
**Composting of invasive weed *Mikania micrantha* Kunth: Toxicity
assessment, plant application and isolation/bioaugmentation of
potential bacterial strain**

A thesis submitted

In partial fulfillment of the requirement for the degree of

Doctor of Philosophy

By

Heena Kauser



**School of Agro and Rural Technology
Indian Institute of Technology Guwahati
Guwahati-781039, Assam, India
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Dedicated to

Amma, Abba, Shahin and Maina

Whose love, blessings, and ongoing inspiration and affection paved the way for
my success





Declaration of Originality

I, Heena Kauser, declare that this thesis titled “**Composting of invasive weed *Mikania micrantha* Kunth: Toxicity assessment, plant application and isolation/bioaugmentation of potential bacterial strain**” and the work presented in it are my own. I confirm that:

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Date:

Signed

Heena Kauser

Registration No.: 176154002





School of Agro and Rural Technology
Indian Institute of Technology Guwahati
Guwahati – 781039, Assam, India

Dr. Meena Khairakpam

Assistant Professor

Email: meena.kh@iitg.ac.in

Phone: +91-361-258-3798

Certificate

This is to certify that the thesis entitled “**Composting of invasive weed *Mikania micrantha* Kunth: Toxicity assessment, plant application and isolation/bioaugmentation of potential bacterial strain**” submitted by Heena Kauser (176154002), a Research Scholar in the School of Agro and Rural Technology, 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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Abstract

Biological invasions have become an increasingly serious problem worldwide due to increasing anthropogenic disturbances and a changing environment. *Mikania micrantha* Kunth is an abhorrent weed that destroys agricultural output. It contains toxic compounds that are detrimental to the natural ecosystem and negatively impact the economic and aesthetic aspects of the environment. *Mikania micrantha* removal by biological, chemical, or mechanical means is still unsuccessful. The traditional approaches such as cutting and uprooting of this weed have increased the problems of organic solid waste. These conventional approaches also did not fully solve the weed problems since the seeds are potent enough to get dispersed by wind or water even after cutting down or uprooting. In North-eastern India, spread of *Mikania* has mostly impacted rubber, tea, and banana plantations, and locals have not established a control strategy to remove this invasive weed. This weed can be managed on-site without discarding into low-lying areas. Thus, local people could use the biomass for the production of compost and utilize in agricultural fields to increase the crop yield of a variety of plants, including vegetables and fruits to attain a sustainable livelihood. There is a paucity of research on *Mikania* composting and vermicomposting, as well as field studies of the compost, which could aid in the paradigm change in the management of such an invasive terrestrial species. This biomass compost can be a substitute for chemical fertilizer in the application of agricultural fields and has the potential to be used for the soil remediation process as well. To accomplish the objectives set after a critical review of the literature, studies on the potential utilization of terrestrial weed *Mikania micrantha* biomass using three different composting technologies were conducted. The novelty of this research work is the reduction of allelochemicals of toxic weed *M. micrantha* and convert it into compost/vermicompost for the agricultural field. Also, this study highlighted the significance of addition of microbial consortia for bioaugmentation study.

The initial study was conducted using six different mix proportions of *Mikania* biomass, cow dung, and sawdust. This study depicts the treatment and management of this plant by in-vessel composting process using a 550 L rotary drum composter (RDC). The trial RD2 with 2.71% has the highest Total Kjeldahl Nitrogen (TKN). Total Organic Carbon (TOC) decreased to 19.72% at the end of the 20th day for the reactor RD2. Another technology

opted is vermicomposting (VC) process which is an excellent technique to convert invasive weed biomass into benign fertilizer. Earthworm species, *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx ceylanensis*, were used to create a 60-day cycle of vermicomposting (VC) process. Among all, *E. fetida* showed a successful growth rate with less mortality rate. The highest Total Kjeldahl Nitrogen (TKN) (3.08%), total phosphorus (TP) (13.24 g/kg) and Potassium (33.25 g/kg) was observed in reactor V_{EF}. However, a fully matured product from a traditional vermicomposting method takes 45-60 days, which is 25-30 days longer than a rotary drum composting technique. Therefore, the use of rotary drum compost would minimize the vermicomposting period by 15-20 days. In this study, in-vessel composting and vermicomposting technologies are proven to be best in time reduction and produce mature, stable, and nutrient-rich compost that is more superior quality than conventional rotary drum compost. *E. fetida* produced vermicompost with total Kjeldahl nitrogen (TKN) content of 3.24%, 12.87 g/kg total phosphorus (TP), and 22.08 g/kg potassium. Out of the three technologies used, rotary drum followed by vermicomposting (RVC) gave best compost quality in limited period of time. To estimate compost quality along with various physico-chemical analysis, toxicity assessment was conducted for the end product. Compost produced from all three technologies showed a germination index (GI) percentage greater than 50%, a recommended value reported for compost application on the field. The highest mitotic index of 68% (25% extract v/v) was recorded for the 60 days vermicompost extract and 65.5% (25% extract v/v) for rotary drum followed by vermicompost as compared to rotary drum compost. Cytotoxicity assessment using *Allium cepa* revealed chromosomal abnormalities such as micronucleated cells, multipolar telophase, c-mitosis, chromosomal bridge, laggard chromosome, chromosomal loss, and sticky chromosome. Out of the three compost products, the vermicompost extract and rotary drum followed by vermicompost had minimum aberrations compared to rotary drum compost. Application of rotary drum compost, vermicompost, and rotary drum followed by vermicompost was evaluated for the performance of *Abelmoschus esculentus* where highest fruit harvest was seen at 25% amended rotary drum compost, 35% amended vermicompost and rotary drum followed by compost. Vermicompost and rotary drum followed vermicompost produced better results in terms of several fruits per plant, fruit weight, and fruit yield than rotary drum compost. Metagenomics study found that *Proteobacteria* was the most abundant phylum in this experiment, followed by *Firmicutes*, *Bacteroidetes*, *Euryarchaeota*, and *Actinobacteria*. Three laccase enzyme-producing

bacterial strains were isolated from the mesophilic phase of rotary drum composting of *Mikania* biomass (*Enterobacter hormaechei* MHK2 (OM149726), *Lysinibacillus fusiformis* MHK3 (OL533643), and *Lysinibacillus fusiformis* MHK4 (OM179766). The bioaugmentation study was conducted using two different methods: Thermophilic composting process followed by bioaugmentation process (TCB) and Vermicomposting process bioaugmented with bacterial consortia (VBB). Compared to the TCB process, the VBB process increased the TKN % and nutrient content during the bioaugmentation process. Phytotoxicity testing revealed a reduction in toxicity after 40 days for both composts. GC-MS analysis revealed that allelochemicals present in *Mikania* biomass were significantly reduced after 40 days of TCB and VBB process.

Weeds continue to be a continual danger to agricultural output, despite decades of advanced weed management measures. Because of the complexity of plant communities, integrated weed management systems may assist decrease economic consequences and enhance weed control procedures. Composting and vermicomposting technologies is a viable option for managing weed and make value-added product from it.

Keywords: Bioaugmentation study; *Mikania micrantha* Kunth; Plant application; Rotary drum composting; Toxicity assessment; Two-stage composting technique; Vermicomposting



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LIST OF ABBREVIATIONS

ANOVA	Analysis of variances
AP	Available phosphorus
APHA	American public health association
C/N	Carbon/ Nitrogen
CD	Cow dung
EC	Electrical Conductivity
FTIR	Fourier-transform infrared spectroscopy
g	gram
GC-MS	Gas chromatography-mass spectrometry
h	hour
kg	kilogram
l	Liter
LS	Laterite soil
M	Molar
<i>M. micrantha</i>	<i>Mikania micrantha</i>
mg	Milligram
NH ₃	Ammonia
NH ₄ ⁺ -N	Ammonical nitrogen
RDC	Rotary drum composting
RVC	Rotary drum followed by vermicomposting
SD	Sawdust
TCB	Thermophilic composting followed by bioaugmentation process
VBB	Vermicomposting process bioaugmented with bacterial consortia
VC	Vermicomposting
XRD	X-ray powder diffraction



Introduction

This chapter discusses the environmental impact of invasive weeds, particularly in the agricultural sector. The chapter also discusses the effect of noxious weed *Mikania micrantha* Kunth, its impact on the environment, and the various control measures implemented. The chapter also focussed on the scope of using *M. micrantha* biomass for composting/vermicomposting process, the importance of compost toxicity assessment, the effect of compost/vermicompost on plant morphological studies, and the effect of bacterial application during the composting process.

1.1. INTRODUCTION

The present worldwide population of 7.7 billion is expected to exceed 9 billion by 2050. To feed this population, global food production will need to be increased by 70 to 100 percent (Chauhan, 2020). However, crop production is hampered by a number of biotic and abiotic constraints, as well as socioeconomic and crop management issues (Ghersa, 2013). Weeds are the most significant biotic constraints to agricultural production in both developing and developed countries. Weeds, along with pathogens (fungi, bacteria, etc.) and animal pests (insects, rodents, nematodes, mites, birds, etc.), present the greatest potential yield loss to crops (Oerke, 2006). Weeds compete with crops for sunlight, water, nutrients, and space. They also serve as a breeding ground for insects and pathogens that attack crop plants. Furthermore, they devastate native habitats, endangering native plants and animals. Invasive alien plant species has gained the global interest of ecologists, biological conservationist, forestry planners, natural resource managers, and social development planners due to their devastating impact on biodiversity and ecosystem functioning. The resulting impact can be termed catastrophic as they ultimately threaten the environmental integrity and, most concerning, the food security of humankind.

The plant invasive species are responsible for altering the nutrient cycle, hydrological regime and survival of other important native species (Mack et al., 2000). According to the habitat, weeds are classified into six classes: terrestrial weed (weeds that grow on land, e.g., *Parthenium hysterophorus*, *Mikania micrantha* etc.), aquatic/water weeds (weeds that grow in water, e.g., *Eichornia crassipes*), submerged weed (Weeds that grow under the water, e.g., *Hydrilla*, *Verticillata*), emerged weeds (weeds which roots are anchored in soil underwater but some parts above the water, e.g., *Sagittaria sagittifolia*), floating weeds (weeds that float above the water having no connection with any part of it with the soil, e.g. *Pistia stratiotes*) and marginal weeds (weeds germinate at the bank of the water body but creeps above the water surface, e.g., *Ipomoea aquatic*, *Eleocharis acicularis* etc. Terrestrial weeds continued to be the major biotic restraint in agriculture and other landscapes due to adaptation and heterogeneity. They have invaded a large area of forest, agriculture and wastelands. Few of these weeds are *Argemone Mexicana*, *Agertaum conyzoides*, *Lantana camara*, *Ipomea carnea*, *Mikania micrantha*, *Parthenium hysterophorus* etc. Out of the mentioned weeds, *Mikania micrantha* Kunth is a weed that is causing a major problem in the north-eastern and southern parts of Indian forest and agricultural land.

Mikania micrantha is a perennial herb or semi-woody vine of Asterasea, native to Central and South America. It has been considered as one of the worst noxious weeds worldwide and nominated among 100 “World’s Worst” invaders (Holm et al., 1977). *M. micrantha* has caused severe economic losses and created serious ecological problems in many countries and regions (Barreto and Evans, 1995). It resulted in serious consequences on farming crops, native plant communities, and natural environments (Holm et al., 1977). Once established, the weed damages or kills other plants by cutting out the light, twinning, and smothering (Huang et al., 2015). Several preventions and elimination strategies have been implemented. None of these techniques were successful in fully eradicating the plant. *M. micrantha* has shown the impact of allelochemicals on native plants that could inhibit the production and regeneration of some native plant species and enable a viable invasion. Allelopathy is characterized as disturbance in plant growth resulting from chemical interactions between plants and other organisms via the release of plant-based bioactive secondary metabolites known as allelochemicals (Latif et al., 2017).

The traditional approaches such as cutting and uprooting of this weed have increased the problems of organic solid waste. These conventional approaches also did not fully solve the weed problems since the seeds are potent enough to get dispersed by wind or water even after cutting down or uprooting. The problems of weeds are mainly faced by the rural

people as their livelihood mostly depends on agriculture. Various novel approaches have been opted for destruction of such kind of obnoxious weed but none of these methods could be successfully adapted due to other environmental effect and many other challenges. Studies were performed on the effective use of such weeds as biomass for the production of compost and vermicompost and the deterioration of allelochemicals. Humans used *Mikania micrantha* to form briquettes formation (Singh and Poudel, 2013) and to feed herbivorous animals. However, over-consumption lead to liver dysfunction and diarrhea. Transformation of the weedy biomass by adopting composting and vermicomposting technologies will be a viable approach to use the end product as organic fertilizer while returning the nutrients like nitrogen, phosphorus and potassium to mother nature at the same time (Saha et al., 2018). Biological, chemical, and respirometry properties must be assessed during the composting process to provide a brief overview of the organic waste degradation process. The biological process includes calculating biological oxygen demand, chemical oxygen demand, and bacterial counts such as the most probable number. Chemical properties include the determination of volatile solids, total organic carbon, pH, electrical conductivity, and nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and sodium. Some respirometry parameters that evaluate compost maturity and stability are the oxygen uptake rate and carbon dioxide evolution rate. The parameters discussed are critical for determining compost quality and degradation rates.

Apart from the evaluation of various physico-chemical and biological parameters during the composting process, the assessment of toxicity is one of the most important criteria for determining the suitability of a compost for agricultural application (Selim et al., 2012). Phytotoxic chemicals, such as heavy metals, may cause toxicity by immature compost (Tam and Tiquia, 1994). Toxicity can be characterized as a delay in germination, inhibition of plant growth, or any other detrimental effect induced by specific compounds (phytotoxins) or by insufficient growth circumstances in the presence of a plant (Barral and Paradelo, 2011). Several investigations have proven that some plant tissues and residues contain organic chemicals that can either stimulate or reduce root growth and development of other neighboring plants (Gartner et al., 1973). In addition, it is well known that the breakdown process of organic residues is connected with changes in the nature and concentration of phytotoxic chemicals in the environment (Patrick et al., 1963). As a result, it is critical to conduct various toxicity assessments of noxious weed compost before applying it to agricultural fields as it contains many allelochemicals that are detrimental to neighboring plants. Several studies have been conducted on the use of

compost/vermicompost using plant models for its growth and fruit yield. Using compost/vermicompost made from a pernicious weed such as water hyacinth, Gajalakshmi, and Abbasi (2002) investigated the effect of it on kitchen gardens containing lady's finger (*Hibiscus esculentus*), brinjal (*Solanum melongena*), cluster bean (*Cyamopsis tetragonoloba*) chili (*Capsicum annum*), and tomato (*Lycopersicon esculentum*). Joshi et al., (2013) reported that vermicomposting helps improve soil quality and enhances plant yield. However, both compost and vermicompost have shown an abundant supply of plant beneficial compounds (Soobhany et al., 2015). Hussain et al. (2020) reported the possibility of using invasive weed *Ipomoea carnea* as an organic fertilizer. The effect of *Ipomoea* vermicompost at four different levels (0, 2.5, 3.75, and 5 tonnes/ha) on the germination, growth, and fruiting of lady's finger (*Abelmoschus esculentus*) was reported. The vermicompost was found to promote the germination and growth of lady's finger at all levels, with the highest results coming from 5 t/ha treatments. The beneficial effect was seen at every step of *A. esculentus* cultivation, from seed germination to vegetative growth phases and fruit output. Due to the vermicompost application, the quality of fruits improved in terms of mineral, protein, and carbohydrate content and a decrease in disease incidence and pest attacks. According to Hussain et al., (2016), vermicompost obtained only from the action of the epigeic earthworm *Eisenia fetida* on *Parthenium* (*Parthenium hysterophorus*) had a favorable effect on the green gram (*Vigna radiata*), ladies finger (*Abelmoschus esculentus*), and cucumber (*Cucumis sativus*).

Very limited studies have been reported on application of bacterial consortia during composting process of invasive weed. Thus, this approach is attractive for addressing the removal of toxic compounds that are present in high concentration during composting process by the application of potential bacterial strain. In compost, microbes and enzymes play an important role in reducing organic matter to simpler organic carbon and nitrogen units. The active microbial population in the compost determines its quality and stability. The optimal environmental variables for microbial viability, such as pH, moisture content, C/N ratio, and temperature, vary depending on the composting methodology used. There have been numerous studies on the isolation and diversity of microbes in composting techniques (Raut et al., 2008; Bhatia et al., 2012). Microorganisms inoculated into composting piles operate as essential drivers during biotransformation, making them a direct choice for modifying microbial metabolic activities during the composting process. Wang et al. (2019) discovered that inoculating *Bacillus stearothermophilus* into compost might significantly reduce ammonia emissions by modifying the structure of the indigenous

bacterial community during the composting process. Moreover, Manu et al. (2017) discovered that microbial inoculums enhanced the decomposition rate of lignocellulose compounds and composting maturity. Zhao et al., (2017) investigated Actinomycetes inoculation, which expedited cellulose and lignin degradation by increasing key enzyme activities. Furthermore, even with the inclusion of inoculum, the composting functioned as well as the mature compost when employed in the premix. Many attempts have been made to improve the composting process by inoculating it with microorganisms, based on the fact that microorganism action is critical to the composting process in determining the quality of the final product and the rate of composting.

The novelty of the current research is to enhance the composting process of invasive weed *M. micrantha* through different composting technologies for quality improvement and time reduction. Furthermore, the study focusses on the application of potential bacterial strain for the bioaugmentation approach of two different composting process to reduce toxicity of the end product and improve the compost quality.

1.2 THESIS ORGANIZATION

The thesis organization is as follows:

Chapter 1 gives a brief description of the problem associated with terrestrial weed, options available for utilization of the weed.

Chapter 2 gives the detailed literature on weed, its problem associated, management strategies obtained, various allelochemical interactions, utilization of the weed employing viable methods, toxicity assessment of the end product, compost/vermicompost application for pot study, bioaugmentation study using microbial strain.

Chapter 3 gives the objectives, need of the study, and scope of the thesis.

Chapter 4 deals with the experimental flowchart of various phases of this study; a collection of the wastes; other feedstock materials; composting methods adopted in the study; toxicity assessment using test plant; pot study experiments; preliminary study on bioaugmentation of terrestrial weed; detailed procedure for physicochemical and biological analyses.

Chapter 5 is about results and discussions of phase-I, including rotary drum composting, vermicomposting and rotary drum followed by vermicomposting of the weed, variation in physicochemical and biological parameters.

Chapter 6 gives the results and discussions of phase-II, including toxicity assessment of the compost produced from three compost technologies discussed in phase I.

Chapter 7 is about results and discussions of phase-III, including a comparative study on the application of compost-produced three compost technologies using the model plant.

Chapter 8 is about results and discussions of phase-III, including metagenomic study, study on the isolation of bacteria, enzyme study, and application of bacteria inoculum during the composting process.

Chapter 9 lists the overall conclusions of the research work and future scope from this study.

Thesis organization flowchart has been illustrated in Fig. 1.1



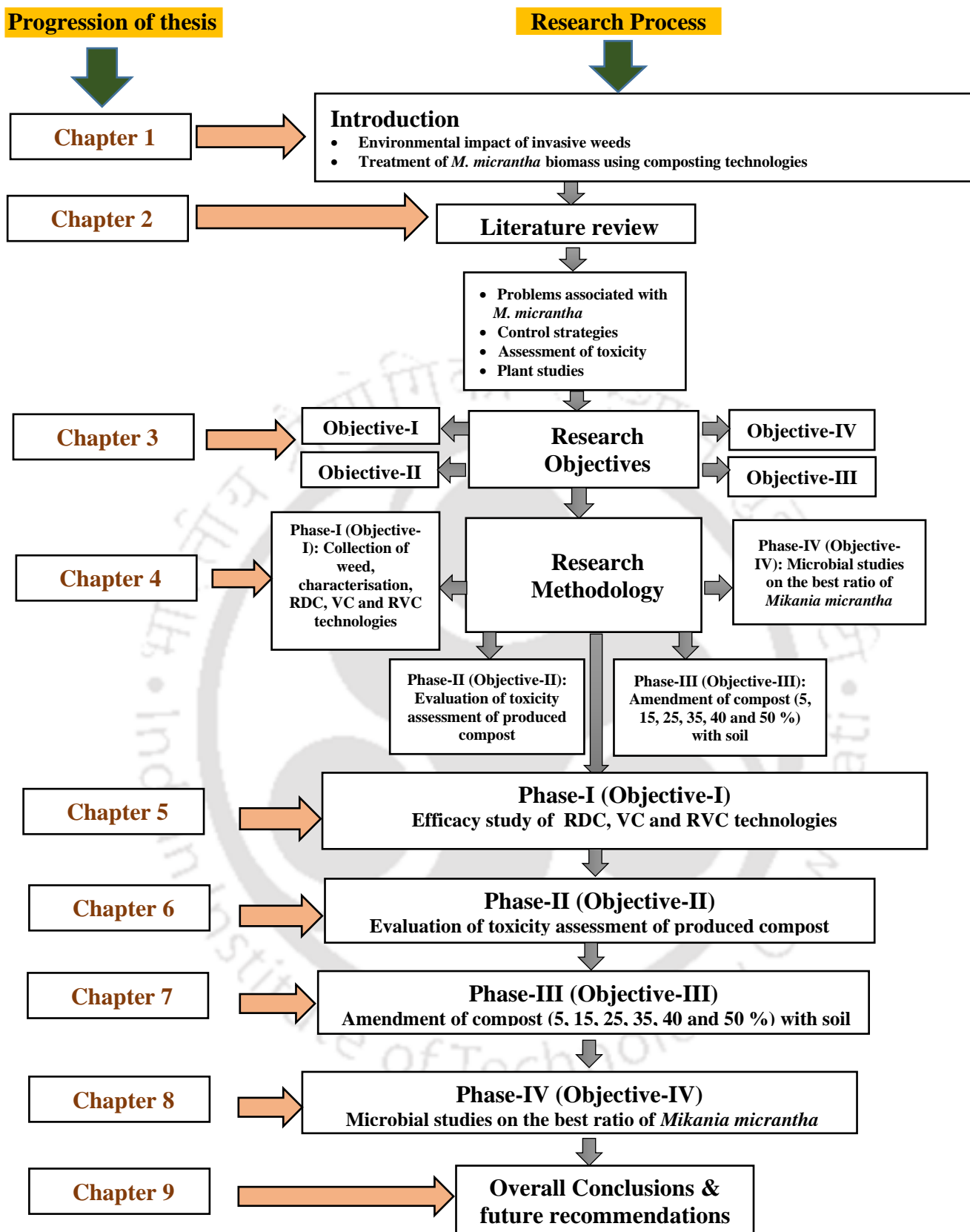


Fig. 1.1. Flowchart of thesis organization





Literature review

This chapter discusses the available relevant literature on the problems associated with the invasive terrestrial weed *Mikania micrantha* Kunth in various parts of the world, including India, control strategies, and utilization as a feedstock for value-added products such as compost and vermicompost. The assessment of toxicity and the usage of compost for plant growth have been thoroughly studied. The chapter also discusses the use of bacterial inoculum for the degradation of organic waste to increase the quality of the compost and reduce the amount of time required for composting process.

2.1 WEED

Weed is a plant that is in the wrong place at the wrong time, causing more harm than positive effect. A weed could be a species that was accidentally planted in the incorrect place and has now become a severe nuisance, whereas another species from the same genus could be a lucrative crop (Adhikari et al., 2004; Lin et al., 2012; Reaser et al., 2007). These plants grow out of place and usually have harmful and objectionable habits (Saha et al., 2018). They can be native or non-native species depending on origin and impact. This plant now establishes and rapidly spreads in the absence of any co-evolved predator or parasite. This has the potential of causing tremendous and often irreversible harm to the environment, economy and human/animal health (Thuiller et al., 2012). These weeds can be agricultural or environmental weeds depending on adverse impacts on food production & native biodiversity or the ecosystem (Benvenuti, 2007; Richardson et al., 2000; Weber, 2017). Multiple factors need to be considered during designing and implementing a weed management/eradication program. The most fundamental requirement of any such plan is to ensure that the rate of weed removal always exceeds the rate of weed proliferation at all population densities. If this condition is not met at any point, the scheme is bound to be

prolonged or, in worst-case scenarios, even fail (Campbell and Gibson, 2001; Lonsdale and Lane, 1994; Wace, 1985; Weaver and Adams, 1996; Whinam et al., 2005). Unlike insect pests, weeds cannot be restricted by mating disruption or sterile male release. Weeds are divided into two main categories in nature: aquatic weeds and terrestrial weeds. Both weeds substantially impact the ecology, necessitating extensive control to keep their expansion to a minimum.

2.1.1 Terrestrial weed

Terrestrial weeds significantly impact ecosystems such as forests, agriculture, and urban areas, necessitating effective management of these invasive plants. Controlling their expansion is difficult due to their rapid adaptation and morphological improvement. The procedures employed to aid the migration or transport of propagules away from the parent plant are weed dispersal mechanisms. Plants have limited mobility and rely on a range of dispersal vectors, including abiotic and biotic vectors, to deliver their seeds (Bridgemohan et al., 2015). Based on the classification of weeds by Life History, there are three primary types of terrestrial weeds:

- a) Annuals: An annual plant's life cycle is one year or fewer from seed to seed.
- b) Biennials: Biennials typically emerge in a rosette stage the first year, develop seeds, then die the following year.
- c) Perennial: Perennial plants live for more than two years and can reproduce multiple times before dying. Year after year, the same root system produces continued vegetative growth in these plants.

2.1.2 Problems associated with weeds

Weeds fight for sunlight, water, nutrients, and space with crops. Furthermore, they are home to insects and viruses that harm crop plants. They also devastate native environments, endangering local plants and animals. Weed emergence time, weed density, weed type are all factors that influence crop yield losses. Weeds can cause a 100% yield loss if left unchecked. Shifts in global climate factors, such as precipitation patterns and environmental parameters such as carbon dioxide and ultra violet exposure, have the potential to alter the species' current distribution (Garrett et al., 2006). Thus according to Rao and Chauhan (2015), weeds are those that have a destructive impact on land, water, and human health. In recent years, environmentalists and others are seeing an increase in the importance of the issue of invasive species, as demonstrated by an increase in major work on the subject (Culliney, 2005). Weed invasions are one of the world's biggest challenges to controlled and natural habitats (Panetta

and Timmins, 2004). India, invasive weeds, particularly *Parthenium hysterophorus* in urban areas, *Lantana camara* in forest areas, *Ageratum conyzoides* in cropland and *Mikania micrantha* Kunth have accumulated a proportion of high toxic pollutants (Saha et al., 2018). Latest studies reveal that the exotic invasion of plants has not only changed the physiochemical properties of the soil but has also deeply altered the quantity, nature, and activity of the microbiota in soil (Callaway et al., 2004). The first critical step of colonization is introducing a region outside its former geographical range (Sharma et al., 2005). Naturalization is the stage of the invasion sequence that follows the introduction after a species has conquered diverse challenges to survival and daily reproduction and has formed populations large enough that the risk of extinction is low due to environmental variation (Mack, 1996; Richardson et al., 2000). Evolutionary metamorphosis has been used as a useful biological intrusion process over 30 years ago (Baker, 1974). Genetic bottlenecks are commonly expected to be linked to biological invasions (Barrett, 1986), as a limited number of individuals removed from further gene expression may be included in the populations introduced (Dlugosch and Parker, 2008). About 40% of Indian flora species are foreign, 25% of which are invasive (Singh, 2005). Economic and environmentally harmful terrestrial weeds that are noticeable in India and many other parts of the world include *Parthenium hysterophorus*, *Chromolaena odorata*, *Lantana camara*, *Mikania micrantha*, *Ageratum conyzoides*, *Galinsoga parviflora*, etc. The role of the environmental effects of terrestrial weeds such as *Parthenium hysterophorus*, *Chromolaena odorata*, and *Lantana camara* has been discussed below, and also, based on the extent of literature, *Mikania micrantha* Kunth has been chosen for comprehensive study, its management, allelopathic effect, and environmental effect has been discussed below.

- ***Parthenium hysterophorus***

Biological intrusion is seen as a key threat to native species caused by alien invasive species (Shabbir and Bajwa, 2006). *P. hysterophorus* (Asteraceae), generally alluded as the congress or carrot grass, is known to be among the 100 disastrous invasive plant species in the world (GISD, 2018). The species belongs to the Asteraceae family of plants and is native to the South of the United States, Mexico and Central and South America as shown in Fig. 2.1. In India, *P. hysterophorus* was wrongly smuggled from Central America along with wheat (*Triticum aestivum*) into Pune in 1956 (Vartak, 1968). However, based on the herbarium 2.1 record, it is claimed that at least one prior adoption occurred about 1800 (Bennet et al., 1978). This weed is prevalent in almost every part of India as shown in Fig. 2.2. Broad resilience, photo-and thermo-insensitivity, drought resilience, strong competitiveness and allelopathy, high seed production capacity, durability of seed in soil

seed banks and small and light seeds capable of long-distance transport through wind, water, birds, vehicles, farm machinery and other animal traffic add to its rapid global growth, cutting through national borders (Saha et al., 2018).



Fig. 2.1. Global aerial distribution of *Parthenium hysterophorus* and retrieved from Adkins and Navie, 2006)



Fig. 2.2. *Parthenium hysterophorus* in the premises of IIT Guwahati, Assam, India

On the development of winter crops, radishes and chickpeas, Singh et al. (2003) investigated the allelopathic effects of unburnt and burnt *P. hysterophorus* residue. *P. hysterophorus* burning decreased germination, biomass formation, plumulus and *Phaseolus mungo* radicle length in fields (Kumar and Kumar 2010). A substantial proportion of

phenolics are estimated in residue extracts as well as in residue-incorporated *Parthenium* dust, the main community of secondary metabolites normally involved in allelopathy (Singh et al., 2005). *Parthenium* has strong allelopathic effects on germination and on the growth of plant species under cultivation. Negative allelopathic effects of *Parthenium* on cultivated plants have been well documented (e.g. *Brassica campestris*, *B. oleracea* and *B. rapa*, *Glycine max*, *Lolium multiflorum*, *Oryza sativa*, *Phaseolus vulgaris*, *Raphanus sativus*, *Cicer arietinum*, *Triticum aestivum*, *Vigna radiata*, and *Zea mays*) (Yadav and Chauhan 1998). By affecting native plant regeneration processes and suppressing the growth of neighbouring plant species through the release of volatile and non-volatile allelopathic plants, *Parthenium* weeds can have an effect on natural biodiversity (Khaliq et al., 2015). *Parthenium* weed can serve as a defense mechanism against microbes and different types of plant-borne predators (nematodes, insects, and mammals), adversely affecting the soil food web and chemistry (Adkins and Shabbir, 2014). Maharjan et al., (2017) recorded that crucifer species (*Raphanus sativus*, *Brassica campestris* and *Brassica oleracea*) were more susceptible to the inhibitory effects of *P. hysterophorus* leaf aqueous extract. At >2% concentration in crucifer species, germination was inhibited. Concerns over these detrimental effects on human health, cattle, agricultural production, and biodiversity are rising in tropical and subtropical areas of the Indian, African and Australian continents (Dhileepan and Senaratne 2009).

- ***Chromolaena odorata***

In a lot of the Paleotropics, *C. odorata* originates from South and Central America and has invaded a wide variety of environments, ranging from tropical rainforests to Savannahs (Raimundo et al., 2007) (Fig. 2.3). In the year 1845, *C. Odorata* was first introduced as an ornamental plant to the Calcutta Botanic Garden in India, and Jamaica was known as India's possible geographical source (Muniappan et al., 2005). It is a medium-sized shrub that reaches 1,5-2 m in height. In its naturalised ranges, the species forms dense, impenetrable single-species, which outshadow other native plants (Goodall and Erasmus 1996). Shrubs typically reach heights of 2-3 m when growing in isolation, but when assisted by other vegetation, they can reach heights of 5-10 m (Fig. 2.4). Widespread invasive shrubs (Rejma'nek and Richardson 2013) are two varieties or biotypes of *C. odorata*, all coming from the Americas. *C. odorata* has fast seed growth, i.e. 20 mm per day (Hills and Ostermeyer 2000), and its seed germination is influenced positively by light intensity (Witkowski and Wilson 2001). Within a brief amount of time, it proliferates to the surrounding places (Holm et al. 1977), and is very well assisted by its numerous modes of

dispersal (wind, animals and human beings). It was first documented as naturalized in South Africa near Durban in 1947 (Hilliard, 1977) and is now widespread in the subtropical eastern and northeastern parts of the world. It is one of the most invasively influenced weeds in the world and is a major weed in Central and Western Africa, India, Australia, the Pacific, and Southeast Asia (McFadyen, 2003). The trees, forests and natural vegetation can be rapidly developed and melted. It is unpalatable and dangerous and can kill domestically ingesting animals (Mandal and Joshi, 2014). *C. Odorata* can reproduce both by sexual and vegetative means and by apomictic means, i.e. without pollination, capable of producing fertile seeds (Rambuda and Johnson 2004).



Fig. 2.3. Global aerial distribution of *Chromolaena odorata* and data retrieved from Joshi (2006) India

C. odorata was possibly accidentally introduced in Australia and West Africa as a contaminant of imported seed or fodder (Zachariades et al. 2009). *C. odorata* in southern Africa has become a troublesome invasive (Zachariades et al., 2011) that can survive fire and acidic soil, but its growth is inhibited by low temperatures (Yadav and Tripathi 1981). *C. Odorata* is believed to rely on the combination of its high reproductive potential (Koutika and Rainey, 2010), high relative growth rate (Ramakrishnan and Vitousek, 1989.), ability to inhibit light competition from native plant growth (Honu and Dang, 2000), and accumulation of native soil pathogens (Mangla et al., 2008). Suwal et al. (2010) have revealed that aqueous extracts of *C. odorata* can contain water-soluble allelochemicals that exert inhibitory effects on the germination and seedling growth of paddy and barnyard grass through Petri dish bioassays.



Fig. 2.4. *Chromolaena odorata* in the premises of IIT Guwahati, Assam, India

- ***Lantana camara***

Lantana camara (Verbenaceae) is a conspicuous, dangerous, yet ornamental plant native to the temperate, tropical, and subtropical parts of the world (Ghisalberti, 2000). In a cross section, the arc of *L. camara* is square, with pithy and short, reverse hooked stems. The invasive *Lantana* is a 2-4 m long ramified shrub. Woody stems can be cross-sectional and hairy when young, but can grow to be cylindrical up to 150 mm thick with age (Mishra, 2015) (Fig. 2.5). Author further reported that *Lantana* is mainly native to subtropical and tropical America, although some taxa are native to Africa and Asia. It now exists in around 50 countries where many plants are grown under the names of hundreds of cultivars (Fig. 2.6). The number of *Lantana* species reported ranges from 50 to 270 unique and subspecific individuals, but a better estimate tends to be 150 species. In most parts of India, the species has been invaluable. The British developed it at the Calcutta Botanical Garden in 1809 as an ornamental plant (Nanjappa et al., 2005). Their presence and distribution is indiscriminate in virtually every region in India, except in the Thar Desert and surrounding areas, including field, grass, fallow and forest (Dobhal et al., 2011).



Fig. 2.5. Global aerial distribution of *Lantana camara* and data retrieved from Goncalves et al., 2014) India



Fig. 2.6. *Lantana camara* in the premises of IIT Guwahati, Assam, India

The strength of natural plants in a specific area is inhibited by allelochemicals present in *Lantana* and eventually results in low productiveness (Sharma et al., 1988). *Lantana* stem, leaf and fruit leachates hinder the growing and seedling of some terrestrial plants (Quan et al., 2009). *Lantana* has essential consequences and is difficult to monitor in the economic and environmental sectors. Constant caution is the secret to effective management of *Lantana*. Continuous management of the new growth is important for progress (Day et al., 2003). Author also added that the capacity to hybridise the varieties of *L. camara* with closely associated species of the genus is the key reasons for *L. camara* weediness and the limited success of

biocontrol. Such agents have been more effective in establishing agents obtained from related *L. camara* species in the countries concerned, or which have a wide host range.

- ***Mikania micrantha* Kunth**

M. micrantha is found in several Asian countries and is well known as one of the world's most dangerous weeds. (Acevedo-Rodriguez, 2005) confirmed that Mexico, Central America, South America, and the Caribbean are the real native sites of *M. micrantha*. *Mikania* has about 450 species, of which is *M. micrantha* has the most harmful environmental impacts (Saikia et al., 2020). It was first introduced in India in 1940, but it was tentatively exploited in Assam in the 1950s during the Second World War (Choudhury, 1972). Author had also noted that it was mistakenly introduced to the fodder grass for cattle feeding. Due to its rapid growth, forests and other natural structures have come under a major threat. The aerial distribution of *Mikania* worldwide and in India has been shown in Fig. 2.7 (a & b).

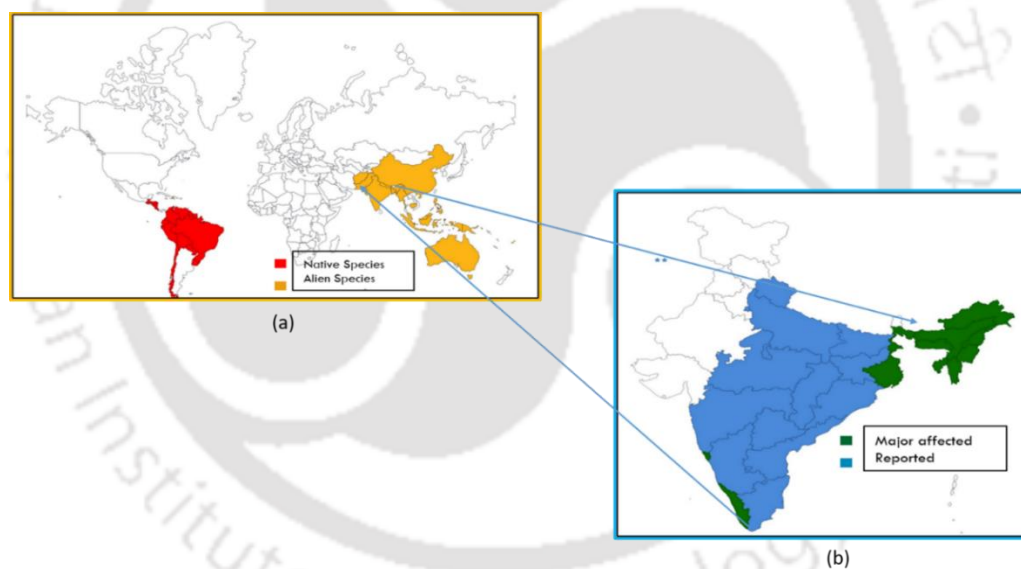


Fig 2.7. (a) Global distribution of *Mikania micrantha* (b) Areas invaded in India (Distribution data retrieved from Iyer et.al., 2019)

M. micrantha is found in a wide range of habitats as it can be spotted on fences, agricultural lands, forests, fallow lands, wetlands, etc., where the habitat is moist, and the land is fertile (Ghale, 2013). *M. micrantha* is distinguishable even in a complex habitat like a forest, as these plants tend to cover up the host plants entirely and can be recognized by the heart-shaped leaves, as shown in Fig 2.8 (Zhang et al., 2004) reported that *M. micrantha* could extend up to 15 m, supporting itself from other plants or any vertical post, such as electric poles, fences, walls, etc. It is a multi-branched plant, annual, scrambling with a 5-

ribbed stem, pubescent or glabrous internodes, and 7.5-21.5 cm in length. With an acute apex and broad base, the 4-13 cm long leaves are opposite, cordate, or triangular (Tripathi et al., 2012). Every floret is bisexual actinomorphic, five petals symphyogenetic to a trumpet-shaped corolla; five stamens, five anthers attached to the stigma; independent filament, embedded in the base of the corolla, matured stamens spreading out of the corolla (Chen et al., 2007). Small, white, or cream-colored flowers can be observed at the nodes having compound corymbose inflorescence panicle (Kim et al., 2021). *M. micrantha* expresses extraordinary vegetative growth from its nodes. Each plant can produce more than 40,000 wind dispersible seeds each year (Kuo et al., 2002). In India, the plant was reported to be troublesome in the northeast and south-west (Parker, 1972), and a classical biological control technique was applied. Still, no satisfactory result was obtainable (Banerjee and Dewanji, 2012).



Fig. 2.8. *Mikania micrantha* spotted on a fence in North-east India

Vaid, (1973) reported on the distinguishing features of species of *Mikania*, namely *M. micrantha*, *M. scandens*, and *M. cordata* (Margolis et al., 1982), and later verified that the only species to be present in India is *M. micrantha* Kunth, in the Western Ghats and the tropical forests of the Northeastern Himalayas (Rameshprabu and Swamy, 2015). Over the years, *M. micrantha* has developed extensively in southern state Kerala and North-eastern state Assam and damaged the forest area and tea farms, affecting its economy (Banerjee and Dewanji, 2012). Economically speaking, the gains due to *M.*

micrantha are meagre compared to the losses it causes to various ecosystems. Apart from this, the plant has also been reported to be found in Tamil Nadu (Ramachandran and Soosairaj, 2008), Uttar Pradesh (Uttar Pradesh State Biodiversity Board, 2009), Andhra Pradesh (Reddy and Raju, 2009), Orissa, and Meghalaya (Banerjee & Dewanji, 2012). It can be commonly spotted climbing on fences, trees, or electric posts, as shown in Fig. 2.9.



Fig. 2.9. Spreading of *Mikania* in IIT Guwahati campus, Assam, India

The invasion of *M. micrantha* in a tea plantation in the North-eastern part of India is shown in Fig. 2.10. The key problem associated with *Mikania* is that they will smother the host plants, compete for nutrients and hinder photosynthesis in the host plants (Saha et al., 2020). Due to its extraordinary growth, *M. micrantha* is usually called ‘mile a minute’ and can grow to 8-9 cm in 24 hours (Choudhury, 1972). It has a cryptic riparian ecosystem that can develop along with reed-like plants by the riverbanks (Barreto and Evans, 1995). Besides the smothering and resource competitive nature of *M. micrantha*, it also contains different secondary metabolites, making them a potential threat. According to (Naibin and Qiaoying, 1999), *M. micrantha* consists of high volumes of α -pinene and β -pinene that can be useful for making an effective insect repellent. *M. micrantha* also contains other secondary metabolic substances, such as β -caryophyllene, which act as allelopathic compounds, influencing the growth and germination of the neighboring plants (Wang et al., 2009). The author further reported that β -caryophyllene had inhibited the germination and seedling production of *B. campestris* and *R. sativus*.



Fig. 2.10. Invasion of *Mikania micrantha* on the tea plantations of Assam, India

There are many secondary metabolites in the leaves and stem of *M. micrantha* that have anti-inflammatory and anti-allergic effects (Rufatto et al., 2012). Leaves and stems of *M. micrantha* also have phenolic compounds that would influence many plants. (Sakachep and Rai, 2021) documented the effects of these phenolic compounds on *Oryza sativa* and *Raphanus*. The author further found that the aqueous extract of *M. micrantha* had a potent inhibitory effect on the development of *Lolium multiflorum* and *Raphanus sativus*. *M. micrantha* has shown the impact of allelochemicals on native plants that could inhibit the production and regeneration of some native plant species and enable a viable invasion. All these characteristics result in a complete ecological disruption. This obviously indicates that the effect on the agriculture sector is disastrous, resulting in a major depreciation of economic output. Even though its uses are reported as curative agents against itches, scorpion bites, stomach aches, etc., from different parts of north-eastern India, the therapeutic evidence is scarce and lacking whatsoever. To control the growth and spread of *M. micrantha*, various methodologies have been tried, but very few are found to be adequately effective.

2.1.3 Allelochemical interactions of weeds

The concept of interference has been proposed by (Harper, 1967) to include all competitors for signalling pathways and allelopathy resulting through the release of harmful chemicals introduced to the ecosystem. The negative influence of the adjoining plant on interaction is called interference by (Muller, 1969), who proposed that two elements, competition, and allelopathy, tend to plant intervention. A broad research on the

allelochemical inhibitory potential of weeds on crop varieties has also been reported. However, the influence of weeds through this mechanism of plant involvement will either be favorable (stimulatory) or destructive (inhibiting) (Qasem and Foy, 2001). Allelopathy is the mechanism by which a plant releases a chemical compound into the atmosphere that prevents development in the same or adjacent environment for another plant. This mechanism is different from the competition because the requisite factor is not exhausted and relies on the inclusion of a detrimental factor. These two mechanisms describe the reaction as any alteration in the habitat arising from the living organism's behavior (Muller, 1969).

Weeds compete for resources with crop plants in agroecosystems, reducing crop yield and quality and resulting in massive financial losses. Weed control is accomplished through a variety of methods (e.g., mechanical and chemical), but these methods are costly and hazardous to the environment. Allelochemicals are the secondary metabolites produced by the plant species. Secondary metabolism is a biosynthetic source of several interesting compounds that can be used in the chemical, food, agronomic, cosmetics, and pharmaceutical industries. Secondary pathways are not required for individual cell survival but benefit the plant as a whole (Adams et al., 2019). Another general feature of secondary metabolism is that it is found in a specific organism or groups of organisms and is an expression of species individuality (Dewick and Rohr, 1998). Secondary metabolism adds chemical diversity to organic molecules with low molecular weight that are linked by the respective pathways; these organic molecules are referred to as secondary metabolites. The schematic diagram of allelopathy processes and factors influencing allelopathy has been shown in Fig. 2.11. Plants biosynthesize three types of secondary metabolites: phenolic compounds, terpenoids/isoprenoids, and alkaloids and glucosinolates (nitrogen- or sulfur-containing molecules, respectively) (Vickers, 2017). The shikimate pathway biosynthesizes phenolic compounds, which are abundant in plants that is found in the chloroplast. As pigments, antioxidants, signalling agents, electron transport, communication, the structural element lignan, and as a defence mechanism, these aromatic molecules play critical roles. Han et al. (2000) found that organic acids, esters, alcohol, aldehyde, phenol, acetone, and hydrocarbons were abundant in the decomposition products of soybean stubs (including organic compounds in rhizosphere soil). Allelochemicals have been identified in some of them. *M. micrantha*'s volatile oil contained (48) phytochemicals, (27) of which were terpenoids, in Peru, its native region. Monoterpenes and sesquiterpenes, alcohols and ketones, and their derivatives were the major phytochemicals identified in

aerial parts of *M. micrantha* in China. (51) phytochemicals were identified in stems and leaves, including -cubebene (12.87%), terpinolene (12.32%), 1,2,3,4 tetrahydro1,1,2,4,4,7-hexamethylnaphthalene (9.49%), -caryophyllene (9.49%), -caryophyllene (7.69%), limonene (4.58%), farnesene (2.46%), (42) phytochemicals were discovered in flowers. Terpinolene levels in flowers, on the other hand, were much lower (0.15%) than in stems and leaves (Ni et al., 2007). Approximately 240 weed species are allelopathic and damage crop growth and development (Singh et al., 2003). The author further added that they are already reducing crop efficiency, congesting water pathways, creating human health problems, and becoming unsightly at places such as fields, parks, pathways, pavements, etc. Usually, weeds are harvested after cutting, but either the plant material persists on the surface of the field or is integrated into the soil for decomposition. Allelochemicals in these residues can influence the growth and development of good crops and may hinder the development of economic crops (Abbas et al., 2017). Weed residue released remains active and accessible for plants afterward, which can influence the germination and development of future crops by disrupting simple plant processes such as cell division interrupting (Deka et al., 2011).

The processes by which allelochemicals inhibit germination and crops from rising seeds have been explored in-depth (Zohaib et al., 2016). The author reported that allelochemicals impact the soil chemistry characteristics by releasing large quantities of phytotoxic chemicals during decomposition. Diverse studies indicate that allelochemical residues of many types of terrestrial weed are released into the soil, impacting the production of related crop plants and the plants of the next year (Altieri et al., 2011). Photosynthesis is affected to such a degree by environmental conditions, temperature, CO₂, water, and microbe concentrations. After treatment with allelochemicals, a decline in CO₂ assimilation was widespread in many plants. The effectiveness of the three major photosynthesis cycles, the stomatological regulation of the supply of CO₂ and the transport of thylakoid electrons (light reactions), and the carbon reduction cycle (dark reactions) would potentially degrade allelochemicals. However, the comprehensive mechanism for reduced allelochemical assimilation remains somewhat elusive in most studies (Zhou and Yu, 2006). Allelopathy is characterized as a disturbance in plant growth resulting from chemical interactions between plants and other organisms via the release of plant-based bioactive secondary metabolites known as allelochemicals (Latif et al., 2017), as shown in Fig 2.12. In order to mediate interspecific interactions, allelochemicals play a significant role (Duke, 2007).

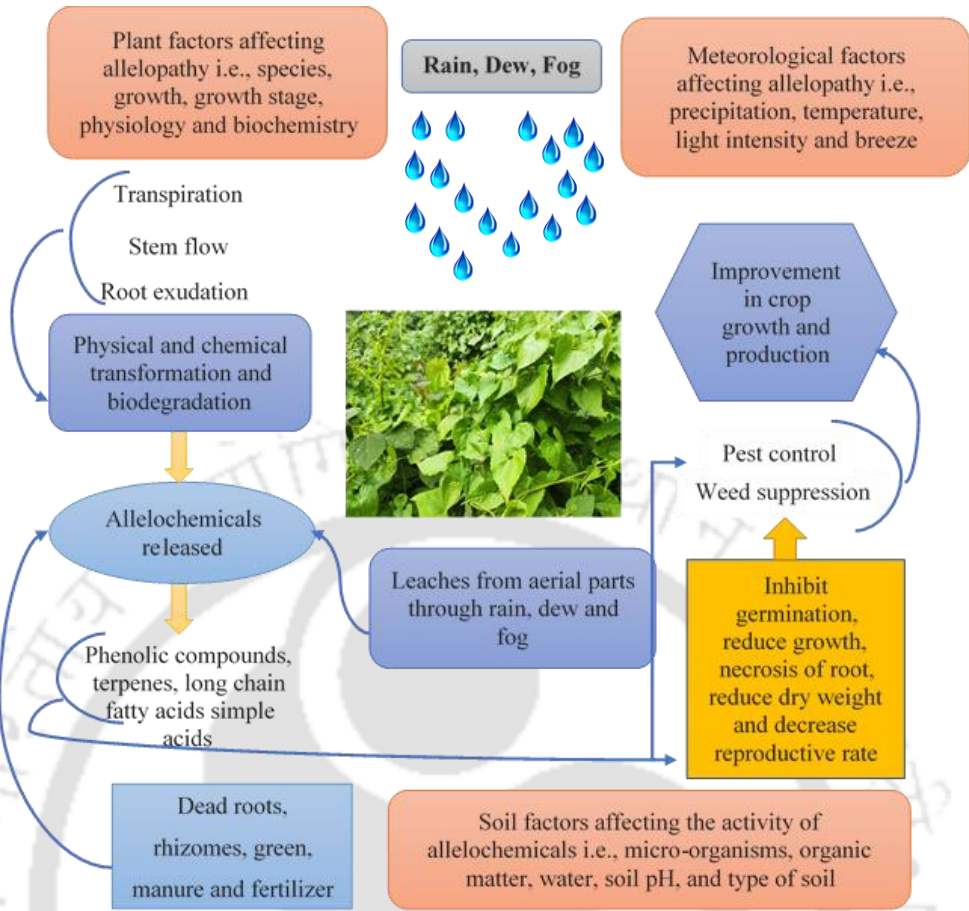


Fig. 2.11. Allelopathy processes and factors influencing allelopathy (Shah et al., 2016)

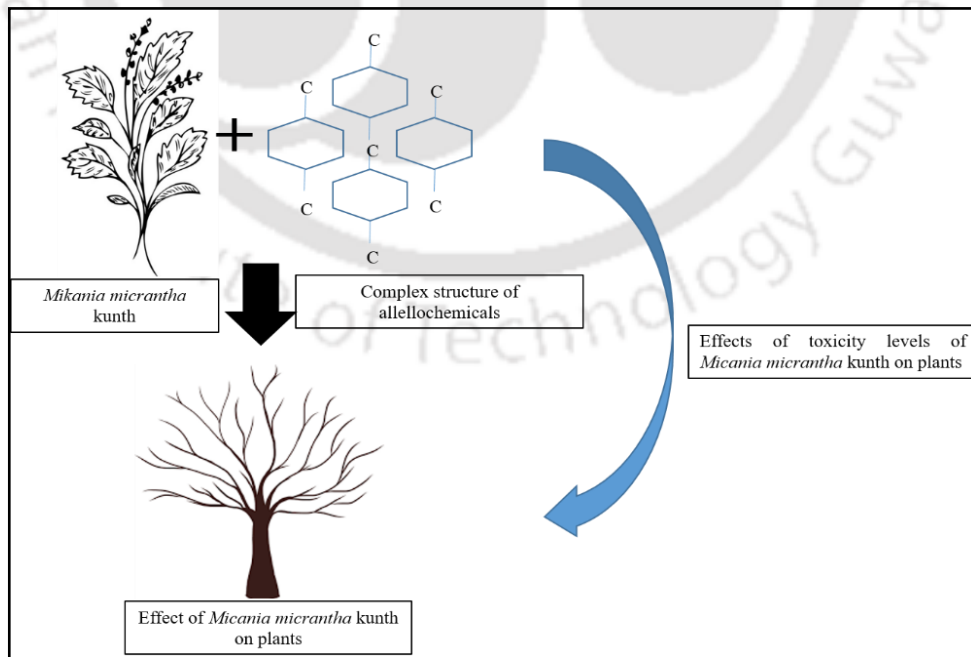


Fig. 2.12. Effects of allelochemicals in growing plants

It is widely considered that leaves have more allelochemical effects than other parts of the plant, and allelochemicals are often soluble in water, and they can therefore easily leach out into the soil (Wu et al., 2015). Allelochemicals of *M. micrantha* affect indigenous plants rather than wild plants, which may be more resistant to allelochemicals in exotic plants from the same area as *M. micrantha* (Callaway and Aschehoug, 2000). Authors further reported that *M. micrantha* leaf extract had major impacts on the seed germination percentage, initial plant growth periods, and germination rate of (26) plants. Its phytotoxic varieties contain phenols, flavonoids, alkaloids, and terpenes that damage seeds, weeds, co-occurring plant species, insects, and pathogens. The potential is dependent on environmental factors and plant tissues and facilitates its successful invasion. High levels of atmospheric CO₂ may improve the biosynthesis and phytotoxicity of allelochemicals in *M. micrantha*, which, if released in bioactive concentrations, could increase its potential allelopathic effect on neighboring native plants (Wang et al., 2010). Authors also stated that terpenoids have several environmental roles, like allelopathy. *M. micrantha* has also been stated to include sesquiterpene lactones, diterpenes, flavonoids, and phenolic compounds mainly responsible for allelopathic responses and antibacterial and anticancer activities. On Hainan Island, two parasitic plants, *M. micrantha* and *Ipomoea cairica*, are widely spread and have badly damaged the ecosystem. In this research, leaf extracts from two weeds were collected and measured for their allelopathic ability on *Chrysanthemum coronarium*. The bioassay of phytotoxicity showed that the suppressed effects of *M. micrantha* on *C. coronarium* development are greater than that of *Ipomoea cairica* when the concentration of the extract is between 50 and 100 mg/ml (Ma et al., 2020). (Ismail and Chong, 2002) reported that the emergence of tomatoes and Chinese cabbage was significantly diminished when *M. micrantha* debris was incorporated into the soil, irrespective of the amount of debris and the decomposition time. Still, by contrast, the emergence of maize and long bean seedlings was not affected. It was also apparent that fresh weight and germination of *Asytasia intrusa*, *Chrysopogon aciculatas*, and *Paspalam conjugatum* was significantly decreased by extracts of *M. micrantha* (Poudel et al., 2019). However, *M. micrantha* decreased root elongation of cabbage in the soil of a greenhouse but stimulated ryegrass's root elongation (Zhou and Huang, 2009). *M. micrantha* rhizosphere soil induces a substantial decrease in the shoot length of *Panicum antidotale* (approximately 21%). Besides, leaf leachate from the *Mikania* genus leads to reduced germination of rice seeds in non-sterile soil (Sheam et al., 2020). Over the past few years, *M. micrantha* has decreased the production of many orchards. *M. micrantha* roots form a

dense cover over the orchard canopy, which allowed this species to replace the coexisting native *Persicaria chinensis* (L.). The overall phenolic content of *M. micrantha* was found to be 4.63 ± 0.37 mg gallic acid equivalent/gm of dry leaves (Lallianchunga et al., 2016). *M. micrantha* releases them via various mechanisms, including volatilization of aerial parts, and decomposition of plant debris in the soil. Its allelochemical varieties include phenolics, flavonoids, alkaloids, terpenes which can affect crops, weeds, co-occurring plant species, insects, and pathogens. The potential is dependent on environmental factors and plant tissues and facilitates its successful invasion. Around 80 phytochemicals have been identified in this species, compositions of which depend on the region of growth and tissue it is present. Creepers in China have monoterpenes and sesquiterpenes, alcohols and ketones, and their derivatives. 51 phytochemicals in stems and leaves (β -cubebene, terpinolene, 1,2,3,4-tetrahydro-1,1,2,4,4,7-hexamethylnaphthalene, β -caryophyllene, α -caryophyllene, limonene, β -farnesene, ocimene, γ -terpinene, δ -cardinol, δ -bisabolene; in order of abundance) and 42 phytochemicals in flowers with much lower content of terpinolene. Phenolics compounds include 3,5-Di-o-caffeoylquinic acid n-butyl ester and 3,4-Di-O-caffeoylquinic acid n-butyl ester, and flavonoids Mikanin, Eupalitin, Eupafolin, Luteolin, and 3,4,5,7-tetrahydroxy-6-methoxyflavone- 3-O- β -D-glcopyranoside are present. Some phenolics, flavonoids, and terpenoids derived from *M. micrantha* have been listed in Table 2.1.

Table 2.1. Different types of compounds extracted from *Mikania micrantha* Kunth

Author, year	Compounds identified
Nicollier and Thompson, 1981	1. Volatile terpenes Sesquiterpene lactones
Boeker et al., 1987	1. Germacra-1 2. 10Z 3. 4E-dien-12 8 α -olides
Cuenca et al., 1988	1. Dihydromikanolide 2,3-epoxy-1-hydroxy-4,9-germacradiene-12,8:15,6-diolide
Ismail and Chong, 2002	1. Vanillic acid 2. Resorcinol 3. Caffeic acid 4. <i>p</i> -Hydroxybenzaldehyde
Zhang et al., 2003	Isobutyl acetate 2-Butanamine

Feng et al., 2004	<ol style="list-style-type: none"> 1. 3,5-Di-o-caffeoylquinic acid n-butyl ester 2. 3,4-Di-o-caffeoylquinic acid n-butyl ester 3. Mikanin 4. Eupalitin 5. Eupafolin 6. 3,4',5,7-Tetrahydroxy-6-methoxyflavone3-o-β-D-glcopyranoside Luteolin
Bakir et al., 2004	Mikanolide
But et al., 2009	<ol style="list-style-type: none"> 1. 3β-acetoxy-1 2. 10-epoxy-4-germacrene-12,8
Xu et al., 2013	6-diolide <ol style="list-style-type: none"> 1. 8,10-dihydroxy-9-benzoyloxythymol 2. 9-isobutyryloxy-10-hydroxythymol 3. 7,8,9,10-tetrahydroxythymol
Bravo-Monzón et al., 2014	7,8,10-trihydroxy-9-E-feruloyloxythymol <ol style="list-style-type: none"> 1. Limonene 2. α-pinene 3. Sesquiterpenes germacrene D
Ríos et al., 2014	β-caryophyllene <ol style="list-style-type: none"> 1. 8-epi-mikanokryptin
Dong et al., 2017	Melampolide 11Hβ-11,13-dihydromicrantholide <ol style="list-style-type: none"> 1. Benzyl 5-O-β-d-glucopyranosyl-2,5-dihydroxybenzoate (7S,8R)-threo-dihydroxydehydrodiconiferyl alcohol 9-acetate
Dong et al., 2017	<ol style="list-style-type: none"> 1. β-d-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-9β-hydroxy-<i>ent</i>-16-kauren-19-oate 2. β-d-glucopyranosyl-15α-(3-methylbutanoyloxy)-9β-hydroxy-<i>ent</i>-16-kauren-19-oate 3. β-d-glucopyranosyl-15α-(2-methylbutanoyloxy)-9β-hydroxy-<i>ent</i>-16-kauren-19-oate β-d-glucopyranosyl-15α-(3-methyl-2-butenoyloxy)-9β-hydroxy- <i>ent</i> -16-kauren-19-oate

Shao et al., (2005) investigated the effects of dihydromikanolide, deoxymikanolide, and 2,3-epoxy-1-hydroxy-4,9-germacradiene-12,8:15,6-diolide, three sesquiterpenoids. By causing root injury and reducing radicle length, these sesquiterpenoids impeded both germination and seedling growth. These sesquiterpenoids are thought to leached down to

the soil by rainfall or gradually released with the decomposition of leaf litter, affecting the growth of the surrounding plants and assisting *M. micrantha* to become a dominant species in new ecosystems.

2.2 VARIOUS MANAGEMENT TECHNIQUES ADOPTED FOR WEED CONTROL

The different prevention and control measures for terrestrial weed management, such as physical, chemical, and biological, can reduce the quantity of weed but not completely eliminate it. Apart from the fact that each of these methods has its drawbacks. People have used mechanical control methods such as plucking the plant, uprooting, burning, and cutting. But this has not resulted in being an appropriate method for the management.

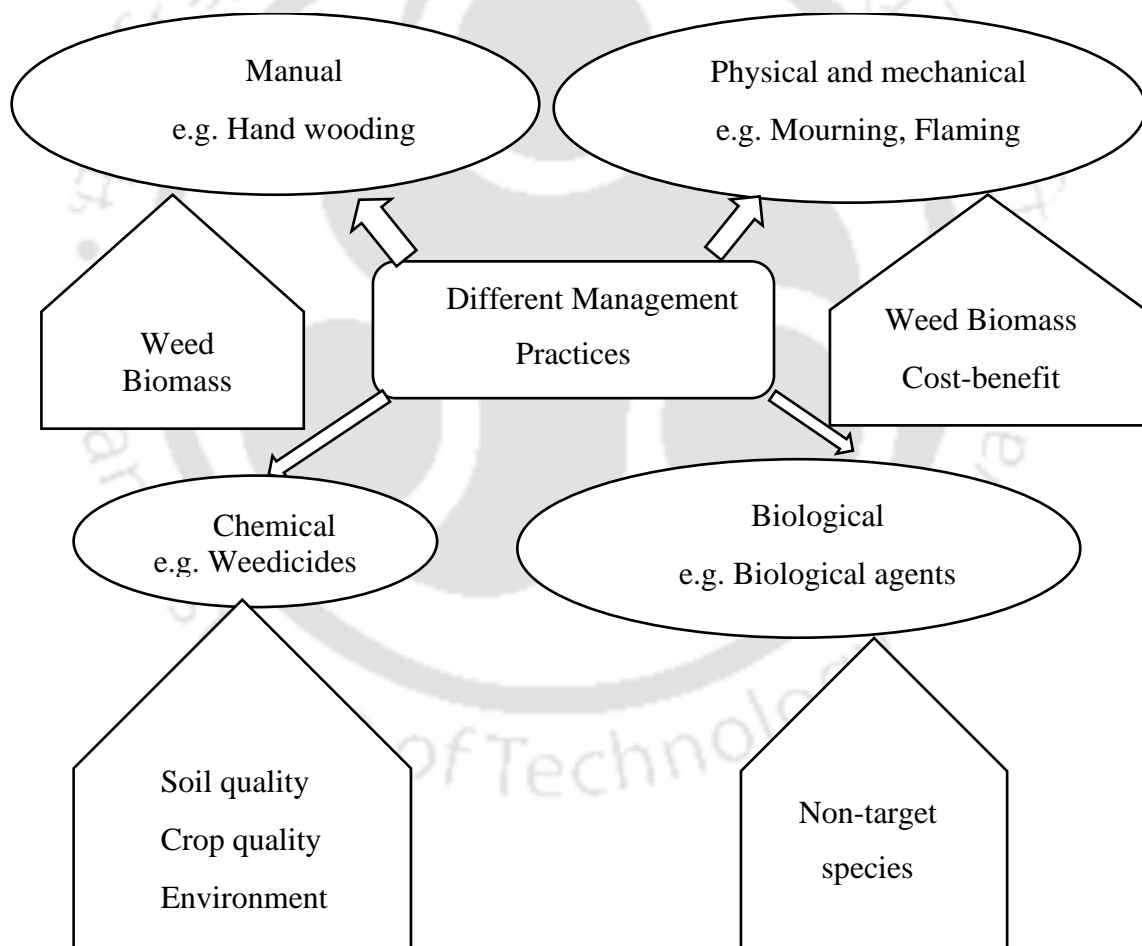


Fig 2.13. Various control strategies have taken on the management of terrestrial weed

Some of the disadvantages of this method are: It requires more hands, which is not possible every time. Plucking and throwing the plants will result in the reproduction of a

new, fully mature plant. Burning will result in the emission of gases that contain harmful secondary metabolites. All these reasons have made this technique an unsuitable one. Many researchers have tried the use of different chemical and biological control methods for eradication of the weed which has its own limitations. Below are descriptions of several terrestrial weed management practices and their implications (Fig. 2.13).

2.2.1 Manual, Physical, Mechanical Methods

People have tried to utilize slashing in plantations manually, but this has been ineffectual and labor-intensive. Because the plant can sprout from even the smallest fragments, all of the stems must be eliminated. Furthermore, *M. micrantha's* crawling and climbing characteristics allow it to enter into the crowns of bushes or trees where mechanical treatments are difficult to apply without causing crop damage (Ellison et al., 2014). Manual cleaning appears to be never-ending labor because if a year is allowed to clean, weed introduction by seeds or plant parts occurs swiftly in the site from the bordering area (Muniappan and Viraktamath, 1993). Every year, a single plant can produce up to 40,000 viable seeds, and even the smallest stem fragment can sprout a new plant in a moist environment (Tiwari et al., 2005). A systematic manual cutting could be a good way to prevent *Mikania* spread, and it would be more fit in community-based forest management, where users contribute willingly to forest management activities (Kuo et al., 2002). According to the study, *Mikania* should be clipped above the ground (1-1.5m) to prevent nutrient and moisture loss, and the leftover part on the ground should be gathered and utilized as mulch over a nearby creeper (Sapkota, 2006). In comparison to other mechanical control methods, this method requires comparatively little labor input. Various studies indicate that the abundance of alien species can be reduced in a small region with a continual effort of manual cleaning (high labour and cost), but this is not possible or sustainable in broad areas. Even in a smaller group, if there is no outside support, the community will not always be active. They lose interest if there is no obvious benefit, as demonstrated in the Dharahara Buffer zone Community Forest User Group in the area (Siwakoti, 2007). In many cases, disposing of weed waste is a challenge. It can be piled along the weeded area's perimeter to allow the still-growing plants to continue to flourish. Littering of a weeded plot (either during transit for safe disposal off-site or by people removing it for fodder) might result in new growth and (perhaps) the spread of the weed to previously uninfested areas. In the case of *Mikania*, a little part of the stem (a node) can grow into a new plant in just a few days (Sankaran et al., 2001). As a result, all garbage

must be i) removed off the plot, (ii) burned as quickly as possible, or iii) utilized for value-added products.

2.2.2 Chemical control technology

Zhang et al., (2004) have reported that herbicides effectively control this plant, but it has a bad effect on the environment. Shen et al., (2013) explained that 2,4-D, Glyphosate, and Atrazine had been reported as an effective herbicide for controlling this plant, but this chemical's potency is less, and regrowth of the plant have been observed. *Mikania* is typically controlled with both pre-emergent and post-emergent herbicides. Herbicides such as glyphosate, 2,4-D, napropamide, and atrazine can be used to reduce *M. micrantha*'s dense growth habit (Palit, 1981). According to (Hu and But, 1994), 0.4% bentazone efficiently inhibited *M. micrantha* germination, and 0.2% picloram/0.4% bentazone was most effective against the growth of 25, 45, and 60-day old seedlings. Furthermore, when competing crops are used in conjunction with a single treatment of bentazone, control can be increased to 90% for up to 3 months, which is preferable to using bentazon alone. Despite spray applications of 2,4-D and glyphosate destroyed above-ground areas of this weed, it had fully recovered after 3 months, but injections of 25% hexazinione and spray applications of 75% sulfometuron methyl killed the entire plant (Zan et al., 2001). *M. micrantha* growth was greatly decreased within 2-3 days of spraying with 0.8 kg gramaxone or 1 kg 2, 4-D amine/ha, according to (Kumar 2005), and the weed did not regenerate for up to 80 days later. Herbicide applications should ideally be made prior to flowering and seed set. While a single, thorough application of any of these herbicides can keep the weed at bay for about a year, most locations will experience regrowth owing to wind-borne seeds, especially after the monsoon starts. Depending on the severity of the re-infestation, annual applications may be required for the next few years. The use of the same herbicides renders the weed flora resistant to them, and evidence of the evolution of resistant species has been found (Auld et al., 1987). Herbicide handling presents several technological challenges, which raises the threat. Excessive application is caused by non-target species, inaccurate herbicide application, and a calibration failure in the spraying apparatus. Herbicide costs include the cost of chemicals and the cost of electricity, machinery, and personnel. Chemical herbicides are being used less frequently due to their accumulation in the food chain, which has detrimental consequences for human health (Al-Samarai et al., 2018).

2.2.3 Biological control technology

Various research has been undertaken around the world to counteract the influence of the *Mikania*. It has been shown that the classical biological method is the most cost-effective, environmentally friendly, and self-sustaining method for controlling this weed. *Mikania micrantha* Kunth (Asteraceae) has been the subject of biological control research since 1978, focusing on insect agents (Poudel et al., 2019). By 1982, host specificity investigations on the first agent, *Liothrips mikaniae* (Priesner) (*Thysanoptera*, *Phlaeothripidae*) from Trinidad, had been completed and the thrips had been released in the Solomon Islands in 1988, and then in Malaysia in 1990 (Day et al., 2011). Neither release resulted in the establishment, and several explanations for this are examined, including the effects of generalist thrips predators and the efficacy of various release tactics (Shichou et al., 2001). Other insect natural enemies were thought to be worth researching for host specificity and effectiveness, but the weevil loss discouraged additional effort. The number of leaves, stem length, bio mass, photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency and chlorophyll content of *M. micrantha* plants that were extirpated by *C. campestris* decreased 2 months after the plants were parasitized by *C. campestris* (Xiong et al., 2003). Extensive studies show that *C. campestris* can reduce the size of *M. micrantha* infestations, land managers should be aware that *C. campestris* is a weed of at least 25 crops, including alfalfa (*Medicago sativa* L.), clover, fava bean (*Vicia faba* L.), beets (*Beta vulgaris* L.), and carrots (*Daucus carota* L.), resulting in significant (Costea and Tardif, 2006). Some chemical and manual control measures are limited when the plant expands over its host. In fact, given to the nature of crop losses and the challenges in attempting to manage them, *C. campestris* was deemed a biological control target (Linke et al., 1992). In 1996, a new initiative was launched in collaboration with the Kerala Forestry Research Institute (India) and Vicosia University (Minas Gerais, Brazil) to assess the weed problem in India's the Western Ghats and develop traditional biological control using exotic coevolved fungal pathogens. *Puccinia spegazzini*, a rust pathogen, has been chosen as the primary option for introduction, and a wide range of neotropical isolates is now being tested in glasshouses (Poudel et al., 2019). The rust has shown 100% specificity for *M. micrantha* (currently 38 non-target species examined), is very destructive (leaf, petiole, and stem infections resulting in cankering and death), and has a wide range of environmental tolerance (Nair, 1988). *M. micrantha* is a major invasive species in Asia, while it is a small ruderal species in its native neotropical habitat (Barreto and Evans, 1995).

As a result, there is substantial evidence that coevolved natural enemies (likely exclusive to a single host taxon) play an important role in managing *Mikania* populations. Biological methods have limited success as the biological agents used become pests in other horticultural crops (Negi et al., 2019). Bio-controlling methods are environmentally friendly, but the application is limited due to host-specific. There is a chance of an attack on neighboring crops (Saini et al., 2014). In a larger area of weed infestation, there is a requirement for a periodical supply of biological agents (Saini et al., 2014). The cost involved in this method is very high. The development cost of the first mycoherbicide was approx USD 2 million. A few antidotes for commercializing the bio-control method are potential in the market, production quantity, and investment cost. The total cost of these management practices was considerably raised to a higher value (Craven et al., 2009).

2.2.4 Studies done on control of *Mikania micrantha* by utilization

Several research have been conducted on the usage of *Mikania* biomass for the generation of value-added end products. Some of the research studies are explained below.

Singh and Poudel (2013) reported the safe use of invasive weed *Mikania micrantha* Kunth for Briquette Fuel production. A survey was done to determine the estimated amount of *Mikania* biomass raw material available. Old rootstocks, runners, and suckers can even regrow from manual cutting. As a result, briquetting using dry biomass of this weed for fuel may be an option for its use. The briquette fuels' physical and fuel qualities and combustion tests were evaluated as an alternative fuel. Various test results reveal that using this plant to make briquette fuel will offer a viable alternative energy source while also assisting in the long-term biodiversity conservation.

Raj and Syriac, (2016) reported about the bio-utilization of invasive weeds like *Eichhornia crassipes*, *Salvinia molesta*, *Parthenium hysterophorus*, *Mikania micrantha* Kunth, *Chromolaena odorata*, *Mimosa invisa*, and *Lantana camara* as these weeds can outcompete native species for space, water, nutrients, and other essential resources, adaptability to a variety of environmental conditions, absence of natural predators and parasites and prolific seed-producing characteristics, once established it is very difficult to control or eliminate. The current approach to invasive alien weed control is based on the eradication principle by usage.

Chaudhuri and Debnath (2019) utilized *Mikania micrantha* Kunth biomass for the vermicomposting process in the ratio 7:3 with cow-dung using *Perionyx excavatus*. Results stated that vermicomposting brought pH to be neutral at the end of the study. A significant

increase in the nitrogen, phosphorus, and potassium content was detected in the final product compared to the initial feedstock. Vermicompost produced from *Mikania micrantha* can be a good source of organic fertilizer utilized for agriculture.

Debnath and Chaudhuri (2020) studied the growth and reproduction of earthworm species *Perionyx excavatus* during the vermicomposting process of *Mikania micrantha* Kunth. The research on the growth and reproduction of composting earthworms may be essential for the production of composting earthworms on a large scale. By using a limited supply of four experimental diets-cow dung alone, cow dung mixed with acacia (*Acacia auriculiformis*) leaf litter, bamboo (*Bambusa polymorpha*) leaf litter, and the terrestrial weed *Mikania micrantha*, the study evaluated the growth and reproduction of *P. excavatus* in a laboratory environment. The performance of *P. excavatus* in terms of growth was greatly improved when grown in an acacia-cow dung mixture. According to the results, the mikania-cow dung mixture had the slowest rate of biomass increase and the slowest rate of reproduction. According to the present findings, cow dung and acacia leaf litter can be employed as vermiculture substrates for *P. excavatus* in vermiculture.

Yadav and Neupane (2021) stated comparison research on the utilization of invasive weed *Mikania micrantha* Kunth and banana tree waste was published, where the authors observed that the fixed carbon content of banana biomass was higher than that of *Mikania micrantha*, which was approximately 11.26%. According to the findings of the study, banana tree waste is more suited for briquette production than the *Mikania micrantha* biomass.

2.3 SUSTAINABLE APPROACH

2.3.1 Biological treatment of terrestrial weed

The world is concerned, especially in the agriculture sector and economic development, by the exponential growth of this terrestrial weed. Different management methods for eradication were implied, but none were entirely effective, as shown in Fig. 2.14. In order to eradicate these weeds, the chemical pesticide has been added, which adds to more environmental issues. The best way to turn this herb into a profitable commodity is by utilizing it. Conversion of this weed into compost as an agricultural fertilizer is possible. Since it is poisonous and toxic to crops and species via environmentally sustainable methods and the development of added-value items, reclamation of the weed is desperately required. Wakjira et al., (2009) reported that until flowering, composting *Parthenium* is a method of minimizing its allelopathic inhibition ability and one way of controlling it

through utilizing. Author further explained that composting of *Parthenium* with other plant materials decreased the allelopathic inhibition effects of *Parthenium* on the rate of emergence of lettuce and longer stretches of radicles and plumules than composting *Parthenium* alone. Rai and Suthar (2020) observed that *Parthenium* mixed with cow dung decreased organic carbon by 45-52% and increased mineral contents. Quansah et al., (2001) reported that three high biomass-producing plants (*Chromolaena odorata*, *Panicum maximum*, and *Pueraria phaseoloides*), considered to be weeds in the fields of farmers in Ghana, were evaluated as sources of nutrients and amendments to organic matter. Jamilah, (2017) clarified that after applying *C. odorata*, there was an improvement in soil chemical properties, compost achieved maximum fertility for rice crops. The intake of nitrogen and other minerals in rice crops has improved due to increased availability of chemical fertilizers followed by a decrease in the dose of *C. odorata* compost. The nutrient uptake potential of rice crops in Pandan Wangi rice is greater and results in a higher forage yield than Cisokan or Red Cempo rice. Panjaitan et al., (2018) reported that the increased fertility of ultisol soils by adding *C. odorata* compost and mycorrhizal inoculation gives plants the optimum absorption of nutrients. The results of this study suggest the residues of these traditional weeds for evaluation in organic composting, together with the release of nutrients to plants as organic fertilizer for plant-parasitic nematode control capacity (Odeyemi et al., 2014). Composts produced by in-vessel compost technology have shown considerable efficacy in improving soil properties, such as soil conductivity, stability, tolerance to erosion, soil fertility, and plant nutrition (Larchevêque et al., 2006). Paradelo and Barral (2012) reported that compost serves as a soil conditioner, increasing organic matter, water content, and the ability for cation exchange in the soil. The microbial study is still in its primitive stage, and more research needs to be focused on this area.

Apart from compost, vermicompost application also improves soil quality in terms of nutrients and physical properties. Vermicomposting is a process by which worms turn organic materials (usually waste) into a humus-like product known as vermicompost (Kumar et al., 2008). Although microorganisms biochemically degrade organic matter, earthworms are the key drivers of the mechanism by promoting aeration conditions, fragmenting the substrate, and thus dramatically microbial operation (Dominguez and Edwards, 2010; Edwards et al., 2019). Vermicomposting technology has been used widely to produce compost using terrestrial weed as the main substrate. The vermicomposting method eliminates the toxicity of weed substrates by altering their chemical composition and rendering non-toxic organic manure with beneficial biological properties (Rai et al.,

2021). Vermicomposting of *Lantana camara*, an invasive terrestrial weed, was done mixing with cow dung that gave a good quality compost (Singh and Kumar, 2017). It was further observed that the use of *Parthenium* and *Lantana* for vermicomposting process increased total nitrogen, phosphorus, potassium, and sodium in all the trails. Earthworm actions are a major factor in weed control as seeds of plant material are destroyed by earthworms due to contraction and various enzymatic processes that lead to seed dormancy.

