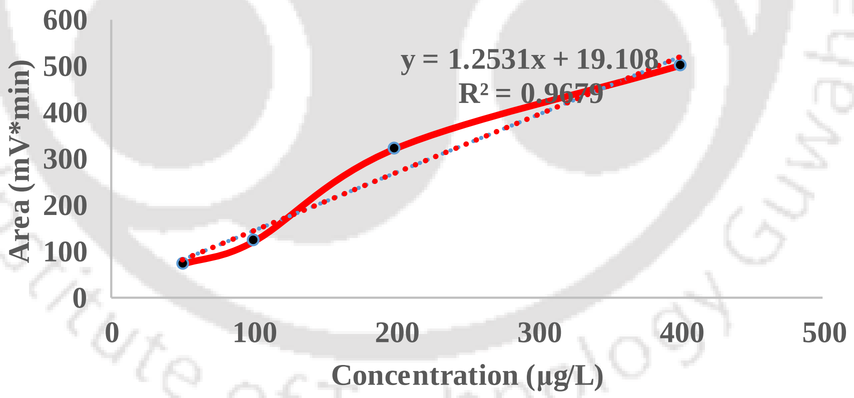


(b)

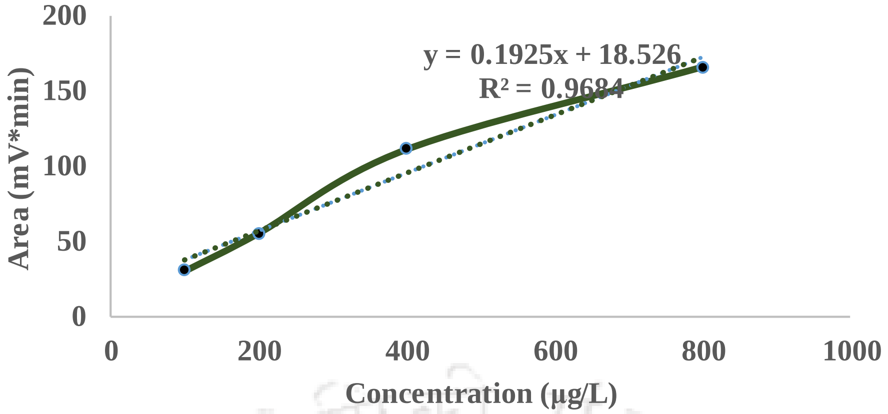
Fig. 6.19. Thermogravimetric analysis of *L. camara* feedstock (a) on initial day; (b) 20th day

6.10. GC-FID

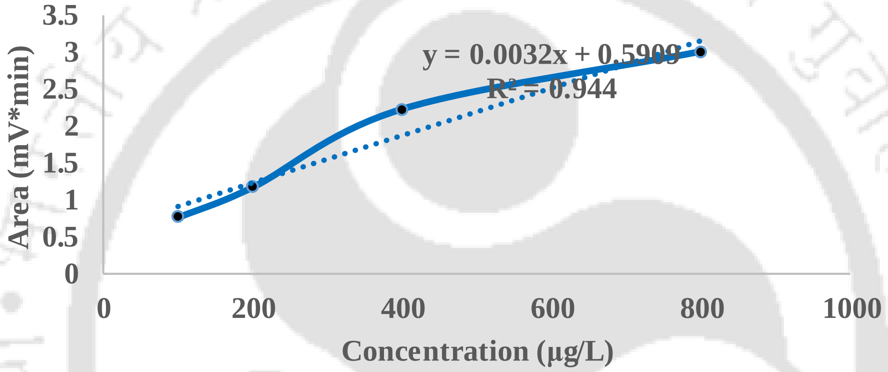
To evaluate the quantitative impact of composting on the breakdown of allelochemicals, GC-FID was utilized as a method for quantifying particular hazardous organic molecules. The FID metric was employed as an indication for this specific reason. Terrestrial weeds possess a distinct class of toxic organic compounds, rendering them inherently dangerous. Ageratochromene serves as the toxic organic compounds for *A. conyzoides*, whereas farnesene and diethyl phthalate are the toxic organic compounds found in *L. camara*, and *P. hysterophorus* respectively. In addition, many additional toxic organic compounds were also specifically addressed, and these compounds were shown to be consistent across all three terrestrial weed species. The results obtained using GC-FID demonstrated notable alterations in the chemical constituents during the progression of composting terrestrial weeds. The content of ageratochromene in the compost sample of *A. conyzoides* collected on the 20th day has exhibited a reduction of 16.3% as compared to the first mixture sample. Significant quantities of caryophyllene oxide and stigmasterol were detected in the methanol extracts of *A. conyzoides*, *P. hysterophorus*, and *L. camara*. The component caryophyllene exhibited a substantial decrease of 57.6, 64, and 60.1% in the compost extracts of *A. conyzoides*, *P. hysterophorus*, and *L. camara* on the 20th day, respectively, in comparison to the first day mixture extracts. In comparison to the first day mix extracts, the 20th day compost extracts of *A. conyzoides*, *P. hysterophorus*, and *L. camara* exhibited a reduction in stigmasterol levels by 10.9%, 51.8%, and 57.8% respectively. Similarly, the concentration of farnesene in the compost sample of *L. camara* on the 20th day exhibited a reduction of 75.5% when compared to the first day mixture extract. In contrast, the concentration of Diethyl phthalate in the compost sample of *P. hysterophorus* on the 20th day exhibited a reduction of 44% when compared to the first day mixture extract. The decrease in levels of harmful organic chemicals can be ascribed to the microbial breakdown facilitated by microorganisms, including actinobacteria, Aspergillus, Penicillium, and Cellulolytic Bacteria, throughout the composting procedure. The deterioration may also be attributed to processes such as transformation, metabolism, volatilization, and chemical interactions. in addition, In conclusion, the study highlights the potential of composting technology as an innovative and ecologically sound approach to mitigating the allelopathic effects caused by weeds on land. Composting is a sustainable method for managing weeds and enhancing ecosystems by efficiently breaking down harmful allelochemicals and transforming them into less harmful molecules. The findings of this research contribute to our understanding of the complex interactions between plants in both natural and farmed environments, thereby aiding in the development of effective strategies to harness the benefits of allelopathy while minimizing its negative effects on plant communities. Fig. 6.20 illustrates the Standard curves of targeted toxic organic compounds in *A. conyzoides*, *hysterophorus*, and *L. camara*. Table 6.11 represents the standard and sample concentration curves of targeted toxic compounds.



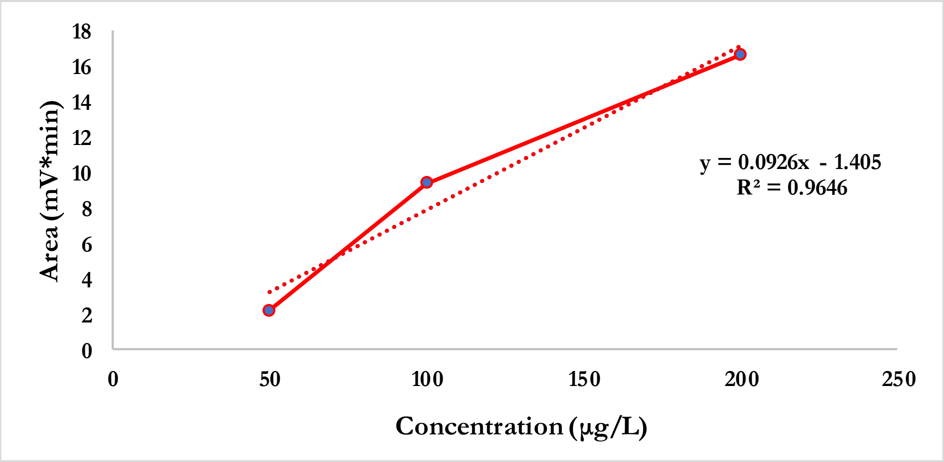
(a) Ageratochromene



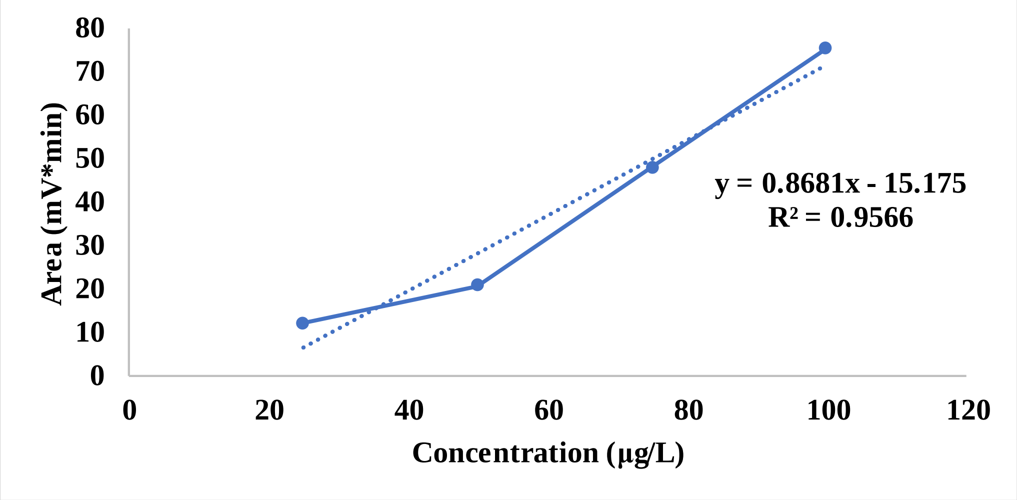
(b) Caryophellene oxide



(c) Stigmasterol



(d) Farnesene



(e) Diethyl Phthalate

Fig. 6.20. Standard curves of targeted toxic organic compounds in *A. conyzoides*, *P. hysterophorus*, and *L. camara*

Table 6.11. Standard and sample concentration curves of targeted toxic organic compounds

Ageratochromene (Precocene I)

Concentration (µg/L)	**Area (mV*min)**	**Concentration (µg/L)**
400	501.3	
200	322.02	
100	120.8	
50	72.12	
A. conyzoides	132.8	90.7
initial day	130.2	**88.7**
20th day	114.2	**75.9**
Caryophyllene oxide		
800	166	
400	111.18	
200	55.4	
100	30.2	
	A. conyzoides	
Raw extract	150	683.0
initial day	145.1	**657.6**
20th day	72.1	**278.3**

	P. hysterophorus	
Raw extract	101.2	579.5
initial day	76.69	302.2
20th day	39.45	108.7
	L. camara	
Raw extract	102.4	436.2
initial day	95.7	401.3
20th day	49.3	159.9
Stigmasterol		
800	3.01	
400	2.23	
200	1.17	
100	0.76	
	A. conyzoides	
Raw extract	2.09	468.5
initial day	2.05	**456.0**
20th day	1.89	**406.0**
	P. hysterophorus	
Raw extract	1.34	**236**
initial day	1.26	**212**
20th day	0.91	**102**
	L. camara	
Raw extract	1.56	304
initial day	1.50	**287**
20th day	0.97	**121**
Farnesene		
200	16.6	
100	9.39	
50	2.2	
Raw *L. camara* extract	7.6	97.2
initial day	7.5	96.2
20th day	5.2	23.5

Diethyl Phthalate		
100	75.43	
75	48	
50	20.7	
25	12.19	
Raw *P. hysterophorus* extract	22.2	43.1
initial day	21.3	42.0
20th day	5.2	23.5

Producing quality compost is the most important job on the organic farm. A lot of the problems I see on farms I visit could be solved by making better compost.

Eliot Coleman
1938-present

7

Application of Terrestrial weeds compost on soil and plants

7.1. APPLICATION OF TERRESTRIAL WEEDS COMPOST IN SOIL: A POT STUDY APPROACH

7.1.1. Soil characterization

The soil in this study is characterized as having a coarse structure due to the high concentration of sand and a relatively low amount of silt and clay. The soil characterization was depicted in Table 7.1. The soil's hue ranges from moderately deep to very deep with grey to moulted grey shades. The pH of the soil is a crucial component that influences the solubility, concentration, and mobility of micronutrients and macronutrients in the soil, which in turn impacts the growth and development of plants. The optimal pH range for agricultural applications is between 6 and 7, and the soil pH in this study falls within this range at 6.74. The SOM of the soil was 0.97% and SOC of the soil was 0.54%, which were relatively low. Lower SOM and SOC levels may result in lower soil fertility, decreased water-holding capacity, and increased erodibility. The CEC of the soil was observed to be 10 c mol/kg. Low CEC can cause soil degradation, lower soil fertility, and reduced crops yields. This is due to the soil's diminished capacity to hold nutrients, which results in nutrient loss and inadequate plant uptake. It can also make soil more prone to erosion and diminish soil water-holding capacity (Jain and Kalamdhad, 2020).

EC is a measure of the concentration of water-soluble salts in the soil solution, and high salinity in soil can adversely affect agricultural productivity. The recommended range of EC for alluvial soil is between 0.2 mS/cm – 0.8 mS/cm, and the EC of the soil in this study is within this range, at 0.38 mS/cm.

The bulk density of the soil is influenced by its texture, SOM, and moisture content. In this study, the bulk density of the soil was found to be 1.49 g/cm^3. SOM is an essential component that supplies nutrients to the soil, but in this case, its concentration was low in the alluvial soil due to frequent rainfall and recurrent floods that wash away the SOM in the study area.

The nitrogen and available phosphorus content of the soil were found to be 0.28% and 0.61 g/kg, respectively. The concentration of chromium in the soil was found to be reasonably high at 1985 ± 11.78 mg/kg, and the DTPA extraction efficiency of chromium was 2.92%. The concentration of lead, a heavy metal, was 412 ± 45.23 mg/kg, which is also quite high. The high concentration of heavy metals in the alluvial soil can be attributed to the deposition of industrial effluents on the sediments of the flood plain in the study area.

In comparison to other HMs, the overall HM concentrations of Fe, Ni, and Pb in soil were high. Table 7.1 shows the HM concentrations. The source was the reason for the build-up of HMs in alluvial soil. The alluvial material was obtained from the Brahmaputra river's embankments downstream. However, the overall HM concentration may not be related to HM toxicity. The toxicity of HM is determined by the bioavailable fractions of HM. The bioavailable fractions of HMs in soil are depicted in Fig. 7.1 (a). When bioavailable portions of HMs such as Pb, Ni, and Cr are higher, they have detrimental consequences. In the current investigation, bioavailable fractions of Pb, Ni, and Cr in alluvial soil had concentrations higher than the CPCB (2005) permitted limits. Fig. 7.1 (b) shows the chemical speciation of alluvial soil. The majority of metallic elements exist in a state of bioavailable and potential mobility, specifically in F1, F2, F3 and F4 fractions. This implies that plants have the ability to uptake heavy metals through their root system. The elements Pb and Cr exhibit a high degree of prevalence at F3 fractions, which may pose a potential hazard to both the soil and plant life.

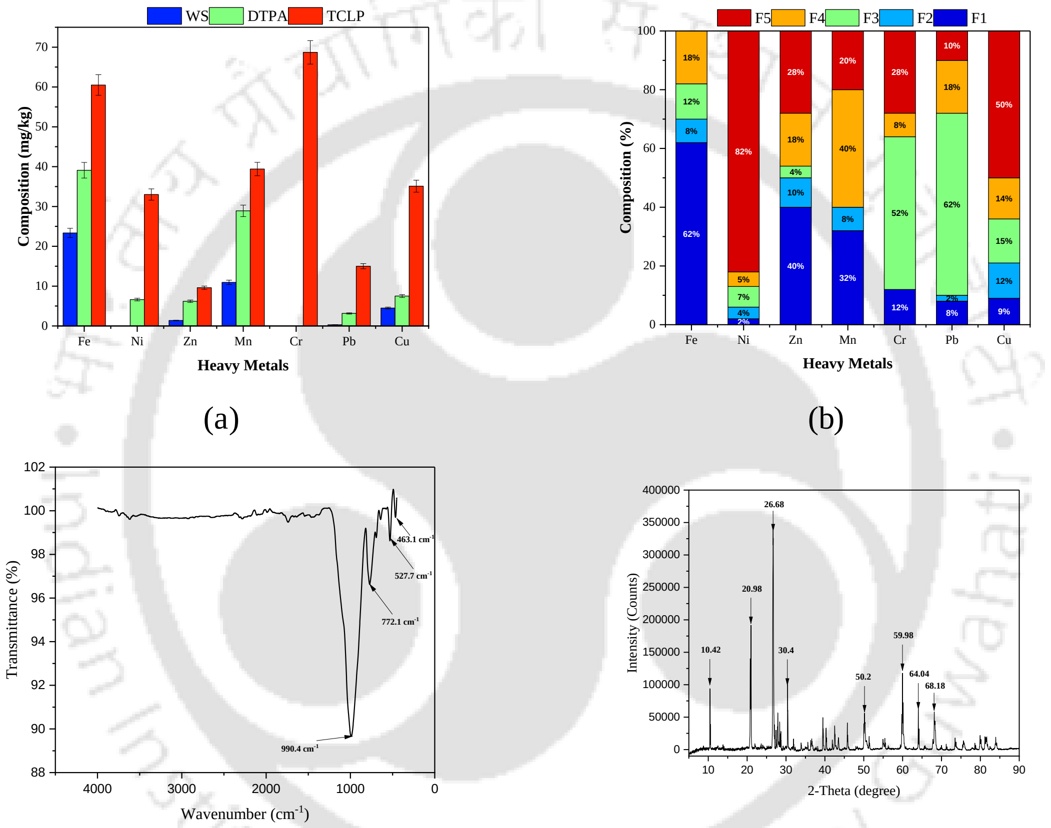
The soil used in the study was alluvial soil. The chemical and mineralogical characterization was performed through FTIR and PXRD analysis (Fig. 7.1 (c) and (d)). The functional groups of the minerals and nutrient associated with the organic compounds was analysed through FTIR. The prominent spectra were found at the wavelengths of 990.4, 772.1, 527.7 and 463.1 cm^{-1}. The spectra 990.4 cm^{-1} could be relatable to aliphatic phosphates (P-O-C stretch), which specifically conforms the potential existence of phosphate mineral in the soil. Aliphatic phosphates are decomposed in soil by microbes in a process through mineralization, which generates inorganic phosphate such as orthophosphate (PO_4^{2-}) that plants may use. Because phosphorus is a crucial macronutrient required for many physiological activities in plants, such as photosynthesis, energy transmission, and cell division, this process is critical for plant growth and development. The spectra could also be relatable to silicate ion (Si–O) (Ren et al., 2020). Furthermore, Silicate ions could increase plant cell structural stability by stiffening cell walls, which could assist minimise heavy metal uptake. Since heavy metal ions frequently bond to cell walls, therefore they could be blocked from entering the plant. Silicate ions would raise the pH of the soil or growth media, reducing heavy metal solubility and making them less accessible for plant absorption. The 772.1 cm^{-1} could be relatable to C-C vibrations, aliphatic chloro compounds (C-Cl stretch) and indicates the occurrence of quartz (Dubale et al., 2022; Ren et al., 2020). The spectra ranging at 527.7 cm^{-1} could be attributed to the Al-O-Si deformation of phyllosilicate mineral (Medina et al., 2017).

