

## **Chapter 1**

### **1. Introduction**

#### **Origin of the problem**

With the advent of the human race, agriculture was the first activity that led to the development of settlements. The settlements gave way to civilizations and now humans have conquered almost every part of terrestrial ecosystems. The increase in human population comes with the added need for food, water, shelter, and other basic necessities. This calls for the major challenges of sustainable crop production without endangering soil health and managing all the wastes produced in the most ecologically sound manner. To compensate for the number of nutrients in agricultural crops, different types of chemicals have been used and the sharp rise in crop production and productivity is known as the green revolution. The remarkable achievement of food security resulted in a significant increase in the use of synthetic fertilizers, which is causing severe loss of soil quality in the forms of degradation of the stable C stock and destructive impacts on soil microbial resources [1]. According to the available data, fertilizer consumption in India is increasing at an average annual rate of about 5% since 1960; while the growth in fertilizer consumption has drastically increased by 16% in between 2015-16 to 2020-21 (IFFCO, 2021). Such a level of dependence on chemical fertilizers would lead to mean further denudation of soil health, food quality, surface and groundwater quality, and predominance of hazardous substances causing a health hazard to the users. These consequences have been well understood on a global scale, which emerged an imperative need to develop and promote the use of organic-based inputs (composts, green manures, biofertilizers, recycling of crop residues, etc.) as the most prolific substitutions of inorganic fertilizers [1].

The vermicomposting technology is emerging as an important one owing to the rapidity of this method and the production of quality compost compared to traditional composting methods [2]. Earthworms and microorganisms greatly help nature maintain a constant flow of nutrients from one system to another and minimize environmental degradation. Epigeic earthworms live on the surface of the soils and feed on organic debris [1]. These earthworms harbor rich concentrations of different kinds of microorganisms, enzymes, hormones, etc. in their intestines [3]. The organic materials

ingested by earthworms are mineralized by these microbes and enzymes in the earthworm gut and eventually released through earthworm excreta [4]. Epigeic earthworms, being organic matter feeders, are most suitable for vermicomposting of biogenic solid wastes [2]. *Eisenia fetida* and *Eudrilus eugeniae* are among those epigeic earthworms whose efficiency in waste degradation is well established [5,6].

*Eisenia fetida*, originally found in cool regions of the world, has also been successfully established in tropical soils [4,2]. The species has unique adaptation qualities owing to its prolific fecundity and highly efficient stress-relieving defense metabolism [7]. The worm prefers aerobic conditions, mesophilic temperature range (25-30°C), near neutral pH (6-7.5), and moderate moisture status (50-60%) to reproduce satisfactorily [1]. The high reproduction rate, voracious feeding habits, and high rate of excretion are the major qualities of *E. fetida* that establish it as one of the most preferred earthworms for the stabilization of varied kinds of complex solid wastes [8]. Moreover, the pollutant remediating potential of *E. fetida* has been well documented in recent literature [9,10,11]. *Eudrilus eugeniae*, being originated from Africa has been widely found in various parts of Asia; the species has been profusely utilized for vermicomposting of solid wastes of varying nature owing to its aggressive growing habit [12,13]. Although the habitat preference of

*E. eugeniae* is quite similar to that of *E. fetida*; the species prefers higher moisture status compared to other epigeic earthworms [2]. The waste degradation efficiency of *E. Eudrilus*, in terms of time and mass, is also far more spectacular than *E. fetida* owing to the aggressive reproduction and feeding behavior of the species [14,45]. Moreover, the prolific pollutant removal potential of this earthworm species during vermicomposting of toxic waste materials has been reported in recent studies [15,16].

The rapid decomposition and nutrient enhancement features of vermicomposting are the results of synergistic functions of earthworms and microorganisms. Several studies have postulated that microbial communities of the feedstocks subjected to vermicomposting are strongly influenced by the presence and activity of earthworms [17,4]. Interestingly, the microbial community structure of vermicomposting beds greatly differs depending upon the nature of feedstock, decomposition stage, earthworm species, and their burrowing habit [18,5,19,20]. Huang et al. (2018) found that the population of Proteobacteria greatly augments in *E. fetida* mediated vermibeds composed of lignocellulosic biomass. Vermibed composed of vegetable refuges has been reported to

