Waste and the Environment: Underlying Burdens and Management Strategies

Series Editor: **Sunil Kumar**

Earthworm Technology in Organic Waste Management

Recent Trends and Advances

Volume Editors

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EARTHWORM TECHNOLOGY IN ORGANIC WASTE MANAGEMENT

RECENT TRENDS AND ADVANCES

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CHAPTER

1

Earthworm-associated bacterial community and its role in organic waste decomposition

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1. Introduction

Charles Darwin (1809−1882) observed the behavior of earthworms and examined their sensitivity to noise, light and sound. He also explored the importance of earthworm activity within the ecosystem, naming earthworms the engineers of soil after observing their interaction with soil microbes and their role in the biogeochemical cycle (Gomez et al., 2015). Approximately 800 genera and 8000 species of earthworms exist on Earth, accounting for 90% of the invertebrate biomass present within the soil (Deka et al., 2011). Earthworms can deliver significant environmental and economic benefits to the world in terms of sustainable land use, food security, and climate change mitigation.

Vermicomposting is a natural process by which organic waste is broken down to form compost by the joint action of earthworms and gut microbes. According to some researchers, semidecomposed organic waste is more suitable for vermicomposting than fresh waste, as excess moisture and electrical conductivity negatively affect earthworms. A bulking agent is necessary to condition organic waste and create favorable conditions for earthworms during the degradation process. Bulking material maintains the initial C-to-N ratio of the entire vermibed and acts as a source of diversified decomposing bacteria (Li et al., 2021a). Inoculation of suitable bacteria into the vermibed enhances organic degradation efficiency and produces nutrient-enriched vermicompost. Previous studies have shown that adding nitrifiers and phosphate solubilizers increases the mineralization process and enriches the nutrient content of the final product (Li et al., 2020a). Earthworms affect waste composition and characteristics by ingesting, altering, and mixing processes. Different rates of digestion and assimilation in earthworms suggest the possible presence of gut microbes. Those microbes help digest organic waste, improving the worm's immunity and the stimulation of soil microbes.

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Earthworm gut maintains the required pH, moisture, and oxygen levels to grow selective microbes (Chen et al., 2018). The movement of earthworms within the soil changes the soil structure and assists in nutrient cycling. The meeting of soil volume with earthworms is called the drilosphere (Samal et al., 2019; Behera and Samal, 2022). The soil in this portion is mixed with energy-rich mucus that activates dormant microbes by the priming effect. Earthworms also promote the production of various plant growth hormones like abscisic acid, cytokinin, auxin, and gibberellins (Li et al., 2020b; Sapkota et al., 2020). Various gut microorganisms have been studied using culturing methods, and recently, modern methods without cultures, such as nanopore sequencing, microarray, 16S rRNA technique, fluorescence in situ hybridization (FISH), random amplification of polymorphic DNA (RAPD), and denaturing gradient gel electrophoresis (DGGE), have been used in the study process. The current chapter focuses on the mechanism of organic waste degradation in vermicomposting, different types of vermicast and their properties, the associated microbial community in the earthworm gut, and detection techniques.

2. Earthworms

Earthworms fall within the phylum Annelida. Their bodies are covered with a thin cuticle through which gas exchange occurs, and the gut is divided into a foregut, midgut, and hindgut. A worm's body has approximately 100 segments, each contracting and relaxing separately. Earthworms are natural hermaphrodites; they reproduce through cross-fertilization and copulation, which depend on feed quality (Yasmin et al., 2022; Zhang et al., 2023). Earthworms prefer to live in organic-rich soil and near water bodies. In addition, the availability of earthworms depends on the pH, C/N, temperature, and moisture of the surrounding (Cui et al., 2022; Jin et al., 2022; Samal et al., 2017a). The vermicomposting process may not be effective if the stocking density of earthworms is too high, and the raw materials should be free from detergents, pesticides, toxic chemicals, and similar substances. Based on feeding and burrowing capacities, earthworms are classified into anecic, endogeic, and epigeic, as shown in Fig. 1.1. Some required properties of composting earthworms are illustrated in Fig. 1.2.

3. Pollutant degradation mechanisms in vermicomposting

In the vermicomposting process, earthworms and microbes work symbiotically to break down organic matter. Degradation is typically a two-step procedure. In the first step, the worm fragments the particles into smaller ones. The surface area of the fragmented particles increases and supports microbial growth. Those microbes further break down the particles to make them finer through aerobic degradation (Fig. 1.3). Biodegradation rates depend on microbial diversity and the nature of waste substances (Mahapatra et al., 2022; Vivas et al., 2009). The rate of decomposition process increases when the organic fraction of the waste content is higher. The movement of earthworms within waste piles makes them porous so that oxygen concentration increases, making them suitable for the growth of aerobic

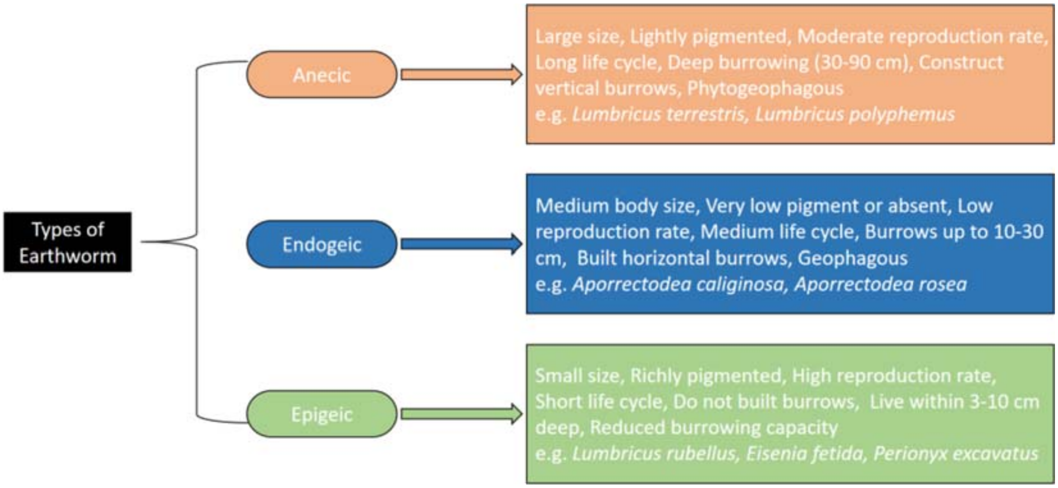

FIGURE 1.1 Earthworm classification based on feeding and burrowing capacities.