The PXRD analysis can give vital evidence on soil composition since minerals play an important role in defining soil qualities such as pH, nutrient availability, and water-holding capacity. Various minerals have varied chemical and physical characteristics, and they can impact soil fertility and plant development in different ways. The spectra of soil sample recorded at 2θ was 10.42°, 20.98°, 26.68°, 30.41°, 50.21°, 59.98°, 64.04° and 68.18°. The spectra between 20° to 30° could be attributed to the phosphate mineral. The spectra also attributes to the presence of magnesium ions in the soil, which may improve struvite crystallisation and prevent nitrogen loss (Li et al., 2020). The other spectra may reveal the occurrence of kaolinite, dolomite, montmorillonite and quartz.

Table 7.1. Initial characterization of alluvial soil

Parameter	**Alluvial Soil**
Moisture content (%)	21.2 ± 1.3
SOM (%)	0.97 ± 0.3
SOC (%)	0.54 ± 0.03
pH	6.74 ± 0.2
EC (dS/m)	0.3 ± 0.03
CEC (c mol/kg)	10 ± 1.3
TKN (%)	0.28 ± 0.05
Bulk Density (g/cm^3)	1.49 ± 0.3
Specific gravity	2.65 ± 0.02
WHC (%)	12.3 ± 1.2
Porosity (%)	44.6 ± 1.5
Total Phosphorus (g/kg)	1.8 ± 0.7
Available Phosphorus (g/kg)	0.6 ± 0.06
K (g/kg)	1.75 ± 0.8
Fe (mg/kg)	22300

Ni (mg/kg)	550
Zn (mg/kg)	126
Mn (mg/kg)	112
Cr (mg/kg)	256
Cd (mg/kg)	0
Pb (mg/kg)	435
Cu (mg/kg)	128



(a) (b)

(c) (d)

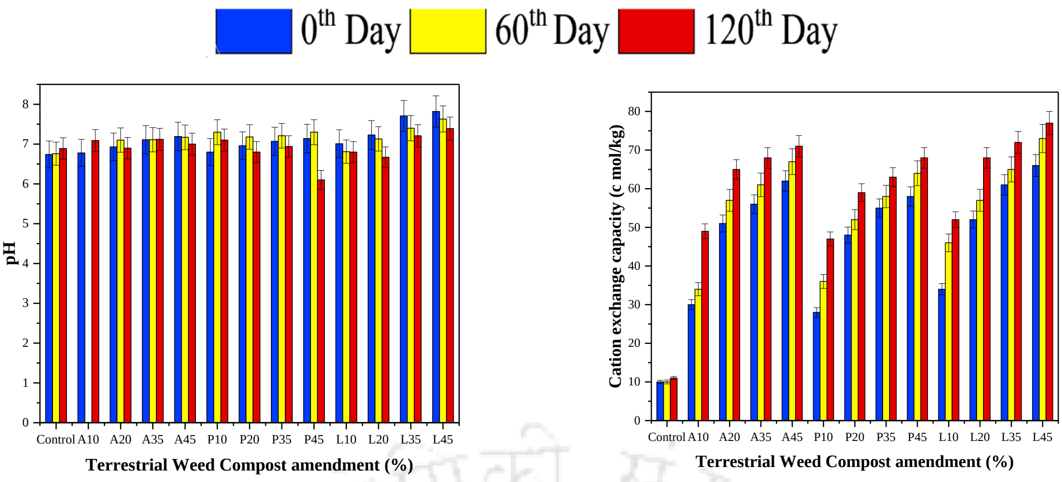
Fig. 7.1. (a) Bioavailable and leachable fractions; (b) Chemical speciation of HMs of alluvial soil; (c) FTIR characterization of alluvial soil; (d) PXRD characterization of alluvial soil

7.1.2. Effects of physical and chemical parameters on soil health

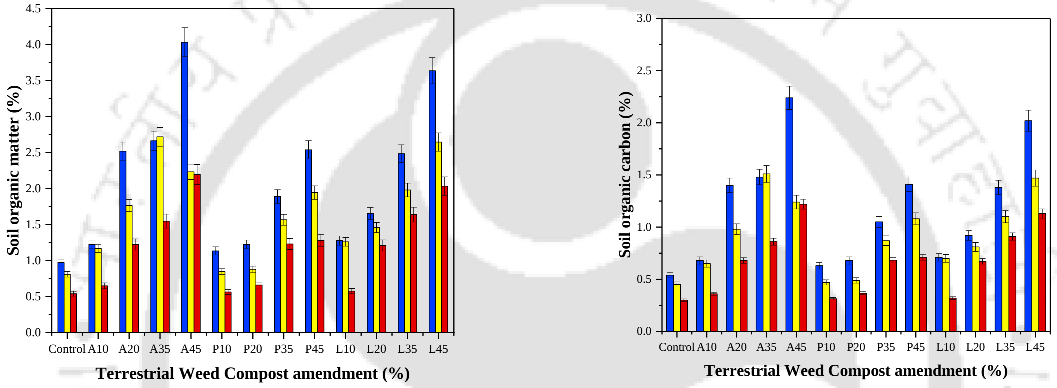
In general, adding compost to soil increases SOM content, which can improve soil fertility and structure. Compost also contains nutrients such as nitrogen, phosphorus, and potassium, which can benefit plant growth. The exact effect of compost on soil properties depends on the amount of compost added and the time elapsed since application. In the current study, TWC was amended with soil at 10, 20, 35 and 45% till 120 days and analysed at initial day, 15th, 30th, 45th, 60th, 90th and 120th days. The CEC of the soil was found to be increasing after the application of compost. Fig. 7.2 (b) illustrates the increasing trends of CEC in the due course of the study. The CEC on 120th day of A10, A20, A35, A45, P10, P20, P35, P45, L10, L20, L35 and L45 were increased by 38.7, 21.5, 17.6, 12.6, 40.4, 18.6, 12.6, 14.7, 34.6, 23.5, 15.2 and 14.2% compared to initial day samples. The application of TWC had the potential to enhance the accessibility of crucial plant nutrients, including nitrogen, phosphorus, and potassium. The provision of nutrients can potentially elicit a growth response in plants and enhance the release of organic compounds from roots, thereby augmenting the soil's CEC through the provision of additional organic matter and microbial activity.

The addition of TWC in soil has varied the SOM concentration in the range of 0.54 to 4.32 %. As the compost amendment raised, the SOM content has been increased in the soil in initial days. But after 120th day, the SOM concentrations were decreased relatively compared to initial day concentrations. The SOM and SOC concentrations on 120th day of A10, A20, A35, A45, P10, P20, P35, P45, L10, L20, L35 and L45 were reduced by 46.1, 51.4, 41.8, 45.5, 50.3, 46, 34.9, 49.5, 54.9, 26.9, 34 and 44% compared to initial day samples. Following the addition of compost, the first burst of microbial activity can induce fast degradation of organic matter, resulting in a drop in SOC (Jain and Kalamdhad, 2020). Fig. 7.2 (c) and (d) illustrates the decreasing trends of SOM and SOC in the study.

The bulk density has been observed within the range of 1.05 to 1.47 kg/m^3. There was no much variations seen in TWC amended with soil. But, the trend of bulk density was found to be increased from initial day to the 120th day (Fig. 7.3 (a)). TWC contains optimal amount of volatile content that serves as a food source for soil microorganisms. When compost is mixed with soil, the microorganisms in the soil start to decompose the organic matter in the compost, which can lead to the release of gases and the formation of stable organic matter. This process can cause the soil to become more compact and, therefore, increase its bulk density. Compost contains a high amount of moisture that can affect the bulk density of soil when it is mixed with it. When compost is added to soil, the water content of the soil can increase, which can lead to the soil becoming more compact and increasing its bulk density.



(a) pH (b) Cation exchange capacity



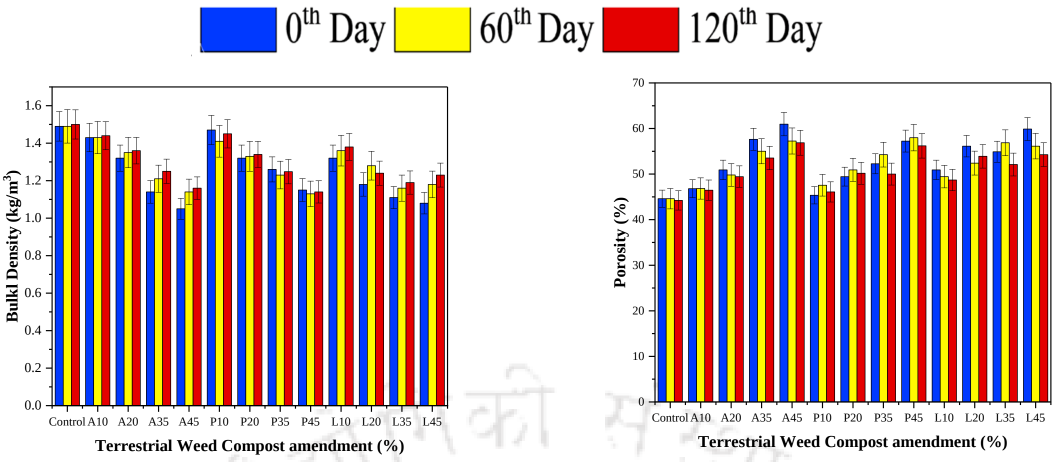
(c) SOM (d) SOC

Fig. 7.2. Evaluation of chemical parameters of the soil at initial, 30th, 60th and 120th days

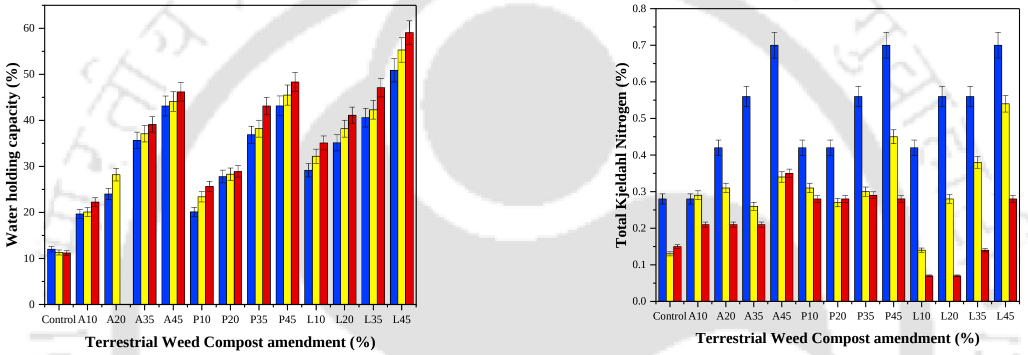
The addition of TWC to soil resulted in a significant increase in porosity, with levels rising from 46.8 to 60.9%, 45.3 to 57.2%, and 50.9 to 59.8% as the compost concentration increased from 10 to 45% (Fig. 7.3 (b)). However, a decreasing trend in porosity was observed over time, with levels gradually declining from the initial measurement to the 120th day, Jain and Kalamdhad (2020) has reported the decreasing trend of porosity in their study. The initial increase in porosity following the application of compost to soil is likely due to the presence of organic matter and the formation of larger pores. However, over time, several factors can contribute to a decrease in porosity. Compost could contain fine particles that can fill in pore spaces, leading to a reduction in porosity. As microorganisms in the soil decompose the organic matter in the compost, they release carbon dioxide, which can cause the soil to become more compact, reducing pore space. Over time, water and other forces can cause the particles in the soil to settle and become more tightly packed, reducing porosity.

WHC refers to a soil's ability to hold water. Soils with high WHC can hold more water, making it available for plant growth and reducing runoff. Soils with low WHC can limit plant growth and productivity, and increase the risk of erosion. The application of composts made from *A. conyzoides*, *P. hysterophorus*, and *L. camara* to soil led to a notable improvement in water holding capacity (WHC), with WHC levels increasing from 19.6 to 43.1%, 20.1 to 43.1%, and 29.1 to 50.8% as the concentration of compost in the soil increased from 10 to 45% (Fig. 7.3 (c)). Over time, the increase in WHC may continue as the organic matter in the compost breaks down further, improving soil structure and increasing the soil's ability to hold onto water (Cooper and DeMarco, 2023). Organic matter in compost can also increase the CEC of the soil, which means that the soil can hold onto more nutrients and water. However, the rate of increase may slow down as the compost becomes more fully integrated into the soil and the soil reaches its maximum capacity for water storage.

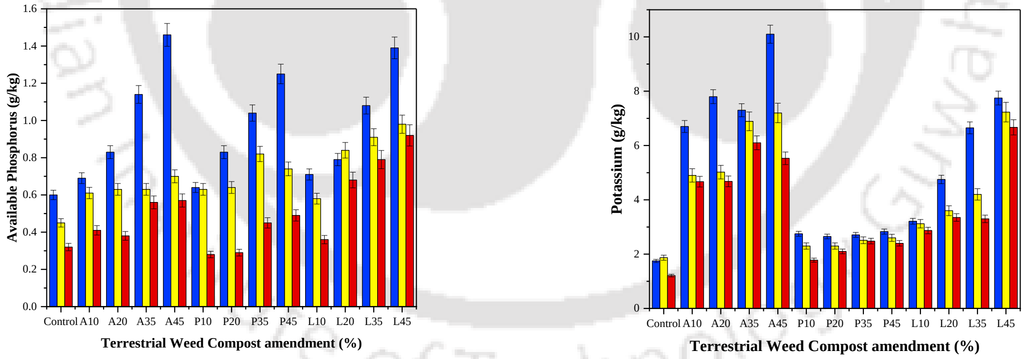
Nutrient content in soil is vital for promoting healthy plant growth and maintaining soil fertility. Nutrients such as nitrogen, phosphorus, and potassium are essential for plant development, while micronutrients such as zinc, iron, and manganese are also necessary for healthy plant growth. Nutrient-rich soil is also more resistant to environmental stressors, such as drought and pests, and can prevent soil erosion, which is essential for maintaining ecological balance. Finally, nutrient-rich soil can support the production of nutrient-dense crops, which is crucial for promoting human health and preventing nutrient deficiencies. In the current study, nutrient content such as TKN, available phosphorus and potassium has found to be reduced after the application. One of the main reasons could be attributed that the nutrients in the compost might become immobilised or locked up in the soil, making them less available to plants. This is because microorganisms in the soil utilise accessible carbon sources, such as organic materials in compost, to meet their energy needs. As a result, the microbes can consume some of the available nutrients, which may become momentarily unavailable for plant uptake. Also, some of the nutrients in the compost may be lost due to leaching, volatilization, or plant uptake. Furthermore, the nutrient concentration of the original organic material used to generate the compost might alter the nutritional content of the completed compost and the soil. As a result, it is critical to monitor nutrient levels in the soil and modify the application of compost or other organic amendments accordingly to maintain optimal nutrient levels for plant growth. Fig. 7.3 (d), (e) and (f) depicts the alterations in the nutrient content of the soil in the study.