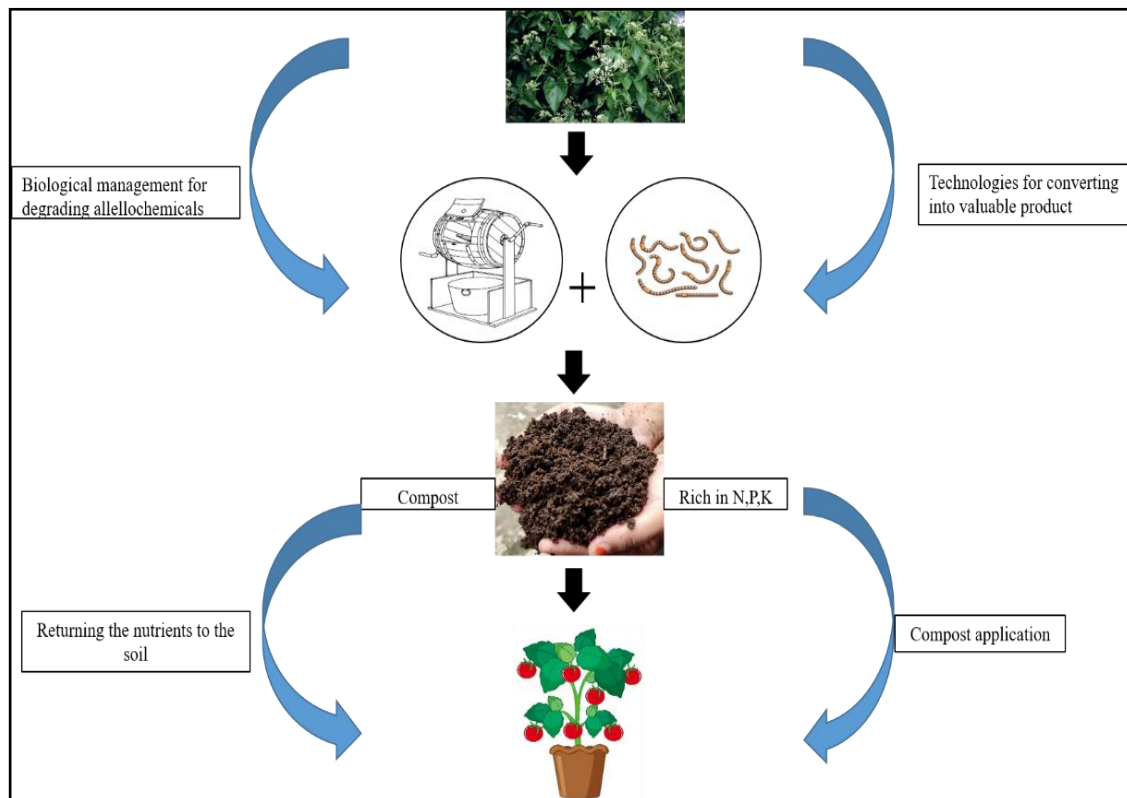


Fig. 2.14 Advantages of compost prepared from weed biomass on crop growth

Vermicomposting of *Parthenium* combined with cow dung at various ratios decreased pH, organic carbon, and C:N ratio but increased total nitrogen content, EC, available phosphorus, total calcium, total potassium, and heavy metals (Yadav and Garg, 2011). Hussain et al., (2016) reported the use of *Parthenium* for the production of vermicompost using earthworm species *E. fetida* that employed the germination of *Vigna radiate*, *Abelmoschus esculentus* and *Cucumis sativus*. Author further added that there was no allelopathic effect of *Parthenium* during the process. Using the vermicomposting process, bioconversion of the *L. camara* increased the nitrogen content and reduced the overall organic carbon content (Devi and Khwairakpam, 2020). Suthar et al., (2013) reported that *Lantana* leaf biomass, after mixing with cow dung in appropriate proportion, can be used

as a suitable substrate for vermicomposting and the phytotoxicity check specifically showed that vermicompost waste mixtures are appropriate for the agronomic purpose. Vermicomposting can turn *Ageratum conyzoids* into a beneficial end product by combining with cow dung to minimize the C / N ratio, total organic carbon, and CO₂ growth, thus also increasing the stability of vermicompost that is rich in nutrients such as total nitrogen, total phosphorus, potassium and calcium (Devi and Khwairakpam, 2020). The mixture of *Ipomoea staphylina* biomass, cow dung, and mushroom spent straw was suitable for producing vermicompost using *E. eugeniae* (Balachandar et al., 2021). Rameshwar & Argaw, (2016) stated that an attempt was made in the research areas of Haramaya University, Ethiopia, to develop the *L. camara* vermicompost with farm waste and various animal manures. Hussain et al., (2015) reported that in addition to plant/animal toxicity in other respects, *Lantana* is known to have a severe negative effect on the environment. Its vermicompost was considered to be good organic fertilizer as it increased germination efficiency and encouraged the development of all three botanical species such as green gram (*Vigna radiata*), female finger (*Abelmoschus esculentus*), and cucumber.

Table 2.2. Work done on destruction of allelochemicals of terrestrial weed through composting and vermicomposting process

Author/year	Study area	Title	Remarks
Olaniyi et al., 2009	Nigeria	Effect of different methods of <i>Chromolaena odorata</i> compost preparation on the growth and yield of cucumber (<i>Cucumis sativa</i>) in southwestern Nigeria	Cucumber production was highest in compost made from <i>Chromolaena</i>
Borah et al., 2009	India	Characterization of vermicompost in relation to weed biomass and earthworm species	The highest production of NPK was found in <i>Ipomoea carnea</i>
Yadav et al., 2011	India	Vermicomposting – An effective tool for the management of invasive weed <i>Parthenium hysterophorus</i>	Parthenium can be a raw material for vermicomposting when mixed with cow dung in a sufficient quantity.
Anbalagan et al., 2012	India	Bio management of <i>Parthenium hysterophorus</i> (Asteraceae) using an earthworm, <i>Eisenia fetida</i> (Savigny) for recycling the nutrients	Vermicomposting of Parthenium mixed with cow dung gave a good C/N ratio
Anbalagan et al., 2012	India	Biomangement of <i>Parthenium hysterophorus</i> (Asteraceae) using using an earthworm, <i>Eisenia fetida</i> (Savigny) for recycling the nutrients	Vermicomposting of <i>Parthenium</i> mixed with cow dung gave a good C/N ratio
Rajiv et al., 2013	India	Vermiremediation: Detoxification of Parthenin toxin from <i>Parthenium</i> weeds	Vermicompost was performed on terrestrial weed
Rajbanshi and Inubushi (1998)	Nepal	Chemical and biochemical changes during laboratory-scale composting of allelopathic plant leaves (<i>Eupatorium adenophorum</i> and <i>Lantana camara</i>)	Nitrogen content increase upto 43 and 29% in eupatorium and <i>Lantana</i> . Significant decrease of allelochemical was observed in terms of seed germination

2.4 COMPOSTING PROCESS

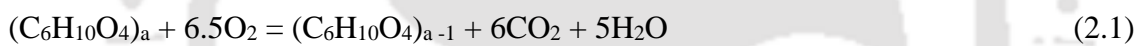
2.4.1 History of Composting and its Technology

The Akkadian empire introduced the use of fertilizer in the Mesopotamian Basin into their farming activities which were recorded in the clay tablets at around 2300 B.C. (Fitzpatrick et al., 2005). In his book titled “De Agri Cultura,” Marcus Porcius Cato, a retired Roman general, described composting in 234-149 BC. It included composting animal dung such as goat, sheep, cattle, and other dung, and plant residue, including stalks of vine, chalk and husk, and leaves of ilex and oak (carryoncomposting.com). He further reported about the composting system designed in 1787 by George Washington, first president of the United States, at his estate of Mount Vernon, Va. Historically, composting was advocated both as the basis of the organic method of gardening and farming and as a methodology for waste management. Ancient composting is also demonstrated by the use of ard (a tool used to incorporate compostable materials into the cultivated lands) in Scotland and China. Several remarkable Americans who supported and practiced compost were George Washington Carver, James Madison, Thomas Jefferson, and George Washington. In the early 18th and 19th centuries, since the U.S. had an abundance of nutrient-rich land, many farmers dismissed the idea of fair agricultural activities. Professor F.H. King of the United States Department of Agriculture visited China, Japan, and Korea at the beginning of the 19th century and published its findings in farmers of forty centuries, describing how manure, canal mud, green manures, and composts are used to maintain soil fertility. (Blum, 1992) explained that earlier, composting to preserve soil fertility was not considered a worthwhile investment method, resulting in a major decrease in the vitality of soil nutrients. Howard (1935) made the breakthrough in composting technique in India. They collaborated with Jackson and Wad and their co-workers to systemize the traditional procedure into a newly reformed Indore process technique (Jackson and Wad, 1934). The Indore Process's invention involved composting animal and vegetable waste layers, alternating with animal manure. However, in 1931 in a short publication entitled “The Waste Products of Agriculture,” he developed a pit composition system. Its 'compost factory' comprised 30-3 large buckets, each 30 ft. by 14 ft. and 2,0 ft. depth and with inclined sides, arranged between the lines of pits in three rows with adequate space for easy cart passage. In 1924 Austrian author Rudolph Steiner presented the ideals of biodynamic farming and stressed composting as a key activity (Steiner, 1924). The Dust Bowl in the United States was one of the largest agricultural disasters of history when dripping and

exhausted farming practices were practiced (Blum, 1992). He further reported that environmental and soil conservation workers extensively explored the creativity and causes of soil vitality that led into the dust bowl in the 1930s. The Beccari composting system, which Giovanni Beccari developed in Italy in the 1920s, was one of the earliest mechanized composting systems. More than 50 such systems were built in Europe during the 1920s and 30s, and five in the United States. It was an effective early mechanized system, later replaced by a more productive system, and no Beccari system is currently in service (Gotaas and Organization, 1956). Indeed, when Howard and his associates, Acharya and Subramanyam, independently developed Indore and Bangalore composting methods, India can take the credit for developing systematic composting methods (Bhide, 1983).

2.4.2 Definition and Purpose

Composting is a microbiological conversion of organic residues of plant and animal origin to manure rich in humus and plant nutrients. During the process, the release of by products such as carbon dioxide, water, and heat (Abbasi & Ramasami, 1999; Bharadwaj, 1995). The simplified chemical formula has expressed the aerobic bioconversion reaction of organic waste in Eq. 2.1.



The reaction (2.1) is strongly exothermic with an assumed heat of formation of the organic matter of 230 kcal/mol. It generates 616 Kcal of heat per mole of organic matter reacted; with comparison, the heat of biodegradation of glucose is -673 kcal/mole to produce carbon dioxide and water (Themelis et al., 2002). Generally, composting systems are of two types: open systems and in-vessel systems. Open composting systems are the first types of systems originated and practiced from the evolution of composting times, including windrow systems, static and household systems. In-vessel systems include tunnel systems, the rotary drum, and the reactor systems of various designs (Gajalakshmi and Abbasi, 2008). Furthermore, based on the oxygen supply system to the composting system, they are classified into two; the agitated and the static system. Normally, in an agitated system, the compost materials are mechanically turned using large machines to supply aeration and release inner temperature, including mixing the materials. Whereas in static systems, the compost heaps are made on a series of perforated tubes connected to a blower which is controlled manually or on a timer basis to supply aeration into the system, so the temperature is maintained within the system (Tchobanoglous, 1993) as shown in Fig. 2.15.

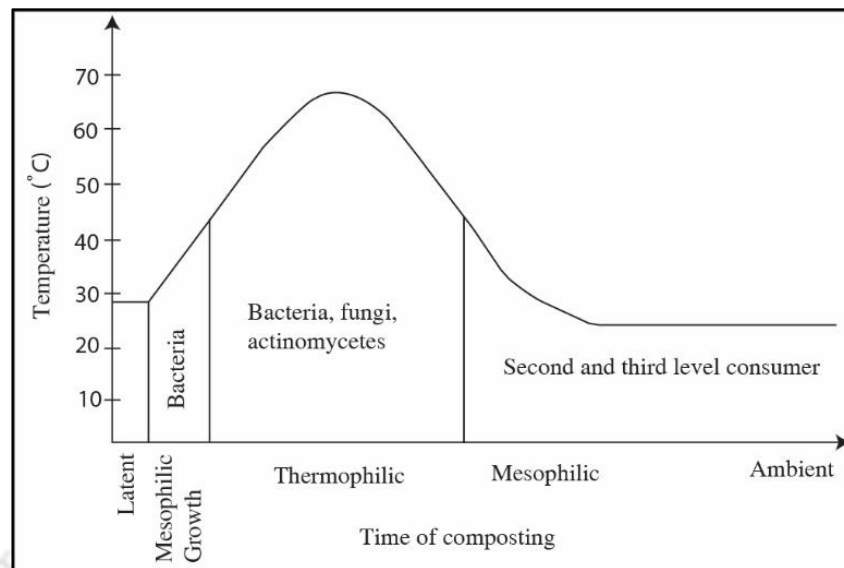


Fig. 2.15 Stages of composting process (Source: Sa'adah, 2015)

Organic matter generally degrades more rapidly and more completely if oxygen is plentiful. This can be explained by the presence of a large amount of free energy produced for microbial growth where the prominent electron-acceptor is oxygen. Oxygen can be incorporated into molecules, devoid of this element, through the action of the widely distributed, non-substrate-specific, and inducible enzymes 'oxygenases.' This is often the first necessary step in metabolic sequences leading to the degradation of molecules resistant to biological attack. Classes of organic micro-contaminants acted upon by oxygenases include saturated alkanes, aromatic hydrocarbons, and halogenated hydrocarbons; anaerobic environments lack this mechanism. Since anaerobic composting produces significantly less free energy (heat) than aerobic composting, a longer period is required for organic decomposition and pathogen inactivation.

2.4.3 Factors affecting the composting process

- **Temperature**

The aerobic decomposition of a gram mole of glucose releases 484 to 674-kilo calories (kcal) energy under controlled conditions, while only 26 kcal are released when it is decomposed anaerobically (CPHEEO, 2016). It was further elaborated that municipal solid waste is known to have good insulation properties, and hence the released heat results in an increase in temperature of the decomposing mass. As some heat loss occurs from the exposed surface, the actual rise in temperature will be slightly less. When the decomposing mass is disturbed, heat loss results in a drop in temperatures. Under properly controlled

conditions, temperatures rise beyond 70°C in aerobic composting. As the compost heats up above 40°C, thermophilic bacteria take over. Members of the genus *Bacillus* dominate the microbial populations during this phase. The diversity of bacilli species is fairly high at temperatures from 50-55°C but decreases dramatically at 60°C or above. When conditions become unfavorable, bacilli survive by forming endospores, thick-walled spores that are highly resistant to heat, cold, dryness, or lack of food. They are ubiquitous and become active whenever environmental conditions are favorable. Once the compost cools down, mesophilic bacteria again predominate. The numbers and types of mesophilic microbes that recolonize compost as it matures depend on what spores and organisms are present in the compost and the immediate environment. In general, the longer the curing or maturation phase, the more diverse the microbial community it supports. Various studies have shown that the activity of cellulose enzyme reduces at more than 70°C, and the optimum temperature range for nitrification is 30°C to 50°C, beyond which nitrogen loss is known to occur. The high temperature also helps destroy some common pathogens and parasites. The heat death of a cell means the thermal inactivation of its enzymes. Without enzymatic activity, a cell cannot function and will die. Pathogens are also destroyed or controlled by competition with other microbes, antagonistic relationships, antibiotic or inhibiting substances produced by microbes, and time of survival (Haug, 1993). But temperature is the only factor that the operator can measure and control during composting. Hence, the US Environmental Protection Agency recommended 53°C for 5 days, 55°C for 2 days, and 70°C for 30 minutes to destroy pathogens.

- **Moisture content**

The maximum permissible moisture content is represented by the numerical value of the operational parameter moisture. As previously indicated, this value changes depending on the substrate. The lowest permitted moisture concentration for efficient composting is around 45%, regardless of substrate. Because conditions in a composting mass are favorable to evaporation, unfavorably low moisture content is a common concern in composting (i.e., water loss) (Mohee and Mudhoo, 2005). The author further reported that ideal moisture content for wastes approaches saturation as long as the material can be adequately aerated to meet the oxygen demand. The moisture level over which oxygen availability becomes insufficient and anaerobic conditions may occur. The optimum moisture content is usually the maximum permitted moisture content. Because the air trapped between particles is the microbial population's principal source of oxygen, interstitial ('pore') volume is a critical element (i.e., the more numerous the pores, the

greater the interstitial volume). The relationship between porosity and moisture arises because the greater the percentage of pore volume occupied by water, the less volume accessible for air and thus for oxygen.

- **pH**

The alkalinity or acidity of a solution is measured using the pH scale. A decrease in the pH value of composting material is associated with an increase in acid generation during the early stages of composting, which is caused by simple organic acids formed during the initial decomposition phase due to the fermentation activity that is taking place. Organic acids generated serve as a source of food for bacteria while also lowering the pH of the environment (Ameen et al., 2016) The subsequent rise in pH after that is an indicator of acid consumption. Buffering is not required, and it may potentially have negative implications. As the breakdown progresses, alkaline pH features become more prominent because proteins are targeted, and ammonia is released. Excessive nitrogen loss as ammonia will result from highly alkaline conditions, whereas failure to warm up the matrix under highly acidic conditions will result from highly acidic starting conditions. pH values that are managed are achieved through proper modifications in feed preparation. (Nakasaka et al., 1993) concluded that the breakdown rate of organic matter was faster in the pH-controlled studies than in the control experiments. The regulation of pH value increased nitrogen loss. High pH values indicate the presence of anaerobic conditions, which are followed by the production of odors and harmful by-products.

- **C/N ratio**

The basic nutrients required for composting microorganisms are carbon (C), nitrogen (N), phosphorous (P), and potassium (K). Microorganisms use carbon for energy and growth, but nitrogen is required for protein formation and reproduction. The carbon-to-nitrogen ratio (C:N ratio) is the ratio of carbon to nitrogen (Kumar, 2011). A proper C:N ratio usually ensures that all other nutrients are present in sufficient proportions. Although initial C:N ratios ranging from 20:1 to 40:1 consistently produce satisfactory composting results, raw materials combined to yield a C:N ratio of 25:1 to 30:1 are ideal for active composting. When the C:N ratio is less than 20:1, the available carbon is fully consumed without stabilizing all of the nitrogen, resulting in the generation of excess ammonia and disagreeable aromas (Li et al., 2011). When the C:N ratio exceeds 40:1, not enough N is available for microbial development, and the composting process slows drastically.

2.4.4 In-vessel composting technique

Rotary drum composters were among the earliest types of in-vessel composting systems to be designed, with engineering techniques that were radically different from other traditional methods previously used in Fig. 2.16 (a & b).

Part (2000) stated that mechanical systems are designed to reduce odor and process time by managing environmental parameters such as airflow, temperature, and oxygen content. In-vessel systems have grown in popularity because of odor control, faster processing, cheaper labor costs, and limited area requirements. The time spent in the tank varies from 1 to 2 weeks, although most systems use a 4- to 12-week curing period after the active composting period.

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



Fig. 2.16. In-vessel composter design (a) Small scale in vessel composter (<https://xactsystemscomposting.com/images/>) & (b) Pilot scale in-vessel composter (<https://sfcenvironment.com/orex>)

Smith et al. (2006) evaluated mechanical rotating drum composting for food waste. Food scraps from a Texas prison were mixed with fine-textured soft-wood shavings in ratios of 2/1, 3/1, and 4/1 to yield 0.4 m³ of compost blend. During 15-day composting testing, all mixes showed volume and weight reductions. During the experiments, all blends reached

thermophilic temperatures ($> 45^{\circ}\text{C}$). The initial pH of all blends was low, reaching 3.55 before returning to compostable levels (6.0-7.5). Compost pH increased as temperature increased towards the thermophilic range. Most nutrients (P, K, Ca, Mg, S, Na, and Zn) rose in concentration during composting, except for N, which declined in some cases. The study concluded that mechanical in-vessel rotating drum composting might be a viable food waste disposal option. The mechanism of the composting process has been illustrated in Fig. 2.17.

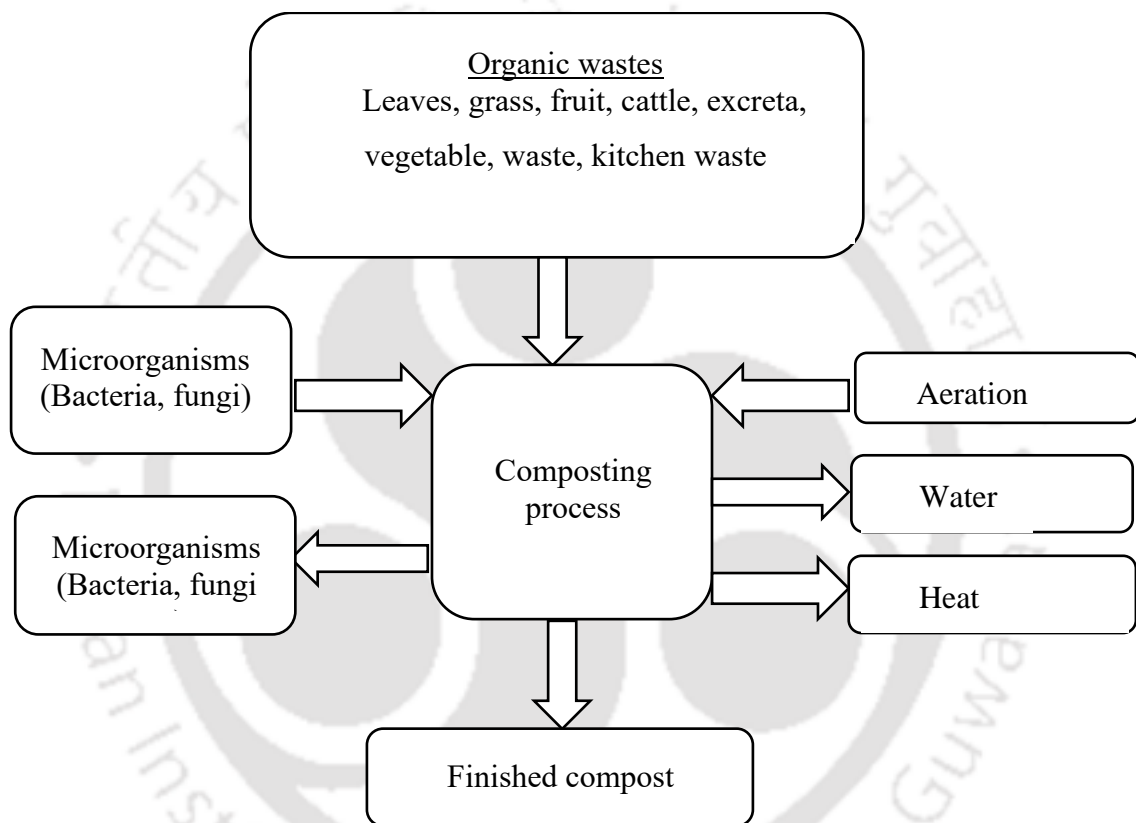


Fig. 2.17. Mechanism of the composting process

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

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

Jain et al. (2018) investigated the physical parameters of solid pulp and paper mill sludge composting in a 550 L rotational drum composter. During the composting process, physical factors such as bulk density, volatile solids, moisture content, free air space, void ratio, ash content, and particle density varied. Bulk density was found to be growing, while free air space was decreasing and was found to be 52% in the final compost. The particle density was found to increase from 610 to 680 kg m³. End compost was tested for nutritional characteristics, which were shown to be increasing throughout composting.

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

Ajmal et al. (2020) investigated and optimized the applied temperature and allowed processing time in accelerating the breakdown and mineralization rates of agricultural waste in-vessel composting. Under the Taguchi technique, a total of nine experiments were carried out with three levels of temperature (55 °C, 65 °C, 75 °C) and time (15 h, 18 h, 22 h) in a pilot-scale composter equipped with temperature, aeration, agitation, and humidity control systems for the composting of poultry manure (PM), vegetable waste (VW), and rice straw (RS).

Maturi et al. (2021) used the in-vessel composting technique to control the invasive weed *Ageratum conyzoides*. The research focused on composting this diverse weed by evaluating the heavy metal toxicity throughout the composting process. The substrate's

volatile solids and biological parameters were elevated and decreased in the final compost product. The mass balance idea assessed the rotary drum composting process's dynamics. The research specifies the dynamics of heavy metals in the rotary drum composting process and shows the proportions of each metal in several distinguished chemical fractions.

2.4.5 Vermicomposting process

Vermicomposting is a sophisticated bio-oxidative composting process that employs earthworms to transform organic wastes into high-quality compost. Different types of earthworms species used in the various studies for the degradation of organic waste has been shown in Fig. 2.18. Vermicomposting, unlike other composting processes, is not an exothermic process. The organic matter holding the majority of the nutrients is transformed into more accessible forms known as vermicast during this process. Initially, the substrate is split into small bits for swallowing, and it then enters the gizzard of earthworms, where it is minced. This mincing increases the surface area of the substrate and allows for microbial action (Chan and Griffiths, 1988). Vermicomposting is the most eco-friendly recycling process to reduce organic waste. This recycling process not only converts organic debris into high quality compost but the chemical changes that the debris undergoes makes the nutrients easily available to the plants. Several epigeics (*Eisenia fetida*, *Eisenia andrei*, *Eudrilus eugeniae*, *Perionyx excavatus*, *Perionyx ceylanesis* and *Perionyx sansibaricus*) have been identified as potential candidates to decompose organic waste materials (Suthar, 2007).

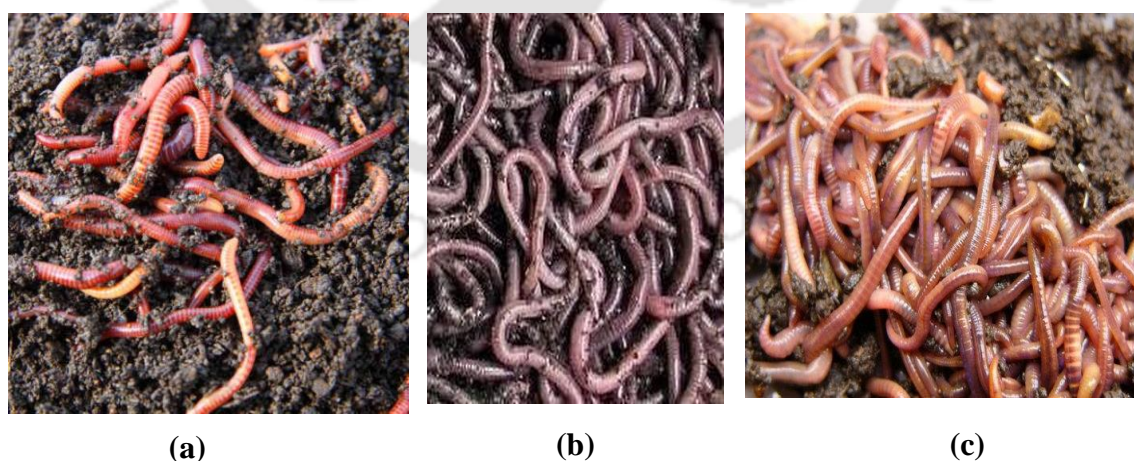


Fig. 2.18. Different types of earthworm species; (a) *Eisenia fetida* (red wiggler worm), (b) *Eudrilus eugeniae* (African Nightcrawler), (c) *Perionyx excavatus*

Some attempts have been made to biodegrade a variety of materials using vermicomposting (Sangwan et al., 2008). The improvement of plant growth with use of vermicompost may result from its nutrients and biologically active substances (Warman and AngLopez, 2010). Many plant growth regulators including auxins, gibberellins, humic acids (Atiyeh et al., 2002) and cytokinins of microbial origin (Tomati et al., 1988) are found in vermicompost. Additionally, the activity of soil enzymes such as urease, phosphomonoesterase, phosphodiesterase and arylsulfatase increases significantly with vermicompost application (Albiach et al., 2000).

Vermicasts are earthworm excretory pellets, whereas vermicompost is a homogeneous mixture of vermicasts and humus like decomposed organic materials in the vermibed (Fig. 2.19). Samal et al. (2019) thoroughly reviewed the ability of vermicasts to improve soil health and regulate soil texture, and fresh vermicasts maintain their stability for an extended period of time. The percentage of waste mixtures (waste materials + amendments) consumed by earthworms determines the stability of the vermicasts. The cast materials' particles form strong bonds with a variety of microbial symbionts and enzymes. Dlamini and Haynes (2004) confirmed that vermicasts ejected by epigeic earthworm species are more stable than vermicasts ejected by endogeic earthworm species.

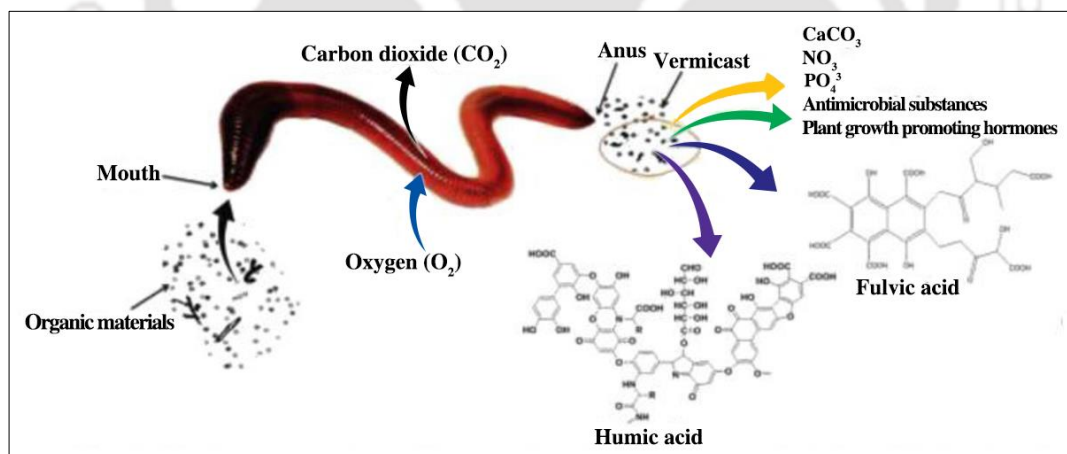


Fig. 2.19. The mechanism of conversion of organic waste into humified material

A temperature range of 15 to 35°C was discovered to be optimal for the growth of *Eisenia fetida* (Dominguez and Edwards, 2004). This temperature variation has a significant impact on earthworm reproduction and activity. In a study on the effect of temperature on earthworms conducted by Frederickson and Howell (2003), it was discovered that low ambient temperatures have a much lower rate of reproduction than

moderate temperatures during processing. As a result, as indicated by (Neuhauser et al., 1988), the temperature has a higher influence on earthworms during the composting process.

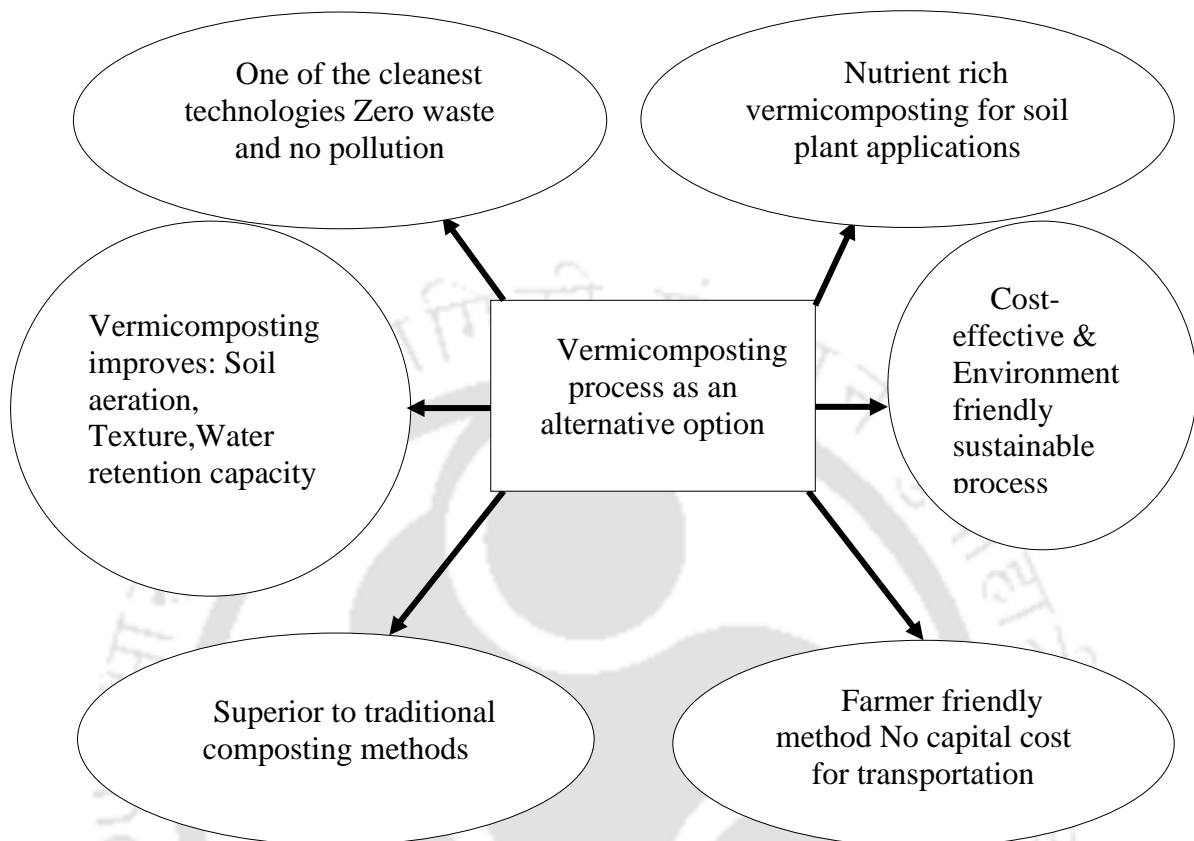


Fig. 2.20. Vermicomposting approach as an alternative technique for organic waste management

Furthermore, as revealed by (Jadia and Fulekar, 2008) in a study on vermicomposting of vegetable waste in a hydro-operating bioreactor, the thermophilic condition during the process favors the inactivation of many hazardous pathogens in the bedding materials. Earthworms are extremely susceptible to anaerobic environments. They use their body wall to draw oxygen in and diffuse carbon-di-oxide out despite their lack of specific respiratory organs. Almost any sort of organic material, including agricultural, urban, or industrial organic material, can be vermicomposted; however, pre-processing such as washing, pre-composting, and macerating may be required to assist vermicomposting (Gajalakshmi et al., 2001). Vermicomposting is a viable approach for the degradation of organic waste is illustrated in Fig. 2.20. Various research has been conducted on the degradation of organic matter using vermicomposting technology because it produces higher-quality compost than traditional composting techniques.

Moisture content, pH, and temperature are all important factors in vermicomposting since they have a direct impact on cocoon development and earthworm growth, ultimately affecting composting stability. Most researchers have established that earthworms have well-defined tolerance limits to the aforementioned characteristics. As a result, if there is a significant difference in these limitations, the composting process may be slowed, and in the worst-case scenario, earthworms may die. It has been proposed that an average moisture content of 50 to 90 % be used to create a suitable environment for earthworms to function successfully on organic matter transformation (Domínguez et al., 2010). Furthermore, the optimal range of these factors changes depending on the species used in the process. The pH of earthworms is relatively sensitive. It has been observed that they can survive in slightly acidic to alkaline conditions, but not below 4.5. (Bhawalkar, 1995) offered a neutral substrate pH for vermicomposting, while (Edwards and Bohlen, 1996) indicated a wide pH range of 5.0 to 9.0 for maximum earthworm growth during the operations. Bhawalkar (1995) recommended a neutral substrate pH for vermicomposting, while (Abduli et al., 2013) indicated a wide pH range of 5.0 to 9.0 for maximum earthworm growth during the operations. (Satchell, 1955) also reported on the classification of taxa based on their acidity tolerance. It was also reported that *Lumbricus terrestris* was not extremely sensitive to pH among the various species classified during his investigation. The C/N ratio is also important in the breakdown process during vermicomposting (Nayak et al., 2013). Possible utilization of organic waste using vermicomposting process has been illustrated in Table 2.3.

Table 2.3. Work done on vermicomposting of different organic waste

Author/year	Title	Remarks
Shanthi et al., 1993	Vermicomposting of vegetable waste.	The controlled laboratory tests used three common species (<i>Pheretima sp.</i> , <i>Eisenia sp.</i> , and <i>P. excavatus</i>). The study concluded that <i>P. excavatus</i> is the best species for vermicomposting vegetable waste.
Elvira et al., 1998	Vermicomposting of sludges from paper mill and dairy industries with <i>Eisenia andrei</i> : a pilot-scale study	Vermicomposting of paper mill sludge was done using <i>Eisenia Andrei</i> to obtain nutrient rich compost.
Benitez et al., 1999	Enzyme activities as indicators of the stabilization of sewage sludges composting with <i>Eisenia foetida</i> .	Studied the evolution of earthworm (<i>Eisenia fetida</i>) biomass and changes in enzyme activities during 18 weeks of sewage sludge vermicomposting.
Bansal and Kapoor, 2000	Vermicomposting of crop residues and cattle dung with <i>Eisenia foetida</i> .	90-day composting experiment with <i>Eisenia fetida</i> on mustard leftovers, sugarcane waste, and calf dung. After 90 days of vermicomposting, C:N ratio got reduced and enhanced mineral N.
Yadav and Karmegam, 2020	Bioconversion of different organic waste into fortified vermicompost with the help of earthworm: A comprehensive.	The research examined into the bioconversion of several types of organic wastes using epigeic earthworm species.
Das et al., 2021	A valorisation approach in recycling of organic wastes using low-grade rock minerals and microbial culture through vermicomposting.	The study investigated the influence of rock mineral addition on organic wastes (water hyacinth and paddy straw) combined with microbial inoculums on vermicompost quality.
Ameen and Al-Homaidan, 2022	Improving the efficiency of vermicomposting of polluted organic food wastes by adding biochar and mangrove fungi.	Over a 90-day composting period, the study evaluated vermicomposting of food waste modified with biochar and cow manure.

2.5 MICROBIAL DIVERSITY AND ENZYME ACTIVITY

In compost, microbes and enzymes play an important role in reducing organic matter to simpler organic carbon and nitrogen units. The active microbial population in the compost determines its quality and stability. The optimal environmental variables for microbial viability, such as pH, moisture content, C/N ratio, and temperature, vary depending on the composting methodology used. There have been numerous studies on the isolation and diversity of microbes in composting techniques (Raut et al., 2008; Bhatia et al., 2012).

2.5.1 Bacteria

Physical decomposers include the micro-organisms that live in or around a compost pile, such as mites, centipedes, sowbugs, snails, beetles, ants, and earthworms. These organisms grind, shred, and chew materials to break them down into smaller bits. The most organic material breakdown is accomplished by micro-organisms such as bacteria, fungus, and actinomycetes, which are often overlooked in a compost pile yet are responsible for most organic material decomposition (Cusworth, 2020). They are referred to as chemical decomposers because they use chemicals produced by their bodies to break down organic substances in their environment. Importantly, a wide range of mesophilic, thermotolerant, and thermostatic aerobic microorganisms, including bacteria, actinomycetes, yeasts, and a variety of other fungi, have been found in abundance in composts and other self-heating organic materials, as well as in soils and other organic materials that do not require external heating (Amner et al., 1988; Faure and Deschamps, 1991; Finstein and Morris, 1975; Strom, 1985; Beffa et al., 1996). During the composting process, a variety of factors influence the microbial community. Under aerobic conditions, the temperature is the most important factor influencing the types of microorganisms present, the diversity of species present, and the rate of metabolic activity. When it comes to chemical decomposers in a compost pile, aerobic bacteria are the most prevalent. In the process of decomposition, they produce heat. In a compost pile, the millions of aerobic bacteria trying to degrade the organic materials generate heat, which causes the pile to heat up. They can grow on soluble proteins and other soluble substrates with relative ease. These bacteria also possess the ability to assault more complicated material by releasing extracellular enzymes (Epstein, 1997). *Bacillus*, *Cellulomonas*, *Pseudomonas*, *Klebsiella*, and *Azomonas* are some of the bacterial species typically found in aerobic breakdown systems and other bacteria (Strom, 1985; Bhatia et al., 2013). Composting occurs at temperatures less than 40°C, and mesophilic bacteria such as *Bacillus* spp. and *Azotobacter* spp. are responsible for CO₂ evolution from the

composting heaps during the early phases of composting. Once the thermophilic stage is reached, these microorganisms are either partially or completely killed, depending on their activity level during this period (Beffa et al., 1996).

So far, various methodologies have been employed to explore the microorganisms that are present during the composting process. The use of conventional plating and the identification of culturable microorganisms are examples of techniques that can be used to determine microbial diversity during composting (Hardy and Sivasithamparam, 1989; Davis et al., 1992) and more recent techniques measuring ATP content (Tseng et al., 1996), microbial biomass (Derikx et al., 1990). It has recently been discovered that direct analysis of phospholipid fatty acid (PLFA) patterns can be used to determine the microbial community composition in compost samples without the need for organisms to be cultured on agar media (Hellmann et al., 1997; Bogg et al., 1998) or the extraction of DNA or rRNA (Kutzner, 2001; Ivors et al., 2000) in compost samples. Composting is the process through which organic matter is processed by a variety of microbes and then transformed into a humus-like compound through humification. Nitrogenous substances go through various biochemical alterations as a result of this process. Ammonium ions are produced in the compost due to the denitrification process, which occurs when proteins and amino acids are present. These ammonium ions will be aerobically transformed into nitrate due to this conversion (Hansen et al., 1990). Compost-derived ammonium-oxidizing and nitrite-oxidizing bacteria of the genera *Nitrosomonas* & *Nitrobacter* and *Nitrobacter* spp. are responsible for these changes (Focht and Verstraete, 1977). During drum composting, Bhatia et al. (2012) discovered the presence of gram-positive rod bacteria and the preponderance of gram-negative bacilli-shaped bacteria. Due to a diverse group of bacteria and a higher temperature, the transformation of organic compounds during the biodegradation of organic waste and the change in the usage of nutrients (organic matter) were observed.

2.5.2 Enzyme activity during the composting process

It is also widely known that enzymes are responsible for most of the biochemical events that occur during composting (Ayuso et al. 1996; Vuorinen 1999, 2000). Amidohydrolases and dehydrogenases are involved in the mineralization of organic N during composting (Bottomley et al., 2020). During composting process, microbes and enzymes play an important role in breaking down organic matter into simpler units of organic carbon and nitrogen. The active microbial population in the compost is responsible for determining the

quality and stability of the finished compost product. The optimal environmental conditions for microbial survival include pH, moisture content, C/N ratio, temperature, and other factors, and these characteristics vary depending on the composting methodology employed to produce the compost. Numerous studies have been conducted on the isolation and microbiological diversity of composting methods (Raut et al., 2008). The release of several types of substrate-based hydrolytic enzymes by microorganisms in the compost increases the overall efficiency of organic material breakdown in the compost. These enzymes help to accelerate the degradation process (Benitez et al., 1999).

Enzymes in compost can be divided into two classes: intracellular and extracellular enzymes (Vuorinen, 2000). Intracellular enzymes catalyze biochemical reactions that take place within the cells' own membrane (Nannipieri et al., 1990). Extracellular enzymes, on the other hand, are enzymes that are purposefully released outside of cells, typically to catalyze the destruction of polymeric substances (such as plant polymers, cellulose, hemicellulose, and lignin) that are too big to pass through the cellular membrane (Sylvia et al., 2005). It is impossible to tell the difference between intracellular and extracellular enzymes in compost suspensions. However, following a brief incubation period, the extracellular groups of enzymes may be allocated more readily, and a considerable part of the enzymes found in soils and composts can be assigned to these groups (Vuorinen, 1999). Cellulases, B-galactosidases, urease, phosphatases, and arylsulphatase are only a few of the significant enzymes that regulate the pace of substrate degradation. N-mineralization is mediated by the enzyme urease, and the enzymes phosphatase and arylsulphatase are responsible for removing phosphate and sulfate groups from organic molecules (Mondini et al., 2002). Once a well-established relationship between enzyme activity, organic matter amount, and organic matter quality are established, compost quality, defined as the degree of breakdown of readily biodegradable organic matter, may be better understood (Garcia et al., 1992; Lasaridi and Stentiford, 1998).

2.5.3 Effect of inoculum addition during the composting process

Microorganisms inoculated into composting piles operate as essential drivers during biotransformation, making them a direct choice for modifying microbial metabolic activities during the composting process (Awasthi et al., 2020). Wang et al. (2019) discovered that inoculating *Bacillus stearothermophilus* into compost might significantly reduce ammonia emissions by modifying the structure of the indigenous bacterial community during the composting process (Wang et al., 2019). Moreover, Manu et al.

(2017) discovered that microbial inoculums enhanced the decomposition rate of lignocellulose compounds and composting maturity. Many studies investigated the isolation of actinomycetes which expedited cellulose and lignin degradation by increasing key enzyme activities (Zhao et al., 2017; Wei et al., 2019). As reported by Ryckeboer et al. (2003), lignocellulosic biomass is difficult to degrade, and the composting process involves many microorganisms (Chroni et al., 2009, Wei et al., 2013), which can be affected by a variety of biochemical and physical factors, efficient composting performance was generally difficult to achieve with lignocellulosic biomass (Cayuela et al., 2009). As a result, many attempts have been made to improve the composting process by inoculating it with microorganisms (López et al., 2006; Sasaki et al., 2006; Obodai et al., 2010), based on the fact that microorganism action is critical to the composting process in determining the quality of the final product and the rate of composting (Elorrieta et al., 2002, Fuchs, 2010). Vargas-Garca et al. (2007) investigated the effects of microbial inoculation (*Bacillus shackletonni*, *Streptomyces thermovulgaris*, and *Ureibacillus thermosphaericus*) and co-composting material on the evolution of humic-like substances throughout windrow composting and concluded that the benefits of inoculation were dependent on the properties of the applied raw materials and microorganisms. It has been demonstrated that inoculating food waste with thermo-tolerant lipolytic bacteria improves breakdown and reduces maturation time in vessel composting (Tsai et al., 2007; Ke et al., 2010). Tsai et al. (2007) showed that inoculation with a thermo-tolerant lipolytic microbe improved food waste breakdown, seed germination rates, and shortened maturation time in an in-vessel composting system. Various studies have been conducted on the uses of bacterial doses for composting process, as discussed in Table 2.4.

Table 2.4. Application of bacterial inoculum for various waste using composting technology

Sl.No.	Authors	Bacterial strain used	Doses	Type of waste	Time Duration
1	Tsai et al., (2007)	<i>Brevibacillus borstelensis</i>	5 litre of inoculum of test 10^7 microbes (>cfu/ml)	Food waste + Saw Dust+ Leaves (35 Kg)	28 days
2	Pan et al., (2012)	<i>Bacillus subtilis</i> & <i>Pseudomonas spp</i>	1% broth inoculum each of 10^7 cfu/ml	Fruit wastes, Vegetable wastes, Leaves, Hay, newspaper, Wheat straw and Rice husks (20 gms)	180 days
3	Rastogi et al. (2020)	<i>Thermoactinomyces vulgaris</i>	10^8 cfu/gm	Inoculum was mixed with food wastes and sawdust, at a ratio of (2:10:1)	60 days
4	Xi et al. (2012)	<i>Nitrobacter</i> and <i>Thiobacillus</i>	10^8 cfu/ml	Municipal solid waste	
5	Abdullah et al. (2013)	<i>Bacillus thermoamylovorans</i> , Mixed <i>Bacillus</i> species (such as <i>B. Brevis</i> , <i>B. coagulans</i> and <i>B. licheniformis</i>)	13 log cfu/gm	Kitchen waste (onion peels, paper) (2kg and 6 kg)	30 days
6	Xu et al. (2019)	<i>Bacillus licheniformis</i> , <i>Aspergillus nidulans</i> and <i>Aspergillus oryzae</i>	1:1:1	Dairy manure and Sugarcane leaves (4:1) 25 kg	45 days
7	Zhao et al. (2016)	<i>Streptomyces sp.</i> and <i>Micromonospora sp.</i>	10^8 cfu/ml	Chicken manure and Maize straw (1:2)	36 days
8	Zhao et al. (2017)	<i>Streptomyces sp</i> and <i>Actinobacteria bacterium</i> (1:1)	10^9 cfu/ml (2% dry weight)	Corn straw and Dairy manure (1:2)	60 days

9	Gou et al. (2017)	<i>Flavobacterium glaciei</i> , <i>Brevundimonas diminuta</i> , <i>Aspergillus niger</i> , <i>Penicillium commune</i>	10 ⁸ cfu/ml	Dairy manure (20kg) and Rice straw(3.8 kg)	54 days
10	Ke et al. (2010)	<i>Thermoactinomyces vulgaris</i>	10 ⁸ cfu/gm	Compost:Food Waste:Saw Dust (2:10:1)	60 days
11	Wang et al. (2016)	<i>Bacillus subtilis</i> and <i>Chaetomium thermophilum</i>	10 ⁸ –10 ⁹ cfu/ml, 10 mL/kg	<i>Sophora flavescens</i> residues: wheat bran:Saw Dust (16:1:4)	60 days

2.6 TOXICITY ASSESSMENT OF COMPOST/VERMICOMPOST

Toxicity assessment is one of the most significant criteria for determining whether compost is suitable for agricultural use and avoiding environmental problems before recycling back to agricultural land (Selim et al., 2012). Phytotoxic chemicals, such as heavy metals, may cause toxicity by immature compost (Tam and Tiquia, 1994). Phytotoxicity can be characterized as a delay in germination, inhibition of plant growth, or any other detrimental effect induced by specific compounds (phytotoxins) or by insufficient growth circumstances in the presence of a plant (Barral and Paradelo, 2011). Excess salt accumulation (Tam and Tiquia, 1994), phenolic compounds (Wong, 1985), ethylene and ammonia (Tam and Tiquia, 1994), and organic acids (Manios et al., 1989) are all examples of chemicals that can slow seed germination and plant growth. Acetic acid is most likely the most harmful organic acid generated from immature compost, although there are additional substances that contribute to the phytotoxic effect of the immature compost as well (Ozores-Hampton, 1998). High levels of salt in the compost and the release of organic acids into the compost have been linked to the inhibition of germination and growth of plants. Germination and growth assays are frequently the most effective methods of determining phytotoxicity (Gariglio et al., 2002). Toxicity is one of the most important criteria to consider when evaluating compost's quality and suitability for agricultural, landscaping, and environmental restoration. This is especially true when evaluating compost used in high-value horticultural applications, as it is in all of its potential applications.

Several investigations have proven that some plant tissues and residues contain organic chemicals that can either stimulate or reduce root growth and development of other neighboring plants (Gartner et al., 1973; Still et al., 1976; Solbraa, 1979). In addition, it is well known that the breakdown process of organic residues is connected with changes in the nature and concentration of phytotoxic chemicals in the environment (Patrick et al., 1963; Still et al., 1976; De Manios et al., 1989). Chemical analysis can be used to identify some harmful components. However, these procedures can be costly and time-consuming to implement. Furthermore, unanticipated pollutants can be present in the compost not found during standard examinations of the material (Barral and Paradelo, 2011).

Moreover, there are currently no analytical methodologies that can be used to determine the effects of synergistic and antagonistic interactions between dangerous substances (Emino and Warman, 2004). However, biological tests are the most realistic and full

method of determining whether composted materials are suitable for use with plants because they allow the evaluation of many phytotoxic variables present in the compost at the same time. In 1981, Zucconi et al. published a paper describing a germination test or index based on cress (*Lepidium sativum*, L.). Warman (1999) conducted a comprehensive assessment of the literature on germination testing and concluded that the written process developed by Zucconi et al. (1981) is difficult to replicate. Following Garglio et al. (2002), who used lettuce as an indication in a plant growth bioassay, Fauci et al. (2002) employed pinto bean and tomato as indicators in a plant growth bioassay starting in 1999. Recently, Smith and Hughes (2001) conducted a study comparing cress germination and cellulolytic activity. During composting, the generation of phytotoxic chemicals is a fleeting phenomenon. It is highest during the first stage when the organic waste is rapidly destroyed and diminishes during the stability stage when "humification" and mineralization predominate. In the last stages of composting, toxicity may decrease due to a variety of causes, primarily the metabolic breakdown of some phytotoxic organic compounds and the integration of certain phytotoxic chemicals into the 'humic acids' fraction (Zucconi et al. 1985). Several studies have been conducted to investigate the toxicity of compost/vermicompost using various plant models before applying it to the agricultural area.

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

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

in promoting cell division and proliferation, which is beneficial to plant health and crop productivity.

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

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

2.7 THE BENEFIT OF COMPOST (SOIL APPLICATION AND PLANT GROWTH)

A related concern is the vast accumulation of organic waste by human activities, which has prompted the development of several alternatives to landfilling and promoting recycling. Composting is one of the most well-known and well-established processes among these possibilities. Composting stabilizes and sanitizes organic waste by accelerating aerobic decomposition under regulated circumstances, resulting in compost. It has been shown that the use of compost can significantly reduce the use of ammonia-type fertilizers, which account for approximately 2% of total natural gas consumption in the United States. Ammonia-type fertilizers are manufactured using approximately 2% of total natural gas consumption in the United States (Schonfeld et al., 2003). Because the compost is predominantly comprised of NPK and other micronutrients, it can be utilized as a fertilizer in various situations. If the amount of ammonia lost during the composting process is decreased, the majority of the nitrogen can be trapped in the compost. The addition of

compost to the soil nourishes it and increases the number of important nutrients and organic matter in the soil. As a result, soil physical qualities such as bulk density and porosity are improved as well as cation exchange capacity and the presence of various chemicals and biological features (Tits et al., 2014; Weber et al., 2007). In addition to lowering the financial burden of acquiring chemical fertilizers, compost can also assist in mitigating the negative environmental impacts connected with the manufacture of chemical fertilizers and their use (Jain and Kalamdhad, 2020). Recent research has explored the significance of compost application in the soil (Ren et al., 2018; Goswami et al., 2017; D'Hose et al., 2014). However, further research is needed. According to many authors, compost-treated soils had statistically significant positive effects on the overall health of the soil when compared to untreated soils in the study. For example, Willekens et al. (2014) reported an increase in the accessible potassium contents of the soil following the application of compost to the soil. By preventing nutrients from seeping into the groundwater, compost has also been beneficial to the soil environment (Grey and Henry, 1999; Li et al., 1997).

Using compost/vermicompost made from a pernicious weed such as water hyacinth, Gajalakshmi, and Abbasi (2002) investigated the effect of it on kitchen gardens containing lady's finger (*Hibiscus esculentus*), brinjal (*Solanum melongena*), cluster bean (*Cyamopsis tetragonoloba*) chili (*Capsicum annum*), and tomato (*Lycopersicon esculentum*). Hussain et al. (2020) reported the possibility of using invasive weed *Ipomoea carnea* as an organic fertilizer. The effect of *Ipomoea* vermicompost at four different levels (0, 2.5, 3.75, and 5 tonnes/ha) on the germination, growth, and fruition of a lady's finger (*Abelmoschus esculentus*) was reported. The vermicompost was found to promote the germination and growth of lady's finger at all levels, with the highest results coming from 5 t/ha treatments. The beneficial effect was seen at every step of *A. esculentus* cultivation, from seed germination to vegetative growth phases and fruit output. Due to the vermicompost application, the quality of fruits improved in terms of mineral, protein, and carbohydrate content and a decrease in disease incidence and pest attacks. According to Hussain et al., (2016), vermicompost obtained only from the action of the epigeic earthworm *Eisenia fetida* on *Parthenium* (*Parthenium hysterophorus*) had a favorable effect on the green gram (*Vigna radiata*), ladies finger (*Abelmoschus esculentus*), and cucumber (*Cucumis sativus*). Various work done on the application of compost/vermicompost on plant model has been illustrated in Table 2.

Table 2.5. Studies on the application of compost on plant for various compost concentration

Sl. No.	Authors	Compost material used	Plant model used	Doses of compost & type of soil	Result
1	Lazcano et al. (2009)	Compost(cow manure) and vermicompost (pig manure)	Tomato(<i>Solanum lycopersicum</i>)	0%, 10%, 20%,50%, 75%, 100%	Doses higher than 50% compost results in plant mortality. 10%,20% and high doses of vermicompost optimum growth
2.	Das et al. (2010)	<i>Eupatorium adhenophorum</i> , <i>Lantana camara</i> , weed mixture (grass and broad leaf weeds) and rice straw were taken as raw material	High yielding rice variety	The microbial inoculants along with cow dung was made into a slurry by mixing fresh cow dung: virgin soil: well rotten compost in a ratio of 1:1:0.5 using appropriate amount of water.	The nutrient uptake and post harvest soil fertility status were found to be significantly improved due to application of various composts.
3.	Gurav and Sinalkar (2013)	Equal amounts of used Tea powder, soil, cow dung	<i>Vigna Radiata</i> , <i>Cicer arietinum</i> , <i>Tagetes</i>	Compost: Soil (1:1, 1:3)	1:1 shows high germination frequency and less germination time and also improved plant growth than the second one (1:3)
4.	Rekha et al. (2018)	Vermicompost	<i>Capsicum annum</i>	50% vermicompost	Number of leaves, number of branches, shoot length all increased.

- | | | | | | |
|----|-------------------------------|---|----------------------------------|---|--|
| 5. | Abdullah Al-Dhabi et al. 2019 | The vegetable waste, such as cabbage, cucumber, cauliflower leaves, and brinjal waste are taken as compost. | Tomato plant | 5.0 kg of vegetable waste was used for the composting process. The microbial consortium, including rhizosphere-associated <i>Streptomyces</i> sp., <i>Candida utilis</i> , and <i>Lactobacillus</i> , were added with vegetable waste | The total weight of tomato increased over 15% compared to the control compost and was statistically significant |
| 6. | Khatun et al. (2019) | Compost | Okra plant | Cattle manure and saw dust were weighed and mixed in three different ratios | The maximum plant height was found in 1:2 of cattle manure and saw dust |
| 7. | Ruvini et al. (2019) | Compost | Soybean (<i>Glycine max</i> L.) | 100% compost, 75% compost with 25% biochar, 50% compost with 50% biochar, 25% compost with 75% biochar, 100% biochar | Combined application of 25% compost with 75% biochar (T4) significantly increased the growth parameters of <i>Glycine max</i> (L.) |

2.8 INFERENCE FROM THE LITERATURE REVIEW

Weed management is one of the biggest challenges in the field of solid waste management. Invasive terrestrial weed has been creating a global menace for decades, and traditional approaches such as cutting and uprooting have added to the problems of organic solid waste. These conventional approaches did not fully solve the weed problem since the seeds are potent enough to get dispersed by wind or water even after cutting down or uprooting. *Mikania micrantha kunth*, *Parthenium hysterophorus*, *Chromolaena odorata*, *Saccharum spontaneum* pose some of the world's most noted terrestrial weed problems. Being one of the most destructive weeds worldwide, *Mikania* is causing havoc in the agricultural field of banana plantations, coffee plantations, tea plantations, and rubber plantations. Many researchers have reported the utilization of *Mikania micrantha* in briquette formation and as fodder for cattle. It is also reported to have an allelopathic potential, and therefore a study on control and treatment of *Mikania micrantha* has become a prominent matter of concern. Meanwhile, the utilization of this plant on a large scale must be studied to address its impact on the agricultural field. It contains many toxic compounds that are detrimental to the natural ecosystem and have negative impacts on economic and esthetic environmental aspects. Remediation techniques that propose composting may provide a novelty in tackling the menace of such dreadful weed.

There is a need to address such concerns, which can only be accomplished through carbon-rich agents. So far, such studies for composting and vermicomposting of *Mikania micrantha* Kunth have been few. The investigation of phytotoxicity, cytotoxicity, and genotoxicity for the final compost product has not been focused on in recent years. Previous research has not adequately examined the use of *Mikania* compost and vermicompost for plant growth studies. The necessity for bioaugmentation research using various forms of organic waste has grown significantly in recent years in order to improve compost quality and shorten compost duration. However, very few pieces of literature are accessible on the bioaugmentation investigation of terrestrial weed composting, which will be a paradigm change for the current research.

2.9 KNOWLEDGE GAPS

- The majority of weed management mainly focusses on chemical and mechanical control strategies but very few have been focused on the utilization of the biomass for the production of value-added product like compost/vermicompost.

- The early research studies were conducted on a global perspective and Indian scenerio, i.e., biodegeradtion of weed biomass using composting and vermicomposting technologies, focused on the production of final product for agricultural field. But very few research focused on the complete toxicity assessment of the product before using for agricultural field.
- The combination of two-stage technologies has not been focused much in the early research for composting and vermicomposting of weed biomass. Therefore, this will give a new insight about the current work.
- Althought very few research has been found on composting and vermicomposting technologies of *Mikania micrantha*, but a complete elaborate study on plant study is very less.
- Bioaugmentation study using bacteria and fungus has been used in several research for different organic substrate in order to enhance the degradation process. However, biaoaugmenattion study for the reduction of weed allelochemicals during composting process is very less as compared to other organic substrate.



Research Objectives

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

3.1 RESEARCH OBJECTIVES

The broad objective of the study was to find out the efficiency of rotary drum composting of *Mikania micrantha* Kunth. The purpose was also to find the best strategy for improved treatment efficiency by performing different composting methodologies. The entire research work is divided into four phases and four objectives as follows:

- **Objective-I:** Performance evaluation of rotary drum composting, vermicomposting and rotary followed by vermicomposting of invasive weed *Mikania micrantha* Kunth.
- **Objective-II:** Toxicity assessment of the compost produced, using Mung bean (*Vigna radiata*) and Onion (*Allium cepa*) as the test plant.
- **Objective-III:** A comparative study on the application of compost on soil based on plant growth using compost produced from three compost technologies.
- **Objective-IV:** Microbial study, isolation of bacteria, enzyme assay and bioaugmentation study.

3.2 NEED OF THE STUDY

India is a vast country with almost 70% of its people depending on agriculture for their livelihood. Any deduction to the gross agricultural output will affect a large population tremendously. The effect of invasive plants on rural communities is much more complex than that of the negative ecological repercussion. More than two-thirds of the population live in rural areas and practice subsistence farming. Forest resources are very important for their survival and daily livelihoods. The abundance of invasive plants usually outcompetes the native ones thereby affecting the delivery of the quantity of native forest

products. Forest products are a major input in the farm household production input in village. The effect of failing to provide quality timber and forest products can cause rural farmers to change their livelihood strategies. This is due to the convoluted linkages between community livelihood and forest; a change in the state of one variable can be expected to have an impact on other. *Mikania micrantha* happens to be one such invasive weed that effects lots of vegetation like rubber plantation, tea plantation and banana plantation in Assam, India. *Mikania micrantha* had already invaded the eastern and the southern parts of the country, so it has become absolutely necessary to stop this unwanted vegetation from spreading to other parts of the country. The main problems associated with *Mikania micrantha* is that it can smother other plant species inhibiting their photosynthesis process and takes up minerals from the surrounding plants. It has the ability to conquer a vast area once it is established and has huge potential to cope up with the environment. All the control measures other than biological treatment is either insufficient or harmful in some ways or other. This study aims to give an eco-friendly solution to this problem. This weed can be managed on-site without discarding into low-lying areas. So, local people could use the biomass for the production of compost and utilize in agricultural fields to increase the crop yield of a variety of plants, including vegetables and fruits to attain a sustainable livelihood. There is a paucity of research on *Mikania* composting and vermicomposting, as well as field studies of the compost, which could aid in the paradigm change in the management of such an invasive terrestrial species. This biomass compost can be a substitute for chemical fertilizer in the application of agricultural fields and has the potential to be used for the soil remediation process as well.

3.3 SCOPE OF THE STUDY

The scope of the research is to collect *Mikania micrantha*, cow manure, and sawdust from various locations and compost them using various methods. The experimental setup for the physico-chemical analysis, biological analysis, microbiological analysis, and learning the instrumentation's procedures were carried out in the first attempt. The effectiveness of the batch rotary drum composter, vermicomposting and rotary drum followed by vermicomposting were also examined using varied ratios of *M. micrantha*, cow dung, and sawdust. Additionally, testing the compost toxicity is an important step in ensuring its safety for usage in agricultural fields. Therefore, it is essential to continue the toxicity trial for compost manufacturing. An essential step for the actual implementation of the product for field usage is the application of compost in soil for

plant development. As microorganisms are the most crucial components for the decomposition of any form of organic waste, microbial investigation throughout the composting process is also an essential factor.





Materials and methods

Different experimental approaches were used to accomplish the stipulated objectives. The research was carried out in different phases using various raw material combinations, and a detailed methodology is given in this chapter.

4.1 EXPERIMENTAL FLOWCHART

In order to accomplish the objectives, the proposed research was carried out in four different phases (Fig. 4.1). **In phase I**, the efficacy of rotary drum composting was investigated using six different ratios of *Mikania*, cow dung, and sawdust. The best ratio obtained from the rotary drum composting (RDC) was used in a vermicomposting (VC) process that used a monoculture of three earthworm species (*Esenia fetida*, *Eudrilus eugienae*, and *Perionyx ceylanesis*) to evaluate the best-performed earthworm species in terms of mortality rate and compost quality. The combination of Rotary drum composting and vermicomposting process (RVC) was used for the composting of *Mikania micrantha* with the best ratio obtained from RDC. As vermicomposting process takes longer period, pre-degradation of the substrate was done using rotary drum composter for 10 days. For this study, all the three earthworm species used were evaluated to find the best-performed earthworm species in terms of mortality rate and compost quality. **In phase II**, the phytotoxicity, cytotoxicity, and genotoxicity assessment of compost produced from three compost technologies were performed using plant models like *Vigna radiata* and *Allium cepa*. **In phase III**, a comparison study conducted on different compost ratios was amended with soil. Later, a plant model like *Abelmoschus esculentus* was used for quality performance, and plant morphology was characterized. **In phase IV**, bacteria were isolated from rotary drum compost, and later metagenomic sequencing was conducted. The efficacy

of the pure bacterial strain was reported based on the enzyme (laccase enzyme) produced. The desired bacterial strain was used in a composting bioaugmentation study. The entire research methodology is illustrated in Fig. 4.1.

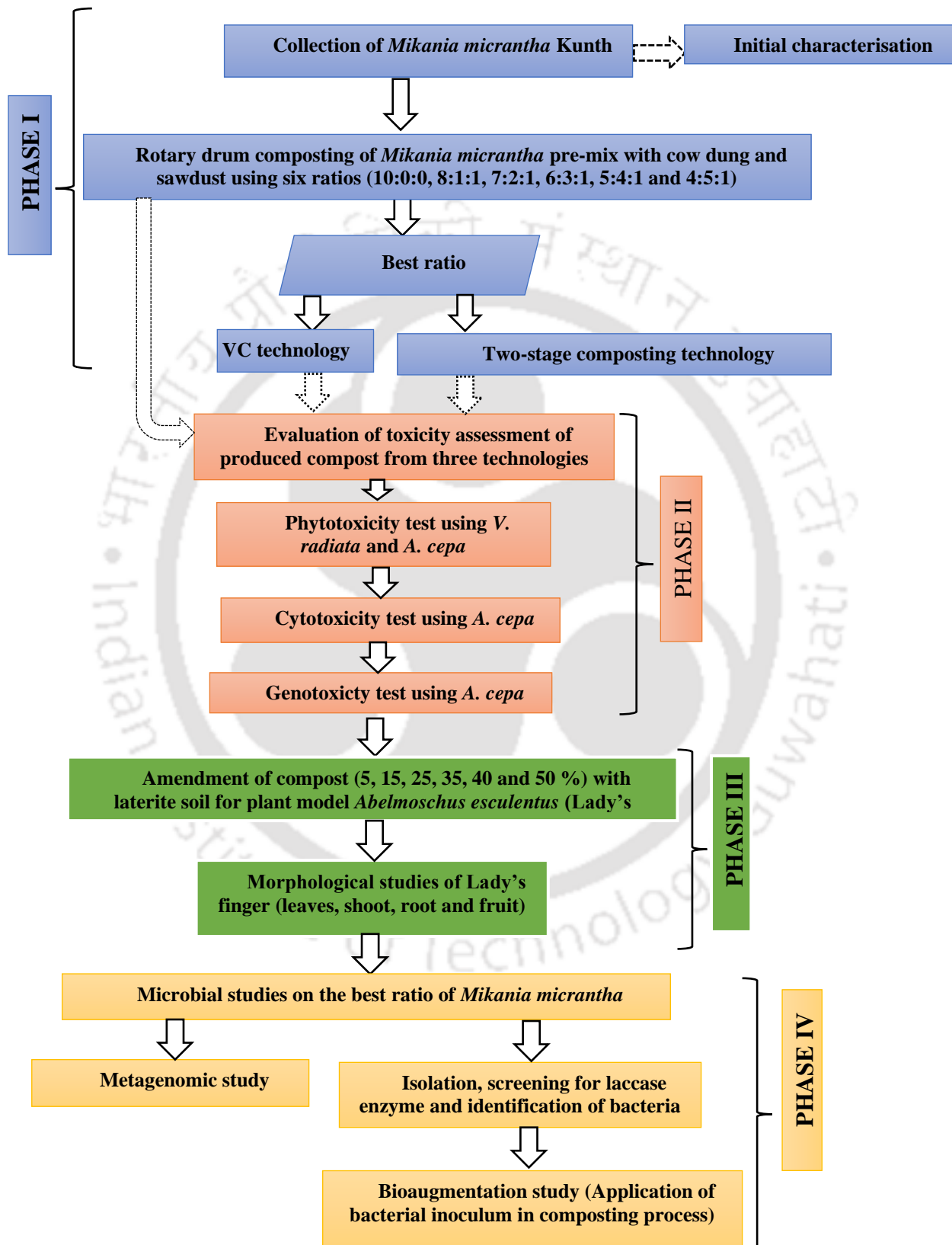


Fig. 4.1. Detailed experimental flowchart

PHASE-I (OBJECTIVE-I): PERFORMANCE EVALUATION OF ROTARY DRUM COMPOSTING, VERMICOMPOSTING AND ROTARY FOLLOWED BY VERMICOMPOSTING OF INVASIVE WEED *MIKANIA MICRANTHA* KUNTH

4.2 COMPOST MATERIAL

Mikania micrantha Kunth was used in different proportions as substrate, cow dung as an inoculum, and sawdust as a bulking agent to prepare waste mixtures. *Mikania micrantha* was collected from Aminagaon, Sualkuchi, and the vicinity of the IITG Campus. Cow-dung was collected from a nearby dairy farm, Amingaon, North Guwahati, and saw-dust were collected from a nearby sawmill of Amingaon market, Assam. The collected *M. micrantha* sample was chopped into 1 to 2 cm size, mixed with cow dung and sawdust. Subsequently, the different waste combinations were mixed before feeding into the reactor.

Fig. 4.2 and 4.3 show the schematic diagram of a pilot-scale rotary drum composter of 550 L capacity; operated by batch mode. The main unit of the composter, i.e., the dimension of the drum was 1.022 m in length and 0.76 m in diameter, fabricated by a 4 mm thick stainless metal sheet.

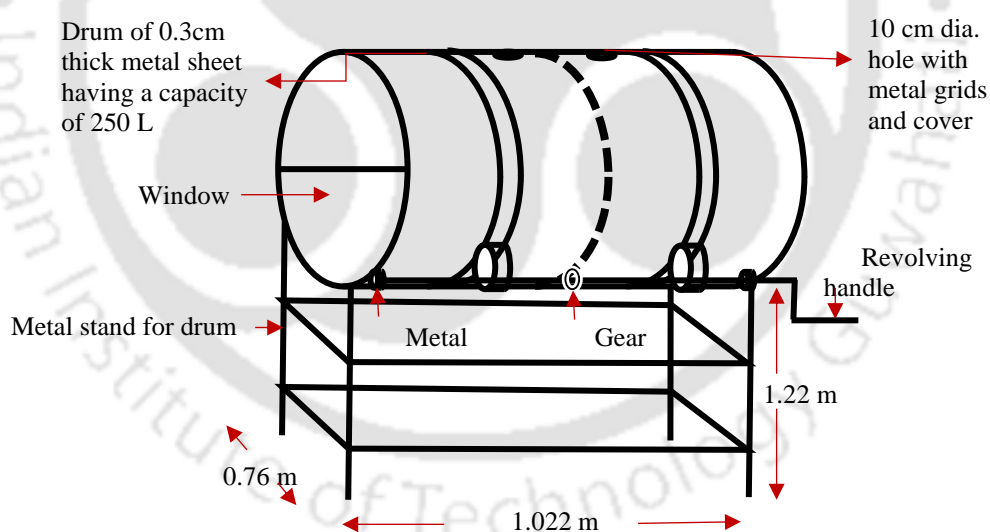


Fig. 4.2 Schematic diagram of Rotary Drum Composter



Fig. 4.3 Pictorial representation of drum composting

Table 4.1 provide the waste combinations of the study and a total of 120 kg waste was used for each ratio in order to accomplish the composting process.

Table 4.1. Details of experimental trials for RDC process

S. No.	Trials	Ratio	Substrate (<i>Mikania</i>)	Inoculum (Cow dung)	Bulking Agent (Saw dust)
1	RD 1	4:5:1	48 kg	60 kg	12 kg
2	RD 2	5:4:1	60 kg	48 kg	12 kg
3	RD 3	6:3:1	72 kg	36 kg	12 kg
4	RD 4	7:2:1	84 kg	24 kg	12 kg
5	RD 5	8:1:1	96 kg	12 kg	12 kg
6	RD 6	10:0:0	120 kg	0 kg	0 kg

The inner side of the drum is painted with an anti-corrosive coating to prevent reacting with the waste. The drum was mounted on four rubber rollers attached to a metal stand and rotated manually with its handle. 40×40 mm angles were welded longitudinally inside the drum to ensure appropriate mixing, agitation, and aeration of the wastes during rotation. In addition, two adjacent holes of 10 cm each are made on top of the drum to

drain out the excess water. The composting period of 20 days was the optimal approach for stabilizing waste material in in-vessel composting reactors. Manual turning was done every 24 h through one complete rotation of the rotary drum to ensure that the material on the top portion moved to the central portion, where it was subjected to a higher temperature and avoided anaerobic conditions. The flow diagram of preparation and mixture of raw materials based on selected ratios is illustrated in Fig. 4.4.



Fig. 4.4 Preparation of the feeding mixture for the rotary drum composter

4.3 VERMICOMPOSTING

4.3.1 Earthworm cultures

Current research work includes three monoculture of earthworm, namely *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx ceylanesis* were collected from Central plantation crops research institute (CPCRI), ICAR, Kahikuchi, Guwahati, Assam. However, *P. ceylanesis*, commonly named 'Jai Gopal,' is a genetically modified species developed at IVRI, Bareilly, UP, India. Earthworm culture was done on a perspex bin, designed especially for culturing, containing 16 to 18 holes for proper aeration and drainage of excess water sprayed on it, as shown in Fig. 4.5. The bin's overall size is 450 mm × 300 mm × 450 mm that has been fabricated in the laboratory, IIT Guwahati. The bedding material contains dried banana trunks, dried betel nut leaves, and fresh cow dung. The first layer contains dried banana trunk and dried betel nut leaves upon which cow dung was layered up to 2-3 cm. Earthworm species were inoculated in the bin for proper growth and reproduction. Water was sprinkled every 2-3 days to maintain the moisture content; the bin was covered

with jute bags and kept in a dark location. Earthworms were cultured for almost 3 months before the experiment to obtain mature species.



Fig. 4.5 Earthworm culture

4.3.2 Vermicompost material

The terrestrial weed, *M. micrantha*, was aggregated from the IIT Guwahati campus, Assam. Cow dung was obtained from the nearby locality of IIT Guwahati, and sawdust was obtained from the sawmill, Amingaon, North Guwahati. Cow-dung and sawdust were mixed properly with *M. micrantha* because cow dung will act as a microorganism inoculum which enhances the degradation process (Paul et al., 2020), and saw dust will act as a bulking agent that helps to trap excess water sprinkled on it so that proper moisture content is maintained throughout the process. *M. micrantha* was shredded to 1-2 cm for proper mixing with cow dung and sawdust.

4.3.3 Set up of vermireactors

Handmade bamboo reactors of size $88.21 \times 104 \text{ mm}^3$ (approx.) were used for operating the entire experiment. The reactors were purchased from the local market at Bijoy Nagar, Kamrup, Assam. As bedding is very important for earthworm acclimatization, all reactors were first arranged to contain newspaper and dried banana trunks, as depicted in Fig 4.6. On top of the bedding material, 0.2 g of semi-dried cow dung was layered to acclimatize the earthworm better. Earlier, it was reported that an earthworm could consume half of its body weight daily (Haimi and Huhta, 1986). Accordingly, the mass of waste enforced for 1.5 kg was estimated based on the best ratio of waste combination obtained from RDC process. A total of 120 numbers of earthworms were used based on 1.5 kg of total waste for each reactor. The experimental details of the trials has been illustrated in Table 4.2. The waste mixture was fed into the bamboo reactor, kept at room temperature with very little

light exposure, as illustrated in Fig. 4.7. Water was sprayed into the reactor on alternate days to keep it moist.

Table 4.2. Details of experimental trials for VC process

Reactors	<i>M.micrantha</i> substrate	Cow dung	Sawdust	Earthworms (number)
V _C	0.75 kg	0.6 kg	0.15 kg	120
V _{EF}	0.75 kg	0.6 kg	0.15 kg	120
V _{EE}	0.75 kg	0.6 kg	0.15 kg	120
V _{PC}	0.75 kg	0.6 kg	0.15 kg	120



Step 1: First layer using

Step 2: Second layer using dried

Fig. 4.6. Different bedding steps used for vermicomposting



Fig. 4.7. Vermireactor setup

4.4 Rotary drum followed by vermicomposting (RVC)

4.4.1 Waste material

For the current study, waste mixture was fed into the Rotary drum reactor (R1) to obtain thermophilic temperature. When the temperature drops to 35-40°C, the partially decomposed material was used for vermicomposting with three different earthworm species, *E. fetida*, *E. eugeniae*, and *P. Ceylanesis*. The current study reduces the time required for vermicomposting by incorporating an in-vessel compost technique for 10 days, followed by a 20-day vermicomposting process and obtaining nutritional ‘vermicast’ from it. This study aims to monitor various physicochemical and biological parameters during the rotary followed by vermicomposting (RVC) process to compare its efficiency to traditional vermicomposting techniques in terms of nutrient quality. Earthworms require more time for acclimatization than the actual process to happen. So a pre-degradation process is conducted in a 550 L capacity Rotary drum composter to acquire a significant rise of temperature (thermophilic phase) and then to vermicompost for 20 days using bamboo bins.

4.4.2 Vermireactor design and earthworm culture

The experimental procedure was carried out in a bamboo reactor of almost $88.21 \times 104 \text{ mm}^3$ (approx.) sizes. Banana leaves that have been degraded for 1–2 weeks have been used as bedding material for vermireactor. 5:4:1 ratio is used for the mixture of waste obtained from a previous study on drum composting of *Mikania*. *Mikania* was collected from the vicinity of the IIT Guwahati campus, Amingaon, Assam, India. Cow dung and sawdust were collected from a local dairy farm near the IITG campus and Amingaon market. A total amount of 1.5 kg waste has been used, including *Mikania* as the first part, cow dung as the second part, and sawdust as the third part. For each reactor, a total of 120 earthworms were used. For the process, three separate earthworm species were used for application in the reactor, namely: applying *E. fetida* (VrEF), applying *E. eugeniae* (VrEE), applying *P. ceylanesis* (VrPC), and without earthworm species (VrC). Wet jute bags were used to cover the vermireactor because earthworms can perform more efficiently with less light exposure. The experimental trials of the study has been illustrated in Table 4.3.

Table 4.3. Details of experimental trials RVC process

Reactors	<i>M. micrantha</i> substrate	Cow dung	Sawdust	Earthworms (number)
VrC	0.75 kg	0.6 kg	0.15 kg	120
VrEE	0.75 kg	0.6 kg	0.15 kg	120
VrEF	0.75 kg	0.6 kg	0.15 kg	120
VrPC	0.75 kg	0.6 kg	0.15 kg	120

PHASE-II (OBJECTIVE-2): TOXICITY ASSESSMENT OF ROTARY DRUM COMPOST, VERMICOMPOST AND ROTARY FOLLOWED BY VERMICOMPOST USING MUNG BEAN (*Vigna radiata*) AND ONION (*Allium cepa*) AS THE TEST PLANT.