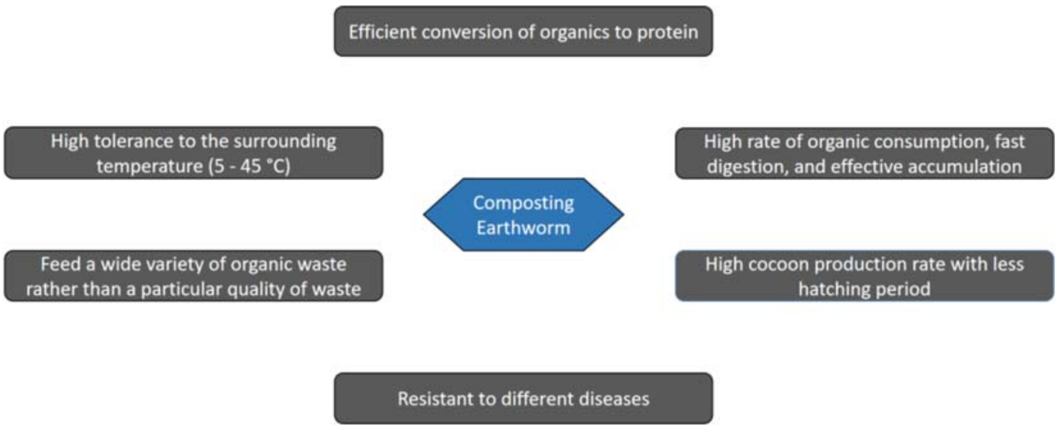

FIGURE 1.2 Properties of composting earthworms.

decomposing bacteria. The secretion of mucus and coelomic fluid conditions the waste substance for degradation. Earthworms also maintain a neutral pH throughout the composting process. Earthworms reportedly can change the pH of organic substances by up to 0.5–2 units during the digestion process. Calciferous glands of earthworms maintain the pH of blood and body tissue. Table 1.1 shows the characteristics of organic waste in the earthworm-associated decomposition process.

Several enzymes secreted from the earthworm gut, like amylase, protease, lipase, and cellulase, accelerate the degradation of various waste components, such as cellulose, hemicellulose, and lignin (Cui et al., 2018; Eastman et al., 2001; Ravindran et al., 2016). Research indicates that enzyme activity is strong in the foregut and midgut of the earthworm.

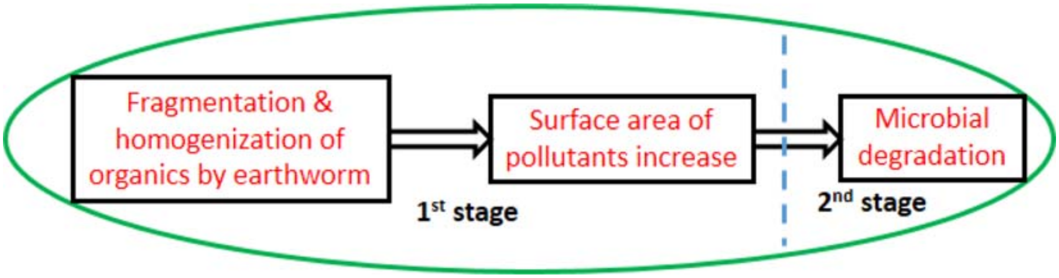

FIGURE 1.3 Organic pollutant degradation in vermicomposting process.

TABLE 1.1 Characteristics of organic waste in earthworm-associated decomposition process.

Organic waste	Earthworm	pH		EC		C/N		GI (%)		Maturity day	References
		Initial	Final	Initial	Final	Initial	Final	Initial	Final		
Paper sludge, tomato waste	*Eisenia fetida*	7.8	9.3	5.3 dS m^{-1}	2.6 dS m^{-1}	24.91	13.1				Gomez et al. (2015)
Chicken manure, wheat straw		6.7	7.9	2750 μS cm^{-1}	3120 μS cm^{-1}	30	18		1.09	42	Chen et al. (2020)
Citronella waste, cow dung	*Perionyx excavatus*	6.8	6.2	0.3 dS m^{-1}	0.61 dS m^{-1}	43	7.4			105	Deka et al. (2011)
Chicken manure, wheat straw		6.4	8.4			31	15	0.25	1.4	50	Chen et al. (2018)
Vegetable waste	*E. fetida*	7.5	6.95	2.65 dS m^{-1}	3.65 dS m^{-1}	48.6	16.7			90	Sharma and Garg (2017)
Cattle manure											
Sludge, manure, cow dung	*E. fetida*	8.1	7.45			16.65	12.6			60	Xie et al. (2016)
Chicken manure, rice straw		6.9	8			30	18	10	90	45	Li et al. (2021a)
Rice straw, paper waste	*E. fetida*	6.8	7.5	6.7 mS cm^{-1}	8.4 mS cm^{-1}	134	31			105	Sharma and Garg (2018)
Dairy manure		7.6	7.8	2.6 mS cm^{-1}	2.2 mS cm^{-1}			38	52	28	Li et al. (2020b)

C/N, carbon/nitrogen; *EC*, electrical conductivity; *GI*, germination index; *pH*, potential of hydrogen.

Dehydrogenase is an indicative enzyme of microbes, and its presence justifies the abundance of microorganisms in vermicompost. Worms also secrete various protein-related substances that mix with composting beds and nitrogenous materials released from the mineralization process. During vermicomposting, earthworms alter organic waste properties through burrowing, casting, and dispersal activities (Samal et al., 2017a). In the active phase of vermicomposting, earthworms alter the physical (pH, particle size, organic content, odor, color, etc.) and microbial (bacteria, fungi, actinomycetes, etc.) characteristics of waste materials (Aira et al., 2016). When the maturation phase of composting arrives, worms move from digested waste to fresh waste. The different characteristics of raw organic waste in the earthworm gut change due to various gut-associated processes. The processes include adding sugar, excretory substances, mucus, and coelomic fluid, as well as microbial community modification. Second-phase cast-associated processes focus on the aging process. Ultimately, the cast becomes biologically active and mature (Samal et al., 2019) (Fig. 1.4).

4. Bacterial diversity in the alimentary canal

Earthworms and microbes share a mutual relationship in which microbes help in the final conversion step of organic waste to compost, and worms modify the microbial community. Earthworm increases the activity of rhizobacteria, e.g., *Bacillus*, *Azotobacter*, *Rhizobium*, and *Azospirillum* (Zhang et al., 2022). These microbes are ingested by earthworms along with organic waste/soil, and the population increases due to favorable conditions in the worm intestine. These bacteria induce plant growth by producing various plant growth hormones, demineralizing nutrients, reducing the growth of pathogenic fungus (producing fungal cell-wall degrading enzymes, chitinases, and glucanases), fixing nitrogen, etc. (Zhong et al., 2023). Gut microbes degrade organic matter and chelate various metallic ions in waste materials. Earthworms like *A. caliginosa*, *Lumbricus terrestris*, and *A. terrestris* contain more aerobic bacteria in their gut than soil. According to one report, after worm treatment in the soil, the

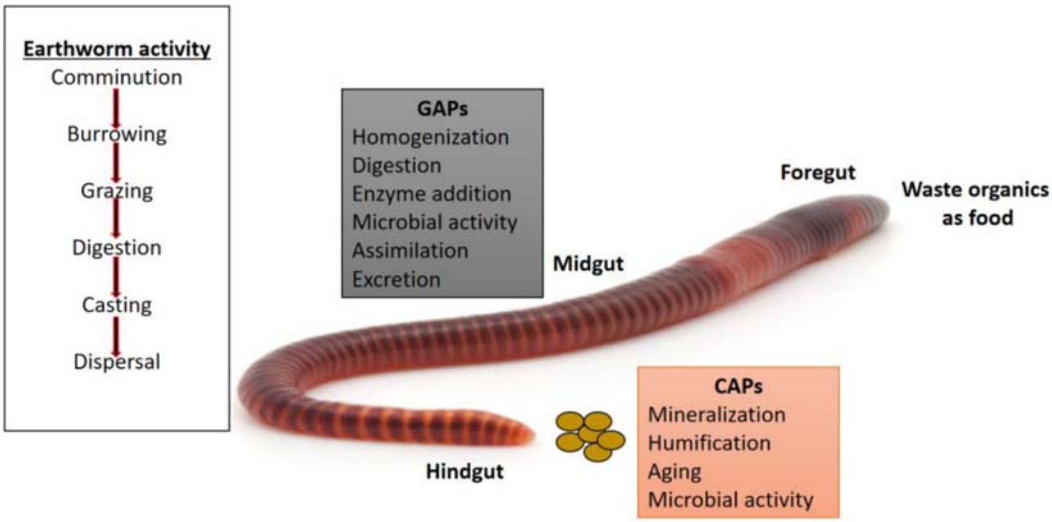

FIGURE 1.4 Gut-associated and cast-associated processes in earthworms.

number of microbes increased by five times. In another report, the number of bacteria and actinomycetes rose by 1000 times while passing through the worm gut (Xu et al., 2021). Overall, it can be said that the number of microbes may increase, decrease, or remain the same after passing through the earthworm gut.