(a) Bulk density (b) Porosity



(c) Water holding capacity (d) Total Kjeldahl Nitrogen



(e) Available Phosphorus (f) Potassium

Fig. 7 3. Evaluation of physical and nutritional parameters of the soil at initial, 30th, 60th and 120th days

7.1.3. Effects of *A. conyzoides* compost (ACC), *P. hysterophorus* (PHC) and *L. camara* (LCC) on Bulk density and WHC of soil: CCD-RSM approach

The CCD-RSM was employed to construct the experimental matrix and optimise outcomes for bulk density and water holding capacity. The optimisation study considered various dependent variables, including bulk density and water holding capacity, as well as independent variables such as the percentage of compost-soil amendment (A) and the duration of sampling (B). The response values of the dependent variables have been documented and can be found in the supplementary file. The analysis revealed a quadratic model, with a total of 13 runs conducted for each substrate. The models exhibited statistical significance, as evidenced by R^2 values ranging from 0.92 to 0.96. The regression model for Bulk density of *A. conyzoides* and soil amendments in actual and coded equations was attained from Equations (7.1) and (7.2).

$$\text{Bulk density} = 1.49 - 0.008\,A + 0.0003B \tag{7.1}$$

$$\text{Bulk density} = 1.30 - 0.16\,A + 0.0197B \tag{7.2}$$

The regression model for WHC of *A. conyzoides* and soil amendments in actual and coded equations was attained from Equations (7.3) and (7.4).

$$\text{WHC} = 9.38 + 0.67\,A + 0.05B \tag{7.3}$$

$$\text{WHC} = 29.7 + 13.5\,A + 3.41B \tag{7.4}$$

The regression model for Bulk density of *P. hysterophorus* and soil amendments in actual and coded equations was attained from Equations (7.5) and (7.6).

$$\text{Bulk density} = 1.51 - 0.005\,A - 0.00005B + 7.97\text{E-}06AB - 0.00007A^2 + 1.96\text{E-}06\,B^2 \tag{7.5}$$

$$\text{Bulk density} = 1.33 - 0.164\,A - 0.006B + 0.009AB - 0.02A^2 + 0.007\,B^2 \tag{7.6}$$

The regression model for WHC of *P. hysterophorus* and soil amendments in actual and coded equations was attained from Equations (7.7) and (7.8).

$$\text{WHC} = 10.7 + 0.67\,A + 0.04B \tag{7.7}$$

$$\text{WHC} = 30.1 + 13.5\,A + 2.54B \tag{7.8}$$

The regression model for Bulk density of *L. camara* and soil amendments in actual and coded equations was attained from Equations (7.9) and (7.10).

$$\text{Bulk density} = 1.44 - 0.013\,A + 0.001B + 0.00003AB + 0.0001\,A^2 - 0.00001B^2 \tag{7.9}$$

$$\text{Bulk density} = 1.28 - 0.117\,A + 0.03B + 0.03AB + 0.04A^2 - 0.05B^2 \tag{7.10}$$

The regression model for WHC of *L. camara* and soil amendments in actual and coded equations was attained from Equations (7.11) and (7.12).

$$\text{WHC} = 15.6 + 0.77\,A + 0.06B \tag{7.11}$$

$$\text{WHC} = 39 + 15.5\,A + 4B \tag{7.12}$$

Effect of ACC on Bulk density and WHC of soil

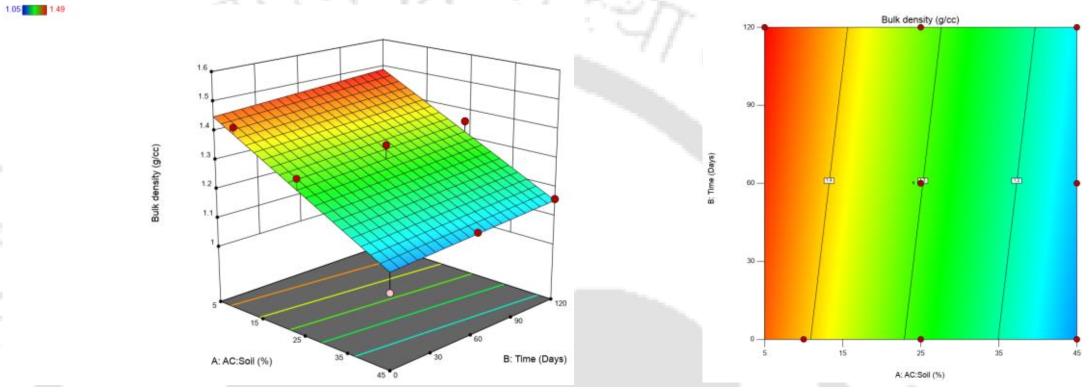
The statistical significance and adequacy of the computational model were evaluated using Analysis of Variance (ANOVA). ANOVA is a statistical method employed to evaluate the presence of a significant difference among a group of variables. Zuo et al. (2020) conducted an evaluation of a specific category of factors by means of assessing their means. The obtained F value for the bulk density model was 52.4, indicating statistical significance at a p-value of less than 0.0001. The statistical analysis revealed that the linear factor (A) and (B) were statistically significant at a p-value of less than 0.05. The validity of the lack of fit (LOF) test was established based on the degrees of freedom (DF) for LOF, which amounted to 6, and the DF for pure error, which was 4. These values exceeded the threshold of 3. The experimental results demonstrated a high level of consistency and precision, as evidenced by the remarkably low coefficient of variance (CV) of 3.13. The obtained coefficient of determination (R^2) was 0.91, which suggests that the regression model effectively explained 91% of the variance observed in the dependent output response. The aforementioned discovery implies that the regression model exhibited a high level of significance.

The obtained F value for the WHC model was 106.1, indicating statistical significance at a p-value of less than 0.0001. The statistical analysis revealed that the linear factor (A) and (B) were statistically significant at a p-value of less than 0.0001. The validity of the lack of fit (LOF) test was established based on the degrees of freedom (DF) for LOF, which amounted to 6, and the DF for pure error, which were 4. These values exceeded the established threshold of 3. The experimental results demonstrated a high degree of consistency and precision, as evidenced by coefficient of variance (CV) of 7.9. The obtained coefficient of determination (R^2) was 0.95, suggesting that the regression model effectively explained 95% of the variance observed in the dependent output response. The aforementioned discovery indicates that the regression model exhibited a high level of significance. The 3D and contour plots for bulk density and WHC of ACC amended soil are depicted in Fig. 7.4 (a).

Effect of PHC and LCC on Bulk density and WHC of soil

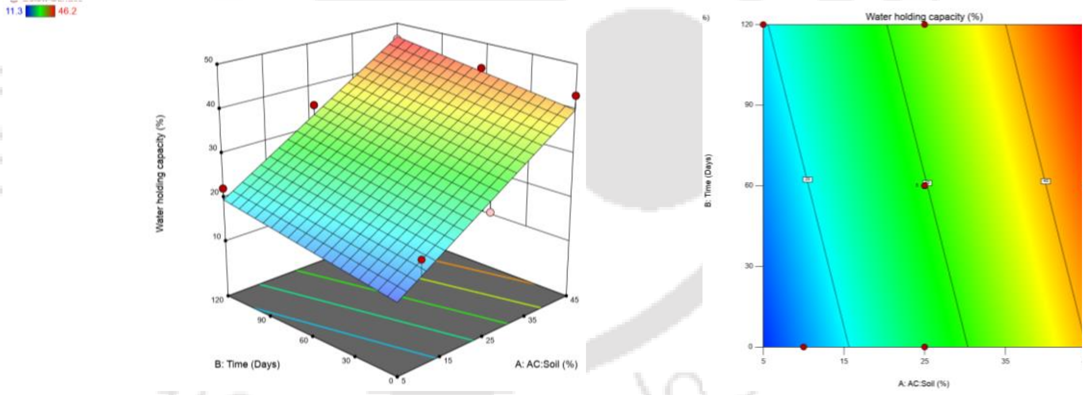
The statistical significance of the bulk density model was confirmed by the obtained F value of 233.4 and 162 for PHC and LCC, which was significant at a p-value of less than 0.0001. The results of the statistical analysis indicate that both the linear factor (B) and quadratic factors (AB) were found to be statistically significant at a p-value of less than 0.0001. Furthermore, it was observed that the interactive variables A^2 and B^2 exhibited statistical significance at p-values lower than 0.05, correspondingly. The LOF test's validity was established through an analysis of the degrees of freedom (DF) for LOF was 3, and the DF for pure error was 4 for both compost amendments. The aforementioned values surpassed the predetermined threshold of 3. The experimental findings exhibited a notable level of uniformity and accuracy, as indicated by the coefficient of variation (CV) of 0.90 and 0.94 for PHC and LCC. The R^2 coefficient obtained for PHC and LCC was 0.99, indicating that the regression model provided a satisfactory explanation for 99% of the variance observed in the dependent output response. The discovery mentioned above suggests that the regression model demonstrated a considerable degree of statistical significance.

The WHC model of PHC and LCC was turned out to be a linear model with $p < 0.0001$. The coefficient of variance was high and the R^2 coefficient obtained for PHC and LCC was 0.91, indicating a satisfactory explanation for 91% of the variance depicted in the dependent output response. The 3D and contour plots for bulk density and WHC of PHC and LCC amended soil are depicted in Fig. 7.4 (b) and (c).



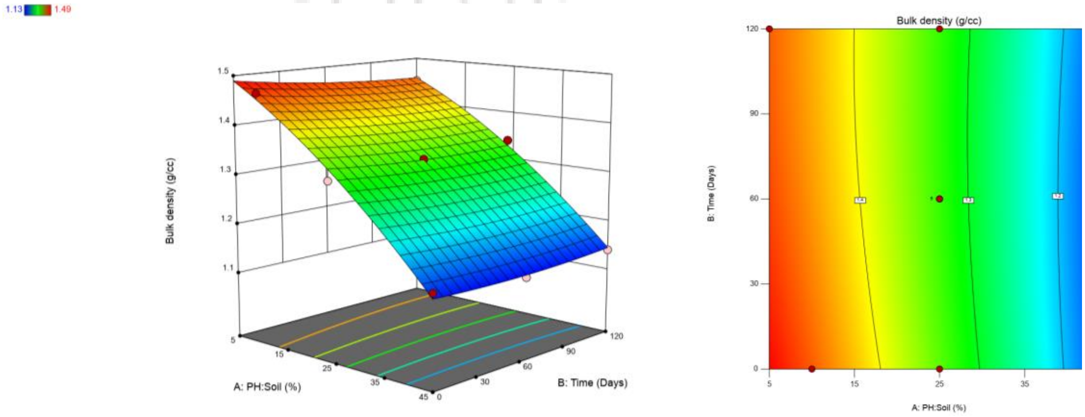
Bulk density

(a)

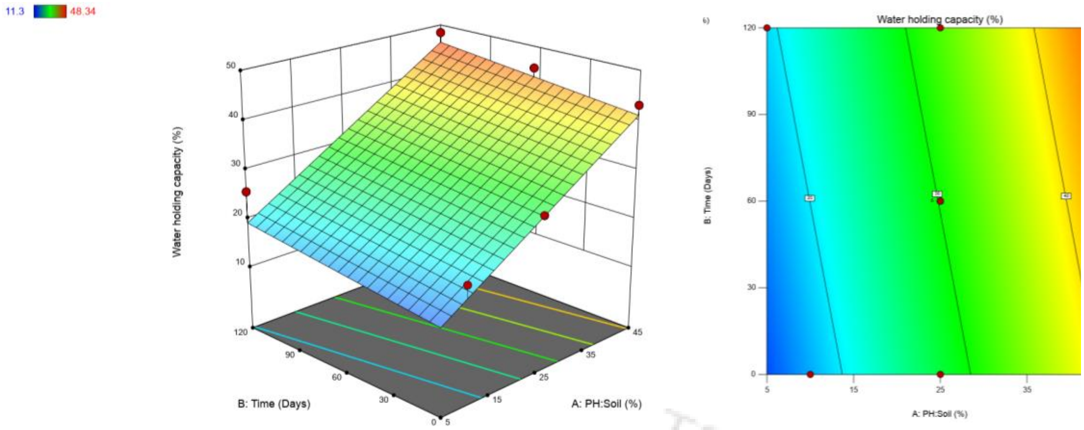


Water holding capacity

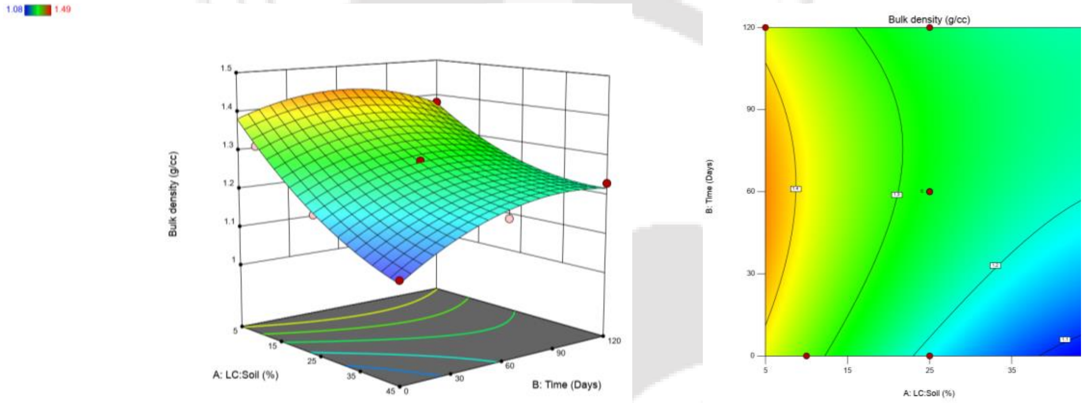
(b)



Bulk density

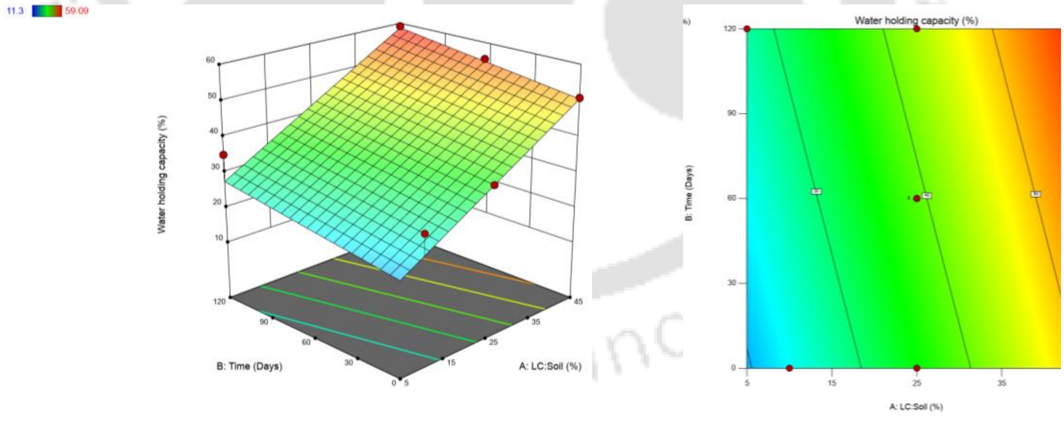


Water holding capacity



Bulk density

(c)



Water holding capacity

Fig. 7.4. 3D and contour plots created through CCD-RSM for bulk density and water holding capacity of soil amended with (a) *A. conyzoides* compost (b) *P. hysterophorus* compost (c) *L. camara* compost

The three amendments, namely ACC, PHC, and LCC, exhibited noteworthy improvement in soil physicochemical characteristics. The analysis of variance (ANOVA) revealed a statistically significant level of model fit for each of the three composts. In comparison to ACC, PHC and LCC demonstrated superior outcomes in enhancing both bulk density and WHC.

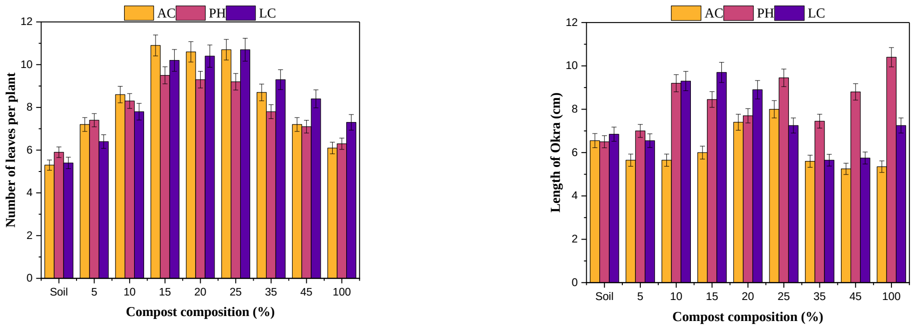
7.1.4. Morphological assessments and Macronutrient dynamics of *Abelmoschus esculentus*

Morphological assessments

Morphological parameters are important to assess in plant studies because they provide information about the physical characteristics of a plant. These characteristics can be used to identify different plant species, evaluate plant health, and monitor plant growth and development (Mastrogianni et al., 2021). In this study, morphological parameters such as germination percentage, root length, shoot length, number of leaves per plant, number of fruits per pot and length of fruits were measured after 120 days (Fig. 7.5 (I)). A total of 6 seeds were sown in each pot in triplicates. The plants grown in soil amended with *A. conyzoides*, *P. hysterophorus*, and *L. camara* composts exhibited a maximum germination percentage of 83.3%, 83.3%, and 100%, respectively, at 20% and 25% levels of compost amendment. The highest number of leaves per plant was observed in the treatment with 15% *A. conyzoides* compost, 15% *P. hysterophorus* compost, and 25% *L. camara* compost amendments. After a 90-day study period, it was observed that the number of leaves on the plant decreased, which could be attributed to leaf senescence, a natural aging process of plant leaves that leads to their gradual deterioration and death. Leaf senescence can be triggered by a variety of factors such as environmental stress, hormonal changes, and nutrient deficiencies, and is a crucial step in the plant's life cycle as it allows for the reallocation of nutrients from older, senescing leaves to younger, growing tissues. The reduction in the number of leaves in the study suggests that the *A. esculentus* plants had reached the end of their growth cycle and had undergone leaf senescence as a natural part of their life cycle (Goswami et al., 2017). The highest number of fruits per pot was observed in the treatment with 15% *A. conyzoides* compost, 15% *P. hysterophorus* compost, and 25% *L. camara* compost amendments. The maximum length of the fruit was observed in the treatment with 25% *A. conyzoides* compost, 20% *P. hysterophorus* compost, and 15% *L. camara* compost amendments. The maximum and minimum fruit length was observed to 11 and 5 cm. The length of fruit is an important trait in plant studies because it can provide valuable information about the plant's reproductive strategy, seed dispersal mechanisms, and ecological interactions. Fruit length is often correlated with the size and number of seeds produced by the plant. In general, longer fruits tend to contain more and larger seeds, which can affect the plant's ability to reproduce successfully.