4.5 TOXICITY ASSESSMENT

Mikania affects the environment by releasing toxic compounds that are harmful to the surrounding plant species. This thereby delays the growth and reproduction of certain native plants and allows itself to become a dominant species in natural plant ecosystems, effectively executing an invasion (Ni et al., 2007). Therefore, this study was also undertaken to evaluate the phytotoxicity by analyzing plant growth before and after composting process.

4.5.1 Phytotoxicity assessment of *Vigna radiata* and *Allium cepa*

The phytotoxicity test was performed using *M. micrantha* fresh aqueous extract and vermicompost extract, as preceded by Matthews and Hastings (1987) modified procedure. Samples were prepared by combining 100g of a single sample with 300 mL of dH₂O and shaking mechanically in a rotary shaker for 24 hours at 120 rpm to achieve a homogenized mixture. After 24 hours of continuous shaking, samples were filtered, and the filtered samples were used for toxicity evaluation. With different dilutions (0, 25, 50, 75, and 100% v/v), *M. micrantha*, rotary drum compost, vermicompost, and rotary followed by vermicompost extract solutions were formulated using dH₂O. As a test plant for toxicity evaluation, *Vigna radiata* (Mung bean) was used for analysis is illustrated in Fig. 4.8. Mung bean was acquired from a certified store in Assam, Guwahati. Mung bean seeds were immersed in HgCl₂ solution (0.1 percent w/v) for proper sterilization for 10 min before starting the experiment and washed thoroughly with dH₂O to eliminate traces of HgCl₂.

Healthy seeds were selected and transferred to petri dishes to evaluate seed germination and sprout length inhibition test (Haq et al., 2016). All the tests were conducted in triplicates; for 4-5 days, the plates were incubated at $25 \pm 1^\circ \text{C}$. After 5 days of incubation, seedling growth and biomass were analyzed (8 h duration of the light cycle and 16 h duration of the dark cycle).



Fig 4.8. Set up for germination Index (GI) test using *Vigna radiata*

Allium cepa (onion) was used to evaluate toxicity. Onion bulb of uniform size was purchased from the local market of Amingaon, Guwahati, Assam. The developed protocol by Haq et al., (2016) was implemented using *A. cepa* phytotoxicity bioassay of *M. micrantha* and vermicompost aqueous extract. The different solutions of *M. micrantha*, rotary drum compost, vermicompost, and rotary followed by vermicompost samples were prepared in the concentrations 25, 50, 75, and 100 % v/v, and tap water was used as control is illustrated in Fig. 4.9.



Fig 4.9. Set up for phytotoxicity test using *Allium cepa*

Onion bulbs were used and placed in the specimen tubes containing 25 ml of the prepared extract. The onion had been put in such a manner that the root tip in the solution is fully dipped. All the samples were analysed in triplicates and were nurtured for 4-5 days keeping in an incubator at 20 ± 2 °C. The processed specimen tubes were regularly irrigated with the extract so that the root tips are dipped in it. After completion of 5 days, root length of onion and dry biomass were measured.

4.5.2 Cytotoxicity and genotoxicity bioassay

For both waste substrate and various staged compost samples, cytotoxicity and genotoxicity studies were undertaken using *A. cepa* as the plant model. Both tests were carried out in meristematic root tip cells based on the mitotic index (MI), and chromosomal aberrations (CA) found in the cells. The uniform-sized test onion bulbs were acquired from a local market near the Indian Institute of Technology, Guwahati. The onion bulbs were peeled and rinsed with distilled water, with special attention paid to the rooted area of the bulbs. In triplicates, the washed bulbs were incubated for 48 hours in dechlorinated water (tap water) at a constant temperature of 23 ± 1 °C. In triplicates, previously grown onion bulbs were rooted in different compost extract solutions (25, 50, 75, and 100 % v/v) for 24h. 4 mM EMS (ethane methyl sulfonate) and tap water and were assigned to positive and negative controls, respectively (Haq and Raj, 2018). The experimental setup of the study has been shown in Fig. 4.10. After 24 hours, root tips were cut to 1-2cm and processed for slide preparation to detect different stages of mitotic cell division. All samples were processed with a defined protocol for MI and CAs analysis (Fiskesjo, 1985). A total of 5000 cells were scored to determine MI (500 to 1000 cells per slide). MI was measured as:

$$MI = \frac{A}{B} \times 100 \quad (4.1)$$

Where, A represents the total number of dividing cells and B represents the total number of measured cells per dosage. A total of 2000 cells per treatment were used to count the number of CA (500 to 1000 cells per slide). Chromosomal aberration (CA) was calculated as

$$CA = \frac{A}{B} \times 100 \quad (4.2)$$

Where, A denotes the total number of abnormal cells and B denotes the total number of counted cells.

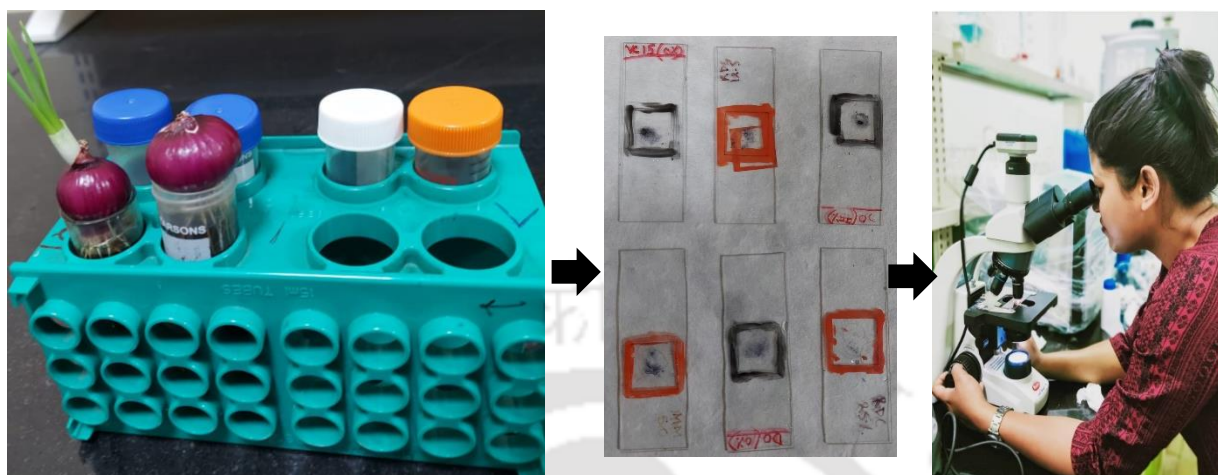


Fig 4.10. Various process involved in the cyto and genotoxicity test of *Allium cepa*

PHASE-III (OBJECTIVE-3): A COMPARATIVE STUDY ON THE APPLICATION OF COMPOST ON SOIL BASED ON PLANT GROWTH USING COMPOST PRODUCED FROM THREE COMPOST TECHNOLOGIES.

4.6 POT EXPERIMENT

4.6.1 Compost preparation

Almost 200 kg of *Mikania* was collected from IIT Guwahati campus, Guwahati, Assam. 150 kg of waste was mixed using the best ratio obtained from RDC process where the first part is *Mikania*, the second part is cow dung, and the third part is sawdust. Cow dung was obtained from a local dairy farm outside Guwahati, Assam, and SD from a sawmill nearby Amingaon. 5:4:1 ratio was used for compost production in 20 days using a rotary drum. Vermicompost was produced using the earthworm species *Esenia fetida* as it gave superior quality compost as compared to the other two earthworm species. About 20 kg of vermicompost was produced based on 120 number of earthworm species for 1.5 kg of waste mixture. Similarly, for the production of rotary drum followed by vermicompost, the waste mixture was fed in to drum composter which was kept for pre-degradation for 10 days. After 10 days degraded substrate was kept for vermicomposting using earthworm species *E. fetida*. A total of 18 kg vermicompost was produced from the pre-degraded waste to use for plant study.

4.6.2 Pot study using *Abelmoschus esculentus* (lady's finger)

Pots of size 10 L were chosen with a height of about 28 cm and an upper diameter of 30

cm as shown in Fig. 4.11. The material of the pot is hard plastic, and a few holes were drilled at the bottom for excess water drainage. A thin layer of gravel with a 2-3 cm height was placed as the base layer. The arrangements of the pot with soil and various compost percentage have been illustrated in Table 4.4. Seeds were purchased from a local seed vendor in Nalbari, Assam, and laterite soil was obtained from the nearby area of the IITG campus. The soil weight was kept constant (4kg) (Jain and Kalamdhad, 2020) and compost was applied in the proportion (0%, 5%, 15%, 25%, 35%, 40% and 50%).

A. esculentus is a warm-season crop that thrives when watered during the summer or rainy season, but it may be grown all year in tropical, subtropical, and temperate climates. It can flourish in a wide range of soil types as long as they are well-drained. The experiment was carried out during February and May, with the mean temperature varying between 22.5°C (lowest) and 31.5°C (highest), which is a suitable climate for the growth of *A. esculentus* in Assam (26°11'59" 91°42'01"E), India. The experiment was carried out in duplicate as illustrated in Fig. 4.12. Pots were put on the open space to ensure that it receives 8-9 h of direct sunlight. 7 seeds were potted in each pot and watered twice daily (morning and evening). The experiment was carried out for a total of 120 days to ensure optimal growth and the acquisition of appropriate morphological characteristics in the plant and the details of it has been illustrated in Fig. 4.13.

The fruit was observed after about 30 days and it was harvested every 2-3 days per week. As compared to control, fruits were more in compost application and by the end of 120 days, almost 36 harvests were done. The length of the fruit was immediately measured, and it rinsed with distilled water before being oven-dried for 24 h at 105°C. For additional investigation, dried samples were ground and sieved through a 0.2 mm mesh screen.

Table 4.4. Details of pot study using various concentration of compost/vermicompost

Soil type	Rotary drum compost	Vermicompost	Rotary drum followed by vermicompost
Laterite soil	(P1 & P2) + 0% compost	(P1 & P2) + 0% compost	(P1 & P2) + 0% compost
	(P3 & P4) + 5% compost	(P15 & P16) + 5% compost	(P27 & P28) + 5% compost
	(P5 & P6) + 15% compost	(P17 & P18) + 15% compost	(P29 & P30) + 15% compost
	(P7 & P8) + 25% compost	(P19 & P20) + 25% compost	(P31 & P32) + 25% compost
	(P9 & P 10) + 35% compost	(P21 & P 22) + 35% compost	(P33 & P34) + 35% compost
	(P11 & P12) + 40% compost	(P23 & P24) + 40% compost	(P35 & P36) + 40% compost
	(P13 & P14) + 50% compost	(P25 & P26) + 50% compost	(P37 & P38) + 50% compost

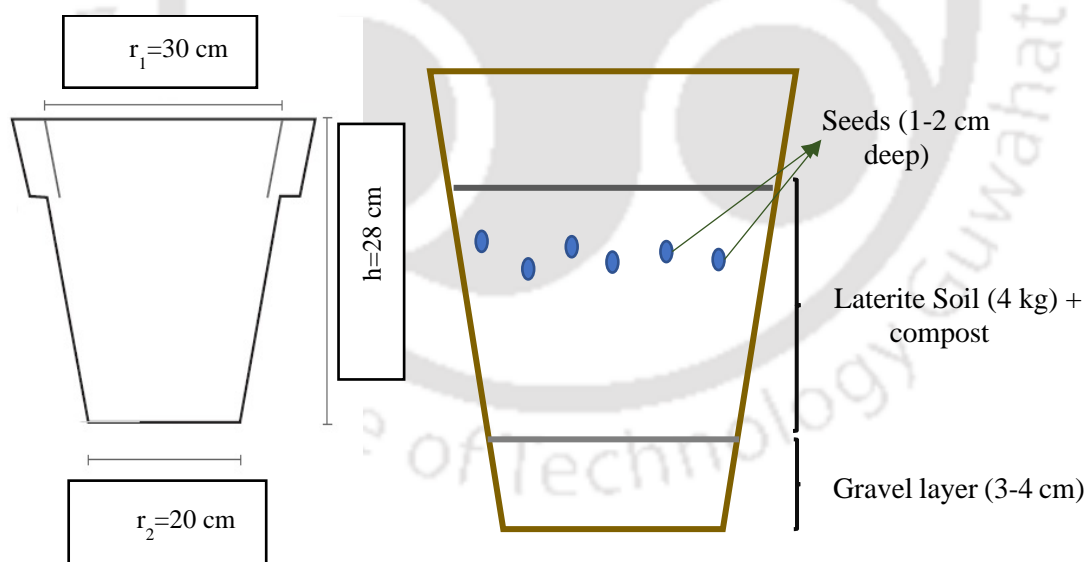


Fig 4.11. Experimental design of pots



Fig 4.12. Pictorial representation of pot experiment



Fig 4.13. Plant morphological studies

PHASE-IV (OBJECTIVE-4): MICROBIAL STUDY, ISOLATION OF BACTERIA, ENZYME ASSAY AND BIOAUGMENTATION STUDY

4.7 MICROBIAL STUDY

The microbial study of *Mikania micrantha* compost was performed by the enumeration and identification of microbial communities followed by the isolation and identification of bacteria by 16S rDNA analysis. The flowchart of microbial study has been illustrated in Fig. 4.14. The first study used outsourced samples (D0, D10, and D20) from the rotary drum composting process for metagenomic sequencing. The next step was to isolate bacteria using an enrichment technique to obtain bacteria that could survive in *Mikania* extract, followed by isolation of pure culture bacteria based on ligninolytic tests.

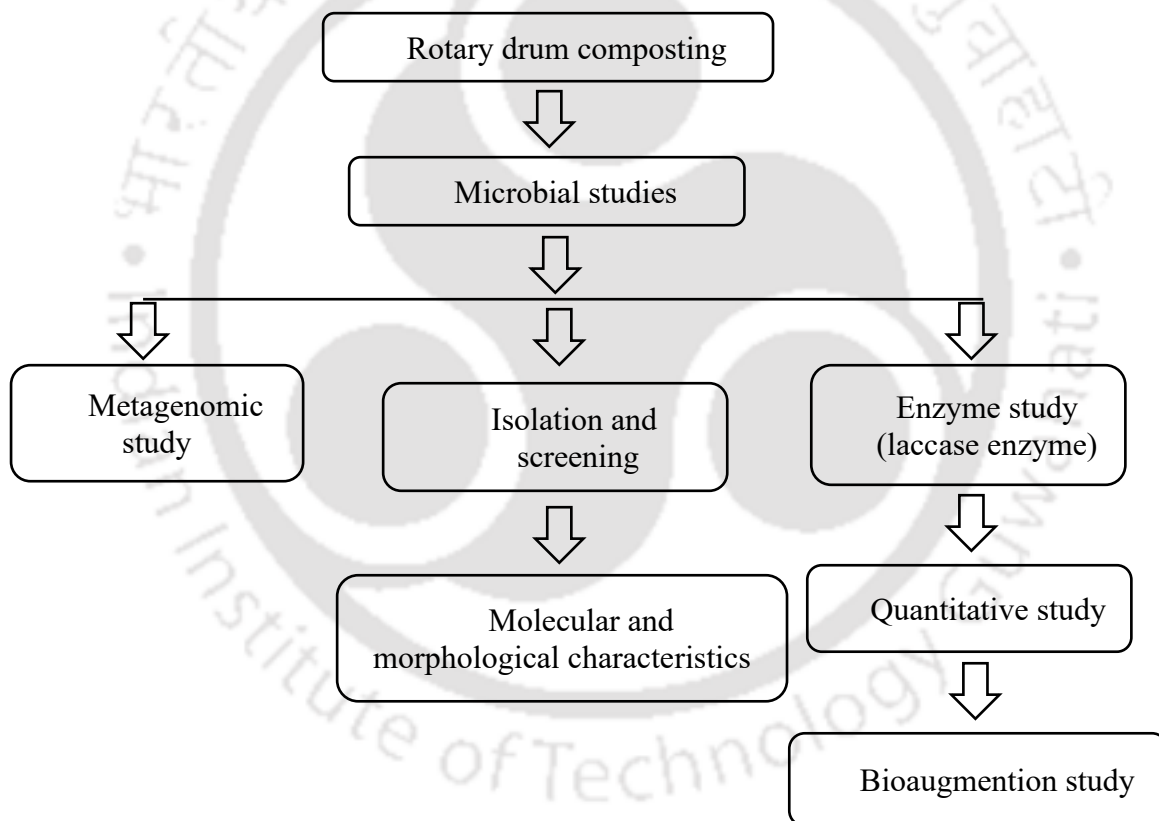


Fig 4.14. Experimental flowchart of microbial study

4.7.1 Metagenomic study and sampling

Sampling was done from RDC at Day 2, Day 10 and Day 20 of the composting process. Sample from each phase (excluding cooling phase) was taken for metagenomics study so that we may come up with diversified bacterial community. Samples were outsourced for the study which contains mixture of *Mikania*, cow dung and saw dust.

The metagenomic DNA was isolated from outsourced samples by commercially available Kit (Nucleospin). The qualities of the isolated metagenomic DNA sample were quantified using NanoDrop. The amplicon libraries were prepared using Nextera XT Index Kit (Illumina inc.) as per the 16S Metagenomic Sequencing Library preparation protocol (Part # 15044223 Rev. B). Primers for the amplification of the bacterial 16S V3-V4 region were designed and synthesized at Eurofins Genomics Lab. Amplification of the 16s gene was carried out. 3µl of PCR product was resolved on 1.2% Agarose gel at 120V for approximately 60 min or till the samples reached 3/4th of the gel. Two forward and reverse primers were used: GCCTACGGGNGGCWGCAG (16S rRNA F) and ACTACHVGGGTATCTAATCC (16S rRNA R). The QC passed amplicons with the Illumina adaptor were amplified using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The amplicon libraries were purified by AMPure XP beads and quantified using Qubit Fluorometer. The amplified libraries were analyzed on 4200 Tape Station system (Agilent Technologies) using D1000 Screen tape as per manufacturer instructions. After obtaining the mean peak sizes from Tape Station profile, libraries were loaded onto MiSeq at appropriate concentration (10-20pM) for cluster generation and sequencing. Paired-End sequencing allows the template fragments to be sequenced in both the forward and reverse directions on MiSeq. The kit reagents were used in binding of samples to complementary adapter oligoes on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

4.7.2 Sample collection and enrichment in *Mikania* extract (1:1, 1:2 and 1:3 w/v)

Samples were collected from mid-area, and end terminals from each phase of composting process (Day 2, Day 10 and Day 20) after manual turning of the rotary drum composter (RDC).

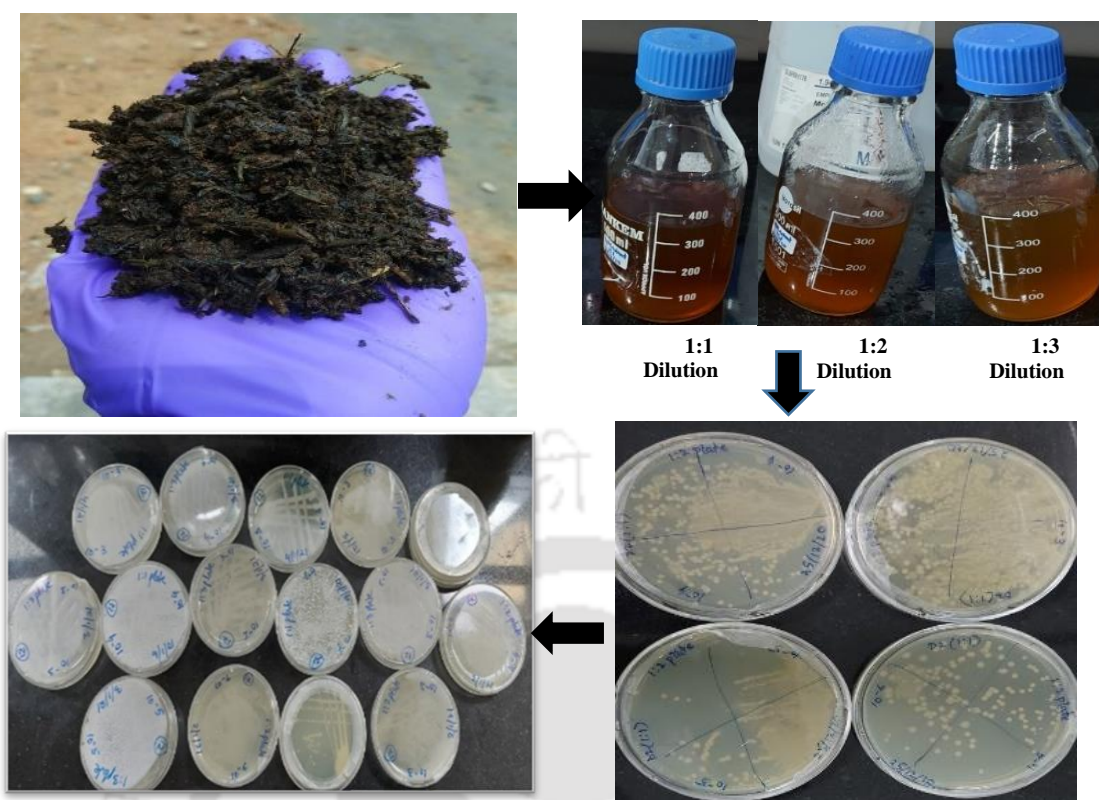


Fig 4.15. Steps involved for isolation of bacteria

Sterile mineral salt media (MSM) containing Na_2HPO_4 (2.4 g/L), K_2HPO_4 (2.0 g/L), NH_4NO_3 (0.1 g/L), MgSO_4 (0.01 g/L), CaCl_2 (0.01 g/L), Glucose (10 g/L) and Peptone (5 g/L) (Haq and Raj, 2018) were amended in varying concentration (1:1, 1:2 and 1:3 w/v) of *Mikania* extract respectively is illustrated in Fig. 4.15. 1g of compost sample from different phase of composting process was added to 99 mL of sterilized MSM media. Experiments were carried out in 250 mL Erlenmeyer flasks and incubated at 120 rpm for 5 days at 35°C. *Mikania* extract with different concentration was used to check the tolerance as well as enrichment and acclimation of the bacteria to higher concentrations of the extract.

4.7.3 Screening of ligninolytic enzyme producing bacteria (laccase enzyme)

To check the presence of laccase enzyme activity from the obtained bacterial strain, different experimental setup has been conducted as shown in Fig. 4.16. Following the optimum operating parameters, the Lac enzymatic activity for the optimum dosage was determined using a guaiacol oxidation test as described by Kalra et al (2013). The reddish-brown hue produced by Lac's oxidation of guaiacol was detected at 450 nm to determine the enzyme activity. Guaiacol (2 mM), sodium acetate buffer (10 mM), and crude enzyme were incorporated in the reaction solution. The reaction solutions were incubated at 30°C for 15 min in triplicates, and the enzymatic activity was measured using a UV-Vis spectrophotometer at 450 nm ($= 0.6740 \text{ M/cm}$) against a blank reagent. The enzyme's

activity was measured in IU/mL, where 1 IU equals the amount of enzyme required to oxidise 1 mol guaiacol per minute is illustrated in Fig. 4.17.



Fig 4.16. Isolation of bacteria in laminar airflow

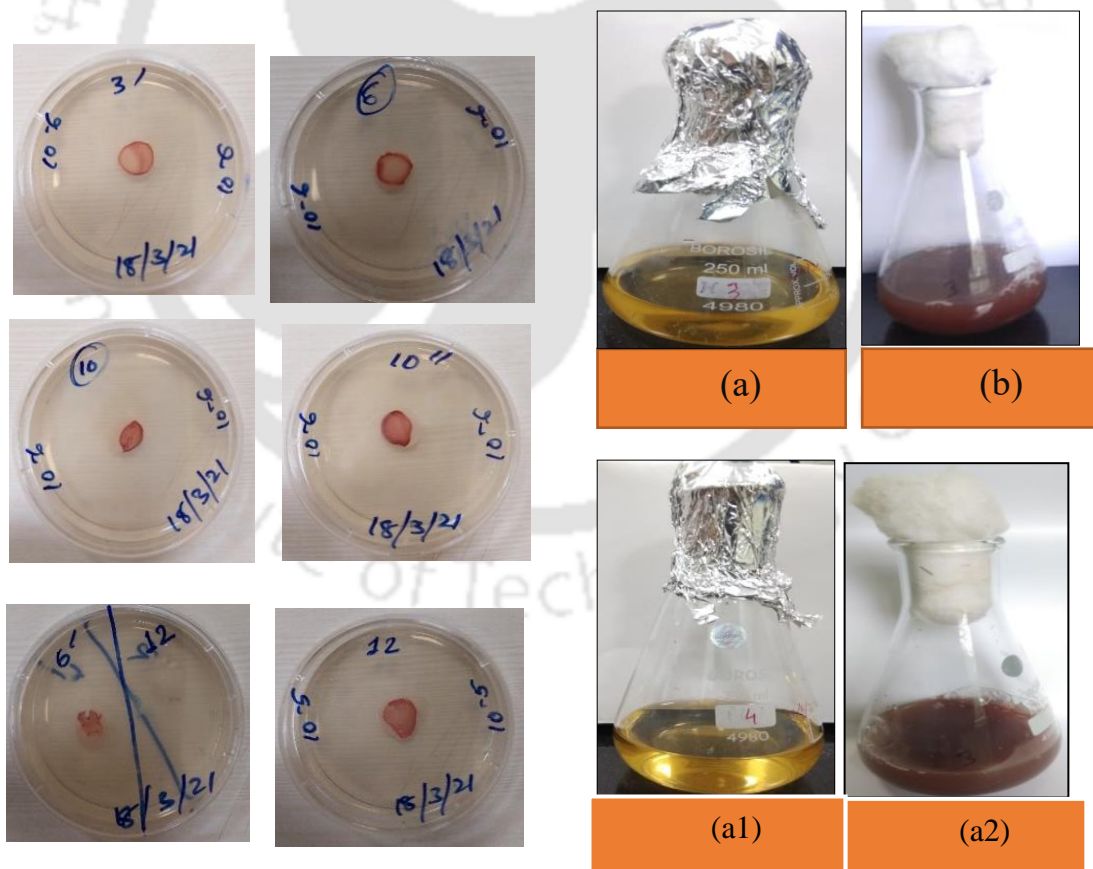


Fig 4.17. Laccase enzyme activity test (a) Before incubation; (b) After 7 days incubation; (a1) Before incubation and (a2) After 7 days incubation

4.7.4 Optimization of Lac enzyme activity: pH, temperature and RPM

Optimum pH for Lac enzyme activity was determined by conducting studies at four distinct pH level (5.0, 6.0, 7.0 and 8.0). The studies were carried out at 35°C and were placed in shaking incubator at 120 rpm. Effect of different incubation temperatures on lac enzyme activity was tested at varied temperature 25, 30, 35 and 40 °C in 250 mL Erlenmeyer flasks and the flasks were observed at regular interval of time. Since the growth of bacteria and the activity of the lac enzyme are both affected by RPM, studies were carried out at various rotational speeds (100, 120, 140, and 180 rpm) to determine the optimal lac enzyme activity. All of the experiments were done in triplicate to ensure optimum activity. Incubation took place for 7 days, and samples were taken every day for testing lac enzyme activity in accordance with the technique described above.

4.7.5 Preparation of bacterial inoculum and its inoculation

The bacterial strain that has shown highest enzyme activity was chosen for the bioaugmentation study. The isolated and purified bacterial strains was inoculated in 100 ml Luria Bertani (LB) broth. Spectrophotometric readings were taken for 16 h at 600 nm within intervals of 1 hour each for study of the growth curve. The bacterial strain was again inoculated in 150 ml LB broth cultures and allow it to grow until the bacterial population reaches around 10^8 CFU/mL. The cultures were then centrifuged at 4000 rpm for 5 minutes and the supernatant is then removed and distilled water is added to the residual bacterial biomass. Then it was inoculated into the reactors at the concentration of 10^8 - 10^9 CFU/mL at 5% (Sarkar et al., 2011) inoculation volume for 2.5 kg of waste mixture (*Mikania*, cow dung and saw dust in the ratio 5:4:1). The control treatment was prepared without worms and bacterial strains additions in order to compare with the inoculated reactors. It was then kept for 30 days under natural condition for degradation activities. Periodically distilled water was sprinkled for proper moisture content.

4.7.6 Application of bacterial strain with optimum lac enzyme activity

A preliminary work was conducted with two different studies, to check the efficacy of obtained bacterial strain on compost mixture.

- **Thermophilic composting followed by bioaugmentation process (TCB):** After completing the optimization test for lac enzyme activity, desired bacterial strain was used for the study of degradation of allelochemicals and also to reduce the time requirement for the composting process. Firstly, mixture of 150 kg *Mikania*, cow-dung and saw dust was mixed in the ratio 5:4:1 as described in section 2.1 and feed in Rotary drum composter. After 6 days, when the temperature drops to 35-40°C, the partially decomposed material was

used for bioaugmentation study using overnight grown bacterial culture having 10^8 CFU/mL. Culture media was used per 2.5 kg of waste for the study of bioaugmentation in bamboo reactor of size $89.11 \times 10^4 \text{ mm}^3$ (approx.). All the set up was conducted in duplicate including control designated as C1 and bioaugmented reactor as B1. Experimental set up of the reactor has been illustrated in Fig. 4.18.

- **Vermicomposting process bioaugmented with bacterial consortia (VBB):** For the vermicomposting process, a 5:4:1 mixture of *Mikania*, cow dung, and saw dust was used with *Esenia fetida*. We conducted an experiment using both bacteria and earthworms because it was previously known that the vermicomposting process takes longer time but produces superior quality. 2.5 kg of waste mixture was used for the experiment, and 10^8 CFU/mL of bacterial culture was inoculated in the reactor. The entire setup was done in duplicate. All the set up was conducted in duplicate including control designated as VC and bioaugmented reactor as VCB.



Fig 4.18. Reactor setup for bacterial inoculation study

4.8 MONITORING AND ANALYSES

4.8.1 Sampling

From each drum, 1 kg (approx. 10-15 cm from the compost surface) of the sample was collected from three different locations. Triplicate specimens were collected from the drum. Sampling was done regularly with two days' gap starting from 0 day to 20 days. 500 g of the collected sample was stored at 4°C and the other half was oven-dried at 105°C and powdered and sieved using 0.2 mm sieve and was kept in dessicator for further physico-chemical analysis.

For vermicomposting and rotary followed by vermicomposting process, an aggregated amount of 110 g of homogenized wet samples was taken on day 0, day 15, day 30, day 45,

and day 60 that is free of juvenile earthworm and cocoons. For rotary followed by vermicomposting, samples were collected on Day 0, Day 10, Day 20 and Day 30. Using a light separation technique, samples were assembled from the top of the reactor to ensure that no adult earthworm, juvenile, or cocoon was included in the sample mixture. Around 60 g of sample was taken from 110 g of the total sample and dried at 105 °C for 24 h. The dried sample was powdered, sieved, and stored in a zip-lock bag for further analysis.

For each bioaugmentation study, 150 g of homogenized wet samples were collected at 5-day intervals. For the various biological analyses, approximately 50 g of sample was taken from a 150 g sample. 100 g samples were oven-dried at 105 °C for 24 h before being ground and sieved for physicochemical analysis.

4.8.2 Parameters analyzed

Different experimental methods were used in the study to accomplish the stipulated objectives. Physico-chemical and biological analysis of the solid waste samples including *Mikania*, cow dung and saw dust were carried out in Solid waste laboratory and School of Rural Technology laboratory. Experimental procedures of the biological, microbiological and physico-chemical parameters were explained below:

- **BIOLOGICAL ANALYSIS**

- ***Soluble Biochemical Oxygen Demand (APHA, 2012)***

About 10 ± 0.1 g 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 h. Then it was filtered using whattman filter paper (Standard filter paper). The supernatant of the samples were taken and analysed for BOD test using BOD₅ test (Eq. 4.3).

$$\text{BOD} = \frac{D_1 - D_2}{P} \times 1000 \times \text{DF} \quad (4.3)$$

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, DF = Dilution factor.

- ***Chemical Oxygen Demand (APHA, 2012)***

About 10 ± 0.1 g 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 h. Then it was filtered using whattman filter paper (Slandered filter paper). The supernatant of samples were taken and analyzed for COD using closed reflux method 1.5 mL of K₂Cr₂O₇ + 2.5 ml of sulfuric

acid reagent were added to cod vial. It was digested for 2 h in COD digester at 150°C and cooled down to room temperature. Then cooled and titrated against ferrous ammonium sulphate using ferroin indicator till color changes from green to wine red.

➤ ***CO₂ evaluation by Soda-Lime method (Kalamdhad et al., 2008)***

About (25 ± 0.1g) of fresh compost sample was taken in 1 L PVC airtight container. About 10 g of oven dried (105°C) soda lime (1.5-2.0 mesh) was taken in a 100 mL beaker and was placed in the above mentioned container. The CO₂ evolution as shown in Eq. 4.4.

$$\text{CO}_2 \text{ evolution} = \frac{W_2 - W_1}{W \times T} \times 1000 \quad (4.4)$$

Where,

W1 = Initial weight of the soda-lime (g), W2 = Final weight of the soda-lime (g), W = Weight of compost sample taken (g), T = Time duration of incubation (h). Then the container with soda-lime beaker was kept in an incubator at 25°C. After 20-24 h the soda-lime was taken out and oven dried it again, then the final weight (W2) g was noted. The difference in mass of soda lime will give the amount of CO₂ absorbed.

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

The oxygen uptake rate (OUR) was performed according to the method described by Lasaridi and Stentiford (1998). The OUR was measured in a liquid suspension of compost (5-8g of compost in 500 ml of distilled water added with CaCl₂, MgSO₄, FeCl₃ 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 O₂ 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 O₂/g VS/h.

➤ ***Microbial analysis***

Lauryl tryptose broth and EC medium respectively were used for total coliforms (TC) and fecal coliforms (FC) analyses in the culture tube by using the most probable number (MPN) method (APHA, 2012).

• **PHYSICO-CHEMICAL ANALYSIS**

Temperature was monitored using a digital thermometer throughout the composting period. The EC and pH was measured in filtered supernatant (BIS: 10158-1982). Volatile

solid (VS) and ash content were also measured according to BIS, 10158-1982. Initial weight of the crucible was taken as W1 g. Weighed (10 ± 0.1 g) ground sample (screened through 0.22 mm sieve) in crucible and kept it in a muffle furnace operating at a temperature of 550-600°C for 2 h. After 2 h crucible was taken out of the muffle furnace and kept in desiccator for 30 m for cooling and then final weight of crucible with sample was taken as W2 g. Volatile solids content of the sample as shown in Eq. 4.5.

$$\text{Volatile Solids} = \left(\frac{2 + W1 - W2}{2} \times 100 \right) \quad (4.5)$$

- pH and Electrical conductivity (IS:10158-1982): About (10 ± 0.1 g) of ground sample (screened through 0.22 mm sieve) was taken and dissolved into 100mL of distilled water. It was kept it in a horizontal shaker for 2 h. The sample was filtered using whattman filter paper. The filtrate sample was used for measuring pH and EC using pH meter and conductivity probe.
- Total organic carbon (TOC) was calculated from VS with a factor of 1.8. Total nitrogen (TN) was analyzed using the Kjeldahl method and NH_4^+ -N using KCl extraction (Tiquia and Tam, 2000). For TN analysis 0.2 g of sample (passed through 0.22 mm sieve) was taken and catalyst mixture (potassium sulphate and cupric sulphate, 5:1) of 3 g was added, and digested with 10 mL conc. H_2SO_4 using digestion equipments at 400°C for 2 h (end color of digested sample was green). After digestion, make the digested sample 100 mL. 10 mL of diluted sample distillate 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 colour.

$$\text{TN}(\%) = \frac{14 \times (S - B) \times N}{\text{Wt. of sample}} \quad (4.6)$$

Where,

S = mL of sulfuric acid used for sample, B= mL of standard sulfuric acid used for blank,
N= Normality of standard sulfuric acid, Wt. = Weight of the sample in g

- For the analysis of NH_4^+ -N, 5 g sample (passed through 0.22 mm sieve) was taken in a reagent bottle to which 50 mL of 2M KCl was added and kept in a horizontal shaker for 2

h. After shaking sample was filtered and supernatant was taken for $\text{NH}_4^+\text{-N}$ analysis using Phenate method of Standard methods (APHA, 2012).

4.8.3 Spectroscopic analysis

To find out the characteristics of the samples different analysis were carried out for the sample before and after composting process. In order to find out the chemical characteristics and functional groups of the compost sample was done by FTIR spectroscopy. The spectra were recorded in a Fourier transform infrared spectrometer with the samples prepared in KBr disc. All spectra were plotted using the same scale on the transmittance axis. The crystalline structure was determined using XRD technique since it provides the most definitive structural information and interatomic distances. Spectroscopic analysis PXRD was performed to know the crystallinity and amorphous nature of the product. Samples were prepared for GC-MS by combining the dried and powdered material with spectroscopic grade methanol in the ratio 1:10 (Hussain et al., 2016) in 250 mL conical flask. Flasks were kept in horizontal shaking incubator at 120 rpm for 24 h. The homogenised mixture was filtered using Whatman no. 41 filter paper. The filtered sample was later kept for evaporation and the residue was once again combined with spectroscopic grade methanol and filtered through 0.45 μm syringe filter before analysing in GC-MS instrument. The samples were processed for the GC-MS analysis following Hussain et al., (2016) procedure. Identification of compounds was done by comparing their mass spectra with that of National Institute of Standards and Technology (NIST) library available with the instrument.

4.8.4 Statistical analysis

The statistical analysis was performed at $p < 0.05$ using one-way variance analysis (ANOVA). Mean and standard deviation from triplicate samples are calculated for each reactor.

4.9 Instrument used

Table 4.5 Instruments used in various analysis

Parameter tested	Instrument	Model/Manufacturer
pH	μ pH system 361	132, Systronics, India
EC	Digital conductivity meter	VSI-04-Deluxe
Na^+ , K^+ & Ca^{2+}	Flame Photometer 128	Systronics

Heavy Metals	AAS	Varian Spectra 55B
Nitrite, Ammonia, chloride, fluoride, sulphate	Spectrophotometer	MRC spectro V-110
Nitrate	UV-Spectrophotometer	CARY 50 Bio, VARIAN
TKN	Kelpus distillation unit	Pelican kelpus – Digital EM VA
COD	Cod digester	Hach DRB 200, Hach, USA
BOD ₅	BOD Incubator	International Commercial Traders
Weight	Weighing balance	SL-234, Denver Instrument
Drying	Hot air oven	ICT, Calcutta, India
VS	Muffle furnace	ICT, Calcutta, India
Sample preservation	Refrigerator	MRC scientific instruments, India
Functional groups	FTIR	Remi Model: Autosorb-IQ MP Perkin elmer spectrum version 10.4.3
Compound identification	GC-MS	GCMS-TQ8030, SHIMADZU
Optical density	UV-VIS spectrophotometer	
Genotoxicity	Compound microscope	Model: BA 210, Make: MOTIC
Microbial analysis	Horizontal Laminar Airflow	
Crystallinity and amorphous	PXRD	Rigaku TTRAX III

Treatment of invasive weed *Mikania micrantha* Kunth using three different compost technologies

This chapter investigates the initial characterization of the substrate used for the biological treatment process using various composting technologies. *Mikania micrantha* Kunth was used as the main substrate, cow-dung as an inoculum, and sawdust as a bulking agent in 6 different ratios for the study. Later, the best ratio was used for the vermicomposting process using three different monocultures of earthworm species. Another study was carried out using the best ratio obtained from RDC process in order to obtain superior compost quality compost in less time, as earthworm aids better quality than the in-vessel technique. The proposed combined technology uses an the in-vessel technique with the vermicomposting procedure for the biological treatment of weed. The two-stage composting technique was used to produce high-quality compost in a shorter amount of time.

5.1 INITIAL CHARACTERIZATION OF *MIKANIA MICRANTHA*, COWDUNG, AND SAWDUST

Table 5.1 summarizes the initial characterization of the weed, cow-dung, and saw-dust. The quantity of moisture present in organic substrates impacts the biological activity and physical structure and has a significant impact on the biodegradation of organic substrates

in a variety of ways (Ahn et al., 2008). According to the literature, microbial activity is suppressed when the moisture content falls below 25%, and aeration can be restricted when the moisture content exceeds 70% (Encarnacion et al., 1995). Cronje et al. (2004) found that most organic substrates compost was best when the moisture content is between 50 and 70%, but some other materials can compost efficiently at a moisture content between 25 and 80% (on a wet basis) outside of this range. The initial moisture content of 87% was recorded in *Mikania*, and for cow dung and saw dust; it was found to be 90 and 12%, respectively. *Mikania* contains a high concentration of essential nutrients. It is likely that compost made from the weed *Mikania* would benefit plants and could be used as a soil conditioner. *Mikania* had a nitrogen content of 2.5%, potassium content of 4.21 g/kg, the sodium content of 3.59 g/kg. The C/N ratio of the organic substrate to be composted is critical because it affects microbial activity, quality, and nutritional value of the end product (Jain and Kalamdhad, 2018). *Mikania* had a C/N ratio of 16.93, cow dung had 34.74, and sawdust 57.35. The initial characterization of the substrate material indicated that it could be composted and vermicomposted, and studies have been carried out further with different composting technologies.

Table 5.1 Initial characterization of the raw materials

Parameters (on dry basis)	Units	<i>Mikania micrantha</i> Kunth (mean \pm St dev)	Cow dung (mean \pm St dev)	Sawdust (mean \pm St dev)	Optimum condition	Reference
Moisture content	%	87 \pm 1.8	90 \pm 2.5	12 \pm 0.2	>70%	(Brinton, 2000)
					50-60	(Brinton, 2000)
Volatile solids	%	77 \pm 2.9	89 \pm 3.7	21 \pm 2.1	% (on dry basis)	
Ash content	%	22.5 \pm 2.5	11 \pm 2.4	79 \pm 0.5	-	-
pH	-	5.90 \pm 0.4	6.8 \pm 0.5	6.7 \pm 0.1	-	-
EC	mS/cm	8.21 \pm 0.2	3.21 \pm 0.02	4.22 \pm 0.2	-	-
TOC	%	42.34 \pm 2.4	48.64 \pm 1.9	11.47 \pm 2.1	-	-
Nitrogen	%	2.5 \pm 0.5	1.4 \pm 0.2	0.2 \pm 0.09	-	-
Ammonia	mg/kg	5.90 \pm 1.1	28 \pm 0.07	0.31 \pm 0.02	-	-
Available phosphorus	g/kg	2.071 \pm 0.8	3.5 \pm 0.2	1.4 \pm 0.4	-	-

Total phosphorus	g/kg	12.183 ± 0.5	4.9 ± 0.4	2.6 ± 0.2	-	-
Sodium	g/kg	3.59 ± 0.3	2.5 ± 0.2	0.9 ± 0.2	-	-
Calcium	g/kg	5.985 ± 0.2	9.2 ± 0.1	2.1 ± 0.2	-	-
Potassium	g/kg	4.21 ± 0.1	0.2 ± 0.2	1.0 ± 0.2	-	-
C/N	-	16.93	34.74	57.35	>30	(Haug, 1993)

5.2 EFFICACY OF ROTARY DRUM COMPOSTER FOR THE TREATMENT OF INVASIVE WEED *MIKANIA MICRANTHA* KUNTH

In the present study, we determine the potency of the in-vessel technique for producing compost of *Mikania* and delve into the possible conditions for making compost of *Mikania* mixing with different proportions of cow dung as inoculum and saw dust as a bulking agent. Based on recent literature, rotary drum composting with different wastes, six different ratios have been taken for composting of *Mikania*.

5.2.1 Physico-chemical analysis

5.2.1.1 Temperature and moisture content

The temperature of the compost and its metabolic activities is a major factor deciding the microbial diversity (Hassen et al., 2001). In all the reactors, namely RD1, RD2, RD3, RD4, RD5, and RD6, an increase in temperature was found during the early stage of the composting process. In all the experiments, the temperature rose simultaneously achieving the peak within 2-4 days. Highest peak was observed in reactor RD6 as compared to other ratios in 2 days as shown in Fig. 5.1. There might be a breakdown of phytochemicals in the reactor RD6 that may lead to an exothermic reaction, which caused high temperatures. At the end of 2 days, 60.1°C temperature was observed in RD6, 58.5°C in RD2, 50.1°C in RD3, and 50.1°C in RD4. In the composting process, temperature from 52 to 60°C is considered to sustain the greatest thermophilic activity (Mohee and Mudhoo, 2005). Good sanitation will be provided by a temperature above 55°C (Venglovsky et al., 2005). Leachate formation was observed in RD6 (control) as no bulking agent was added. It is stated that high temperatures may not always signify the degradation of the substrate and temperature range, but the degradation of the waste is dependent on the substrate used (Hazarika et al., 2018). Though RD6 attained high temperatures in this study, it did not attain the proper degradation process later.

Thermal process and conversion, as stated by (Bian et al., 2019), are the most important composting factors affecting water reduction and biomass reduction. Moisture content is an important contributing factor in the circulation and transportation of gases and nutrients needed for various microorganisms' physiological and metabolic cycle (Liang et al., 2003). The degradation mechanism could be affected by excessive moisture as anaerobic conditions grow. The final compost moisture content varies depending on a substrate's initial moisture content (Jain et al., 2018).

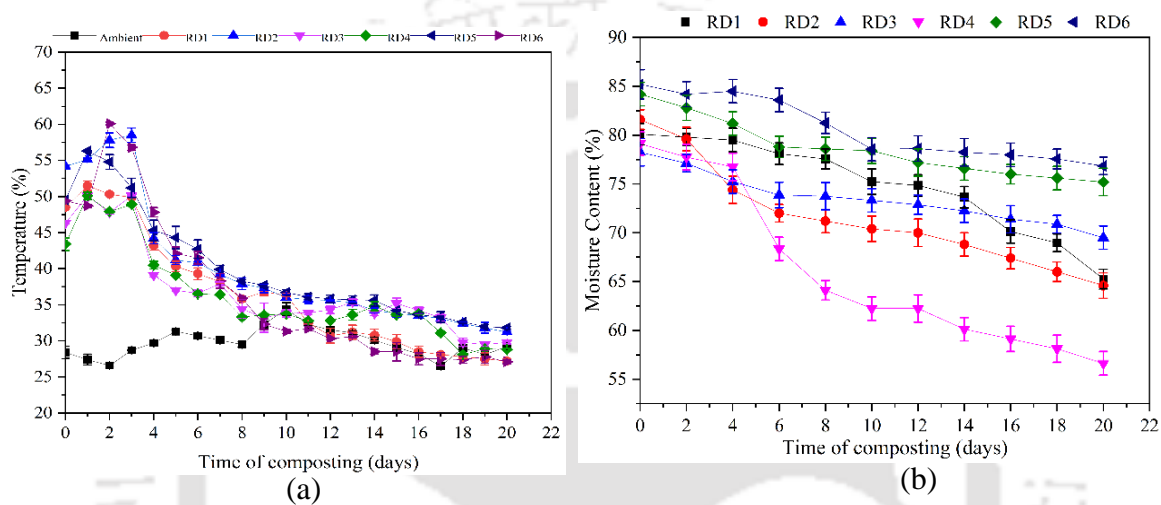


Fig. 5.1. Variation in a) Temperature b) Moisture content during RDC process

Fig. 5.1 (b) indicates a decrease in humidity during the composting process. The highest peak of reduction is reached in RD3, where there is a moisture change up to 6 days, suggesting the equilibrium moisture status of microbes (after 6 days). The graph shows that the humidity content in the reactor RD6 was high despite having more temperature. In some situations, where the surface has a higher temperature, the low moisture content may not be present. Since RD6 has more substrate, the moisture created is trapped in the drum and cannot be evaporated due to its low porosity. A composting process with low moisture will minimize microorganism activity, and high moisture content will impede gas diffusion (Bian et al., 2019). The moisture content criteria differed greatly with respect to different tests and during the composting process at $p < 0.05$.

5.2.1.2 Volatile solids (VS), total organic carbon (TOC), and ash content

The degradation of organic compounds during the composting process is assessed by reducing the volatile solids content. Organic waste stabilization and maturation involve widespread humification, a complex series of processes of conversion of organic matter

into refractory organic compounds that resemble those found in natural soils (Sánchez-Monedero et al., 1999). Reduction is observed primarily during the first week of the thermophilic cycle as thermophilic bacteria massively decompose organic matter. RD2 had a maximum percentage reduction of 48.59%, followed by 19.58, 21.97, 24.49, 31.06, and 31.74% for trial RD1, RD3, RD4, RD5, and RD6, respectively, as illustrated in Fig. 5.2.

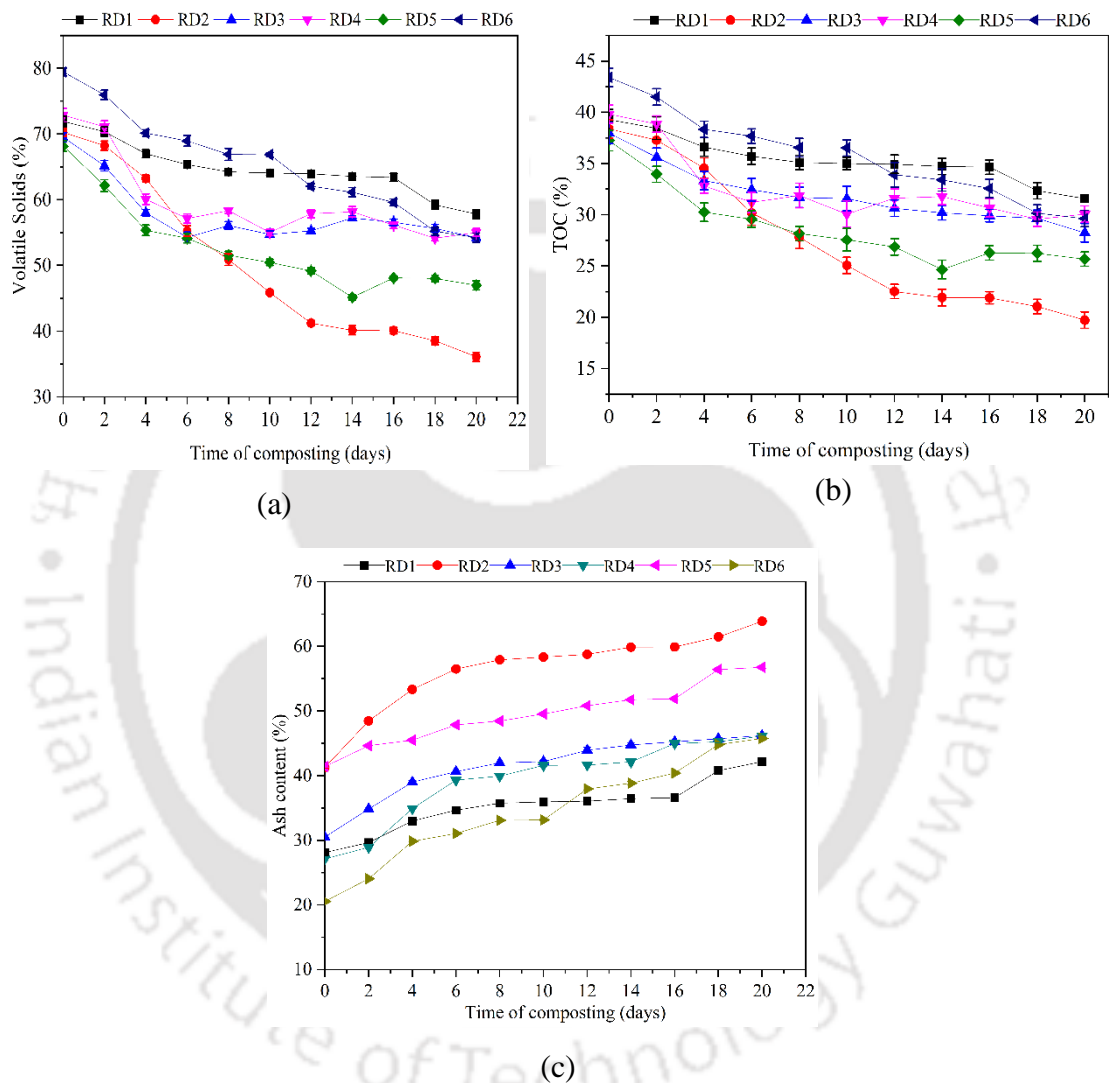


Fig. 5.2. Variation in a) Volatile solids b) Total organic carbon (TOC) c) Ash content during the RDC process

Fig. 5.2 demonstrates the deterioration of TOC (on dry basis) during the composting process. As drop-in volatile solids, TOC degradation in the reactor RD2 is more prominent from 38.37% to 19.72%. Barrington et al. (2002) reported that compounds such as protein, cellulose, and hemicellulose caused the mineralization of organic compounds during the composting cycle. Further, he stated that around 60-65% of total organic compounds

converted by microbial activity and the left portion by microbial cells to carbon dioxide are degraded. In the start, the gross organic carbon content of RD1, RD3, RD4, RD5, and RD6 was 39.28, 37.97, 39.81, 37.24, and 43.41%, respectively, which got reduced to 31.59, 28.24, 30.06, 25.67, and 29.63% respectively at the end of the composting process. Jain et al. (2018) reported that ash content is an important factor in evaluating the nutrient dynamics during the composting process. Ash content was recorded as 40.80, 61.47, 45.72, 45.24, 56.42, and 44.80% for RD1, RD2, RD3, RD4, RD5, and RD6 in the final compost product. In all the reactors, the ash content was increased periodically, but compared with all the reactors, RD2 showed high ash content, which presumes that there was high degradation in the reactor, as shown in Fig. 5.2. Data were analyzed by ANOVA that shows the ash content varied significantly with different mix proportions ($p < 0.05$).

5.2.1.3 pH and EC

Monitoring of the pH after every alternate day showed that the pH value in the composting process increased from 7.24, 7.10, 6.85, 6.48, 7.0, and 7.21 to 7.91, 8.13, 8.30, 8.41, 8.50, and 8.39 in the reactor RD1, RD2, RD3, RD4, RD5, and RD6, respectively shown in Fig. 5.3. The organic matter decomposition leads to ammonium ion formation (NH_4^+), which increases the pH value (Pramanik et al., 2007). pH is an important factor affecting microbial activity in the degradation of organic compounds during composting (Yang et al., 2019).

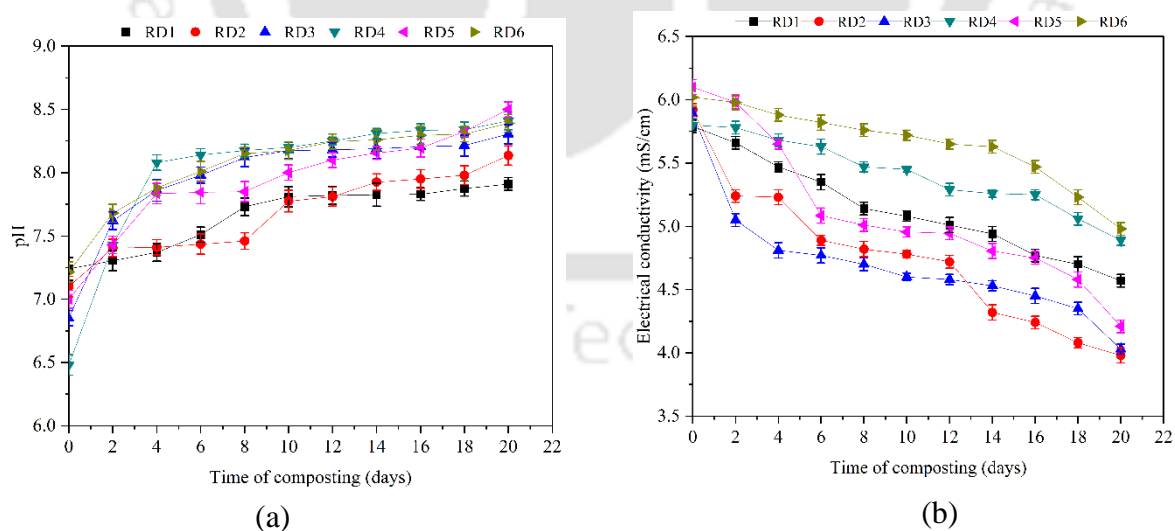


Fig. 5.3. Variation in a) pH b) Electrical conductivity during the RDC process

The pH values of all trails for agro use were within the desired range of 7.0-8.5 (Maso and Blasi, 2008). The metabolism of microorganisms is more at pH 7.5-8.5 during the composting process (Jumnoodoo and Mohee, 2012). The EC value reflects the degree of

salinity in the compost, highlighting the phytotoxic impact of soil application on plant growth (Hazarika and Khwairakpam, 2018). With the evolution of decomposition, changes in EC were observed in all trials, as shown in Fig. 5.3. The initial EC values of 5.79, 6.02, 5.89, 5.80, 6.10, and 5.92 mS/cm, respectively, in RD1, RD2, RD3, RD4, RD5, and RD6 was decreased to 4.57, 4.98, 4.03, 4.89, 4.21 and 3.98 mS/cm throughout composting. During the composting process, the decrease in EC is attributed to ammonia volatilization, which accumulates mineral salts in the compost material that releases humic substances (Wong et al., 1995). Agriculture soil (Bhamidari and Pandey, 1996) accepts an EC value of > 4 dS/m in the compost. Variation in different reactors was observed over time, which was significant ($p < 0.05$) since EC depends on the concentration of cow dung that influences the mineralization process (Singh and Kumar, 2017).

5.2.1.4 Nitrogen profile

The total nitrogen (TN) and ammonical nitrogen ($\text{NH}_4^+\text{-N}$) time progressions are shown in Fig. 5.4. Studies have shown that nitrogen is immobilized during composting and transferred to humus as a material; it can also be used as organic material with a gradual nutrient release. Owing to the net loss of biomass in terms of CO_2 and water loss due to evaporation, TN rises during composting. In the first stage of composting, nitrogen is influenced by proteolytic bacteria (Zorpas et al., 2000).

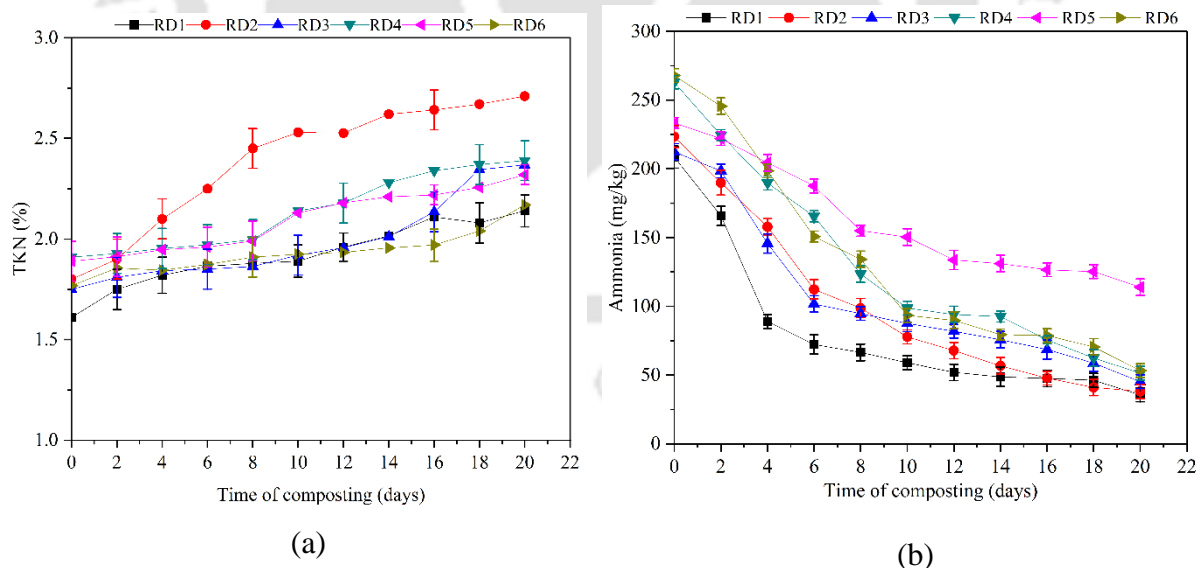


Fig. 5.4. Variation in a) Total kjeldahl nitrogen b) Ammonia during RDC process

The loss of nitrogen to the atmosphere generally occurs at a high composting temperature. TKN was recorded as 2.14, 2.71, 2.51, 2.36, 2.39, and 2.17% in RD1, RD2, RD3, RD4, RD5, and RD6, respectively, after 20 days of the composting process with

respect to initial values. The percentage increase in TKN was recorded as 32.91, 50.56, 35.31, 25.13, 22.75, and 22.73% in RD1, RD2, RD3, RD4, RD5, and RD6, respectively. The concentration of $\text{NH}_4\text{-N}$ increased immediately after the feeding of the reactors. During the thermophilic phase of the composting process, $\text{NH}_4\text{-N}$ concentration decreased to 53.30, 35.59, 38.09, 45.20, 51.42, and 134.9 mg/kg in RD1, RD2, RD3, RD4, RD5, and RD6, respectively. A result indicates that there is a significant decrease in $\text{NH}_4\text{-N}$ concentration in all trials. In the initial stages of the composting process, the organic nitrogen will change its phase to ammoniacal nitrogen. During the maturation stage, due to high aeration, a substantial increase of pH loss of CO_2 in the process will decrease ammoniacal nitrogen (Kalamdhad et al., 2009). However, the presence of nitrate is not detected during the 20 days composting process of *Mikania* in all trials. Statistical analysis using ANOVA showed significant variation of both the parameters during composting process $p < 0.05$.

5.2.1.5 Total and available phosphorus (TP and AP)

Phosphorus (P) is another essential nutrient that plant absorbs. Total phosphorus (TP) was higher in RD2, increasing from 9.28 g/kg to 12.35 g/kg.

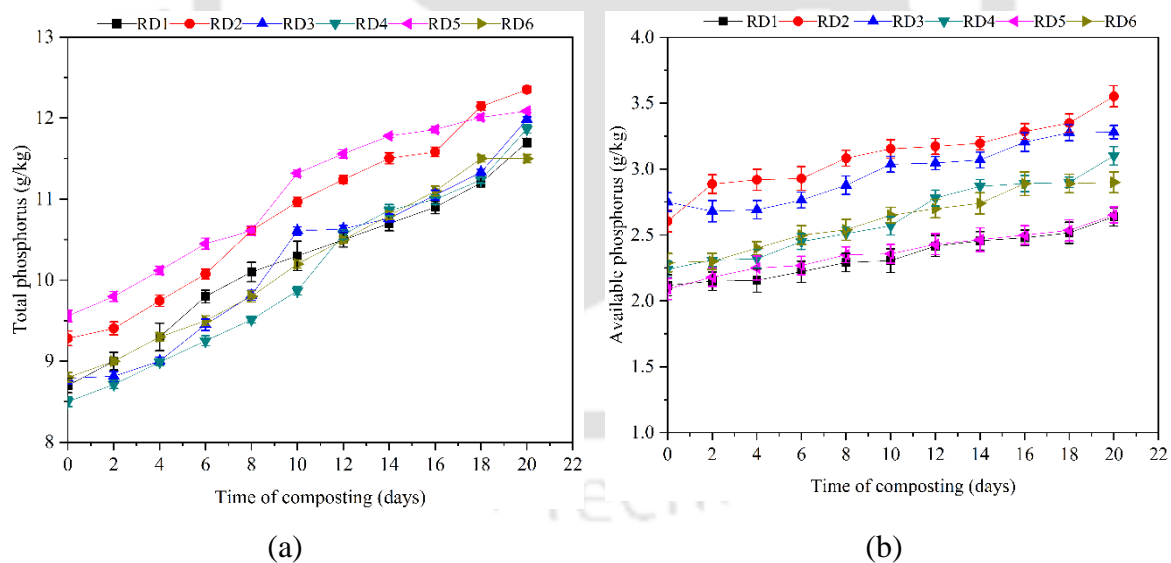


Fig. 5.5: Variation in a) Total phosphorus and b) Available phosphorus during RDC process

Total phosphorus was found to be 11.70, 11.98, 11.86, 12.08, and 11.50 mg/kg in RD1, RD2, RD3, RD4, RD5, and RD6, respectively. Available phosphorus (AP) was found 2.63, 3.55, 3.28, 3.10, 2.65, and 2.90 g/kg in RD1, RD2, RD3, RD4, RD5, and RD6, respectively in Fig. 5.5. RD2 has a maximum amount of total phosphorus and available phosphorus as compared to other parameters. An increase in phosphorus is due to bacterial mineralization

and loss of organic biomass during the composting process (Jakubus, 2016). However, different types of microorganisms would act to solubilize phosphorus at a particular pH during composting (Hameeda et al., 2008). Significant variations ($p < 0.05$) in nutrients (TP and AP) for all reactors have been observed.

5.2.1.6 C/N ratio

One of the most important factors influencing compost quality is the initial carbon-nitrogen (C/N) ratio. The optimum ratio is generally within the range of 19-30 parts of available carbon to 1 part of available nitrogen (Michel et al., 1996). He further added that a C/N ratio above 30 might slow microbial activity, which is regarded as bad for composting. A C/N ratio lower than 18 may lead to the loss of nitrogen in the atmosphere in the form of ammonia. In this study, the C/N ratio initially was 24.40, 21.32, 20.41, 20.98, 17.99, and 21.81 for RD1, RD2, RD3, RD4, RD5, and RD6. Here, for reactor RD1, RD2, RD3, RD4, and RD6 C/N ratio was within the range of 19-30, which is generally considered an ideal microbial diet for microorganisms except for reactor RD5, where C/N was found to be 17.99. The C/N ratio for the final compost was in the range of 7-14. The lowest C/N ratio was found in RD2, where the reduction was highest compared to other reactors. There was an optimum reduction in the RD2 without any leachate formation with proper nutrient contents. The increased nitrogen content and oxidation of organic matter lead to a decrease in C/N over time (Devi and Khwairakpam, 2020). As suggested by CPHEEO (2000), the C/N ratio of the final product should be < 20 .

5.2.1.7 Macronutrients

Potassium is the mineral required for plant productivity after nitrogen and phosphorus. Fig. 5.6 shows the time course of potassium (K), sodium (Na), and calcium (Ca). The elements such as K, Na, and Ca followed the increasing trend for all trials during 20 days of composting. Potassium, sodium, and calcium were observed as 31.88, 5.80, 8.45 g/kg for RD1, 33.8, 6.40, 9.60 g/kg respectively for RD2, 31.54, 6.40, 9.03 g/kg respectively for RD4, 28.18, 5.78, 9.01 g/kg respectively for RD5 and 27.8, 5.40, 8.05 g/kg respectively for RD6. Trial RD2 had the maximum increase of potassium content; trial RD2 had the maximum increment of sodium content, and trial RD5 had the maximum increment of calcium content. The increase of nutrients occurs due to the decomposition of the substrate and mineralization (Hazarika and Khwairakpam, 2018). Although similar results have been found in all the reactors, in reactor RD2 high amount of nutrients was observed in comparison to other reactors. Significant variations ($p < 0.05$) in nutrients (K, Ca, and Na) for all reactors have been observed.

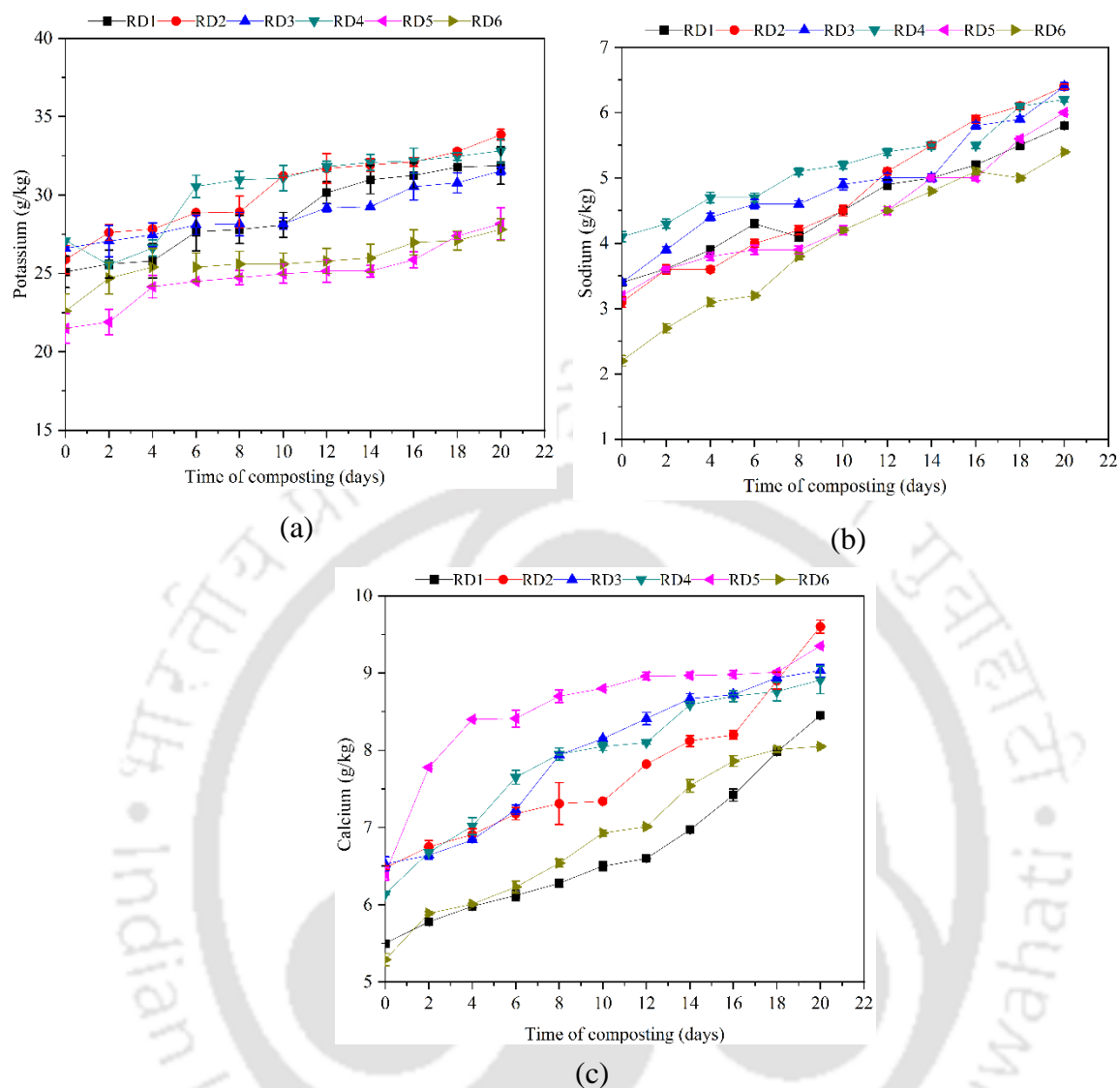


Fig. 5.6. Variation in a) Potassium b) Sodium and c) Calcium during RDC process

5.2.2 Biological parameters

5.2.2.1 sBOD and sCOD

Composting is an aerobic process that helps in the degradation of organic matter. An increase or decrease of BOD and COD depends on the degradation of organic matter. Fig. 5.7 shows that sBOD and sCOD decreased in all trials. Maximum reduction of sBOD was found in RD2 (from 330.23 mg/L to 41.2 mg/L) followed by RD6 (from 372.96 mg/L to 55.5 mg/L). The decline in biological content reduces the BOD and COD, ultimately reducing the carbon dioxide emission. Maximum reduction of sCOD was found in RD2 (from 471.6 mg/L to 75.8 mg/L) and least in RD5 (from 522.67 mg/L to 128 mg/L). Both the parameters exhibited significant variation during the process at $p < 0.05$.

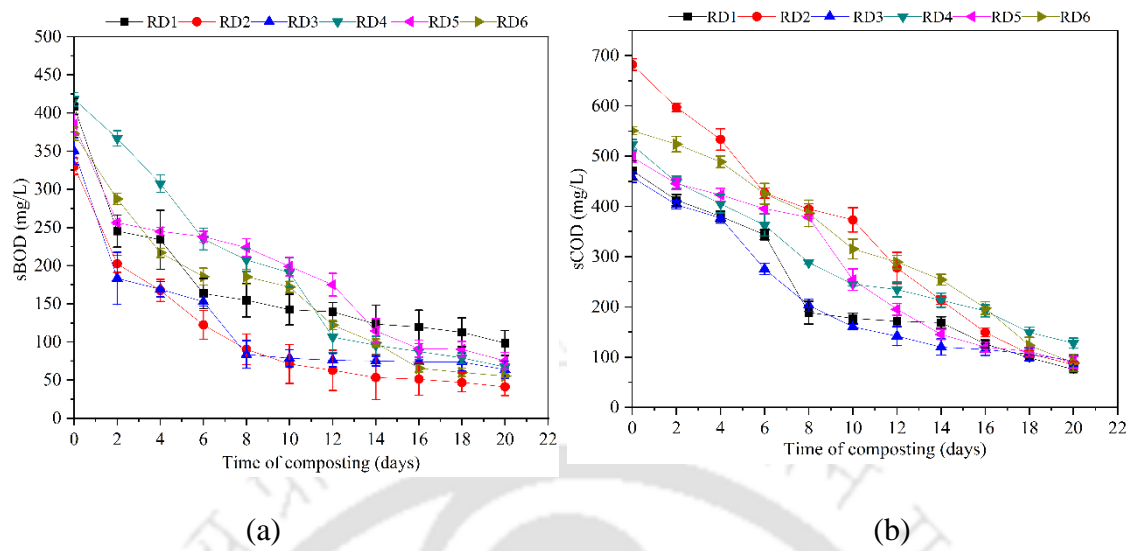


Fig. 5.7. Variation in a) sBOD b) sCOD during RDC process

5.2.2.2 OUR and CO₂

During the decomposition of organic matter, gaseous products are emitted like carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), carbon monoxide (CO), and ammonia (NH₃) which has a mostly negative effect on climate change on Earth. Fig. 5.8 (a & b) show a change in oxygen uptake rate during 20 days of composting processes.

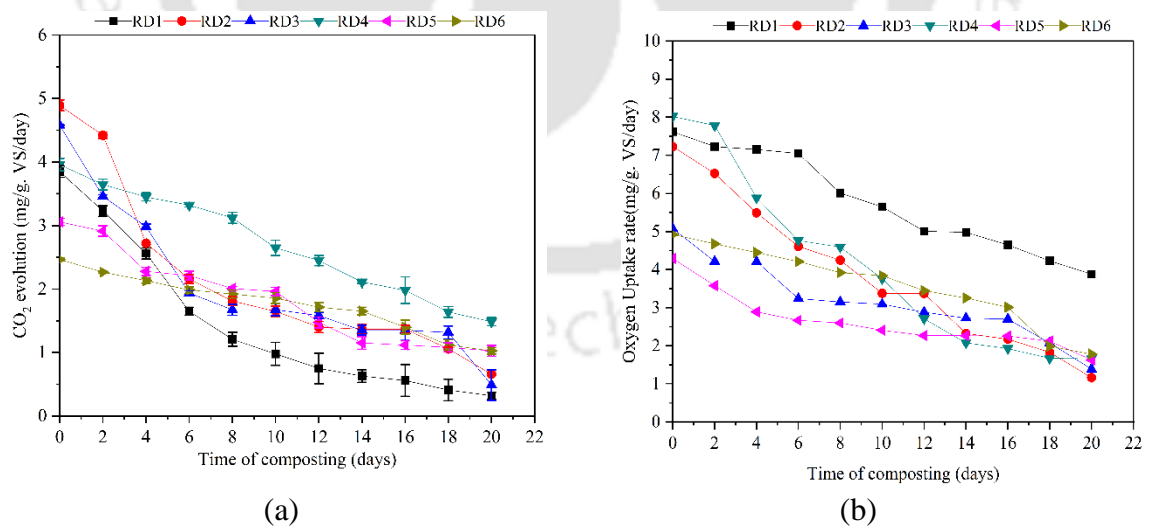


Fig. 5.8. Variation in a) CO₂ evolution and b) oxygen uptake rate (OUR) during RDC process

The oxygen uptake rate (OUR) of RD1, RD2, RD3, RD4, RD5 and RD6 decreased from 7.61, 7.23, 5.07, 8.02, 4.29 and 4.92 mg/gVS/day to 3.88, 1.16, 1.39, 1.67, 1.62 and 1.78 mg/gVS/day respectively, whereas CO₂ evolution rates in (Fig. 6c) decreased from 2.46, 3.85, 4.89, 4.58, 3.96 and 3.05 mg/g.VS/day to 1.02, 0.32, 0.65, 0.45, 1.48 and 1.02 mg/g VS/day. The CO₂ evolution showed a significant difference over the period at p<0.05.

5.2.2.3 Estimation of total coliform and fecal coliform bacteria

Hygiene standards are commonly considered to mark the value of compost by the coliform presence (Nartey et al., 2017). The major contributing factor in the recent study is the achievement of thermophilic temperatures, which minimize both total and fecal coliform. In reactor RD2, which observed the second highest thermophilic phase for long periods of time, the maximum coliform reduction was tracked. Due to high temperature (58°C), the decrease of total coliforms is shown in RD2 than in other reactors. In reactor RD2 and RD6 after the thermophilic phase, a significant reduction in fecal coliforms have been found too. The observed decrease was due to the high temperature during the thermophilic process and adverse conditions (Hassen et al., 2001) shown in Table 5.2.

Table 5.2 MPN test for total and faecal coliform bacteria of RDC process

Reactors	Proportion	Total coliforms			Fecal coliforms		
		0 day	10 day	20 day	0 day	10 day	20 day
RD1	4:5:1	7.5E+06	9.5E+03	7.5E+04	7.5E+04	8.5E+02	6.5E+01
RD2	5:4:1	2.3E+06	2.5E+02	1.5E+05	1.3E+04	1.5E+01	1.3E+01
RD3	6:3:1	2.3E+04	2.3E+03	7.3E+04	2.3E+04	9.3E+03	5.5E+01
RD4	7:2:1	4.3E+04	7.3E+03	7.3E+02	2.3E+06	4.3E+01	4.3E+03
RD5	8:1:1	2.3E+04	7.5E+06	9.3E+01	2.3E+04	2.3E+03	4.3E+01
RD6	10:0:0	7.5E+07	9.3E+05	7.5E+04	4.3E+06	2.1E+04	2.3E+02

5.3 FATE OF INVASIVE WEED *MIKANIA MICRANTHA* KUNTH USING VERMI TECHNOLOGY EMPLOYING THREE MONOCULTURE OF EARTHWORM SPECIES

The purpose of the current work is to ensure the safe treatment of *M. micrantha* and the development of high-quality compost through the use of vermicomposting technology. The novelty of the current study is that it uses *M. micrantha* biomass, which has been found in large quantities in the north-eastern part of India, and evaluates various physicochemical and biological analyses during the process. The overall objective of this research is to determine whether the finished product is suitable for field use based on compost quality. The use of *Mikania* biomass for value-added products like vermicompost using earthworm species like *E. fetida*, *E. eugeniae*, and *P. ceylanensis* has received very little attention.

5.3.1 Physico-chemical analysis

5.3.1.1 Moisture Content

Water has been splashed every alternate day to maintain a moisture content of about 60%, as it is important for proper acclimatization of the earthworm. The worms enjoy a humid atmosphere, and the atmospheric temperature, which can be detrimental to them, was about 30-35°C.