Khambata and Bhat (1953) reported the presence of *Pseudomonas oxalaticus* (Oxalate-degrading bacteria) in the gut of *Pheretima* worm species. Jolly et al. (1993) observed the presence of several endogenous microflorae in the guts of *L. terrestris* and *Octolasion cyaneum* by scanning electron micrographs. The intestine of the earthworm *Eisenia fetida* contains several anaerobic N_2-fixing bacteria, such as *Clostridium butyricum, C. beijerinckii*, and *C. paraputrificum*. The guts of *Lumbricus rubellus* and *Octolasion lacteum* contain greater numbers of aerobic and anaerobic bacteria and nitrifiers-denitrifiers. Earthworms stimulate and create favorable conditions for the growth of microbes through burrowing action. Vivas et al. (2009) informed that recent vermicompost contained more numbers of Actinobacteria and Gammaproteobacteria, while conventional compost contained more α-Proteobacteria and Bacteroidetes. Molecular and culture-dependent analyses of the bacterial community of vermicompost showed the presence of *Firmicutes, Actinobacteria*, α-, β-, and γ-Proteobacteria, and Bacteroidetes. Sapkota et al. (2020) used 16S rRNA gene amplicon sequencing for bacteria and 18S rRNA gene amplicon sequencing for eukaryotes to assess the overall microbial community in the earthworm guts of *Aporrectodea*, *Allolobophora,* and *Lumbricus* species. Extensive eukaryotic diversity was observed along with fungi and metazoan species, and the dominant bacteria species included Firmicutes, Acidobacteria, Proteobacteria, and Verrucomicrobia. Some researchers have reported that hemicellulolytic bacteria such as Proteobacteria, Actinobacteria, and Firmicutes in the anterior intestine of *E. fetida*. Zhang et al. (2023) treated PCB-contaminated soil using earthworms and nano-zero-valent iron. It was observed that earthworms formed certain metabolites such as formamide, S-(2-hydroxyethyl) glutathione and 16-hydroxypalmitic acid, which accelerates the PCB-degrading capacity of PCB-degrading microbiota (*Novosphingobium* and *Achromobacter*) present in earthworm gut.

Various studies have shown an increase in microbial number and diversity in vermicompost due to advantageous conditions in the digestive tract of worms and the ingestion of nutrient-rich foods, which are the energy source for the growth of microbes. Certain bacteria are found in large numbers, particularly in worm guts and casts, such as *A. hydrophila* in *E. fetida*, *Pseudomonas* in *L. terrestris*, and Actinobacteria in *L. rubellus* (Singh et al., 2015). Enzyme quantification and characterization have correlations with the microbial population in compost and provide information regarding compost maturity. The maximum enzyme activity is usually observed around 30–35 days of composting and 45–50 days of vermicomposting (Jolly et al., 1993; Li et al., 2021b). Table 1.2 shows different microorganisms present in the earthworm gut.

Several bacterial species, such as *Pseudomonas*, *Azoarcus*, *Spiroplasm*, and *Acaligenes*, are found in earthworm guts and cast (Samal et al., 2019; Xie et al., 2016). Various species of Firmicutes (*Bacillus cereus, B. benzoevorans, B. licheniformis*, *B. megaterium*), Actinobacteria (*Microbacterium* spp., *Cellulosimicrobium cellulans, Microbacterium oxydans*), Proteobacteria (*Pseudomonas libanensis, Pseudomonas* spp., *Sphingomonas* sp., etc.), and yeasts (*Geotrichum* spp. and *Waynea californica*) have been found from vermicompost (Biswas et al., 2018; Li et al., 2021a; Gómez-Brandón et al., 2008). Pinel et al. (2008) reported the presence of a novel nephridial symbiont, *Verminephrobacter eiseniae* from *E. fetida.* Different families of bacteria,

TABLE 1.2 Various microorganisms in the earthworm gut.

Earthworm	Techniques	Identified microbes	References
Eisenia andrei	16S rRNA pyrosequencing and metagenomic analysis	Cyanobacteria, Firmicutes, Actinobacteria, Hydrogenedentes, Latescibacteria, Planctomycetes	Aira et al. (2016)
Eisenia fetida, *Perionyx excavatus*	16S rDNA-based clonal survey	Cyanobacteria, Firmicutes, Actinobacteria, xylan degraders, chitin degraders, ammonia oxidizers, sulfate reducers, cellulose degraders, dehalogenators	Singh et al. (2015)
P. excavatus, *Eudrilus eugeniae*, *Polypheretima elongata*	16S rRNA gene sequencing	Cyanobacteria, nitrite reducer, ammonia oxidizers, Firmicutes, Bacteroidetes, sulfate reducer, dehalogenators	Thakur et al. (2021)
Metaphire posthuma	16S rRNA gene sequencing	*Bacillus licheniformis*, *Bacillus megaterium*, *Staphylococcus haemolyticus*	Biswas et al. (2018)
Pheretima guillelmi	16S rRNA gene sequencing	*Rhizobium*, *Flavobacterium*, *Streptomyces* *Microbacterium*, *Pseudomonas*, *Aeromonas*, *Bacillus*, *Cellvibrio*, *Ensifer*, *Paracoccus*	Hu et al. (2018)
E. fetida	16S rRNA gene sequencing	*Aeromonas veronii*, *Pseudomonas*, *Aeromonas*, *Aeromonas caviae*, *Aeromonas hydrophila*	Wang et al. (2022)
Aporrectodea, *Allolobophora*, *Lumbricus*	16S rRNA gene sequencing	Actinobacteria, Cyanobacteria, Verrucomicrobia, Acidobacteria, Firmicutes	Sapkota et al. (2020)
Lumbricina sp.	16S rRNA gene sequencing	Bacillaceae, Hyphomicrobiaceae, Xanthobacteraceae, Nocardioidaceae	Zhang et al. (2022)
E. fetida	16S rRNA gene sequencing	Firmicutes, Actinobacteria, Cyanobacteria	Ordoñez-Arévalo et al. (2022)
E. fetida	16S rRNA gene sequencing	*Novosphingobium*, *Achromobacter*	Zhang et al. (2023)
E. fetida	16S rRNA gene sequencing	Verrucomicrobiota, Actinobacteriota, Firmicutes, Myxococcota, Cyanobacteria	Zhong et al. (2023)
Metaphire guillelmi	16S rRNA gene sequencing	*Alicyclobacillus*, *Planctomycetes*, *Cyanobacteria*, *Bacteroidetes*, *Sphingobacterium*, *Aeromonas*	Zhu et al. (2021)
Amynthas robustus	16S rRNA gene sequencing	*Virgibacillus*, *Streptomyces*, *Brevibacterium*, *Streptacidiphilus*, *Dermacoccus*, *Bacillus*	Xu et al. (2021)
Metaphire californica *Amynthas phaselus*	16S rRNA gene sequencing	*Cytophagaceae*, *Comamonadaceae*, *Oxalobacteraceae*	Jin et al. (2022)

such as *Flavobacteriaceae*, *Moraxellaceae*, *Sphingobacteriaceae*, *Microbacteriaceae* and *Aeromonadaceae*, were reported to be present in the earthworm gut (Chen et al., 2018; Sharma and Garg, 2018). The microbial community of earthworm gut and cast are very active and can digest various organic substances such as cellulose, sugars, chitin, lignin, starch, and polylactic acids (Gomez et al., 2015).