Macronutrient dynamics in A. esculentus

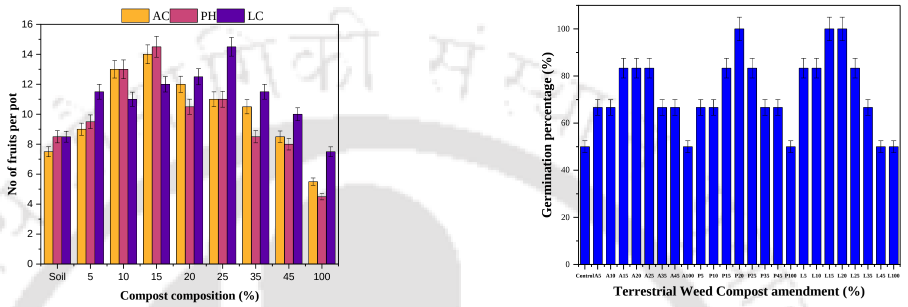
Analysing nutrient content in *A. esculentus* is important when compost is amended with soil at increasing percentages because it can help growers to optimize plant growth and productivity. The macronutrients K, Na, and Ca are essential for plant growth and development, and their availability in the soil can affect the nutrient content in *A. esculentus*. The alterations in nutrient content was depicted in Fig. 7.5 (II). The concentrations of K, Na, and Ca in *A. esculentus* fruit cultivated in soil enriched with increasing proportions of *A. conyzoides* compost demonstrated a significant rise ranging from 56.9 to 67%, 5.2 to 25%, and 17.8 to 57.3%, respectively, relative to *A. esculentus* plants grown in unamended soil. The concentrations of K, Na, and Ca in *A. esculentus* fruit grown in soil amended with increasing percentages of *P. hysterophorus* compost showed a significant increase of 60 to 68.8%, 2.7 to 18.1%, and 42.6 to 54%, respectively, relative to *A. esculentus* fruit grown in soil alone. The levels of potassium K, Na, and Ca in *A. esculentus* fruit grown in soil enriched with increasing proportions of *L. camara* compost demonstrated a significant increase of 51.7 to 76.2%, 1 to 43.7%, and -39.9 to 9.5%, respectively, relative to *A. esculentus* fruit grown in unamended soil. The consistent content of macronutrients was observed to remain stable within the range of 15% to 25% amended compost, which was also reported by Kauser et al. (2022). The sodium and calcium content of *A. esculentus* fruit grown in *P. hysterophorus* and *L. camara* compost were found to be low compared to *A. esculentus* fruit grown in unamended soil. Potassium, sodium, and calcium are essential minerals required by *A. esculentus* for healthy growth and development. These minerals play important roles in different physiological processes in the plant. Potassium is important for maintaining the osmotic balance in cells, regulating water uptake, and facilitating the movement of nutrients within the plant. It also contributes to the plant's tolerance to various abiotic and biotic stresses such as drought, salinity, and diseases. Sodium is not an essential nutrient for plants, but it can be beneficial in small quantities. It can enhance the plant's resistance to pests and diseases and help regulate the plant's water balance. Calcium is important for the structural integrity of cell walls, and it is involved in the regulation of various physiological processes such as cell division and expansion. It also plays a role in the activation of enzymes and the regulation of membrane permeability.



I

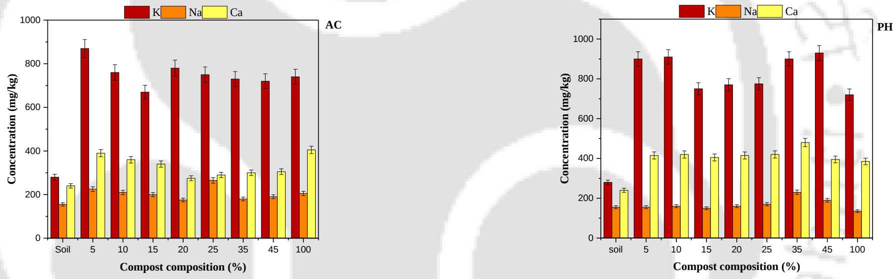
(a) Number of leaves per plant

(b) Length of Okra



(c) Number of fruits per pot

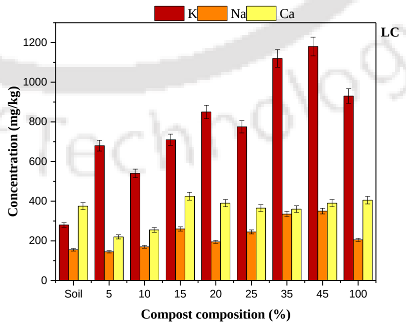
(d) Germination percentage



(d) Nutrient content in *A. esculentus* grown in *A. conyzoides* compost

(e) Nutrient content in *A. esculentus* grown in *P. hysterophorus* compost

II



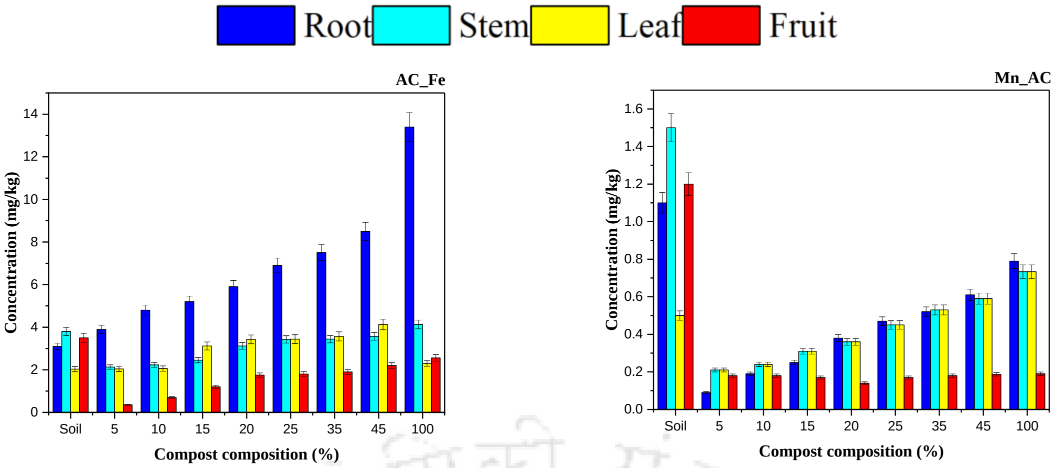
(f) Nutrient content in *A. esculentus* grown in *L. camara* compost

Fig. 7.5. (I) Morphological parameters of *Abelmoschus esculentus* plant after harvesting the plant after 120 days; (II) Nutritional parameters of *A. esculentus* fruit after 120 days.

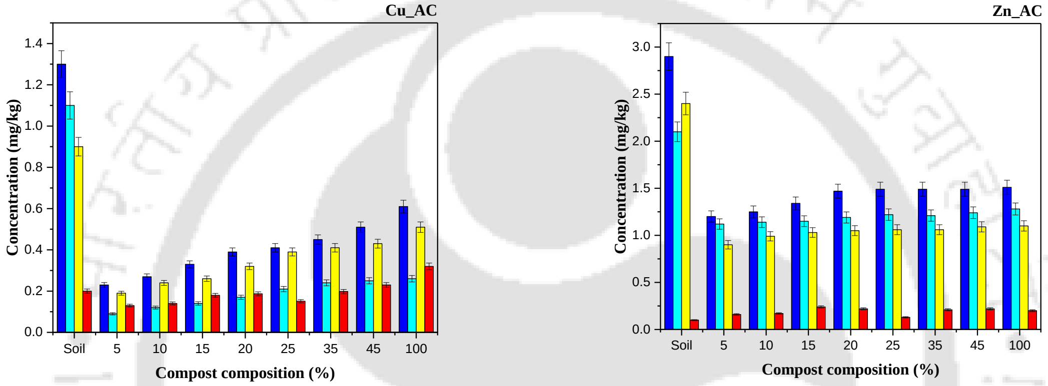
7.1.5. Heavy metals (HMs) characterization in *A. esculentus*

HM assessment in different parts of a plant is important because heavy metals are toxic to both plants and humans. Plants can absorb heavy metals through their roots from contaminated soil, water, or air, and can accumulate these metals in their tissues. When humans consume these plants, they can also be exposed to the heavy metals, which can lead to health problems. Heavy metal assessment in plant tissues is important for several reasons. First, it allows us to identify plants that have been exposed to heavy metals, which can help us to identify contaminated areas and prevent further exposure. Second, it allows us to monitor the uptake and accumulation of heavy metals in plants, which can help us to develop strategies to reduce the risk of human exposure. Finally, it allows us to assess the potential health risks associated with consuming plants that have been exposed to heavy metals. The current study aimed to characterize the accumulation of HMs in different parts of *A. esculentus* (okra) plants, including the root, stem, leaf, and fruit, under varying compost concentrations in soil. The results indicated that the accumulation of HMs in the plants increased with increasing compost concentration in the soil. Specifically, iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) showed similar trends, with the root section of the plant accumulating the highest content of HMs compared to other parts of the plant. The *A. esculentus* plants grown in soil showed Mn concentrations of 1.15 mg/kg in the root, 1.5 mg/kg in the stem, 0.5 mg/kg in the leaf, and 1.2 mg/kg in the fruit. Similarly, the plants showed Cu concentrations of 1.3 mg/kg in the root, 1.1 mg/kg in the stem, 0.9 mg/kg in the leaf, and 0.2 mg/kg in the fruit, and Zn concentrations of 2.8 mg/kg in the root, 2.1 mg/kg in the stem, 2.3 mg/kg in the leaf, and 0.3 mg/kg in the fruit. In contrast, *A. esculentus* plants grown in compost-amended soil showed lower concentrations of Mn, Cu, and Zn in their plant system. It is important to note that the HMs Cu and Zn were found to be within permissible limits, whereas Fe and Mn did not have any permissible limit, as per the standards set by the Food Safety and Standards Authority of India (FSSAI, 2011). In addition to Fe, Mn, Cu, and Zn, the current study also investigated the accumulation of other HMs, namely Ni and Pb, in the fruits of *A. esculentus* plants grown under different amendments. The results showed that the concentrations of Ni and Pb followed a similar pattern, with higher concentrations observed in fruits of *A. esculentus* plants grown in root alone. Specifically, the concentrations of Ni in fruits ranged between 0.6 to 1.3 mg/kg in all amendments of TWC, whereas the concentrations of Pb in fruits ranged between 0 to 0.13 mg/kg in all amendments of TWC. Notably, the concentrations of Ni were within the permissible limits (1.5 mg/kg) set by the FSSAI (2011) . Moreover, the concentrations of Pb was also in permissible limits (0.1 mg/kg) set by FSSAI (2011) in TWC amendments of 25, 35, 45 and 100%, indicating a potential health risk associated with the consumption of *A. esculentus* fruits grown in TWC amendments more than 20%.

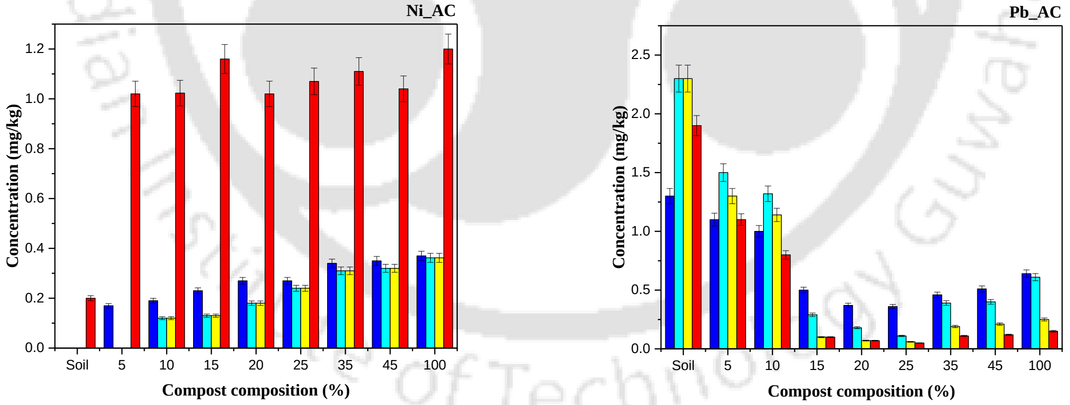
Heavy metals can enter the food chain through plant uptake from soil. The pathway for heavy metal uptake by plants is through the roots. When heavy metals are present in soil, they can bind to soil particles or dissolve in soil water. Plants can take up heavy metals in two ways: through the roots and through the leaves. The process of root uptake involves the movement of heavy metals from the soil into the plant roots. Heavy metals can enter the plant through passive diffusion or active transport mechanisms. Once inside the roots, heavy metals can move to other parts of the plant, such as stems, leaves, and fruits, through the xylem and phloem transport systems. Once heavy metals enter the plant, they can accumulate in different plant tissues, including fruits. The degree of accumulation depends on various factors, including the concentration of heavy metals in the soil, the type of heavy metal, the plant species, and the stage of plant growth. The accumulation of heavy metals in the fruits of the *A. esculentus* plant can pose a risk to human health if the fruits are consumed. Therefore, it is essential to monitor soil quality and limit exposure to contaminated soils to prevent heavy metal uptake by plants. Additionally, good agricultural practices, such as soil testing, crop rotation, and proper disposal of waste materials, can help reduce the risk of heavy metal contamination in fruits and vegetables. The accumulation of Pb in the fruit of a plant compared to other heavy metals such as Fe, Mn, Ni, Cu, and Zn can be influenced by several factors. One of the key factors is the chemical properties of the heavy metals, as each metal has different chemical properties that can affect its mobility and bioavailability in the soil and plant system. Pb is known to be more mobile and soluble in soil than other heavy metals like Fe, Mn, Ni, Cu, and Zn, which means that it can more easily enter the plant through root uptake or foliar absorption. Furthermore, Pb can accumulate in fruit tissues due to its unique properties, such as its high atomic number and ionic size, which make it easy for it to be absorbed by the plant and transported to the fruit. Fig. 7.6, 7.7 and 7.8 depicts the concentrations of heavy metals in various parts of *A. esculentus*.



(a) Iron (Fe) (b) Manganese (Mn)

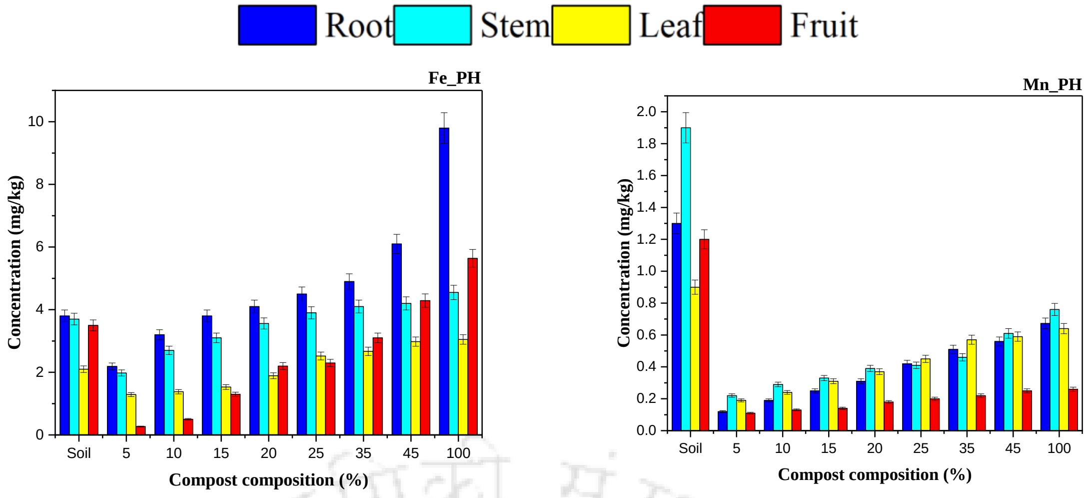


(c) Copper (Cu) (d) Zinc (Zn)

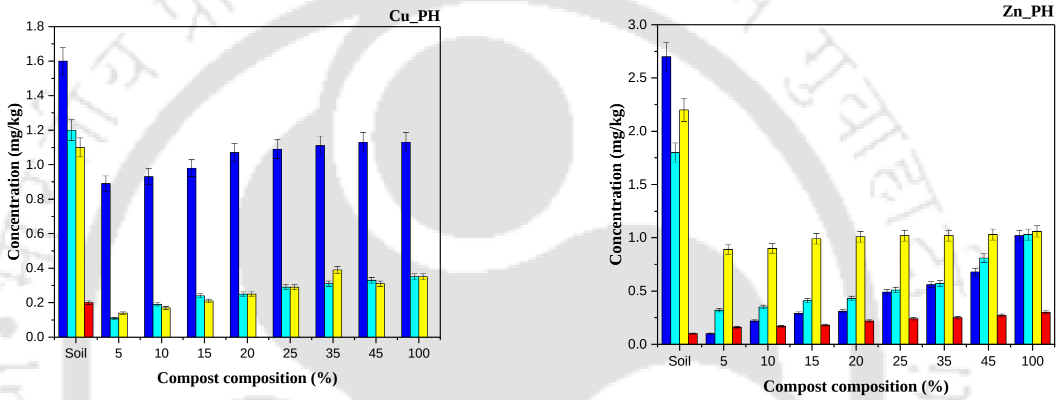


(e) Nickel (Ni) (f) Lead (Pb)

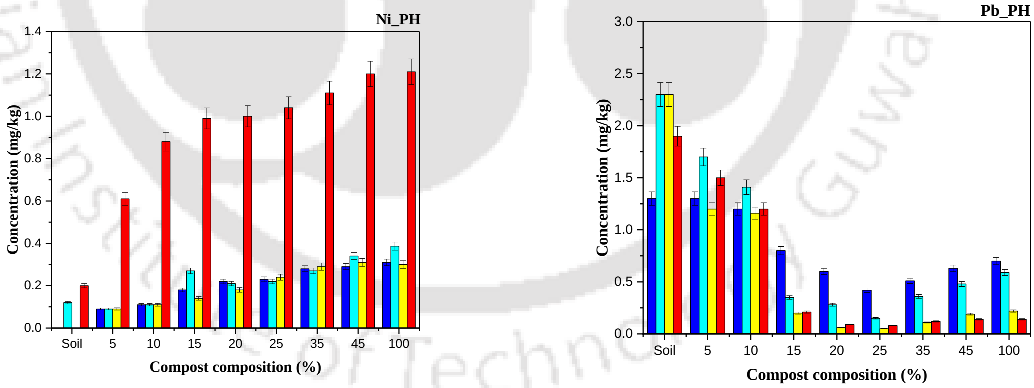
Fig. 7.6. Evaluation of heavy metals in distinct parts of *Abelmoschus esculentus* plant after amending *A. conyzoides* compost in the soil