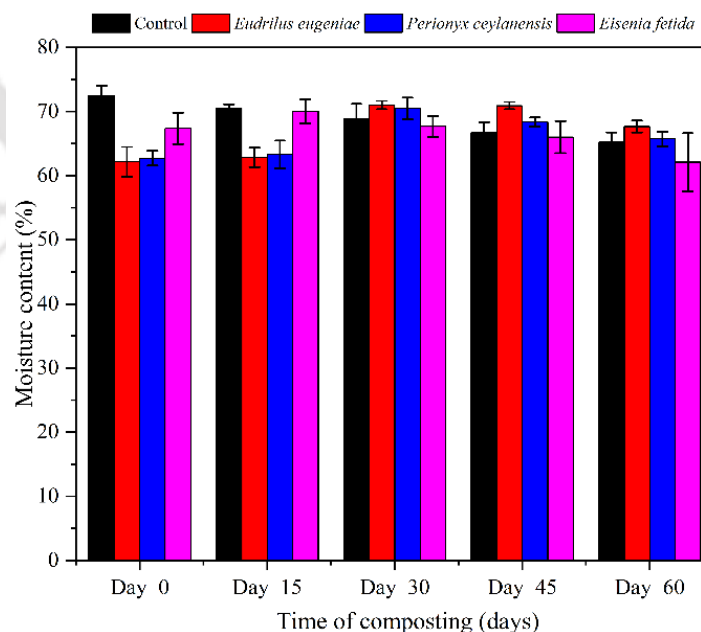


Fig. 5.9. Variation in Moisture content during VC process

Regular application of water is important to maintain the reactors at lower temperatures. Therefore, the moisture content was preserved between 60-70% during the entire vermicomposting process, as shown in Fig 5.9. Too humid conditions can cause pores in the reactors to be blocked, leading to less oxygen supply for the earthworms. Significant variation of moisture content was observed during the vermicomposting process ($p < 0.05$) and with distinct vermireactors ($p < 0.05$).

5.3.1.2 pH and Electrical Conductivity (EC)

Earthworms are especially susceptible to pH; therefore, soil and waste pH is also a limiting factor in the performance and number of earthworms. The pH range from acidic to basic highly depends on the substrate material, which further attributes to the production of organic matter and ammonia. However, in this study, pH was initially found to be increased on day 30th and then abstained until the end of the treatment. A slight decrease of pH is found in all the reactors though it is found in the optimum range is illustrated in Fig. 5.10, which may be due to the bioconversion of organic substance to organic acids and transitional compounds during vermicomposting (Bhat et al., 2015). The final pH was found to be 6.97, 7.21, 7.11, and 7.07 for the reactor V_C , V_{EF} , V_{EE} , and V_{PC} , respectively, which is in the neutral range (Devi and khwairakpam, 2020) except for reactor V_C . In the meantime, earthworms keep the pH within a neutral range by secreting Ca and NH_3 through the intestine to neutralize carboxy and phenolic groups of humic acids during the VC period (Hu et al., 2021). pH identification is important throughout the process of VC since the sustainability of different earthworms varies with pH, and substrates with more acidic pH are not ideal for VC because basic pH has shown faster degradation (Khare et.al., 2005). The EC values of all experiments were seen to be decreased throughout the process of VC except for the reactor V_{PC} as shown in Fig. 5.10(b). The original EC values were 4.19, 4.13, 3.62 mS/cm and eventually decreased to 4.04, 3.58, 3.56 for the reactor V_C , V_{EF} , and V_{EE} , which may be due to mineral absorption in the reactors containing earthworms (Singh and Kaur, 2015). While for the reactor V_{PC} , a marginal EC rise was seen from 3.33 to 3.53 mS/cm. The overall EC values obtained in the reactors inoculated with earthworm did not reach the threshold of 4 dSm⁻¹ assumed to be adequate for VC to be used for agricultural practice (Lasaridi et.al., 2006). As reported by (Saez et al., 2021) the decrease in EC may be due to ammonia volatilization and the accumulation of insoluble salts. Majlessi et.al. (2012) explained that EC measures soluble ion concentration or compost salinity. The authors further reported that the mineralization of nitrogen and the development of organic acids might also develop the compost's salinity. Plant phytotoxicity is based on the high

salinity of the compost, which is why it is highly advised that the EC compost does not surpass 4 dSm^{-1} , which is satisfied by the results obtained. Significant variations of pH and EC have been observed throughout the vermicomposting process ($p < 0.05$).

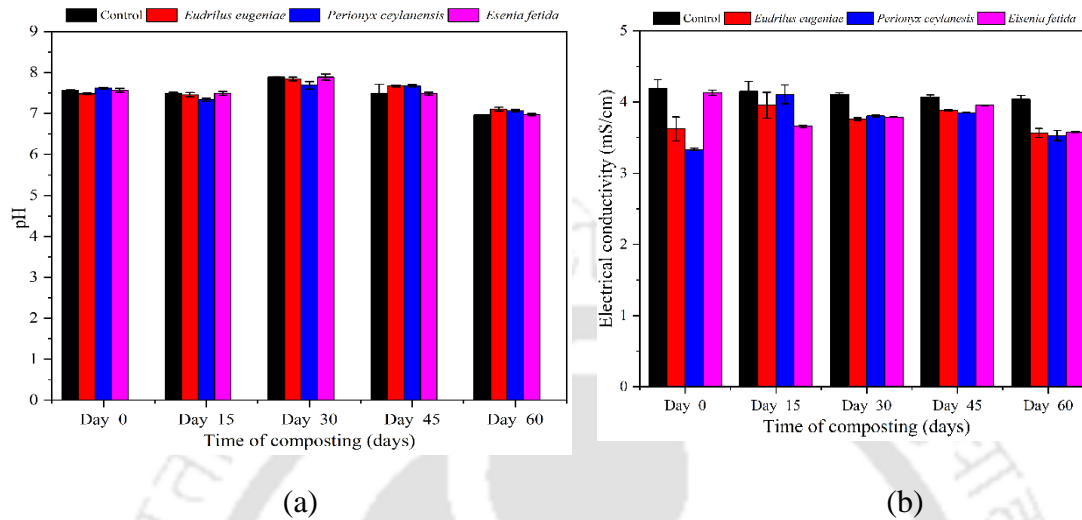


Fig. 5.10. Variation in a) pH b) Electrical conductivity during VC process

5.3.1.3 Volatile solids, Ash content, and Total organic carbon

Organic matter consists of easily accessible substances that the composting microbiota can use automatically, and polymeric organic compounds are required to be treated enzymatically so that microorganisms will use them as sources of carbon and nitrogen (Jurado et. al., 2015). Researchers have further reported that organic matter decomposition is entirely a biological process by which various microorganisms use organic substrate molecules as nutrients at various aerobic processes. Many volatile solids are carbonaceous forms, where the TOC directly interacts with the volatile solids. The portion of the solid that was left behind is the ash content after the volatile solids vaporize. With composting period, the content of volatile solids declined. The reduction was mainly seen within the first 15 days when more food was available for the worms, and the reduction steadily stabilized between 45 and 60 days of vermicomposting. The overall reduction was 16.52 % for V_C , 51.89% for V_{EF} , 46.75% for V_{EE} , and 40.70% for V_{PC} . VS losses were evident throughout the process of vermicomposting in all the reactors, as shown in Fig. 5.11. The declination rate continues as the vermicomposting process advances in the reactors steadily with the absorption of usable carbon supplies and the substrate's stabilization.

TOC was found to be decreasing in all the reactors from 38.03, 40.74, 39.81, and 39.22 to 31.74, 19.60, 21.20, and 23.25% for V_C , V_{EF} , V_{EE} , and V_{PC} , respectively, throughout the

process of VC as depicted in Fig. 5.11. TOC is mainly reduced due to carbon reduction done by microbial metabolism and respiration that comes out in the form of CO₂ and the equalization of organic matter by earthworms (Vig et al., 2011; Hait and Tare, 2011). TOC reduction was observed more in inoculated worm reactors than in the control reactor. The highest reduction was found in the reactor V_{EF}, followed by V_{EE} and V_{PC}. The observed findings are confirmed by various authors who have also reported a carbon loss of 20-45% in CO₂ throughout the VC process (Elvira et al., 1998). Bernal et al. (2009) reported that vermicomposting or composting allows the organic substrate to be partly mineralized, contributing to carbon losses in the process. A significant prognostic factor for the decomposition and mineralization of the vermicompost material is the ash content (Khwairakpam and Bhargava, 2009). In all the reactors, Ash content increased throughout the vermicomposting process Fig. 5.11.

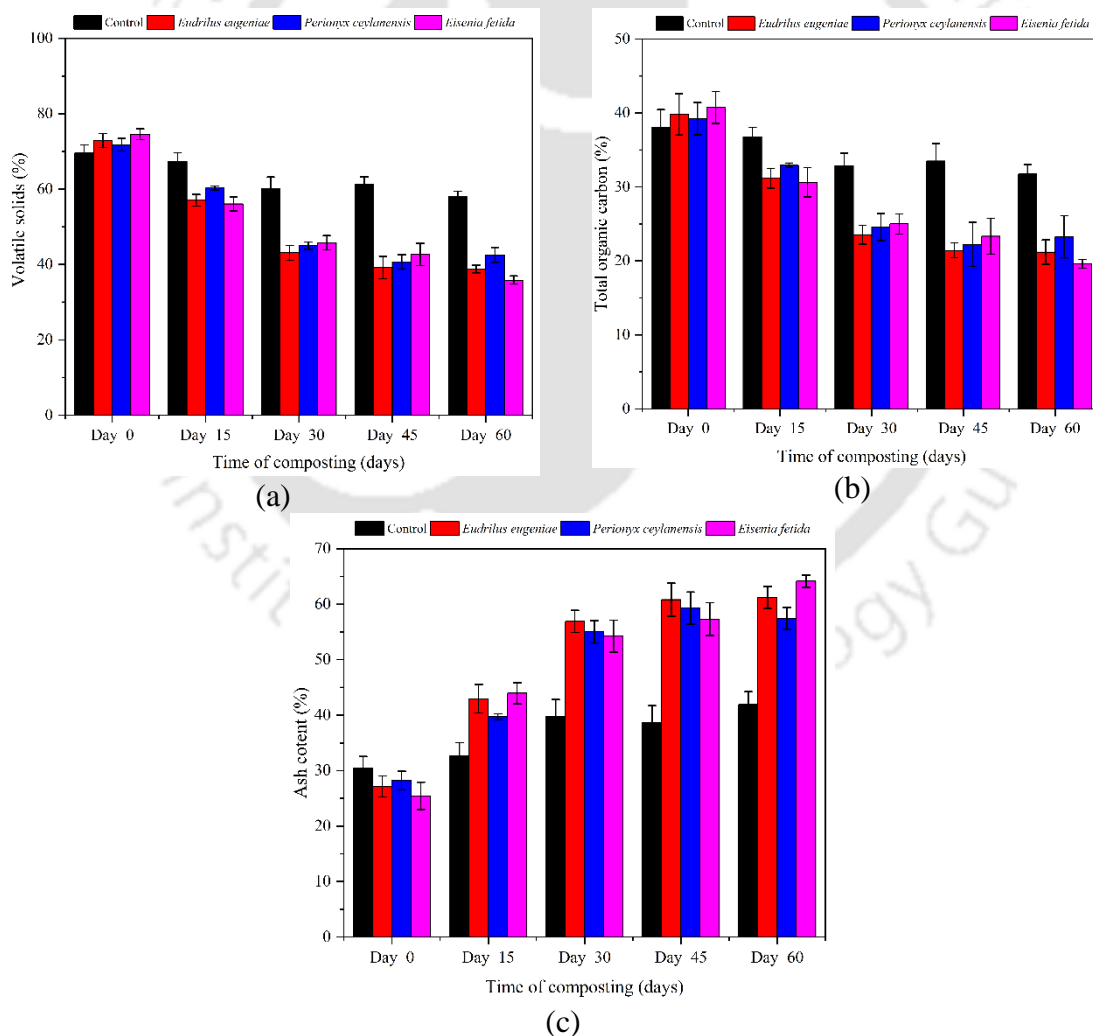


Fig. 5.11. Variation in a) Volatile solids b) Total organic carbon (TOC) c) Ash content during VC process

The increment was found in the range of 30.4-41.9% for V_C , 25.44-64.13% for V_{EF} , 27.133-61.2% for V_{EE} , and 28.22-57.44 V_{PC} . Higher final ash content was observed in the earthworm inoculated reactors, which can be due to the higher volatilization rate and organic matter mineralization in the presence of earthworms (Gupta and Garg, 2008). VS, TOC, and Ash content varied during the process of vermicomposting ($p < 0.05$) and in different vermireactors ($p < 0.05$).

5.3.1.4 Total Kjeldahl Nitrogen and Ammoniacal Nitrogen

Initial TKN content was found to be 1.14, 1.21, 1.07, and 1.23% for V_C , V_{EF} , V_{EE} , and V_{PC} , respectively, which was later increased to 1.4, 3.08, 2.87, and 1.75% during 60 days of the vermicomposting process as illustrated in Fig. 5.12. This rise in nitrogen content during VC has been stated, referring to the secretion of excretory compounds, such as mucus, nitrogenous excretory compounds, growth-stimulating hormones, and enzymes, by earthworms during the process (Tripathi and Bhardwaj, 2004). TKN was observed to increase significantly at the end of the vermicomposting phase in all the reactors except for control which shows a stagnant increase throughout the process. Due to the use of substrates by microbes and worms and their biochemical cycles and the loss of water by evaporation during the mineralization of organic matter, the reduction of dry matter may have led to a relative increase in nitrogen (Viel et al., 1987).

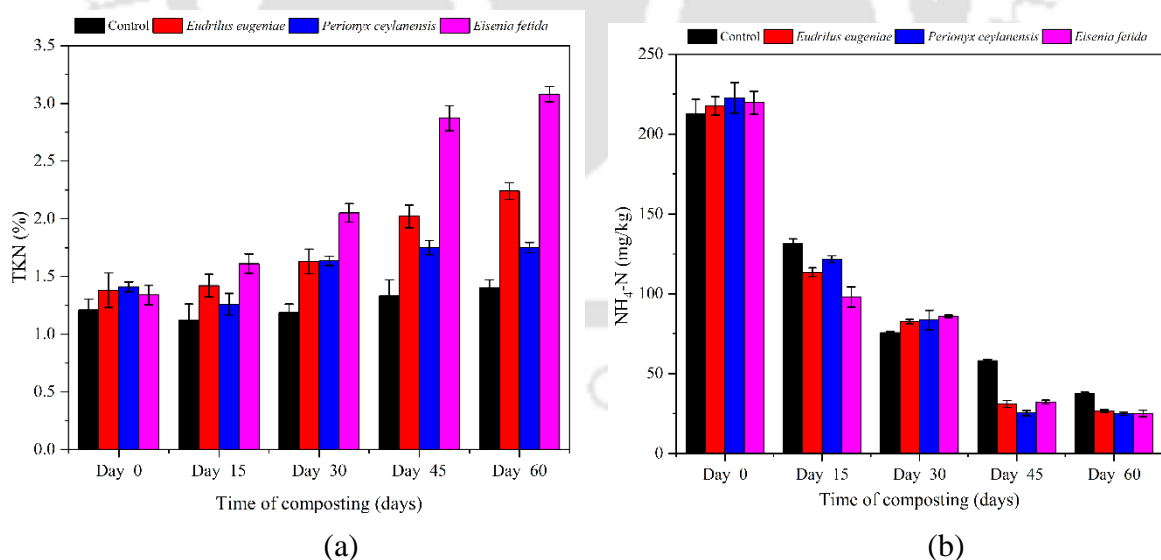


Fig. 5.12. Variation in a) Total Kjeldahl nitrogen b) Ammoniacal Nitrogen during VC process

The vermireactor V_{EF} provided the highest TKN content of 3.24%, followed by V_{EE} with 2.87%. This may be attributed to nitrogen enrichment by adding microbial-induced

nitrogen from mucus and nitrogen waste from earthworms (Khwairakpam and Bhargava, 2009). Many studies have reported the improvement of sufficient amount of nutrients of compost and vermicompost produced from weed biomass (Ananthavalli et al., 2019). It is also stated that the quality of VC and its nutrient content depends highly on the type of biomass or substrate used (Soobhany et al., 2015).

$\text{NH}_4^+\text{-N}$ decreased at the end of the process with better oxidation of organic matter and organic nitrogen mineralization. Initial ammonia nitrogen was found to be 212.84, 219.67, 217.64, and 222.65 mg/kg for V_C , V_{EF} , V_{EE} , and V_{PC} , respectively, and was finally found to be reduced to 37.74, 24.98, 26.68, and 24.86 mg/kg at the end of 60 days as shown in Fig. 5.12. Lv et al. (2018) stated that when organic nitrogen is transformed into ammonia and then oxidized into nitrate by nitrifying bacteria in the presence of oxygen, $\text{NH}_4^+\text{-N}$ is produced. $\text{NH}_4\text{-N}$ emission during the VC process was observed after 15 days of the process. There have already been studies of reductions in $\text{NH}_4\text{-N}$ content during VC (Hobson et al., 2005). During composting, the volatilization of ammonia has been reported to be mainly influenced by factors such as initial ammonia concentrations, moisture content, and pH (Pagans et al., 2006). Reduction of $\text{NH}_4^+\text{-N}$ was found preferably lower in all the reactors at the end of the vermicomposting process. During the 60 days of vermicomposting process of *M. micrantha*, the presence of nitrate was not observed. Prominent variation in TKN and $\text{NH}_4^+\text{-N}$ was observed in different days of vermicomposting ($p < 0.05$) and different reactors ($p < 0.05$)

5.3.1.5 Total phosphorus (TP) and Available phosphorus (AP)

TP was found to increase throughout the vermicomposting process, as illustrated in Fig 5.13. The initial TP was 8.23, 7.13, 7.07, and 6.15 g/kg for the reactors V_C , V_{EF} , V_{EE} , and V_{PC} , whereas the initial AP in all the reactors was found to be 1.98, 2.29, 2.31, and 2.05 g/kg. Pramanik et al. (2007) stated that acid production during the decomposition of organic matter by microorganisms is primarily responsible for the solubilization of insoluble phosphorus, which subsequently increases AP in vermicompost. During composting and vermicomposting, various forms of microorganisms were documented to the role of phosphorous solubilization at different pH (Hameeda et al., 2006). Specifically, phosphate solubilizing bacteria are reported to convert insoluble phosphates into soluble forms through the mechanism of acidification, chelation, exchange of reactions, and gluconic acid development (Rodriguez and Fraga, 1999). At the end of 60 days, the final TP was found to be 9.66, 13.24, 13.59, and 13.52 for V_C , V_{EF} , V_{EE} , and V_{PC} , respectively, while the final AP was found to be 2.67, 3.78, 3.25, and 2.86 g/kg, which maybe because as organic waste

passes through the gut of the earthworm, phosphorous is transferred to usable forms, therefore final content increase (Lee, 1992). Significant variation of TP and AP was observed during the process of vermicomposting ($p < 0.05$) among different reactors ($p < 0.05$).

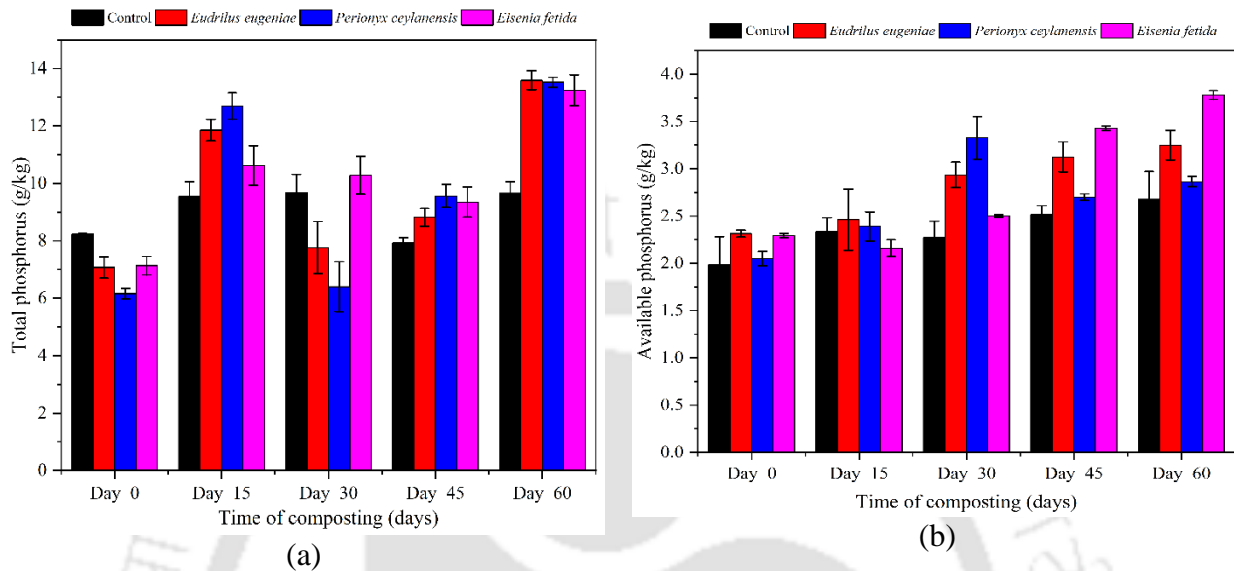


Fig. 5.13. Variation in a) Total phosphorus b) Available phosphorus during VC process

5.3.1.6 Macronutrients (Sodium, Potassium, and Calcium)

Potassium (K) content has been shown a significant rise in all reactors. The K concentration was found to be increased from 28.47, 28.9, and 29.20 g/kg to 33.25, 27.38, and 24.49 for V_{EF} , V_{EE} , and V_{PC} , respectively, as shown in Fig. 5.14, which is attributed to the physical degradation of organic waste by earthworm's enzymatic activity and gut microorganism (Bhattacharya and Kim 2016). The concentration of Na was found to increase for the reactors V_{EF} , V_{EE} , and V_{PC} from 2.12, 2.15, and 2.12 g/kg to 4.12, 3.41, and 2.98 at the end of 60 days. There was an improvement in the concentration of calcium in all the reactors. Calcium concentrations rose from 3.47, 4.67, 3.9, and 4.02 g/kg to 4.27, 6.45, 4.47, and 4.61 g/kg control at the end of 60 days, respectively.

The increase in macronutrients can be due to the impact of pH change and the association between microbial and organic materials in the intestine of the earthworm. In order to increase their availability, metals bind to ions and other carbonates, as explained by Suthar (2009). Increased calcium content can be due to the metabolism of calcium by the earthworm digestive tract that added calcium carbonate (segregated by the calciferous glands of the earthworms) during vermicomposting (Spiers et al., 1986). Significant variation of nutrients (K, Ca, and Na) was observed during the vermicomposting process

($p < 0.05$) among different vermicreators ($p < 0.05$).

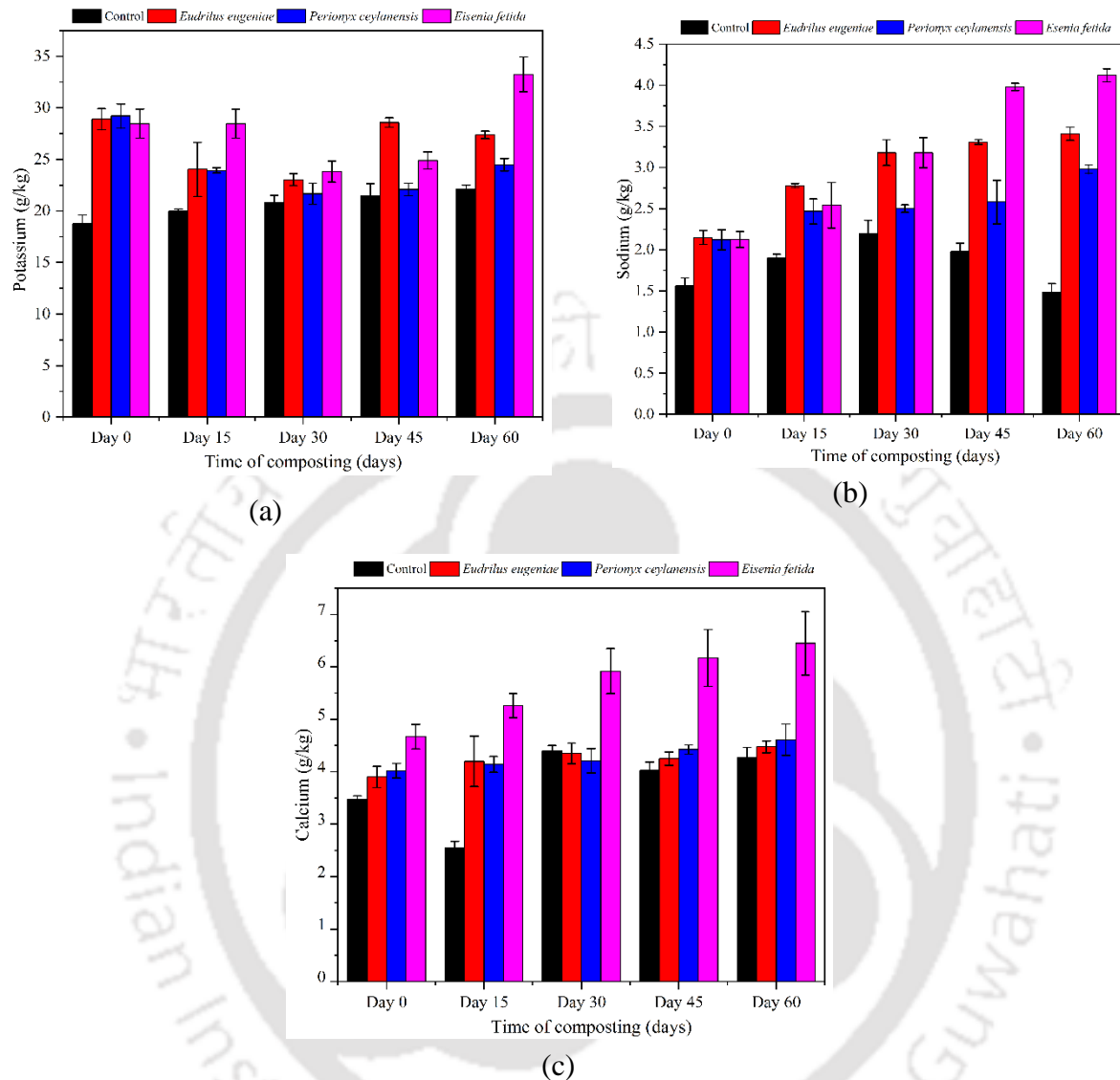


Fig. 5.14. Variation in a) Potassium b) Sodium and c) Calcium during VC process

5.3.1.7 C/N ratio

The C/N ratio is a strong organic stability factor in the process of composting and vermicomposting. In all the studies, the C/N ratios decreased from 30.17 to 6.04 for the reactor V_{EF} , 31.45 to 7.38 for V_{EE} , 31.88 to 13.28 for V_{PC} 33.36 to 22.67 for V_C . The reduction of the C/N ratio is due to the increase in TKN percentage and decrease in the TOC content of the reactors. A reduction in C/N ratio >20 suggests an advanced organic material stabilization stage, representing an appropriate degree of organic waste maturity. However, researchers have proposed different ideal C/N ratios ranging from <12 to <25 , but even the optimum amount depends on the original raw material (Majlessi et al., 2012).

The reduction in the C/N ratio can also rise as the earthworm population increases as a function of time (Ndegwa and Thompson, 2000), which led to a rapid decrease in organic carbon due to increased oxidation of organic matter. The release of carbon dioxide (CO₂) in the respiration process, mucus, and N excrements production increases N levels and lowers the C/N ratios. Composts with a high C/N ratio can cause soil alteration and immobilize nitrogen (Hoitink and Boehm, 1999), and those with a low C/N ratio can cause ammonium toxicity (Epstein, 1997). The C/N ratio corresponds to the degree of maturity, and therefore a decrease in the C/N ratio implied an increase in the level of maturity of the vermicompost. The results showed that highly matured compost was produced in the reactors inoculated with earthworms.

5.3.2 Biological and stability parameters

5.3.2.1 sBOD and sCOD

The present analysis found that sBOD and sCOD had a decreasing trend in all the reactors, as shown in Fig. 5.15.

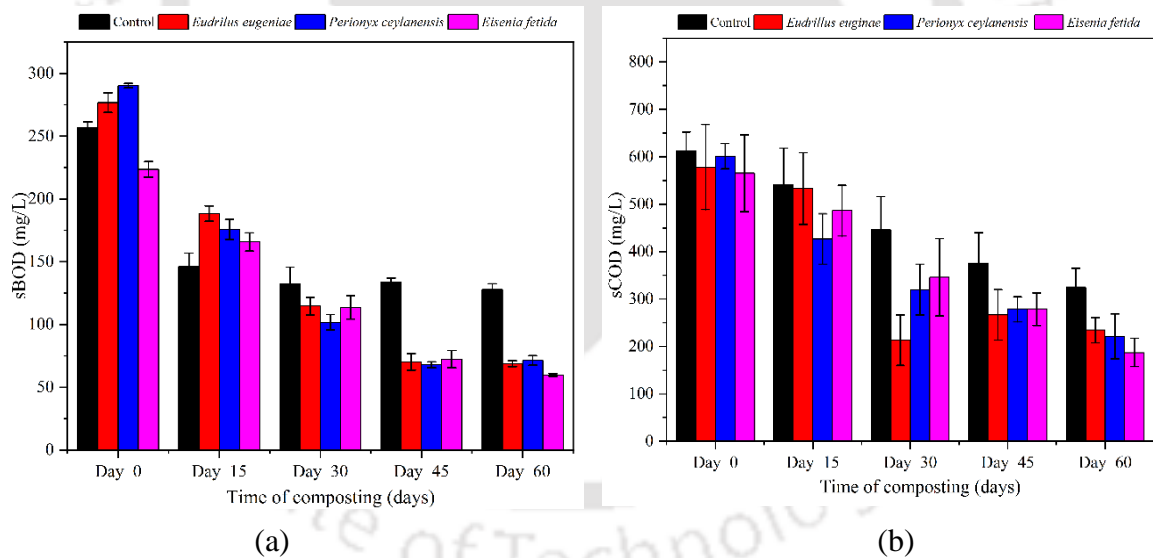


Fig. 5.15. Variation in a) sBOD b) sCOD during VC process

Maximum reduction of BOD was found in the reactor V_{EF} (from 223.65 to 59.8 mg/L), followed by V_{EE} (from 276.8 to 68.8 mg/L). The reduction pattern depends on the substrate used and earthworm species. Maximum reduction of sCOD was found in the reactor V_{EF} (from 565.4 to 187.4 mg/L) followed by reactor V_{PC} (from 601.2 to 221.45 mg/L). The V_C reactor had a poor decline in sBOD and sCOD, which can be due to the lack of readily available carbon, which subsequently slowed down the microbial activity and impacted the

degradation rate (Paul et al., 2020). The decrease of sBOD and sCOD depends on the degradation of organic matter during the vermicomposting process. Variation of sBOD and sCOD was observed among different vermireactors ($p < 0.05$) during the different periods of vermicomposting ($p < 0.05$).

5.3.2.2 OUR and CO₂ evolution

Composting is a natural process that proceeds until it is stabilized and mature, free of pollutants and odors. Compost stability can be inferred by the evolution of CO₂, where carbon extracted can be calculated directly from compost (Paul et al., 2020). The oxygen uptake rate (OUR) is the stability parameter that includes organic compounds that promote microbial respiration during the active process of the composting mixture. As a result, high oxygen intake rates induce instability (Said-Pullicino et al., 2007). Large organic molecules are broken down into smaller, soluble ones as composting begins, and more substrates may temporarily become usable. With composting period, microbial activity decreased, as demonstrated by a reduction in all trial values, as shown in Fig. 5.16. For both reactors, OUR was found to decrease by many folds. The percentage reduction in OUR for earthworm test species *E. fetida* was 92.67, followed by 91.3 for earthworm test species *P. ceylanensis*. For the earthworm species *E. eugeniae*, the percentage reduction was 87.73, and for the control reactor, where no earthworm was used, the percentage reduction was 73.57. The most direct technique for compost stability is the rate of evolution of CO₂ as it calculates carbon extracted directly from compost.

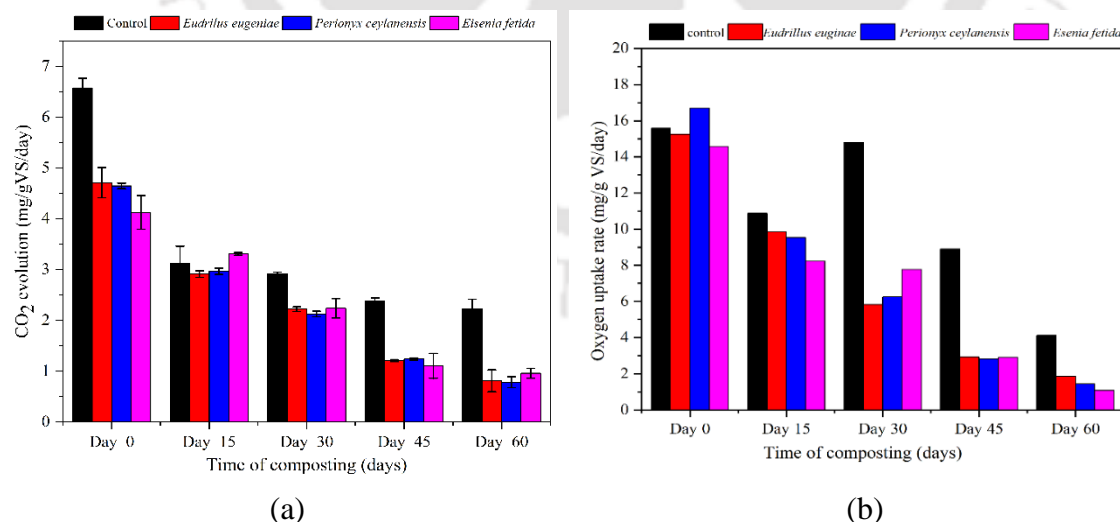


Fig. 5.16. Variation in a) CO₂ evolution b) Oxygen uptake rate during VC process

The highest decrease in the rate of respiration activity was observed during the initial phases of vermicomposting periods. The reduction trend for all the trials appeared to be

similar; however, the different substrate combinations likely affect the earthworm species, as seen from the results as shown in Fig. 5.16. The highest CO₂ reduction was observed in *P. ceylanensis* (83.21%), followed by *E. eugeniae* (82.84%), *E. fetida* (76.81%), and least in the control reactor (51.30%) at the end of the composting period. A lower CO₂ evolution rate indicates the stability of the end product of compost. Significant variation of CO₂ evolution was observed among different vermicoreactors (p<0.05) during the different periods of vermicomposting (p<0.05).

5.3.2.3 Estimation of total coliform and fecal coliform bacteria

Coliform bacteria's presence is often used as an indicator of the overall sanitary quality of soil and water environments. Le Minor (1984) reported that *E. coli* is the most representative bacterium in the group of fecal coliforms. It is reported that fecal coliform decreases during VC as they enter the earthworm's food chain (Khwaitrakpam and Bhargava, 2009). The average number of total coliform bacteria decreased from 7.5×10^8 to 5.5×10^3 MPN/g dry weight in V_C, while it decreased from 4.3×10^4 to 1.3×10^3 , 7.5×10^6 to 1.5×10^3 , and 9.3×10^2 to 3.3×10^2 MPN/g dry weight in V_{EF}, V_{EE}, and V_{PC}, respectively as shown in Table 5.3.

Table 5.3. MPN test for total and faecal coliform bacteria of VC process

Reactors	Proportion	Total coliforms			Fecal coliforms		
		0 day	30 day	60 day	0 day	30 day	60 day
V _C	5:4:1	7.5E+08	9.3E+03	5.5E+03	7.5E+04	9.3E+01	1.5E+04
V _{EF}	5:4:1	4.3E+04	2.3E+04	1.3E+03	3.3E+06	1.3E+03	1.5E+01
V _{EE}	5:4:1	7.5E+06	4.3E+04	1.5E+03	6.5E+02	2.3E+04	1.3E+03
V _{PC}	5:4:1	9.3E+02	4.3E+08	3.3E+02	4.5E+05	4.3E+01	2.3E+03

Similarly, fecal coliform decreased from 7.5×10^4 to 1.5×10^4 MPN/g dry weight in VC, while it decreased from 3.3×10^6 to 1.5×10^3 , 6.5×10^2 to 1.3×10^3 , and 4.5×10^5 to 2.3×10^3 MPN/g dry weight in V_{EF}, V_{EE}, and V_{PC}, respectively. There was a decreasing

trend in all the reactors during the vermicomposting process. The reduction was very less in control than the reactors inoculated with earthworms.

5.3.3 Growth and development of earthworm

The growth of earthworm biomass during the vermicomposting period is shown in Table 5.4. The European breed, *E. fetida*, has shown steady growth in composting. However, the highest growth was seen between day 15 and day 30. After day 60, the average final count of *E. fetida* was 204 adults, 117 juveniles, and 31 cocoons per 100 g of vermicompost. The steady rise in population by *E. fetida* suggests that the substrate mix used is favorable to the earthworm species, and biodegradation happened at a good pace. The African breed, *E. eugeniae* showed stable growth throughout the composting process. However, the growth was seen maximum between day 15 to day 30. After day 60, the average final count of *E. eugeniae* was 174 adults, 92 juveniles, and 2 cocoons per 100 g of vermicompost. The Indian cross breed, *P. ceylanensis* showed an unsteady growth throughout the composting process.

The growth seems to drop between day 15 to day 30. But again, the growth tends to increase after day 30. After day 60, the average final count of *P. ceylanensis* was 108 adults, 15 juveniles, and 8 cocoons per 100 g of vermicompost. Studies have shown that substrate form, digestibility, and quality strongly affect the sustainability, growth rate, and reproduction rate of earthworms (Tripathi and Bhardwaj, 2004). The difference between the development of biomass and cocoon in various vermireactors may be attributed to the feed's biochemical consistency, which was one of the key factors in deciding cocoon production initiation (Flack and Hartenstein, 1984). Overall, the earthworm species *E. fetida* proved to be the best because of the rise in biomass, and juveniles hatched relative to the other earthworm species.

Table 5.4. Growth and development of earthworm species during VC process

		DAYS				
		<i>Eisenia fetida</i> (V_{EF})				
Results	0	15	30	45	60	
Adults	120	129	157	185	204	
Juveniles	0	7	71	99	117	
Cocoons	0	10	11	24	31	
(Number per 100 g)						
		<i>Eudrilus euginae</i> (V_{EE})				
Adults	120	118	121	117	104	
Juveniles	0	5	70	88	92	

Cocoons (Number per 100 g)	0	4	5	3	2
<i>Perionyx ceylanensis</i> (V _{PC})					
Adults	120	112	83	97	108
Juveniles	0	3	4	6	15
Cocoons (Number per 100 g)	0	11	22	10	8

5.3.4 Spectroscopic analysis (X-ray diffraction)

X-ray diffraction is used to examine the crystallinity and amorphous nature of cellulose or lignin in the substrate (Arumugam et al., 2018).

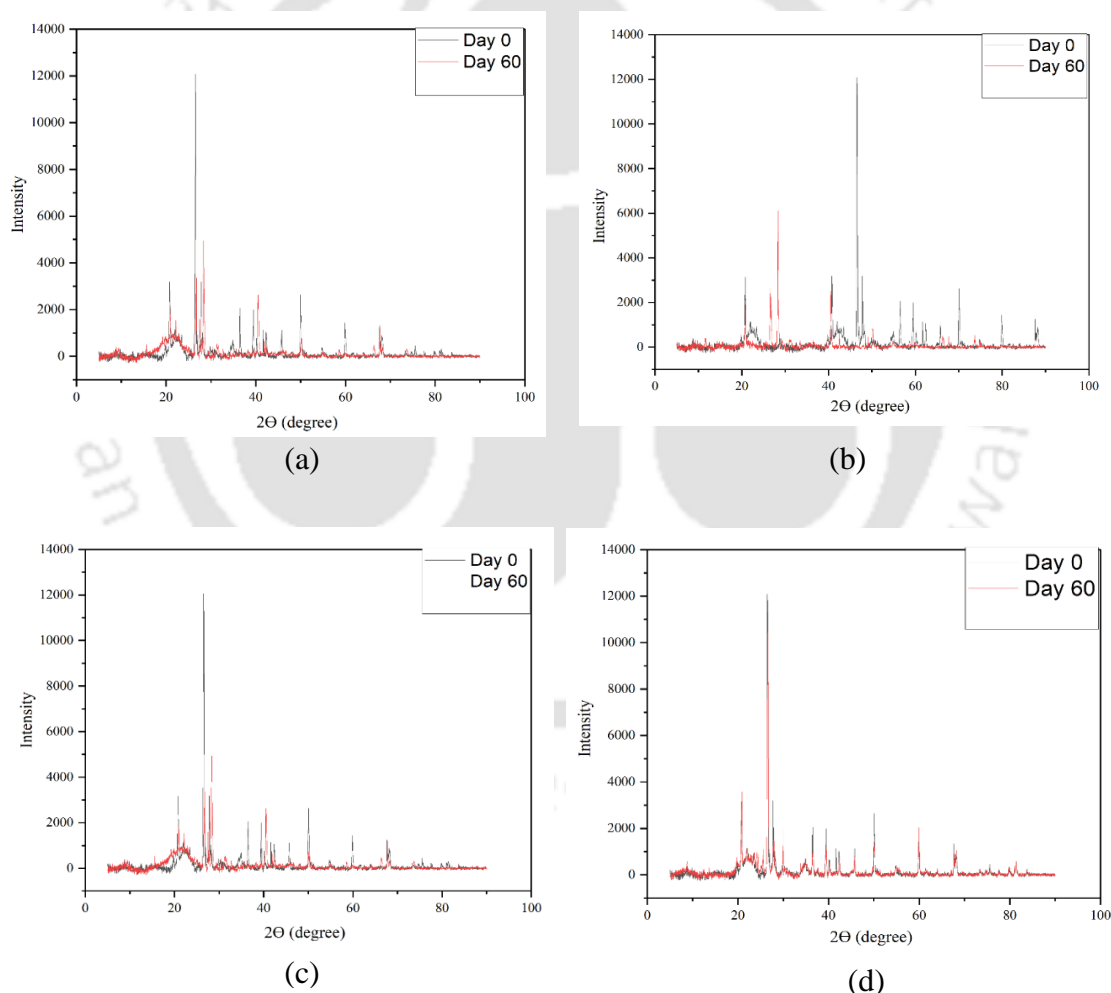


Fig. 5.17. XRD spectra for the dried vermicompost samples of a) *Esenia fetida* (V_{EF}) b) *Eudrilus euginae* (V_{EE}), c) *Perionyx ceylanensis* (V_{PC}), and d) control (V_C)

Sharp peaks depicted are usually used to identify crystalline substances. Sharp peaks between 20°-30° may be ascribed to hemicellulose, lignin xylans, and other non-

cellulosic polysaccharides that were witnessed in all of the reactors on Day 0 in this study (Li et al., 2020). According to Paul et al. (2020), the peaks appear sharp due to strong H-bonding between the compounds, but they may recede after the vermicomposting period, indicating H-bonding breakdown. After 60 days of vermicomposting, as shown in Fig. 5.17. Sharp peaks were seen to decline that may be attributed to the degradation of organic matter, resulting in a decline in the C/N ratio (Biyada et al., 2020). In comparison to the reactors V_{PC} and V_C , the reactors V_{EF} and V_{EE} produced almost identical results. According to XRD data, earthworms and microbes assisted in the breakdown of crystalline substances during the vermicomposting process (Ravindran et al., 2019).

5.4 BIOLOGICAL TREATMENT OF *MIKANIA MICRANTHA* KUNTH USING THE TWO-STAGE COMPOSTING TECHNIQUE TO REDUCE THE TIME PERIOD OF VERMICOMPOSTING

The novelty of the current study lies in reducing the time required for vermicomposting by incorporating an in-vessel compost technique for 10 days, followed by a 20-day vermicomposting process and obtaining nutritional ‘vermicast’ from it. This study aims to monitor various physicochemical and biological parameters during the Rotary followed by vermicomposting (RVC) process to compare its efficiency to that of traditional vermicomposting techniques in terms of nutrient quality. This study employs terrestrial weed biomass *Mikania micrantha* because no extensive research has been conducted on this weed biomass using a two-stage composting technique. Earthworms require more time for acclimatization than the actual process to happen. So a pre-degradation process is conducted in a 550L capacity Rotary drum composter to acquire a significant rise of temperature (thermophilic phase) and then to vermicompost for 20 days using bamboo bins.

5.4.1 Physico-chemical analysis

5.4.1.1 Temperature

Microorganisms play an important role in composting, whereas earthworms major in vermicomposting. Vermicomposting occurs at room temperature instead of composting, which has a thermophilic phase that ensures material sanitation and maximum degradation (Fornes et al., 2012). In the present experimental setup, a temperature profile was recorded to evaluate the completion of the thermophilic stage. The maximum temperature reached during this stage was 54.3°C on day 2 in the reactor R1, as shown in Fig. 5.18. The thermophilic stage was almost over by day 6. But the rotary drum composting was continued till day 10 to ensure further degradation of the organic compounds and obtain

the optimum temperature required for the earthworms.

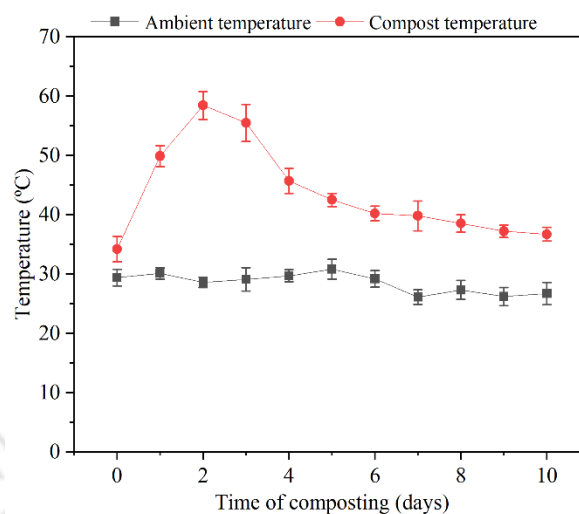


Fig. 5.18. Variation in temperature profile during rotary drum composting

The temperature begins to drop after the fourth day of composting, and on the tenth day, it was measured at 37-38°C, which is advisable for the vermicomposting process (Reinecke and Kriel, 1981). The sudden temperature increase is mainly due to the (exothermic reaction) heat released during the composting process and major losses of the components like lipids, sugar, and starches mainly done by extremophiles thermophilic bacteria. The rate of oxidative decomposition of various waste is greatest during composting in the thermophilic phase of temperature range 50-60°C (Belyaeva and Haynes, 2009), which may shorten the time required for vermicomposting as maximum reduction occurs during this phase and earthworm gets degraded material which is more useful for making better quality vermicompost. Microorganisms play an important role in composting, whereas earthworms major in vermicomposting. Vermicomposting occurs at room temperature instead of composting, which has a thermophilic phase that ensures material sanitation and maximum degradation. In the present experimental setup, a temperature profile was recorded to evaluate the completion of the thermophilic stage. During this stage, the maximum temperature reached was 54.3°C on day 2 in reactor R1. The thermophilic stage was almost over by day 6. But the rotary drum composting was continued till day 10 to ensure further degradation of the organic compounds and obtain the optimum temperature required for the earthworms. The temperature begins to drop after the fourth day of composting, and on the tenth day, it was measured at 37-38°C, which is advisable for the vermicomposting process. The rate of oxidative decomposition of various waste is greatest

during composting in the thermophilic phase of temperature range 50-60°C (Varma and Kalamdhad, 2015), which may reduce the time required for vermicomposting as maximum reduction occurs during this phase and earthworm gets pre-degraded material, which is more useful for making better quality vermicompost. Similar studies have been studied by Vicentine et al., (2021), where a combination of composting technology has been used to stabilize cattle manure.

5.4.1.2 Moisture content

The reactors were splashed with water every day as the moisture content of at least 60% was maintained throughout the whole process. The moisture content at the end of rotary drum composting was below 70% as shown in Fig. 5.19.

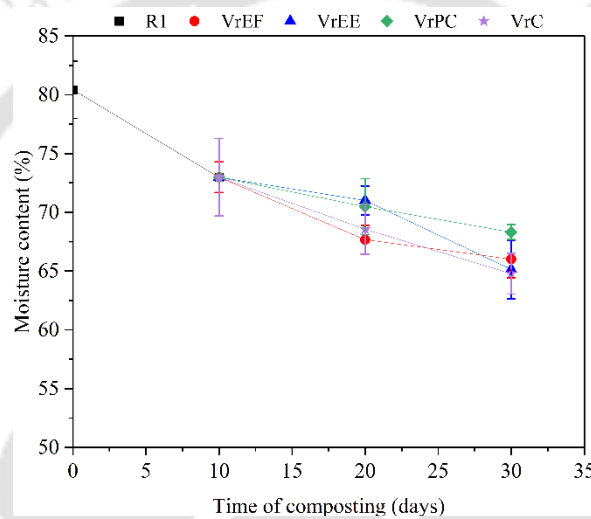


Fig. 5.19. Variation in moisture content during RVC process

The worms prefer a moist environment. And also the ambient temperature was at around 30-35°C which can be harmful for the worms. In order to keep the reactors at lower temperatures regular application of water is vital. Throughout the span of vermicomposting technique, the moisture content was maintained between 60-80%. Too wet conditions can lead to blocking of pores in the reactors leading to less oxygen supply for the worms.

5.4.1.3 pH and EC

The substrate used in the vermicomposting process significantly impacts pH variation. Deviation of pH towards acidic and alkaline conditions during the process is responsible for the production of acids and NH₃ based on the raw material used (Varma et al., 2015). In reactor R1, pH rose from 7.20 to 7.90 during the first 10 days. The final pH value was in the range of 7.60, 7.99, 7.94, and 7.97 for VrEF, VrEE, VrPC, and VrC, respectively, as shown in Fig. 5.20. The pH values during the vermicomposting had a decreasing trend.

Sharma and Garg (2020) achieved a comparable neutral pH value while vermicomposting *Parthenium* plants after 90 days of vermicomposting (pre-degradation for 3 weeks). The author reported that pH between 7-7.5 is mostly obtained due to the formation of PO_4^{3-} , NO_3^- and CO_2 emissions. Also, it was reported that the condition is mainly gained due to various compounds secreted by earthworms that play a significant role in neutralizing the $\text{R}-\text{CO}_2\text{H}$ group and $\text{C}_6\text{H}_5\text{OH}$ group. pH in all the reactors was in the range of acceptable range of 6.5 - 8.0 (Zhang and Sun, 2015). The initial EC values were found to be 5.42 mS/cm, which was reduced to 5.08 mS/cm in the reactor R1, whereas during vermicomposting, the final values were reduced to 3.21, 3.78, 3.45, and 4.65 mS/cm in the reactor VrEF, VrEE, VrPC, VrC as shown in Fig. 5.20(b). The EC values of all the samples after 30 days were lower than the initial days due to organic acid decomposition leading to higher pH and precipitation of soluble salts. At $p < 0.05$, pH and EC differed significantly between tests during the RVC process. In addition to pH, EC is an important parameter when compost toxicity is seen, as EC above 4 ds/m for soil application is not recommended (Karak et al., 2013).

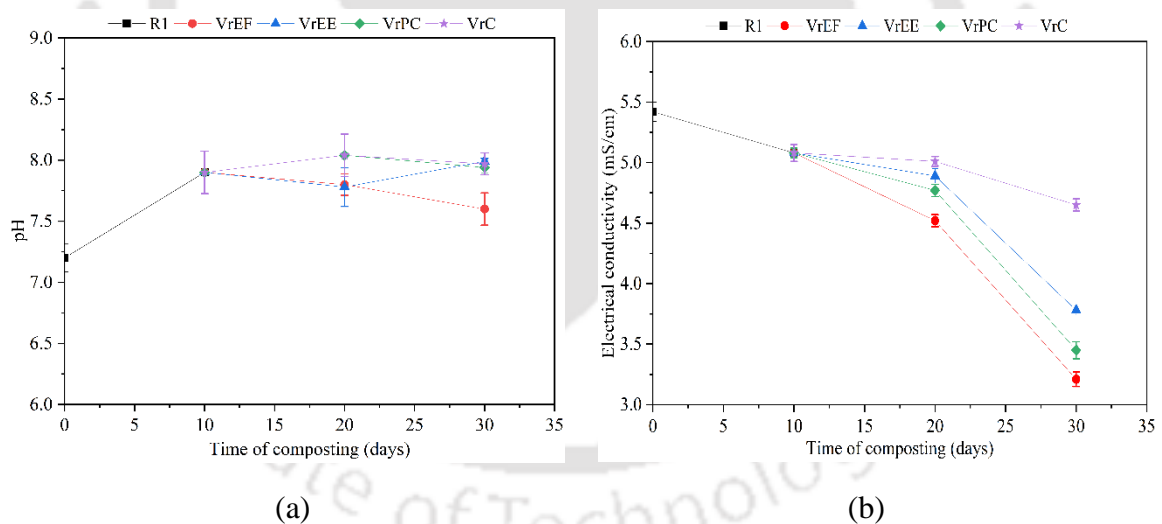


Fig. 5.20. Variation in a) pH and b) Electrical conductivity during RVC process

5.4.1.4 Volatile solids (VS), total organic carbon (TOC), and ash content

Volatile Solids normally represent the amount of organic matter in wastes as shown in Fig. 5.21. The greater the concentration of volatile solids represents organic matter. The TOC directly relates with the volatile solids as much of the volatile solids are carbonaceous. After the volatile solids vaporize, the fraction of solid left behind is the ash content.

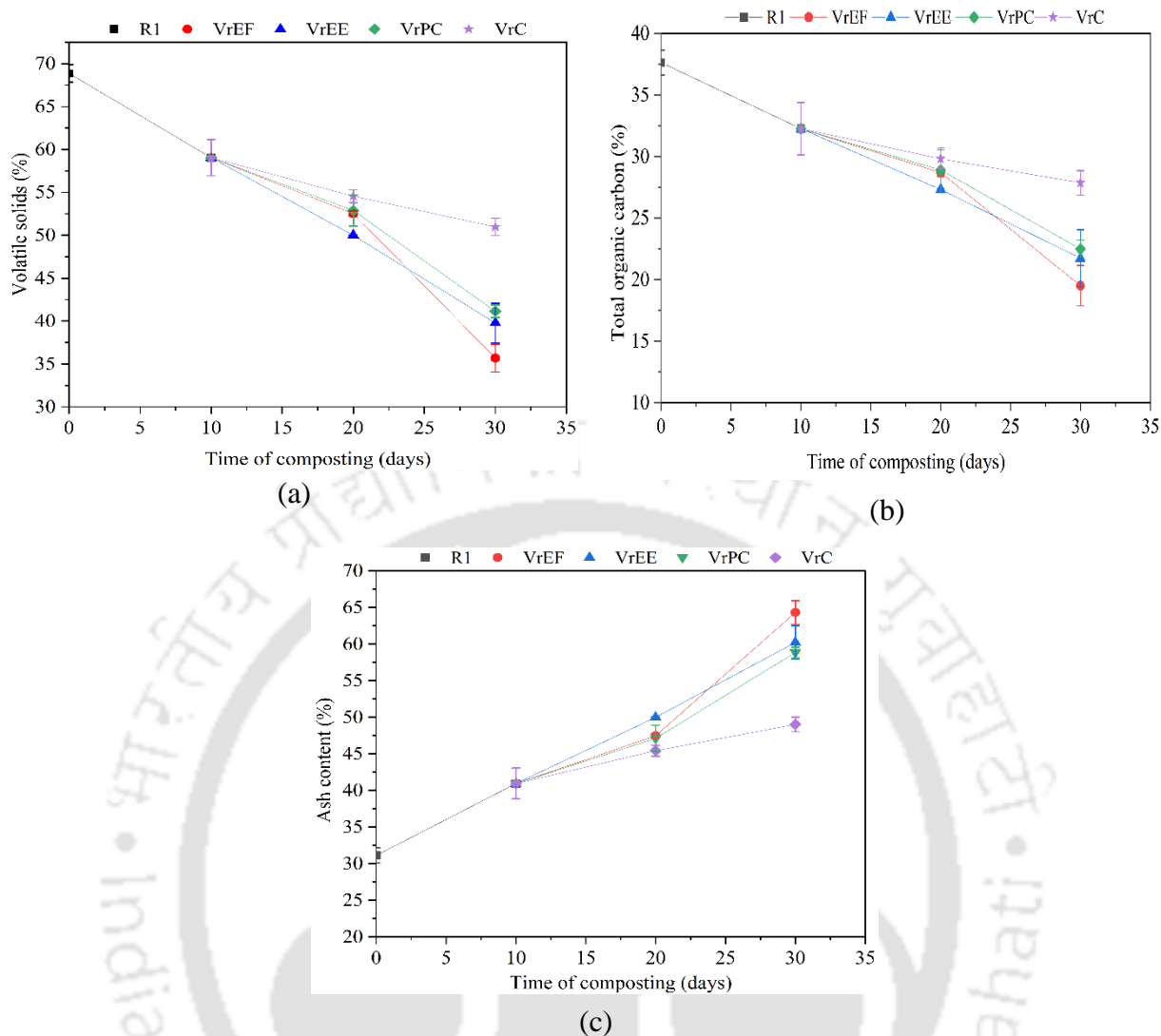


Fig. 5.21. Variation in a) Volatile solids and b) Total organic carbon and c) Ash content during RVC process

The VS reduction was observed to be 59.04% after 10 days of rotary drum composting, and it was further observed that after vermicomposting, it was reduced to 35.69% for the reactor VrEF, 39.78% for VrEE, 41.15% for VrPC, and 50.98% for VrC. Microorganisms usually utilize substrate for the building of cells (anabolic reaction) and the production of energy (catabolic reaction).

Throughout the vermicomposting process, reduction of TOC was observed due to the degradation of organic matter by earthworms and microorganisms in all the reactors. Earthworms contain a variety of enzymes such as proteases, lipases, cellulases, amylases, alkaline phosphatases, dehydrogenases, and ureases that contribute to the breakdowns of organic compounds (Usmani et.al., 2019). In reactor R1, initial TOC was observed to be 37.6% that has further declined to 32.27% at the end of 10 days, having a thermophilic

phase of almost 4 days. Reduction of TOC for the reactor VrEF, VrEE, VrPC, and VrC was recorded as 28.69, 27.32, 28.89, and 29.81%, respectively, during the first 10 days of vermicomposting. At the end of the vermicomposting process, TOC reduction was observed as 19.50, 21.73, 22.48, and 27.85% for the reactor VrEF, VrEE, VrPC, and VrC, respectively. Devi and Khwairakpam (2020) reported a 38.5% reduction in TOC after 45 days of vermicomposting with the invasive weed *A. conyzoides*. Ash content is inversely related to the volatile solid content, and it increases throughout the composting process. So the reactors VrEF had the highest ash content after 30 days of rotary-vermicomposting followed by VrEE, VrPC, and VrC.

5.4.1.5 Nitrogen profile

Compost quality is determined in part by the amount of nutrients present. Reactor VrEF, VrEE, VrPC had significantly higher nutrient contents ($p < 0.05$). TKN content was increasing throughout the process of vermicomposting process. During the first 10 days of rotary drum composting, a slight increase of TKN was observed due to the reduction of substrate. After 10 days of rotary drum compost, the partially degraded substrate was used for vermicomposting for almost 20 days. At the end of the first 10 days of vermicomposting, TKN was recorded as 2.54, 2.03, 1.91, 1.59% for the reactor VrEF, VrEE, VrPC, and VrC, respectively, as shown in Fig. 5.22. It was seen that after the next 10 days of vermicomposting, it increased to 3.24, 2.89, 3.02, and 1.79%. The current study attained 3.24% (reactor VrEF) of TKN within 30 days of the vermicomposting process. Total nitrogen increased during the first ten days, which was due to a decrease in the carbon content of the substrate as a result of CO_2 loss, as well as the action of azotobacteria that fix nitrogen from the atmosphere (Zorpas et al., 2003). An increase of TKN was also observed due to earthworm activity that inclines the nitrogen production through microbially mediated nitrogen transformation by addition of mucus and nitrogenous substances by earthworm (Gunadi et al., 2002). The author further reported that nitrogen is abundant in amino acids, which are the basic components of proteins and nucleic acids. Ammonia, which can be procured from the breakdown of protein in the compost, is used to meet the microbial need for nitrogen, mostly done by hydrolyzing enzyme protease. Nitrogen is then retrieved from the carbon chain as $\text{NH}_4^+\text{-N}$, which is volatilized and released into the atmosphere (Ginkel, 1996). *Mikania*, cow dung, and sawdust is good source of protein that was degraded by help of earthworms and microorganisms during the process. Devi and Khwairakpam (2020) observed a similar trend of increase of TKN during vermicomposting of *Ageratum conyzoids* using *Esenia fetida*. Increment in TKN may also

be due to the loss of organic compounds as CO₂ and water loss through evaporation (Viel et al., 1987).

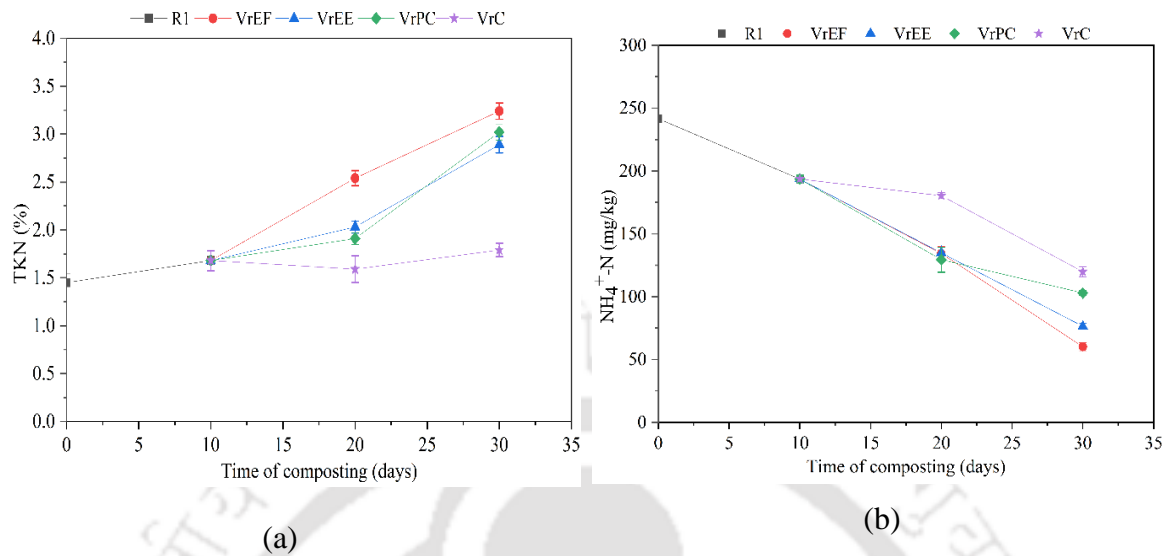


Fig. 5.22. Variation in a) TKN and b) NH₄⁺-N during RVC process

All the earthworm species have shown positive results on the degradation of organic compounds and increment of TKN throughout the process of vermicompost. Total nitrogen is typically influenced by proteolytic bacteria and the temperature where nitrogen is released into the atmosphere through volatilization (Wagner et al., 1990). The ammoniacal nitrogen was observed to decrease throughout the process. Initially, ammoniacal nitrogen decreased from 241.54 to 193.52 mg/kg during the rotary drum composting. Finally, it was observed to reduce to 60.22, 76.34, 50.40, and 119.75 mg/kg for VrEF, VrEE, VrPC, and VrC, respectively, at the end of 30 days.

5.4.1.6 Total and available phosphorus (TP and AP)

Mineralization of organic phosphorus increases TP during composting process Fig. 5.23. All the trials throughout the process observed an increase in TP concentration. Earthworms pass organic materials through their guts, which transform phosphorus into a form that plants can use (Lee, 1992). Earthworms benefit from the pre-degraded materials as they can easily digest them fast. The pre-degraded waste material has helped earthworms properly digest and acclimatization, eventually increasing the nutrient content. Similar findings have been reported, with the augmentation of TP and its mineralization during vermistabilization of organic substrates is being attributed to phosphatase and phytase activity by a gut microorganism of earthworm (Parthasarathi et al., 2016). Maximum TP was recorded for the reactor VrEF (12.87 g/kg) followed by VrEE (10.45 g/kg) and VrPC (9.45 g/kg). In the reactor VrC a gradual decrease of TP was observed, which may be due

to the leaching of the nutrient from the vermireactor. Since the substrate was pre-degraded before the vermicomposting process, cutting the vermicomposting time didn't affect the final product's nutrient content. AP was also observed to be highest in reactor VrEF (3.65 g/kg) followed by 2.94 g/kg for VrPC, 2.91 g/kg for VrEE, and 2.47 g/kg for VrC at the end of 30 days of the process.

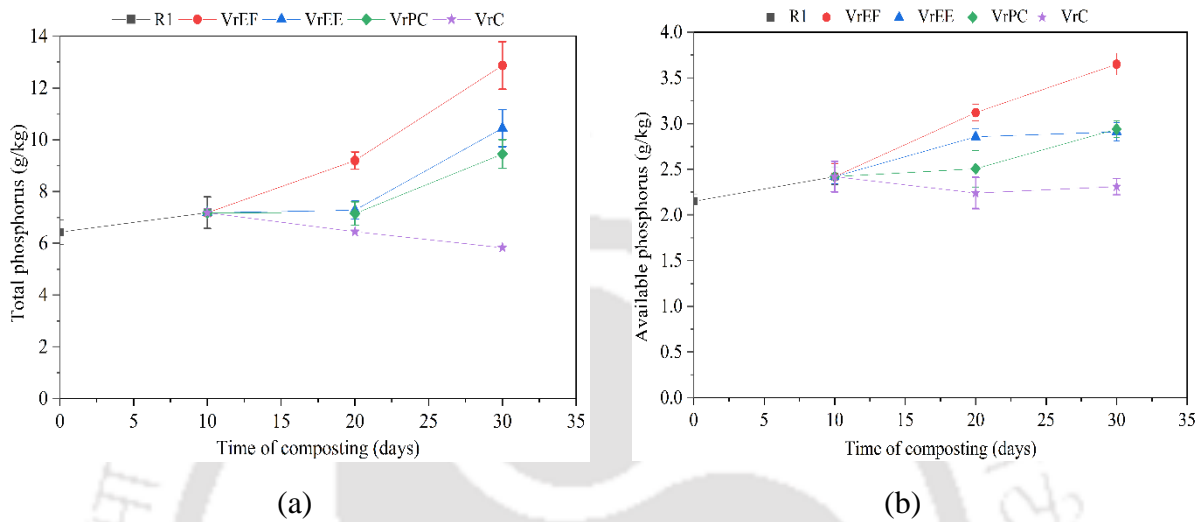


Fig. 5.23. Variation in a) Total phosphorus and b) Available phosphorus during RVC process

5.4.1.7 Macronutrients (Sodium, Potassium, and Calcium)

The concentration of Na increased from 1.53 to 2.02 g/kg during the rotary drum composting. The final concentration of Na after vermicomposting was 2.81, 2.34, 2.58, and 2.41 g/kg for VrEF, VrEE, VrPC, and VrC respectively at the end of the 30th day is illustrated in Fig. 5.24. K concentration was also found to be increasing throughout the process. K concentration increased from 19.89 to 20.72 g/kg during 10 days of rotary drum composting. During the vermicomposting phase, the value of potassium concentration increased to 26.78, 22.28, 24.65, and 22.14 g/kg in VrEF, VrEE, VrPC, and VrC respectively at the end of 30. The value of Ca concentration increased from 1.54 to 1.82 g/kg during the first 10 days of rotary drum composting. The concentration of Ca after the vermicomposting was found to be 3.16, 3.86, 3.45, and 2.45 g/kg for VrEF, VrEE, VrPC, and VrC respectively at the end of 30. The action of pH change and microbial activity on organic matter as it passes through the earthworm gut can be attributed to increased macronutrients (Kouba et al., 2018). Varma et al. (2016) also reported the rise of nutrient content during vermicomposting of the aquatic weed water hyacinth. Reactor VrEF has

demonstrated better results than other reactors in nutrient concentration. $p < 0.05$, nutrient content differed significantly between tests during the RVC process.

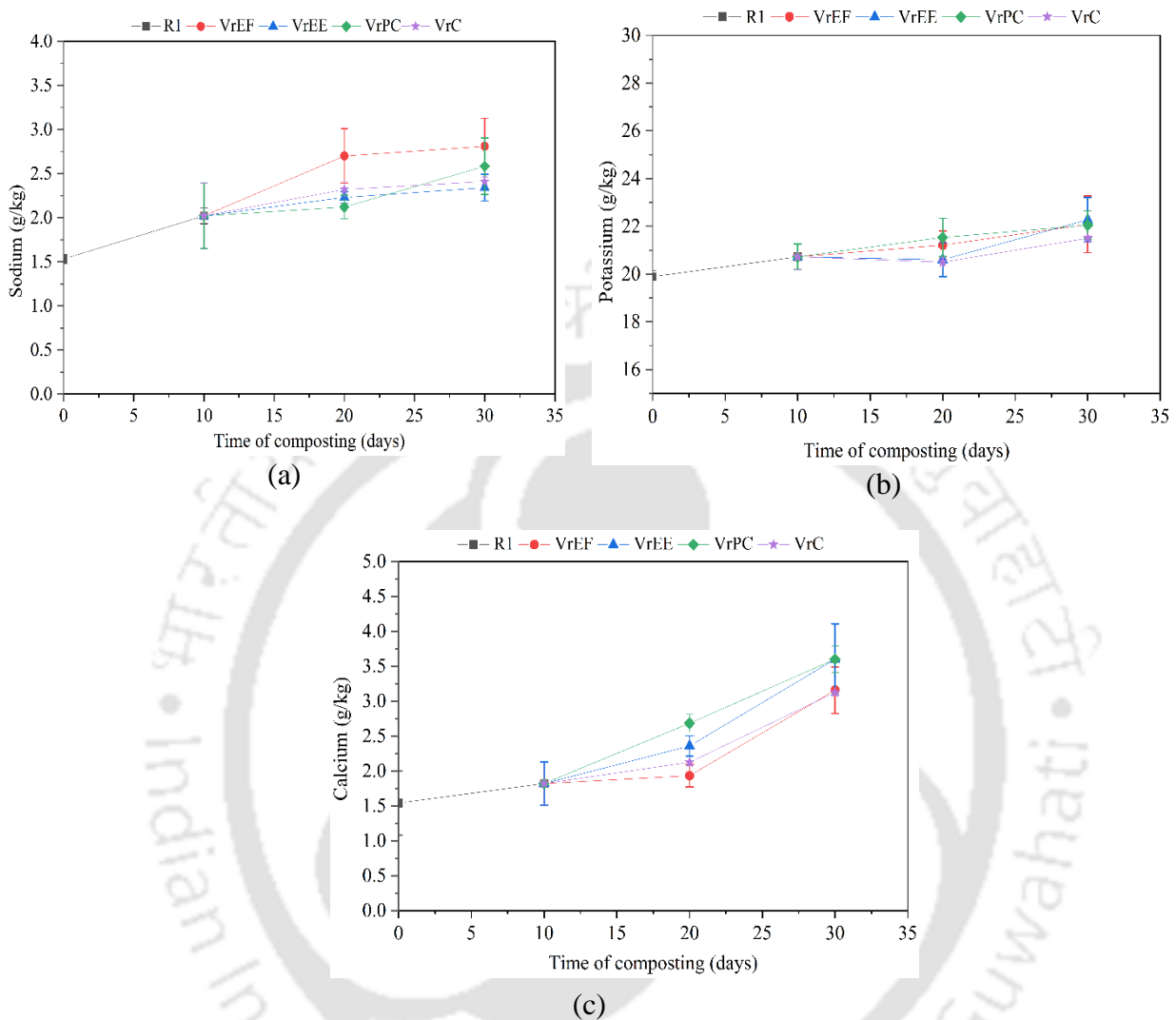


Fig. 5.24. Variation in a) Sodium, b) Potassium and c) Calcium during RVC process

5.4.1.8 C/N ratio

During the 10 days of rotary drum composting, the C/N ratio dropped from 25.95 to 19.20. In the vermicomposting trials, the C/N dropped from 19.20 to 6.01 for VrEF, 19.20 to 7.52 for VrEE, 19.20 to 7.44 for VrPC, and 19.20 to 15.56 in the VrC reactor. And a decline in the C/N ratio to less than 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes. The C/N ratio is used as an index for the maturity of organic wastes and is a very important parameter because plants cannot assimilate N unless the ratio is in the order of 20 or less. The C/N ratio obtained in the final compost indicates a superior maturity of the produced compost.

5.4.2 Biological and stability parameters

5.4.2.1 sBOD and sCOD

The decreasing trend in the concentration of sBOD was observed in all the trials during the RVC process that is an important parameter for compost quality (Kalamdhad and Kazmi, 2007). For reactor R1, it was observed that sBOD decreased to 208.42 mg/L from 356.5 mg/L after the first 10 days. However, when the pre-degraded waste was vermicomposted, it was observed that the sBOD concentration decreased from 208.42 mg/L to 46.4 mg/L for reactor V_rEF, 208.42 mg/L to 90.2 mg/L for reactor V_rEE and 208.42 mg/L to 72.2 mg/L for reactor V_rPC as shown in Fig. 5.25(a).

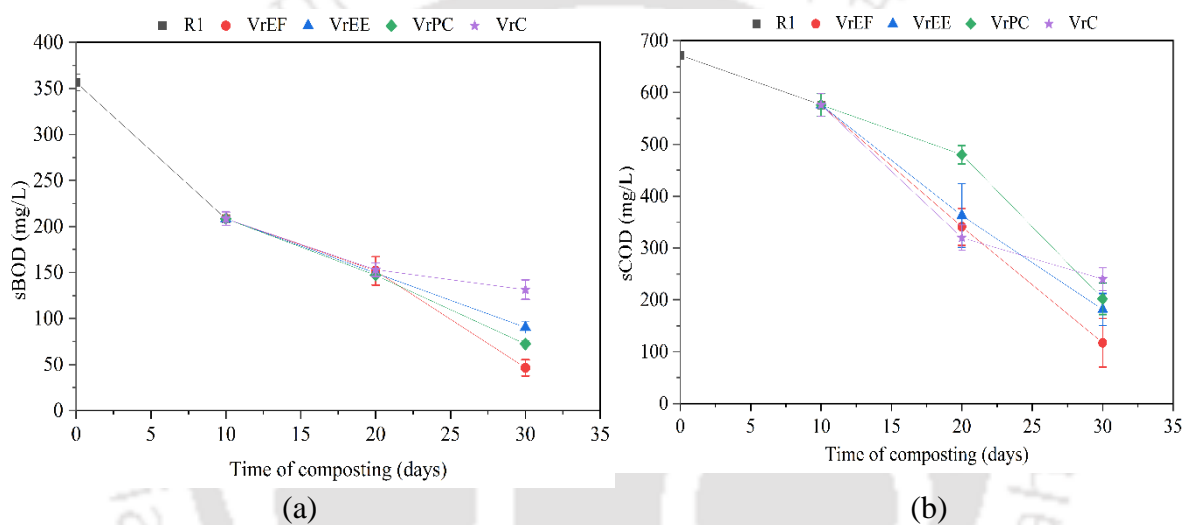


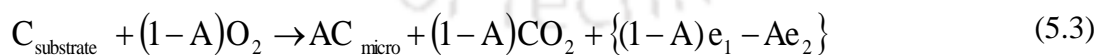
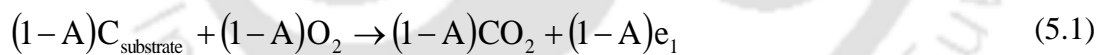
Fig. 5.25. Variation in a) sBOD and b) sCOD during RVC process

sBOD reduction during the RVC process was due to the degradation of an organic compound by earthworm and microorganisms that attends stabilization which can be further used for the crop in soil. Comparisons among the different techniques and trials with rotary drum composting and vermicomposting showed that sCOD was decreased from 672 mg/L to 576 mg/L in the first 10 days for reactor R1. It was reduced further to 117.34 mg/L, 181.34 mg/L, 202 mg/L, and 240 mg/L for the reactor V_rEF, V_rEE, V_rPC, and V_rC, respectively, as shown in Fig. 5.25(b). As Jain et.al. (2018) described, organic matter deterioration determines whether sBOD and sCOD increase or decrease. Biological content reduction reduces sBOD and sCOD, reducing carbon dioxide emissions, suggesting the compost has undergone stabilization. sBOD and sCOD analyzed varied differently at $p < 0.05$ during the RVC process.

5.4.2.2 OUR and CO₂ evolution

OUR is the most appropriate parameter for determining compost or vermicompost stability. Microorganisms will consume organic matter for their metabolic process, resulting in high oxygen consumption rates conferring compost instability (Said-Pullicino et al., 2007). As the organic matter decreases, less oxygen consumption will occur by a microorganism that confers compost stability. The rate of OUR evolution was minimum in the first 10 days of the composting process. However, it was increased once the pre-degraded waste was vermicomposted where earthworm and microorganisms helped in the degradation of organic matter, resulting in a decrease of OUR (Paul et al., 2020). Since conventional vermicomposting takes more than 45 to 60 days, the current study reduced the duration and achieved OUR value >1 for the VrEF reactor followed by VrEE and VrPC, which shows vermicompost stability within 20 days as shown in Fig. 5.26(a).

CO₂ evolution in the vermireactors was observed to be decreasing, as shown in Fig. 5.26(b). The breakdown of organic matter is primarily represented by the amount of O₂ consumed and CO₂ released, which is dependent on the chemical composition of the substrate used. CO₂ evolution was observed to be decreasing as the TOC decreased during the RVC process. Ginkel, (1996) described the catabolic and anabolic reaction that takes place during the degradation of the organic compound. The reaction is shown as follows, where equation (1) represents a catabolic reaction, (2) represents an anabolic reaction, and equation (3) represents the combination of catabolic and anabolic reaction.



Where,

e_1 denotes the catabolic reaction's energy in kJ mol C⁻¹

e_2 denotes energy required to build microbial bodies from organic biomass in kJ mol C⁻¹

and, A represents the yield in microbial-C/ mol of substrate-C.

On the right-hand side of the equation in reaction (3), the term ($A C_{\text{micro}}$) describes the microbial cell yield, the second term ($(1-A) CO_2$) describes the CO_2 yield, and the third term $\{(1-A) e_1 - A. e_2\}$ describes the heat produced during the degradation. In this study, during the first 10 days of composting process microbes plays an important role where the maximum amount of heat is produced due to the degradation of the organic compound, after 10 days earthworms play an important role to make the mixture stable and mature. The RVC process has helped to achieve that within 30 days because the conventional vermicomposting process takes more time to degrade organic compounds. At $p < 0.05$, CO_2 evolution differed significantly between tests during the RVC process.