5. Vermicast

Vermicast is the soil mass produced after the digestion of organic waste materials by the earthworm. In the digestion process, the organic substance is completely digested and rearranged within the worm gut. The existing chemical bond within an organic substance breaks down, and a new chemical bond is formed (Cui et al., 2018; Deka et al., 2011). The properties of vermicast vary from one species to another in terms of cast size, moisture content, humic acid, fulvic acid, pH, hormone, bulk density, and conductivity. Vermicast in the soil is beneficial for soil health, as it adds organic matter, increases water retaining capacity, improves soil structure, increases cation exchange capacity (CEC), helps form soil aggregates, enhances soil fertility, improves soil aeration, and prevents soil crusting. The four types of casts produced by different earthworms (Samal et al., 2019) are as follows:

- Small casts are joined together to form a flattened or globular cast. It is typically produced by anecic and endogeic worm species.
- Initially, the casts are like slurries or liquid mass, but when dried, the original shape becomes visible.
- Several globular casts are deposited and form a towerlike structure above ground.
- These are granular casts produced below the soil surface. All species of worms produce these types of casts.

The initial three casts are large and compact, but the last one is smaller and looser. The outer layer of compacting cast is covered by 10–20 mμ thick clay minerals that reduce aeration and microbial activity. The availability of N and P is higher in vermicast than in surrounding soil due to the mineralization of bound nutrients by microbes. Extractable P present in vermicast act as a P source for plants. Earthworm gut release phosphatase enzyme and phosphate solubilizer, which helps in phosphate mineralization. The majority fraction of P in orthophosphate form is adsorbed to metal hydroxides. The concentrations of other nutrients, such as Ca, Mg, K, and Na, increase during gut transit. Earthworms prefer to feed on organic substances with a higher C-to-N ratio, which indicates a low degree of decomposition. Subsequently, the produced cast has a low-value C-to-N ratio. In vermicast, NH_4^+ contributes to the salinity level of earthworms, which is generally repelled if the level rises more than 0.5%. If the fresh waste salinity level is high, vermicast salinity will be higher, too. Within 1–2 weeks, all NH_4^+ ions within the cast are nitrified.

5.1 Physical properties

Earthworms feed on half-decomposed organic substances and soil minerals to improve the palatability of the ingested substance. The composition of the feeding substance varies from earthworm species to species. The choice of ingested materials depends on the biochemical properties of organic waste, its size, shape, moisture content, age and initial microbial content

(Samal et al., 2017b). The newly formed cast may be washed out by rain and wind, but when it dries, it becomes stable and soil texture changes. Plant roots can uptake nutrients from the dried cast when damaged, so root systems penetrate easily. Cast present underground contain moisture so that roots can break the layer easily and absorb nutrients. If earthworms ingest more organic material along with soil, the cast will become soft. Fungal hyphae and microbial mucilage are sticky and keep the cast particle intact. With more organic content in ingested material, more microbes will be found in vermicast. Secretion of various liquids from microbes makes the cast more stable (Hu et al., 2018; Yasmin et al., 2022). Dlamini and Haynes (2004) reported that the cast of *L. rubellus* was more stable than that of *Aporrectodea caliginosa*, as the former ingested more organic food.

5.2 Microbial properties

Fresh vermicast contains a variety of survived microorganisms, but as the age of the cast increases, various other microbes also start to flourish. Different decomposing and nitrifying-denitrifying bacteria grow slowly. The carbon assimilation rate is higher in fungi than in bacteria, and the digestion rate of fungi is also higher than that of earthworms. In the earthworm gut, fungi are digested more rapidly than bacteria. In addition, the carbon assimilation rate is higher in fungi than in bacteria. Fungal spores that escape the worm gut transit grow fast outside in the cast (Sapkota et al., 2020). It has been observed that fungal species like *Alternaria* and *Cladosporium* decreased, and species like *Trichoderma* increased (Zhang et al., 2022; Zhu et al., 2021). A few algal species also slowly flourish in old vermicast-containing soil. Some protozoa divide by grazing on survived bacteria and fungi. The priming effect of earthworms helps activate inactive bacteria during gut transit. Microbial respiration and decomposition are higher in worm cast than in normal soil. Dominguez (2004) found various α-, β-, δ- and γ- *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Acidobacteria*, and *Flavobacterium*.

In the vermicomposting process, the earthworm breaks down all the organic waste, kills pathogenic microbes by secreting body fluid having antibacterial properties, adds favorable microbes to the compost pile and throws the digested waste to the top layer of the piles. Simultaneously, earthworms also kill pathogenic microbes and add beneficial microbes to the compost pile, changing the dynamics of microorganisms (Thakur et al., 2021). Since the pathogen level is quite low in vermicast, it is considered a type A biosolid. During the vermicomposting process, several biochemical modifications happen, such as adding sugar and mucus, soluble carbon, and nitrogenous excretory products. A five-times increase in microorganism content was observed by Munnoli et al. (2002) while composting potato peel waste. Kavian and Ghatnekar (1996) observed a 40-times increase in microbes in dairy sludge vermicompost. Ravindran et al. (2016) found an increase in bacteria, fungi, and actinomycetes in tannery sludge after the composting process. A study conducted by Eastman et al. (2001) observed a reduction in coliform bacteria in organic waste compost. Bhatia et al. (2013) reported a reduction of bacteria from 2.3×10^4 to 2.5×10^2 CFU g^{-1} during full-scale rotary drum composting. Xu et al. (2021) examined the remediation process of DDT contamination by earthworms in both drilosphere and nondrilosphere matrices. Ten microbial species—*Streptomyces*, *Virgibacillus*, *Brevibacterium*, *Dermacoccus*, etc.—were identified in the earthworm drilosphere as helping to break down DDT.

6. Vermiwash

Vermiwash is the aqueous fraction of vermicompost enriched with nutrients, vitamins, microorganisms, enzymes, plant growth hormones, etc. It can be applied as fertilizer to enhance crop productivity and suppress various plant diseases. This arises from the presence of antimicrobial components and pesticidal and antifungal activity in the vermiwash. Vermiwash is more suitable for plant application than vermicompost as the macro and micronutrients can reach the root area easily. The combined application of vermiwash and pesticides becomes more effective against thrips, mites, and other insects. Fig. 1.5 explains the components and benefits of Vermiwash. Akinnuoye-Adelabu et al. (2019) reported that vermiwash and worm mucus prevent the growth of the fungal species *Fusarium graminearum* in wheat fields. Vermiwash contains decomposing bacteria that suppress pathogenic bacteria and fungi by secreting various metabolites. It can be used raw or by diluting up to 5%–10% for foliar spray.