elevate various bacterial communities [21]. Activation of bacterial groups in lignin-rich straw-based vermibeds by *E. fetida* has facilitated the identification of several operational taxonomic units [22]. As such, the presence of earthworms rapidly alters the microbial community profile within a week's time. Wang et al. (2016) and Zhou et al. (2016) observed that bacteria and fungi diversity in vermibeds sharply changes after three weeks of earthworm incorporation, which may also strongly correlate with earthworm fecundity. In contrast, Mucoromycota fungi (e.g., *Mortierella*) superseded the Ascomycota and Basidiomycota groups in lignocellulosic waste-based vermicomposting systems [23]. In fact, the changes in microbial profile result in the controlled release of useful enzymes in vermicomposts. For example, Castillo et al. (2013) recorded that activation of Actinobacteria via earthworm intestinal release resulted in a steep rise in chitinase activity in the vermibeds composed of olive mill residues. Elevated activity of cellulose-degrading enzymes via release of earthworm gut-associated fungal groups has resulted in rapid degradation of cellulosic substrates [24]. Moreover, the augmented introduction of Basidiomycota group of fungi in vermicomposts activated extracellular enzymes through oxidative transformation, which in turn enhanced lignin breakdown [25].

Recent advances in molecular phylogenetic analytical techniques have indeed facilitated researchers in studying microbial profiles in composting systems. Amplification of 16S rRNA genes using polymerase chain reaction (PCR)-based techniques is widely utilized for studying bacterial profiles. The studies related to microbial profiling in earthworm inhabitation are mostly focused on soil matrixes; in the domains of soil-feeding endogeic earthworms. However, such efforts in the zones of epigeic earthworms, especially in artificially-reared systems (i.e., vermireactors) are limited in the literature. The DNA can be directly extracted from compost and vermicompost beds and amplified via PCR [26]. Bacterial community structure and size during vermicomposting and composting could be extensively assessed using real-time PCR and denaturing gradient gel electrophoresis (PCR-DGGE)-based sequencing of 16S rRNA gene fragments (Vivas et al., 2009)[27]. Pyrosequencing of 16S rRNA genes has also been utilized to study the dimensions and diversity of bacterial taxa in composting beds [28,29]. However, bioinformatic analysis of the PCR products of 16S rRNA amplified gene fractions using the Illumina platform could provide greater scope to generate useful information on microbial profile structure. Budroni et al. (2020), using such high-throughput sequencing (i.e., Illumina) platform, recently observed that pH and

organic C status largely regulated microbial diversity in vermibeds. On the other hand, phospholipid fatty acid (PLFA) analysis provides scope for qualitative and quantitative estimation of all types of microbial functional groups including fungi, actinomycetes, anaerobes, etc. [30]. PLFAs being integral components of microbial cell membranes are readily synthesized in synchrony with microbial proliferation and rapidly degenerate as soon as the microbes die and cannot be detected in microbe-generated molecules, thus the PLFA analysis provides true ‘footprints’ of the current microbial groups [31]. Nevertheless, the arrays of molecular techniques need to be used for widening their scope and applications for generating new knowledge on microbial community profiles in vermicomposting systems, which finally determines the quality of the finished compost. As microbial profiles of vermicomposting systems are strongly influenced by earthworm species and feedstock properties and palatability, novel information can be generated from every similar study even though the study appears to be repetitive.

Earthworms are capable of detoxifying hazardous compounds and elements in order to establish a conducive habitat. Quite a few earthworm species (*Lumbricus rubellus*, *Lampitoma mauritii*, *Nicodrilus caliginosus*, etc.) including *E. fetida* immobilize toxic metals like Cd in their intestines through induction of metallothionein or other metal-induced proteins [32,33,9,34]. Metal removal efficacy of *E. eugeniae* has also been observed earlier [15]. However, using an innovative metal budgeting equation Paul et al. (2020) recently suggested that toxic metals like Cr are largely detoxified by *E. eugeniae* through a humic compound mediated chelation pathway in the cotton textile sludge-based vermicomposting system. Liebeke et al. (2015) revealed that earthworms (*Lumbricus terrestris*) remediate the toxic impacts of polyphenols by releasing some specific surface-active compounds. Few studies have also reported polycyclic aromatic hydrocarbons (PAHs) removal efficiency of some earthworm species (*Lumbricus rubellus*, *Metaphire guillelmi*, *Amyntus gracilis*, *Apporrectodea caliginosa*, and *Eisenia andrei*) including *E. fetida* in soil [35,36,37,38]. The PAHs, considered among the most hazardous organic pollutants that cause severe impacts on human health [39], are often detected in solid wastes of modern industrial settlements [40]. In contrast, the PAH removal efficacy of earthworms has not been extensively studied so far. Particularly, the PAH-removal capacity of *E. eugeniae* has rarely been reported in the literature.