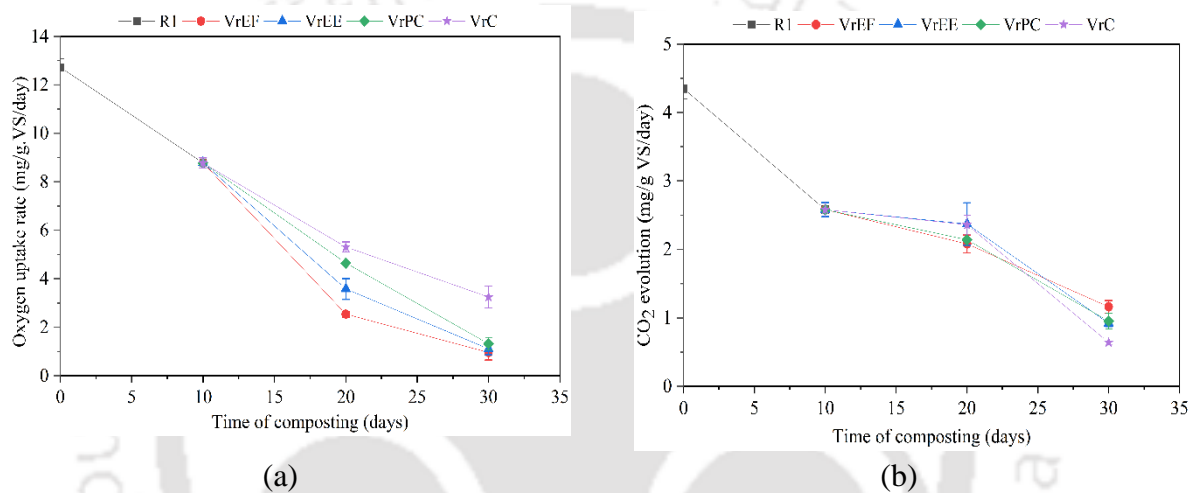


Fig. 5.26. Variation in a) OUR and b) CO_2 evolution during RVC process

5.4.2.3 Estimation of total coliform and fecal coliform bacteria

Hygiene standards are commonly considered to mark the value of compost by the coliform presence (Nartey et al., 2017). Total coliform and faecal coliform bacteria are shown in Table 5.5. There was an increase in the microbial population during the process in which it was observed that after 20 days, there was a sudden decrease in the microbial population. This is due to the degradation process in which earthworm plays a major role in the decrease of coliform and fecal coliform bacteria.

Table 5.5. MPN test for total and faecal coliform bacteria of RVC process

Reactors	Total coliforms			Fecal coliforms		
	0 day	10 day	30 day	0 day	10 day	30 day
VrC	9.3E+07	9.5E+04	9.3E+01	8.3E+04	7.5E+04	4.6E+02
VrEF	6.4E+04	7.5E+03	2.3E+01	5.3E+02	4.4E+02	2.3E+01

V _r EE	7.5E+03	9.3E+03	1.5E+04	6.4E+02	7.5E+02	1.5E+01
V _r PC	6.3E+02	5.5E+02	2.3E+01	4.4E+06	4.4E+02	1.5E+03

5.4.3 Growth and development of earthworm

The development of earthworm species used in the vermiconversion of organic waste materials is a good indicator of a successful vermicomposting process. The current study emphasized the growth and breeding of earthworm species *E. fetida*, *E. euginae*, and *P. ceylanesis*. Except for the reactor VrPC, where there was a decline in earthworm growth, the other two species showed an increasing trend in the growth and development during vermicomposting. A typical vermicomposting process takes 45-60 days for full maturity, and the growth of earthworm species varies accordingly. However, in the current study, due to the pre-degradation of waste, earthworms could feed it easily without much acclimatization, for which the number of worms increased more rapidly within 30 days. At the end of the 30th day, earthworms were evaluated in terms of growth rate, cocoon production, juvenile, and adult number, as shown in Table 5.6. It has been observed that in the reactor VrEF, the number of adult earthworms increased to 341 (juvenile 116 and cocoons 23/100 g) at the end of 30 days, followed by reactor VrEE with 296 (juvenile 96 and cocoons 14/100 g) number of earthworms at the end of 30 days. In reactor VrPC, the earthworm number was decreased to 112 (juvenile 18 and cocoons 11/100 g) at the end of 30 days. Despite feeding degraded material, earthworms were seen coming out of the reactor during the initial days of the process. The mortality of *P. ceylanesis* could be due to the weed biomass that has been mixed with cow dung and sawdust, making it unsuitable for it to survive. An upsurge in the metabolic activity of microorganisms associated with the earthworm's gut, as well as an escalation in the growth of earthworm biomass, characterize the mesophilic hydrolytic stage of the vermicomposting process (Vivas et.al., 2009). Throughout the vermicomposting process, earthworm species increased in number, with *E. fetida* showing a large number of adults, cocoons, and juveniles. Pre-degrading or thermo-composting of biomass helped better nourish and grow the earthworms.

Table 5.6. Growth and development of earthworm species during RVC process

	Days		
	<i>Eisenia fetida</i> (V _r EF)		
Results	10	20	30
Adults	120	198±5.56	341±2.45
Juveniles	0	33±4.54	116±2.53
Cocoons (No.s per 100 g)	0	9±1.95	23.67±5.31
<i>Eudrilus eugeniae</i> (V _r EE)			
Adults	120	170±2.62	296±7.21
Juveniles	0	22±2.45	24±2.41
Cocoons (No.s per 100 g)	0	96±3.68	14±1.92
<i>Perionyx ceylanensis</i> (V _r PC)			
Adults	120	138± 4.21	112±2.05
Juveniles	0	10±0.94	18±2.16
Cocoons (No.s per 100 g)	0	12±2.05	11±2.49

5.4.4 Fourier transform infrared spectroscopy (FTIR)

The FT-IR spectra of vermicompost using earthworm species and without earthworm has been shown in Fig. 5.27. O-H stretching was observed at 3685 cm⁻¹ and 3306 cm⁻¹, which signifies a strong bond representing mainly phenolic compounds (Hussain et al., 2015). The next peak at 2992 cm⁻¹ is attributed to aliphatic C-H stretching of fatty acids and lipids (Hussain et al., 2016). The peak at 1490 cm⁻¹ indicates the presence of C=C aromatic compounds in the sample (Mochochoko et al., 2013). The peak at 1034 and 842 cm⁻¹, according to the author, primarily represents C-O stretch and CH in-plane deformation, which is consistent with our findings. The peaks in the reactor VrEF are shallower than in the other reactors after treatment, owing to degradation by various types of enzymes found in the earthworm gut and microflora. The FT-IR graphs show a significant reduction during the vermicomposting process.

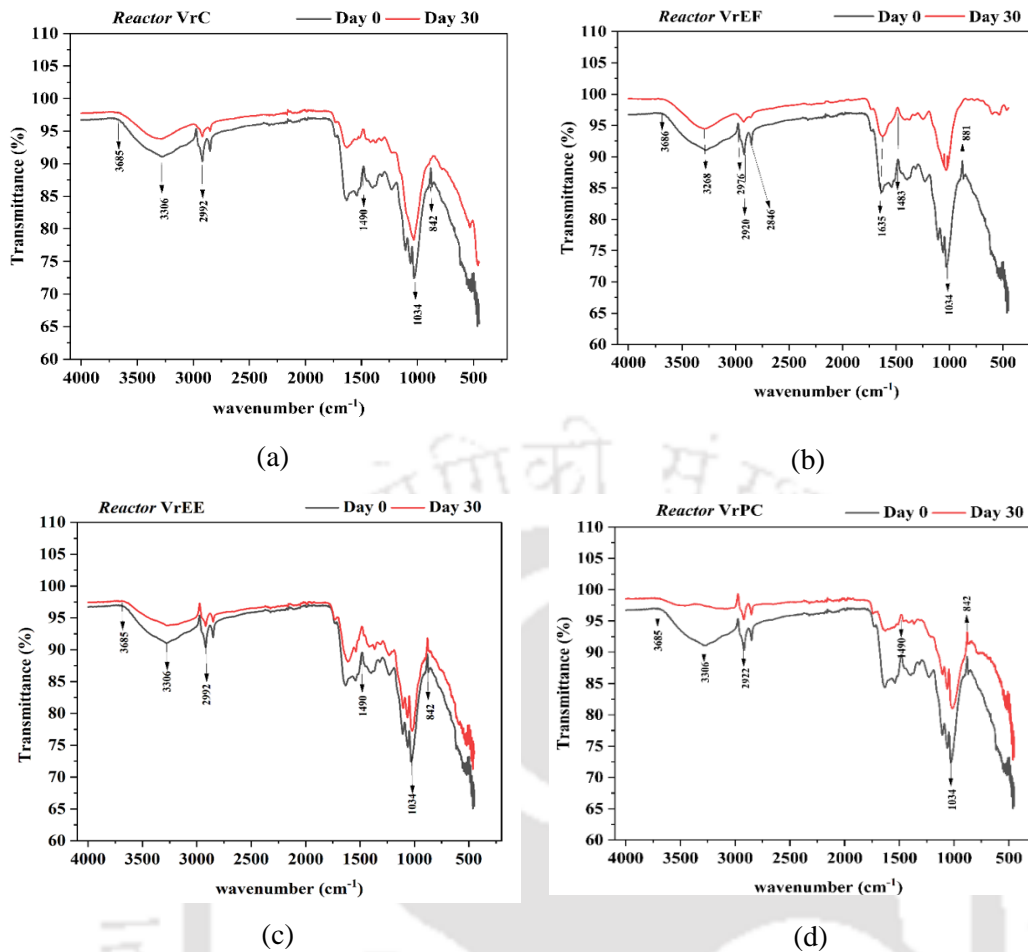


Fig. 5.27. FTIR spectra of dried vermicompost samples (a) VrC (b) VrEF (c) VrEE and (d) VrPC during RVC process

5.5 SUMMARY OF PHASE I

This study gives a comprehensive idea about the management of invasive terrestrial weed *Mikania* with the help of a specially designed rotary drum composter, vermicomposting and rotary drum composting followed by vermicomposting.

- In 20 days, the revolving drum composter will yield healthy compost. The ratio 5:4:1(RD2) was effective and suitable for the composting process in terms of texture and compost quality. It has achieved a temperature of 58.5°C that develops an appropriate thermophilic phase during the process. The highest TKN was observed in this ratio, with 2.71% as compared to other reactors. The decrease in TOC was observed highest in reactor RD2 with 19.72%, whereas the lowest reduction was found in reactor RD1 with 31.59%. There was a significant reduction in sBOD, sCOD, CO₂ evolution rate, and OUR values and the prominent increase in nutritional properties (total and available phosphorus, sodium, potassium, calcium) in the reactor RD2.

- The ratio 5:4:1 was found to be the suitable ratio for the vermicomposting process, and the percentage rise of TKN for the reactor V_{EF} was 58.6, potassium 14.58, and total phosphorus 46.14. Reactors V_{EE} and V_{PC} showed declining trends in potassium and total phosphorus concentration. The percentage reduction in CO_2 evolution for the reactor V_{EF} was 77.08% and OUR 92.67%. In all reactors, except for the V_C reactor (without earthworm), the removal percentage of sCOD and sBOD was found to be almost identical. In contrast to previous work on rotary drum composting of *M. micrantha*, it was discovered that compost made with VC has a higher nutrient quality, a finer texture, and is more efficient on test plants. However, the time required for VC is longer than that required for RDC process. Combining both technologies can speed up the process and cut downtime.
- The rotary drum composting and the vermicomposting combination proved to be a better solution than the other two techniques. The European Species *E. fetida* was the best earthworm species among the three species. Through the two-stage composting process, *E. fetida* achieved 3.24% TKN with considerable decreases in organic carbon and enhanced total phosphorus up to 12.87 g/kg. The reduction in sBOD, sCOD, and CO_2 evolution rate was greater in the earthworm-aided reactor. The earthworm growth and development were higher for the species *E. fetida* and *E. eudrilus*. The FTIR spectra showed a significant transition of the complex organic compound into a simpler form.
- With respect to various physico-chemical analysis conducted during composting process using three technologies, it was found that highest nutrient content was achieved in RVC process followed by VC process and RDC process.
- Fecundity of the earthworm was dependent on the substrate mixture. Highest cocoon, juvenile and adult earthworm was obtained for the species *E. fetida* followed by *E. eudrilus* and *P. ceylanesis*.
- The values obtained for the aforementioned parameters are within the range allowed by the FCO standard (Moisture content-15-25%, VS- >20%, C/N- <20, pH-6.5-7.5, EC- <4.0 dS/m, Phosphorus- 0.5% minimum, Potassium- 1.0% minimum, Nitrogen- 0.5% minimum) (FCO, 1985).

This chapter explores a variety of composting techniques in an effort to expedite the natural decomposition of the invasive plant. Out of all the three processes used, vermicomposting and rotary drum followed by vermicomposting gave best results as compared to rotary drum composting process in terms of compost quality. However, before applying compost to soil or for plant study, standard experiments for various toxicity assessments should be

incorporated in order to know more about its toxicity level before applying it to field. These experiments should be carried out in order to ensure that the compost is safe to use. The next chapter will cover the various toxicity tests that were performed on the three different types of compost that were produced using three distinct methods.



Toxicity evaluation of the compost using *Vigna radiata* and *Allium cepa* L.

This chapter insights the phytotoxicity evaluation of compost produced from three different technologies of the composting process that has been discussed in Phase I. The collective study of physico-chemical analysis and toxicity tests is more beneficial to estimate compost quality. Hence, compost samples were assessed for phytotoxicity. The phytotoxicity test using mung bean-based seed germination and sprout length has been considered the best toxicity assay method.

6.1 PART-I: RESULTS AND DISCUSSIONS OF THE TOXICITY ASSESSMENT OF THE COMPOST OBTAINED FROM BEST RATIO OBTAINED FROM THE ROTARY DRUM COMPOSTING STUDY

6.1.1 Phytotoxicity evaluation of rotary drum compost

To estimate compost stability, a combined review of physicochemical analysis and biological toxicity tests are most often used. Hence, compost samples were tested for phytotoxicity. The phytotoxicity test was performed on mung beans (*Vigna radiata*), based on seed germination and sprout duration, has been deemed one of the most effective and commonly used tool as a test seed for toxicity testing (Wang and Keturi, 1990). The seed germination test for phytotoxicity is very sensitive, and it can be easily inhibited by any toxic agent present in the atmosphere or substrate. Using mung bean (*Vigna radiata*), seed

germination inhibition (Germination index percentage, GI%) or induction in mung bean after exposure to various concentrations of *Mikania* extract and compost samples. In the control (tap water) solution, minimum to no seed germination inhibition was observed. Allelochemicals must enter the target plant's root system through the soil in order to produce a phytotoxic impact (Inderjit 2001). However, establishing allelopathic interference is dependent on a number of parameters, including the concentration, mobility, and permanence of allelopathic chemicals. Indeed, allelochemicals are transformed by the complex of chemical, physical, and biological properties of the soil environment, which determines their phytotoxic degree (Blum 2006). Allelochemicals may impact soil features, particularly biological ones. The seed germination index was reported only up to 50% dosage in *Mikania* extract. There was a significant decrease in GI%, root length, shoot length, and biomass index from 25% to 100% concentrations of *Mikania* extract. As the duration of composting went on, the rate of GI and other characteristics of *Vigna radiata* in compost extract increased, whereas seed germination was observed throughout all the same concentrations in the compost sample. At 100% concentration, the GI% was observed to be 90% in the compost sample. In comparison to control, the highest root growth of early seedling of mung bean (1.60 ± 0.02 cm) was observed at 25% concentration of the *Mikania* sample. Whereas, in the compost sample, the root length of the mung bean was increased at the same concentration was 3.01 ± 0.02 cm at the end of Day 10. At the end of 20 days, the root length was found to be increased to 4.98 ± 0.06 cm in 25% concentration of compost extract. As the concentration of compost samples increased for Day 20 sample, the highest growth was observed in 25% compost extract (4.98 ± 0.06 cm). There was no proper growth of mung bean seed after 50% concentration of *Mikania* extract and Day 0 samples, and gradual inhibition of growth was also found in the extracts. The shoot length reduction was observed highest at 100% of *Mikania* extract and Day 0 extract. The value was increased on Day 10 of the composting process, and the highest value was observed in 25% concentration (2.45 ± 0.01 cm). At the end of 20 days of the composting process, the highest shoot length was observed in 25% (2.74 ± 0.03 cm). The biomass of mung bean in raw *Mikania* extract was reduced throughout the concentration from 25 to 100% and in Day 0 extract. It was observed to be increased after 10 days of the composting process, and the highest biomass was observed in 25% of compost extract (2.01 ± 0.02 cm). At the end of 20 days of the composting process, it was found to increase compared to Day 0 and Day 10. Ni et al. (2007) reported about allelochemicals present in *Mikania* that are toxic to crop plants and can have a toxic effect on crop plants which could be another cause of seed

inhibition in the current study. The inhibition of seed germination might be the consequence of disturbed osmoregulatory system due to high salinity and phytotoxins present in *Mikania* extract samples, causing osmotic stress and toxicity in plants (Bybordi and Tabatabaei, 2009).

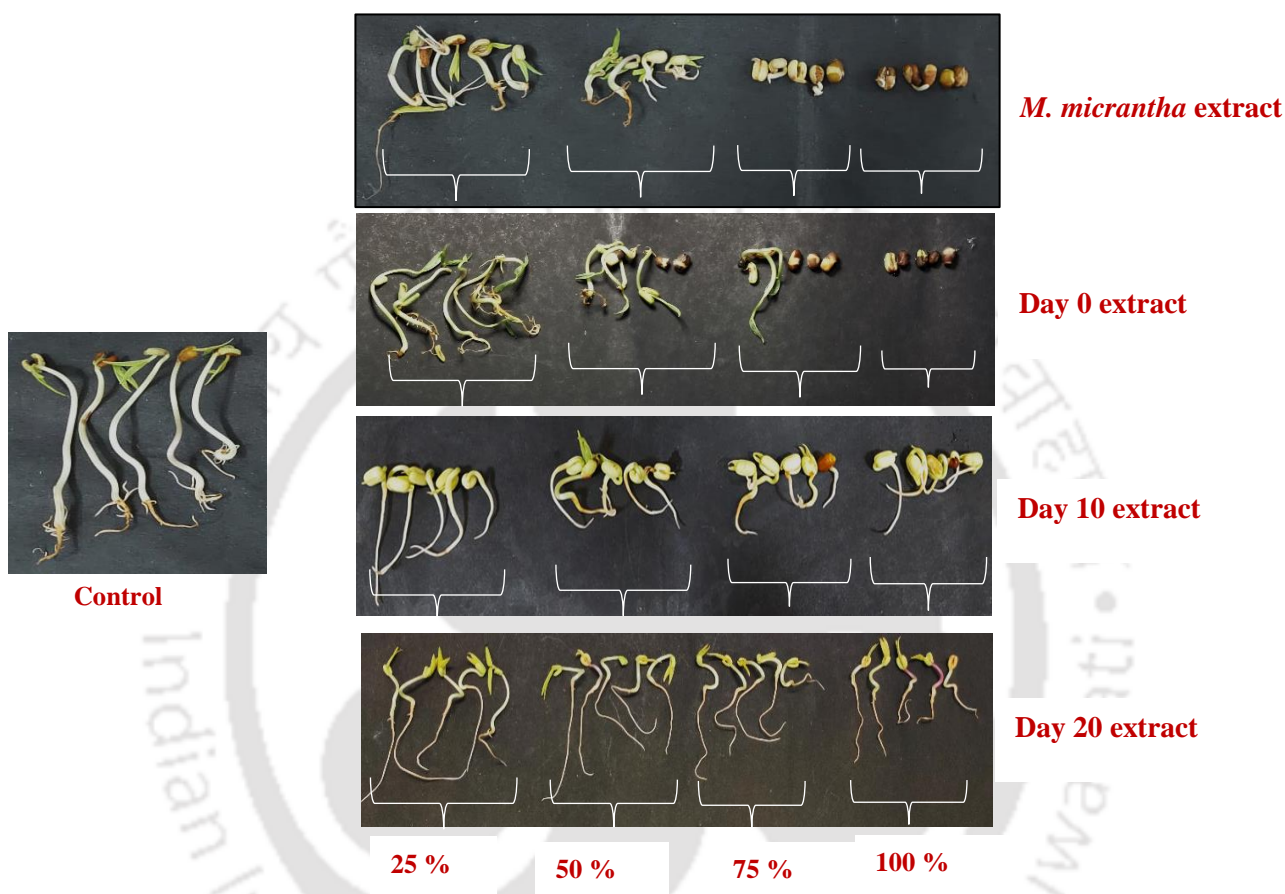


Fig. 6.1 Germination Index % of Mung bean (*Vigna radiata*) during RDC process

Table 6.1. Phytotoxicity test results of *Vigna radiata* in *M. micrantha* extract and compost extract during various phases of RDC process

(a) <i>Mikania micrantha</i> extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
Control	100 ± 0.08	3.33 ± 0.01	2.76 ± 0.04	3.12 ± 0.07
25%	83.1 ± 0.04	1.60 ± 0.02	0.98 ± 0.05	0.34 ± 0.08
50%	50 ± 0.02	1.12 ± 0.04	0.66 ± 0.08	0.14 ± 0.06

75%	36.67 ± 0.05	0.78 ± 0.04	0.40 ± 0.06	0.10 ± 0.04
100%	26.67 ± 0.02	0.06 ± 0.00	0.1 ± 0.01	0.09 ± 0.02
(b) Day 0 extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
25%	86.7 ± 0.21	1.42 ± 0.03	1.74 ± 0.04	1.85 ± 0.05
50%	69.8 ± 0.39	0.82 ± 0.04	1.55 ± 0.03	1.77 ± 0.04
75%	57.8 ± 0.19	0.53 ± 0.01	1.17 ± 0.05	1.72 ± 0.02
100%	40.1 ± 0.26	0.37 ± 0.03	0.58 ± 0.07	1.58 ± 0.04
(c) Day 10 extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
25%	90.1 ± 0.17	2.02 ± 0.04	1.54 ± 0.04	1.78 ± 0.08
50%	76.67 ± 0.24	1.5 ± 0.1	1.10 ± 0.08	1.01 ± 0.07
75%	70.0 ± 0.51	1.12 ± 0.09	0.98 ± 0.01	0.89 ± 0.01
100%	63.34 ± 0.18	0.84 ± 0.14	0.65 ± 0.08	0.68 ± 0.01
(d) Day 20 extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
25%	93.3 ± 0.26	4.98 ± 0.06	2.74 ± 0.03	2.45 ± 0.12
50%	83.34 ± 0.4	4.01 ± 0.02	2.51 ± 0.06	2.23 ± 0.1
75%	80 ± 0.3	4.09 ± 0.01	2.42 ± 0.1	2.30 ± 0.3
100%	76.67 ± 0.2	1.53 ± 0.01	2.12 ± 0.07	2.03 ± 0.2

A. cepa test is a standard test for rapid and sensitive screening of chemicals and pollutants representing environmental hazards. The root tip is often the first and foremost part of a plant that comes into contact with chemicals/pollutants found in water or soil. The root tip system of *A. cepa* has particularly shown sensitivity to harmful effects of environmental hazards (Bhat et al., 2015). *A. cepa* test introduced by Levan, (1938) has been used frequently and validated by several workers for testing chemical pollutants posing hazardous environmental effects (Morales and Jordao, 2001). The consequence of diverse concentrations of *Mikania* and compost samples on the root growth and length of *A.*

cepa were evaluated as shown in Fig 6.2. Primarily, the onion bulbs were embedded in different concentrations of *Mikania* and compost samples (25-100% v/v) and controlled to monitor the root growth of *A. cepa*. Results showed that root growth inhibition was found at all tested concentrations of *Mikania* samples. The growth length of the root of the onion bulb in various concentrations of extract has been illustrated in Table 6.2. The growth of the onion bulb in 0% to 100% *Mikania* extract was 8.5 ± 0.25 to 0.1 ± 0.02 cm, and at Day 0, the length root was inhibited as the concentration of the sample was high. The maximum root length was observed in 25 % concentration (2.01 ± 0.05 cm) of the Day 0 sample. At the end of 20 days of the composting process, the root length increased and recorded highest as 9.5 ± 0.04 cm. The end product exhibited a positive result for root length compared to *Mikania* extract, Day 0, and Day 10 samples.



Fig. 6.2. Phytotoxicity assessment test using *Allium cepa* during RDC process

Table 6.2. Phytotoxicity test results of *Allium cepa* in *M. micrantha* extract and compost extract during various phases of RDC process

(a) Root lengths of <i>Allium cepa</i>				
Concentration	Raw <i>Mikania</i> extract (cm)	Day 0 (cm)	Day 10 (cm)	Day 20 (cm)
Control		8.5 ± 0.2		
25%	1.5 ± 0.05	2.01 ± 0.05	6 ± 0.04	9.5 ± 0.04
50%	1 ± 0.04	0.8 ± 0.02	6.25 ± 0.06	9.0 ± 0.06
75%	0.2 ± 0.02	0.45 ± 0.03	5.5 ± 0.07	8.0 ± 0.05
100%	0.1 ± 0.02	0.15 ± 0.01	5.25 ± 0.04	6.6 ± 0.04
(b) Biomass Index of <i>Allium cepa</i>				
Concentrations (v/v)	Raw <i>Mikania</i> extract (g)	Day 0 (g)	Day 10 (g)	Day 20 (g)
Control		1.1 ± 0.02		
25	0.09 ± 0.01	0.07 ± 0.04	0.10 ± 0.04	1.85 ± 0.05
50%	0.13 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	1.77 ± 0.04
75%	0.12 ± 0.05	0.10 ± 0.01	0.09 ± 0.08	1.72 ± 0.02
100%	0.09 ± 0.07	0.06 ± 0.04	0.07 ± 0.05	1.58 ± 0.04

6.1.2 Cytotoxicity and genotoxicity assessment using *A. cepa*

Mitotic Index (MI) was calculated by the total number of dividing cells in the cell cycle. The cytotoxicity of environmentally toxic substances and compost samples may be determined by evaluating the cell death rate, abbreviated as MI (Chowdhury et al., 2015). Root growth inhibition of *A. cepa* may be interpreted as a sign of toxicity resulting from cellular damage or a block in cell division. MI can be used to characterize an organism's cell mortality during the mitotic stage of the cellular cycle (Haq and Kalamdhad, 2021). It was evident that the reduction in MI of *A. cepa* in *Mikania* samples was due to increased concentrations, as shown in Table 6.3. MI of negative control with dechlorinated water was 72.15%. The reductions in MI were observed from 44.24 to 22.72% in the *Mikania* extract sample from 25 to 100% concentrations. The drops may be due to the cytotoxic impacts of the *Mikania* extract, which may induce indeterminate prophase, resulting in an erratic

mitotic phase and therefore suppressing division during interphase (El Fels et al., 2016). The MI in the compost sample from D0 and D20 was observed in 100% concentrations with 31.92 and 39.37%, respectively. The increasing MI trends in the Day 20 compost sample suggest that the cytotoxic impacts of *Mikania* extract are efficiently reduced due to composting activity. When *A. cepa* induced with *Mikania* extract demonstrated substantial variance in MI compared to compost sample and control ($p < 0.05$). A decrease in MI in *A. cepa* has previously been linked to pesticide exposure (Saxena et al. 2005). The cytotoxic potential of the test compound has been linked to a decrease in MI (Samka-kincl et al. 1996; Radic et al. 2010). According to Fiskesjo (1988), the effect of toxic chemicals on spindle apparatus resulted in a significant decrease in MI. The mitotic index is thought to be a reliable tool for detecting cytotoxic pollutants in the environment (Grover and Kaur 1999; Chandra et al. 2005).

Table 6.3. Cytotoxicity assessment (Mitotic index %) of *M. micrantha* extract and compost extract during various phases of RDC process

	Concentration (%)	Total cells	Dividing cells	MI (%) (Mean \pm SD)
Control	0	887	639	72.04 \pm 4.57
<i>Mikania</i> extract	25	947	418	44.13 \pm 5.12
	50	982	318	32.38 \pm 2.45
	75	898	234	26.05 \pm 0.87
	100	946	214	22.62 \pm 1.24
RDC (Day 0)	25	988	575	58.19 \pm 2.46
	50	972	409	42.07 \pm 4.78
	75	986	364	36.91 \pm 2.14
	100	991	316	31.88 \pm 1.42
RDC (Day 10)	25	1056	528	50.00 \pm 2.15
	50	1123	498	44.34 \pm 1.78
	75	998	398	39.87 \pm 2.60
	100	878	312	35.53 \pm 1.52
RDC (Day 20)	25	988	635	64.27 \pm 1.54
	50	992	516	52.01 \pm 2.10
	75	956	468	48.95 \pm 4.12

In the current study, chromosomal aberration (CA) of *A. cepa* showed both aneugenic (including c-mitosis, multipolar telophase, and micronucleated cell) and clastogenic abnormalities (chromosomal bridge) (Bhat et al., 2015). The CA (%) in meristematic cells of roots grown in *Mikania* extract was significantly lower than in control RDC extract, indicating that the extract had a toxicity effect.

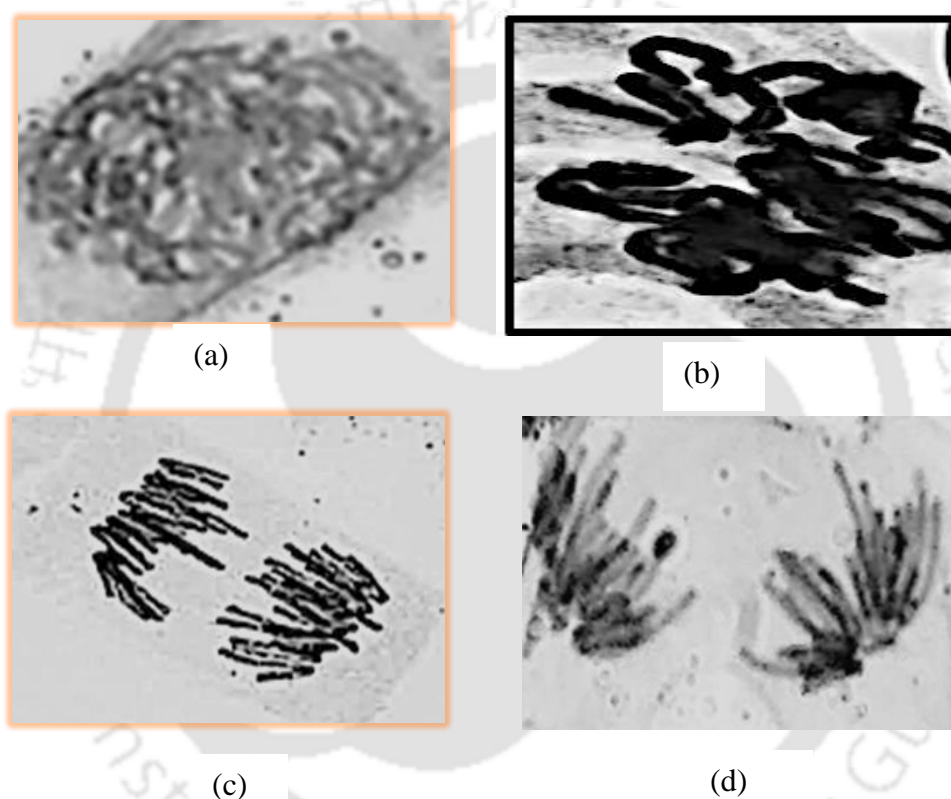


Fig. 6.3: Different stages of mitosis cell division in *A. cepa* a) Prophase b) Metaphase c) Anaphase d) Telophase

According to Shao et al. (2005), *Mikania* is composed of a complex mixture of sesquiterpenes that inhibit the growth of a wide range of seedlings which may also be the cause of toxicity in the roots of *A. cepa*. CA (%) for the raw extract of *Mikania* was in the range of (9.70-16.09%) as the concentration of aqueous extract increased from 25%-100%. The maximum number of CA was seen at 75% and 100% concentration of *Mikania*. Fig. 6.3 depicts the various stages of mitotic cell division discovered during the current study for all three composts produced using different composting processes. As illustrated in Fig.

6.4, the principal CA observed during the genotoxicity assessment of all compost products produced using various compost processes include micronucleated cells, multipolar telophase, c-mitosis, chromosomal bridge, lagging chromosome, chromosomal loss, and sticky chromosome.

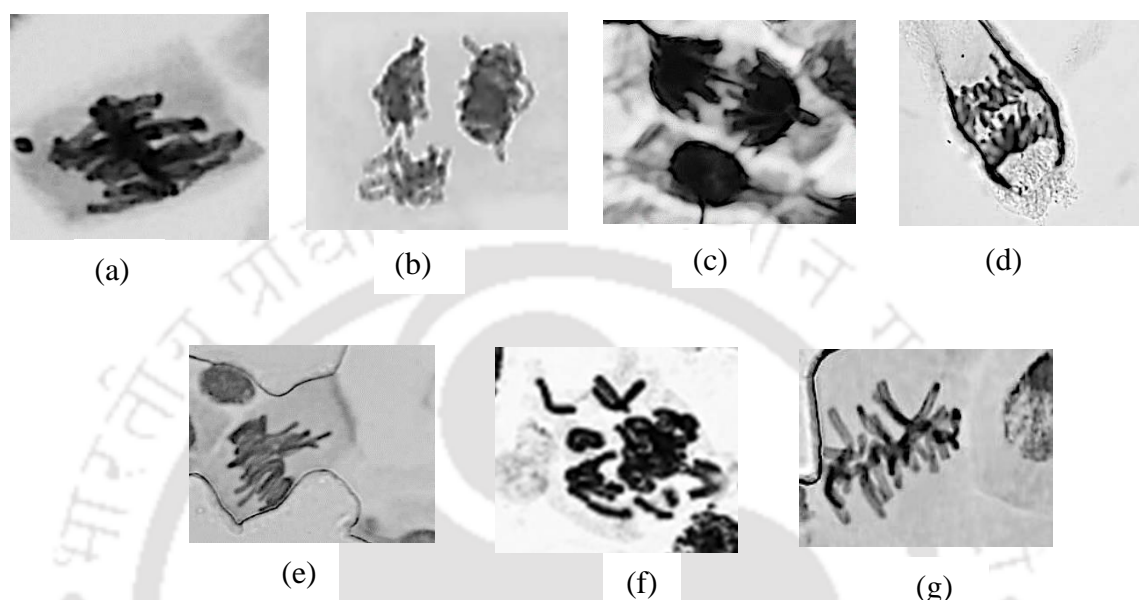


Fig. 6.4. Different chromosomal aberrations in *A. cepa* a) Nuclear bud b) Multipolar telophase c) Chromosomal bridge d) Vagrant chromosome e) Laggard chromosome f) Chromosomal loss and g) Sticky chromosome

No prominent CA was observed in the cell of *A. cepa* exposed to tap water (control). Multipolar telophase, chromosomal bridge, and c-mitosis were observed almost in all the concentrations of compost extract. From Day 0 to Day 20, there was a significant drop in the percent of aberrant cells in the RDC sample. Maximum CA was observed in the pre-composted RDC extract, but a decrease in aberration frequencies was observed in the post-composted *Mikania* extract (RDC sample). According to Auerbach (1962), micronuclei were believed to be an indicator of a real mutation effect; hence, the high percentage of micronuclei caused by the examined *Mikania* compost may be indicative of their mutagenic effects. In general, the formation of micronuclei in root meristems is a sign of chromosome breakage and disruption of the mitotic process due to anomalies in the spindle, which can occur in a variety of ways (Grover and Kaur, 1999). The chromosomal bridge was observed maximum in 100% concentration (16.09) of raw extract of *Mikania* as compared to other concentrations of the extract as shown in Table 6.4. These findings lend further credence

to the hypothesis that the stickiness of chromosomes may result in insufficient separation of daughter chromosomes as a result of cross-linkage of chromo-proteins during the separation process, which resulted in the formation of sub-chromatid linkages between chromosomes, which allowed them to remain connected by bridges (Migid et al., 2007). It has been illustrated how *A. cepa* undergoes several phases of mitotic cell division and how its chromosomal aberration changes after exposure to various extracts. It is possible that the raw extract of *Mikania* and RDC (Day 0) induced chromosome bridges originating during anaphase and telophase were formed by rupture and blending of chromatid materials or that the unequal exchange of chromatids during translocation (Barman et al., 2021). On the other hand, the aneugenic effect shows faulty chromosomal segregation and inhibition of cytokinesis as a result of a dysfunctional mitotic spindle during cell division, as demonstrated by the results of this study. This study has demonstrated that the rotary drum composting technique can reduce *Mikania* toxicity during the mitotic cell division of *A. cepa*.

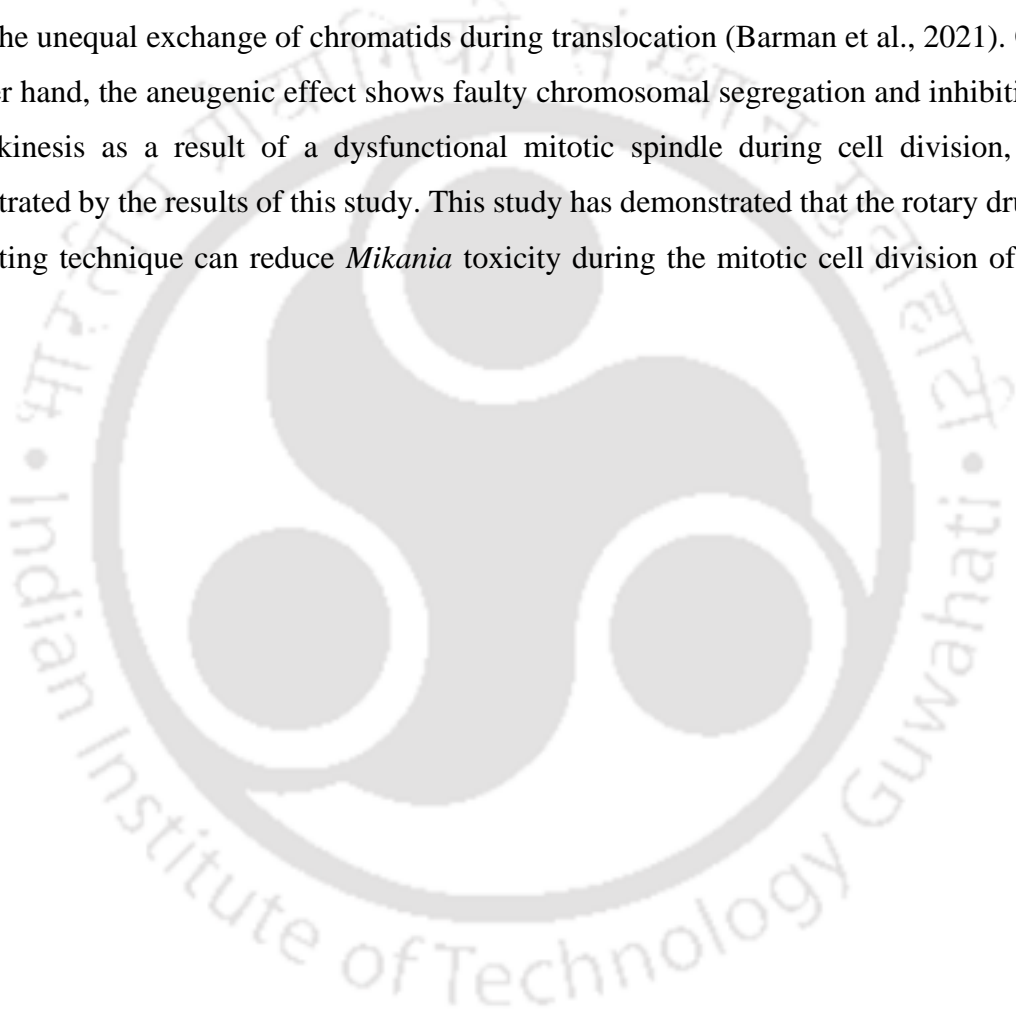


Table 6.4 Genotoxicity assessment of *M. micrantha* extract and compost extract during various phases of RDC process

	Concentration	Micro nucleated cells	Multipolar telophase	Chromosomal bridge	Vagrant chromosome	Laggard chromosome	Chromosomal loss	Sticky chromosome	Aberrant cells (%) (Mean \pm SD)
Control	0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Mikania</i>	25	20.1 \pm 1.1	4.7 \pm 0.3	20.7 \pm 1.31	17.4 \pm 1.2	12.4 \pm 1.2	8.7 \pm 1.0	7.8 \pm 1.4	9.70 \pm 2.1
	50	23.1 \pm 1.2	7.4 \pm 0.1	25.8 \pm 0.9	20.1 \pm 0.1	20.1 \pm 2.4	15.7 \pm 2.4	10.2 \pm 0.9	12.47 \pm 1.8
	75	25.7 \pm 1.2	9.5 \pm 0.2	28.7 \pm 0.8	22.4 \pm 0.8	18.7 \pm 4.0	18.1 \pm 4.1	12.7 \pm 2.1	15.09 \pm 4.2
	100	22.1 \pm 2.1	10.5 \pm 0.3	30.1 \pm 1.2	25.8 \pm 1.2	25.4 \pm 3.1	22.7 \pm 2.7	15.4 \pm 1.4	16.09 \pm 4.8
RDC (D0)	25	11.1 \pm 1.2	4.7 \pm 1.2	7.1 \pm 0.2	20.1 \pm 2.0	2.7 \pm 0.8	25.4 \pm 2.4	3.4 \pm 0.9	7.55 \pm 1.2
	50	11.1 \pm 0.8	6.7 \pm 0.9	10.1 \pm 1.0	24.7 \pm 4.2	2.5 \pm 0.9	29.8 \pm 3.4	5.4 \pm 1.2	9.31 \pm 2.4
	75	4.5 \pm 0.7	5.4 \pm 1.0	8.7 \pm 1.0	21.1 \pm 2.1	18.7 \pm 2.5	20.7 \pm 2.8	27.8 \pm 2.4	10.84 \pm 3.1
	100	25.4 \pm 0.8	9.2 \pm 2.0	10.6 \pm 1.0	28.7 \pm 0.8	1.8 \pm 0.8	32.1 \pm 5.4	6.1 \pm 0.4	11.50 \pm 2.5
RDC (D10)	25	10.2 \pm 0.8	3.8 \pm 0.8	10 \pm 0.9	18. \pm 2.0	1.4 \pm 0.4	19.51 \pm 2.1	6.1 \pm 0.2	6.55 \pm 1.0
	50	10.2 \pm 1.0	7.8 \pm 0.6	7.7 \pm 0.5	12.4 \pm 1.8	17.5 \pm 2.4	21.5 \pm 2.4	24.6 \pm 1.4	9.05 \pm 0.8
	75	11.5 \pm 2.0	9.2 \pm 1.1	9.7 \pm 0.2	8.7 \pm 1.0	2.1 \pm 0.8	15.4 \pm 2.4	18.7 \pm 2.0	7.54 \pm 0.7
	100	14.7 \pm 1.8	11.7 \pm 1.2	10.2 \pm 0.7	6.7 \pm 0.6	1.1 \pm 0.5	18.7 \pm 0.1	21.4 \pm 4.0	9.62 \pm 1.0
RDC (D20)	25	4.7 \pm 0.6	2.4 \pm 0.8	2.4 \pm 0.2	9.24 \pm 1.2	15.5 \pm 1.8	14.7 \pm 0.5	21.4 \pm 2.0	7.12 \pm 2.1
	50	10.2 \pm 0.6	5.7 \pm 0.8	2.1 \pm 0.6	12.4 \pm 2.0	16.0 \pm 3.2	14.5 \pm 2.1	20.1 \pm 2.0	8.18 \pm 1.1
	75	14.5 \pm 0.2	7.4 \pm 2.1	3.17 \pm 0.8	14.1 \pm 1.5	10.2 \pm 1.1	11.4 \pm 1.4	14.7 \pm 1.0	7.90 \pm 1.2
	100	10.2 \pm 0.5	8.7 \pm 1.7	5.8 \pm 0.2	12.5 \pm 2.1	17.2 \pm 1.4	11.1 \pm 1.0	19.8 \pm 2.4	8.7 \pm 1.4

6.2 PART-II: RESULTS AND DISCUSSIONS OF THE TOXICITY ASSESSMENT OF THE COMPOST OBTAINED FROM VERMICOMPOSTING PROCESS USING *Esenia fetida*

6.2.1 Phytotoxicity evaluation of vermicompost

Phytotoxicity bioassays have garnered increasing attention from the world's environmental groups. The consequences of phytotoxicity created by the organic waste resulted from a mixture of many elements, including heavy metals, ammonia, salts, and organic acids of low molecular weight (Zucconi et al., 1985). Therefore, it is important to determine terrestrial weed toxicity from the prepared compost, as it contains certain secondary metabolites that are harmful to plant species. Along with assessing other Physico-chemical and biological parameters, it is crucial to know the use of plant seeds to suggest vermicompost maturity and non-toxicity. Several allelochemicals in *M. micrantha* need to be degraded during the vermicomposting process, so the phytotoxicity assay is necessary to validate the outcome obtained from vermicompost.

Based on compost quality, texture, and earthworm mortality rate, it is found that *E.fetida* was more suitable for *M. micrantha* composting process. Hence, compost sample of Day 0, Day 30, and Day 60 and raw *M.micrantha* aqueous extract was taken for this phytotoxicity assay. Two plant species that are suitable for phytotoxicity tests are taken, namely *Vigna radiata* (mung bean) and *Alium cepa* L. (onion) (Izhar Haq et al., 2016). The seed germination test is considered one of the simplest short-term, sensitive and cost-effective methods of phytotoxicity evaluation for wastewaters (Rusan et al., 2015; Lyu et al., 2018). Seed germination is a very sensitive process, likely to be disturbed by the substances present in the environment. Zucconi et al., (1985) suggested that GI >50% is a manifestation of high caliber compost in terms of maturity and phytotoxicity. Seed germination of mung bean upon exposure to different *M. micrantha* extract and vermicompost extract concentrations is shown in the Table 6.5. The effect of *Mikania* before and after the composting process on the growth of mung bean seedling following 5 days has been evaluated. In comparison to control, the highest root growth of early seedling of mung bean (1.60 ± 0.02 cm) was observed at 25% concentration of the *Mikania* sample. The seed degraded and rotten at 75 and 100% *Mikania* extract. The GI of mung bean was found to be 83.1, 50.0, 36.67, and 26.67% for 25, 50, 75, and 100% *Mikania* extract concentration respectively. Seed germination inhibition was observed as the concentration of extract increased. This may be due to the phytochemicals present in *M.*

micrantha, which inhibits mung bean growth (Ismail and Chong, 2002). There is a very contradictory result in the growth of mung bean when grown in the aqueous vermicompost extract. There was increasing growth of mung bean when the concentration of vermicompost increased. The GI of mung was found to be 100, 96.0, 98.0 and 100% for 25, 50, 75 and 100% of Day 60 vermicompost extract. It was evident that the degradation of phytochemicals occurred during the vermicomposting process. The effect of mung bean growth in the aqueous extract of *M. micrantha* and vermicompost is shown in Fig 6.5. The average root length, shoot length, and biomass weight are found to be 3.33 ± 0.01 cm and 2.76 ± 0.04 cm and 3.12 ± 0.07 g, respectively, for control. But a decreasing trend in the length of mung beans was found as the concentration of aqueous extract of *M. micrantha* increased. The lowest root length (0.06 ± 0.00 cm), shoot length (0.1 ± 0.01 cm), and biomass weight (0.09 ± 0.01 g) were recorded at 100 % of *Mikania* extract. At the same time, there was a positive result in the growth of mung bean in an aqueous vermicompost extract. There was an increasing trend in the root length and shoot length of mung in vermicompost extract after Day 30 of vermicomposting process. At 25%, root length, shoot length, and biomass weight was 3.18 ± 0.02 cm, 2.53 ± 0.03 cm, and 2.21 ± 0.98 g whereas, at 100%, it was found to be 5.55 ± 0.02 cm, 3.44 ± 0.06 cm, and 2.32 ± 0.87 g, respectively. The increasing trend is due to the degradation of phytochemicals and increased nutrient content of vermicompost at 60 days.

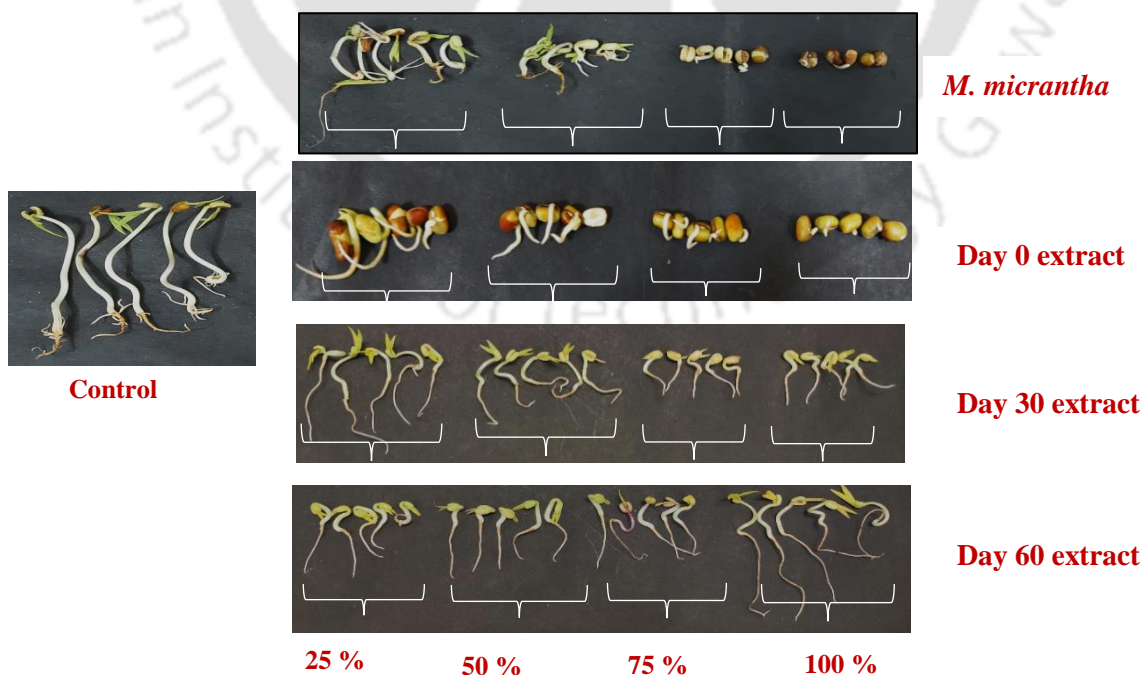


Fig. 6.5 Germination Index % of Mung bean (*Vigna radiata*) during VC process

Table 6.5. Phytotoxicity test results of *Vigna radiata* in *M. micrantha* extract and compost extract during various phases of VC process

(a) <i>Mikania micrantha</i> extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
Control	100 ± 0.08	3.33 ± 0.01	2.76 ± 0.04	3.12 ± 0.07
25%	83.1 ± 0.04	1.60 ± 0.02	0.98 ± 0.05	0.34 ± 0.08
50%	50 ± 0.02	1.12 ± 0.04	0.66 ± 0.08	0.14 ± 0.06
75%	36.67 ± 0.05	0.78 ± 0.04	0.40 ± 0.06	0.10 ± 0.04
100%	26.67 ± 0.02	0.06 ± 0.00	0.1 ± 0.01	0.09 ± 0.02
(b) Day 0 extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
25%	80.0 ± 1.12	1.0 ± 0.24	0.54 ± 0.21	0.98 ± 0.02
50%	64.4 ± 1.52	0.44 ± 0.15	0.21 ± 0.04	0.82 ± 0.04
75%	46.0 ± 2.1	0.32 ± 0.08	0.18 ± 0.01	0.68 ± 0.04
100%	32.0 ± 1.41	0.21 ± 0.07	0.09 ± 0.01	0.51 ± 0.01
(c) Day 30 extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
25%	90 ± 0.4	4.01 ± 0.02	2.45 ± 0.01	2.01 ± 0.02
50%	80 ± 0.3	3.11 ± 0.04	1.42 ± 0.02	1.98 ± 0.01
75%	76.67 ± 0.2	1.75 ± 0.02	2.27 ± 0.04	1.45 ± 0.04
100%	73.34 ± 0.4	1.30 ± 0.04	1.58 ± 0.02	1.24 ± 0.07
(d) Day 60 extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
25%	100	3.18 ± 0.02	2.53 ± 0.03	2.21 ± 0.98
50%	96.0	4.06 ± 0.02	3.27 ± 0.2	2.29 ± 0.62
75%	98.0	4.39 ± 0.03	3.61 ± 0.3	2.34 ± 0.92
100%	100	5.55 ± 0.02	3.44 ± 0.06	2.32 ± 0.87

Further, the phytotoxic effect of sample extract was measured in the root growth inhibition test using *Allium cepa*. Root growth inhibition in *A. cepa* root has been considered as a toxicity indicator since it may result from inhibition of the cell division (Egito et al., 2007). *A. cepa* test is a standard test for rapid and sensitive screening of chemicals and pollutants representing environmental hazards. The root tip is often the first and foremost part of a plant that comes into contact with chemicals/pollutants found in water or soil. The root tip system of *A. cepa* has particularly shown sensitivity to harmful effects of environmental hazards (Bhat et al., 2015). The result of *A. cepa* growth in different concentrations of *M. micrantha* and vermicompost extract is shown in Fig. 6.6.



Fig. 6.6. Phytotoxicity assessment test using *Allium cepa* during VC process

The effect of the growth of *A. cepa* was observed in the aqueous extract of *Mikania* and vermicompost extract after 5 days of incubation at $20 \pm 2^\circ\text{C}$ (Izhar Haq et al., 2016) as shown in Table 6.6. The growth of onion bulb in 25% to 100% *Mikania* extract was 1.5 to 0.1 cm, whereas the growth was increasing after the vermicomposting process, and it was observed that at Day 30 of vermicomposting process, the growth of onion bulb was

recorded 9.1-6.4 cm for 25-100% concentration of the sample extract. At the end of 60 days of the vermicomposting process, root length was observed even more, which signifies the degradation of allelochemicals present in the *Mikania* plant. A maximum root length of 10.4 ± 0.05 cm was recorded in 100% Day 0 vermicompost extract.

Table 6.6. Phytotoxicity test results of *Allium cepa* in *M. micrantha* extract and compost extract during various phases of VC process

(a) Root lengths of <i>Allium cepa</i>				
Concentration	Raw <i>Mikania</i> extract	Day 0	Day 30	Day 60
Control			8.5 ± 0.2	
25%	1.5 ± 0.05	2.25 ± 0.03	9.1 ± 0.02	8.1 ± 0.09
50%	1 ± 0.04	1.7 ± 0.04	8.5 ± 0.04	8.6 ± 0.08
75%	0.2 ± 0.02	2.1 ± 0.05	7.1 ± 0.05	9.2 ± 0.07
100%	0.1 ± 0.02	1.5 ± 0.07	6.4 ± 0.07	10.4 ± 0.05
(b) Biomass Index of <i>Allium cepa</i>				
Concentrations (v/v)	Raw <i>Mikania</i> extract	Day 0	Day 30	Day 60
Control			1.1 ± 0.02	
25%	0.09 ± 0.01	0.34 ± 0.01	1.04 ± 0.01	1.2 ± 0.05
50%	0.13 ± 0.02	0.21 ± 0.01	0.98 ± 0.02	0.92 ± 0.04
75%	0.12 ± 0.05	0.19 ± 0.02	0.91 ± 0.05	0.81 ± 0.02
100%	0.09 ± 0.07	0.18 ± 0.01	0.78 ± 0.04	1.12 ± 0.04

6.2.2 Cytotoxicity and genotoxicity assessment using *A. cepa*

Mitotic activity and chromosomal behavior were observed in four different concentrations of vermicompost extract at different phases of a vermicomposting process using *A. cepa* as a biological system (Bhatta and Sakya, 2008). The results indicated that *Mikania* extract at a higher concentration significantly increased cell frequency compared to control, and this increase was dose-dependent. The MI of the negative control with dechlorinated water was 72.15 ± 4.57 . The reductions in MI were observed from 44.24 to 22.72% in the *Mikania* extract sample from 25 to 100% concentrations. The drops may be due to the cytotoxic impacts of the *Mikania* extract, which may induce indeterminate prophase, resulting in an erratic mitotic phase and therefore suppressing division during

interphase (El Fels et al., 2016). The percentage of MI was significantly increased in the post vermicompost extracts as compared to the initial *Mikania* extract, as shown in Table 6.7. At 100% concentration of vermicompost extract, MI percentage was observed to be (52.0%) while the MI percentage at 100% concentration of *Mikania* extract was observed to be (22.62%). The interference in the normal cell cycle leads to a decrease in dividing cells which reduces MI (Sharma and Vig, 2012). According to El-Ghamery et al. (2000), the reduction in the mitotic index could be due to protein synthesis or DNA inhibition. Compared to D20 of rotary drum compost extract, vermicompost performed better at 100% concentration than rotary drum compost because of the metabolic conversion of highly toxic forms to nontoxic forms has been demonstrated in *E. fetida* via mitochondrial and cytoplasmic fractions (Fischer and Koszorus, 1992). Jain et al. (2004) discovered a decrease in MI in initial fly ash mixtures while increasing the mitotic index in post vermicomposted fly ash mixtures. Srivastava et al. discovered MI in the final vermicomposted mixtures of municipal sludge (2005).

Table 6.7. Cytotoxicity assessment (Mitotic index %) of *M. micrantha* extract and compost extract during various phases of VC process

	Concentration (%)	Total cells	Dividing cells	MI (%) (Mean \pm SD)
Control	0	887	639	72.04 \pm 4.57
<i>Mikania</i> extract	25	947	418	44.13 \pm 5.12
	50	982	318	32.38 \pm 2.45
	75	898	234	26.05 \pm 0.87
	100	946	214	22.62 \pm 1.24
	VC (Day 0)	25	1012	575
VC (Day 0)	50	989	404	41.00 \pm 2.01
	75	956	334	35.00 \pm 1.74
	100	972	271	28.10 \pm 1.02
	VC (Day 30)	25	898	431
VC (Day 30)	50	942	394	42.21 \pm 2.01
	75	961	383	40.01 \pm 3.25
	100	926	341	37.05 \pm 1.21
	VC (Day 60)	25	996	677
VC (Day 60)	50	896	520	58.01 \pm 1.25
	75	852	480	57.31 \pm 2.58
	100	892	463	52.00 \pm 1.54

The occurrence of CA was more prominent in 100% concentration of *Mikania* extract after 24 h of exposure, with the mild frequency of aberrations observed in vermicompost

extract as shown in Table 6.8. The major CA was noted as micronucleated cells, multipolar telophase, c-mitosis, chromosomal bridge, laggard chromosome, chromosomal loss, and sticky chromosome, as shown in Fig 6.3. There was no CA found in control (0 %) and in *Mikania* extract it was found highest in 100% concentration (16.0 ± 4.8) whereas at 25 % it showed (9.70%) aberrations, 50% showed (12.4%) aberrations and at 75% it showed (15.0 ± 4.2 %) aberrations. It was observed that the increase in the concentration of *Mikania* extract had a toxic effect on the plant cell. An almost similar result was observed in the D0 sample extract that showed CA in the range of 7.0 % to 11.1 % for 25 %, 50 %, 75 %, and 100 % extract. As the vermicomposting process preceded, the effect of CA on the cell was observed to be less, which was in the range of 4.7-6.5% for the D30 sample. According to Bhat et al. (2014), the percent aberration was higher (30.8 %) after the initial exposure of press mud sludge, but it was reduced to 20.3 % after vermicomposting with *E. fetida*. Stickiness in the chromosomes may be due to DNA condensation or entanglement of chromatin fibers (Osterberg et al. 1984; Chauhan et al. 1999). Bridges in the chromosome may be due to stickiness or dicentric chromosome formation (Jabee et al. 2008). Breaks in chromosomes result from the fragile site breakage (Lukusa and Fryns 2008). Vagrant in chromosomes indicates spindle poisoning (Rank 2003). *E. fetida's* chlorogocyte cells and intestinal microorganisms can detoxify the genotoxicity of wastes (Srivastava et al. 2005). According to the findings of this study, initial feed mixtures of *Mikania* waste have cytotoxic/genotoxic potential that decreases at the end of vermicomposting. The results also revealed that the increase in root length and mitotic index and the decrease in chromosome aberrations in the post vermicompost extracts indicate that *E. fetida* can detoxify *Mikania* toxicity.

Table 6.8. Genotoxicity assessment of *M. micrantha* extract and compost extract during various phases of VC process

	Concentration	Micro nucleated cells	Multipolar telophase	Chromosoma l bridge	C-mitosis	Laggard chromosome	Chromosom al loss	Sticky chromosome	Aberrant cells (%) (Mean ± SD)
Tap water (Control)	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
<i>Mikania extract</i>	25	20.1 ± 1.1	4.7 ± 0.3	20.7 ± 1.31	17.4 ± 1.2	12.4 ± 1.2	8.7 ± 1.0	7.8 ± 1.4	9.70 ± 2.1
	50	23.1 ± 1.2	7.4 ± 0.1	25.8 ± 0.9	20.1 ± 0.1	20.1 ± 2.4	15.7 ± 2.4	10.2 ± 0.9	12.4 ± 1.8
	75	25.7 ± 1.2	9.5 ± 0.2	28.7 ± 0.8	22.4 ± 0.8	18.7 ± 4.0	18.1 ± 4.1	12.7 ± 2.1	15.0 ± 4.2
	100	22.1 ± 2.1	10.5 ± 0.3	30.1 ± 1.2	25.8 ± 1.2	25.4 ± 3.1	22.7 ± 2.7	15.4 ± 1.4	16.0 ± 4.8
VC (D0)	25	9.87 ± 1.8	5.8 ± 1.0	7 ± 0.8	19.8 ± 2.4	4.1 ± 0.5	20.4 ± 1.1	4.1 ± 0.9	7.0 ± 0.4
	50	10.1 ± 3.1	7.1 ± 1.1	7.8 ± 1.0	20.1 ± 2.7	5.1 ± 0.7	28.7 ± 2.9	4.7 ± 1.0	8.4 ± 1.1
	75	11.8 ± 1.8	8.2 ± 2.7	8.9 ± 1.5	22.4 ± 2.7	5.8 ± 0.2	30.1 ± 1.7	9.8 ± 1.1	10.1 ± 2.5
	100	12.5 ± 1.5	9.2 ± 1.4	11.2 ± 1.7	24.5 ± 1.8	6.1 ± 0.1	32.1 ± 2.5	12.5 ± 2.4	11.1 ± 1.8
VC (D30)	25	9.12 ± 2.8	2.7 ± .8	8.7 ± 2.0	13.47 ± 2.4	4.1 ± 0.8	2.1 ± 0.9	2.2 ± 0.8	4.7 ± 1.0
	50	10.1 ± 2.7	3.8 ± 0.9	9.8 ± 0.8	15.87 ± 3.4	5.5 ± 0.09	3.8 ± 0.5	2.5 ± 0.5	5.4 ± 1.1
	75	11.1 ± 2.4	4.7 ± 0.5	9.9 ± 0.6	16.7 ± 1.5	6.8 ± 1.0	4.2 ± 0.7	3.1 ± 0.1	5.8 ± 0.7
	100	12.4 ± 1.5	7.4 ± 0.7	10.7 ± 1.0	16.9 ± 2.7	6.1 ± 0.8	4.0 ± 0.6	3.2 ± 0.1	6.5 ± 0.8
VC (D60)	25	5.5 ± 2.4	1.7 ± 0.5	1.2 ± 0.7	6.4 ± 1.8	2.5 ± 0.2	2.1 ± 0.2	1.0 ± 0.05	2.0 ± 0.2
	50	6.1 ± 1.8	2.8 ± 0.6	1.8 ± 0.2	6.5 ± 0.6	2.8 ± 0.3	2.5 ± 0.1	1.1 ± 0.4	2.6 ± 0.1
	75	7.2 ± 0.8	3.4 ± 0.2	2.1 ± 0.1	7.1 ± 0.5	3.1 ± 0.4	3.1 ± 0.7	2.1 ± 0.1	3.3 ± 1.0
	100	8.1 ± 0.9	4.1 ± 0.7	2.2 ± 0.2	8.2 ± 0.2	4.1 ± 0.9	3.0 ± 0.1	2.5 ± 0.2	3.6 ± 0.9

6.3 PART-III: RESULTS AND DISCUSSIONS OF THE TOXICITY ASSESSMENT OF THE ROTARY FOLLOWED BY VERMICOMPOST (RVC)

6.3.1 Phytotoxicity evaluation of rotary drum followed by vermicompost

Environmental toxicity assessment of samples using a plant seed germination test is considered one of the simplest short-term methods (Wang, 1985). Seed germination is a very sensitive process likely to be disturbed by inhibitory substances in the growing environment. In the present study, mung bean seeds were germinated in different concentrations of *Mikania* and compost extracts. The number of seeds germinated in each concentration was observed. The effect of different concentrations of compost samples on early seeding growth (5 days) is apparent in Fig. 6.7.

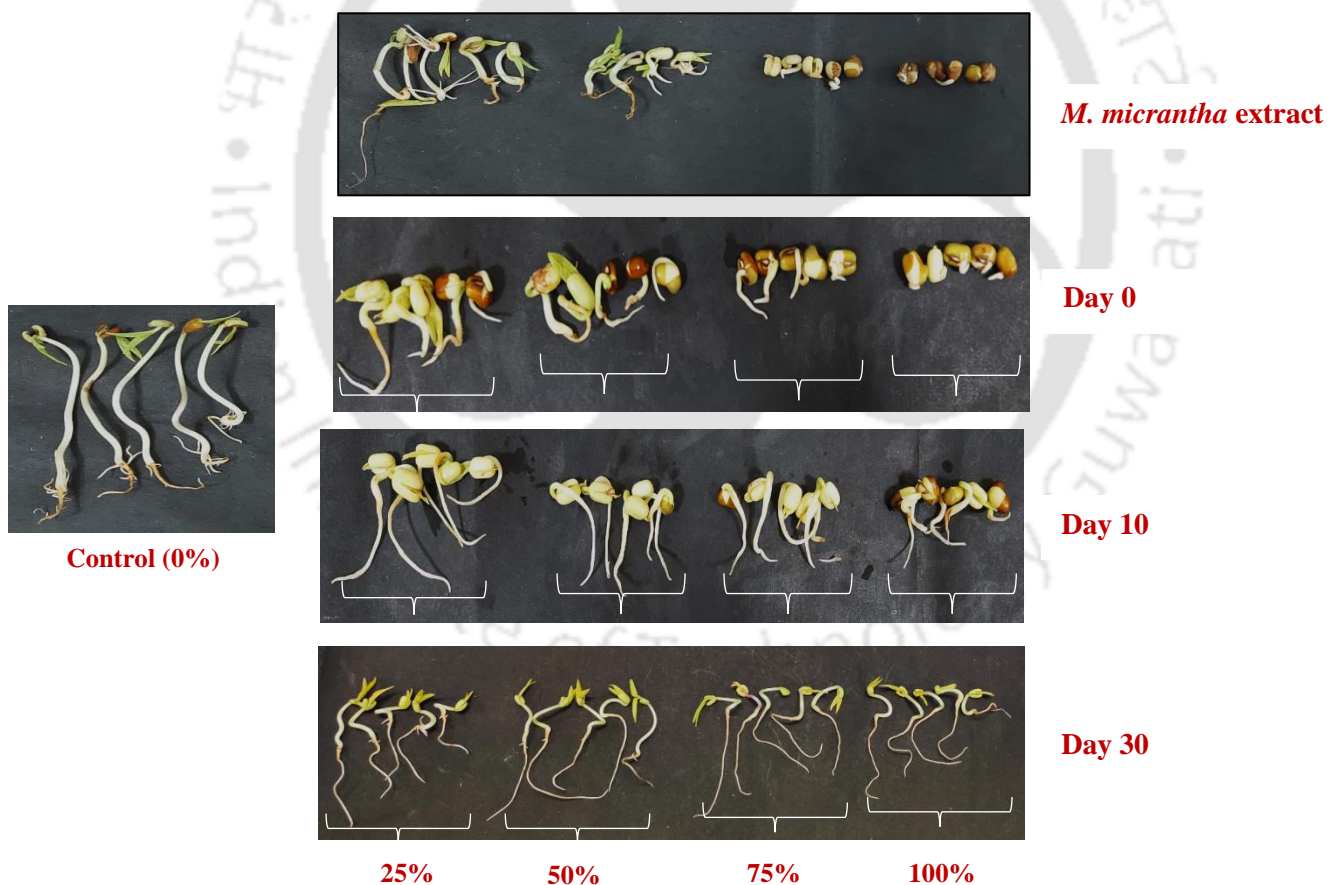


Fig. 6.7 Germination Index % of Mung bean (*Vigna radiata*) during RVC process

The result indicates no seed germination inhibition observed up to 50 % (v/v) compost concentration. *Mikania* extract and Day 0 compost extract exhibited the highest inhibition of mung bean compared to control, as shown in Table 6.9. Compared to controls, root lengths of 5-day-old seedlings were highest at 100 % (6.7 ± 0.01 cm) concentration of Day 30 vermicompost sample. The shoot length for the same was recorded as 3.61 ± 0.2 cm. As the pre-degraded sample was vermicomposted after rotary drum composting, significant changes in seedling growth were observed. The pre-degraded substrate significantly affected the vermicomposting process as acclimatization takes more time for earthworms. Compared to control, more growth was observed in vermicompost, which may be due to the nutrient availability. The significant reduction in root length, shoot length, and biomass of seedling at 100 % *Mikania* and Day 0 extract may be correlated with the cumulative effect of excess amount of EC, BOD, COD, and allelochemicals present. This observation confirms the results obtained by others working on various crop plants (Medhi et al., 2011; Dutta and Boissya, 1997).

Table 6.9. Phytotoxicity test results of *Vigna radiata* in *M. micrantha* extract and compost extract during various phases of RVC process

(a) <i>Mikania micrantha</i> extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
Control	100±0.08	3.33±0.01	2.76±0.04	3.12±0.07
25%	83.1±0.04	1.60±0.02	0.98±0.05	0.34±0.08
50%	50±0.02	1.12±0.04	0.66±0.08	0.14±0.06
75%	36.67±0.05	0.78±0.04	0.40±0.06	0.10±0.04
100%	26.67±0.02	0.06±0.00	0.1±0.01	0.09±0.02
(b) Day 0 extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
25%	80.0±1.12	1.0±0.24	0.54±0.21	0.98±0.02
50%	64.4±1.52	0.44±0.15	0.21±0.04	0.82±0.04
75%	46.0±2.1	0.32±0.08	0.18±0.01	0.68±0.04
100%	32.0±1.41	0.21±0.07	0.09±0.01	0.51±0.01

(c) Day 10 extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
25%	70±1.12	5.46±0.05	3.86±0.05	1.12±0.02
50%	64.5±2.41	4.53±0.04	2.64±0.04	1.71±0.04
75%	56.1±1.54	3.33±0.02	2.71±0.03	1.09±0.01
100%	46.0±1.28	4.19±0.01	3.6±0.02	1.10±0.05
(d) Day 30 extract				
Conc (v/v)	GI	Root Length (cm)	Shoot Length (cm)	Biomass (g)
25%	93.33±0.2	3.18±0.01	2.53±0.04	2.21±0.02
50%	96.7±0.4	4.06±0.02	3.27±0.1	2.29±0.03
75%	87.5±0.08	4.55±0.02	3.44±0.04	2.32±0.04
100%	100±0.4	6.7±0.01	3.61±0.2	2.45±0.02

The effect of the growth of *A. cepa* was observed in the aqueous extract of *Mikania* and RVC aqueous extract after 5 days of incubation at $20 \pm 2^\circ\text{C}$ (Izhar Haq et al., 2016). The result of *A. cepa* growth in different concentrations of *M. micrantha* and RVC extract is shown in Fig 6.8. Inhibition of root growth was observed in the aqueous extract of *Mikania* as the concentration increased. The average root length of *A. cepa* was 1.5 cm, 1.0 cm, 0.2 cm and 0.1 cm in 25 %, 50 %, 75 % and 100 % concentration of *Mikania* extract. There was a decreasing trend in the growth of onion as the concentration of *Mikania* increased. The growth was found to increase in RVC extract, as shown in Table 6.10. At Day 10 of the rotary drum composting process, sample extract showed an increase in the growth of the onion bulb. The average growth was recorded as 6.0 cm, 6.25 cm, 5.50 cm and 5.25 cm in 25 %, 50 %, 75 % and 100 % concentration. Whereas average root length was found to be 8.75 cm, 10.05cm, 9.85cm and 10.35 cm in 25 %, 50 %, 75 % and 100 % concentration of RVC (Day 30) extract. The reduction of root length was observed beyond 50 % of *Mikania* extract while there was an increasing trend in root length of onion in RVC extract. Thus, the dosage applied determined the toxicity of composts, and all composts were deemed non-toxic because germination rates exceeded 50 % (Toundou et al., 2021).



Fig. 6.8. Phytotoxicity assessment test using *Allium cepa* during RVC process

The assessment of phytotoxicity concluded that the *M. micrantha* extract was detrimental to the growth of *V. radiata* and *A. cepa*. But it showed excellent results for developing *V. radiata* and *A. cepa* when the weed is degraded for 10 days before vermicompost. Thus, the dosage applied determined the toxicity of composts, and all composts were deemed non-toxic because germination rates exceeded 50 % (Toundou et al., 2021). The assessment of phytotoxicity concluded that the *M. micrantha* extract was detrimental to the growth of *V. radiata* and *A. cepa*. But it showed excellent results for the development of *V. radiata* and *A. cepa* when the weed is vermicomposted.

Table 6.10. Phytotoxicity test results of *Vigna radiata* in *M. micrantha* extract and compost extract during various phases of RVC process

(a) Root lengths of <i>Allium cepa</i>				
Concentration	Raw <i>Mikania</i> extract	Day 0	Day 10	Day 30
Control		8.5±0.2		
25%	1.5 ± 0.05	2.25 ± 0.03	6.0 ± 0.03	8.75 ± 0.05
50%	1 ± 0.04	1.7 ± 0.04	6.25 ± 0.02	10.05 ± 0.07
75%	0.2 ± 0.02	2.1 ± 0.05	5.50 ± 0.01	9.85 ± 0.05
100%	0.1 ± 0.02	1.5 ± 0.07	5.25 ± 0.01	10.35 ± 0.04
(b) Biomass Index of <i>Allium cepa</i>				
Concentrations (v/v)	Raw <i>Mikania</i> extract	Day 0	Day 10	Day 30
Control		1.1 ± 0.02		
25%	0.09 ± 0.01	0.34 ± 0.01	0.88 ± 0.02	1.04 ± 0.01
50%	0.13 ± 0.02	0.21 ± 0.01	0.91 ± 0.04	0.93 ± 0.06
75%	0.12 ± 0.05	0.19 ± 0.02	0.75 ± 0.01	1.07 ± 0.05
100%	0.09 ± 0.07	0.18 ± 0.01	0.62 ± 0.03	1.12 ± 0.02

6.3.2 Cytotoxicity and genotoxicity assessment using *A. cepa*

Mitotic Index is important indicators in environmental pollution monitoring, particularly for assessing contaminants with toxic and cytotoxic potential (Hoshina, 2002). Smaka-Kincl et al. (1996) demonstrated that a decrease in the MI of *A. cepa* meristematic cells could be used to determine the presence of cytotoxic agents in the environment and, as a result, can be used as a sensitive test to estimate pollution levels. It was evident that the reduction in MI of *A. cepa* in CH samples was due to increased concentrations, as shown in Table 6.11. The MI of the negative control with dechlorinated water was 72.15 %. The MI in the RVC samples from D0, D20, and D30 at 100 % concentration was 38.71, 41.58, and 54.87 % respectively. The outcome was similar to that of the vermicomposting process (D60). During the vermicomposting process, earthworms degraded waste material over a longer period. The reactor received fresh waste, and the earthworm required more time to acclimate and degrade the substrate. However, when it was pre-degraded using a rotary drum for 10 days, the degradation was greater, and after 20 days of vermicomposting with *E. fetida*, the final product showed a similar result in 100 % concentration. The comparison was even better with rotary drum compost (D20); the MI in the RDC sample was 64.27-39.36 %, whereas the MI in the RVC sample showed in the range of 65.5-54.87 % for 25,

50, 75, and 100 % concentration. CA is defined by changes in either chromosomal structure or total chromosome number, which can occur spontaneously and as a result of exposure to physical or chemical agents (Russel, 2002). Several factors, including DNA breaks, inhibition of DNA synthesis, and replication of altered DNA, can cause structural chromosomal alterations. The numeric CA, such as aneuploidy and polyploidy, results from abnormal chromosome segregation, which can occur spontaneously or as a result of aneugenic agents (Albertini et al., 2000).

Table 6.11. Cytotoxicity assessment (Mitotic index %) of *M. micrantha* extract and compost extract during various phases of RVC process

	Concentration (%)	Total cells	Dividing cells	MI (%) (Mean ± SD)
Control	0	887	639	72.04 ± 4.57
<i>Mikania</i> extract	25	947	418	44.13 ± 5.12
	50	982	318	32.38 ± 2.45
	75	898	234	26.05 ± 0.87
	100	946	214	22.62 ± 1.24
	RVC (Day 0)	25	989	546
	50	1001	487	48.65 ± 4.12
	75	987	405	41.10 ± 2.58
	100	925	358	38.71 ± 1.98
RVC (Day 10)	25	957	562	58.72 ± 4.12
	50	897	462	51.42 ± 2.47
	75	1024	499	48.74 ± 1.41
	100	987	410	41.58 ± 2.01
RVC (Day 30)	25	936	614	65.5 ± 1.87
	50	975	598	61.41 ± 2.11
	75	958	562	58.70 ± 1.87
	100	975	535	54.87 ± 1.54

The occurrence of chromosomal abnormalities was more prominent in 100 % concentration of *Mikania* extract after 24 h of exposure, with the mild frequency of aberrations observed after 24 exposures in vermicompost extract as shown in Table 6.12. The major CA was noted as micronucleated cells, multipolar telophase, c-mitosis, chromosomal bridge, laggard chromosome, chromosomal loss, and sticky chromosome, as shown in Fig 6.3. CA, such as chromosome bridges and breaks, indicate a clastogenic action, whereas chromosome losses, delays, adherence, multipolarity, and C-metaphases indicate an aneugenic action (Leme and Marin-Morales, 2009). CA of the composting process at D0 was observed in the range of 7.3-12.0 % as the sample concentration

increased from 25-100 %. The highest amount of cellular and nuclear abnormalities were observed in the sample. Micronucleated cells can still be formed by other processes such as polyploidization, which results from the removal of excess DNA from the main nucleus in an attempt to restore normal ploidy conditions (Fernandes et al., 2009). According to Leme et al. (2008), micronucleus size can be an effective parameter for assessing clastogenic and aneugenic effects in *A. cepa* because this species has a symmetric karyotype that is homogeneous in terms of chromosomal size, with large and few chromosomes ($2n = 16$). As a result, a large micronucleus would indicate an aneugenic effect caused by chromosome loss, whereas a small micronucleus could indicate a clastogenic action caused by chromosome break which can be seen in the current study. CA effect on the cell of *A. cepa* upon exposure of D30 vermicompost sample was in the range of 3.0-3.8 % as the concentration of the sample increased from 25-100 %. There was clear evidence that the pre-degraded substrate aided earthworms in decomposing faster than the vermicomposting process, and the toxicity effect was also reduced.