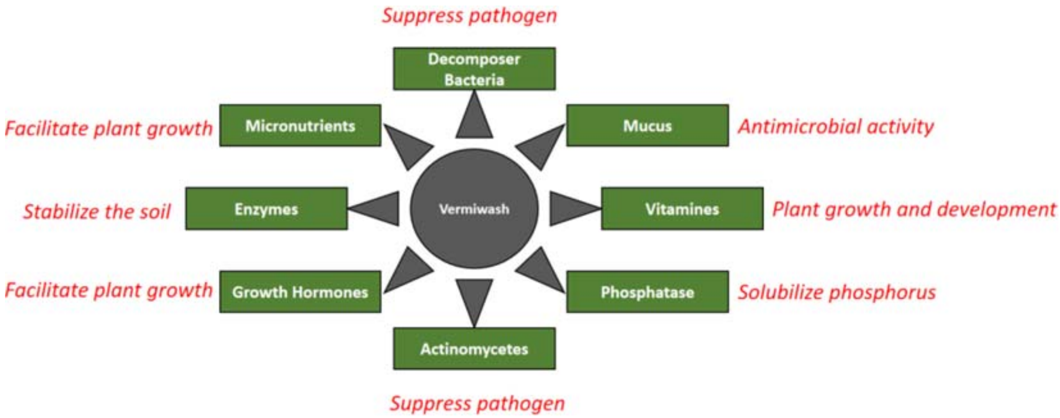

FIGURE 1.5 Components and benefits of vermiwash.

Vermiwash can be prepared easily by designing one vermibed, as shown in Fig. 1.6. The bottom layer consists of a coarse bed that facilitates vermiwash flow. Next, a layer of sand is placed, and the third layer consists of soil inoculated with earthworms. Above the soil layer, cow dung is kept, and the top layer is covered by half-decomposed substances, such as leaves, straw, and grasses, to prevent direct exposure to sunlight. Water sprinkling is necessary to prevent the organic from drying out and facilitate the decomposition process by the earthworm. Finally, vermiwash will be collected at the bottom of the system or vermibed (Fig. 1.6).

7. Molecular techniques to detect earthworm gut microbes

Recently, SEM and epifluorescence microscopy techniques have been used rapidly for identifying earthworm gut microbes. SEM and Whole-Cell Hybridization technique is used to study the gut microbial characterization of *L. terrestris* (Jolly et al. 1993). Other techniques

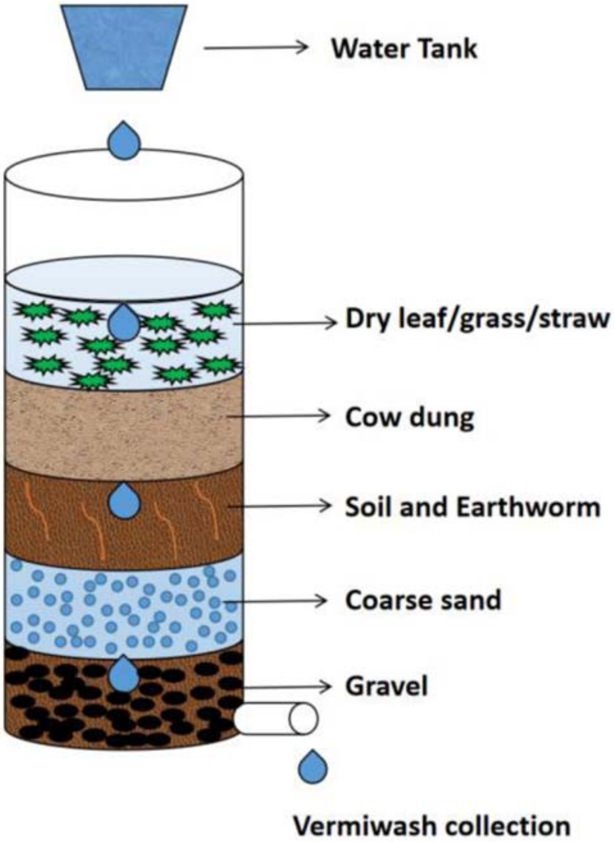

FIGURE 1.6 Vermiwash preparation.

used to study earthworm gut microbes include 16S rDNA technique, FISH, and DGGE. Schramm et al. (2003) reported the presence of *Acidovorax* bacteria in the gut of *L. terrestris*, *A. tuberculata, O. lacteum*, and *E. fetida* using the FISH technique. Aira et al. (2016) used the 16S rRNA pyrosequencing technique to characterize the microbial communities of vermicast of *Eisenia andrei*, and the dominant species identified were *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*, etc. Singh et al. (2015) identified more Actinobacteria and Firmicutes in the gut of *Perionyx excavatus and E. fetida* using 16S rDNA-based clonal survey analysis. Other microbes were also identified in the worm's gut, such as xylan degraders, chitin degraders, ammonia oxidizers, nitrogen fixers, and cellulose degraders. Thakur et al. (2021) studied different bacterial community structures in the alimentary canal of *Polypheretima elongata, P. excavatus*, and *Eudrilus eugeniae* through the 16S rRNA gene amplification process. Diversity analysis using 16S amplicon sequencing revealed that the dominant phyla were Proteobacteria, followed by Actinobacteria, Firmicutes, and Bacteroidetes. Fluorescent microscopy is also used to study bacterial species within each intestinal segment of the earthworm. Biswas et al. (2018) isolated three phosphate-solubilizing bacteria, such as *B. licheniformis, B.*

megaterium, and *S. haemolyticus* from the gut of *Metaphire posthuma* using 16 S rRNA gene sequencing. Hu et al. (2018) found silicate-solubilizing bacteria in the gut of *Pheretima guillelmi*, which plays a key role in silicon weathering in the crop field. Twenty bacterial strains were isolated from the gut walls using 16S rRNA analysis, among which three species solubilized silicate in the soil. Fischer et al. (1997) studied the gut of *O. borincana* using trace electronic microscopy and observed the abundance of the genus *Bacillus.* He observed the favorable environment for microbes in the posterior part of the worm. Though very few molecular techniques are being used to identify earthworm gut microbes, several other methods are available for use in the detection process, such as long-read sequencing, nanopore sequencing, next-generation sequencing, microarray, RAPD, amplified ribosomal DNA restriction analysis, and ribosomal intergenic spacer analysis.

8. Conclusion

Various categories of bacteria in the earthworm gut act symbiotically with earthworms to degrade organic waste and convert it into value-added products. Gut microbe development is a natural selection process that survives various enzymatic and biological activities. Gut microbes produce exoenzymes that degrade organic substances to release beneficial nutrients for plant growth. Microbial activity in vermicast affects soil crumb structure and stability. Worms play a major role in inoculating organic waste with microbes. Most microbes in organic waste are in the dormant stage with limited metabolic activity, but when they pass through the gut, they become active. The presence of earthworms in waste material increases soil microbial respiration and the microbial degradation of organic matter. Of all the worm species on Earth, *E. fetida* is the one most preferred for waste degradation. During the waste degradation process, the earthworm population increases; the surplus worms can be sold in the composting market and used in medicine and protein-rich animal feed.