Numerous articles published over the decades on vermicomposting and earthworm-mediated systems have significantly contributed to the knowledge base and developed a clear understanding of the process as a whole. The true application of knowledge is possible by transforming the knowledge in technology development. Vermicomposting has also evolved into an acceptable technology for converting biogenic solid wastes into organic fertilizers. However, the majority of technical optimization of the process has been focused on earthworm species, feedstock composition, maturity indices, and end-product quality. Efforts have also been made to develop suitable medium to large-scale vermireactors for dealing with solid wastes [41,42]. Paczka et al. (2020) showed that modification of vermireactors designs significantly influenced earthworm activity, which resulted in alterations of end-product quality. Abbasi (2015) [42] claimed to develop a prototype for a large-scale rapid-action mechanized vermicomposting reactor that enabled the process to produce ready vermicompost within a few hours. However, there is no report on portable vermireactors for household use, which could be highly suitable and handy for the city-dwelling population to recycle their daily waste in kitchen gardens, growing houseplants, and amusement parks. Therefore, the broad goal of the present research endeavor was to optimize vermitechnology for rapid conversion of lignocellulosic wastes and to assess the PAH degrading efficiency of commonly used earthworm species. Spent mushroom straw and vegetable wastes are among the mostly widely generated waste materials [43]. Hence, these wastes were considered for technological optimization under the purview of the current study. Under the perspectives of the reviewed literature, specific research gap and research questions have also been formulated.

### **Research gaps**

- ❖ Understandings on the variations in microbial diversity and community profiles in different earthworm species mediated vermicomposting systems are yet inadequate.
- ❖ Stocking density of earthworm and end product quality of feedstock are yet to be optimized for lignocellulosic waste based vermicomposting systems.
- ❖ Little is known about organic pollutant removal efficiency of earthworm with special reference to toxic compounds like polycyclic aromatic hydrocarbons (PAHs).

- ❖ Designing and application of engineered vermireactors with high efficiency and suitability for high quality vermicomposting preparation has been rarely attempted so far.

### **Research questions**

Under the purview of the identified research gaps, the research questions were drawn as below:

- ❖ How to appreciate the influence of earthworm species on microbial community structure in the feedstocks and how such interactions determine the maturity and quality of the end products?
- ❖ Does the initial stocking density of earthworms stimulate or modulate the microbial activity, their community profiles, nutrient mobility, and metal availability in lignocellulosic waste-based vermicomposting systems?
- ❖ What are the possible mechanisms of earthworm-induced detoxification/immobilization of PAHs during vermicomposting?
- ❖ Can we expedite the vermicomposting process by developing mechanized continuous user-friendly vermireactor without compromising the end-product quality?

### **Research objectives**

The identified research questions enabled me to formulate the objectives of the research and carry out the study in systematic manner. The objectives are specified as below:

1. To evaluate the variations in spent mushroom straw decomposition and microbial community structure in response to different earthworm species during vermicomposting.
2. To evaluate the variations in spent mushroom straw decomposition and microbial community structure in response to different earthworm species during vermicomposting.
3. To study the polyaromatic hydrocarbons (PAHs) detoxification routes in *Eisenia fetida* and *Eudrilus eugeniae* mediated vermicomposting system.
4. To study the impact of vermireactor improvisation on bio-waste sanitization, microbial diversity, and nutrient bioavailability during vermicomposting.