(a) Iron (Fe) (b) Manganese (Mn)

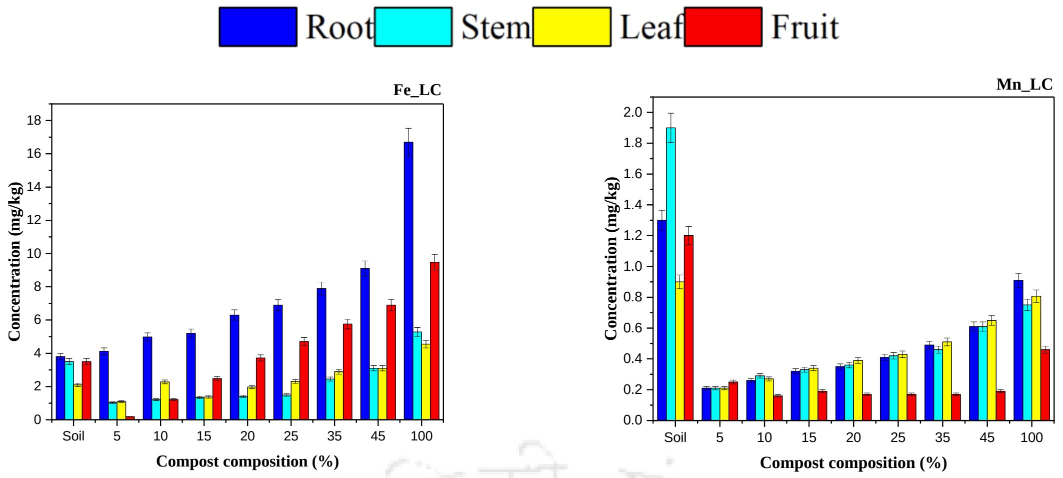


(c) Copper (Cu) (d) Zinc (Zn)

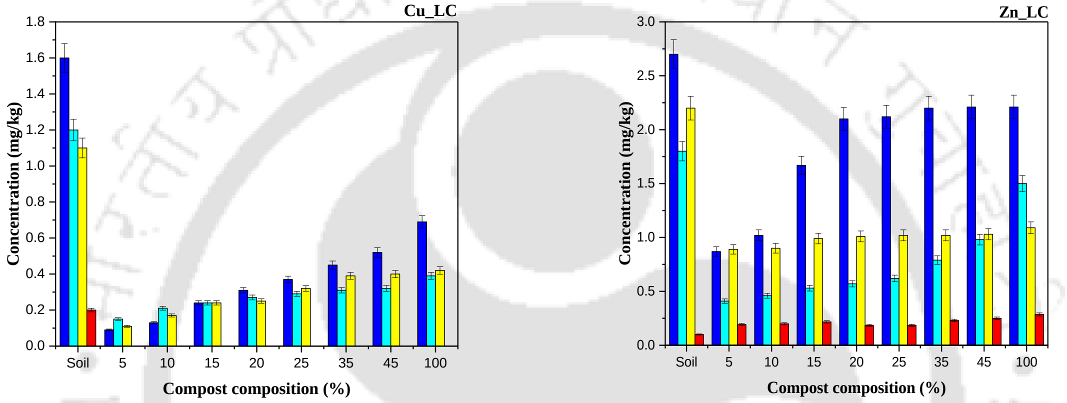


(e) Nickel (Ni) (f) Lead (Pb)

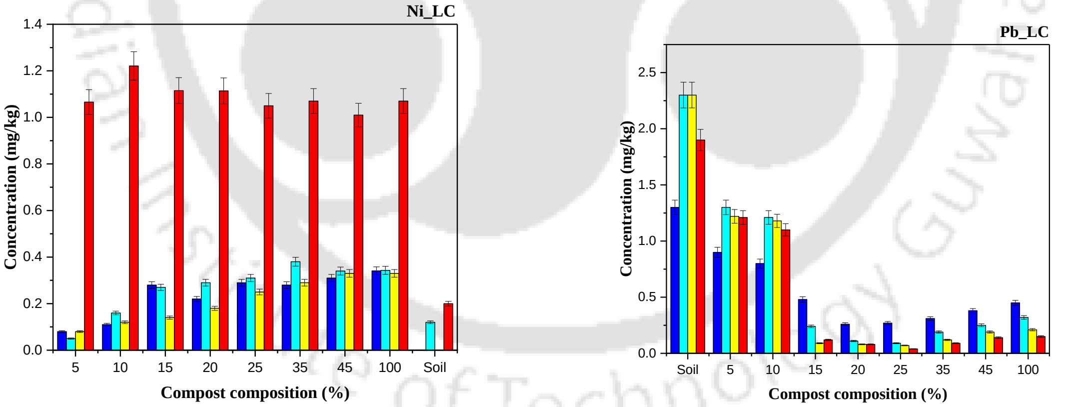
Fig. 7.7. Evaluation of heavy metals in distinct parts of *Abelmoschus esculentus* plant after amending *P. hysterophorus* compost in the soil



(a) Iron (Fe) (b) Manganese (Mn)



(c) Copper (Cu) (d) Zinc (Zn)



(e) Nickel (Ni) (f) Lead (Pb)

Fig. 7.8. Evaluation of heavy metals in distinct parts of *Abelmoschus esculentus* plant after amending *L. camara* compost in the soil

7.1.6. Significance of nutrients in *A. esculentus*

According to FSSAI (2011), *A. esculentus* is expected to possess certain mineral content per 100 g, including potassium (299 mg), calcium (81 mg), magnesium (57 mg), iron (0.8 mg), and phosphorus (57 mg). The fruit of *A. esculentus* exhibits optimal nutrient concentrations when cultivated in soil supplemented with TWC amendments. The fruit's nutrient content exhibited optimal levels until a 25% amendment of TWC was introduced. However, subsequent increases in nutrient concentrations were observed, indicating that an amendment percentage ranging from 15% to 25% of terrestrial weed compost is most favourable for soil application.

Potassium in *A. esculentus* being a crucial mineral that serves a fundamental role in the maintenance of optimal cardiovascular health, the regulation of blood pressure, the facilitation of muscular functionality, and the promotion of comprehensive neural function. In addition to its nutritional value, *A. esculentus* was also rich in calcium content, which plays a vital role in promoting optimal bone and dental health (Phonglosa et al., 2015). Calcium plays a crucial role in various physiological processes, including muscle contraction, blood clotting, and the maintenance of optimal nerve function. Magnesium found to be high in *A. esculentus,* it is a vital mineral that plays a significant role in more than 300 enzymatic reactions within the human body. Its functions encompass facilitating energy production, supporting muscle functionality, aiding nerve transmission, and promoting the maintenance of an optimal immune system. Although the iron content in okra was comparatively modest in relation to certain alternative sources, it nevertheless plays a role in fulfilling the body's iron needs. Iron is a crucial element required for the synthesis of haemoglobin, a protein responsible for the transportation of oxygen to every cell within the human body. The aforementioned factors are of utmost importance in the context of energy generation, optimal immune system functioning, and cognitive maturation. Available phosphorus content in *A. esculentus* was moderate and its an essential mineral required for the development and upkeep of optimal bone and dental health. Additionally, it exerts influence on energy metabolism, DNA synthesis, and cellular repair. Fig. 7.9 depicts the growth and germination of *A. esculentus*.

25th Jan, 2022 8th Feb, 2022

Fig. 7.9. Growth and germination of *A. esculentus* in terrestrial weeds compost amended soil

7.2. APPLICATION OF TERRESTRIAL WEEDS COMPOST IN SOIL: A FIELD SCALE APPROACH

The recognition of the significance of SOM is imperative in various domains, including the retention of nutrients, maintenance of soil structure, and availability of water for plants. The depletion of SOM is a common occurrence when intensive cultivation is implemented, as it is greatly influenced by agricultural management practises. There has been a resurgence of interest in the utilisation of organic soil amendments, including plant residues, manures, and composts, as a result of the financial implications and environmental risks associated with the application of chemical fertilisers. Composts present various benefits in comparison to plant residues, such as reduced volume, slower decomposition rates, and the opportunity for waste recycling (Bernal et al., 2009). Composts possess a diverse range of characteristics that can be altered in order to achieve the intended impacts on soil properties and plant growth, contingent upon the particular feedstocks and processing techniques employed. However, it is imperative to establish a comprehensive comprehension of the correlation between the characteristics of compost and their impact on the well-being of soil and plants. The utilisation of waste-driven bioproducts as agricultural fertiliser has the potential to result in the retention of antibiotics within the soil, which can subsequently be absorbed by plants and subsequently enter the food chain. This process poses a potential risk to human health over time. In order to mitigate adverse environmental impacts, it is imperative to ensure the proper disposal of organic waste and various forms of animal excrement (Wang et al., 2022). Fig. 7.10 depicts the process of ploughing and cultivation of *A. esculentus and S. lycopersicum* in soils after amending with ACC, PHC, and LCC.

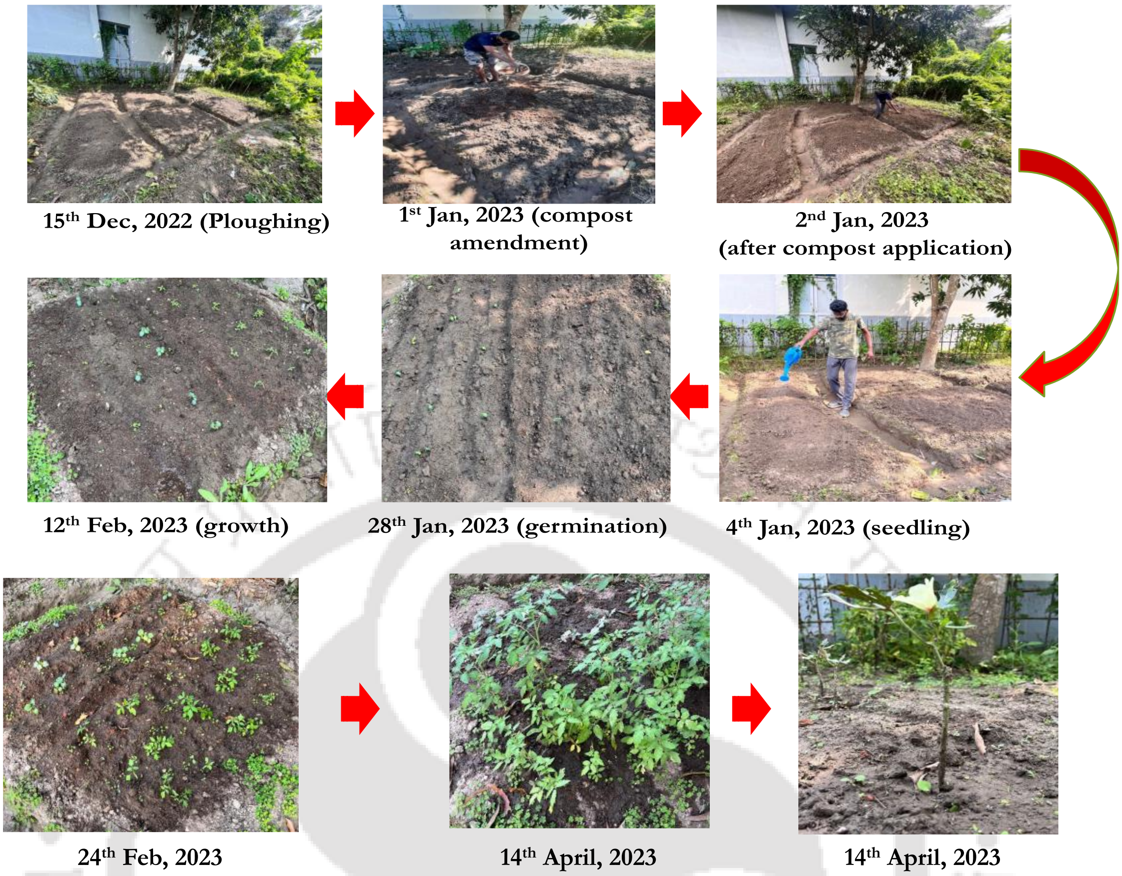


Fig. 7.10. Field applicability study of *A. esculentus and S. lycopersicum* plants in ACC, PHC, and LCC amended soils

7.2.1. Soil Characterization

The soil in this investigation exhibits a coarse structure as a result of its elevated sand content and comparatively diminished quantities of silt and clay. The soil characterization was presented in Table 7.2. The coloration of the soil varies from moderately deep to very deep, exhibiting shades of grey to mottled grey. The soil's pH is a fundamental factor that exerts influence over the solubility, concentration, and mobility of both micronutrients and macronutrients within the soil matrix. Consequently, this pH-dependent phenomenon significantly affects the growth and developmental processes of plants. The pH range considered optimal for agricultural applications typically falls between 6 and 7. In the present study, the soil pH value was found to be 6.9, which falls within this recommended range. The SOM content was measured to be 10.2%, indicating a relatively low level. Similarly, the soil SOC content was determined to be 5.66%, also indicating a relatively low concentration. Reduced levels of SOM and SOC can lead to diminished soil fertility, a decline in water retention capacity, and an elevation in susceptibility to erosion. The CEC of the soil was determined to be 15 c mol/kg. A diminished CEC has been found to be associated with soil degradation, decreased soil fertility, and diminished crop yields. The cause of this phenomenon can be attributed to the reduced nutrient retention capacity of the soil, leading to nutrient depletion and insufficient absorption by plants. According to Jain and Kalamdhad (2020), the presence of this substance can also increase the susceptibility of soil to erosion and reduce its capacity to retain water.

EC serves as an indicator of the concentration of water-soluble salts present in the soil solution. It is worth noting that excessive salinity in the soil can have detrimental effects on agricultural productivity. The optimal range of EC for alluvial soil typically falls between 0.2 mS/cm and 0.8 mS/cm. In the present study, the EC of the soil under investigation is within this recommended range, measuring at 0.29 mS/cm.

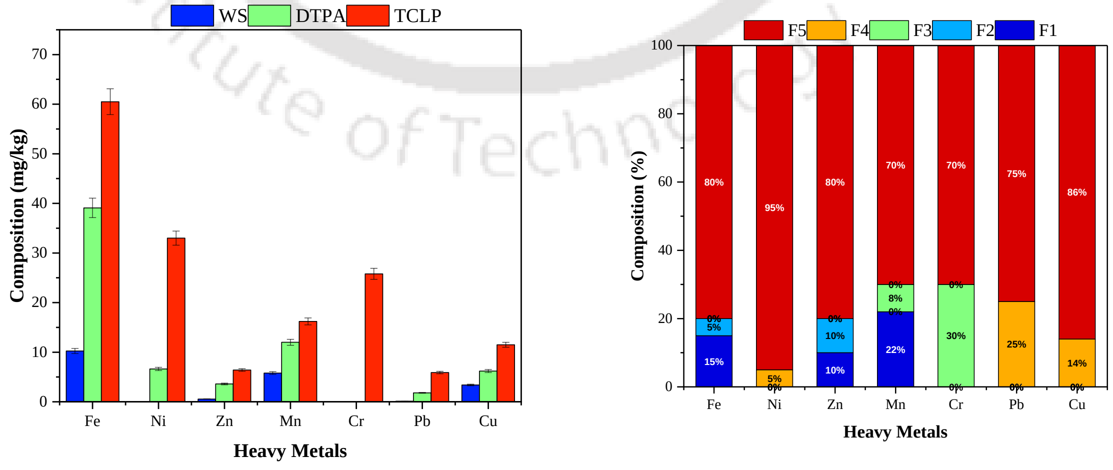
The density of soil is primarily affected by its texture, SOM, and the amount of moisture it contains. The present study determined the bulk density of the soil to be 1.54 g/cm^3. SOM is a crucial constituent responsible for providing nutrients to the soil. However, in the specific context being discussed, the concentration of SOM was found to be low in the alluvial soil. This can be attributed to the frequent occurrence of rainfall and recurrent floods in the study area, which result in the erosion and removal of SOM from the soil. The soil was determined to have a nitrogen content of 0.54% and an available phosphorus content of 1.01 g/kg.

The concentrations of Fe, Ni, and Pb in soil were detected and analysed. Table 7.2 presents the concentrations of HMs. The primary cause for the accumulation of HMs in alluvial soil can be attributed to the source. Nevertheless, it is important to note that the concentration of HM does not necessarily correlate with its toxicity. The assessment of HMs toxicity relies on the bioavailable fractions of HM. Fig. 7.11 (a) illustrates the bioavailable fractions of HMs in soil. The bioavailability of HMs in the soil collected downstream of the Brahmaputra river was found to be lower in comparison. Fig. 7.11 (b) illustrates the chemical speciation of alluvial soil. The majority of metallic elements have less ability to be bioavailable and exhibit immobile characteristics, particularly in the form of F5. This suggests that plants possess a lower capacity to absorb heavy metals via their root system.