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CHAPTER

2

How do earthworms affect the microbial community during vermicomposting for organic waste recycling?

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1. Introduction

The global population has increased dramatically. Sustainable agricultural practices are increasingly recognized as critical to meeting future global agricultural demands (Prasad et al., 2019). Crop production must be expanded using more sustainable and eco-friendly methods to ensure future food security while reducing environmental pressure on agroecosystems. Because microorganisms participate in a variety of soil processes, the soil microbial community directly and indirectly affects crop production (Rodriguez-Campos et al., 2014). Soil microorganisms are effectively involved in the decomposition and recycling of organic matter (Hayat et al., 2010). As a result, the effective management of microbial communities is essential for optimizing soil quality and crop production.

As microorganisms have limited mobility, they mainly need vectors for their movement through the different layers of the rhizosphere (Edwards, 2004). Indeed, earthworms, commonly called "soil engineers," are burrowing animals that intervene in the dynamics of bacterial populations and ensure, through intestinal transit, their dispersion in the soil

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(Aira et al., 2018; Picón et al., 2015). They multiply by five the number of bacteria in the surrounding soil (McLean et al., 2006). They are also important in temperate terrestrial ecosystems because they fertilize soils by changing their physicochemical and biological properties (Edwards, 2004; Eisenhauer and Scheu, 2008; Al-Maliki et al., 2020). These annelids increase plant biomass by 75% on average, with an overall increase of 36% for seed yield and 57% for aerial parts (Brown et al., 2000). The richness of earthworm casts in microbial populations has already been reported. In addition, cutaneous excreta play an interesting role in the dispersion of plant growth-promoting bacteria (Yakkou et al., 2021, 2022). Moreover, depending on the earthworm species, earthworms can have a balanced, destructive, or constructive impact on microbial richness and diversity (Egert et al., 2004; Furlong et al., 2002; Koubová et al., 2015). Given these characteristics, the interactions between earthworms and microorganisms are complex and difficult to comprehend.

The abundant population of earthworms in the soil provides an ideal environment for increased activity levels or the multiplication of selected microorganisms in the gut. Therefore, earthworm-microorganism associations drive the conversion of organic materials into humus-like material known as vermicompost. Vermicomposting improves soil organic matter content, oxygen consumption, moisture retention, plant permeability and water retention, nutritional content, and crop yield and durability (Kim et al., 2004, 2011; Picón et al., 2015).

Vermicomposting is the process by which earthworms and microorganisms break down organic waste. In this composting system, earthworms act with microorganisms as greedy feeders, digesting and mixing the substrate to accelerate decomposition and change organic wastes' physical, chemical, and biological properties (Domínguez and Gómez-Brandón, 2012). Recent research has shown the potential for earthworms' compost as a sustainable resource in organic agriculture, contributing to increased food production and reduced pollution (Bhat et al., 2017). The research demonstrates that earthworms, with the aid of microorganisms, can break down the organic material contained in some harmful industry wastes (pressmud and bagasse), converting them into rich fertilizers. In addition, much research has been conducted to demonstrate that microorganisms in vermicompost actively combat the potential negative impact of environmental stressors (Kazeminasab et al., 2016; Cui et al., 2018; Mupambwa and Mnkeni, 2018; Zhou et al., 2022a).

Through the complex mechanism of vermicomposting, the substrate containing microbial communities passes through the earthworm's gut and is digested, and its structure and function are modified (Aira et al., 2009). Recent research emphasizes the importance of the earthworm digestive tract as a significant influence on microbial ecosystems. The earthworm digestive tract is seen as a natural laboratory that acts to increase microbial diversity and abundance (Knapp et al., 2009). It is noteworthy that during vermicomposting, earthworms not only ingest soil microorganisms but also release secretions containing compounds that modify microbial populations' growth and activity. These secretions create a favorable environment for microbial growth, allowing for more functional microbes with greater capabilities and higher metabolic activity (Lv et al., 2018). It has been demonstrated that vermicomposting modifies microbial populations' activity levels and functional diversity. Furthermore, earthworms selectively modify microbial biomass, favoring those with certain metabolic pathways (Cui et al., 2018, 2019).

Significant increases in macronutrients can be observed in the final vermicompost products, while other chemical components are reduced, indicating the effectiveness of earthworms in managing the nutrient ratios of soil (Bhat et al., 2017). Understanding how these changes affect existing microbial communities is essential, as well as how they are altered by earthworm activity.

Overall, through a mix of direct impacts (effects brought on by direct earthworm activity) and indirect effects, earthworms change the microbial community (effects caused by indirect earthworm activity) (Aira and Dominguez, 2011). Nevertheless, little is known regarding the influence of the indirect effects on microbial community functioning and the interactions between earthworms and microorganisms through vermicomposting.

The purpose of the current chapter is to improve the use of vermicomposting as a waste-recycling method by gaining an understanding of how earthworms affect the microbiota. This is especially important in light of the increasing amount of waste produced by humans that could be diverted to vermicomposting and therefore managed in a more sustainable way. Thus, it is crucial to explore the roles of earthworms and other factors in the vermicomposting process to make it a more reliable, efficient, and sustainable waste management technique. Understanding and controlling the different parameters' evolutions during vermicomposting can help create optimal conditions for waste management.

2. Earthworm−microorganism interactions: Selectivity and diet

2.1 Bacteria

Earthworms and microorganisms engage in synergistic interactions that contribute to soil transformation. The interaction with bacteria ingested from soil and passing through the intestine is one of the most well-studied interactions (Byzov et al., 2007; Huerta Lwanga et al., 2018; Kim et al., 2004; Nechitaylo et al., 2010; Tikhonov et al., 2016). The activity and fate of microorganisms in the gut of earthworms and their castings have been extensively studied using both culture-based and molecular techniques (Picón et al., 2015). Some microorganisms are not affected by the digestive enzymes of earthworms, giving them a greater capacity to degrade complex substances in organic soil matter during vermicomposting (Picón et al., 2015). At least a portion of the earthworm's diet during vermicomposting consists of microorganisms; therefore, earthworm activity can potentially affect vermicomposting performance.

Earthworms recognize and selectively consume particular microorganisms. In response to microbial activity and metabolites, it seems likely that endogenous earthworm species congregate in distinct soil regions (Hughes et al., 1994). This behavior could enhance earthworm reproductive performance and population density. Earthworm activity clearly affects microbial ecosystems, possibly changing the numbers and activities of both diseases and beneficial microbes. Litter-decomposing microorganisms, mycorrhizal fungi, nitrogen fixers, biological control agents, and mesofauna numbers and activity are increased by earthworm activity (Zhao et al., 2022). Table 2.1 represents the ecological role of bacteria isolated from various earthworm species.

Because of the lack of oxygen, the intestine is populated by anaerobic and facultative anaerobic bacteria such as *Proteobacteria, Firmicutes, Bacteroides* and *Actinobacteria* (Drake and Horn, 2007; Horn et al., 2003). The bacterial composition of the gut of earthworms varies

TABLE 2.1 Ecological function of bacteria isolated from the intestine of various earthworm species.