## Plan of research

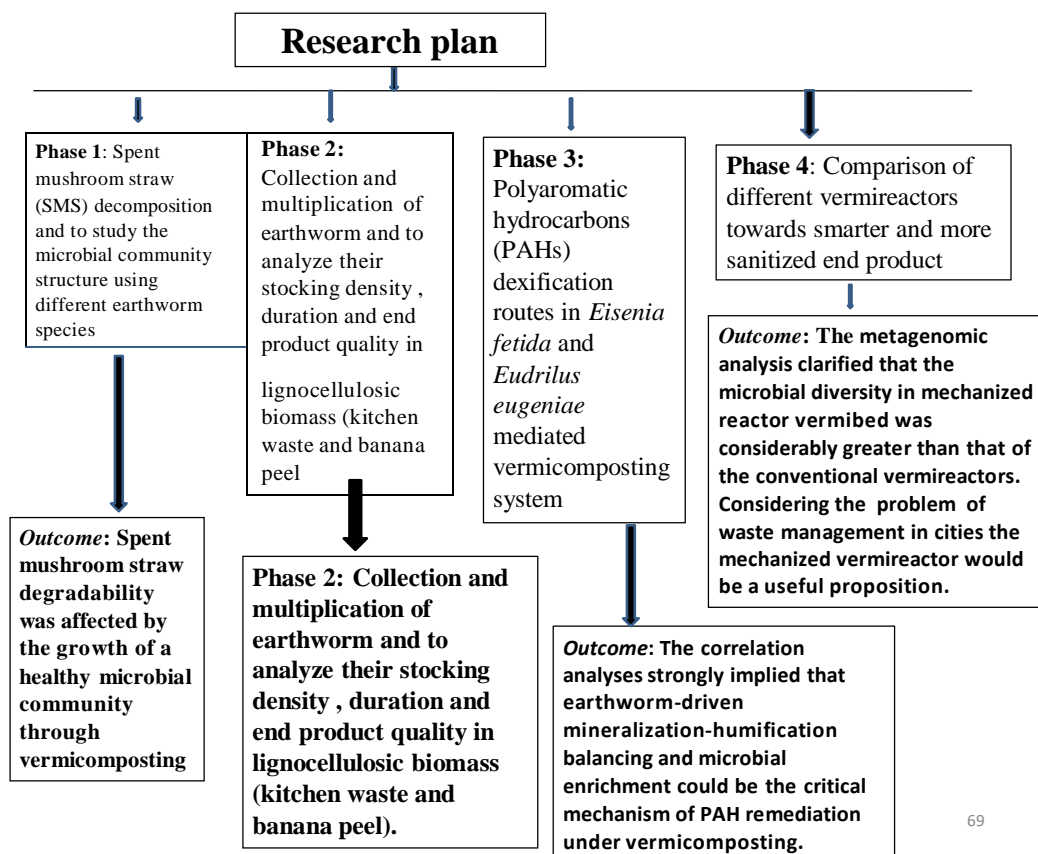
The study was performed in phased manner as shown in **Fig. 1.1**. Overall, various experiments were designed under following four phases:

*Phase I:* In this phase, spent mushroom straw (SMS) was taken as the representative candidate of lignocellulosic biowaste and undergone for vermicomposting with three different earthworm species (*Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus*). Subsequently, the decomposition process was monitored with special reference to microbial community structure.

*Phase II:* In the second phase, kitchen vegetable waste and fallen banana stem, as representatives of lignocellulosic waste, were vermicomposted with four different initial stocking densities of two earthworm species (*Eudrilus eugeniae* and *Eisenia fetida*). Eventually, the decomposition process was studied in regard to microbial community structure, nutrient bioavailability dynamics, humification rate, and metal availability.

*Phase III:* The third phase was executed to appreciate the removal/detoxification kinetics of 13 PAHs during vermicomposting with a mixed population of *Eisenia fetida* and *Eudrilus eugeniae*.

*Phase IV:* In this phase, a prototype of continuous-flow mechanized vermireactor was designed and prepared and its efficacy was evaluated in comparison with traditional vermicomposting reactors in regard to nutrient enrichment, microbial growth, enzyme activity, and solubility of potentially toxic metals.