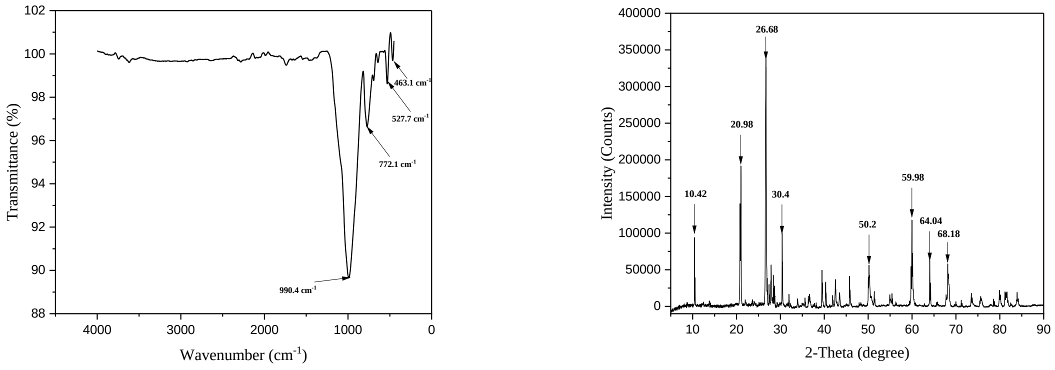
Table 7.2. Initial Characterization of soil used in the field study

Parameter	**Control**
Moisture content (%)	21.50 ± 0.4
Soil Organic matter (%)	10.2 ± 0.2

pH	6.24 ± 0.03
EC (mS/cm)	0.56 ± 0.02
Soil organic carbon (%)	1.20 ± 0.4
Bulk density (kg/m^3)	1.38 ± 0.2
Specific Gravity	2.42 ± 0.02
Porosity (%)	42.97 ± 2.6
WHC (%)	13 ± 1.3
Cation exchange capacity (CEC) (c mol/kg)	16.3 ± 2.4
TKN (%)	0.23 ± 0.01
Available phosphorus (g/kg)	0.61 ± 0.02
Total phosphorus (g/kg)	1.73 ± 0.4
Na (g/kg)	1.42 ± 0.7
K (g/kg)	3.21 ± 0.42
Iron (mg/kg)	3234 ± 11.4
Nickel (mg/kg)	23 ± 2.3
Zinc (mg/kg)	29 ± 3.1
Manganese (mg/kg)	57 ± 1.6
Chromium (mg/kg)	8.3 ± 1.1
Cadmium (mg/kg)	0
Lead (mg/kg)	28.4 ± 2.5
Copper (mg/kg)	12.4 ± 0.3



(a) (b)



(c) (d)

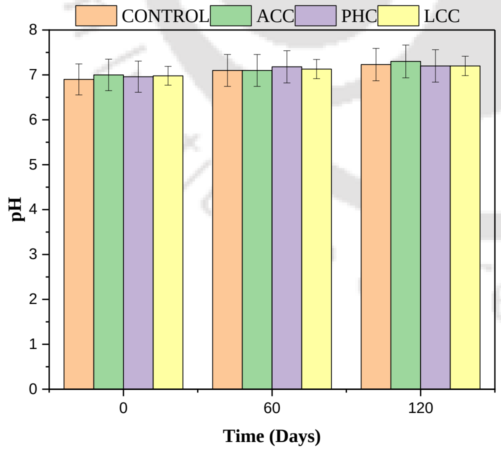
Fig. 7.11. (a) Bioavailable and leachable fractions; (b) Chemical speciation of HMs of alluvial soil; (c) FTIR characterization of alluvial soil; (d) PXRD characterization of field soil

7.2.2. Effects of physical and chemical parameters on soil health

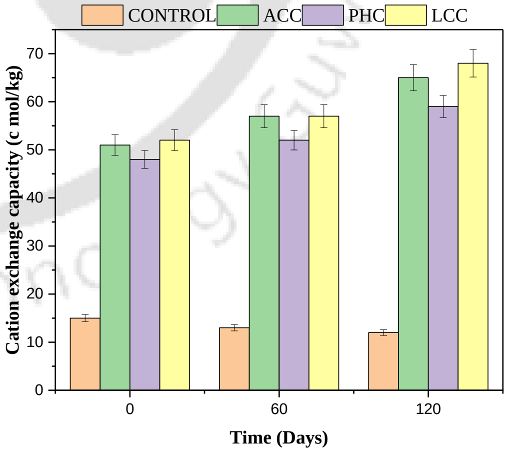
In a general context, the incorporation of compost into soil has been observed to augment the SOM content, thereby potentially enhancing both soil fertility and structure. Compost is enriched with essential nutrients, including nitrogen, phosphorus, and potassium, which have the potential to enhance plant growth. The specific impact of compost on soil characteristics is contingent upon the quantity of compost incorporated and the duration that has transpired since its application. In the present investigation, TWC was supplemented with soil at a concentration of 20% (w/w) for a duration of 120 days. The samples were collected and analysed on the initial day, as well as on the initial, 30th, 60th, and 120th days. The soil's CEC was observed to exhibit an increase subsequent to the application of compost. Fig. 7.12 depicts the upward trajectory of CEC observed throughout the duration of the investigation. On the 120th day of the experiment, the CEC of the soil samples treated with ACC, PHC, and LCC were observed to have increased by 21.5, 18.6, and 23.5 respectively, in comparison to the initial day samples. The utilization of TWC exhibited the capacity to augment the availability of essential plant nutrients, such as nitrogen, phosphorus, and potassium. The introduction of nutrients has the potential to stimulate plant growth and increase the release of organic compounds from roots, thereby enhancing the soil's CEC through the addition of extra organic matter and microbial activity.

The introduction of AAC, PHC, and LCC into the soil has resulted in fluctuations in the concentration of SOM, leading to a reduction of 38, 34.6, and 30.7% in the samples collected on the 120th day compared to the samples collected on the initial day. With the addition of compost as an amendment, there has been an observed increase in SOM content during the initial period. However, it was observed that after the 120th day, the concentrations of SOM exhibited a relative decrease compared to the concentrations on the initial day. This decline in SOM concentrations can be attributed to the initial burst of microbial activity triggered by the addition of compost, which leads to accelerated degradation of organic matter. Consequently, this degradation process causes a reduction in SOC levels (Jain and Kalamdhad, 2020). Fig. 7.12 (c) and (d) depict the declining patterns observed in SOM and SOC within the scope of the investigation.

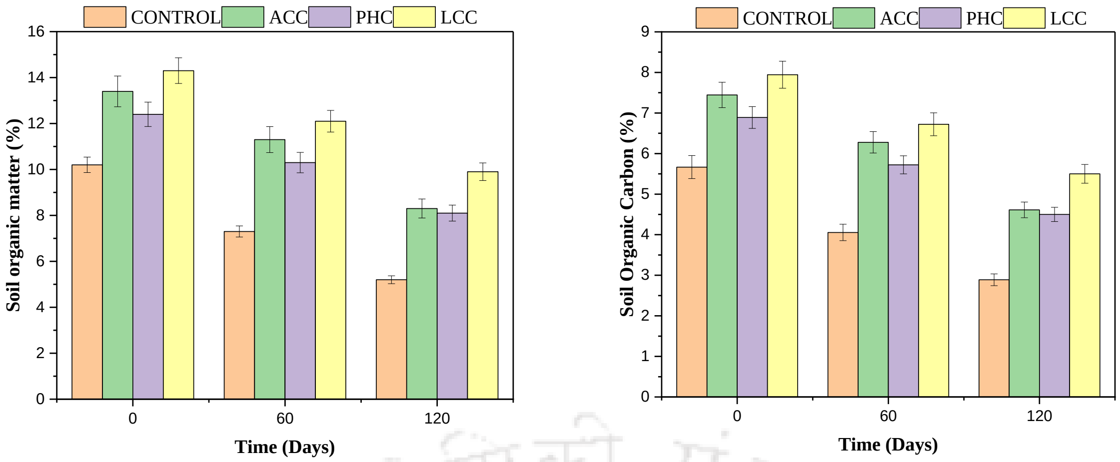
The observed bulk density values range from 1.31 to 1.38 kg/m^3. Limited variations were observed in the TWC when amended with soil. However, it was observed that there was an increase in bulk density from the initial day to the 120th day, as depicted in Fig. 7.13 (a). The TWC possesses an optimal quantity of volatile content, which functions as a nourishing substrate for soil microorganisms. When compost is incorporated into soil, the microorganisms present in the soil initiate the decomposition process of the organic material within the compost. This decomposition process may result in the emission of gases and the formation of stable organic matter. This process has the potential to induce compaction in the soil, resulting in an elevation of its bulk density. The presence of a significant moisture content in compost can have an impact on the bulk density of soil when it is incorporated into the soil matrix. The introduction of compost into soil has the potential to augment the soil's water content, thereby inducing an increase in soil compaction and bulk density.



(a)



(b)



(c) (d)

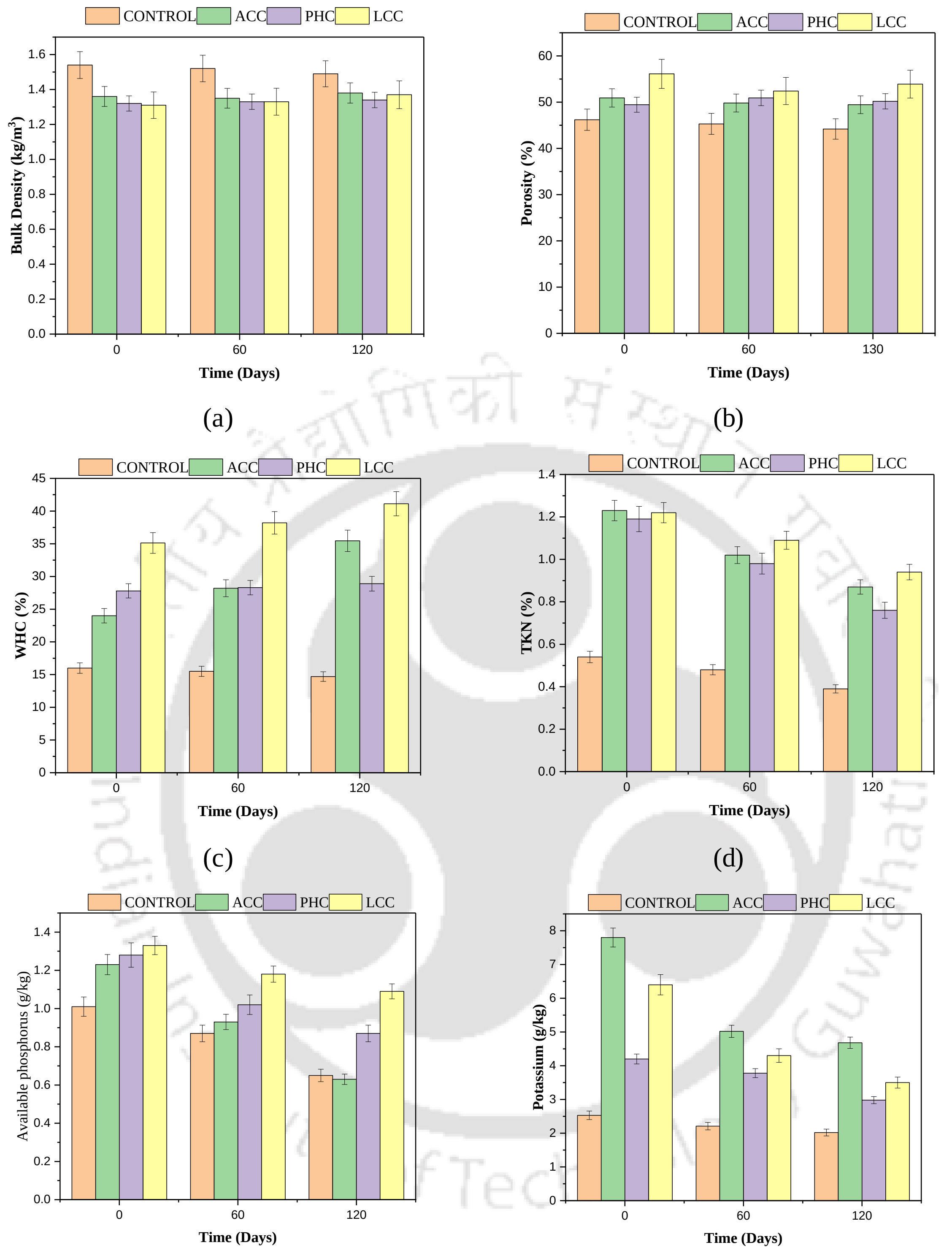
Fig. 7.12. Characterization of soil amended with TWC from initial day to 120th day (a) pH, (b) CEC, (c) SOM (d) SOC

*ACC: *A. conyzoides* compost, PHC: *P. hysterophorus* compost, LCC: *L. camara* compost

The introduction of TWC into the soil led to a notable reduction in porosity over time. Specifically, the porosity decreased from 50.9 to 49.4%, 49.4 to 50.1%, and 56.1 to 53.9% in the 120th day sample compared to the initial day (see Fig. 7.13 (b)). In their study, Jain and Kalamdhad (2020) have documented a decline in porosity. The observed rise in porosity subsequent to the application of compost to soil can be attributed to the introduction of organic matter and the subsequent development of larger pores. Nevertheless, as time progresses, various factors may contribute to a reduction in porosity. The presence of fine particles within compost has the potential to occupy pore spaces, thereby causing a decrease in overall porosity. The decomposition of organic matter in compost by soil microorganisms results in the release of carbon dioxide, leading to soil compaction and a subsequent reduction in pore space. Over a period of time, the infiltration of water and various external forces can induce compaction of soil particles, leading to a decrease in porosity.

WHC is a term used to describe the capacity of soil to retain water. Soils possessing a high WHC have the ability to retain larger quantities of water, thereby facilitating its availability for plant growth while concurrently mitigating runoff. Soils characterized by a low WHC have the potential to impede plant growth and productivity, while concurrently elevating the susceptibility to erosion. The utilization of ACC, PHC, and LCC on soil resulted in a significant enhancement in WHC. Specifically, the WHC levels exhibited an increase from 24 to 35.5%, 27.8 to 28.9, and 35.1 to 41.1% in the 120th day sample as compared to the initial day sample (refer to Fig. 7.13 (c)). Over the course of time, it is plausible that the augmentation in water holding capacity (WHC) could persist due to the progressive decomposition of organic matter within the compost. This process would subsequently enhance the soil's arrangement and augment its capacity to retain water (Cooper and DeMarco, 2023). The presence of organic matter in compost has the potential to enhance the CEC of the soil, thereby enabling it to retain a greater quantity of nutrients and water. Nevertheless, it is plausible that the rate of growth could decelerate as the compost becomes more thoroughly assimilated into the soil and the soil attains its utmost potential for water retention.

The presence of essential nutrients in soil plays a crucial role in facilitating optimal plant development and sustaining the overall fertility of the soil. Essential nutrients, including nitrogen, phosphorus, and potassium, play a crucial role in facilitating plant development, while the presence of micronutrients such as zinc, iron, and manganese is imperative for promoting optimal and robust plant growth. Soil that is abundant in nutrients exhibits enhanced resilience against environmental stressors, including drought and pests, and plays a crucial role in mitigating soil erosion, thereby contributing to the preservation of ecological equilibrium. In conclusion, the presence of soil that is abundant in nutrients is essential for facilitating the growth of crops that are high in nutritional value. This factor plays a critical role in safeguarding human well-being and mitigating the risk of nutrient insufficiencies. The present investigation has revealed a decrease in nutrient content, specifically TKN, available phosphorus, and potassium, subsequent to the application. One of the primary factors contributing to this phenomenon can be attributed to the potential immobilization or sequestration of nutrients within the compost, resulting in reduced accessibility for plants. This phenomenon occurs due to the utilization of available carbon sources by soil microorganisms, such as organic materials found in compost, in order to fulfil their energy requirements. Consequently, the microorganisms have the ability to metabolize a portion of the accessible nutrients, leading to a temporary unavailability of these nutrients for plant absorption. Additionally, it is possible for certain nutrients present in the compost to be lost as a result of processes such as leaching, volatilization, or uptake by plants. Moreover, the nutritional composition of the initial organic matter utilized for compost production may impact the nutrient concentration of the final compost and subsequently affect the nutritional properties of the soil. Therefore, it is imperative to closely monitor the nutrient levels in the soil and adjust the application of compost or other organic amendments accordingly in order to sustain optimal nutrient levels for the growth of plants. Fig. 7.13 (d), (e), and (f) illustrate the changes in the nutrient composition of the soil as observed in the study.