Type of earthworm	Earthworm species	Isolated bacteria	Proven role of bacteria	References
Epigeic	*Eisenia foetida*	*Dechloromonas denitrificans* *Flavobacterium denitrificans* *Paenibacillus anaericanus* *Paenibacillus terrae*	Production of N_2O	Horn et al. (2005)
		Rhodococcus sp. and *Bacillus* sp.	Biodegradation of dichlorodiphenyltrichloroethane (DDT)	Mudziwapasi et al. (2016)
	Eudrillus eugeniae	*Bacillus tropicus* *Bacillus aerius* *Bacillus safensis* *Bacillus xiamenensis* *Bacillus subtilis*	Production of amylase nitrate reductase cellulase, xylanase and protease	Utekar et Deshmukh (2019)
	Perionyx excavatus	*Mycobacterium monacense*, *Stenotrophomonas maltophilia*, *Acinetobacter* sp., and *Alcaligenes faecalis* *Stenotrophomonas maltophilia* and *Acinetobacter* sp. *Chryseobacterium indologenes*	Cellulose degradation	Dey et al. (2018)
Endogeic	*Metaphire posthuma*	*Staphylococcus haemolyticus* and *Bacillus licheniformis* *Bacillus megaterium*	Phytohormone (AAI) production phosphate solubilization Heavy metals resistance	Biswas et al. (2018)
		B. licheniformis	Extracellular polymeric compounds production Resistance to metals (Cu and Zn) Germination and growth promotion of *Vigna radiata* plants	Biswas et al. (2018)
	Glyphidrilus spelaeotes	*Chromobacterium piscinae* *B. megaterium*, *Alcaligenes faecalis*, and *Sphingomonas* sp. *Pseudomonas aeruginosa Acinetobacter* sp.	Cellulose degradation	Dey et al. (2018)
Anecic	Pheretima guillelmi	*Microbacterium paludicola* *Flavobacterium gilvum* *Aeromonas hydrophila* *Pseudomonas lutea* *Flavobacterium granuli* *Streptomyces azureus* *Bacillus drentensis* *Rhizobium leguminosarum Paracoccus* *Cellvibrio mixtus* *Microbacterium paludicola* *Bacillus cytotoxicus* *Rhizobium leguminosarum* *Streptomyces flavovariabilis* *Flavobacterium gilvum* *Ensifer adhaerens*	Increasing silicon availability in soil and its absorbance by corn	Hu et al. (2018)

according to species, living habits, and characteristics of the surrounding environment. As illustrated in Fig. 2.1, *Lumbricus* sampled from various habitats displayed different gut bacterial compositional characteristics. *Proteobacteria, Firmicutes, Bacteroides, Verrucomicrobia,* and *Actinobacteria* dominated gut phyla in *L. rubellus* retrieved from arsenic-rich soils, representing 50%, 30%, 6%, and 3% of total bacteria, respectively (Knapp et al., 2009). The intestinal bacteria of *L. terrestris*, the most prevailing, collected from grasslands were *Actinobacteria* (36%), *Proteobacteria* (25%), *Planctomycetes* (9%), *Verrucomicrobia* (9%), *Acidobacteria* (4%), *Chloroflexi* (4%), and *Firmicutes* (3%) (Pass et al., 2015). Similar research has been conducted on *Eisenia foetida*, widely used for vermicompost production. The dominant bacteria in the gut of *E. foetida*-high agricultural soils were *Proteobacteria* (44%–50%) and *Actinobacteria* (6%–9%) (Wang et al., 2017). Despite the differences in bacterial composition between earthworms and the environments, it is obvious that the *Proteobacteria, Firmicutes, Actinobacteria*, and *Bacteroidetes* are the most detected phyla in the earthworm gut (Drake and Horn, 2007).

The role of bacteria in earthworm species' diets, as well as the degree of species-specific feeding and digestion patterns, remains mostly unclear.

Certain bacterial species can be digested, others may be unaffected and live in the earthworm's intestine, and still others may develop or become more active. In the case of using *E. fetida* species for vermicompost, 19 species of bacteria were digested in the gut, and this dietary supplement significantly boosted the earthworm's development rate (Flack and Hartenstein, 1984). However, Morgan and Morgan (1988) discovered that just 2 of the 12 bacterial species examined permitted *E. fetida* to maintain weight; the earthworms lost weight or

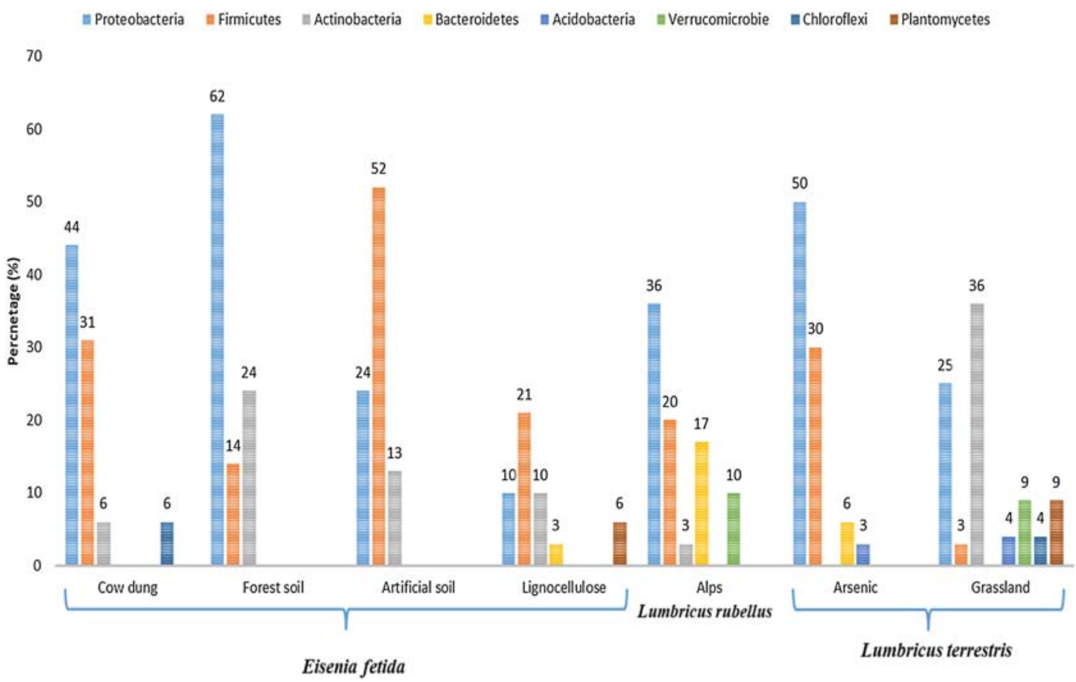

FIGURE 2.1 The percentage of bacterial communities isolated from the earthworm gut (Sun et al., 2020).

perished while feeding on other species. Thorpe et al. (1993) demonstrated that in the absence of competition from other microbes in the gut of *L. terrestris*, the bacterium *Pseudomonas fluorescens* may proliferate. Doube et al. (1994) revealed that rhizobia and *Pseudomonas corrugata* survived transit through *Aporrectodea trapezoides* and *A. rosea* intestines. Different earthworm species can have different effects on the same bacteria. For example, Schmidt et al. (1997) supplied the same dose of *P. corrugata* inoculum to four species of earthworms. The density of *P. corrugata* was detected in fresh castings of *A. longa* was 10 times greater than that of *L. rubellus*, *A. caliginosa*, and *L. terrestris* castings.