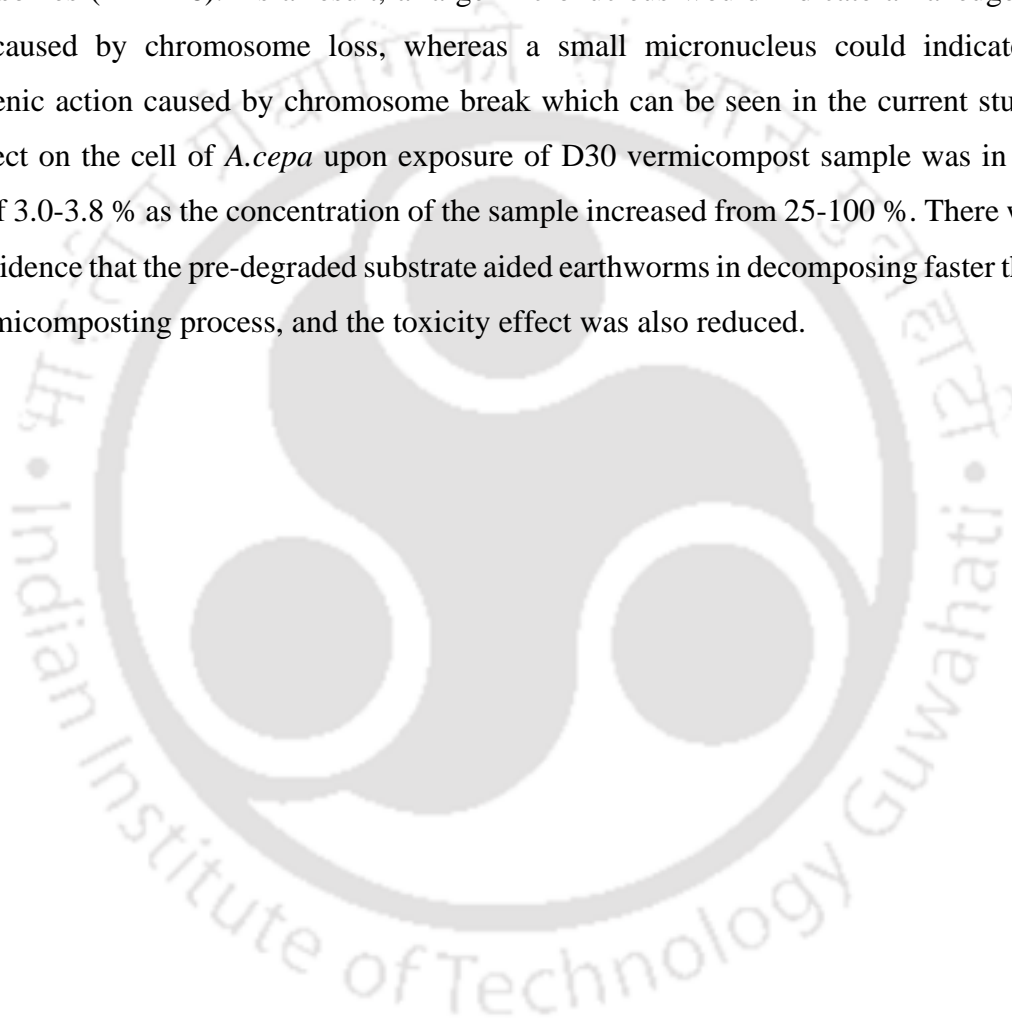


Table 6.12. Genotoxicity assessment of *M. micrantha* extract and compost extract during various phases of RVC process

	Concentration	Micro nucleated cells	Multipolar telophase	Chromosomal bridge	C-mitosis	Laggard chromosome	Chromosomal loss	Sticky chromosome	Aberrant cells (%) (Mean \pm SD)
Tap water (Control)	0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Mikania extract</i>	25	20.1 \pm 1.1	4.7 \pm 0.3	20.7 \pm 1.31	17.4 \pm 1.2	12.4 \pm 1.2	8.7 \pm 1.0	7.8 \pm 1.4	9.70 \pm 2.1
	50	23.1 \pm 1.2	7.4 \pm 0.1	25.8 \pm 0.9	20.1 \pm 0.1	20.1 \pm 2.4	15.7 \pm 2.4	10.2 \pm 0.9	12.4 \pm 1.8
	75	25.7 \pm 1.2	9.5 \pm 0.2	28.7 \pm 0.8	22.4 \pm 0.8	18.7 \pm 4.0	18.1 \pm 4.1	12.7 \pm 2.1	15.0 \pm 4.2
	100	22.1 \pm 2.1	10.5 \pm 0.3	30.1 \pm 1.2	25.8 \pm 1.2	25.4 \pm 3.1	22.7 \pm 2.7	15.4 \pm 1.4	16.0 \pm 4.8
RVC (D0)	25	8.97 \pm 0.9	6.1 \pm 0.75	6.9 \pm 0.7	17.8 \pm 1.2	6.4 \pm 1.1	21.4 \pm 1.0	4.7 \pm 0.7	7.3 \pm 0.8
	50	9.25 \pm 1.1	6.8 \pm 0.88	7.0 \pm 0.5	19.8 \pm 2.7	7.1 \pm 0.9	27.8 \pm 1.7	5.1 \pm 0.65	8.2 \pm 0.57
	75	12.4 \pm 1.2	9.0 \pm 0.9	7.2 \pm 0.2	21.8 \pm 1.8	8.0 \pm 0.7	31.4 \pm 2.5	8.8 \pm 0.7	9.9 \pm 1.0
	100	14.1 \pm 3.4	10.1 \pm 2.4	9.8 \pm 1.1	24.6 \pm 3.4	8.2 \pm 0.7	32.5 \pm 1.9	11.9 \pm 0.69	12.0 \pm 0.87
RVC (D10)	25	9.8 \pm 1.1	4.0 \pm 0.87	9.8 \pm 1.7	17.8 \pm 1.7	1.8 \pm 0.1	18.7 \pm 1.7	7.0 \pm 0.87	7.1 \pm 1.2
	50	10 \pm 0.8	8.1 \pm 1.0	10.0 \pm 1.4	11.8 \pm 1.2	10.5 \pm 0.4	20.2 \pm 0.87	22.5 \pm 2.3	10.3 \pm 1.7
	75	11.9 \pm 0.7	8.5 \pm 0.98	9.8 \pm 1.7	9.1 \pm 1.4	10.1 \pm 1.2	15.8 \pm 1.0	19.2 \pm 1.4	8.2 \pm 0.8
	100	12.8 \pm 0.5	10.9 \pm 2.4	10.1 \pm 2.1	12.4 \pm 1.8	11.1 \pm 0.8	20.1 \pm 2.7	20.9 \pm 1.0	9.9 \pm 0.8
RVC (D30)	25	6.1 \pm 0.65	2.1 \pm 0.5	4.1 \pm 0.87	7.1 \pm 1.4	3.7 \pm 0.5	3.5 \pm 0.65	2.0 \pm 0.6	3.0 \pm 0.1
	50	6.5 \pm 0.8	2.7 \pm 0.2	4.8 \pm 0.4	7.2 \pm 1.1	3.8 \pm 0.1	3.8 \pm 0.4	2.4 \pm 0.4	3.2 \pm 0.2
	75	6.9 \pm 1.0	2.8 \pm 0.45	5.0 \pm 0.7	8.1 \pm 0.98	3.7 \pm 0.2	4.0 \pm 0.5	2.9 \pm 0.1	3.4 \pm 0.1
	100	7.8 \pm 0.9	3.9 \pm 0.4	6.1 \pm 0.9	8.8 \pm 0.75	4.0 \pm 0.1	4.8 \pm 0.8	3.2 \pm 0.6	3.8 \pm 0.3

6.4 SUMMARY ON PHASE II

The summary of various toxicity evaluations of the compost produced from three compost technologies has been given below:

- Vermicompost and rotary drum, followed by vermicompost, produced the best results for *Vigna radiata* seed germination and *Allium cepa* root length. The compost had a GI of greater than 50%, which is the recommended value for using compost on the farm. *A. cepa*, on the other hand, grew much faster on 100% vermicompost concentration than RDC and RVC. The phytotoxicity results showed that seed germination and crop germination trends increased during the composting process and vermicomposting, indicating that the allelochemicals and toxic compounds present in *Mikania micrantha* were reduced or transformed during the composting process.
- The cytotoxicity and genotoxicity results revealed that exposure onion root to various concentrations of compost sample resulted in mitotic effect and chromosomal abnormalities. Compared to compost samples, the raw extract of *Mikania* had extremely high chromosomal aberrations. The results showed that the toxic effect of *Mikania micrantha* is more pronounced in rotary drum compost than in vermicompost and rotary, followed by vermicompost because earthworms assisted in the degradation of a greater number of phytotoxic chemicals present in *Mikania micrantha*. Cytotoxicity and genotoxicity are useful parameters for toxicity testing of compost made from noxious weeds.

The chapter investigated different toxicity assessments on the compost that was produced, which demonstrates quite clearly that it is safe to utilise the compost for plant study. All of the compost samples were found to have GI values more than 50%, which is the minimum acceptable level for incorporation into soil. The application of composts in varied proportions with soil in pot using test plant models will be covered in the next chapter.

Amendment of compost with laterite soil for the plant model *Abelmoschus esculentus* (Lady's finger)

This chapter enlightens the use of *Mikania* biomass for the production of compost and vermicompost and the monitoring of the growth of *Abelmoschus esculentus* (Okra) post the compost application. The current study mainly focuses on the compost produced using the in-vessel technique within 20 days, vermicompost produced in 60 days using *Esenia fetida*, and rotary followed by vermicompost production using in-vessel composter for 10 days for pre-degradation of the waste followed by vermicompost using *Esenia fetida* for the next 20 days. Eventually, a comparison study was carried out for plant growth and fruit yield using compost produced by three compost technologies.

7.1 IMPACT OF RDC COMPOST ON MORPHOLOGICAL FEATURES OF TEST PLANT *A. esculentus* (Okra)

7.1.1 Early growth and germination percentage

Daily observations were made on germination emergence for 10-15 days. A considerable increase in all growth parameters was recorded in RDC treated seeds, compared to control plants' growth parameters after 30 days of germination. Flowering was observed in all of the pots by the fourth week following seeding, as shown in Fig. 7.1, with fruit appearing first in the pots that had been amended with compost. Significant differences

at $p < 0.05$ among seedling growth parameters of okra grown in controls and germination media having various RDC concentrations are shown in Fig. 7.2.



Fig 7.1. Flowering stage of RDC amended pots

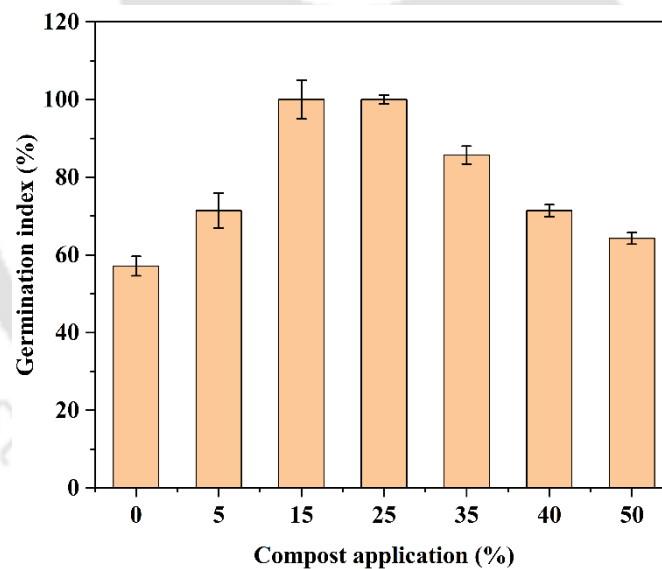


Fig. 7.2. Effect of different treatments of RDC compost on Germination Index

All fruits were of identical height in the first 1-2 days. However, later fruits were longer in pots containing a higher concentration of RDC compost. When comparing RDC compost applications to the control, there was a statistically significant increase in the germination percentage. The application of RDC compost differed with a different concentration on germination of *A. esculentus*. The enhanced nutritional content of RDC compost may be

responsible for the positive reaction in the germination percentage. Within 15 days of the experiment, germination was observed in all the pots except for the control pot. About 100% germination was observed in 25% application of both RDC compost. But as the percentage of RDC was increased gradual decrease in germination was observed at 35%, 40%, and 50%. Several studies have suggested that the germination process of seeds is regulated internally by the seed composition and genotype of the plant species; however, some environmental factors such as water content, temperature, and light are also known to play a significant role in the germination process of seeds (Kucera et al., 2005). In the current study, it was quite evident from much research that *Mikania* exerts a negative effect on soil and has inhibitory properties on vegetation, but RDC compost showed no such antagonism on the germination of *A. esculentus* up to 25% concentration of compost application. Therefore, it can be concluded that the effect of allelopathy has decreased for the weed during rotary drum composting. The phytotoxicity assessment study was carried out in our previous study, which encountered that the extract of *Mikania* weed harms the germination of Moong bean, whereas the weed has a positive effect on the growth of Moong bean after it has been composted

7.1.2 Morphological features of *A. esculentus* (root length, shoot length, no. of leaves, yield, and length of fruit)

The mean no. of leaves, no. of plants per pot, shoot length, root length, and fruit yield have been depicted in Fig. 7.3. *A. esculentus* growth was dramatically increased when RDC compost was administered at lower concentrations of 15 and 25% than 40 and 50%. Though germination of the seed was about 74 and 64% in 40% and 50% RDC amended soil, only 4-5 plants became matured and survived till 120 days. Also, the plants cultivated in soil treated with RDC flowered earlier and produced a greater quantity of flowers than the plants grown in control soil. Nutrients provided in the form of NPK affected the growth of the *A. esculentus* plant. The number of leaves per plant, the shoots' length of the roots, as shown in Fig. 7.4, and the number of fruits increased in correlation with the rise in compost proportion. But at 50% of compost, there was a slight decrease in the number of leaves and fruits per plant. Lowering of the number of leaves was observed after 90 days of the study, which may be attributed to the senescence of leaves (Goswami et al., 2017).

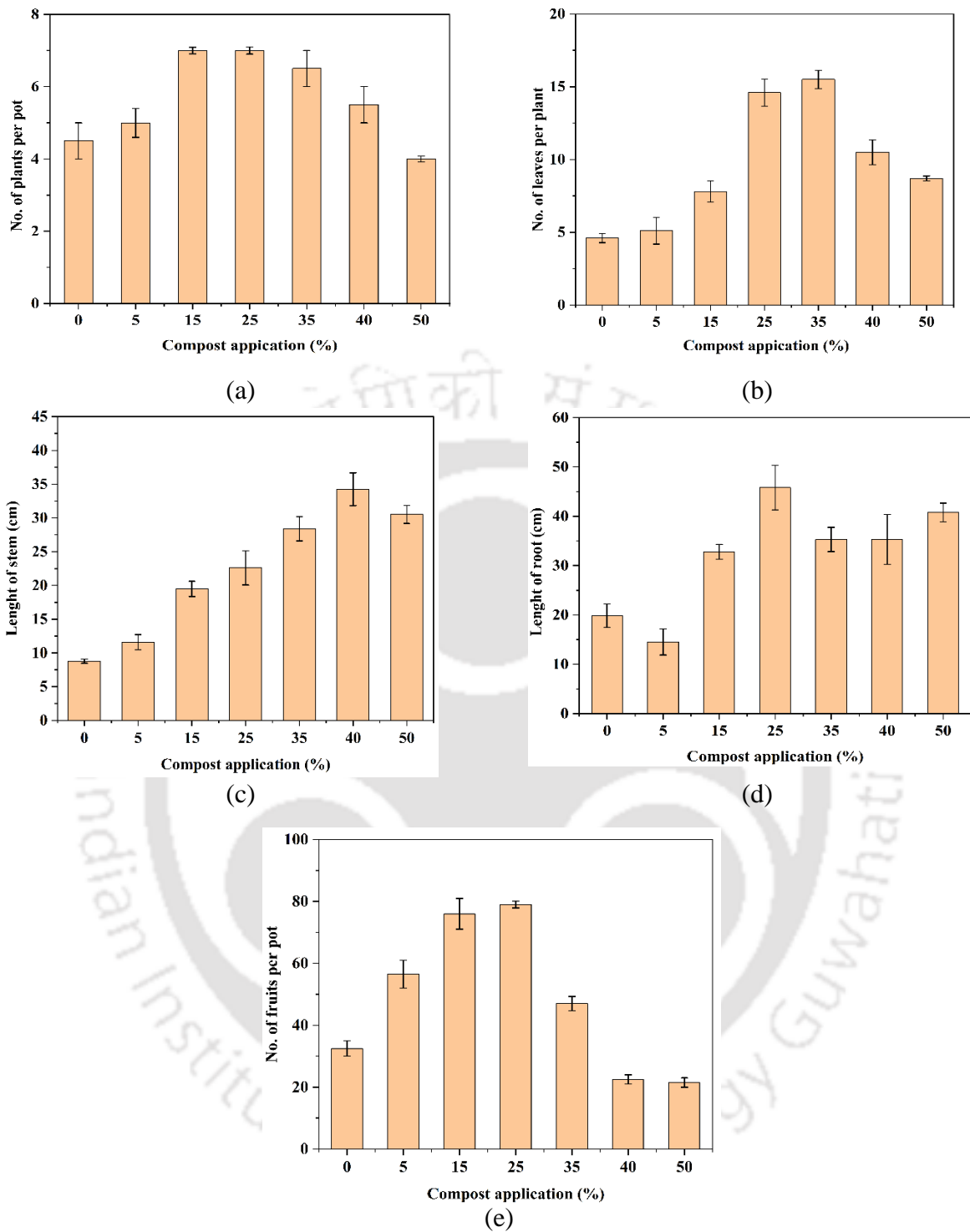


Fig. 7.3. Effect of different treatments of RDC compost on plant morphology a) No. of plants per pot b) No. of leaves per plant c) Shoot length d) Root length and e) Fruit yield

Fruit development started around four weeks after seeding in all pots containing RDC amended soil and non-amended soil for 120 days. There was a significant difference in

the effect of compost on the yield of fruit. Subsequently, for RDC amended soil yield was recorded at 25% (32.5 ± 2.1) as shown in Fig. 7.4. As Maynard (2005) described, a higher yield of fruit is directly proportional to the number of nutrients that the fertilizer contains. The fruit length was comparatively higher in RDC amended soil than control, and the fruit texture was relatively hard when the amendment percentage increased. As the concentration of RDC increased, leaf liner was observed, which is a pest that affects leaves and inhibits the fruit's growth (Patnaik et al., 2020).

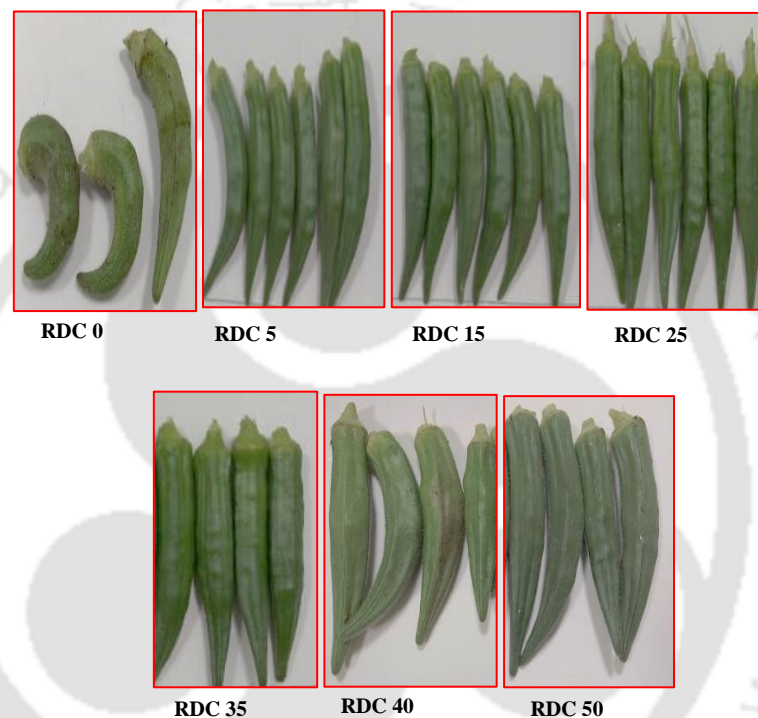


Fig. 7.4. Fruit yield of *A. esculentus* plant after RDC amendment in soil



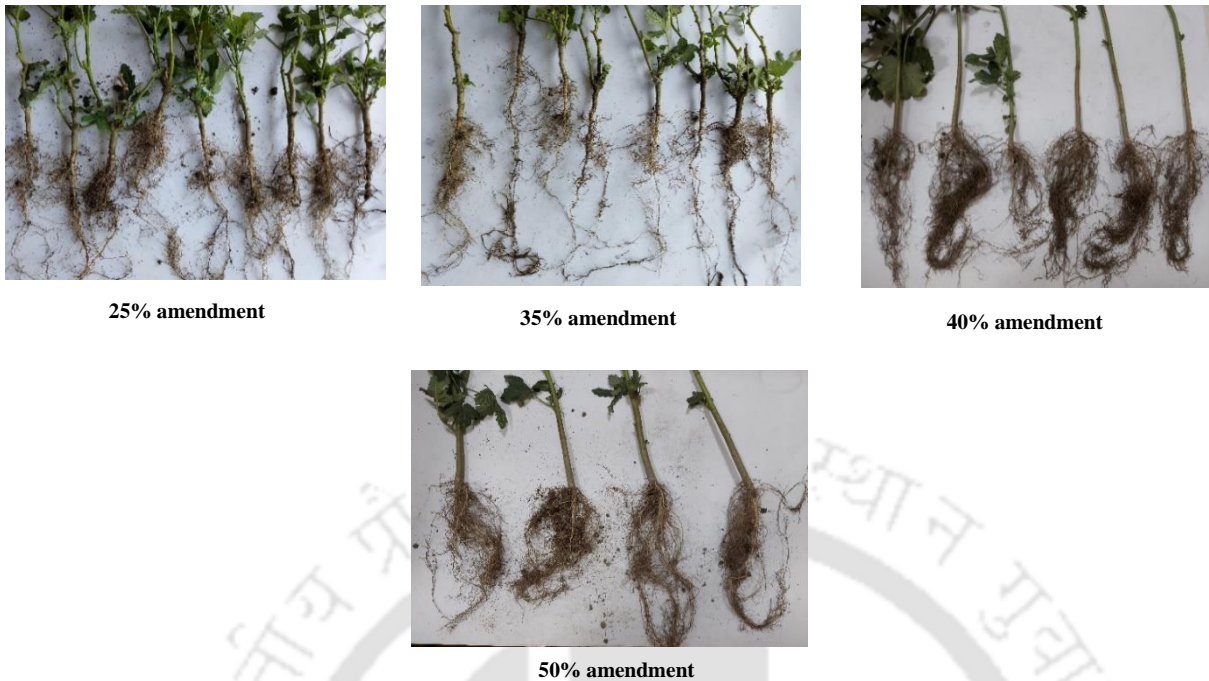


Fig. 7.5. Effect on shoot length and root length after amendment of various concentration of RDC

7.1.3 Macronutrients in the fruit of *A. esculentus*

Substantially significant growth performance was observed when *A. esculentus* was grown with RDC than control. The response of the fruit of *A. esculentus* varied slightly with a significant effect on the growth parameters and nutritional value, as shown in Table 7.1. The changes between the treatments were less at first but became more apparent as the experiment progressed. Following the application of RDC compost on *A. esculentus*, macronutrients such as sodium, potassium, phosphorus, and calcium were recorded in the fruit. RDC amended soil has higher Ca and K at 25% and Na at 35% compost. The fruit of *A. esculentus* is a good source of Ca that helps in bone development and makes less chance of fractures (Grubben and Denton, 2004). As it was observed that the application of RDC on soil did not follow any trend on the increase of nutrients, even higher nutrient content was recorded at 25% and 35% rather than 50%, which supports the data reported by Olaniyi et al., (2010), where author reported a similar trend of highest nutrient content was achieved at an optimum level of fertilizer application. Goldman et al. (1990) reported that crop quality could also be affected by aeration, watering, and other agricultural techniques that tend to affect the nutrient content of the fruit. Singh (2013) explained that P is a component of the energy molecule adenosine triphosphate (ATP) and thus plays an important role in

photosynthesis. Early stimulation of the root system through efficient translocation to the root or certain growth stimulation compounds formed on account of the protoplasmic activity of tops in phosphorus fed plants, when enhanced absorption of nitrogen and other nutrients and their utilization, could explain the beneficial influence of phosphorus in early stages of growth. Similarly, the role of potassium in stomata opening and, as a result, regulating CO₂ entry is well understood. Potassium is important for controlling the water economy in plants and improving drought tolerance. Potassium ions significantly impact relative water content, osmotic potential, and transpiration rate, allowing plants to extract moisture from the soil more efficiently (Mengel et al., 1984).

Table 7.1. Macronutrient content in the fruit of *A. esculentus* post application of compost produced from RDC process

	Na (g/kg)	K (g/kg)	Ca (g/kg)	P (g/kg)
Control	0.24 ± 0.01	1.69 ± 0.04	0.98 ± 0.01	0.35 ± 0.01
RDC (5%)	0.41 ± 0.04	3.12 ± 0.05	1.12 ± 0.04	0.21 ± 0.04
RDC (15%)	0.58 ± 0.07	1.89 ± 0.07	1.42 ± 0.05	0.24 ± 0.01
RDC (25%)	0.41 ± 0.01	1.78 ± 0.02	2.05 ± 0.02	0.31 ± 1.01
RDC (35%)	0.87 ± 0.02	1.21 ± 0.01	1.40 ± 0.04	0.39 ± 0.02
RDC (40%)	0.21 ± 0.00	1.56 ± 0.03	1.87 ± 0.21	0.40 ± 0.04
RDC (50%)	0.41 ± 0.01	1.71 ± 0.01	1.75 ± 0.02	0.51 ± 0.04

7.2 IMPACT OF VC COMPOST ON MORPHOLOGICAL FEATURES OF TEST PLANT *A. esculentus* (Okra)

7.2.1 Early growth and germination percentage

To encounter that *A. esculentus* were treated with vermicompost exhibited early seed germination and an increase in all growth parameters. Our findings were consistent with those of Atiyeh et al. (2000), who reported the benefits of vermicompost as bedding media for promoting seed germination, seedling growth, and plant productivity. Our research found that vermi-compost has a higher NPK level than RDC, which promotes early seed germination and increases all germination and growth parameters. Previous research found

that earthworms increase nitrogen levels and nutrient accessibility to plants, which not only promotes plant growth but also changes the vegetative structure (Tripathi et al., 2013; Jusselme et al., 2013; Mudrak et al., 2012; Ilieva-Makulec and Makulec 2007; Chaoui et al., 2003). The augmentation of the soil with VC enabled significantly greater germination success of lady's finger seeds in comparison to controls: 90-100% seeds germinated in fertilized soil in comparison to only about 57% in the control soil. Flowering was observed in all of the pots by the third week following seeding as shown in Fig. 7.6, with fruit appearing first in the pots that had been amended with VC. However, flowers took longer to appear on control pots than on plots treated with VC. Significant differences at $p < 0.05$ among seedling growth parameters of Okra grown in controls and germination media having various VC concentrations as shown in Fig. 7.7. A number of studies have suggested that the germination process of seed is regulated internally by the seed composition and the genotype of the plant species, however, some of the environmental factors such as water content, temperature, and light also are known to have a definitive role (Kucera et al., 2005; Hussain et al., 2015). Further, several chemicals such as nitrate, ammonium, phenols, methanol, ethanol, acetone, ethyl ether, gibberellins, cytokine etc. singly or in combinations, are known to foster germination (Hussain and Abbasi, 2018). All these compounds are contained in the VC derived from organic substrates (Hussain and Abbasi, 2018). The present studies also indicate that VC may be acquiring similar attributes which led to be enhancement in the germination success of lady's finger. 100% germination of seed was observed in the pot amended with 25% and 35% of VC concentration as compared to control, 5%, 15%, 40% and 50%. Number of plants survived till 120 days were highest in almost all the reactors except the control. Shafique et al. (2021) reported that the significantly increased plant growth is due to the highly porous texture of vermi-compost and rich in nutrients and minerals, which play important role in the enhancement of physiochemical properties of soil, have a great impact on the movement of nutrients, water, and air to stimulate the plant growth and have a significant effect on the absorption efficiency. It has been well recorded by Gajlakshmi and Abbasi (2002), who employed water hyacinth as their compost material to do a comparison between compost and vermicompost. According to the author, VC exhibited more significant growth as compared to compost. The decrease of germination for RDC may be attributed to more C/N than that of VC as decomposition of organic matter is more in VC than that of RDC (Abdelhamid et al. 2004; Ojo et al. 2014).



Fig. 7.6. Flowering stage of VC amended pots

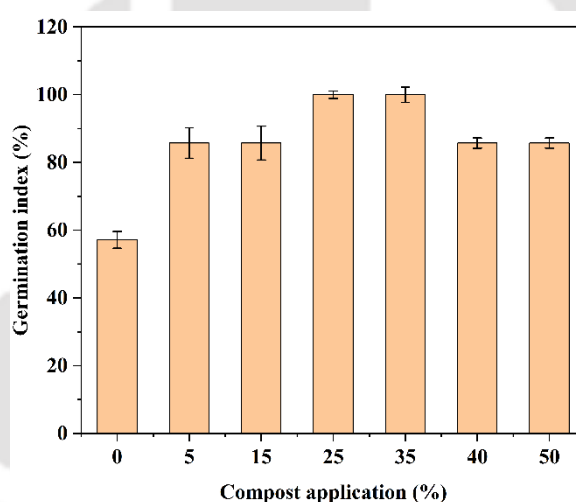


Fig. 7.7. Effect of different treatments of VC compost on Germination Index

7.2.2 Morphological features of *A. esculentus* (root length, shoot length, no. of leaves, yield, and length of fruit)

The mean number of leaves, number of plants per pot, shoot length, root length, and fruit yield have been depicted in Fig. 7.8. The effect of compost extract processing parameters on the number of leaves of the vegetables is presented in Fig. 7.8(a). *A. esculentus* with vermicompost fertigation produced significantly higher leaves (10 per plant) than control (4 per plant). The highest number of leaves was recorded for 35% VC treated soil, while the lowest was observed at 5% VC treated soil. The number of leaves of *A.esculentus* plant increased with increased VC amendment but decreased with compost.

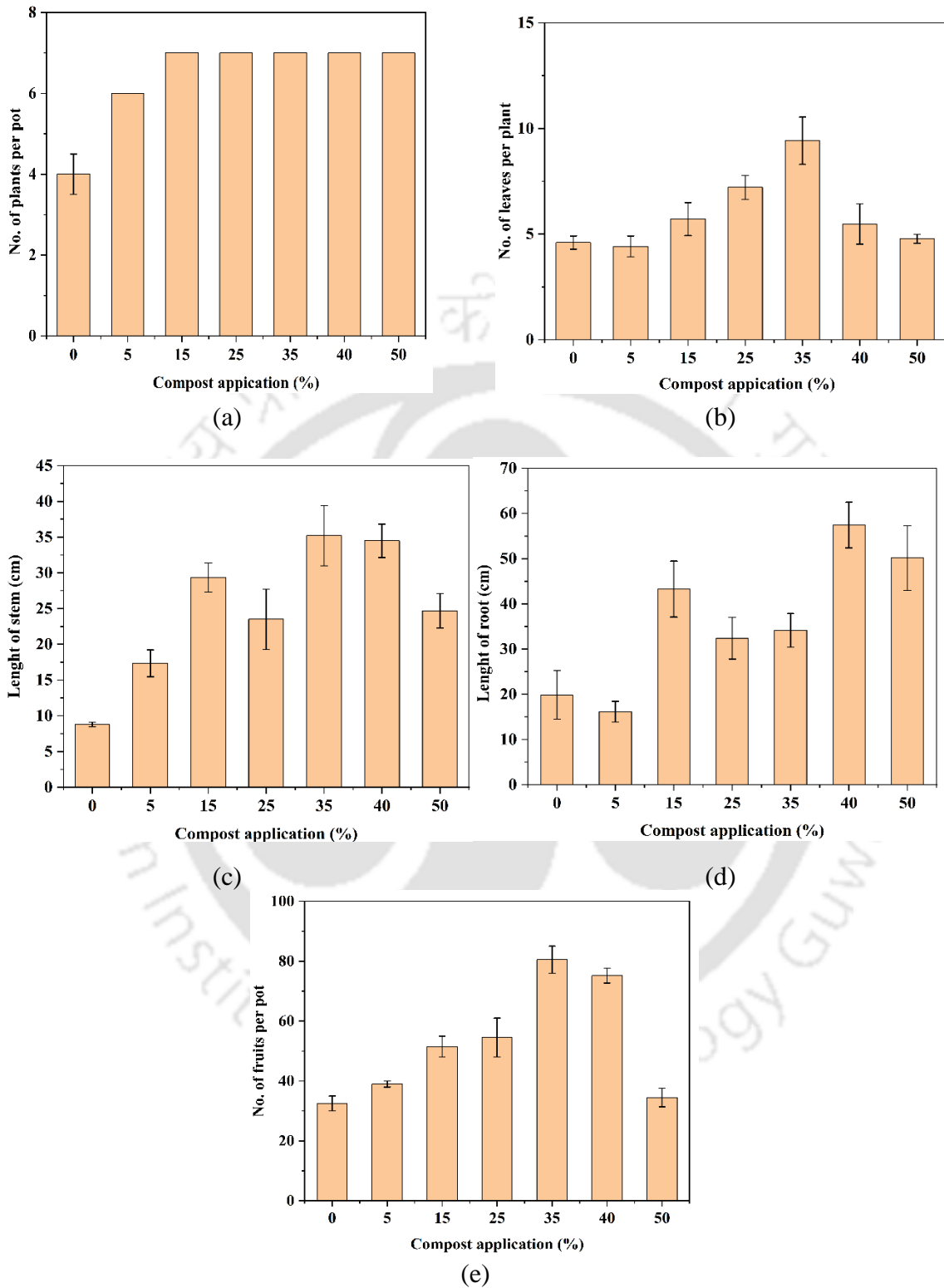


Fig. 7.8. Effect of different treatments of VC compost on the plant morphology a) No. of plants per pot b) No. of leaves per plant c) Shoot length d) Root length and e) Fruit yield



Fig. 7.9. Fruit yield of *A. esculentus* plant after VC amendment to soil

The study revealed 30% vermicompost concentration showed maximum seedling growth, i.e., no. of leaves, no. of plants per pot, shoot length, root length, and fruit yield. Makkar et al. (2017) reported that substituting soil with 60% (v/v) vermicompost improved the performance of *Linum usitassimum* seeds performance and morpho-physiological parameters of seedling compared to control treatments. Lowering of no. of leaves was observed after 90 days of the study, which may be attributed to the senescence of leaves (Goswami et al., 2017). The increased morphological parameters upon VC application may be attributed to plant stimulating hormones present in it (Singh and Varshney, 2013). The yield of *A. esculentus* fruit had a curvilinear relationship with the amendment of VC (Fig. 7.8 (e)). VC amended soil slightly increased the number of fruit, and the highest value was recorded at 35% (92 ± 5.65). There was a significant difference in the effect of both fertilizers on the yield of fruit. As Maynard (2005) described, a higher yield of fruit is directly proportional to the number of nutrients that the fertilizer contains. In the current study, NPK was highest in VC than that of RDC, and also highest TOC reduction was observed in VC (Ojo et al., 2014), which may have been attributed to the improved growth of vegetative plants.

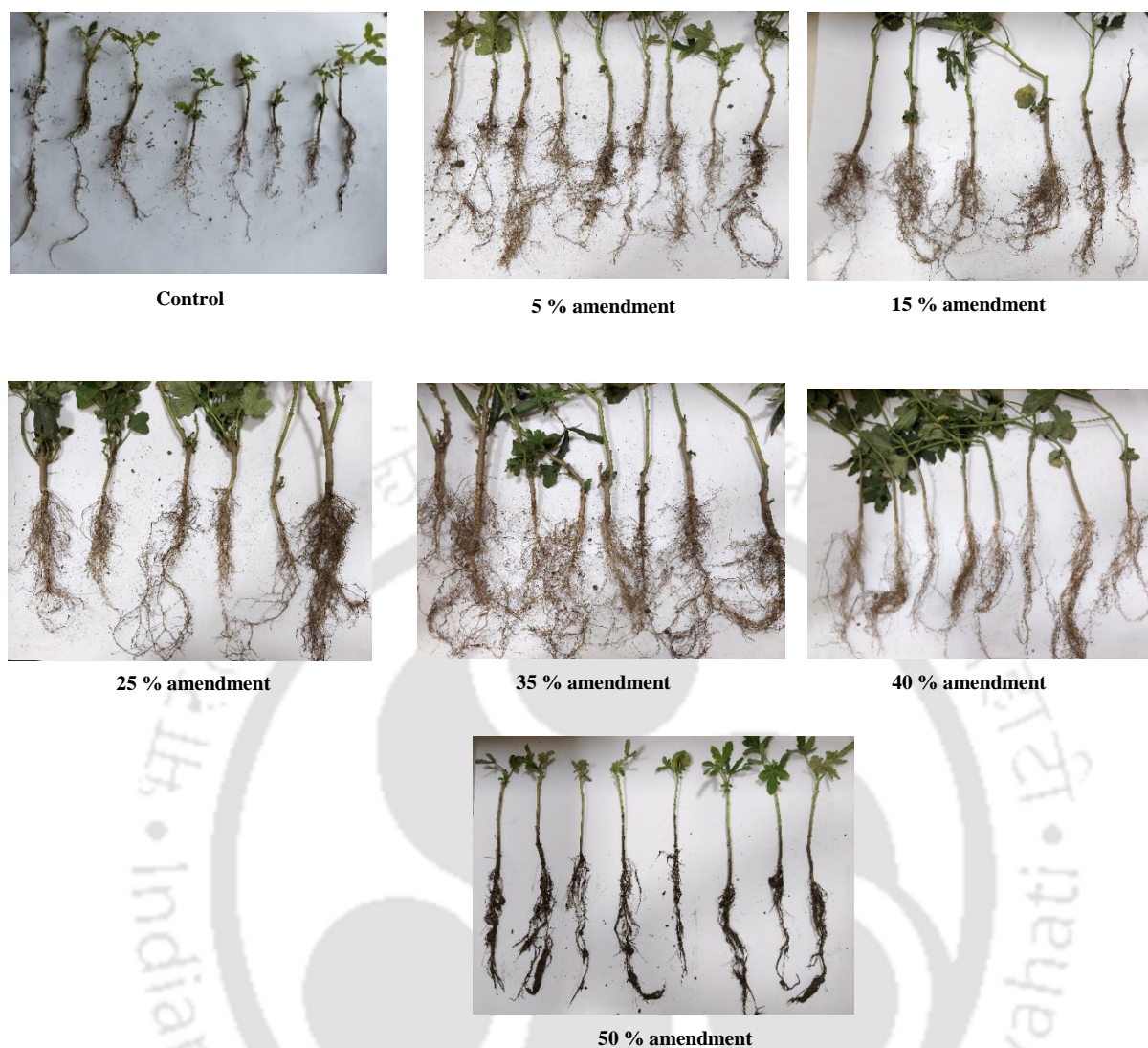


Fig. 7.10. Effect on shoot length and root length after the amendment of various concentrations of VC

7.2.3 Macronutrients in the fruit of *A. esculentus*

The findings of this study revealed that VC treatments had a significant impact on okra quality, as shown in Table 7.2. As the concentration of VC increased, the overall quality of okra improved, including nutrients like potassium, phosphorus, sodium, and calcium. The trend of nutrient potassium in the fruit of VC amended soil was $VC_{40\%} > VC_{35\%} > VC_{50\%} > VC_{25\%} > VC_{15\%} > VC_{5\%} > \text{Control}$. Potassium is an essential nutrient for plant growth, and vermicompost contains an abundance of it (Mondal et al., 2015), which aided in its increase in the fruit. Colpan et al., (2013) discovered similar results on improving potassium in the fruit of tomato after vermicompost application. Calcium was found to be increased after 50

% amendment of VC compared to other treatments. The trend of Ca follows in the order VC_{50%} > VC_{40%} > VC_{35%} > VC_{25%} > VC_{15%} > VC_{5%} > Control. It was observed that the application of VC on soil followed increasing Ca content as the VC concentration increased. Treatment with varying concentrations of VC resulted in higher nutrient uptake, which could be attributed to improved synchronization between nutrient release and uptake in plants. The low nutrient uptake in the control pots could be attributed to the lower yield. The uptake of nutrients increased in the other treatments because the increased availability of nutrients with the application of VC accelerated the physiological and metabolic activities. Furthermore, organic fertilizer may have increased the availability of native micronutrients via chelation (Rathore, 1995), resulting in increased biomass production and uptake. Phosphorus is an essential nutrient that is frequently added to soil to increase crop yield (Sims et al., 2000).

Table 7.2. Macronutrient content in the fruit of *A. esculentus* post application of compost produced from VC process

	Na (g/kg)	K (g/kg)	Ca (g/kg)	P (g/kg)
Control	0.24 ± 0.01	1.69 ± 0.04	0.98 ± 0.01	0.35 ± 0.01
VC (5%)	0.64 ± 0.02	3.87 ± 0.01	2.01 ± 0.41	0.74 ± 0.01
VC (15%)	0.82 ± 0.01	4.02 ± 0.01	2.11 ± 0.25	0.87 ± 0.00
VC (25%)	1.01 ± 0.08	4.28 ± 0.02	2.82 ± 0.12	0.92 ± 0.01
VC (35%)	1.27 ± 0.02	5.06 ± 0.12	3.74 ± 0.40	0.87 ± 0.04
VC (40%)	0.92 ± 0.04	5.24 ± 0.04	3.52 ± 0.87	1.02 ± 0.02
VC (50%)	0.82 ± 0.02	4.78 ± 1.54	4.98 ± 1.01	0.82 ± 0.02

When available P levels are low, microbial biomass and roots may increase the production of phosphatases, which are responsible for P mineralization (Chabot et al., 1996). On the other hand, P uptake depended on plant demand, which in turn depended on N uptake, perhaps more than on soil P availability. The data revealed a significant difference between treatments. The maximum phosphorous (1.02 g/kg) was observed in VC_{40%} treatment, followed by (0.87 g/kg) in VC_{35%} treatment, and the minimum (0.35 g/kg)

in control. The data obtained were consistent with the work of Salvi et al., (2015), who obtained similar results. Since the use of vermicompost, the Na content of okra fruit has increased significantly. The VC_{35%} treatment had the highest Na content of okra, followed by the VC_{25%} treatment, and the control treatment had the lowest Na content of okra. The results demonstrated that the application of vermicompost increased the Na content of okra.

7.3 IMPACT OF RVC COMPOST ON MORPHOLOGICAL FEATURES OF TEST PLANT *A. esculentus* (Okra)

7.3.1 Early growth and germination percentage

Germination is an internally regulated process that is primarily influenced by genotype, though external factors such as light period, temperature, moisture, and the presence of certain chemical compounds can also influence this process, either promoting or inhibiting it (Lazcano et al., 2010). This information is integrated through signaling via multiple hormones that promote or inhibit germination (Finkelstein, 2004). The use of a rotary drum followed by vermicompost (RVC) positively affected *A. esculentus* (Okra) seed germination and plant growth, as shown in Fig. 7.12. It is worth noting that after 15 days of seeding, significant seed germination was observed in the compost amended pots compared to the control. Organic fertilizer acts as a suitable physical medium for seed germination as well as preventing desiccation due to its high water holding capacity, and several studies have shown that the addition of organic manure increases plant cover in burnt soil, reducing the risk of soil erosion (Larcheveque et al., 2005). Even though RVC was produced using both rotary drum composting and vermicomposting techniques, the result was significantly higher than that of rotary drum compost and slightly different from that of vermicompost. The different treatments assayed significantly influenced the germination percentage of the seedlings independently; the addition of RVC to the germination media produced a 100% germination at 35% and 40% concentration of RVC compared to the control treatment. Early flowering was observed in the treatment 25-50 % after 15 days of seeding compared to the control treatment. Compost produced using earthworm species has been shown to stimulate the germination of various ornamental and horticultural plant species, including green gram and tomato plants (Zaller, 2007). Seed germination was found to be lowered at 50% amendment of RVC, and as discussed by Weil and Kroontje (1979), when organic manures above agronomic rates are applied, there might be a release of phytotoxic quantities of NH₃, NO₃, and salts. Okra plant mortality can occur

depending on the type of organic amendment applied. Cautioned that reliance solely on organic inputs for crop production would raise the problem of procurement and toxicity, especially at high application rates (Obi and Ebo, 1995).

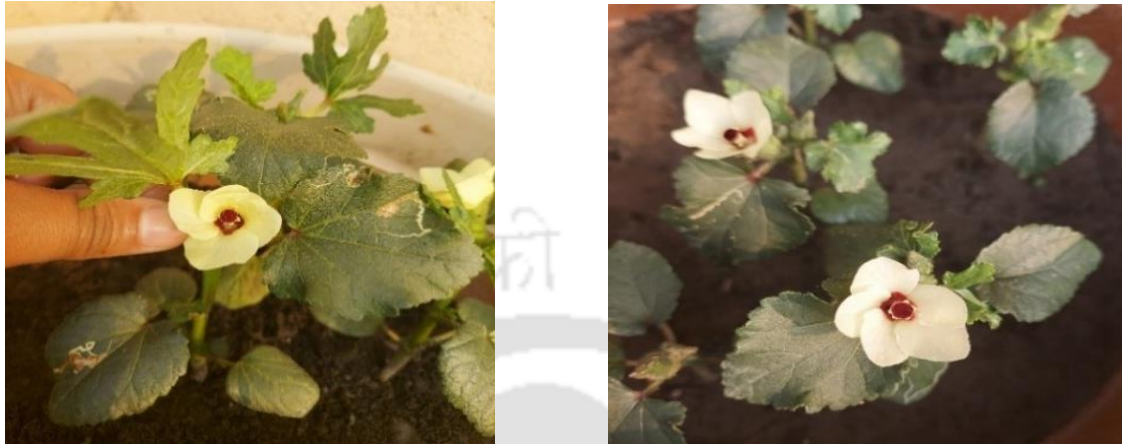


Fig. 7.11. Flowering stage of RVC amended pots

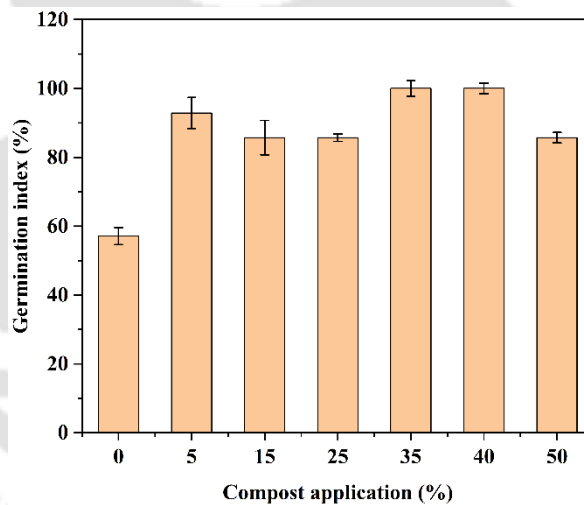


Fig. 7.12. Effect of different treatments of RVC compost on Germination Index

7.3.2 Morphological features of *A. esculentus* (root length, shoot length, no. of leaves, yield, and length of fruit)

According to the findings of this study, different RVC percentages have a significant effect on the growth of okra plants, as shown in Fig. 7.13. Compared to the control treatment, plants that received only RVC compost performed significantly better in growth. This growth enhancement could be due to direct stimulation of growth by improving

nutrient efficiency or indirectly through their effect on plant cation exchange capacity (Epstein et al., 1976).

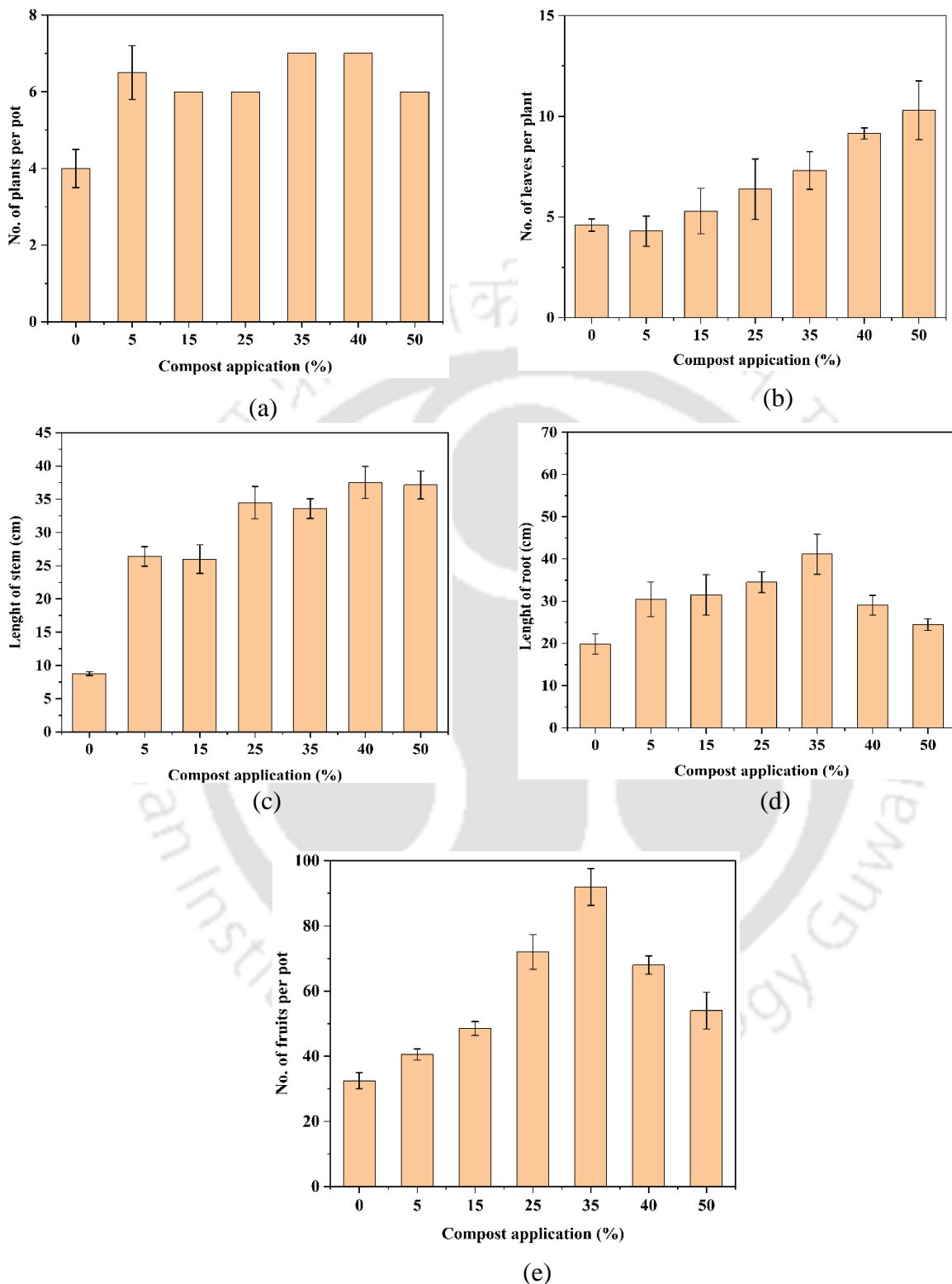


Fig. 7.13. Effect of different treatments of RVC compost on plant morphology a) No. of plants per pot b) No. of leaves per plant c) Shoot length d) Root length and e) Fruit yield

The higher nutrient content percentage in RVC may also have played an indirect role by improving soil fertility, modifying soil physical and chemical conditions, and directly by acting as powerful chelators and increasing nutrient availability to the plants (Pizzeghello et al., 2002; Ingham, 2003). The highest number of leaves was recorded for 50% RVC treated soil, while the lowest was observed 5% RVC treated soil. It was observed that the number of leaves of the *A. esculentus* plant increased with increased RVC amendment compared to control. Fruit yield of *A. esculentus* at various concentration of RVC amended pots have been illustrated in Fig. 7.14.

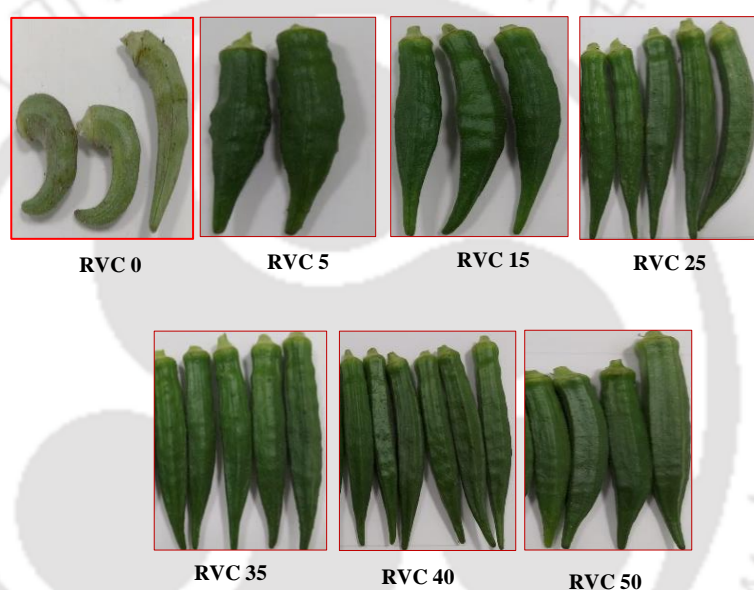


Fig. 7.14. Fruit yield of *A. esculentus* plant after RVC amendment to soil

The maximum average value obtained for fruit yield of 92 at 35% RVC treatment for *A. esculentus* plant as compared to control, which was approx. 32 numbers per pot. The result showed that RVC application has a profound effect on the vegetative development of plants and ensures vigorous growth, hence increasing yield. The higher yield from the pot with the organic fertilizer could be because the nutrients (especially NPK) were readily available (Oyewusi and Osunbitan, 2021). Also, it was observed that fresh yield increased with increasing compost, although at >40%, the yield was found declining. This indicates that sufficient soluble nutrients for marketable vegetable yield can be met in the range of compost used. The highest root length was observed at 35% (41.15 ± 4.75 cm) amended RVC, and the lowest was observed in control (19.87 ± 2.37 cm) (Fig. 7.15). As reported by Balaji et al. (2020), vermicompost has a high humic acid content, which aids in root

development by increasing the effectiveness of the root system and thus increasing plant growth. The entire mechanism was accomplished by increasing the permeability of the cell membrane, which stimulated nutrient uptake. Humic acid also played a beneficial role in developing soil microbial characteristics, resulting in the high production of organic acids and thus an improvement in soil quality. The microbes in the vermicompost may produce plant growth regulators such as auxin, cytokinins, gibberellins, and many metabolites that the plants can use (Gholami et al., 2018). In the current study, treatment doses of 35% RVC compost had a significant effect on plant growth when compared to the control.



Fig. 7.15. Effect on shoot length and root length after the amendment of various concentrations of RVC

7.3.3 Macronutrients in the fruit of *A. esculentus*

The content of Na, Ca, K and P in okra fruit has been illustrated in Table 7.3. All treatments significantly increased the nutrient content in the fruit compared to the control treatment. The maximum content of P in okra fruit was observed in the treatment RVC_{40%} (1.41 g/kg) followed by RVC_{35%} (1.21 g/kg), and the minimum was observed in the control treatment (0.35 g/kg). Okra fruit is a traditional valuable nutrient vegetable that contains potassium and phosphorus, both of which aid in regulating physiological activities in the body. Hossain (1996) found that N and P contents in okra were improved in the combined use of organic fertilizer with N, P, and K. The results indicated that N and P contents were significantly increased in okra due to the application of vermicompost. Similarly, Na content was significantly increased with the application of vermicompost. The highest concentration of Na increased in the treatment RVC_{35%} 1.12 g/kg followed by RVC_{40%} (0.98 g/kg) and lowest in control.

Table 7.3. Macronutrient content in the fruit of *A. esculentus* post application of compost produced from RVC process

	Na (g/kg)	K (g/kg)	Ca (g/kg)	P (g/kg)
Control	0.24 ± 0.01	1.69 ± 0.04	0.98 ± 0.01	0.35 ± 0.01
RVC (5%)	0.57 ± 0.00	2.87 ± 0.98	1.87 ± 0.08	0.81 ± 0.02
RVC (15%)	0.67 ± 0.01	3.87 ± 0.45	1.91 ± 0.05	1.01 ± 0.01
RVC (25%)	0.85 ± 0.04	4.05 ± 0.78	2.01 ± 0.01	0.99 ± 0.04
RVC (35%)	1.12 ± 0.11	4.52 ± 0.05	2.85 ± 0.02	1.21 ± 0.01
RVC (40%)	0.98 ± 0.01	5.07 ± 0.84	3.01 ± 0.57	1.41 ± 0.02
RVC (50%)	0.81 ± 0.02	5.11 ± 0.87	2.45 ± 0.08	1.08 ± 0.05

Different treatments maintained available P status slightly higher than the control. In terms of P content, the levels of vermicompost did not differ significantly. The increase in available P content of soil due to vermicompost incorporation can be attributed to both direct P addition and solubilization of native P via the release of various organic acids. As observed by Sharma et al. (2005), a similar improvement in available P status resulted from the use of organic fertilizer. Increasing Ca was observed in the treated pot with vermicompost, and the maximum was observed in the treatment RVC_{40%}, followed by the

treatment RVC_{35%}. K was found higher in the treatment RVC_{50%}, followed by the treatment RVC_{40%}. Gupta et al., (2019) found higher levels of K when organics and fertilizers were used together in the high hills of the North-Western Himalayas. According to Ansari (2008), vermicompost releases nutrients slowly and steadily into the soil, allowing plants to absorb the available nutrients. The use of vermicompost improves nutrient availability for plants while also increasing vegetable productivity.

7.4 SUMMARY ON PHASE III

The following observations have been made from phase III:

- As compared to the control, the experiments showed that the use of *Mikania* composts and vermicomposts has a significant capacity for promoting plant growth and affecting germination rate, shoot length, marketable yield, shoot, and root length. During vermicomposting process, organic wastes are converted into usable form of nutrients by bio-oxidation and stabilization with the synergism of earth worms and microorganisms which makes the end product more nutrient content than that of compost.
- Okra plants grown in 35% vermicompost/soil mixtures and rotary drum compost followed by vermicompost/soil mixtures produced more fruits than rotary drum compost and control. In terms of fruit yield, shoot length, and length, the addition of vermicomposts into soil consistently outperformed by the addition of composts equivalent.
- The use of all three composts may have increased the availability of native micronutrients via chelation, resulting in higher biomass production and uptake. Among these three composts, vermicompost and rotary drum followed by vermicompost were found to be superior in terms of fruit quality, possibly because it added a higher amount of nutrients to the soil, resulting in higher yields and uptakes with higher yields treated plots.
- For the successful growth of the plant *A. esculentus* and increased fruit yield, rotary drum compost of 15 to 25% is recommended, whereas vermicompost and rotary followed by vermicompost of 35% and 40% are recommended.

The chapter presented an overarching perspective on the incorporation of varying amounts of compost into soil for the purpose of promoting plant development. The fact that the experiment in the pot was effective demonstrates that field research may be conducted in the future for varying proportions of compost to soil ratio. In the next

chapter, we will discuss a novel strategy for the breakdown of organic biomass that contains allelochemicals by employing bacterial monoculture. The general microbiological investigation will be covered in the discussion part of the chapter.





Microbial diversity analysis of *Mikania micrantha* compost and bioaugmentation studies during the composting process

This chapter enlightens the microbial diversity study of the compost produced using the best trial of the rotary drum composting process. The metagenomic study has been done on three samples of different phases of the rotary drum composting process which shows the diversification of bacterial community during the process. This work also explains the isolation of laccase-producing bacteria from the rotary drum composting process and the application of the isolated bacterial inoculum for the different composting process.

8.1 METAGENOMIC STUDY ON THE BEST TRIAL OF *MIKANIA MICRANTHA* COMPOST

8.1.1 Microbial succession (DNA) from the best trial

This study focuses on the enumeration and identification of different bacterial communities in the degradation of *Mikania micrantha*. The best combination of waste materials i.e., *Mikania*, cow dung, and sawdust in the proportion 5:4:1 were used for the rotary drum composting process. Three samples (Day 2, Day 10, and Day 20) were collected from three different phases of the composting process.

- **Metagenomic DNA Isolation, Qualitative and Quantitative analysis**

The metagenomic DNA was isolated from the received compost samples by a commercially available Kit (Nucleospin). The qualities of the isolated metagenomic DNA sample were quantified using NanoDrop.

- **Preparation of 2 x 300 MiSeq library**

The amplicon libraries were prepared using Nextera XT Index Kit (Illumina inc.) as per the 16S Metagenomic Sequencing Library Preparation protocol. Primers for the amplification of the bacterial 16S V3-V4 region were designed and synthesized at Eurofins Genomics Lab. Amplification of the 16s gene was carried out. 3µl of PCR product was resolved on 1.2% Agarose gel at 120V for approximately 60 min or till the samples reached 3/4th of the gel.

Table 8.1. Primers used in the present study

Primers	Sequence
16S rRNA F	GCCTACGGGNGGCWGCAG
16S rRNA R	ACTACHVGGGTATCTAATCC

The QC passed amplicons with the Illumina adaptor were amplified using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The amplicon libraries were purified by AMPure XP beads and quantified using Qubit Fluorometer.

- **Quantity and quality check (QC) of the library on Agilent 4200 Tape Station**

The amplified libraries were analyzed on a 4200 Tape Station system (Agilent Technologies) using D1000 Screen tape as per manufacturer instructions.

- **Cluster Generation and Sequencing**

After obtaining the mean peak sizes from the Tape Station profile, libraries were loaded onto MiSeq at an appropriate concentration for cluster generation and sequencing. Paired-End sequencing allows the template fragments to be sequenced in both the forward and reverse directions on MiSeq. The kit reagents were used in the binding of samples to complementary adapter oligoes on the paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

- **QC on Agarose gel**

The metagenomic DNA was isolated from the received soil and water samples by a commercially available Kit (Nucleospin). The qualities of the isolated metagenomic DNA sample were quantified using NanoDrop. The QC passed samples were processed for first amplicon generation followed by NGS library preparation using Nextera XT Index Kit (Illumina Inc.). The QC passed libraries were sequenced on Illumina MiSeq platform using 2 x 300 bp chemistry. The intensity of bands in the gel represents the reproducibility of the DNA extraction method as well as PCR amplicons (Fig. 8.1).

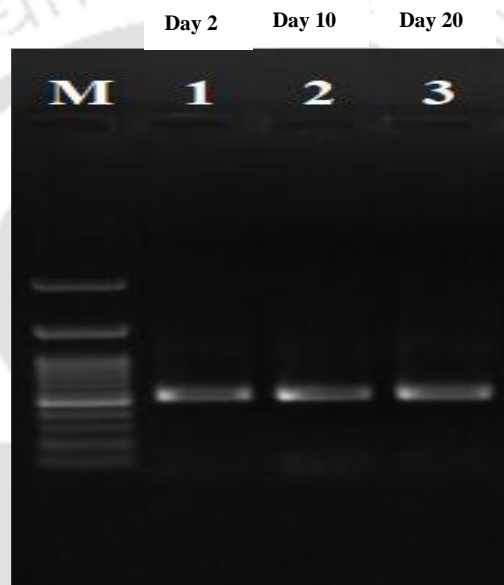


Fig. 8.1. QC of the first amplicon generated on 1.2% Agarose gel

8.1.2 Taxonomic hits distribution

The taxonomic domains, phyla, class, order, family, genus, and species distributions for the annotations are shown below. The predicted proteins and ribosomal RNA genes in the compost sample have been assigned to the appropriate taxonomic level.

- **Comparative Analysis between samples at the phylum level**

16S rRNA sequencing High-throughput sequencing revealed the diversity of the microbial community in two different samples at the phylum level. The microbial community diversity and abundance in Day 2, Day 10, and Day 20 samples are shown in Fig. 8.2 and Table 8.2.

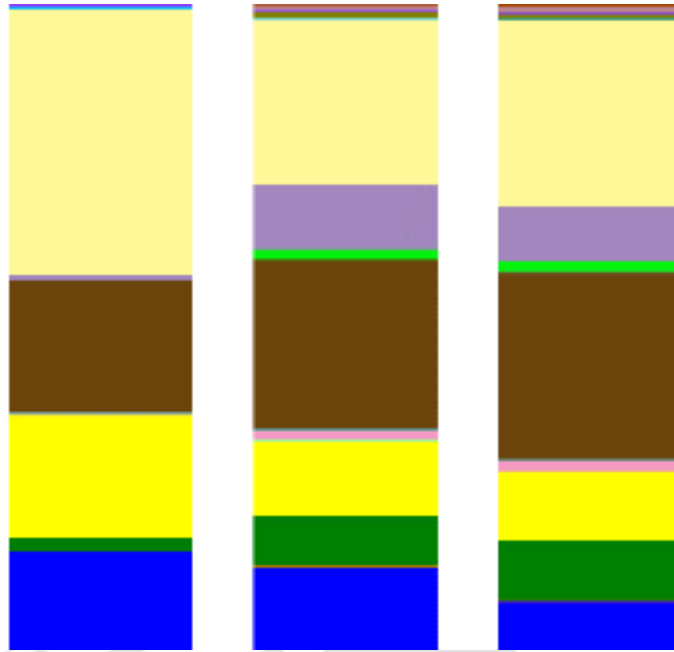


Fig. 8.2 Stacked Bar chart showing the relative abundance of each phylum within each sample

The major bacterial phyla, which are *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Euryarchaeota*, *Chloroflexi*, *Actinobacteria*, etc. were observed in all samples. *Proteobacteria* was the most abundant phylum accounting for 40.98% of all OTUs. *Firmicutes* (18.88%) were the second most frequent group in sample 1 (Day 2). The other biggest groups were *Bacteroidetes*, 15.35% *Euryarchaeota*, etc. as represented in Fig. 8.2. At the initial days of the composting process, *Proteobacteria* and *Firmicutes* are the dominant phyla and in this stage, the temperature was recorded to be $>55^{\circ}\text{C}$. The results are following the previously reported data since *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* are commonly reported bacterial phyla in composting process (Galitskaya et al., 2017; Wei et al., 2018). Phylum *Firmicutes* are known to grow at high temperatures and are widely distributed especially in the thermophilic phase of composting of agricultural biomass (Zhang et al., 2016). Meanwhile, *Actinobacteria* is also considered thermophilic/thermotolerant and also plays important role in terms of the breakdown of organic materials. The *proteobacteria* and *firmicutes* are the most abundant bacterial phyla in the composting process, soil, sediments, and rumen. Cow dung is the primary source of proliferation of these microorganisms in compost (Yamamoto et al., 2011). Most members of *Bacteroidetes* and *proteobacteria* phyla are facultatively or obligately anaerobic and heterotrophic. It has been reported that *Bacteroidetes* are abundant following the

thermophilic stage and play a major role in compost maturation by degrading complex polymers (Neher et al., 2013).

Table 8.2 Relative abundance of each phylum within each sample

Legend	Taxonomy	1(%)	2(%)	3(%)
	k_Bacteria;p_Proteobacteria	41	25.5	28.6
	k_Bacteria;p_Firmicutes	20.4	26.1	28.7
	k_Bacteria;p_Bacteroidetes	18.9	11.5	10.5
	k_Archaea;p_Euryarchaeota	15.4	12.7	7.5
	k_Bacteria;p_Actinobacteria	2	8	9.1
	k_Bacteria;p_Tenericutes	0.8	0.4	0.5
	k_Bacteria;p_Plantomycetes	0.6	9.9	8.3
	k_Bacteria;p_Chloroflexi	0.3	1.4	1.5
	k_Bacteria;p_TM7	0.1	0.7	0.7
	k_Bacteria;p_Gemmatimonadetes	0	1.6	1.7
	k_Bacteria;p_Verrucomicrobia	0	0.6	0.6
	k_Bacteria;p_[Thermi]	0	0.8	1

- **Comparative Analysis between samples at class level**

Bacteria may be present as active or dormant cells, or as spores, throughout the composting process. During the composting process, only their number and level of activity change (Gentleman et al., 2004). *Bacillales* and *Actinobacteria* microorganisms, which are known to be critical for an efficient composting process, were abundant enough in the waste to inoculate the subsequent composting process. The taxonomic distribution of bacteria at the class level has been shown in Fig.8.3. The most abundant class is *Gammaproteobacteria* (24.5%) followed by *Betaproteobacteria* (13.01 %), *Methanomicrobia* (11.69 %), *Bacilli* (11.15%), etc. (Table 8.3). *Flavobacteria* were found during the higher temperatures of natural compost, while *Arthrobacter sp.* was found during the high-temperature process in cellulose-decomposing strain compost (Liu et al., 2011). *Bacillus sp.* was thought to be the dominant species during the middle and late stages of composting. *Flavobacteria*, which include opportunistic pathogens, are common in composts. Its presence in composts has

also been reported, as has the importance of these *Flavobacteria* in the degradation of phenolic and chlorinated compounds (Danon et al., 2008).

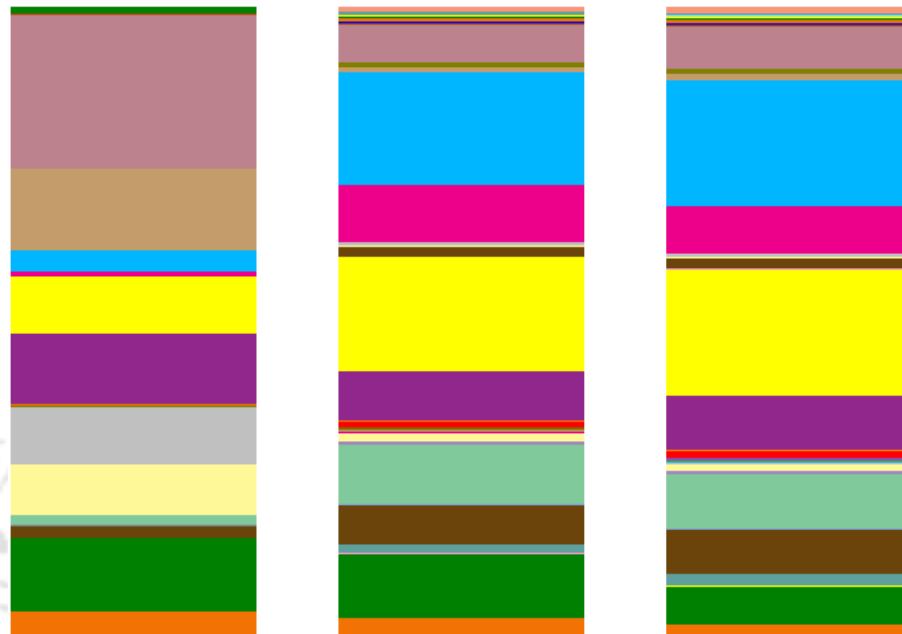






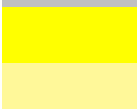


Fig. 8.3. Stacked Bar chart showing the relative abundance of each class within each sample

Table 8.3. Relative abundance of each class within each sample

Legend	Taxonomy	1(%)	2(%)	3(%)
	k_Bacteria;p_Proteobacteria;c Gammaproteobacteria	24.5	5.9	6.6
	k_Bacteria;p_Proteobacteria;c Betaproteobacteria	13	0.9	1
	k_Archaea;p_Euryarchaeota;c Methanomicrobia	11.7	10.1	6
	k_Bacteria;p_Firmicutes;c_Bacilli	11.1	7.7	8.6
	k_Bacteria;p_Bacteroidetes;c Sphingobacteriia	9.3	0.1	0.1
	k_Bacteria;p_Firmicutes;c_Clostridia	9.2	18.3	20.1
	k_Bacteria;p_Bacteroidetes;c	8	1.2	1.2

Flavobacteriia			
k_Archaea;p_Euryarchaeota;c Methanobacteria	3.7	2.6	1.5
k_Bacteria;p_Proteobacteria;c Alphaproteobacteria	3.4	17.9	20
k_Bacteria;p_Actinobacteria;c Actinobacteria	1.8	6.2	7
k_Bacteria;p_Bacteroidetes;c Bacteroidia	1.6	9.2	8.4
k_Bacteria;p_Tenericutes;c_Mollicutes	0.8	0.4	0.4
k_Bacteria;p_Planctomycetes;c Planctomycetia	0.6	9.1	7.6
k_Bacteria;p_Chloroflexi;c Thermomicrobia	0.2	0.9	1
k_Bacteria;p_Actinobacteria;c Acidimicrobiia	0	1.4	1.7
k_Bacteria;p_Bacteroidetes;c Cytophagia	0	0.7	0.6
k_Bacteria;p_Gemmatimonadetes;c Gemm-5	0	1.5	1.7
k_Bacteria;p_Planctomycetes;c Phycisphaerae	0	0.7	0.6
k_Bacteria;p_Proteobacteria;c Deltaproteobacteria	0	0.8	0.9
k_Bacteria;p_[Thermi];c_Deinococci	0	0.8	1

- **Comparative Analysis between samples at the order level**

To kill pathogens and weed seeds, heating is required to enable the development of a thermophilic population of microorganisms capable of degrading the more recalcitrant compounds (Boulter et al., 2000). Due to their ability to form endospores during the thermophilic stage, *Bacillus sp.* was able to survive in the compost pile. *Bacillus sp.* was found to be prevalent genera present throughout the composting process (Ishii et al., 2000 and Kumar et al., 2011) as well as the most prevalent bacterial taxon recovered from

compost feedstock (Ryckeboer et al., 2003). Taxonomic hit distribution at order level shows that compost has *Pseudomonadales*, *Burkholderiales*, *Methanosarcinales*, *Lactobacillus*, etc. as shown in Fig. 8.4. It was found from the report that 15.5% *Pseudomonadales* at the initial days of the composting process was decreased to 3.5% on day 10 of the composting process. *Burkholderiales* were found to be highest in Day 2 (12.9% abundance) followed by *Methanosarcinales* (11.1% abundance in Day 2) as shown in Table 8.4. *Burkholderiales* and *Methanosarcinales* are very pre-dominant bacterial communities present during composting process (Rademacher et al., 2012).

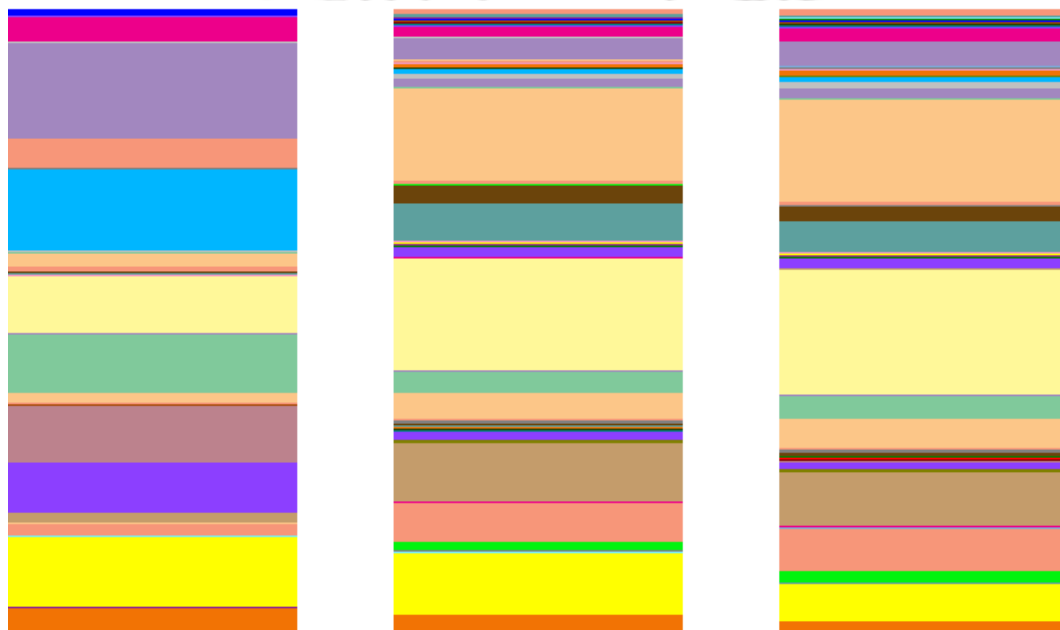


Fig. 8.4. Stacked Bar chart showing the relative abundance of each order within each sample

Table 8.4. Relative abundance of each order within each sample

Legend	Taxonomy	1(%)	2(%)	3(%)
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudo monadales	15.5	3.5	3.9
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o Burkholderiales	12.9	0.8	0.8
	k_Archaea;p_Euryarchaeota;c_Methanomicrobia;o Methanosarcinales	11.1	9.9	5.8
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales	9.4	3.4	3.6

k_Bacteria;p_Bacteroidetes;c_Sphingobacteriia;o Sphingobacteriales	9.3	0.1	0.1
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales	9.2	18.2	19.9
k_Bacteria;p_Bacteroidetes;c_Flavobacteriia;o Flavobacteriales	8	1.2	1.2
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o Enterobacteriales	4.5	0.2	0.2
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o Xanthomonadales	4.1	1.5	1.9
k_Archaea;p_Euryarchaeota;c_Methanobacteria;o Methanobacteriales	3.7	2.6	1.5
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o Rhizobiales	2.1	14.8	16.5
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o Actinomycetal	1.8	6.2	7
k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales	1.6	9.2	8.4
k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales	1.4	4.1	4.7
k_Bacteria;p_Tenericutes;c_Mollicutes;o Acholeplasmatales	0.8	0.4	0.4
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o Caulobacterales	0.7	0.6	0.5
k_Bacteria;p_Planctomycetes;c_Planctomycetia;o Pirellulales	0.3	6.1	5.1
k_Bacteria;p_Planctomycetes;c_Planctomycetia;o Planctomycetales	0.3	2.8	2.3
k_Bacteria;p_Chloroflexi;c_Thermomicrobia;o_JG30-KF- CM45	0.2	0.5	0.6
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o Sphingomonadales	0.2	0.8	0.9
k_Bacteria;p_Actinobacteria;c_Acidimicrobiia;o Acidimicrobiales	0	1.4	1.7
k_Bacteria;p_Bacteroidetes;c_Cytophagia;o_Cytophagales	0	0.7	0.6

k_Bacteria;p_Gemmatimonadetes;c_Gemm-5;o__	0	1.5	1.7
k_Bacteria;p_Planctomycetes;c_Phycisphaerae;o Phycisphaerales	0	0.5	0.4
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o Rhodospirillales	0	1.3	1.6
k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o Myxococcales	0	0.7	0.7
k_Bacteria;p_[Thermi];c_Deinococci;o_Deinococcales	0	0.8	1

8.1.3 Heatmap

Heatmap images are generated to visualize the operational taxonomic unit (OTU) table at different Taxonomic Levels where each row corresponds to an OTU and each column corresponds to a sample. The higher the relative abundance of an OTU in a sample, the more intense the color at the corresponding position in the heatmap. Red contributes a low percentage of OTUs to the sample while purple contributes a high percentage of OTUs.

A comparative study on the relative abundance of bacterial genera is represented through heatmap in Fig. 8.5. A very diverse community of microorganisms has been detected on days 2, 10, and 20. The highest operational taxonomic unit (OTU) belonged to mainly 2 phyla as *Proteobacteria* and *Firmicutes*. The relative abundance of phylum *Proteobacteria* is 41% on day 2, 25.5% on day 10, and 28.6% on day 20 shown in Table 8.5. The results are per the previously reported data since *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* are commonly reported bacterial phyla in composting process (Galitskaya et al., 2017; Wei et al., 2018). Phylum *Firmicutes* are known to grow at high temperatures and are widely distributed especially in the thermophilic phase of composting of agricultural biomass (Zhang et. al., 2016). Meanwhile, *Actinobacteria* is also considered thermophilic/thermotolerant and also plays important role in terms of the breakdown of organic materials. *Pseudomonas* has been famously reported as plant disease-suppressive bacteria, that can improve the composting quality during the maturation phase (Wei et al., 2018). It is found to be 15.5% for day 2, 3.5% on day 10, and 3.9% on day 20.