(a) (b) (c) (d) (e) (f)

Fig. 7.13. Geotechnical characterization of soil amended with TWC during the study till 120 days (a) Bulk density, (b) Porosity, (c) WHC, (d) TKN, (e) Available phosphorus, and (f) Potassium

*ACC: *A. conyzoides* compost, PHC: *P. hysterophorus* compost, LCC: *L. camara* compost

7.2.3. Morphological assessments and Macronutrient dynamics of *A. esculentus and S. lycopersicum*

Morphological assessments

The evaluation of morphological parameters holds significance in plant studies due to its ability to provide insights into the physical attributes of a plant. The aforementioned attributes can be employed for the purpose of distinguishing various botanical taxa, assessing the well-being of plants, and overseeing their progression and maturation (Mastrogianni et al., 2021). The present investigation involved the assessment of various morphological parameters, including germination percentage, root length, shoot length, number of leaves per plant, number of fruits per pot, and length of fruits. These measurements were conducted after a period of 120 days, as depicted in Table 7.3. In each plot, a combined total of 30 seeds of *A. esculentus* and *S. lycopersicum* were planted. The germination percentage of plants cultivated in soil supplemented with ACC, PHC, and LCC reached a maximum of 83.3, 83.3, and 100% in *A. esculentus* and 83.3, 86.6, and 93.3%, respectively. The maximum number of leaves per plant was observed on the 60th day. Following a 90-day observational period, it was noted that there was a reduction in the quantity of leaves on the plant. This decline can be attributed to leaf senescence, a naturally occurring physiological process in which plant leaves progressively deteriorate and ultimately perish. Leaf senescence can be induced by various factors, including environmental stress, hormonal fluctuations, and nutrient insufficiencies. This process plays a vital role in the plant's life cycle by facilitating the redistribution of nutrients from ageing leaves to developing tissues. The observed decrease in leaf count within the experiment indicates that the *A. esculentus* and *S. lycopersicum* plants had reached the culmination of their growth cycle and experienced leaf senescence as an inherent component of their life cycle (Goswami et al., 2017). The mean height of *A. esculentus* plants in soil amended with ACC, PHC, and LCC was found to be 102, 86, and 102 cm, respectively. In contrast, *S. lycopersicum* plants exhibited mean heights of 122, 126, and 127 cm in the same soil conditions, respectively. The mean vertical root length of *A. esculentus* plants in soil amended with ACC, PHC, and LCC was recorded as 35, 36, and 29 cm, respectively. In contrast, *S. lycopersicum* plants exhibited mean vertical root lengths of 56, 48, and 39 cm in the same soil amendments, respectively. The assessment of plant height and vertical root length is of great significance in agricultural studies owing to its capacity to provide valuable insights into crop well-being, growth dynamics, nutrient uptake, reactions to environmental stressors, and overall yield. The monitoring of these parameters plays a crucial role in enabling the process of making informed decisions and implementing efficient management strategies to ensure the long-term viability and achievement of crop production. A total of 35, 39, and 45 fruits of *A. esculentus* and 28, 24, and 27 fruits of *S. lycopersicum* were harvested from each plot of soil amended with ACC, PHC, and LCC, respectively, after a period of 75 days (referred to as the 1st harvesting). The observed range of fruit length for *A. esculentus* was between 9 and 15 cm. The mean fruit biomass of *A. esculentus* in soil treated with ACC, PHC, and LCC amendments was recorded as 0.02, 0.03, and 0.02 kg, respectively. In contrast, the mean fruit biomass of *S. lycopersicum* in soil treated with ACC, PHC, and LCC amendments was recorded as 0.04, 0.06, and 0.03 kg, respectively. The measurement of fruit length and biomass holds significance in the field of plant studies due to its potential to yield valuable insights into various aspects of a plant's reproductive strategy, mechanisms of seed dispersal, and ecological interactions. There is frequently a positive association between fruit length and biomass, and the size and quantity of seeds generated by the plant. Typically, fruits with greater length exhibit a higher propensity for harbouring an increased quantity and size of seeds, thereby influencing the plant's reproductive efficacy.

Macronutrient assessment

The analysis of nutrient content in *A. esculentus* and *S. lycopersicum* assumes significance in the context of compost amendment with soil at varying proportions, as it enables growers to enhance plant growth and productivity in an optimal manner. The macronutrients K, Na, and Ca play a crucial role in the growth and development of plants. The presence and abundance of these nutrients in the soil can have an impact on the nutrient composition of *A. esculentus* and *S. lycopersicum*. The changes in nutrient composition were illustrated in the Table 7.3. The concentrations of K, Na, and Ca in *A. esculentus* fruits exhibited a statistically significant increase of 49, 18, and 20% respectively when cultivated in ACC. Similarly, when cultivated in PHC, the concentrations of K, Na, and Ca showed an increase of 54.8, 9, and 4% respectively. Furthermore, when cultivated in LCC, the concentrations of K, Na, and Ca demonstrated an increase of 55.9, 16.8, and 20.5% respectively. These findings indicate a notable difference in the concentrations of these elements compared to *A. esculentus* grown under control soil. The concentrations of K, Na, and Ca in *S. lycopersicum* fruits exhibited a statistically significant increase of 43.9, 30.9, and 25.8% respectively when cultivated in ACC. Similarly, when cultivated in PHC, the concentrations of K, Na, and Ca showed a significant rise of 41.1, 33.7, and 23.9% respectively. Furthermore, when cultivated in LCC, the concentrations of K, Na, and Ca demonstrated a significant increase of 42.9, 32.4, and 21.9% respectively, compared to *S. lycopersicum* grown under control soil. The levels of Na and Ca in the fruits of *A. esculentus* and *S. lycopersicum*, cultivated in soils amended with PHC, were observed to be relatively lower when compared to soils amended with ACC and LCC. K, Na, and Ca are vital minerals that are necessary for the healthy growth and development of *A. esculentus* and *S. lycopersicum*. These minerals are integral to various physiological processes within the plant. Potassium (K) plays a crucial role in the maintenance of cellular osmotic balance, regulation of water uptake, and facilitation of nutrient transport within plants. Additionally, it plays a role in enhancing the plant's ability to withstand a range of abiotic and biotic stresses, including drought, salinity, and diseases. Sodium (Na) is not considered an indispensable nutrient for plants; however, it can confer certain advantages when present in limited amounts. The application of this technique has the potential to augment the plant's capacity to withstand pests and diseases, as well as facilitate the regulation of its water equilibrium. Calcium (Ca) plays a crucial role in maintaining the structural integrity of cell walls and is also involved in regulating a range of physiological processes, including cell division and expansion. Additionally, it is implicated in the facilitation of enzyme activation and the modulation of membrane permeability (Kauser et al., 2022).

Table 7.3. (a) Morphological parameters of *A. esculentus* in ACC, PHC, and LCC amended soils, (b) Morphological parameters of *S. lycopersicum* in ACC, PHC, and LCC amended soils

(a)

Parameters	ACC	PHC	LCC	Soil
Germination Index Percentage (%)	95	90	90	90
Height (cm)	102 ± 7	86 ± 3	126 ± 5	61 ± 5
Vertical length of root (cm)	35 ± 4	36 ± 3.2	29 ± 4.2	16 ± 2
Horizontal Length of the root (cm)	13 ± 4	15 ± 3.1	12 ± 2	6.5 ± 1.3
Number of fruits per site	32	36	33	28
K (mg/kg)	550 ± 10.2	620 ± 12.1	635 ± 11.3	280 ± 10.3
Ca (mg/kg)	150 ± 9.2	136 ± 9.3	148 ± 13.2	123 ± 10.2
Na (mg/kg)	300 ± 11.3	254 ± 11.5	302 ± 10.4	240 ± 9.3

(b)

Parameters	ACC	PHC	LCC	Soil
Germination Index Percentage (%)	85	90	85	75
Height (cm)	122 ± 8	126 ± 8	127 ± 11	64 ± 5
Vertical length of root (cm)	56 ± 5.3	48 ± 4.5	39 ± 4.1	11 ± 4.6

Horizontal Length of the root (cm)	11 ± 2.3	10 ± 2.1	12 ± 3.5	8 ± 2
Number of fruits per site	48	47	49	31
K (mg/kg)	332 ± 11.2	316 ± 12.4	326 ± 15.2	186 ± 12.1
Ca (mg/kg)	142 ± 13.1	148 ± 14.2	145 ± 12.8	98 ± 11.3
Na (mg/kg)	120 ± 14.8	117 ± 11.5	114 ± 10.7	89 ± 7.3

HMs characterization in A. esculentus and S. lycopersicum

The levels of HMs present in the fruits of *A. esculentus* and *S. lycopersicum* were determined to be within the acceptable limits as reported by the FSSAI (2011). Table 7.4 illustrates the concentrations of HMs in the fruits of *A. esculentus* and *S. lycopersicum* plants. The concentrations of Fe, Mn, Zn, and Pb in the fruits of *A. esculentus*, which were grown in soils amended with ACC, PHC, and LCC, ranged from 3.9 to 4.1, 1.2 to 1.5, 1.3 to 2.1, and 0.01 to 0.02 mg/kg, respectively. The concentrations of Fe, Mn, and Zn in the fruits of *S. lycopersicum*, when grown in soils amended with ACC, PHC, and LCC, ranged from 3.5 to 3.7, 0.5 to 0.9, and 0.65 to 0.76 mg/kg, respectively. HMs such as Cd and Cr were not identified in the fruits of *A. esculentus*. In contrast, the presence of HMs such as Ni, Pb, Cr, Cd, and Cu was not observed in the fruits of *S. lycopersicum* plants that were exposed to soils amended with ACC, PHC, and LCC. The application of compost has the potential to modify the soil's pH. The solubility and mobility of HMs in soil can be influenced by specific pH levels. In certain instances, the application of compost has the potential to increase the pH levels of the soil and making the HMs to precipitate on to the soil, thereby diminishing the accessibility of HMs for absorption by plants. Compost is comprised of organic matter and possesses functional groups that have the capability to form complexes with heavy metals, thereby diminishing their mobility within the soil. The phenomenon referred to as metal immobilisation has the capacity to restrict the movement of HMs from the soil to the roots of plants.

Table 7.4. (a) Heavy metal concentrations in *A. esculentes* fruit in ACC, PHC, and LCC amended soils, (b) Heavy metal concentrations in *S. lycopersicum* fruit in ACC, PHC, and LCC amended soils

(a)

Parameters	**Control**	**ACC**	**PHC**	**LCC**
Ni (mg/kg)	0	0.05	0.02	0.03
Pb (mg/kg)	0.03	0.02	0.01	0.02

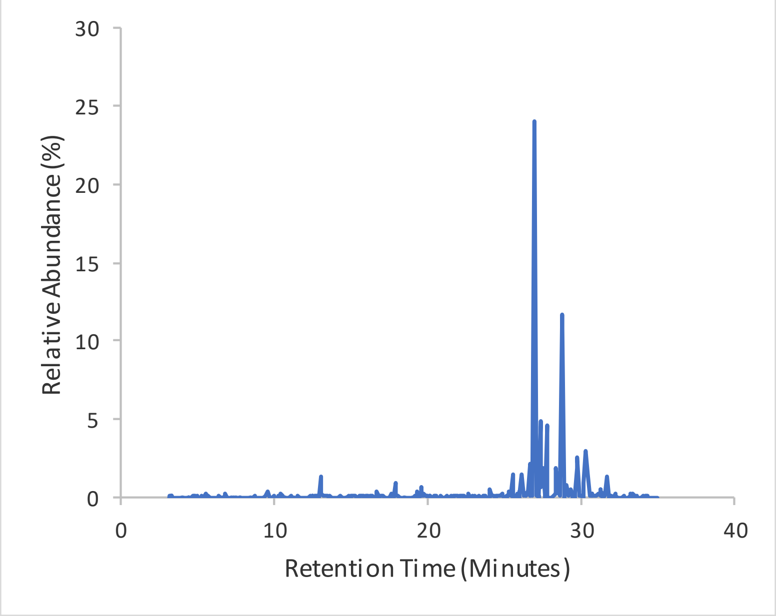
Fe (mg/kg)	5.2	4.1	4.3	3.9
Mn(mg/kg)	2.1	1.5	1.3	1.2
Zn(mg/kg)	3.7	2.1	1.8	1.3
Cu (mg/kg)	1.13	0.8	0.73	0.63
Cd (mg/kg)	0	0	0	0
Cr (mg/kg)	0	0	0	0

(b)

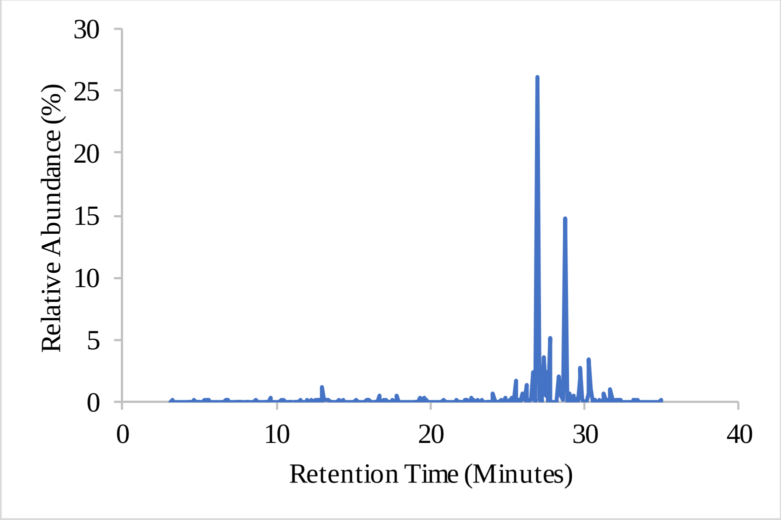
Parameters	**Control**	**ACC**	**PHC**	**LCC**
Ni (mg/kg)	0	0	0	0
Pb (mg/kg)	0	0.01	0.01	0.01
Fe (mg/kg)	3.2	3.6	3.5	3.7
Mn(mg/kg)	1.3	0.9	0.5	0.8
Zn(mg/kg)	1.25	0.76	0.67	0.65
Cu (mg/kg)	0	0	0	0
Cd (mg/kg)	0	0	0	0
Cr (mg/kg)	0	0	0	0

7.2.4. Assessment of toxic organic compounds in *A. esculentus and S. lycopersicum*

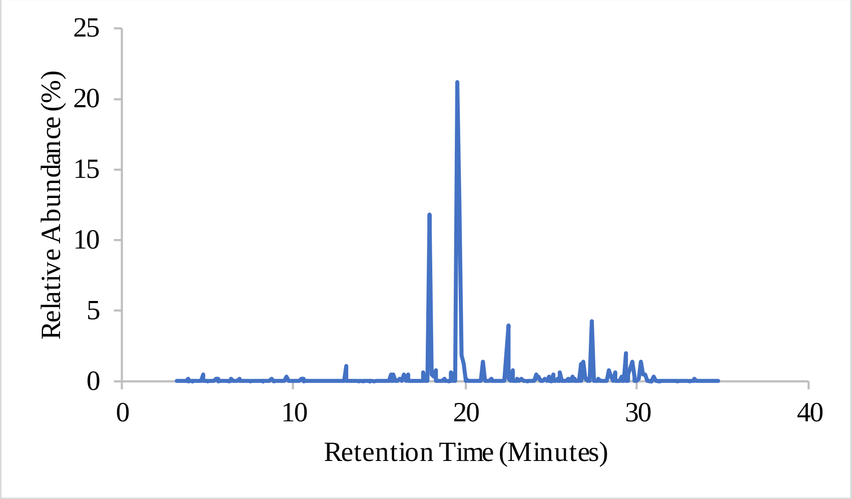
The analysis of fruits of *A. esculentus* and *S. lycopersicum* cultivated in various amendments of TWC with soil has been conducted to examine the presence of toxic organic compounds. This investigation utilised a novel method for identifying compounds by the application of GC-MS. The presence of toxic organic compounds in terrestrial weeds and their compost samples was found to be absent in the fruits of *A. esculentus* and *S. lycopersicum* plants, as per the observations made. Plants possess many biological systems that serve to mitigate the absorption and accumulation of toxic compounds. The processes encompass selective transporters and barriers that serve to impede the ingress of detrimental chemicals into the plant's vascular system. Plants also possess enzymatic systems and metabolic pathways that facilitate the detoxification or conversion of hazardous substances into less deleterious forms. The process of detoxification can take place in different plant tissues, hence minimising the likelihood of hazardous chemicals reaching the fruit. Organic compounds such as Rhodopin, Vitamin-E, and Oleic acid have been detected in the extracts of *A. esculentus* and *S. lycopersicum* fruits. Theses organic compounds are very much crucial for human beings, as the fruits of these are edible materials. Vitamin E is a very effective antioxidant that plays a crucial role in safeguarding cells from the detrimental effects of free radicals, hence mitigating the risk of chronic ailments and the ageing process. Oleic acid, a monounsaturated fatty acid, has been widely recognised for its beneficial effects on cardiovascular health. Consumption of this substance has been shown to have the potential to decrease levels of undesirable low-density lipoprotein (LDL) cholesterol, hence mitigating the likelihood of developing cardiovascular disease. Oleic acid has been shown to exert possible effects on appetite regulation and satiety, which might potentially contribute to the management of body weight. Rhodopin, a flavonoid compound, has antioxidant properties by effectively counteracting detrimental free radicals inside the human body. Fig. 7.14 and Table 7.5 depicts the GC-MS results of fruits of *A. esculentus* and *S. lycopersicum.*