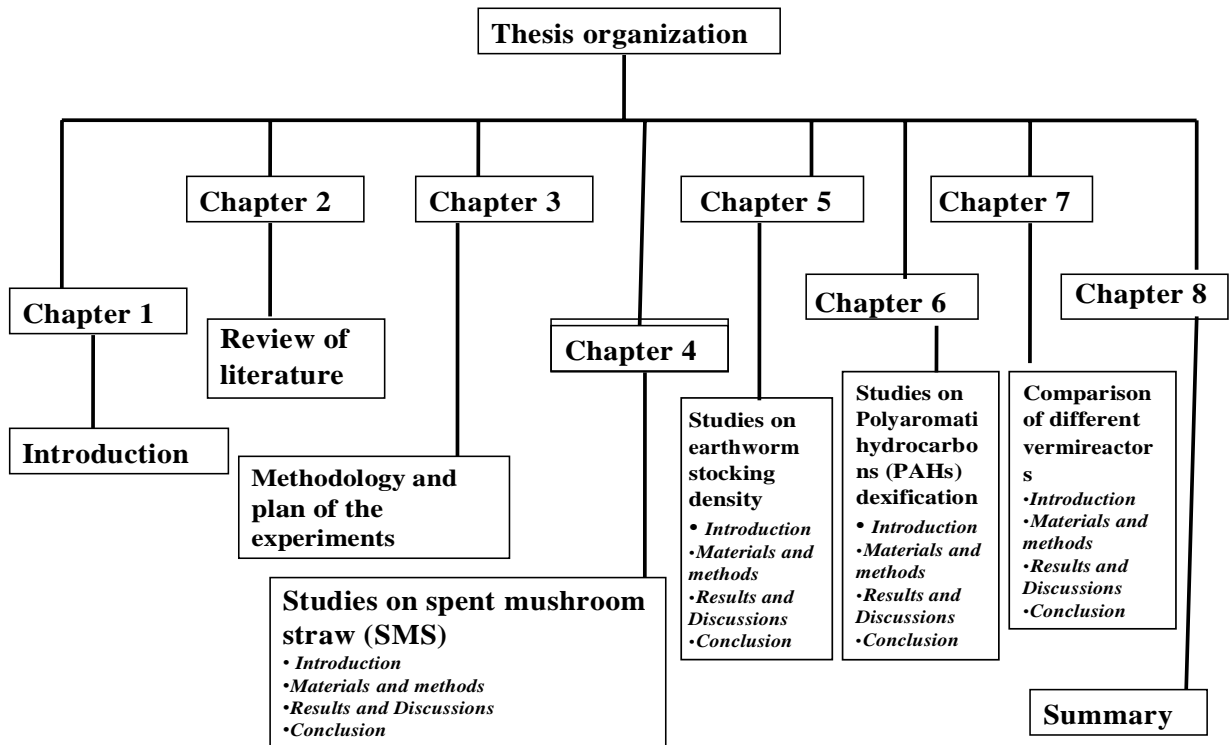
**Fig. 1.1:** Schematic representation of the overall research plan

### Chapter arrangement of the thesis

**Fig. 1.2** Illustrates the organization of the thesis. Overall, the research and realizations of the present endeavor have been detailed in various chapters as given below:

*Chapter 1:* Introduction and backgrounds of the research problem, identified research gaps, research questions, and the major objectives of the study have been presented under the purview of available scientific knowledge in the first chapter. A short description of the research plan has also been outlined.





**Fig. 1.2:** Schematic representation of the thesis organization

*Chapter 2:* The second chapter showcases an exhaustive review studied literature from critical viewpoints before undertaking the research.

*Chapter 3:* This chapter explains the methodological approaches undertaken to achieve the identified objectives of the research. The chapter also elucidates the resources used and the quality control and assurance measures taken in course of the analyses.

*Chapter 4:* This chapter presents a detailed description of the comparative assessment of lignocellulosic (i.e., SMS) feedstock valorization efficiency of three earthworms species. The chapter presents the need of the study under light of relevant published literature in the introduction, then describes the details of the methods, and critically discusses the experimental findings. The chapter basically addresses the first objective of the research endeavor.

*Chapter 5:* The experiments related to role of initial stocking density of earthworms for improving valorization efficiency of vermitechnology via optimization of microbial community structure have been described in detail in this chapter; addressing the second

objective of the research. An introduction with a brief review, followed by details of adopted methods, and a thorough discussion of acquired results of different experiments are the major components of this chapter.

*Chapter 6:* This chapter showcases the scientific knowledge gained through focused experimentation on PAH-degradation kinetics in vermicomposting system. Addressing the third objective of the research, the chapter originates with an introduction and brief review of literature, followed by minute details of the experimental, and ends by discussing the major findings of different experiments.

*Chapter 7:* The experiments and processes adopted to develop a novel continuous-flow mechanized vermireactor have been elucidated in this chapter. Like the previous chapters, this chapter also begins with a brief introduction discussing the critical aspects under the light of the available literature, followed by a detail accounts of the unique features of the developed reactor and experiments undertaken for performance assessment of the developed reactor in comparison with conventional vermicomposting systems, and ends with a critical discussion of the results acquired from the experiments.

*Chapter 8:* This concluding chapter basically summarizes all vital findings and major implications of the research and presents a brief account of future scope of research.

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