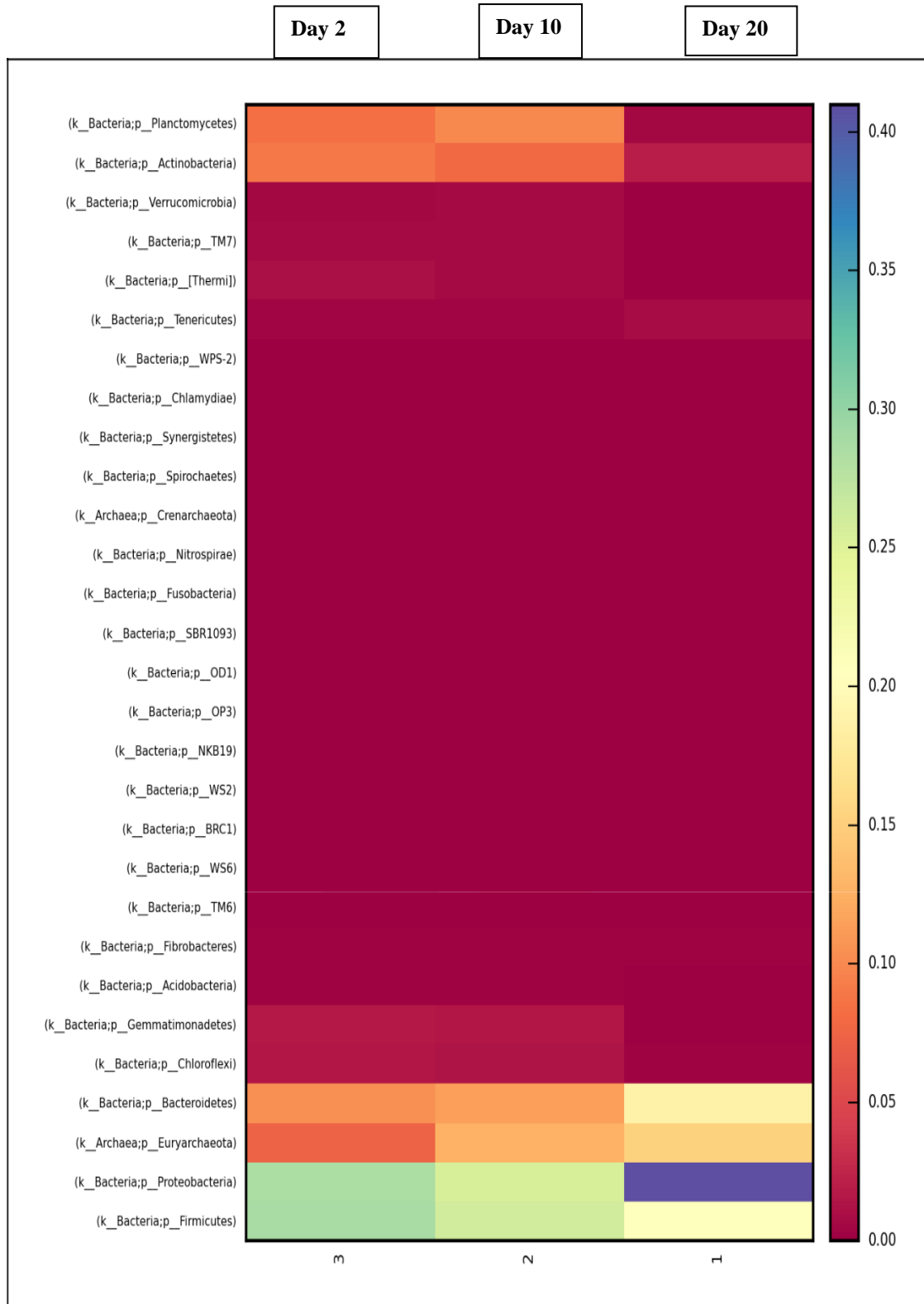


Fig. 8.5 Heatmap at Phyla level of RDC compost at the different time periods

Table 8.5. Most abundant taxonomy identified in the samples at different taxonomic levels

Sample	Day 2 (%)	Day 10 (%)	Day 20 (%)
Phylum	Proteobacteria (40.98)	Firmicutes (26.05)	Firmicutes (28.75)
Class	Gammaproteobacteria (24.50)	Clostridia (18.29)	Clostridia (20.08)
Order	Pseudomonadales (15.49)	Clostridiales (18.20)	Clostridiales (19.95)
Family	Comamonadaceae (12.63)	Clostridiaceae (13.30)	Clostridiaceae (14.36)
Genus	Comamonas (12.18)	Methanosarcina (9.84)	Unclassified Genus from Porphyromonadaceae Family (7.65) Unclassified species Porphyromonadaceae Family (7.65)
Species	Unclassified Species from Comamonas Genus (12.10)	Unclassified Species from Porphyromonadaceae Family (8.36)	Unclassified Species

8.2 ISOLATION AND IDENTIFICATION OF BACTERIA DURING ROTARY DRUM COMPOSTING OF *MIKANIA MICRANTHA* KUNTH

Composting is now the most widely used technology for waste stabilization, and it is also widely employed for bio-conversion of both aquatic and terrestrial weeds. The goal of the composting process is to produce a mature and stable product that may be utilized as an organic fertilizer in agricultural fields. To the best of the knowledge, no extensive study of the use of bacterial inoculum during the composting of terrestrial weed has yet been done by any previous studies. Bioaugmentation appears to have significant advantages in terms of increasing compost maturity, reducing the composting timing, and increasing compost quality (Ballardo et al., 2020). The role of microbial populations in composting processes is widely been established. During composting process, numerous studies on bacterial populations, actinobacteria, and fungus have been extensively studied in accordance to their application part (Chandna et al., 2013). The author further reported that it is critical to

investigate the culturable bacterial variety in-depth to identify new bacteria that may be used to improve and speed up compost preparation. Zhang et al (2019) reported that the bioaugmentation process entails intricate interspecific connections between foreign strains and the native microbial consortia present during the composting process that helps in the enhancement of biodegradation of organic matter. Many studies have been depicted previously for the safe use of terrestrial weed for the production of biofertilizer and their application for plantation using various composting techniques, and yet very little has been asserted on the study of allelochemical degradation during the process and also reducing the processing time. The current study focused on the employment of a novel bacterial strain throughout the composting process to reduce toxicity in *Allium cepa* and improve the quality of the compost.

8.2.1 Isolation, screening, and characterization of bacteria from rotary drum composting of *Mikania micrantha* Kunth

- **Isolation of bacterial strain**

The enrichment method was used to isolate bacterium from the compost. For that MSM (Na_2HPO_4 , 2.4 g/L; K_2HPO_4 , 2.0 g/L; NH_4NO_3 , 0.1 g/L; MgSO_4 , 0.01 g/L; CaCl_2 , 0.01 g/L; Glucose, 10g/L and Peptone, 5 g/L) media were dissolved in three different concentrations (1:1, 1:2 and 1:3) of *Mikania* extract. For the isolation of enriched bacterial strains was serially diluted to 10^{-8} and 50 μL of this solution was spread plated on enriched media agar plates having 1.8% agar as the gelling agent (Fig. 8.6).

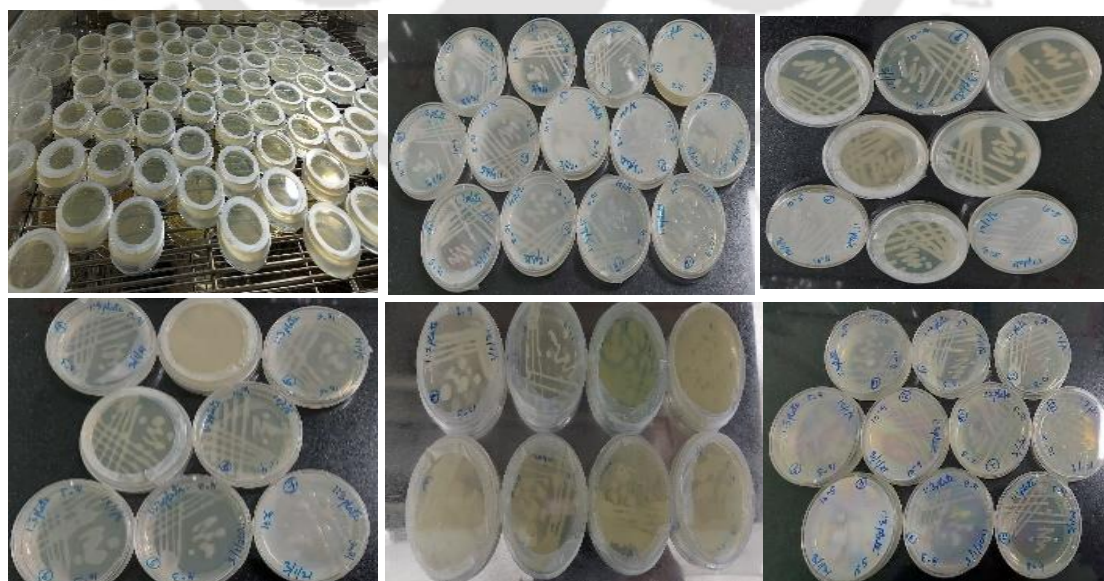


Fig. 8.6. Different bacterial isolates from rotary drum compost of *M. micrantha*

The colonies were repeatedly streaked on enriched media agar plates to get single colonies. The total number of colonies obtained from the compost was 158. Out of 158 isolates, 26 isolates were selected based on their morphology. The colors of the strains were mostly, white, yellow, cream, and slimy. All the distinguishable 26 isolates were undertaken for morphological and biochemical tests as described in Fig. 8.7 and Table 8.6. The purity of isolated colonies was also studied microscopically by Gram staining. All the 26 isolates were named MHK1, MHK2, MHK3, MHK4...MHK26.

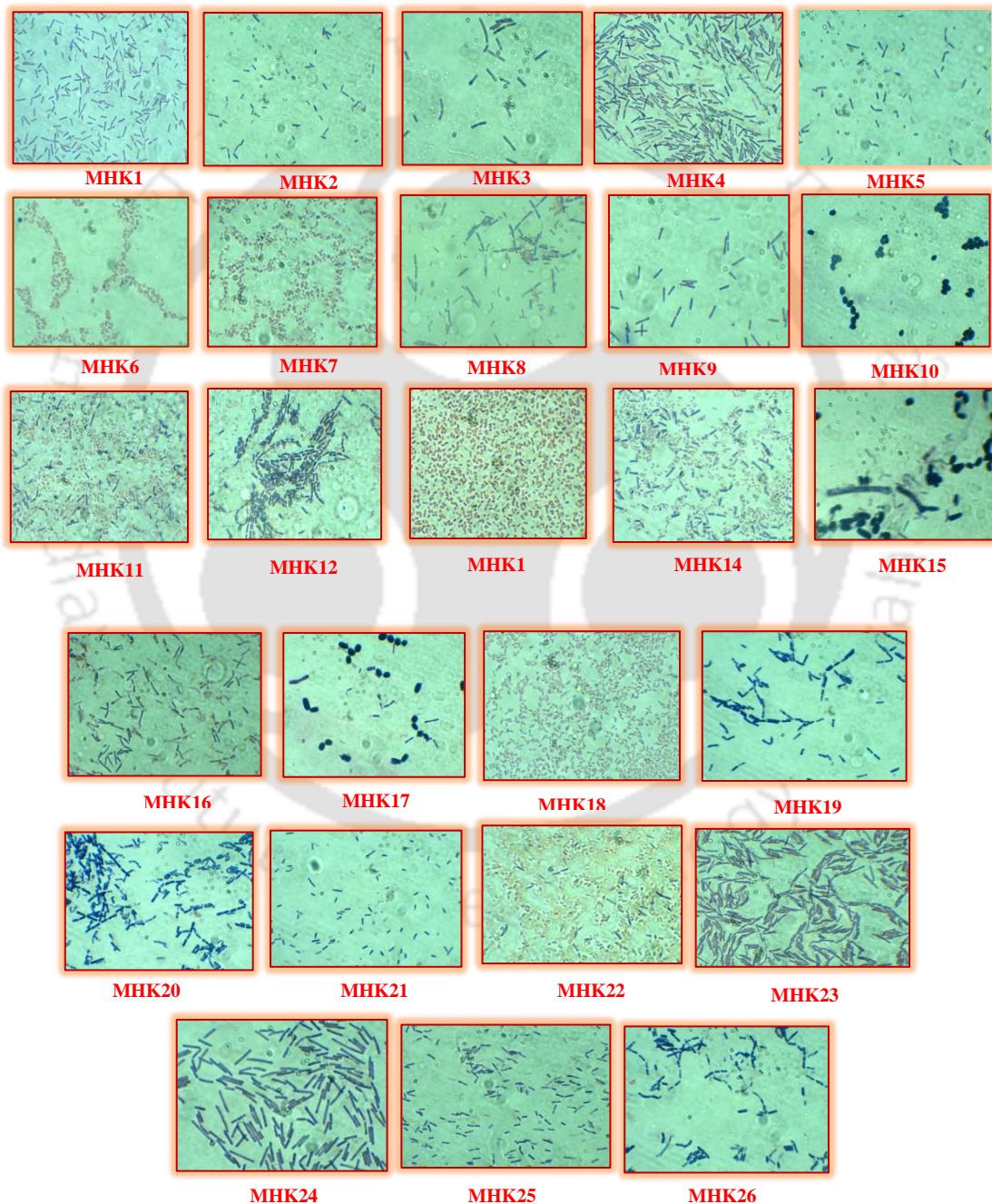


Fig. 8.7. Gram-staining of the isolated bacterial strain

Table 8.6. Gram-staining of the bacterial isolates

Strain name	Morphology
MHK1	Rod shaped
MHK2	Rod shaped
MHK3	Rod shaped
MHK4	Rod shaped
MHK5	Rod shaped
MHK6	coccus
MHK7	Rod shaped
MHK8	Rod shaped
MHK9	Rod shaped
MHK10	Coccus (contaminated)
MHK11	Rod shaped and coccus (contaminated)
MHK12	Rod shaped
MHK13	Rod shaped
MHK14	Rod shaped and coccus (contaminated)
MHK15	Rod shaped and coccus (contaminated)
MHK16	Rod shaped
MHK17	Rod shaped and coccus (contaminated)
MHK18	Rod shaped
MHK19	Rod shaped
MHK20	Rod shaped
MHK21	Rod shaped
MHK22	Rod shaped
MHK23	Two types of rod shaped strain (contaminated)
MHK24	Rod shaped
MHK25	Rod shaped
MHK26	Rod shaped

Out of the 26 bacterial isolates, 6 strain were found contaminated with fungus and was later discarded. The remaining 21 isolates were used for the screening of the laccase enzyme. Laccase enzyme was targeted for screening of the isolated pure culture. Laccase, an important enzyme with four copper ions, uses dioxygen as an electron acceptor and

oxidizes phenolic or non-phenolic compounds related to lignin polymer, generating radical intermediates (Sun et al., 2020; Meng et al., 2021). Plants biosynthesize three types of secondary metabolites: phenolic compounds, terpenoids/isoprenoids, alkaloids, and glucosinolates (nitrogen- or sulfur-containing molecules, respectively) (Santos-Sanchez et al., 2019). Allelochemicals are reported to be the major source of phenolic compounds, terpenoids, and aromatic compounds. Plants produce phenolic compounds via the shikimic and acetic acid (polyketide) metabolic pathways. They are just one type of secondary metabolite implicated in plant allelopathy (Li et al., 2010). Isolated bacterial strains were screened for laccase enzyme production in the current study because laccase enzyme can degrade phenolic compounds and bacteria producing laccase enzyme can be used in the bioaugmentation study.

- **Screening and characterization of the bacterial isolates for laccase enzyme**

To check the presence of laccase enzyme activity from the obtained bacterial strain, a different experimental setup has been conducted. Laccase and laccase-like enzymes are widely distributed and play a variety of biological roles such as lignification, delignification, pathogenicity, detoxification, morphogenesis and sporulation, rhizomorph growth, and development, polymerization of melanin precursors, and spore coat resistance (Strong and Claus, 2011). Nguyen et al., (2016) reported that laccases can oxidize a variety of aromatic and non-aromatic compounds, including substituted phenols, inorganic ions, and a variety of non-phenolic compounds. Laccase has been investigated for use in a variety of biotechnological processes due to its broad substrate spectrum, use of readily available oxygen as the final electron acceptor, and lack of a need for cofactors or peroxide. These properties have sparked considerable interest in laccase's ability to transform or degrade a variety of toxic compounds found in polluted soils and wastewaters. Yoshida (1883) discovered laccase in plants after observing that the latex of Chinese or Japanese lacquer trees (*Rhus* sp.) hardened quickly in the presence of air. After being isolated and purified, the catalyst was named about a decade later (Bertrand, 1894). Laccase enzymes were later discovered in a variety of other plant tissues (Bligny and Douce, 1983; Ranocha et al., 1999). Plant laccases, despite their widespread distribution, have not been extensively studied or characterized. Fungal laccases were discovered in the late 19th century (Bertrand, 1896) and have been found in the majority of basidiomycetes and ascomycetes (Baldrian, 2006). In yeasts, a multicopper enzyme with phenoloxidase activity has been discovered (Augustine et al., 2008; Stoj and Kosman, 2003). Some phenoloxidases with laccase-like enzymatic properties have been isolated from insect larval and adult cuticles (Dittmer et al.,

2004). So far, relatively few prokaryotic laccases have been studied, despite rapid progress in genome analysis indicating that their genes are widespread in bacteria. A laccase-like enzyme was discovered in the spores of a *Bacillus sphaericus* strain (Claus and Filip, 1997), and the *Bacillus subtilis* spore coat protein cot A was the first prokaryotic laccase whose crystal structure was determined (Enguita et al., 2003). Agrawal et al., (2018) described the structure of laccase enzyme containing four copper and its types as shown in Fig. 8.8.

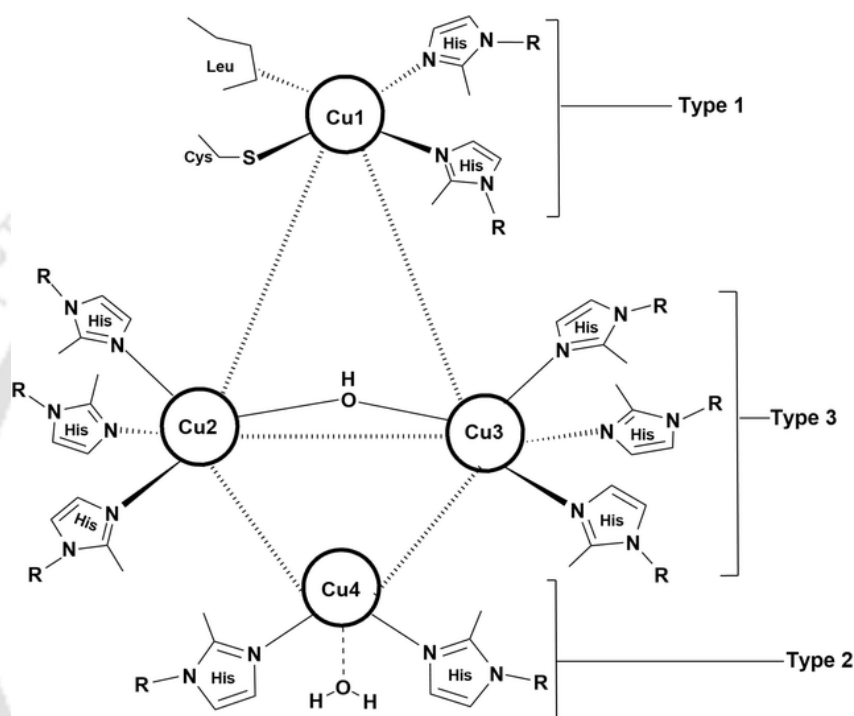


Fig. 8.8. Structure of laccase enzyme and copper centers of the enzyme (Agrawal et al., 2018)

- **Mechanism of Laccase enzyme**

Laccase oxidation is resistant to many pollutants. Using redox mediators (Husain and Husain, 2007) is one way to broaden their range of action. Laccase can oxidize organic compounds into free radicals, which are known as mediators. Because they are less specific, these radicals can oxidize other pollutants, broadening the range of compounds that these enzymes can potentially degrade. Mediators are frequently described as "electron shuttles" that can be reduced back to their parent compound during the oxidation of a pollutant after being oxidized to radicals by laccase (Fabbrini and Gentili, 2002) as shown in Fig. 8.9.

Laccase catalyzes two types of basic reactions: direct oxidation and in-direct oxidation. Direct oxidation occurs when a substrate is oxidized to the corresponding radical as a result of direct interaction with a copper cluster (Matera et al., 2008). Direct oxidation is not

possible in some reactions because laccase can only oxidize compounds whose ionization potential does not exceed the redox potential of the T1 copper ion (Morozova et al. 2007). However, the limitation can be overcome by using a mediator, which is a two-step process in which an enzyme catalyzes the oxidation of the mediator, followed by the oxidized mediator oxidizing the substrate. However, certain characteristics of the mediator must be present for the reaction to proceed without interruption: (a) the reaction must proceed without interruption; (b) it must be a good substrate for laccase in both its oxidized and reduced forms; (c) it must be stable; it must not inhibit the enzymatic reaction, and (d) conversion must be cyclic (Johannes and Majcherczyk 2000).

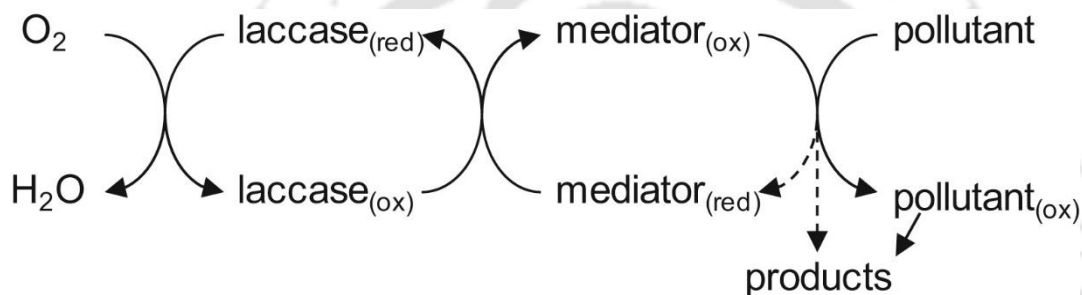


Fig. 8.9. Ideal laccase-mediator reaction model (Margot et al., 2015)

Following the optimum operating parameters, the Lac enzymatic activity for the optimum dosage was determined using a guaiacol oxidation test as described by Kalra et al (2013). The reddish-brown hue produced by Lac's oxidation of guaiacol was detected at 450 nm to determine the enzyme activity. Guaiacol (2 mM), sodium acetate buffer (10 mM), and crude enzyme were incorporated in the reaction solution. The reaction solutions were incubated at 30 °C for 15 minutes in triplicates, and the enzymatic activity was measured using a UV-Vis spectrophotometer at 450 nm (= 0.6740 M/cm) against a blank reagent. The enzyme's activity was measured in IU/mL, where 1 IU equals the amount of enzyme required to oxidize 1 mol guaiacol per minute. For bacterial growth, culture broth (2 mL) was centrifuged (8000xg for 10 min at 4°C) to separate the cell biomass. The bacterial biomass was resuspended into the same amount of distilled water. The absorbance of the cell suspension was measured at 600 nm. Out of 21 bacterial strains, 9 isolates showed a positive result for laccase enzyme in Petri plates as shown in Fig. 8.10.

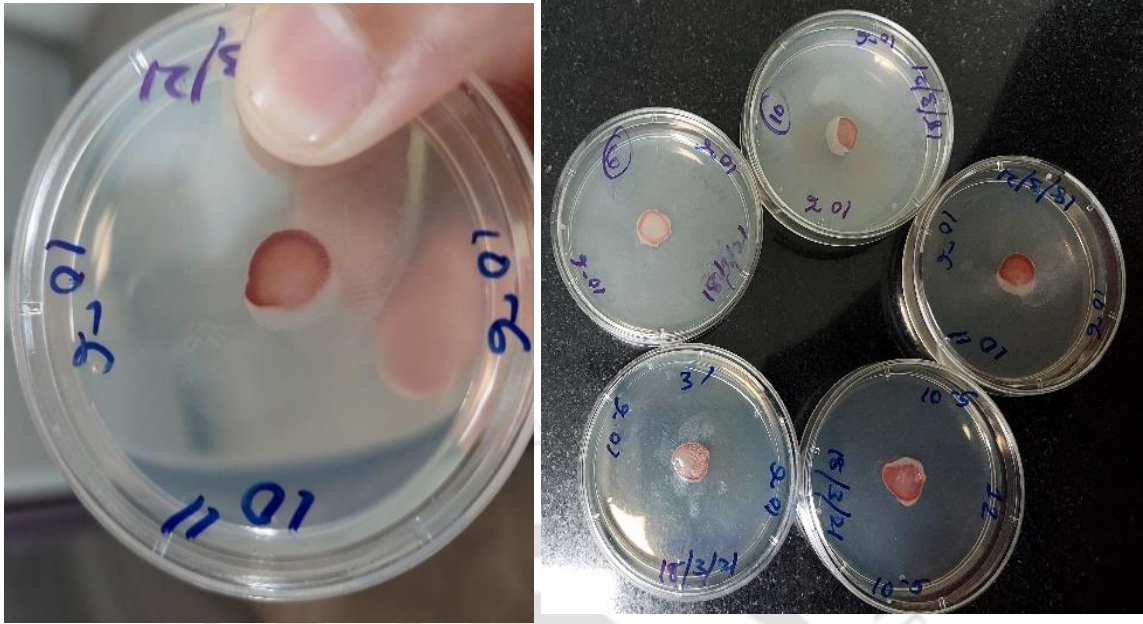
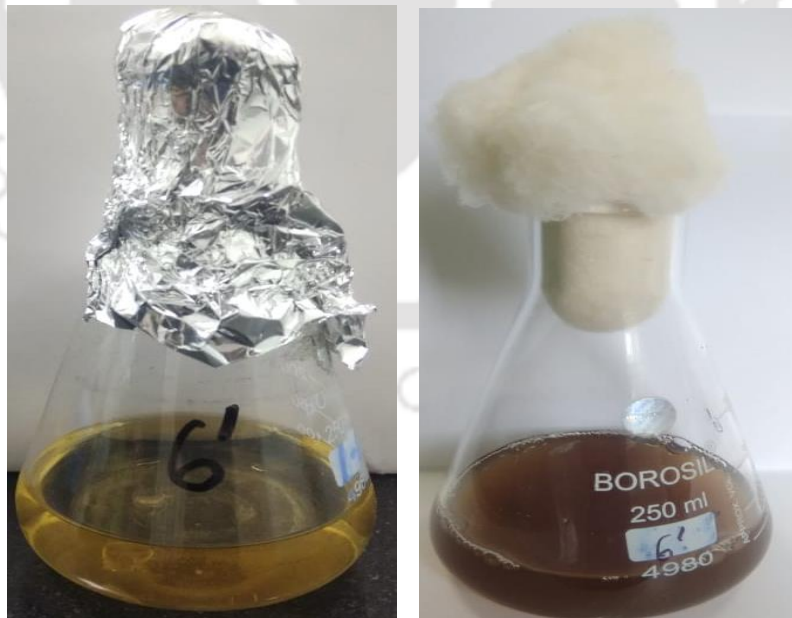


Fig. 8.10. Positive lac enzyme test on Petri plates

The isolates that tested positive for lac enzyme were then tested in broth. Out of the 9 isolates that tested positive for laccase, only 3 (MHK2, MHK3, and MHK4) isolates were discovered to be positive in broth as shown in Fig. 8.11. Finally, the 3 isolates were taken for identification.



Before incubation

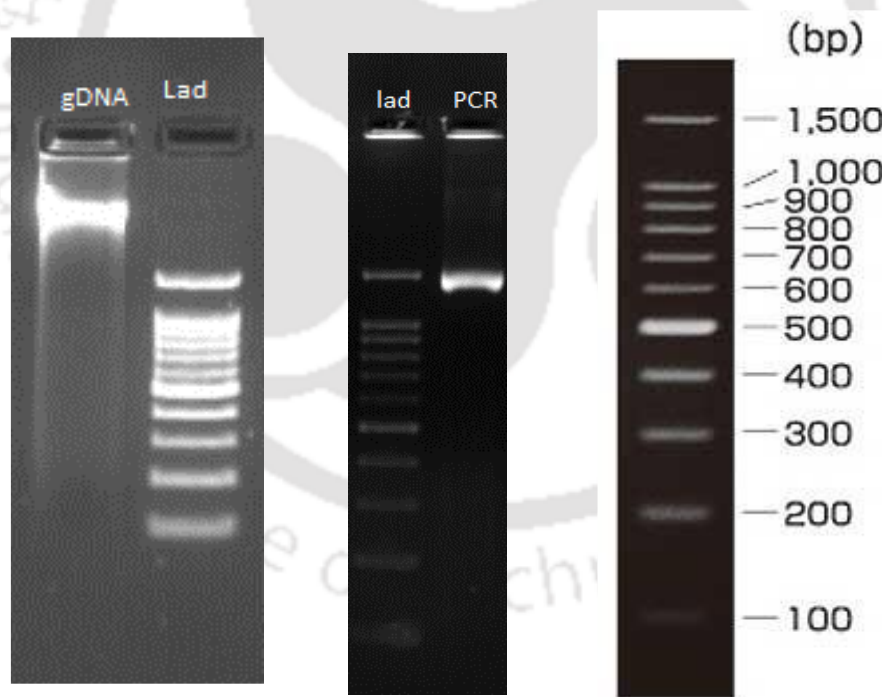
After incubation

Fig. 8.11. Laccase positive test on broth culture

- **Identification of the bacterial isolates**

Three morphologically distinct laccase producing bacterial strains were isolated from the rotary drum composting of *Mikania micrantha* Kunth. Isolated strains were identified as *Enterobacter hormaechei* MHK2 (OM149726), *Lysinibacillus fusiformis* MHK3 (OL533643) and *Lysinibacillus fusiformis* MHK4 (OM179766).

A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose gel (Fig. 8.12). Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The pure culture samples MHK2, MHK3 and MHK4 showed high similarity with *Enterobacter hormaechei* and *Lysinibacillus fusiformis* based on nucleotide homology and phylogenetic analysis (Fig. 8.13). The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2- parameter model (Kimura, 1980).



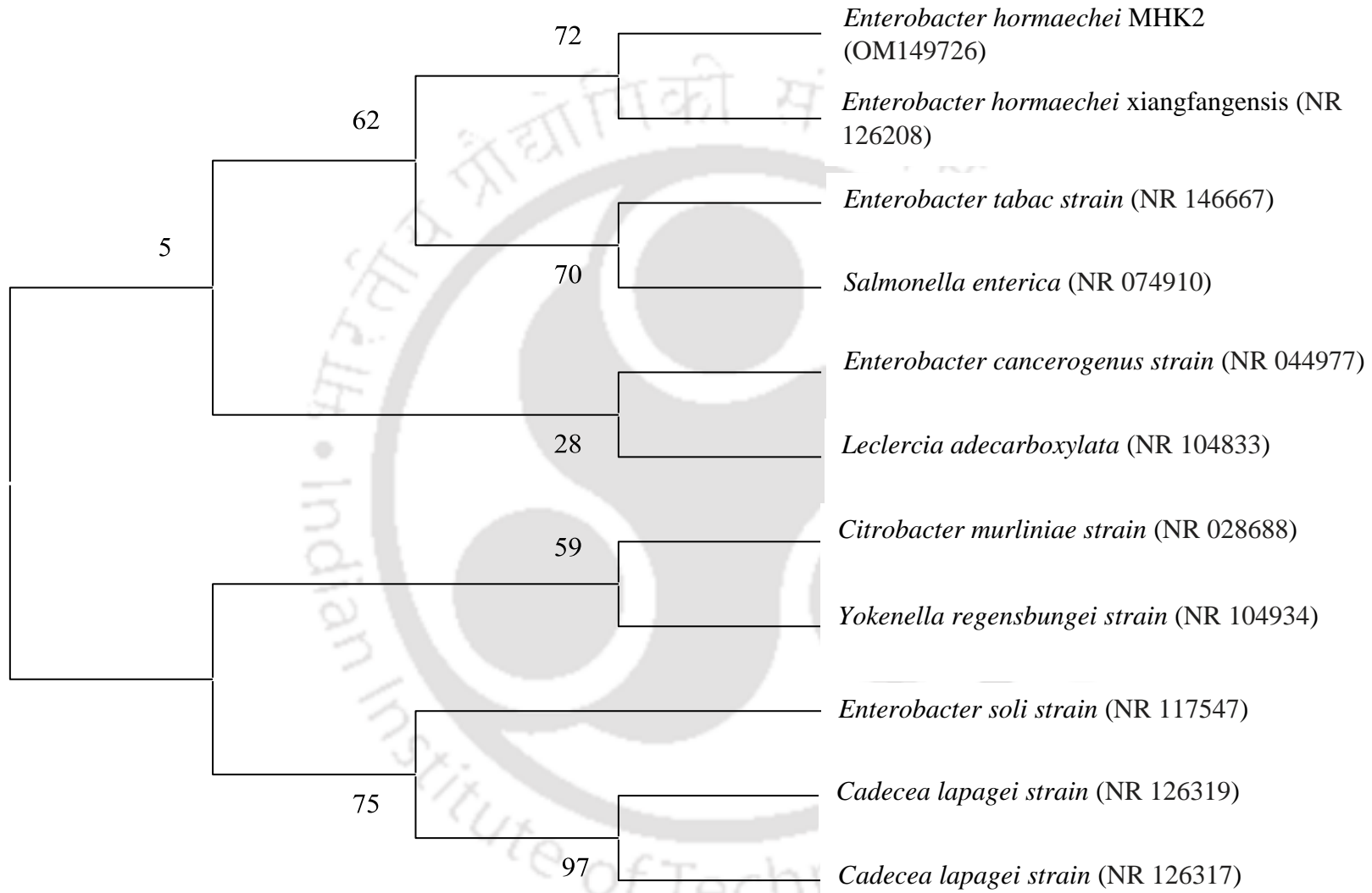
Ladder specification

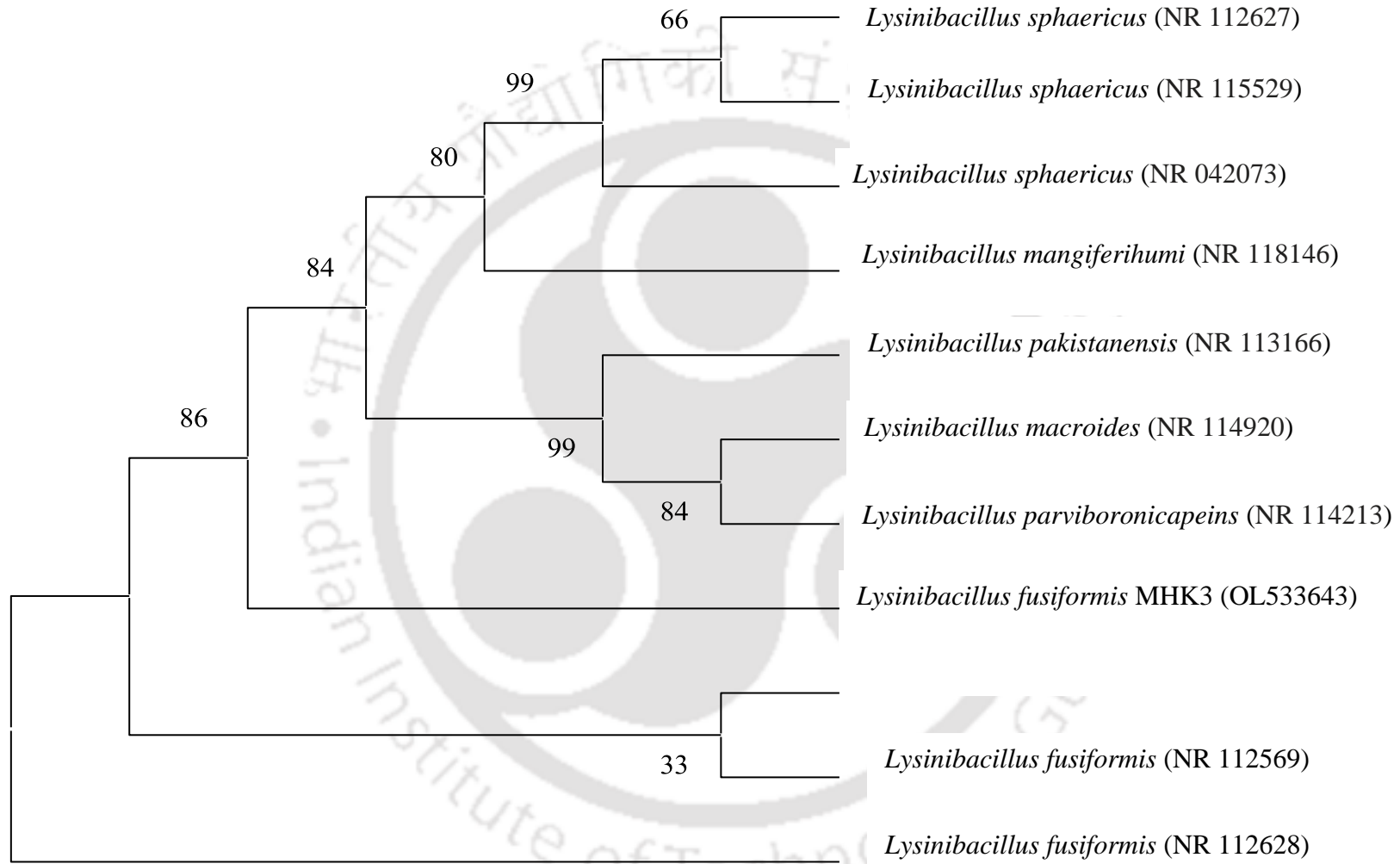
Fig. 8.12. gDNA and 16S Amplicon QC data for the bacterial isolates

The bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage

of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. All positions containing gaps and missing data were eliminated. There were a total of 1434 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2015).







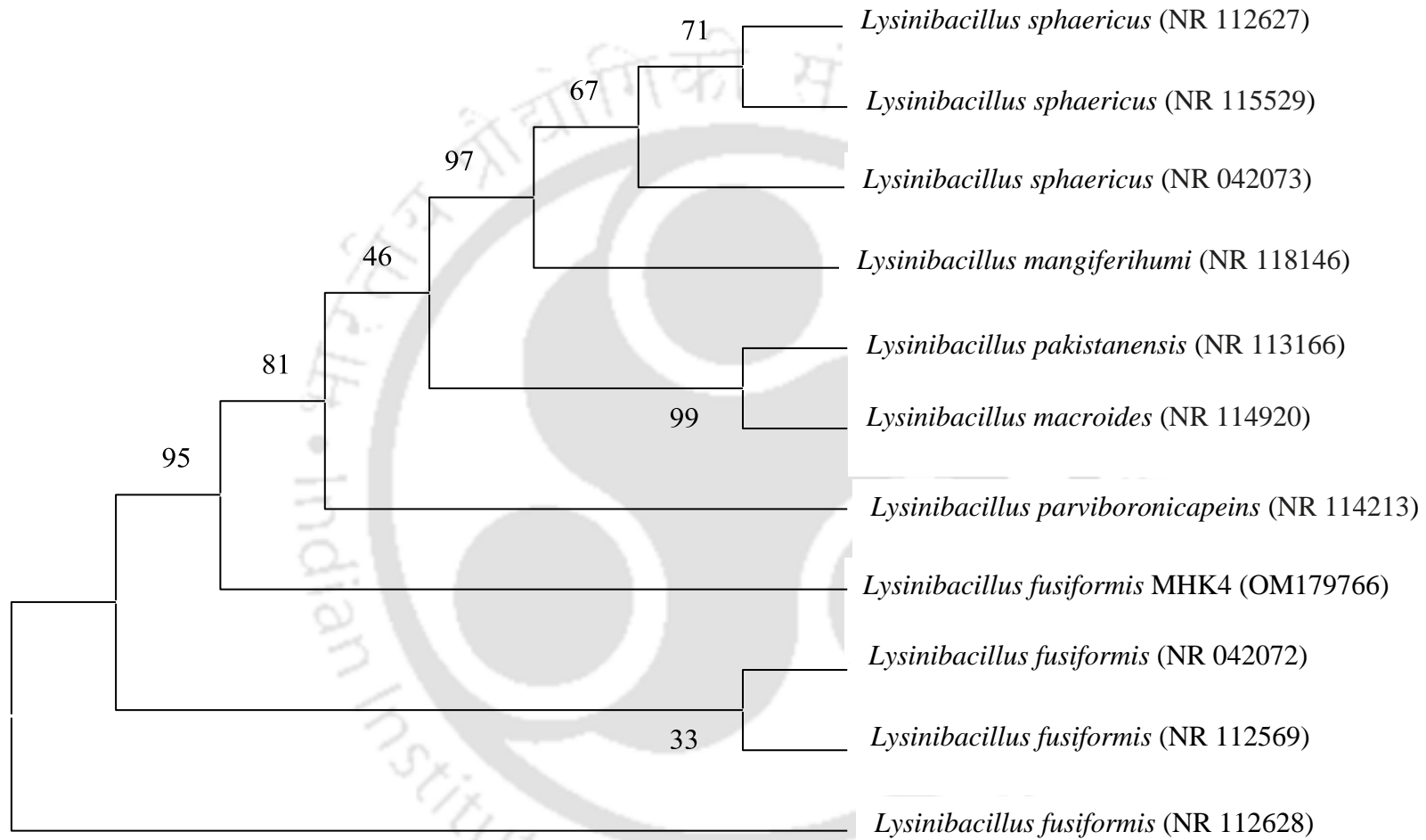


Fig. 8.13. Phylogenetic tree of the bacterial isolates

- **Optimization of Lac enzyme activity: pH, temperature, and RPM**

The three isolated bacterial strains were used for the optimum Lac enzyme activity at four distinct pH levels (5.0, 6.0, 7.0, and 8.0), the effect of different incubation temperatures (25°C, 30°C, 35°C, and 40°C) on Lac enzyme activity and RPM at 80, 100, 120 and 140. The study was carried out to get the bacterial strain that produced the highest enzymatic activity which was later used for bioaugmentation study. All of the experiments were done in triplicate to ensure optimum activity. Incubation took place for 7 days, and samples were taken every day for testing lac enzyme activity following the technique. The growth curve of the three bacterial isolates has been isolated in Fig. 8.14.

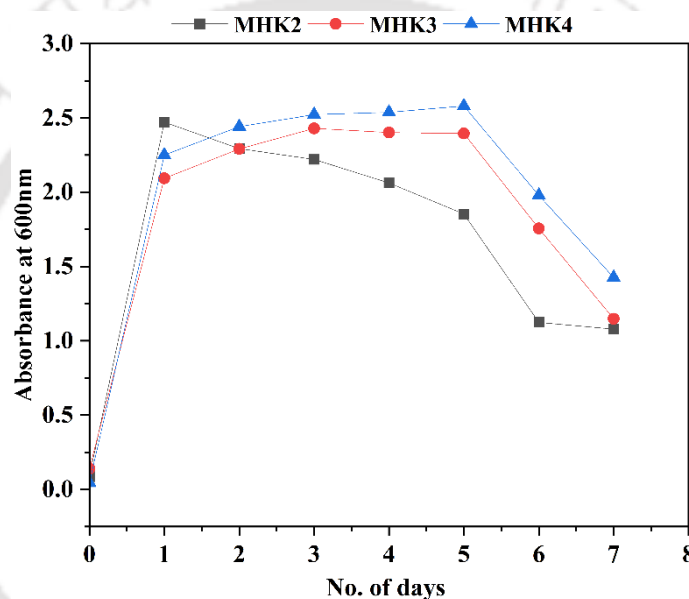


Fig. 8.14. Growth curve of the bacterial isolates

pH is important during the degradation or utilization of xenobiotic compounds or any other type of substrate by microorganisms. Optimal pH stimulates the production of enzymes in microorganisms, which aid in the utilization of nutrients. However, even minor pH changes can make these enzymes vulnerable (Bera et al., 2019). Extreme pH can cause enzyme denaturation and loss of catalytic activity (Banerjee and Ghoshal, 2010). As a result, the organismal metabolism ceases to function, resulting in microbial obliteration. As a result, maintaining an optimal initial pH is critical for the proper activity of the enzyme. A wide range of pH (5.0, 6.0, 7.0, and 8.0) was considered for the present study to record the highest lac enzyme activity. Studies were carried out at a temperature of $30 \pm 2^\circ\text{C}$ and RPM of 120. Fig. 8.15 illustrates the activity of lac enzyme for the three isolated bacterial strains at different pH and 7 days of incubation time.

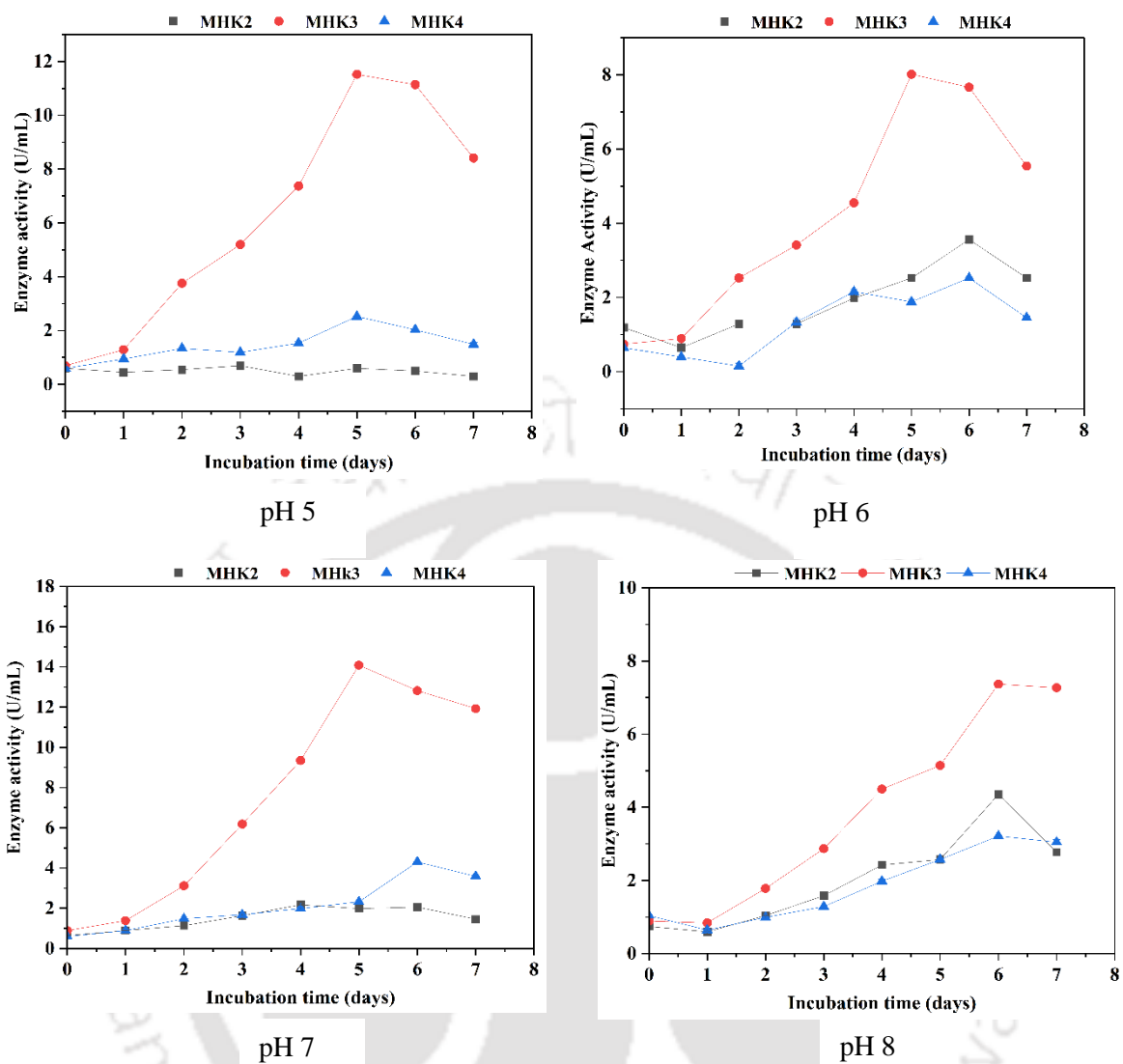


Fig. 8.15. Laccase enzyme activity at different pH

It was observed that maximum enzyme activity (>12 U/mL) was observed for the strain MHK3 at pH 7. However, at acidic pH (5 and 6), the lowest enzyme activity was observed. Out of the three bacterial strains, MHK2 exhibited the lowest enzyme activity as compared to MHK3 and MHK4. The study concludes that the strain MHK3 is a neutrophilic bacterium that exhibited the highest enzyme activity. Fig. 8.16 depicts the effect of temperature on laccase activities. The temperature profile improved the stability of the laccase activity's optimum temperature value. For all three strains, the optimal temperature for the highest enzyme activity was found to be between 30°C and 35°C (MHK2, MHK3, and MHK4).

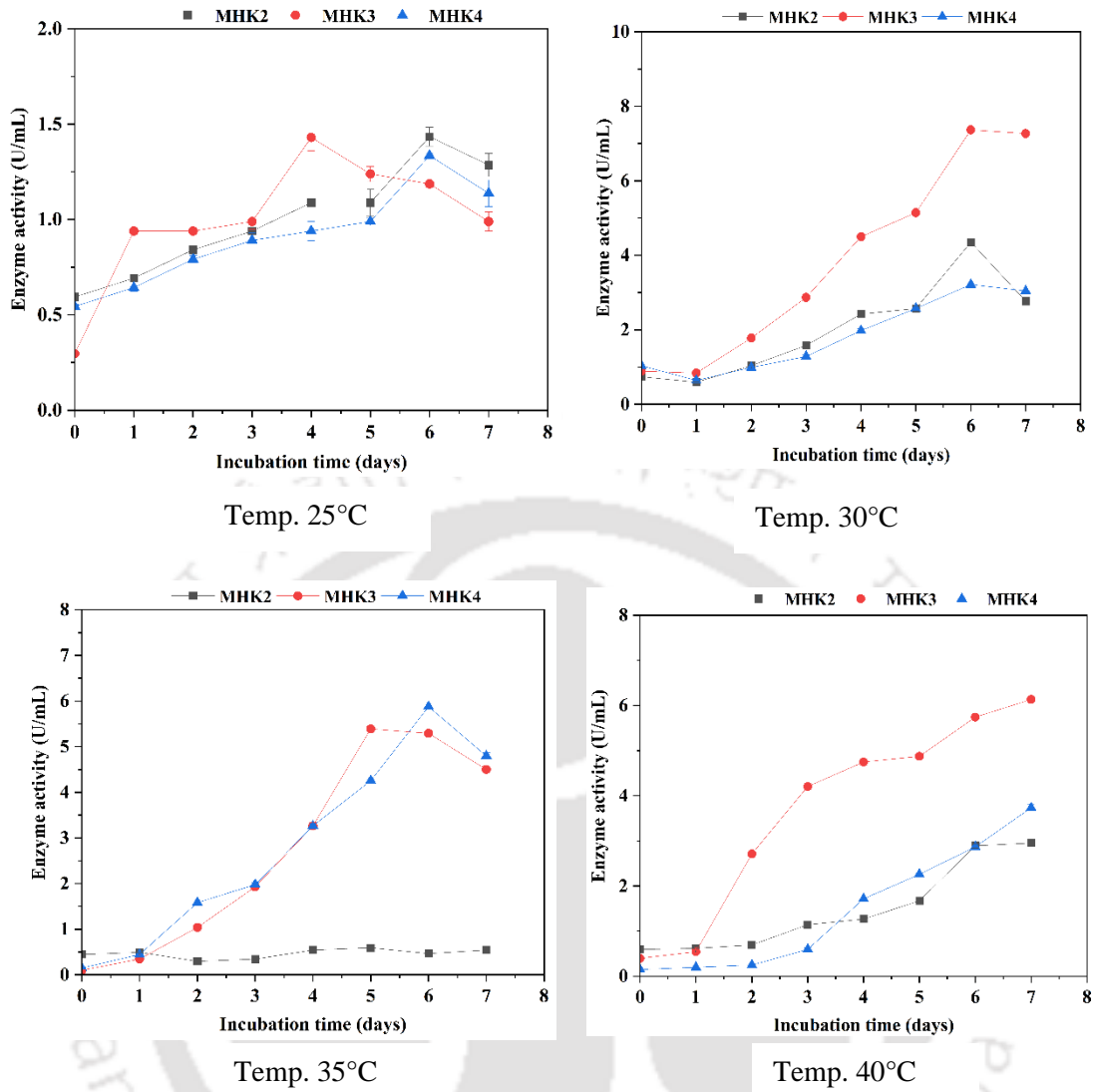


Fig. 8.16. Laccase enzyme activity at different temperature

It demonstrates that laccase activity was highest in the mesophilic temperature range. The temperature has a significant impact on enzyme activity because it changes the physical and chemical properties of the enzyme. At 30°C, strain MHK3 demonstrated the highest enzyme activity, followed by strains MHK4 and MHK2. Rotation per minute (RPM) impacts the growth of bacteria which affects the enzyme activity as well. RPM optimization was carried out at 80, 100, 120, and 140 as shown in Fig. 8.17. It was observed that maximum enzyme activity was observed at RPM 120 followed by 140, 100, and 80. MHK3 showed the highest enzyme activity at RPM 120 as compared to the other two bacterial strains. Out of the three laccase-producing enzyme bacterial isolates, *Lysinibacillus*

fusiformis MHK3 (OL533643) was selected for the bioaugmentation study since it showed the highest enzyme activity among all at pH 7, temperature 30°C, and RPM 120.

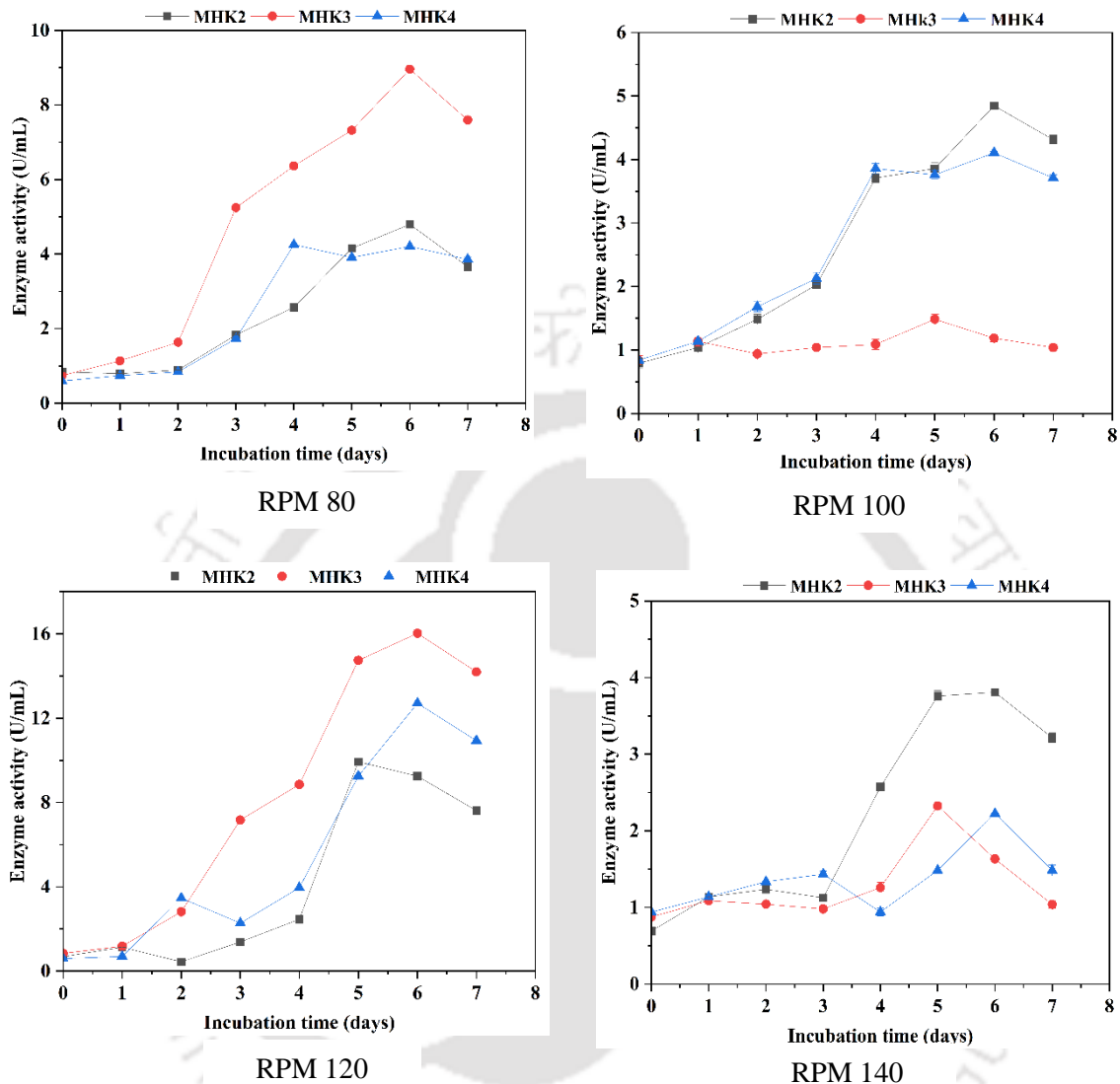


Fig. 8.17. Laccase enzyme activity at different RPM

8.3 BIOAUGMENTATION STUDY OF THE BACTERIAL ISOLATES *LYSINIBACILLUS FUSIFORMIS* MHK3 (OL533643) DURING THE COMPOSTING PROCESS

Bioaugmentation is the addition (augmentation) of specialized microbial cultures to a given environment to perform a specific remediation task. These specialized microbial cultures are typically grown separately under well-defined conditions to perform a specific remediation task (Alvarez and Illman, 2006). There have been two distinct bioaugmentation approaches developed over the years. One approach is based on the injection of microorganisms with the desired catabolic potential into the host's

environment to complement or replace the host's native microorganism population. A specific metabolic niche is occupied by the selected bacteria or consortia in the contaminated environment, allowing them to survive and outcompete the native microorganisms. Secondly, a large concentration of cells that act as catalysts briefly and degrade a significant amount of the target contaminant before becoming inactive or perishing is added (Vogel and Walter, 2002).

Natural noxious weeds are extremely toxic, and it is critical to evaluate them after and before composting to ensure that they are not contaminated. The use of terrestrial weed biomass for the bioconversion of products such as compost and vermicompost has been the subject of numerous studies; however, only a small number of studies have been conducted specifically on bioaugmentation study of the composting process of noxious weed. The application of bacteria not only improves the reaction of the composting process but also has the added benefit of detoxifying the harmful chemicals present in the weed substrate. The purpose of this study is to investigate the use of the bacterial strain *L. fusiformis* MHK3 in two different composting processes, as well as to determine the degradation time and toxicity reduction of the final composted product.

8.3.1 Thermophilic composting followed by bioaugmentation process using *Lysinibacillus fusiformis* MHK3 (OL533643) (TCB)

For the current study, *Mikania micrantha*, cow dung and saw dust was mixed in the ratio 5:4:1 and was pre-degraded in a rotary drum composter for 10 days to accomplish thermophilic degradation. The strain *Lysinibacillus fusiformis* MHK3 (OL533643) was inoculated as per the procedure described in section 4.7.5. Various parameters evaluated during the process have been discussed below.

8.3.1.1 Temperature and moisture content

The temperature has been recognized as a primary and pivotal element in substrate degradation, and it has been taken into account in the context of substrate pre-degradation. Temperature profiling and monitoring are essential for determining and comprehending the expected degradation kinetics in the composting process (Bernal et al., 2009). Fig. 8.18(a) depicts a 10-day temperature profile during the composting process. Microbial activity, which began within the first hours of operation, caused the temperature to rise (Kalamdhad et al., 2009). On the 2nd day of the process, the maximum temperature of 54.5°C was increased, then gradually decreased after the 4th day. Before the bio-augmentation study,

the primary goal of drum composting was to achieve thermophilic substrate breakdown. Achieving the thermophilic stage in most composting processes is followed by the mesophilic stage, in which ambient temperatures are reached after the initial rise in temperature and achievement of the thermophilic stage (Pottipati et al., 2021). The temperature rise is related to the rotary drum's active functioning as well as the amount of substrate utilized. The thermophilic temperature persisted for 3-4 days before steadily reducing to the mesophilic temperature range. As reported by Ahmed et al. (2007), *Lysinibacillus fusiformis* MHK3 (OL533643) strain exhibits greater activity at the mesophilic range of 35-37°C, for which pre-degraded substrate was afterward bio-augmented once the temperature dropped below 40°C.

Evaporation occurs as a result of the heating of the composting material, which results in moisture loss throughout the composting process. However, the formation of heat and moisture as a result of the increased metabolic activity of bacteria throughout the decomposition process foreshadows this outcome (Maturi et al., 2021).

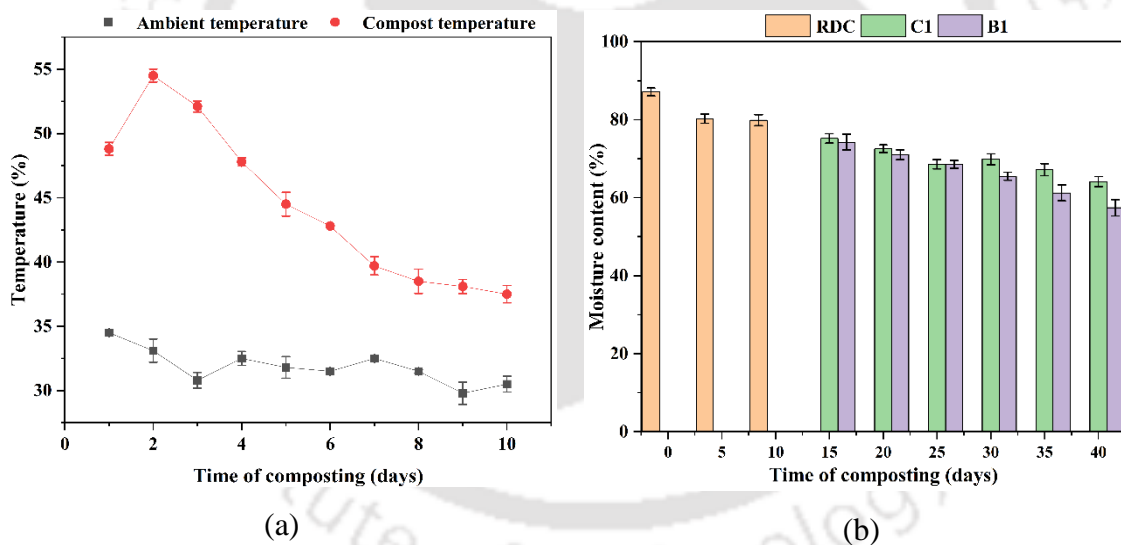


Fig. 8.18. Variation in a) Temperature and b) Moisture content during bioaugmentation process

Moisture content is crucial for the bio-activity of microorganisms; however, breakdown of organic matter results in the development of leachate, which must be avoided by adding bulking agents such as sawdust, dried leaves, or rice husk. In this study, it was discovered that the initial moisture content of the mixture was 87.21%, which was mostly due to the substrate *Mikania*, which had an initial moisture content of 88.74% (Fig. 8.18b). After 10 days of the rotary drum composting process, moisture content was recorded as 79.85%. The

moisture content recorded in the reactor C1 and B1 reactors were 64.12 and 57.41%, respectively, after the pre-degraded substrate was bio-augmented. Moisture levels had decreased in both the control and bacteria-treated reactors, it was discovered.

8.3.1.2 Volatile solids (VS) and total organic carbon (TOC)

As the composting process proceeds, due to active microbial action on organic content, higher VS reduction was observed in reactor B1 in comparison to reactor C1. VS reduction was observed throughout the composting process as shown in Fig. 8.19a. During the rotary drum composting process, VS reduced from 77.52% to 70.25% within 10 days time period. VS decreased from 67.51% to 56.41% for C1 during the composting process, while it decreased from 52.74% to 44.06% for reactor B1. Maximum VS degradation was observed in reactor B1 when compared to reactor C1. A significant amount of TOC was lost as CO₂, as well as through the consumption of available carbon as a source of energy by microorganisms (Khwaitrakpam and Bhargava, 2009). TOC content in all composts decreased clearly as composting progressed as shown in Fig. 8.19b, owing to microbial mineralization of organic carbon.

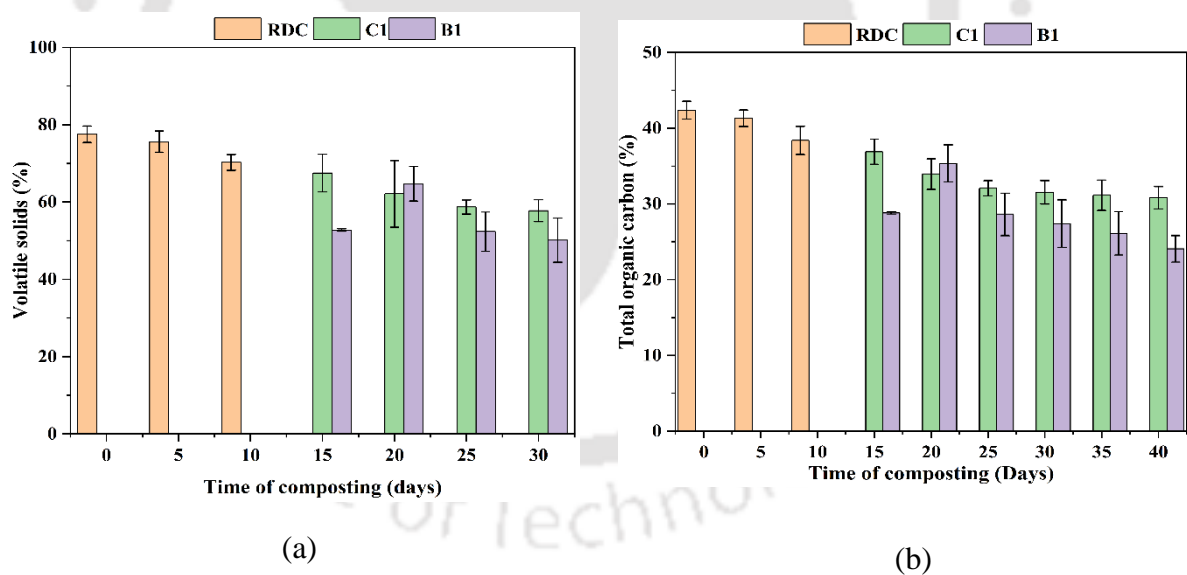


Fig. 8.19. Variation in a) Volatile solids and b) Total organic carbon during bioaugmentation process

This is consistent with the findings of Chan et al. (2016). During the 10-day rotary drum composting process, the initial TOC values were 42.36, 41.31, and 38.31%. TOC after a 40-day composting process was higher in the C1 reactor (30.82%) than in the reactor B1

with the bacterial consortia *L. fusiformis* MHK3 (OL533643) (24.07%). TOC contents decreased significantly in ultimate compost, with the fastest declining speed in the bio-augmented reactor, indicating the highest bio-degradation rate of organic waste.

8.3.1.3 pH and Electrical conductivity (EC)

The initial low pH, typical of *Mikania* residues, quickly shifted to values within the composting range (Miller, 1992). The pH reached neutrality (7.73) during the thermophilic phase due to organic nitrogen mineralization; after that, the pH increased to slight alkalinity during the bioaugmentation process as shown in Fig. 8.20a. According to Smith et al. (2006), compost pH generally increased during the composting process. The volume of ammonia released due to protein degradation increased as the pH level increased during composting. Increased aeration in the drum from daily turning tends to lower CO₂ levels in the compost, which tends to raise pH (Haug, 1993). During Day 0, pH was recorded as 6.87 but after 40 days of the whole process, it was increased to a neutral value of 8.01 and 8.11 for C1 and B1. Lopez et al. (2002) have also reported the same range of initial (pH 6.58) and final (pH 8.2 to 8.83) in aerobic composting of sun-dried green bean, pepper, and cucumber plants that corroborate the present findings.

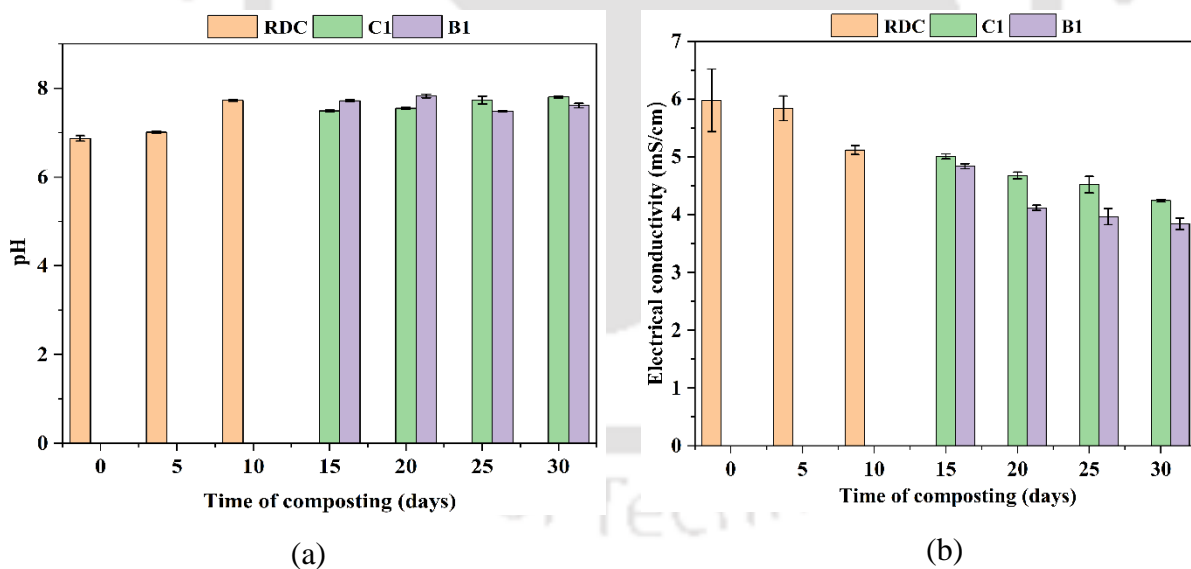


Fig. 8.20. Variation in a) pH and b) Electrical conductivity during bioaugmentation process (b)

During the composting process, alkaline pH is the most beneficial, as acidic conditions destroy the majority of microorganisms. As a result, when the composting process is finished, the pH of the composting mixture is nearly neutral (Ameen et al., 2016). EC was recorded 5.98 mS/cm at Day 0, after 10 days it was seen reduced to 5.12 mS/cm as shown in Fig. 8.20b. Post bacterial application, EC was reduced to 3.54 mS/cm at the end of 40

days of the process. EC in C1 has been reduced to 4.08 mS/cm at the end of 40 days. There was clear evidence of a reduction in EC with and without treatment. Bacterial inoculation had more effect on the degradation of organic compounds that mediated the reduction of EC. It was reported that compost having EC of more than 4 mS/cm is not suitable for application in the agricultural field (Karak et al., 2013).

8.3.1.4 Total Nitrogen and nutrient content (Total phosphorus, TP; Available phosphorus, AP; Sodium, Na; Potassium, K and Calcium, Ca)

The carbon content decreased and the nitrogen content increased in the majority of the composting mixtures due to a concentration effect caused by the degradation of labile organic carbon compounds and the release of CO₂, resulting in a reduction in the overall weight of the composting mass (Pandey et al., 2009). Nitrogen and phosphorous have long been thought to be the most important nutrients in the compost. According to research, nitrogen is immobilized and converted to humus-like material during composting; it can also be used as an organic material with a slow nutrient release (Preusch et al., 2002). Fig. 8.21 shows the TKN trend throughout the composting process.

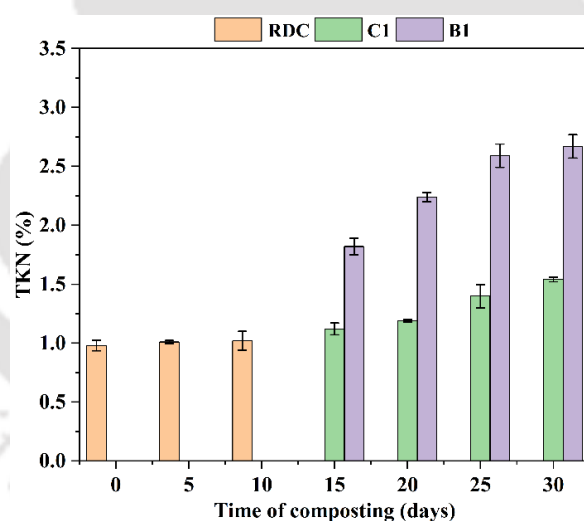


Fig. 8.21. Variation in Total Kjeldahl Nitrogen during the bioaugmentation process

TKN increased from 0.98% to 1.02% during the initial 10 days of the rotary drum composting process. After 10 days, the bio-augmented pre-degraded substrate exhibited the highest TKN percentage of 2.98 as compared to the control reactor (2.08%). The increase in TKN may be due to net loss of dry mass as CO₂ evolution and moisture loss by the generation of heat by microbial action on organic matter. Furthermore, nitrogen-fixing bacteria might also contribute to the increase in TKN in the later stage of composting

(Bishop and Godfrey, 1983). A drastic change in the increase of nitrogen content was observed after 20 days of composting process in the inoculated reactor. This may be due to the addition of bacterial inoculum for the composting process. It has previously been reported that *Lysinibacillus sp.* can fix atmospheric nitrogen as ammonia for plants (Tan et al., 2015; Shabanamol et al., 2017; Tian et al., 2019), as nitrogen fixation by bacteria is attained through the reactions catalyzed of a complex enzyme system known as nitrogenase, which is interpreted by Nif genes. *Lysinibacillus spp.* has Nif genes and generates nitrogenases (Park et al., 2005). The increase in phosphorous content is thought to be due to organic material mineralization. Phosphorus dynamics can be explained by the loss of dry mass, which raises the total concentration, as well as by microbial mineralization, which makes it more available in the form of available phosphorus (Jakubus, 2016). TP and AP level increased during the first days of rotary drum composting, from 8.05 g/kg to 0.87 g/kg to 1.98 g/kg, following moisture loss (Fig. 8.22 a & b). After 40 days, the final TP in the bio-augmented reactor was 10.87 g/kg, and the AP was increased to 4.75 g/kg. For reactor C1, TP was measured to be 10.05 g/kg and AP was measured to be 3.75 g/kg. At $p < 0.05$, phosphorus dynamics varied significantly. The analysis of macronutrients is critical because compost end users are primarily agricultural and determine the compost's fate for commercial application (Hazarika and Khwairakpam, 2018). The use of synthetic fertilizers has become popular due to their instant availability of nutrients, which can, however, be supplemented more appropriately by compost (Weber et al., 2007).

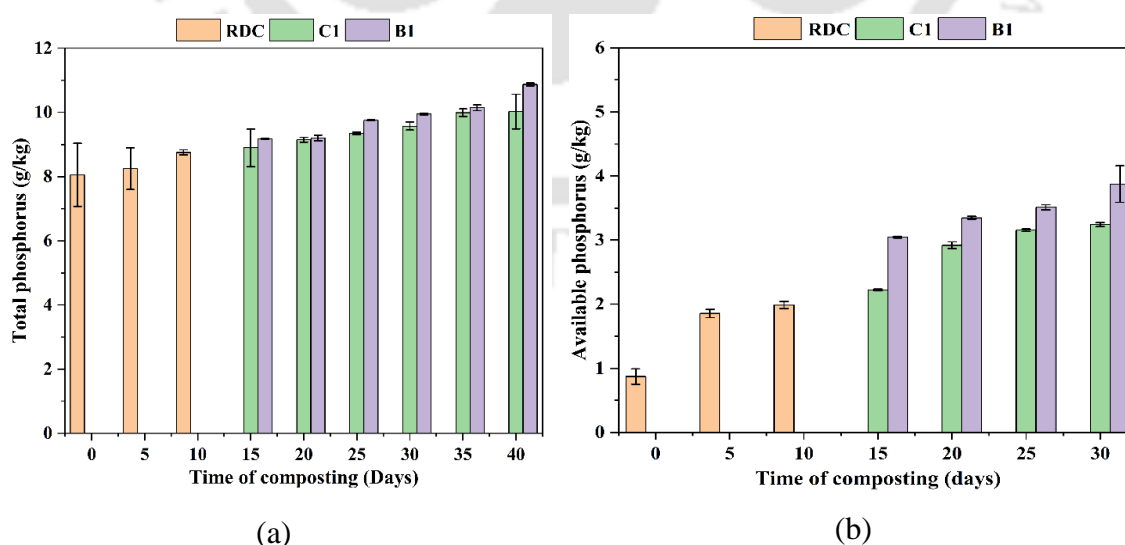


Fig. 8.22. Variation in phosphorus dynamics a) Total phosphorus and b) Available phosphorus during the bioaugmentation process

As shown in Fig. 8.23 (a, b & c), the total concentration of nutrients (Na, K, Ca) was examined in the current study, and a gradual increase was observed due to dry mass loss. During the first 10 days of the rotary drum composting process, Na levels increased from 1.12 to 1.72 g/kg. At the the end of 40 days, it was discovered that it had increased from 2.75 to 3.51 g/kg for the bio-augmented reactor compared to the control (which had increased from 2.12 to 3.01 g/kg). Similarly, Ca was found to increase from 5.0 to 7.37 g/kg during the first 10 days of the rotary drum composting process, but it increased from 7.92 to 10.87 g/kg after the pre-degraded substrate was bio-augmented, compared to the control (7.87 to 9.89 g/kg) at the end of 40 days. Potassium increased in value throughout the composting process, with the highest value recorded in the reactor bio-augmented with bacterial strain (up from 30.3 to 45.62 g/kg).