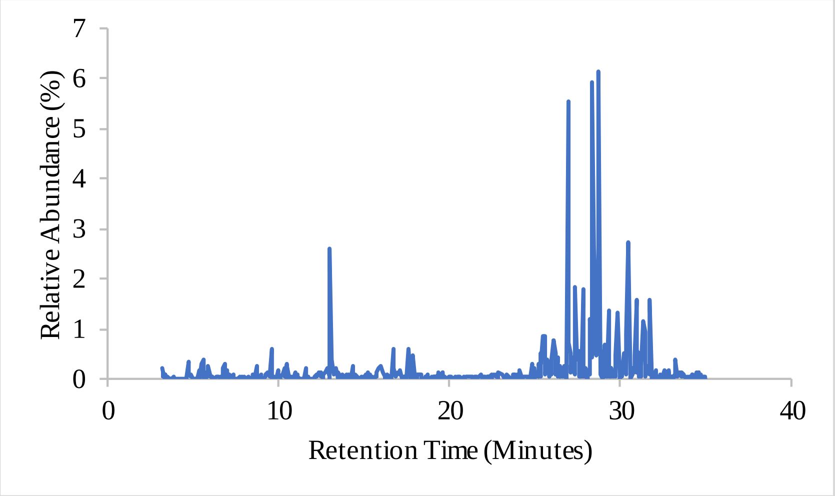
(a) ACC_Okra



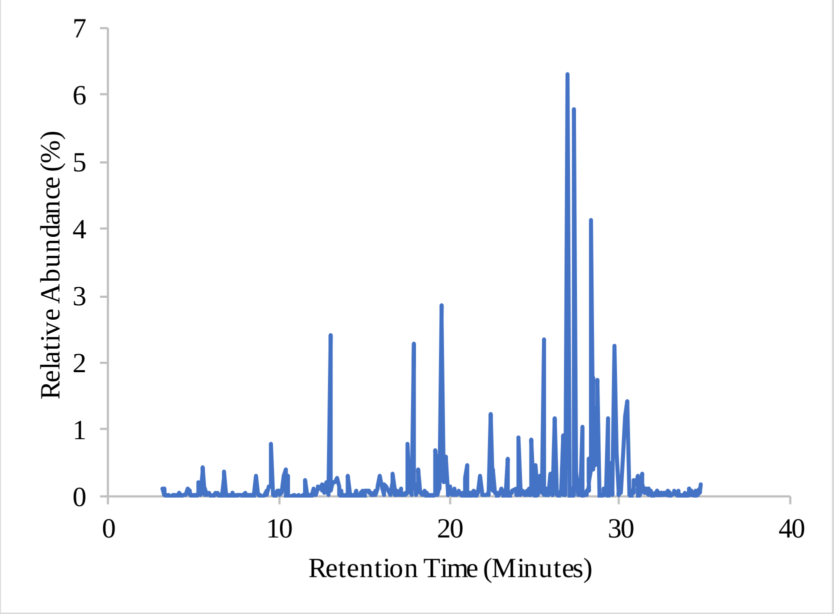
(b) PHC_Okra



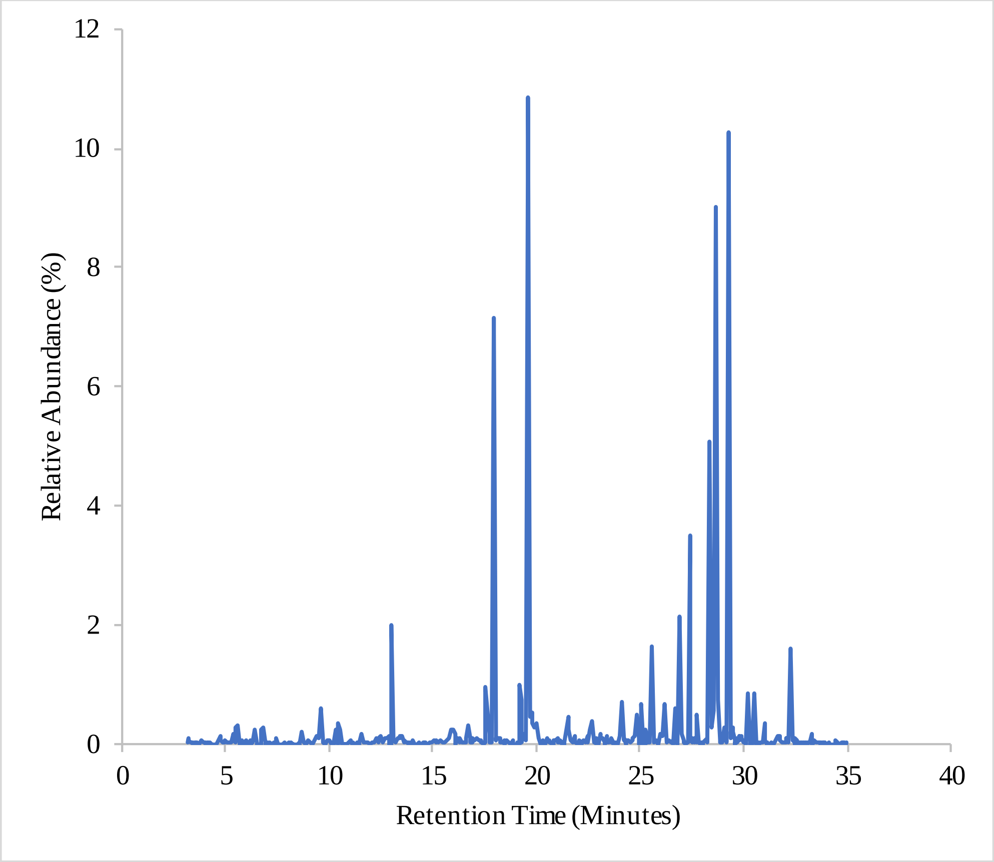
(f) LCC_Okra



(c) ACC_Tomato



(b) PHC_Tomato



(e) LCC_Tomato

Fig. 7.14. GC-MS analysis of fruits of *A. esculentus* and *S. lycopersicum* plants ACC: *A. conyozides* compost, PHC: *P. hysterophorus* compost, LCC: *L. camara* compost

Table 7.5. List of organic compounds detected in the fruits of *A. esculentus* and *S. lycopersicum* plants

Organic Compounds	ACC	PHC	LCC	Control
Ageratochromene	-	-	-	-
Farnesene	-	-	-	-
Diethyl Phthalate	-	-	-	-
Stigmasterol	-	-	-	-
Caryophyllene oxide	-	-	-	-
Phenol	+	+	+	+
Vitamin E	+	+	+	+
Beta-Sitosterol	+	+	+	-
Rhodopin	+	+	+	+
Tetratricontane	+	+	+	-

Oleic Acid	-	-	+	-
Cis-Vaccenic acid	-	+	-	-

ACC: *A. conyozides* compost, PHC: *P. hysterophorus* compost, LCC: *L. camara* compost

◆

Nature demands a gift for everything that it gives, so what we have to keep doing, is returning [leaves & compost materials] back to the soil, then we're continuously giving the gifts to nature, because we have a return cycle..

Geoff Lawton
1954-present

8

Conclusions and Future Recommendations

8.1. SUMMARY AND CONCLUSIONS

The key conclusion insights were made from the experimental results, the biological management of the most intrusive terrestrial weeds *A. conyzoides*, *P. hysterophorus* and *L. camara* can be managed by in vessel composting technique "Rotary drum composting". The highest temperature occurred in composting process found in *A. conyzoides*, *P. hysterophorus* and *L. camara* was 58, 59.5, and 59.9°C, which indicates the maximum degradation of organic matter in thermophilic phase. The reduction in volatile content in the *A. conyzoides*, *P. hysterophorus* and *L. camara* final compost products was 35.1, 35 and 32.4% from the initial day of composting process, which indicates most of the organic matter got converted into inorganic substances in composting process. The TKN of *A. conyzoides*, *P. hysterophorus* and *L. camara* final compost products increased to 3.2, 2.4 and 2.7%. The reduction in lignin, hemicellulose and cellulose of *A. conyzoides*, *P. hysterophorus* and *L. camara* was 61.7, 47.1 and 40.1%; 43.5, 50.7 and 57.3%; 49.1, 50.3 and 48.5% were observed in the final compost compared to the initial day bend.

The order of water-soluble metal concentration in the composted *A. conyzoides*, *P. hysterophorus*, and *L. camara* was: Fe > Mn > Zn >Ni > Cr > Pb> Cu > Cd; Fe > Mn > Pb > Ni > Cr > Zn > Cd > Cu; Fe > Mn > Ni > Pb > Cr > Zn > Cu > Cd, respectively. The sequence of metal contents in DTPA extracts at the end of 20 days composting of *A. conyzoides* was Fe > Mn > Cu > Cr > Zn > Pb > Cd > Ni. The sequence of metal contents in TCLP extracts at the end of 20 days composting of *A. conyzoides* was Fe > Mn > Pb > Cr > Zn > Cu> Ni > Cd. The ascending sequence of bioavailable fractions in composted *P. hysterophorus* in water soluble, DTPA and TCLP portions was Fe < Cu < Pb < Mn < Zn < Ni < Cr < Cd; Pb < Mn < Cr < Cu < Zn < Ni < Cd < Fe; and Pb < Cr < Mn < Cu < Fe < Cd < Zn < Ni. Whereas in composted *L.*

camara, the descending order of heavy HMs concentration. The order of water-soluble metals in composted *L. camara* was found to be as follows: Fe > Mn > Zn > Pb > Cu > Cr > Ni > Cd. The metal concentration sequence observed in the DTPA extracts of composted *L. camara* on initial and 20th day was Fe > Mn > Zn > Pb > Cu > Ni> Cr > Cd. The sequence of metal concentrations in TCLP extract in the initial day and at the end of 20 days composting of *L. camara* was Fe > Mn > Pb > Zn > Cu > Ni> Cr > Cd. Even though total metal concentration is an important indicator of pollution risks, but it essentially provides little or no indication of their specific bioavailability or mobility in soil or plant. The water soluble, DTPA extractable, TCLP extractable heavy metals showed a very little percentage of the total metal concentration, also its concentration.

The potentiality of phytotoxicity and cyto-genotoxicity inhibition in *V. radiata* and *A. cepa* plant models was reduced after 20 days of composting and showed a maximum reduction in 25% concentrated 20th day compost extract. The Cytotoxicity and Genotoxicity results has given a improved version of toxicity results in determining the status of cell growth, NA and CA in test plant *A. cepa* against the *A. conyzoides, P. hysterophorus, L. camara* and various phased compost extracts. The MI found in 100% *A. conyzoides, P. hysterophorus, L. camara* extract were 17.5, 18.6, and 22.2% and in the 25% 20th day compost extracts was 70.6, 72.1, and 67.3%, which indicates an increase in proper cell divisions during composting process. As a whole the NA and CA has decreased during composting process. The GC-MS, GC-FID findings indicate that the toxic organic compounds such as ester, phenol, furan and pentacyclic compounds have been reduced and transformed during the composting process.

The experimental results yielded significant insights, indicating that compost derived from terrestrial weeds through in-vessel composting can be utilised as an effective soil conditioner to enhance plant productivity. On the 120th day of A10, A20, A35, A45, P10, P20, P35, P45, L10, L20, L35 and L45, the CEC values experienced an increase of 38.7, 21.5, 17.6, 12.6, 40.4, 18.6, 12.6, 14.7, 34.6, 23.5, 15.2 and 14.2%, respectively, when compared to the initial day samples. The utilization of TWC exhibits the capability to augment the availability of essential botanical macronutrients, such as nitrogen, phosphorus, and potassium. The supply of nutrients has the potential to stimulate plant growth and increase the secretion of organic compounds from roots, leading to an increase in the soil's CEC by providing more organic matter and promoting microbial activity. The TWC has enhanced the soil health by increasing the CEC, porosity and WHC in the range between 12.6 to 40.4%, 45.3 to 60.9% and 19.6 to 50.8%. An inverse relationship was observed between the percentage of TWC amendment and the bulk density of the soil. The treatment that incorporated amendments of 15% *A. conyzoides* compost, 15% *P. hysterophorus* compost, and 25% *L. camara* compost exhibited the greatest number of fruits per pot. The treatment comprising of 25% *A. conyzoides* compost, 20% *P. hysterophorus* compost, and 15% *L. camara* compost amendments exhibited the highest fruit length. The range of fruit length was recorded as 5 to 11 cm. The study found that an amended ratio of 20 to 25% TWC yielded optimal results in terms of nutrient availability, heavy metal absorption, and morphological characteristics of *A. esculentus*. The levels of HMs found in the fruits of *A. esculentus* were within the permissible limits as reported in FSSAI. After conducting a thorough analysis of the impact of TWC amendments on diverse soil parameters and plant yield, the authors recommend the utilisation of the pioneering TWC products on alluvial soil, at rates of 20% and 25% correspondingly, as a means to enhance soil quality and augment crop productivity.

The best ratio revealed from pot study has been implemented onto the field scale. The CEC of the soil has been increased in the range of 18.6 to 23.5% after 120 days of study. SOM and SOC has been decreased in the range of 30.7 to 38% after 120 days of study. Furthermore, the HMs concentration in the fruit parts of *A. esculentus* and *S. lycopersicum* has been reduced compared to the HMs accumulation in plants grown in pots. The presence of toxic organic compounds has not been observed in the fruits of *A. esculentus* and *S. lycopersicum*. Conversely, the fruits have been detected with few organic compounds, including vitamin E, rhodopin, and oleic acid.

The current study holds significant potential as an efficient waste management technique in agricultural farmlands, particularly where terrestrial weeds pose a problem. One of the key findings of this study is the successful production of a non-toxic compost product from these previously toxic terrestrial weeds. This result is particularly vital as it offers a sustainable solution for the disposal of these weeds, while also providing a valuable resource for agricultural purposes. By converting toxic weeds into a non-toxic compost product, this study can contribute to the development of toxicity parameters in compost characterization. This information can be of great assistance to policy makers in formulating guidelines and regulations related to composting practices and waste management in agricultural settings. Ultimately, this study has the potential to make a positive impact on sustainable agriculture and environmental management.

8.2. FUTURE RECOMMENDATIONS

The results of this investigation are very encouraging for a comprehensive understanding of bio-converting the terrestrial weeds into a value-added agricultural product. To enhance and expand the findings of this thesis, however, it is suggested that future research be conducted in the following areas.

- A certain study could be implemented to assess the mechanism of transformations and reductions of toxic organic compounds in microbiology aspect.
- Isolation of microorganisms in the composting process, which are responsible for the biodegradation of toxic organic compounds in composting terrestrial weeds.
- Utilization of isolated bacteria in remediating hazardous waste sites.
- Application of mixed bacterial consortia for the purpose of biodegradation and mitigation of substrate toxicity can be investigated.

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Annexure 1

PUBLICATIONS

Published articles

- **Maturi, K.C.**, Banerjee, A., Kalamdhad, A.S., 2021. Assessing mobility and chemical speciation of heavy metals during rotary drum composting of Ageratum conyzoides. Environ. Technol. Innov. 24, 101871. https://doi.org/10.1016/j.eti.2021.101871
- **Maturi, K.C.**, Haq, I., Kalamdhad, A.S., 2022. Biodegradation of an intrusive weed Parthenium hysterophorus through in-vessel composting technique: toxicity assessment and spectroscopic study. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-022-21816-4
- **Maturi, K.C.**, Haq, I., Kalamdhad, A.S., 2022. Insights into the bioconversion of Ageratum conyzoides into a nutrient-rich compost and its toxicity assessment: nutritional and quality assessment through instrumental analysis. Biomass Convers. Biorefinery. https://doi.org/10.1007/s13399-022-02532-y
- **Maturi, K.C.**, Haq, I., Kalamdhad, A.S., 2022. Performance assessment of in-vessel composter through heavy metal immobilization and humification of Parthenium hysterophorus. Bioresour. Technol. 360, 127626. https://doi.org/10.1016/j.biortech.2022.127626
- **Maturi, K.C.**, & Kalamdhad, A.S. (2023). Bioconversion of Lantana camara into an agricultural bioproduct through rotary drum composter. Biomass Conversion and Biorefinery, 1-16. https://doi.org/10.1007/s13399-023-05137-1
- **Maturi, K.C.**, & Kalamdhad, A.S. (2023). Comprehensive assessment of composting process of organic substrates using science mapping techniques. Bioresource Technology Reports, 101718. https://doi.org/10.1016/j.biteb.2023.101718

Book Chapters

- **Maturi, K.C.**, Haq, I., Kalamdhad, A.S., 2022. Composting techniques: utilization of organic wastes in urban areas of Indian cities, in: Advanced Organic Waste Management. Elsevier, pp. 43–55. https://doi.org/10.1016/B978-0-323-85792-5.00002-2
- **Maturi, K.C.**, Haq, I., Kalamdhad, A.S., 2022. Integrated terrestrial weed management and generation of valuable products in a circular bioeconomy, in: Biomass, Biofuels, Biochemicals. Elsevier, pp. 41–64. https://doi.org/10.1016/B978-0-323-88511-9.00007-0

Articles (Submitted)

- **Maturi, K.C.**, Haq, I., Kalamdhad, A.S., 2023. Reduction of toxic allelochemicals and production of nutrient rich compost from green waste through in-vessel composting technique **(Under review)**
- **Maturi, K.C.,** Kalamdhad, A.S., 2023. Bioconversion of an invasive terrestrial biomass into an agricultural bioproduct through Rotary Drum Composter: Heavy metals and toxicity assessments **(Under review)**
- **Maturi, K.C.**, Kalamdhad, A.S., 2023. Insights into the translocation mechanism of heavy metals in *Abelmoschus esculentus* through compost amended alluvial soil: Enhancement of soil's nutrient and engineering properties **(Under review)**

International Conferences

- **Maturi, K.C.**, Kalamdhad, A.S., 2020. "Toxicology studies on terrestrial weeds" oral presentation at **International conference on waste management (Recycle 2020)** held at **Indian Institute of Technology Guwahati**.
- **Maturi, K.C.**, Kalamdhad, A.S., 2021. "Bioconversion of terrestrial weeds into a nutrient rich non toxic value added product" poster presentation at **International Conference on Sustainable Biowaste Management 2021 (SBM-2021) Held at Hong Kong Baptist University (Online).**
- **Maturi, K.C.**, Kalamdhad, A.S., 2022. "Insights into the biodegradation of *Parthenium hysterophorus* into a value-added product through in-vessel composting technique" oral presentation at **North East Research Conclave 2022 (NERC-2022)** held at **Indian Institute of Technology Guwahati**.
- **Maturi, K.C.**, Kalamdhad, A.S., 2022. "Evaluation of morphological parameters during terrestrial weeds compost applicability in *Abelmoschus esculentus*" Flash talk and poster presentation at **International Conference on Biotechnology, Sustainable Bioresources and Bioeconomy (BSBB- 2022) held at Indian Institute of Technology, Guwahati.**
- **Maturi, K.C.**, Kalamdhad, A.S., 2023. "**Assessment of biodegradation of *Parthenium hysterophorus* using in-vessel composting technique**" poster presentation at **Research and Industrial Conclave 2023 (RIC-2023) held at Indian Institute of Technology, Guwahati.**
- **Maturi, K.C.**, Kalamdhad, A.S., 2023. "Investigation of morphological characteristics of Abelmoschus esculentus during terrestrial weed compost amendment in soil" poster

presentation at **International conference on solid waste management held at Hong Kong Baptist University.**

- **Maturi, K.C.**, Kalamdhad, A.S., 2023. "**Terrestrial weeds management and their utilization towards a circular bioeconomy**" oral presentation at **International conference on waste management held at Indian Institute of Technology, Guwahati.**

Awards

- Selected as a **Research intern** at Polytechnique Montreal University, Canada in the academic year 2022 – 2023.
- Awarded with **international travel grant** from science and engineering research board, to attend an international conference on solid waste management at Hong Kong Baptist University from 31st May to 4th June, 2023.