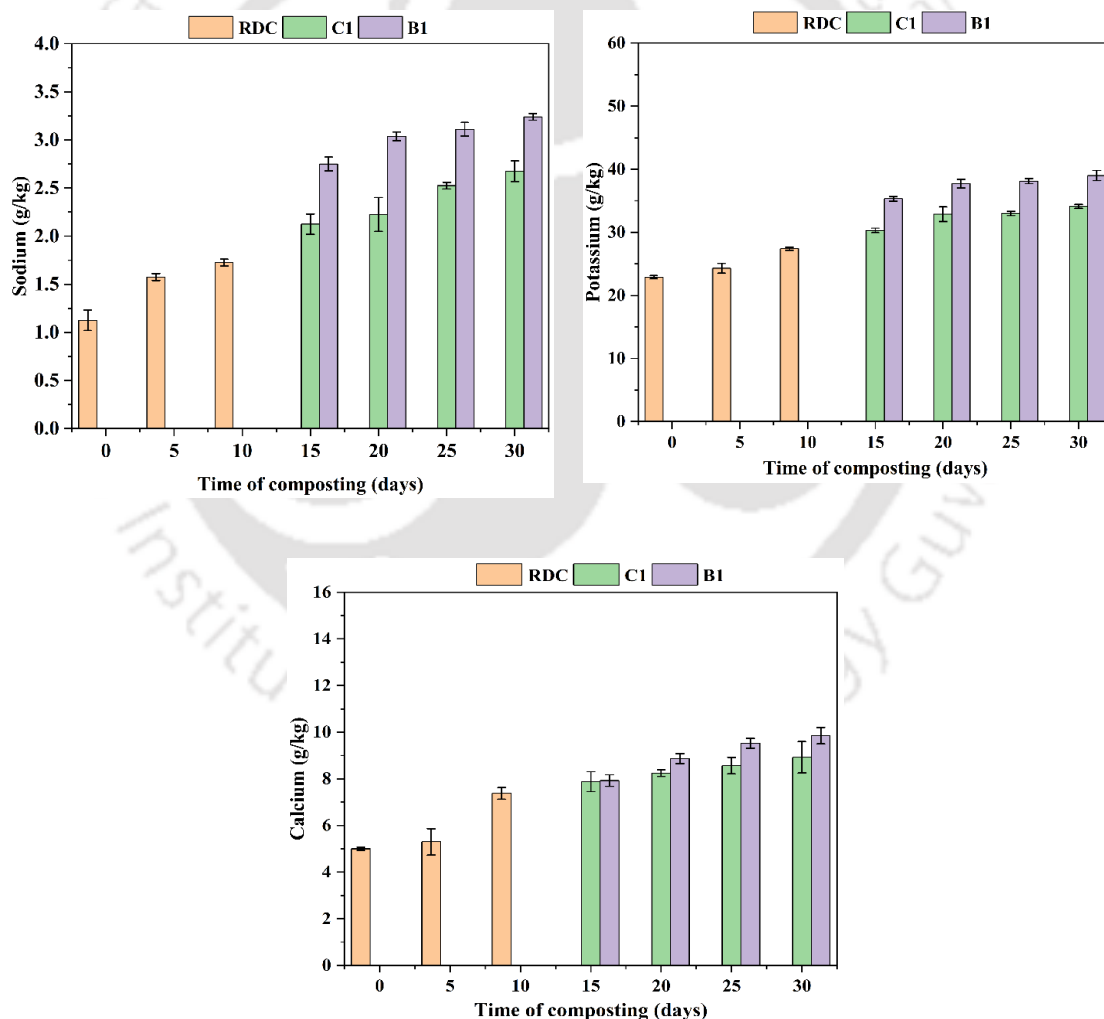


Fig. 8.23. Variation in a) Na, b) K, c) Ca during the bioaugmentation process

8.3.1.5 Soluble BOD, COD, and CO₂ evolution

The decreasing trend in the concentration of sBOD was observed in all the trials during the TCB process which is an important parameter for compost quality (Kalamdhad and Kazmi, 2007). For reactor C1, it was observed that sBOD decreased to 210.6 mg/L from 338.7 mg/L after the first 10 days. However, when the pre-degraded waste was treated with the bacterial strain *L. fusiformis* MHK3 (OL533643) (reactor B1), it was observed that the sBOD was decreased from 186.0 mg/L to 78.52 mg/L whereas the reactor C1 had reduction from 192.9 to 117.41 mg/L as shown in Fig.8.24(a). sBOD reduction during the TCB process was due to the degradation of an organic compound by the inoculum doses and microorganisms that attends stabilization which can be further used for the crop in soil (Wang et.al., 2004). The reduction profile of sCOD has been illustrated in Fig. 8.24b. During the rotary drum composting for the first 10 days, sCOD was decreased from 768.14 to 541.87 mg/L. It was reduced further to 201.41 mg/L from 487.31 mg/L for C1 and B1 sCOD was reduced from 385.61 to 124.87 mg/L. As described by Jain et.al., (2018), organic matter deterioration determines whether sBOD and sCOD increase or decrease. Biological content reduction reduces sBOD and sCOD, reducing emissions of carbon dioxide, suggesting the compost has undergone stabilization. sBOD and sCOD analyzed varied differently at $p < 0.05$ during the TCB process. Compost stability can be evaluated using CO₂ evolution, which is the most direct method because it measures carbon that is derived directly from the compost under consideration. The evolution of CO₂ has a direct relationship with aerobic respiration (Kalamdhad et al., 2009). The higher CO₂ evolution rate during the initial 10 days of rotary drum composting was found to reduce from 5.37 to 3.70 mg/g VS/days as shown in Fig. 8.24c. Later, when the pre-degraded mixture was bio-augmented, the maximum reduction of CO₂ evolution rate was found to be 0.81 mg/g VS/days as compared to control (1.28 mg/g VS/days). The degradation of CO₂ evolution is due to the degradation of organic matter during the TCB process. The amount of O₂ consumed and CO₂ released during the breakdown of organic matter is primarily represented by the chemical composition of the substrate used. During the TCB process, CO₂ evolution was observed to be decreasing as the TOC decreased. After 40 days of the composting process, CO₂ evolution was stagnant at around 0.81 mg/g VS/days which may be due to maximum possible degradation by the bacterial consortia *L. fusiformis* MHK3 (OL533643).

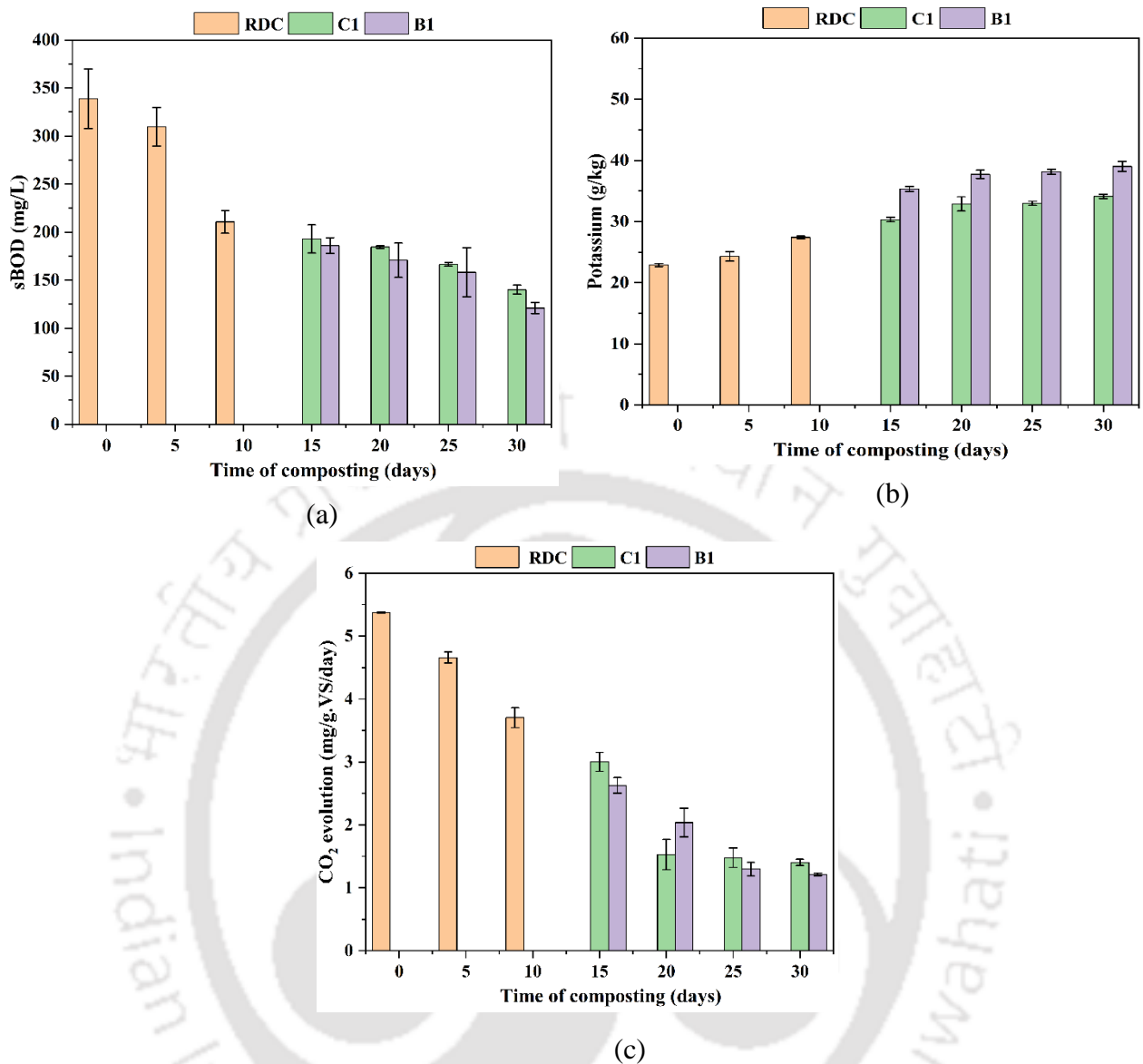


Fig. 8.24. Variation in a) sBOD b) sCOD and c) CO₂ evolution during the bioaugmentation process

8.3.1.6 Toxicity evaluation of compost before and after bacterial treatment using *Allium cepa* bioassay

Plant bioassay systems are extremely useful in biological testing and have several advantages over mammalian and microbial systems. Some advantages include similarities in the chromosomal morphology of plants and mammals, a similar response to mutagens, being less expensive, and taking less time. Among the various plant systems, *A. cepa* is the most advantageous plant species because it is easy to handle, cost-effective has a good correlation with other toxicity tests, and has a high sensitivity to environmental chemicals.

It has frequently been used as an effective standard species for assessing the phytotoxicity of toxic compounds. The phytotoxic effect of both the compost was assessed using an *A. cepa* root growth inhibition test as shown in Table 8.7.

Table 8.7. Phytotoxicity assessment of compost produced during TCB process

(a) Root lengths of <i>Allium cepa</i>			
	Day 0	Day 20	Day 40
Without treatment (C1)			
Concentrations			
25	1.98 ± 0.02	9.1 ± 0.55	11.4 ± 0.98
50	1.21 ± 0.01	5.8 ± 0.71	9.8 ± 0.09
75	0.98 ± 0.01	4.8 ± 0.20	4.1 ± 0.52
100	0.91 ± 0.02	3.5 ± 0.20	3.9 ± 0.24
With bacterial treatment (B1)			
25	2.01 ± 0.02	10.2 ± 1.02	12.1 ± 0.38
50	1.28 ± 0.02	8.7 ± 0.87	11.8 ± 0.41
75	1.01 ± 0.04	6.9 ± 0.58	12.1 ± 1.02
100	0.87 ± 0.01	4.1 ± 0.27	10.1 ± 1.41
(b) Biomass Index of <i>Allium cepa</i>			
	Day 0	Day 20	Day 40
Without treatment (C1)			
Concentrations			
25	0.81 ± 0.04	2.599 ± 0.08	2.207 ± 0.11
50	0.12 ± 0.02	1.74 ± 0.08	1.702 ± 0.18
75	0.08 ± 0.01	1.419 ± 0.21	0.709 ± 0.02
100	0.05 ± 0.01	0.962 ± 0.04	0.889 ± 0.01
With treatment (B1)			
25	0.87 ± 0.02	2.13 ± 0.62	1.71 ± 0.18
50	0.25 ± 0.01	1.91 ± 0.74	2.60 ± 0.24
75	0.18 ± 0.04	2.12 ± 0.58	3.00 ± 0.25
100	0.05 ± 0.01	1.90 ± 0.12	2.92 ± 0.18

Root growth inhibition in *A. cepa* roots has been considered a toxicity indicator because it may result from cell division inhibition. The effect of different concentrations of compost on *A. cepa* root growth and length is depicted in Fig. 8.25 (sample C1) and Fig. 8.26 (sample

B1). Compare to sample C1, B1 showed significant growth in the root length at concentration 25-50%.



Fig. 8.25. Phytotoxicity assessment test using *Allium cepa* for the sample control (C1)

Maximum growth for the sample C1 was observed at Day 40 in the concentration 50% (9.8 ± 0.09 cm). For sample B1, maximum growth was observed in the concentration 75% (12.1 ± 1.02 cm). The results of the study demonstrate that there was a significant amount of toxicity present, which resulted in the onion growth being inhibited during the first few days. In contrast, the use of a bacterial strain significantly reduced the toxicity, and the presence of growth was observed to be particularly prominent in sample B1. It is undeniable that the strain *L. fusiformis* MHK3 (OL533643) demonstrated degradation of toxicity, which occurs as a result of the presence of phytochemicals in the invading plant.



Fig. 8.26. Phytotoxicity assessment test using *Allium cepa* for the sample B1

8.3.1.7 FTIR analysis of the compost sample

The comparison of FTIR spectra of RDC, C1, and B1 samples is shown in Fig. 8.27. It clearly shows a significant peak difference to different functional groups in the compost samples. At the beginning of the composting process, a predominance of the peaks or bands at 3215, 2943, 1640, 1340, and 1043 cm^{-1} was observed, which shows that both composts were rich in aromatic, phenolic, aliphatic, and polysaccharide structures. Peaks in the FTIR spectrum of the RDC sample represent C-O stretch and C-H in-plane deformation. The next peak at 1641 cm^{-1} is attributed to the presence of aromatic compounds in the sample

(Hussain et al., 2016). The reduction in the intensity of bands may be attributed to the reduction of aromatic, and aliphatic carbons by microbial consortia. The peak at 2943 cm^{-1} indicates aliphatic C-H stretching of fatty acids and lipids. Further, the peak at 1642 cm^{-1} represents the aromatic group. The peaks at 1562 and 1400 cm^{-1} denoted the C-H stretching of aromatic ring vibration occurred at 1600 cm^{-1} whereas the peak at 1075 cm^{-1} for C-OH stretching vibrations. On the other hand, the FTIR spectrum of the B1 sample showed marked differences as the intensity of bands was reduced as compared to C1. These results suggest that the bacterial lac enzyme system might be responsible for the degradation.

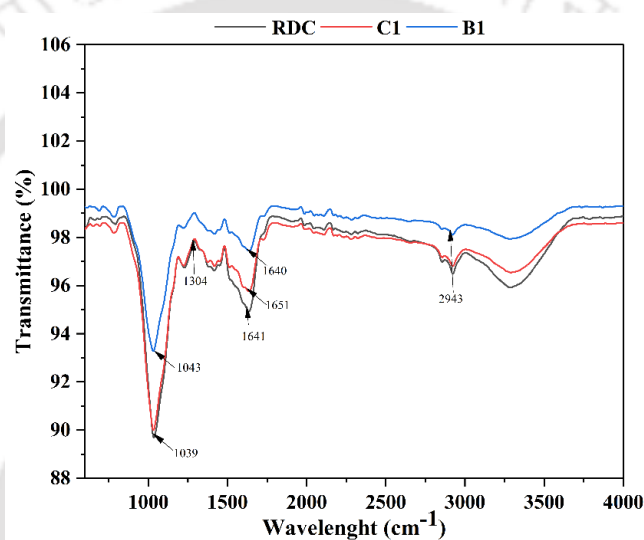


Fig. 8.27. FTIR spectra of the compost sample (RDC, C1 and B1)

8.3.1.8 GC-MS analysis of *Mikania* and compost samples

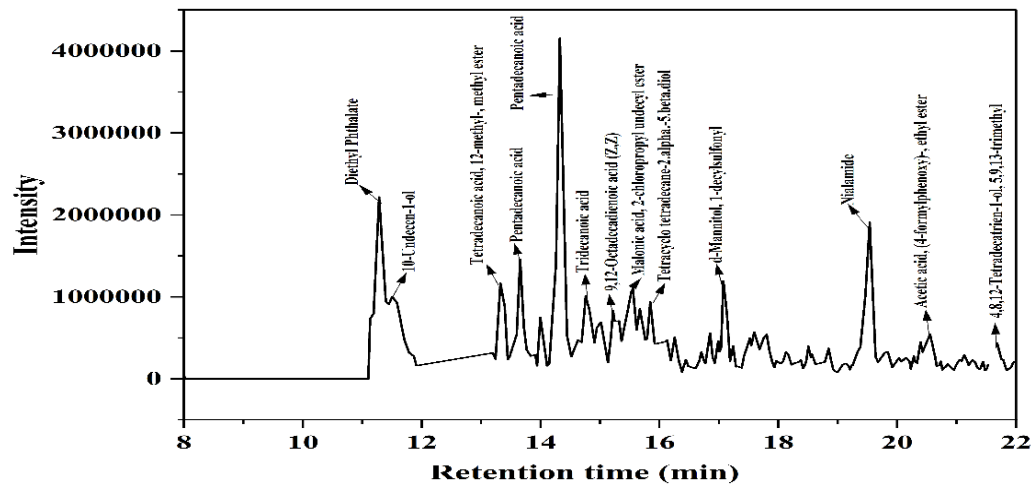
The comparative analysis of *Mikania*, control and bacteria-treated samples has been illustrated in Table 8.8. The results of GC-MS spectra are shown in Fig. 8.28. There are more than 30 peaks in the GC-MS spectrum of *Mikania*. *Mikania* extraction revealed mostly the ester group, acidic group, and alcohol group of compounds. The maximum peak area is shown by Diethyl Phthalate (4.52%), followed by 1-Pentanol, 2-methyl (4.48%), Bicyclo [5.3.0]decane, 2-methylene (2.72%), 4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl- (2.31%), 3-(n-Propylamino)-2,1-benzisothiazole (1.58%), Glycidol (1.51%), 6-Amino-9-(2-hydroxyethyl) purine (1.44%), tetramethyl-4-vinyl (1.39%), Pentadecanoic acid (1.23%), 9,12-Octadecadienoyl chloride, (Z,Z)- (1.22%), 9,10-Anthracenedione, 2-methyl- (1.21%), 1,3,5-Triazine-2(1H)-thione, 1-(2,3-dimethylphenyl)tetrahydro-5-(1-phenylethyl)

(1.18%), Nialamide (1.02%). The other compounds had shown a peak of less than 1% area percentage. Diethyl phthalate is a toxic allelochemical found in invasive weeds. According to Kapanen et al. (2007), diethyl phthalate was found to be less toxic as a pure compound than when mixed with compost, implying a synergistic effect of unknown toxic compounds or the release of compounds due to diethyl addition. Hexadecaonic acid methyl ester is an organic fatty acid compound that is also known as palmitic acid methyl ester and linolenic acid (Aji et al., 2015). Okwu and Ighodaro (2009) discovered that plant fatty acids and alcohols undergo esterification reactions, resulting in esters. Pyridine compounds are a class of alkaloids, nitrogen-containing chemical compounds widely found in plants, that contain a pyridine ring. It is the most toxic, flammable liquid with a fishy smell. GC-MS analysis of untreated sample revealed the presence of various types of organic compounds including Diethyl Phthalate (4.52%), 1-Pentanol, 2-methyl (4.48%), Bicyclo [5.3.0] decane, 2-methylene-5-(1-methylvinyl)-8-methyl (2.72%), Betulin (1.58%), Glycidol (1.51%), 6-Amino-9-(2-hydroxyethyl) purine (1.44%), 1,3,5-Triazine-2(1H)-thione, 1-(2,3-dimethylphenyl) tetrahydro-5-(1-phenylethyl)- (1.18 %), Acetic acid, (4-formylphenoxy)-, ethyl ester (1.08%). GC-MS analysis of post-treatment showed the presence of new peaks suggesting that these peaks belong to degradation products. Also, the initial peaks observed in the untreated sample were mostly absent or were at much-reduced levels. The pre-degraded sample treated with the bacterial strain *Lysinibacillus fusiformis* MHK3 (OL533643) confirms the degradation of certain compounds and transformation of compounds. The bacterial strain was capable of using the phytochemicals present in the sample for its growth and metabolism and can be used for bioaugmentation of *Mikania* substrate for effective reduction of allelochemicals.

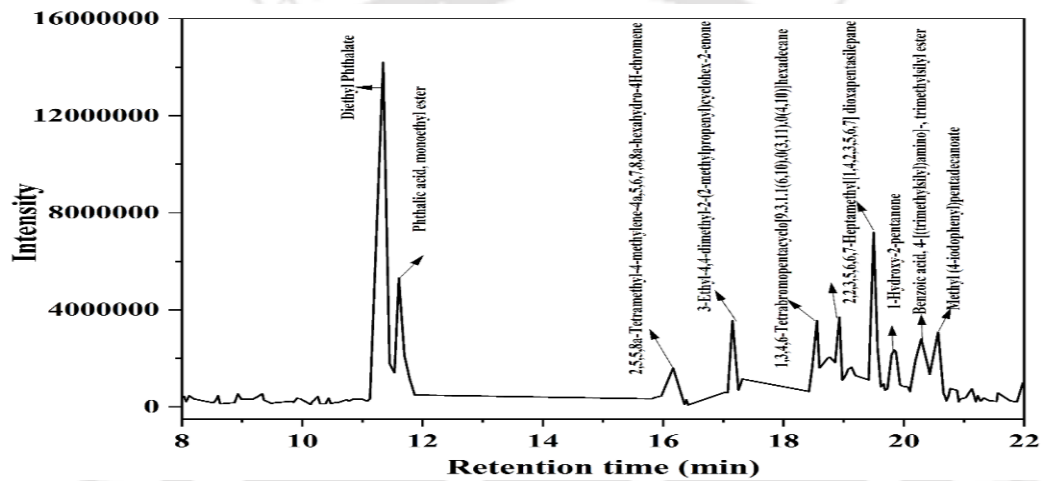
Table 8.8. Compound identification extracted by dichloromethane from *Mikania*, C1 and B1 samples

Retention time in min	Compounds	<i>Mikania</i>	C1	B1
11.28	Diethyl Phthalate	+	+	+
11.60	10-Undecan-1-ol	-	-	+
11.61	Benzoic acid, 4-formyl-	-	+	-
13.32	Pentadecanoic acid	+	-	-
13.65	Tridecanoic acid	+	-	-

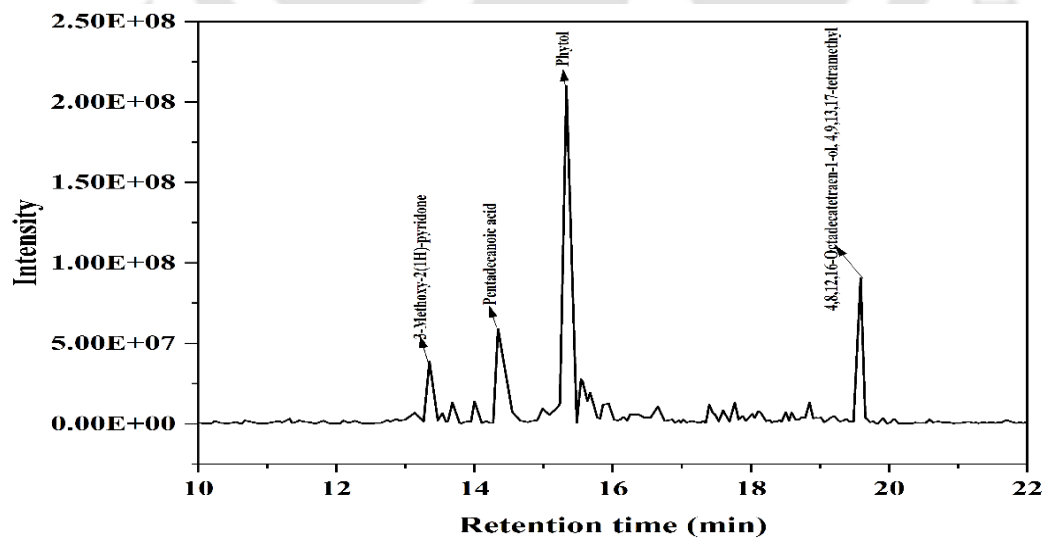
	Tetradecanoic acid, 12-methyl-, methyl			
13.52	ester			
14.26	10-Undecenoyl chloride	+	-	-
14.32	Pentadecanoic acid	+	-	-
14.81	9,12-Octadecadienoyl chlo	+	-	-
15.21	9,12-Octadecadienoic acid (Z,Z)			
15.55	1-Bromo-5-nonene, (Z)-	+	-	-
15.57	Malonic acid, 2-chloropropyl undecyl ester			
15.66	Octadecanoic acid	+	-	-
15.84	9,10-Anthracenedione	+	-	-
16.01	5-(7a-Isopropenyl-4,5-dime	-	+	-
17.07	d-Mannitol, 1-decylsulfonyl			
17.59	7-Hexadecanoic acid			
17.07	10-Undecen-1-ol	+	-	-
17.13	But-3-enal, 2-methyl-4-(2,6,6	-	+	-
17.15	3-Ethyl-4,4-dimethyl-2-(2	-	-	+
17.45	Isolongifolene, 7,8-dehydro	-	+	-
18.55	Stigmasterol trimethylsilyl	-	-	+
18.77	2(1H)-Naphthalenone, 3,4,4a	-	+	-
18.92	7,7,9,9,11,11-Hexamethyl-3	-	-	+
19.21	Aminoguanidine	-	+	-
19.46	Betulin	+	-	+
19.48	9,19-Cyclolanostan-3-ol, 24	-	+	-
19.54	4,8,12-Tetradecatrien-1-ol	+	-	+
19.54	,8,12,16-Octadecatetraen	-	+	-
19.81	Dimethyl{bis[(4,8,8-trimethyldecah	-	+	-
20.27	Pyridine, 2,6-bis(1,1-dimethylethyl)	-	+	-
20.28	1,3,4,6-Tetrabromopentac	-	-	+
20.54	3-(n-Propylamino)-2,1-be	-	+	-
20.56	3-Isopropyl-6a,10b-dimethyl-	-	-	+
22.48	Bicyclo[5.3.0]decane, 2-me	+	-	-
22.49	Ambrein	-	+	-



(a)



(b)



(c)

Fig. 8.28. GC-MS spectra of a) *M. micrantha* b) Control (C1) and c) Bacterial treated (B1) samples during the bioaugmentation process

8.3.2 Vermicomposting process bio augmented with bacterial consortia

Lysinibacillus fusiformis MHK3 (OL533643)

Mikania, cow dung, and sawdust were combined in a 5:4:1 ratio for the vermicomposting process, with the earthworm species *Esenia fetida* serving as the primary composting agent. Bacterial consortia were added to the reactor. 2.5 kg of the waste mixture was used in the reactor, and 5% of the inoculum doses were added to the waste mixture. The reactors set up contain control (with only earthworm) (VC) and bacterial inoculated reactor (earthworm and bacterial inoculum) (VCB).

8.3.2.1 Moisture content

Water is sprinkled into the reactor at regular interval of time since earthworms require an adequate amount of water for their growth and the proper degradation of organic matter in the reactor. The initial moisture content of approximately 88% was recorded for both VC and VCB. For the VC and VCB reactor moisture content at the end of 40 days was observed to be 68.78% and 68.21% respectively (Fig. 8.29). Due to regular sprinkle of water moisture was almost constant in both the reactor system.

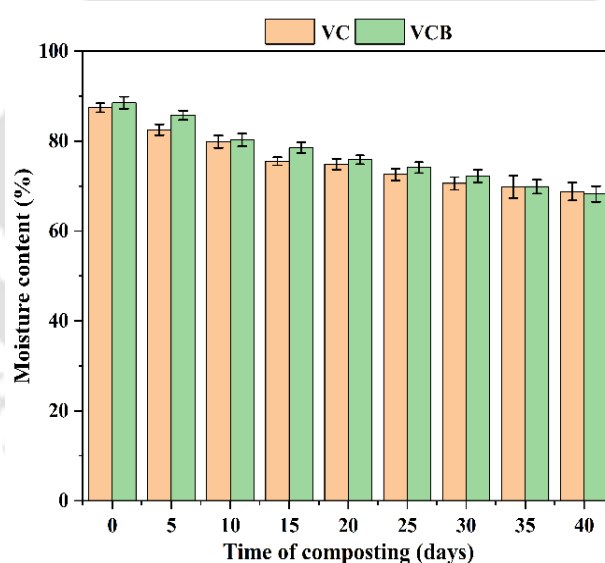


Fig. 8.29. Variation in moisture content during the bioaugmentation process

8.3.2.2 Volatile solids (VS) and total organic carbon (TOC)

A volatile solid represents the amount of organic matter in the wastes. The higher the concentration of volatile solids more is the more organic matter. It is beneficial in assessing the biologically inert organic matter. The content of volatile solids decreased by about

28.06% for the reactor VC at the end of 40 days whereas for the reactor VCB the percentage reduction was found to be 38.76% (Fig. 8.30a). The inoculation of the bacterial consortium into the earthworm treatments resulted in better results. This suggests that the use of isolated bacteria and earthworms at the same time has a synergistic effect (Koolivand et al., 2020). Typically, a decrease in TOC content was observed in vermicompost. In our study, Fig. 8.30(b) shows that the TOC in VCB was significantly lower (26.14%) than in VC (29.65%). Pattnaik and Reddy (2010) reported a reduction in TOC during the vermicomposting process. According to the authors, the loss of carbon in the form of carbon dioxide during the decomposition process was attributed to both the earthworm and microbial respiration. Our findings indicate that the greatest reduction in TOC occurred in VCB, which could be attributed to the presence of microbial consortia. Sharma (2003) has reported a 20-45% reduction in TOC during vermicomposting of municipal or industrial wastes, which supports the observed results.

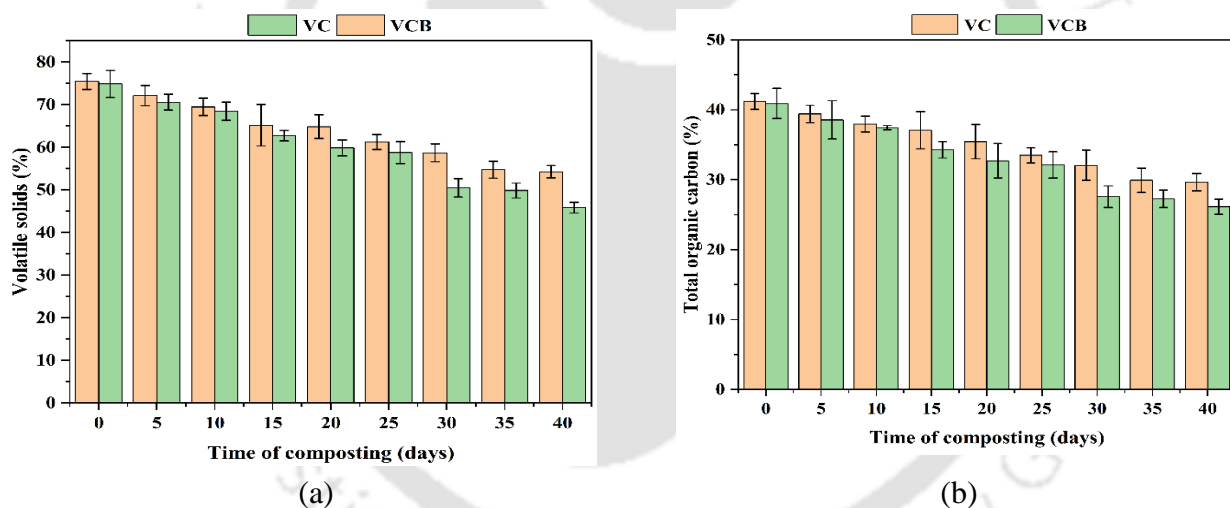


Fig. 8.30. Variation in a) Volatile solids and b) Total organic carbon during the bioaugmentation process

8.3.2.3 pH and Electrical conductivity (EC)

The changes in pH that occur throughout the vermicomposting process are dynamic and dependent on the substrate (Lim et al., 2011). Both the VC and VCB reactors showed a slight increase in pH values, which was observed in our study as shown in Fig. 8.31(a). At the end of 40 days, the pH of the reactor VC was found to be in the range of 6.75-8.12, and the pH of the reactor VCB was found to be in the range of 6.58-8.01. Li et al. (2011) also found evidence for an increase in pH. According to the authors, the mineralization of proteinaceous materials during the process of vermicomposting may be responsible for the

pH shift observed during the process. The decrease in EC could be due to mineral absorption in earthworm-infested reactors as shown in Fig. 8.31(b). While the reactor VC had a marginal value greater than 4 mS/cm, the final value for the VCB reactor was 3.54 mS/cm, which could be due to both the earthworm and the inoculated earthworm. The overall EC values of 4 mS/cm are assumed to be adequate for the use of VC in agricultural practice (Lasaridi et al., 2006). According to Saez et al., (2021), the decrease in EC could be caused by ammonia volatilization and the accumulation of insoluble salts.

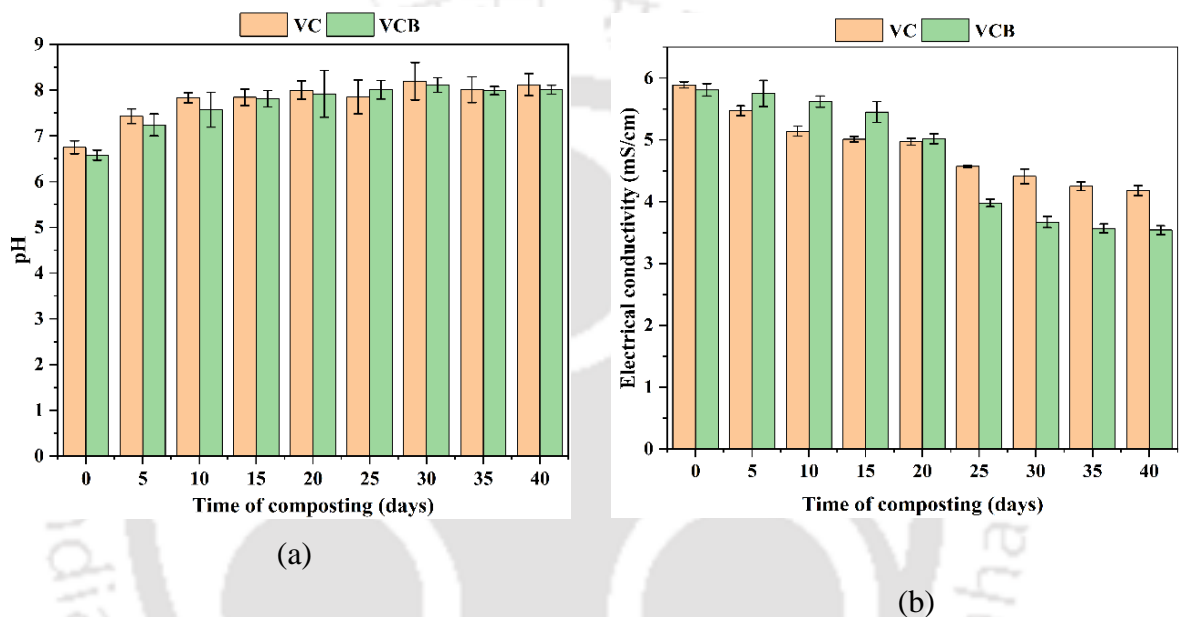


Fig. 8.31. Variation in a) pH and b) Electrical conductivity during bioaugmentation process

8.3.2.4 Total Nitrogen and nutrient content (Total phosphorus, TP; Available phosphorus, AP; Sodium, Na; Potassium, K and Calcium, Ca)

The vermicomposting process showed a significant increase in the total nitrogen content which may be due to the mineralization of organic matter. Nitrogen is an essential element required for successful plant growth (Liu et al., 2014). For an organic agricultural system, continuous application of manure increases the nitrogen (N), phosphorus (P), potassium (K), and calcium content in the soil. Inorganic nitrogen is released and absorbed by plants after organic fertilizers are applied to soils and mineralization begins (Ginting et al., 2003, Watts et al., 2010). The initial TKN content in both VC and VCB was between 1.47 and 1.01%. However, the final product increased in VC and VCB by 2.57 and 3.11%, respectively shown in Fig. 8.32. When compared to VC, the TKN value in VCB obtained

at the end of the process was higher. That is, in VCB, the process is completed in less time than in VC. This is due to the addition of a consortium in VCB, which helps to improve the process. According to Tripathi and Bhardwaj (2005), the increase in TKN content can be attributed to the earthworms' release of mucus, nitrogenous excretory substance, growth-stimulating hormones, and enzymes. Furthermore, Plaza et al. (2007) stated that nitrogen-fixing bacteria mineralize the carbon-rich material, increasing the nitrogen content of the vermicompost. The TP content in the feed mixture increased significantly during the vermicomposting process. According to the data in Fig. 8.33(a), the amount of TP increased up to 39.17% in VCB and 27.29% in VC.

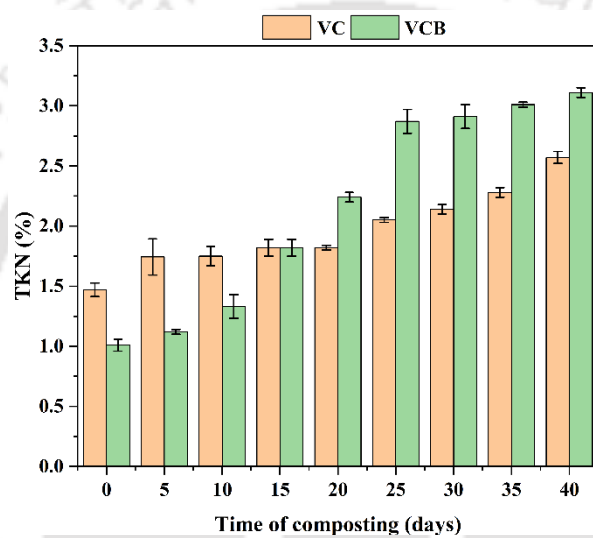


Fig. 8.32. Variation in Total Kjeldahl Nitrogen during the bioaugmentation process

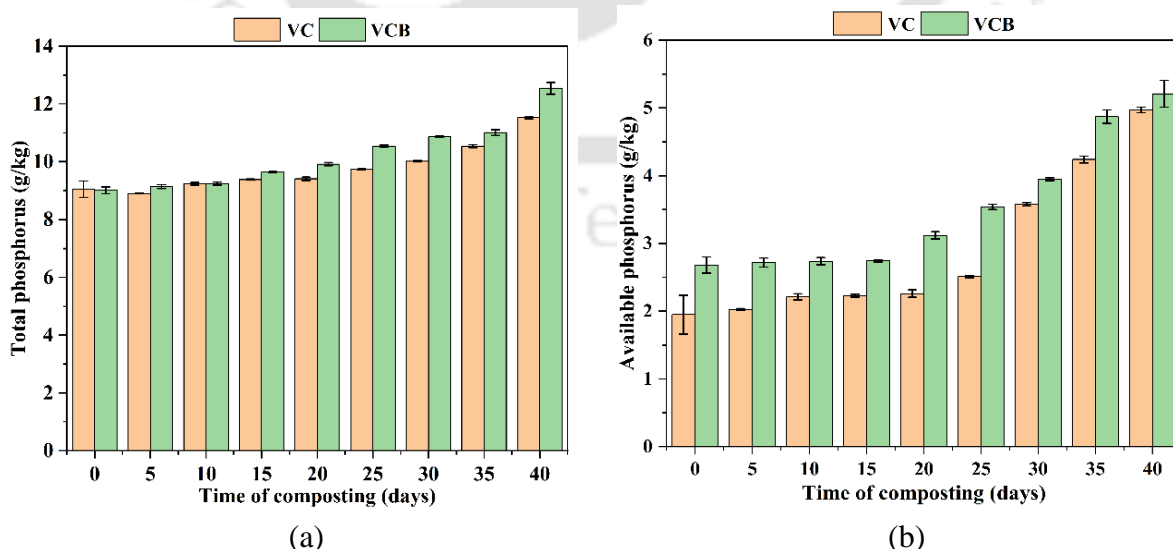


Fig. 8.33. Variation in phosphorus dynamics a) Total phosphorus and b) Available phosphorus during the bioaugmentation process

The activity of earthworms and phosphate-solubilizing microbes during mineralization and phosphorus mobilization causes an increase in TP (Ravindran et al., 2014). The increase in TP could be attributed to both earthworms and microbes, according to the researchers. VCB had a higher level of AP than VC due to the addition of microbial consortia (Fig. 8.33b). Because the phosphorus cycle is a slow process, it takes a long time for the phosphorus to reach the soil. As a result, vermicompost with a high available phosphorus content aids in improving soil fertility (Arumugam et al., 2018). There was an increase in sodium content in VC and VCB. At the end of 40 days, the Na content ranged from 3.3 g/kg to 4.95 g/kg for the reactor VC and increased from 3.2 g/kg to 5.75 g/kg for the reactor VCB (Fig. 8.34a). Sodium can stimulate plant growth and can be used as an alternative in cases where potassium is deficient (Ansari and Sukhraj, 2010). Due to the action of microorganisms, the complete degradation of the substrate mixture was accomplished in a comparatively shorter time in the VCB reactor with higher nutrient content. An earlier study used earthworms to effectively convert *Mikania* biomass into vermicompost (Karthika et al., 2015). In the current study, we discovered that vermicompost, in conjunction with a microbial consortium, can produce high-quality compost while also managing *Mikania* biomass by degrading it in a short period. After the vermicomposting process was completed, the potassium concentration in the reactor VC increased significantly from 21.54 g/kg to 32.45 g/kg whereas for the reactor VCB it increased from 22.74 g/kg to 41.87 g/kg as shown in Fig. 8.34(b). The percentage increase was observed maximum in the reactor VCB (50.64) than the reactor VC (84.12). Organic matter mineralization and biomass loss may have influenced K concentrations in vermibeds. The potassium increase results are consistent with previously reported results on organic waste vermicomposting (Balachandar et al., 2020). The K content increased rapidly for the first 10 to 20 days of vermicomposting before slowing down until the end of the observation. This implies that potassium was released rapidly during the initial rapid organic matter decomposition rates in vermibeds, and that the subsequent slowdown was caused by mobilization of liable K sources by earthworms and associated microbes in other metabolic operations (Gusain and Suthar, 2020). Potassium mineralization in vermicompost is a complex process that is influenced by several factors, including feedstock type, K mobility in the substrate, leaching to bottom layers, and earthworm assimilation rate (Orozeo et al., 1996). Ca concentration during the vermicomposting process increased significantly and the maximum increase was observed in the reactor VCB (11.75 g/kg) at the end of 40 days as compared to the reactor VC (8.15 g/kg) as shown in Fig. 8.34(c). A more rapid increase of Ca

content was observed after 15 days of the process in the reactor VCB. This could be attributed to the release of Ca by earthworm fecal substances, as well as the solubilization of organically-bound Ca via the actions of organic acids formed later in the degradation process (Wei et al., 2018). It should be noted that humic acids formed during the initial phase of organic matter degradation hold a significant amount of Ca via chelation by $-COOH$ and $-OH$ groups found on it, and degradation of such acids in the later phase releases total Ca in the substrate. Earthworm ingestion converts plant tissue-bound Ca into soluble forms via enzymatic solubilization, and further Ca release is performed by fungal and actinomycetes invading worm casts (Domnguez, 2004). Earthworm calcium glands release calcium during the organic waste digestion process, which contributes to the total pool of Ca in worm casts. Ca in manure is a nutrient source in the soil for plants and microbial supply, which aids in buffering acidic soils and improving soil physical structures.

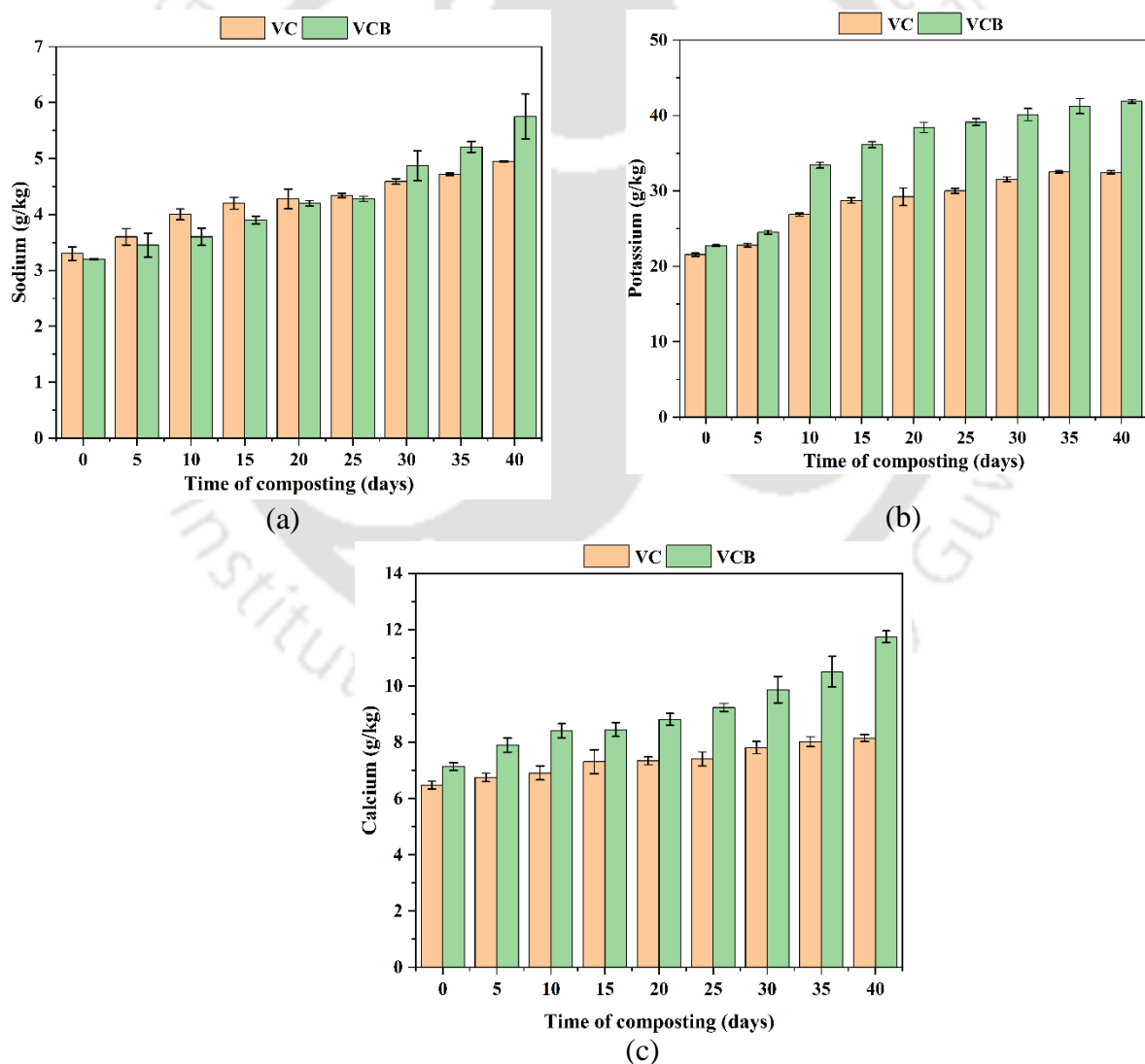


Fig. 8.34. Variation in a) Sodium b) Potassium and c) Calcium during the bioaugmentation process

8.3.2.5 Soluble BOD, COD, and CO₂ evolution

The decreasing trend in the concentration of sBOD was observed in all the trials during the vermicomposting process which is an important parameter for compost quality. For reactor VC, it was observed that sBOD decreased to 88.45 mg/L from 209.6 mg/L after 40 days whereas for VCB reactor the reduction was observed from 203.6 mg/L to 61.25 mg/L (Fig.8.35a). sBOD reduction during the vermicomposting process was due to the degradation of an organic compound by the inoculum doses and earthworms. The reduction profile of sCOD has been illustrated in Fig. 8.35b. During the vermicomposting process, sCOD was decreased from 471.6 mg/L to 128.78 mg/L for the reactor VC. For the reactor VCB, it was reduced from 458.1 mg/L to 91.78 mg/L at the end of 40 days. As described by Jain et.al., (2018), organic matter deterioration determines whether sBOD and sCOD increase or decrease. Biological content reduction reduces sBOD and sCOD, reducing emissions of carbon dioxide, suggesting the compost has undergone stabilization. sBOD and sCOD analyzed varied differently at $p < 0.05$ during the vermicomposting process. Compost stability can be evaluated using CO₂ evolution, which is the most direct method because it measures carbon that is derived directly from the compost under consideration. The evolution of CO₂ has a direct relationship with aerobic respiration (Kalamdhad et al., 2009). CO₂ evolution for the reactor VC was recorded to be reduced from 5.11 to 1.45 mg/g VS/days whereas for the reactor VCB it was reduced from 5.14 to 0.87 mg/g VS/days as shown in Fig. 8.35c. The higher reduction was observed in the reactor VCB which might be due to the synergistic action of earthworm and inoculum doses. The degradation of CO₂ evolution is due to the degradation of organic matter during the TCB process. In general, the TOC content begins to decline during the initial phase of vermicomposting and gradually diminishes at the end (Karmegam and Daniel, 2009), which corresponds to the rate of CO₂ evolution (Gusain and Suthar, 2020). This is primarily due to the stabilization of organic matter, which indicates the completion of the composting process; thus, the reduction in TOC and C/N ratio associated with the respiratory activity is regarded as significant vermicompost maturity parameters (Biruntha et al., 2020). The amount of O₂ consumed and CO₂ released during the breakdown of organic matter is primarily represented by the chemical composition of the substrate used. During the TCB process, CO₂ evolution was observed to be decreasing as the TOC decreased.

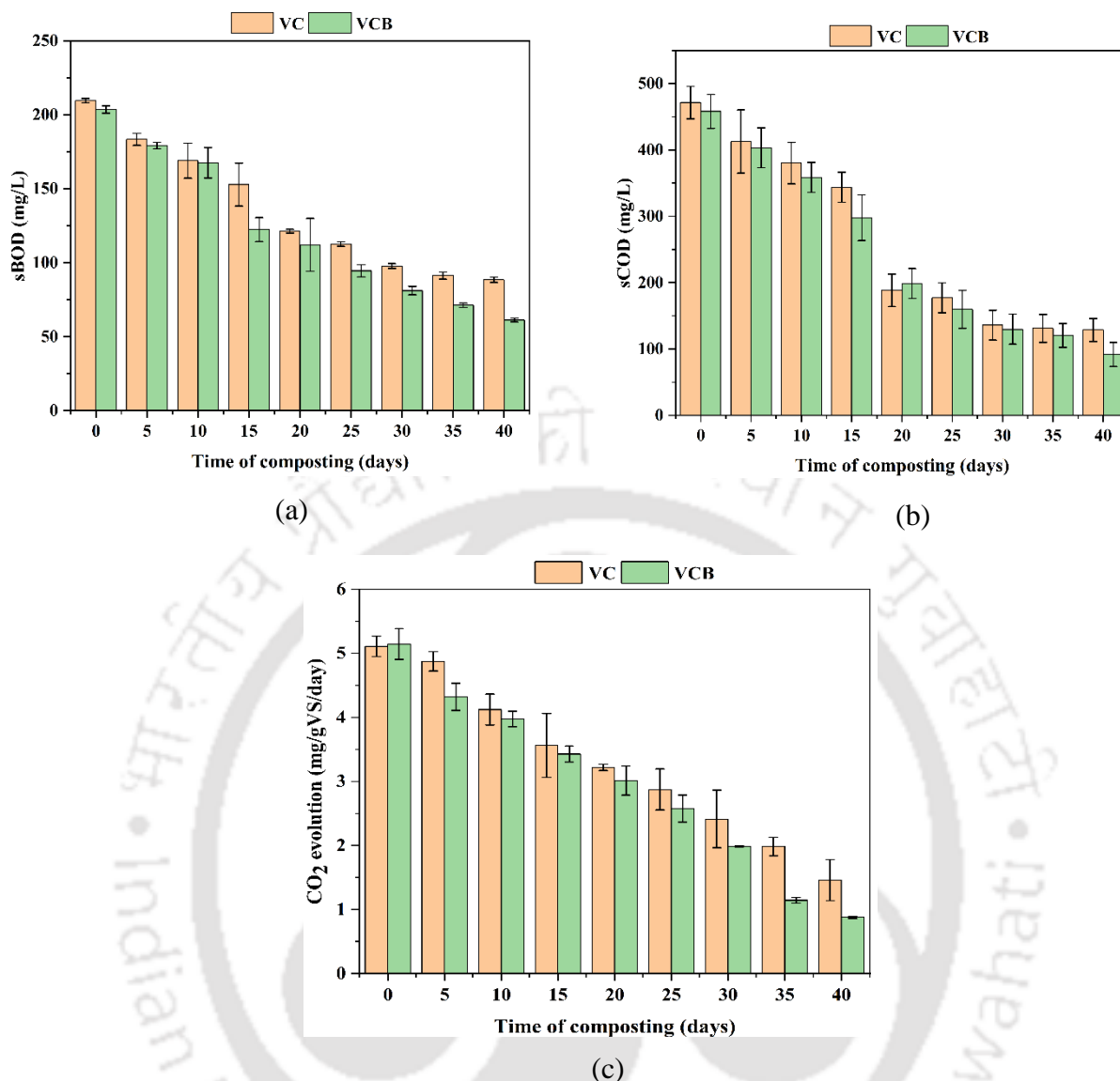


Fig. 8.35. Variation in a) sBOD b) sCOD and c) CO₂ evolution during the bioaugmentation process

8.3.2.6 Growth and development of earthworm

The growth of earthworm biomass during the vermicomposting period is shown in Table 8.9. Although growth was observed in both trials, there was not much significant observation was made during the vermicomposting process between the reactor VC and VCB. Growth is observed more between the days 20-40 days, as earthworm requires time for proper acclimatization if the waste is fresh and has toxic content into it. The European breed, *E. fetida*, has shown steady growth in the composting process. After day 40, the average final count of *E. fetida* was 268 adults, 57 juveniles, and 38 cocoons per 100 g of vermicompost for the reactor VC. The steady rise in population by *E. fetida* suggests that the substrate mix used is favorable to the earthworm species and biodegradation happened

at a good pace. For the reactor VCB, the growth was found to be 287 adults, 68 juveniles, and 41 cocoons per 100 g of vermicompost. According to Neuhauser et al. (1980), when *E. fetida* was fed less than its maintenance level, it lost weight at a rate that depended on the quantity and nature of its ingestible substrates. According to Kale and Krishnamoorthy (1981), earthworms exhibit enhanced growth and reproduction abilities when fed a substrate with more readily available nutrients. Suthar (2007) identified the nitrogen content of the substrate as an important factor in cocoon production.

Table 8.9. Earthworm population throughout the VBB process

	Days				
	VC				
Results	0	10	20	30	40
Adults	200	205	221	247	268
Juveniles	0	10	18	38	57
Cocoons (No.s per 100 g)	0	4	15	20	38
	VCB				
Adults	200	204	248	275	287
Juveniles	0	11	29	51	68
Cocoons (No.s per 100 g)	0	7	10	25	41

8.3.2.7 Toxicity evaluation of compost before and after bacterial treatment using *Allium cepa* bioassay

Several toxicity bioassays have been developed to monitor the harmful effects of pollutants. Levan's (1938) *A. cepa* test has been widely used and validated by several researchers for testing chemical pollutants that pose a risk to the environment (Fiskesjo, 1985). *A. cepa* root growth inhibition could be regarded as an indicator of toxicity, which could be the result of cellular damage or cell division inhibition (Fiskesjo, 1985). The effects of different vermicompost concentrations on *A. cepa* root growth and length are depicted in Fig. 8.36. The onion bulbs were embedded in different concentrations of VC and VCB (Fig. 8.37) samples (25-100% v/v) along with a control to monitor the root growth of *A. cepa*, and the results showed that root growth was inhibited initially in Day 0 but when the substrate was processed using vermicomposting process, a significant amount of growth in the root was observed (Table 8.10). VC and VCB showed prominent growth at the concentration 25% and 50% on Day 20. When both the samples were

compared, apart from root length, there was a significant increase of biomass for the sample VCB (3.04 g in the concentration 100%).



Fig. 8.36. Phytotoxicity assessment test using *Allium cepa* in VC sample



Fig. 8.37. Phytotoxicity assessment test using *Allium cepa* in VCB sample

Table 8.10. Phytotoxicity assessment of vermicompost produced during VBB process

(a) Root lengths of <i>Allium cepa</i>			
	Day 0	Day 20	Day 40
Concentrations	VC		
25	1.1 ± 0.02	11.5 ± 1.01	12.9 ± 0.21
50	0.98 ± 0.01	8.7 ± 0.92	8.5 ± 0.87
75	0.52 ± 0.01	7.4 ± 0.18	7.9 ± 1.02
100	0.24 ± 0.02	6.1 ± 0.41	8.1 ± 0.85

VCB			
25	1.1 ± 0.02	6.9 ± 0.25	7.8 ± 0.21
50	0.98 ± 0.01	7.3 ± 0.84	10.4 ± 0.52
75	0.52 ± 0.01	7.1 ± 0.98	11.1 ± 0.14
100	0.24 ± 0.02	8.1 ± 0.52	11.8 ± 0.86

(b) Biomass Index of <i>Allium cepa</i>			
	Day 0	Day 20	Day 40
Concentrations		VC	
25	0.11 ± 0.01	2.69 ± 0.51	2.65 ± 0.61
50	0.18 ± 0.02	2.15 ± 0.28	2.75 ± 0.07
75	0.12 ± 0.04	1.79 ± 0.12	2.11 ± 0.25
100	0.15 ± 0.02	1.02 ± 0.08	1.98 ± 0.15

VCB			
25	0.11 ± 0.01	2.93 ± 0.18	2.01 ± 0.05
50	0.18 ± 0.02	2.21 ± 0.24	2.34 ± 0.11
75	0.12 ± 0.04	1.74 ± 0.08	2.50 ± 0.24
100	0.15 ± 0.02	1.67 ± 0.08	3.04 ± 0.71

8.3.2.8 FTIR analysis of the vermicompost sample

FTIR analysis is a useful tool for determining the maturity and stability of compost and vermicompost (Gupta and Garg, 2009). Different band spectra analysis aids in the prediction of functional group shifts and provides information about the chemical composition of feed substrates and vermicomposts. Band spectra of the Day 0 sample had peaked at 1047, 1203, 1365, 1649, 1725, 2135, 3350 cm^{-1} which represents alcohols, phenols, fatty acids aromatic rings, carboxylic acids, polysaccharides and CN, CH_2 groups (Mago et al., 2021) as shown in Fig. 8.38. A small peak between 1800-2500 cm^{-1} represents $-\text{COOH}$ stretch of carboxylic acid. A strong peak at 1047 cm^{-1} represents the alcohol and phenolic group and a prominent decrease of the intensity of the peak was observed in the sample VC (D40) and VCB (Day 40). The degradation of organic compounds was observed in the vermicompost sample, although there was a greater shift in the sharp band observed in the VCB sample, which may be due to the addition of bacterial consortia that affected the degradation of the organic compounds.

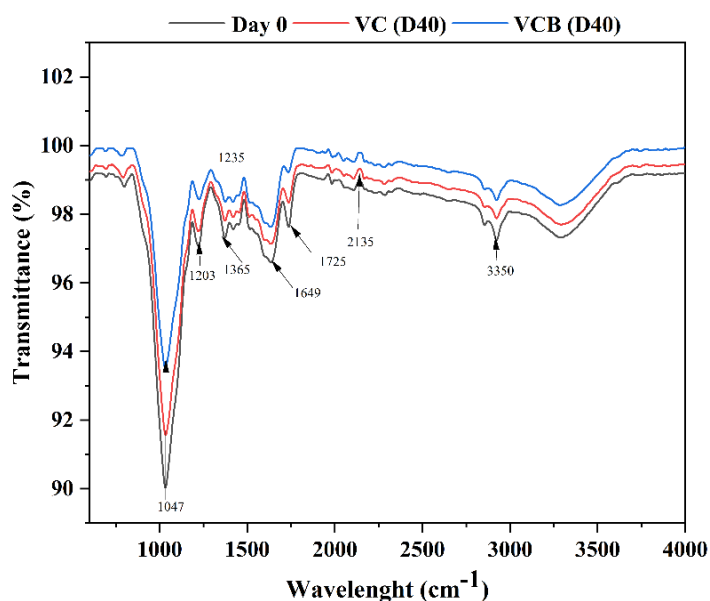


Fig. 8.38 FTIR spectra of vermicompost samples (VC and VCB)

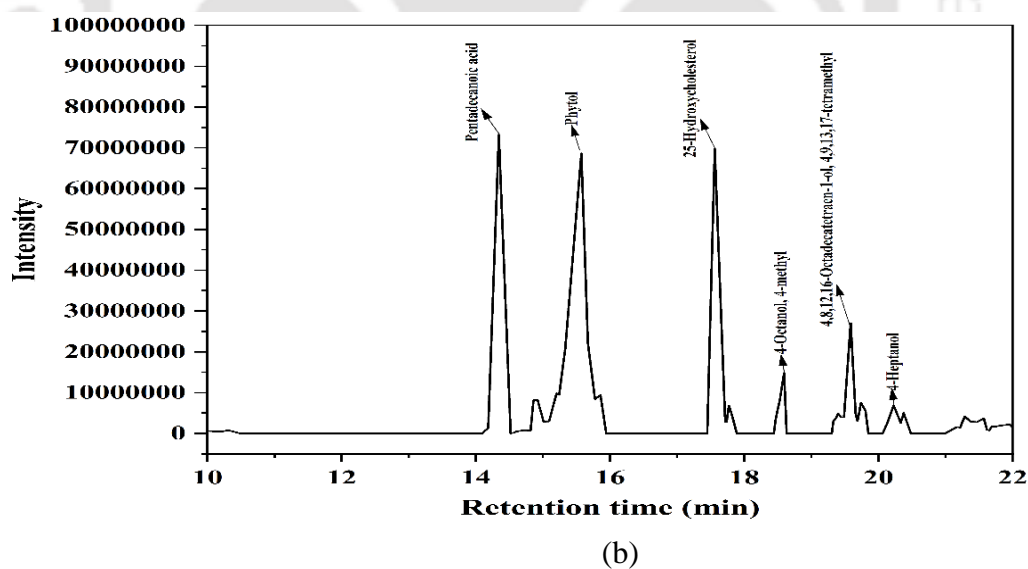
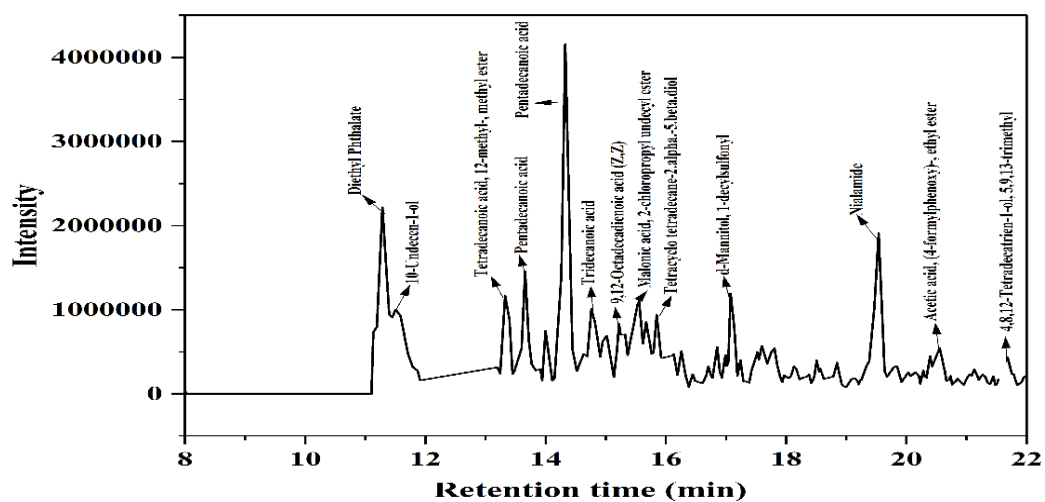
8.3.2.9 GC-MS analysis of *Mikania* and vermicompost samples

Fig 8.39 (a, b & c) depicts the comparative analysis of *Mikania*, VC, and VCB samples. GC-MS spectra of *Mikania* revealed that it contains 32 peaks, which are listed in Table 8.11. Diethyl Phthalate has the highest peak area (4.52%), followed by 1-Pentanol, 2-methyl (4.48 %), Bicyclo [5.3.0]decane, 2-methylene (2.72%), 4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl- (2.31%), 3-(n-Propylamino)-2,1-benzisothiazole (1.58%), Glycidol (1. tetradecane-2.alpha.-5.beta.diol-10-one, 1,4.alpha.,6,14-tetramethyl-4-vinyl (1.39%), Pentadecanoic acid (1.23 %), 9,12-Octadecadienoyl chloride, (Z,Z)- (1.22%), 9,10-Anthracenedione, 2-methyl- (1.21%), 1,3,5-Tri (1.02%). The other compounds had peaks of less than 1%. The chemical footprint of the samples in terms of vermicomposting is revealed by the GC-MS study. At the end of 40 days, 14 peaks were recorded for the sample VC. There were 20 peaks found in the sample VCB, with the maximum peak being pentadecanoic compound, followed by 9,12,15-Octadecatenoic acid (Z,Z,Z), phenol, 2,4-dicholorobenzenesulfonate, 4-Nitrocinnamic acid, 2-Butoxyethyl 22,2,3,3,3-pentafluoropropanoate. Vermicompost is found to contain fatty acids, alcohols, alkanes, alkenes, and nitrogenous compounds. Peak intensity was observed to be reduced following the vermicomposting process for both the reactor VC and VCB. This indicates that the phytochemicals present in the *Mikania* plant were sufficiently degraded (Hussain et al., 2016). The addition of bacterial strains along with earthworm species may have aided in the transformation of various compounds.

Table 8.11. Compound identification extracted by dichloromethane from *Mikania*, VC, and VCB sample

Retention time in min	Compounds			
		<i>Mikania</i>	VC	VCB
11.28	Diethyl Phthalate	+	-	-
11.60	10-Undecan-1-ol	+	-	-
11.61	Benzoic acid, 4-formyl-	-	+	-
13.32	Pentadecanoic acid	+	-	-
13.65	Tridecanoic acid	+	-	-
13.52	Tetradecanoic acid, 12-methyl-, methyl ester	+	-	-
14.26	10-Undecenoyl chloride	+	-	-
14.32	Pentadecanoic acid	+	+	+
14.81	9,12-Octadecadienoyl chlo	+	-	-
15.21	9,12-Octadecadienoic acid (Z,Z)	+	-	-
15.24	Phytol	-	+	-
15.55	1-Bromo-5-nonene, (Z)-	+	-	-
15.57	Malonic acid, 2-chloropropyl undecyl ester	+	-	-
15.66	Octadecanoic acid	+	-	-
15.84	9,10-Anthracenedione	+	-	-
16.01	5-(7a-Isopropenyl-4,5-dime	-	+	-
17.07	d-Mannitol, 1-decylsulfonyl	+	-	-
17.59	7-Hexadecanoic acid	+	-	-
17.07	10-Undecen-1-ol	+	-	-
17.13	But-3-enal, 2-methyl-4-(2,6,6	-	-	-
17.15	3-Ethyl-4,4-dimethyl-2-(2	-	-	+
17.45	Isolongifolene, 7,8-dehydro	-	-	-
18.55	Stigmasterol trimethylsilyl	+	-	-
18.77	2(1H)-Naphthalenone, 3,4,4a	+	-	-
18.92	7,7,9,9,11,11-Hexamethyl-3	-	-	-
19.21	Aminoguanidine	-	+	+
19.46	Betulin	-	-	-
19.48	9,19-Cyclolanostan-3-ol, 24	+	-	-
19.54	4,8,12-Tetradecatrien-1-ol	+	-	-
19.54	,8,12,16-Octadecatetraen	-	+	-
19.81	Dimethyl{bis[(4,8,8-trimethyldecah	-	+	-

20.27	Pyridine, 2,6-bis(1,1-dimethylethyl)	-	+	-
20.28	1,3,4,6-Tetrabromopentac	-	-	+
20.54	3-(n-Propylamino)-2,1-be	-	+	-
20.56	3-Isopropyl-6a,10b-dimethyl-	-	-	+
22.48	Bicyclo[5.3.0]decane, 2-me	+	-	-
22.49	Ambrein	-	-	-



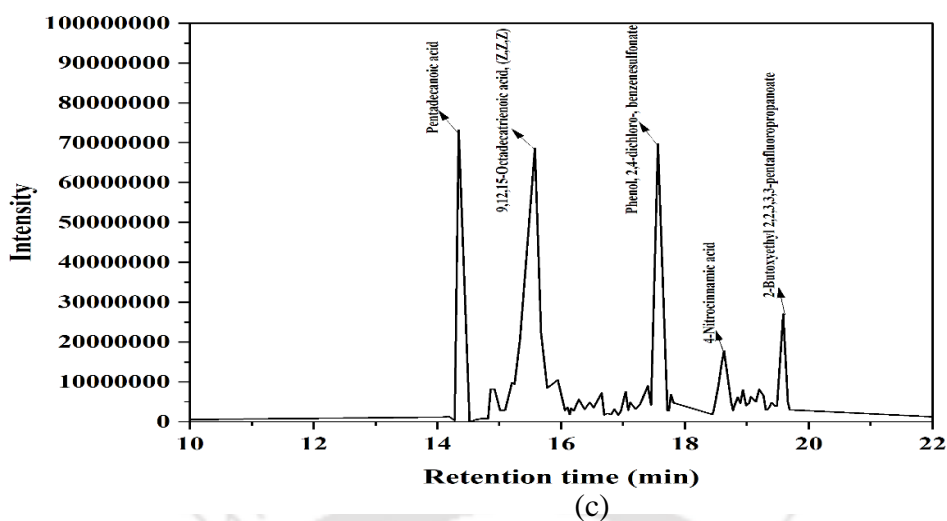


Fig. 8.39. GC-MS spectra of a) *M. micrantha* b) Control (VC) and c) Bacterial treated (VCB) samples during the bioaugmentation process

8.4 SUMMARY OF PHASE IV

This study gives a comprehensive idea about the bacterial community present during rotary drum composting of *M. micrantha*. The possible isolation of bacteria producing laccase enzyme was emphasized and the bioaugmentation study using the bacterial strain was conducted. The overall findings from the study are given below:

- Highest phylum during rotary drum composting was observed for phylum Proteobacteria (41, 25.5 and 28.6%) followed by Firmicutes (20.4%, 26.1% and 28.7%), Bacteroidetes (18.9, 11.5 and 10.5%), Euryarchaeota (15.4, 12.7 and 7.5%) and Actinobacteria (2, 8 and 9.1%) at D2, D10 and D20.
- Three laccase-producing bacterial strains were isolated from rotary drum composting process: *Enterobacter hormaechei* MHK2 (OM149726), *Lysinibacillus fusiformis* MHK3 (OL533643), and *Lysinibacillus fusiformis* MHK4 (OM179766).
- The strain *Lysinibacillus fusiformis* MHK3 performed best in the laccase activity optimization study (OL533643). At pH 7, the strain MK3 had the highest enzyme activity of more than 12 U/mL. Similarly, the strain MHK3 achieved the highest enzyme activity of >16 U/mL at 30°C.
- Bioaugmentation could be a useful tool for improving the performance of the composting process and the quality of compost made from *Mikania* biomass. *Lysinibacillus fusiformis* MHK3 (OL533643) exhibited a wide range of metabolic and physiological abilities,

making it suitable for use as a consortium in the bioaugmentation process. Thermal composting followed by a bioaugmentation process was used in the study, and mature compost was produced on Day 40. The thermophilic degradation lasted nearly 5 days, with the highest temperature reaching 54.5°C. This ratio had the highest TKN of 2.92% higher than the control. The highest reduction in TOC was observed in reactor B1 (24.07%), while the lowest reduction was observed in reactor C1 (30.8 %). In reactor B1, there was a significant decrease in sBOD, sCOD, and CO₂ evolution rate values, as well as a significant increase in nutritional properties (total and available phosphorus, sodium, potassium, and calcium).

- Maximum degradation was observed in bacterial inoculated reactor VCB for the vermicomposting process augmented with bacterial consortia *Lysinibacillus fusiformis* MHK3 (OL533643). Although the results for the reactors VC and VCB were quite comparable, the reactor VCB had the highest TKN of 3.11%. When compared to the control, the reactor VCB achieved the highest percentage reduction in TOC (36.10%). The reactor VCB achieved a percentage increase in nutrients (44.34% Na, 45.68% K, 39.23% Ca, and 28.14% total phosphorus). Out of the two studies on bioaugmentation processes, the vermicomposting process augmented with bacterial strain demonstrated more organic carbon degradation with good nutrient quality at the end of 40 days.
- FTIR analysis revealed functional group changes during the composting process. Even though the changes were more noticeable for the study vermicomposting process bio augmented with bacterial consortia as both earthworm and bacterial strain resulted in the final product.
- For the substrate *M. micrantha*, GC-MS analysis revealed several esters, alcohol, fatty acid, and phenol groups. However, a significant decrease was observed in post-composting samples, where several compounds were transformed during the process. Out of the two studies, the vermicomposting process augmented with bacterial strain showed a greater reduction in allelochemicals than the TCB process.



Overall conclusions & future recommendations

This chapter has mainly drawn significant conclusions from the various studies done on composting processes, the toxicity assessment of the end product, plant application and bacterial application study during the composting process. Furthermore, the recommendations for future work are also presented here.

9.1 OVERALL CONCLUSIONS

- **In Phase-I**, performance evaluation of the compost product generated from three different compost technologies revealed that, vermicomposting and rotary drum followed by vermicompost gave better results in terms of enhancement of nutritional parameter (TKN, TP, Na, Ca and K) and reduction of biological parameters (sBOD, sCOD, CO₂ evolution and OUR). However, in terms of time reduction, rotary drum followed by vermicompost gave superior quality of compost in less time. The European species *Eisenia fetida* was observed to be the best earthworm species for vermicomposting and rotary followed by vermicomposting process of *M. micrantha* among the three.
- **In Phase-II**, the phytotoxicity assessment done on the three compost produced showed a positive result on the Germination index percentage of *V. radiata* and root growth of *A. cepa*. The best results for *V. radiata* seed germination and *A. cepa* root length were obtained using rotary drum compost, vermicompost and rotary drum followed by vermicompost. All the compost had a GI greater than 50%, which is the recommended value for using compost on the farm. The results of the tests show that *Mikania* extract has a toxic effect on root growth. Allelochemicals were degraded during the composting process, resulting in the growth of *V. radiata* and

A. cepa. The results of the cytotoxicity and genotoxicity tests revealed that exposure of compost extract on onion roots to various concentrations of compost sample resulted in mitotic effects and chromosomal abnormalities. *Mikania* raw extract had extremely high chromosomal aberrations when compared to compost samples. Mitotic index percentage was more in vermicompost and rotary followed by vermicompost as compared to rotary drum compost and abnormality of the chromosome was found less in vermicompost and rotary drum followed by vermicompost.

- In **Phase-III**, the study demonstrates the effects of *M. micrantha* compost and vermicompost on *Abelmoschus esculentus*. It was discovered that vermicompost and rotary drum compost followed by vermicompost produced a higher yield of fruit for the plant *Abelmoschus esculentus* than the application of rotary drum compost. The okra fruit contained an adequate amount of nutrients. This study found a viable solution to use *Mikania* biomass and recycle it in a sustainable way using rotary drum composting and vermicomposting.
- In **Phase-IV**, the use of the lac enzyme-producing bacteria *Lysinibacillus fusiformis* MHK3 (OL533643) in composting technology improved compost quality and reduced toxicity. In both investigations, vermicomposting followed by bioaugmentation produced higher-quality compost in terms of different physicochemical and biological characteristics. In addition, the phytotoxicity test demonstrated that the vermicompost extract promoted greater root growth. The bioaugmentation process resulted in the breakdown of allelochemicals found in the substrate *Mikania*, with the best results obtained in the vermicomposting process augmented with the bacterial consortium *Lysinibacillus fusiformis* MHK3 (OL533643).

The current research work concludes that, *Mikania micrantha* is a viable substrate for composting and vermicomposting. Toxicity testing has shown the compost quality and its safe usage in soil for plant growth. The application of compost/vermicompost in various ratios to soil has a significant influence on okra plant development, suggesting that *Mikania* compost may be utilized in the field. A microbiological investigation revealed the usage of bacterial inoculum for allelochemical breakdown throughout the composting process. The primary problems of utilizing weed are handling in bulk amount and storage issues. The

allelochemical impact is also important since this is the major toxic molecule present in the plant that must be destroyed throughout the procedure. Overall, the research work can be summarised by stating that *Mikania* compost can be made on-site by farmers rather than dumping in an open area, and that the utilization of this specific biomass will assist them in making their own compost/vermicompost to be used as an organic fertilizer, as well as finding a sustainable livelihood by selling the product.

9.2 FUTURE RECOMMENDATIONS

- Study on the application of compost/vermicompost on farmyard land using various plant models.
- Study on the use of mixed bacterial consortia for biodegradation of organic substrate can be implemented for future research.
- Optimization study on the use of bacterial doses for the composting process of *M. micrantha*.
- Application of immobilization technique for the microbial study is highly recommended for the future work.



Appendix

PUBLICATIONS

Publications

1. **Kauser, H.**, Pal, S., Haq, I., & Khwairakpam, M. (2020). Evaluation of rotary drum composting for the management of invasive weed *Mikania micrantha* Kunth and its toxicity assessment. *Bioresource Technology*, 3130 123678.
2. **Kauser, H.**, & Khwairakpam, M. (2021). Fate of invasive weed *Mikania micrantha* Kunth using vermitechnology employing three monoculture of earthworm species. *Bioresource Technology Reports*, 16, 100827.
3. **Kauser, H.**, & Khwairakpam, M. (2022). Organic waste management by two-stage composting process to decrease the time required for vermicomposting. *Environmental Technology & Innovation*, 25, 102193.
4. **Kauser, H.**, Saumya, S., Haq, I., & Khwairakpam, M. (2022). Biological treatment of Climbing Hempweed biomass through optimized composting technologies -Toxicity assessment and morphological study of *Abelmoschus esculentus*. *Journal of Environmental Management*, 319, 115631.

Book Chapters

1. **Kauser, H.**, & Khwairakpam, M. (2022). Composting and vermicomposting of obnoxious weeds-A novel approach for the degradation of allelochemicals. *Advanced Organic Waste Management*, 175-192.

International Conferences

1. **Kauser, H.**, Khwairakpam, M., (2019) “Rotary drum composting of Invasive terrestrial weed *M. micrantha*” oral presentation in the international conference on solid waste 2019 (ICSW 2019) held on Nov13-16, 2019, Hangzhou, China.
2. **Kauser, H.**, Khwairakpam, M., (2018) “A review on management of terrestrial weeds and its utilization for agricultural purpose” poster presentation on “Research Conclave’18, held on March 8-11 at Indian Institute of Technology, Guwahati.
3. **Kauser, H.**, Khwairakpam, M., (2019) “Aerobic In-vessel composting of an invasive weed, *M. micrantha* Kunth” first prize for oral presentation on “Research Conclave’19, held on March 14-17 at Indian Institute of Technology, Guwahati.

4. **Kauser, H.**, Khwairakpam, M., (2020) “Efficacy of Rotary drum composter for the treatment of Mikania micrantha, an invasive terrestrial weed”, oral presentation in the international conference Recycle 2020, held at Indian Institute of Technology, Guwahati.

National Conferences

1. **Kauser, H.**, Khwairakpam, M., (2019) “Rotary Drum Composting of M. micrantha kunth, an invasive terrestrial weed” **second prize for Poster presentation** on “National Environmental Conference 2019” held on 31st January - 2nd February at Indian Institute of Technology, Bombay.

Awards/Achievements

1. **Kauser, H.**, Khwairakpam, M., (2019) “Rotary Drum Composting of Mikania micrantha kunth, an invasive terrestrial weed” **second prize for Poster presentation** on “National Environmental Conference 2019” held on 31st January - 2nd February at Indian Institute of Technology, Bombay
2. **Kauser, H.**, Khwairakpam, M., (2019) “Aerobic In-vessel composting of an invasive weed, Mikania micrantha kunth” **first prize for oral presentation** on “Research Conclave’19, held on March 14-17 at Indian Institute of Technology, Guwahati.
3. **Won a partial scholarship in ISWA-SWIS Winter School 2020** held on January 13th-24th, 2020, at the **University of Texas at Arlington, Arlington, Texas, USA.**



4. **Kauser, H.**, Khwairakpam, M., (2022) “Biological treatment of invasive weed Climbing Hempweed using two-stage composting technology” **second prize for oral presentation** on “Research and Industrial Conclave’22” held on 20-22 January at Indian Institute of Technology, Guwahati.

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