2.2 Fungi

In the case of fungi, it appears that several species, especially toxin- or antibiotic-producing fungi such as *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp., are harmful to earthworms (Edwards and Fletcher, 1988; Morgan and Morgan, 1988), which explains why earthworms have a detrimental effect on the vast majority of fungi species. Studies involving six species of earthworms and more than 10 soil and litter fungal species demonstrated that earthworms prefer and partially digest rapidly growing fungal species, which are typically associated with the earliest stages of decomposition (of cellulolytic fungal species and those that consume soluble carbohydrates, e.g., *Trichoderma* spp.) (Bonkowski and Schaefer, 1997; Moody et al., 1996). The survival of fungal spores in the earthworm gut varies significantly based on the spore's characteristics and the environment within the earthworm's intestine (Brown, 1995). Moody et al. (1996) found that no *Fusarium lateritium* spores survived transit through *L. terrestris* and *A. longa*; *Mucor hiemalis* spores were more seriously impacted by passage through the intestines of *L. terrestris* than through *A. longa* intestines (10% vs. 28%). The passage of spores of another fungal species (*Chaetomium globosum*) through the intestines of two species of earthworms did not affect their viability.

Reddell and Spain (1991) demonstrated that spores of an actinomycete, *Frankia* sp., and over 20 species of mycorrhizal fungal groups remained viable after passing through the *Pontoscolex corethrurus* digestive tract.

2.3 Protozoa

Some species of earthworms rely heavily on soil protozoa for nutrition. Until protozoa were introduced, *E. fetida* could not mature in soil that had been sterilized and repopulated by bacteria and fungi (Zirbes et al., 2012). The addition of protozoa to the diet of earthworms led to significant weight gains, according to Flack and Hartenstein (1984). In addition, the densities of the endogenous earthworm *A. caliginosa* rose in soils with plenteous amebas (Bonkowski and Schaefer, 1997). Similar to *A. trapezoides* and *E. fetida*, *A. trapezoides* and *E. fetida* preferred soils with flagellated and ciliated protozoa and positively reacted to the protozoa's living fluid (Edwards, 2004). This suggests that earthworms can also react to protozoan activity products, presumably low molecular weight chemicals. However, nothing is known about how protozoa affect earthworm activity.

3. Microbial abundance and diversity changes during vermicomposting

By altering the microenvironment, earthworms can quickly increase the activity and quantity of functional microorganisms in vermicomposting (Brandón et al., 2019; Huang and Xia, 2018; Monroy et al., 2009; Rosado et al., 2022). This process can lead to more efficient mineralization of organic matter, resulting in improved compost quality and accelerated composting processes (Huang and Xia, 2018; Monroy et al., 2009). However, although Wu et al. (2019) found many bacteria in the original organic waste, the number of active functional microorganisms was rather low (Wu et al., 2019). This calls into question the relationship between variety and function, as well as the role of earthworms in this regard. The simultaneous high abundance and low activity could be explained by intense population regulation (Hunt et al., 2013). Furthermore, earthworm castings supplementing numerous accessible nutrients for microbial metabolisms may increase bacterial numbers throughout the vermicomposting (Domínguez et al., 2010). It was demonstrated that earthworms affected both the abundance (abundance-based coverage estimator (ACE), Chao 1) and diversity (Shannon index) of microbial communities in maize stover and cow dung substrates (Chen et al., 2022). This shows the importance of earthworms in promoting microbial diversity and stability. In addition, using high earthworm densities shows that the values of Chao1 and ACE are more significant, indicating that earthworm density positively influences microbial abundance. The Shannon index trended downward during the precomposting stage. After 30 days of vermicomposting, the Shannon indices were greater than those of the composting treatment (without earthworms), indicating that earthworms altered microbial structure and increased microbial diversity. At later stages (between 45 and 60 days) of vermicomposting, Shannon index values were at their lowest (Chen et al., 2022). However, in research by Huang et al. in 2022, no significant difference was found between the Shannon and Simpson indices of raw organic waste and the vermicomposting product. Despite the fact that the earthworm species in both studies were the same, Huang's vermicomposting method included room drying, which might affect microbial communities.

On the other hand, vermicomposting may provide a unique form of microbial environment for certain bacteria not present in the original raw organic waste. In Huang et al.'s study (2022), operational taxonomy units (OTUs) accounted for 64.97% of the original organic waste and 71.42% of the vermicomposting, implying that most microorganisms in the vermicomposting process came from the original organic waste. However, it is interesting to note that some of the microorganisms in vermicomposting were not present in the original organic waste. Thus, the vermicomposting OTU was 11.7% unique compared with the original organic waste.

In prior pilot-scale vermicomposting studies using plant-derived materials, the presence of earthworms decreased the microbial activity (Rosado et al., 2022; Nogales et al., 2020). In fact, on a short-term basis, epigeic earthworms are known to alter microbial communities by fragmenting and consuming new organic debris during the earthworm gut transit, which, later, increases the accessible surface area for microbial colonization and accelerates the breakdown rate of organic matter during vermicomposting (Domnguez et al., 2010). This might explain why there were no significant differences in microbial biomass carbon in the presence of earthworms during a 63-day research using reactors fed with raw marc. Regardless of the absence of differences in microbial biomass carbon, decreased respiration values were recorded after 14 days of vermicomposting (Gómez-Brandón et al., 2022).

Gómez-Brandón et al. (2022) did not notice any variations in microbial respiration compared to the control (without earthworms), which was consistent with the results of the same research team's previous study (Gómez-Brandón et al., 2011). They proved that the activity of *E. andrei* enhanced the short-term stability of grape marc, as shown by reductions in microbial biomass and activity relative to the control and suggesting that the microbial biomass and activity during vermicomposting may be restricted or managed. However, it relies on other factors, such as the vermicomposting stage, earthworm density, and organic waste type.

At the early stage of vermicomposting, the bacterial abundance was found to be two times greater than composting without earthworms. However, after 63 days, the bacterial and fungal abundances decreased with earthworm presence. These findings align with those of Brandón et al. (2019), who observed a fast rise in bacterial richness and diversity in Menca distilled and raw marc during the first stages of vermicomposting (first 14 and 28 days, respectively). This can be explained by the fact that the first phase of vermicomposting is the active phase (Vuković et al., 2021), which includes the simultaneous actions of earthworms and beneficial microbes to break down organic compounds through metabolic activities. Thereby, their physical and soil biological activities are altered.

In contrast to fungus, bacteria take a more opportunistic approach to nutrient use by quickly consuming freshly created labile substrates. After the process, it is possible that epigeic earthworms indirectly lowered bacterial and fungal abundances by depleting the resources needed by microorganisms due to the accelerated breakdown of organic matter as vermicomposting develops (Gómez-Brandón et al., 2022). Contrary to the findings of the most recently cited studies, Zhou et al. (2022b) discovered that the total bacteria increased in both vermicomposting and composting systems over 90 days, from 58.08 107 to 97.74 107 CFU g1 and 59.15 107 to 84.43 107 CFU g1, respectively. Composting raised the proportion of total bacteria by 42.70%, which was much less than vermicomposting (68.30%). It was hypothesized that the increase in total bacteria was due to nutrient-rich pig dung promoting the proliferation of microorganisms. The vermicomposting procedure had the most diversified collection of bacteria to enable the enzymatic breakdown of the pig manure (Hrebeckov et al., 2019), as shown by the more pronounced rise in the total number of bacteria. However, the total number of fungi declined dramatically from the beginning of the vermicomposting process to 30 days; a significant increase was obtained from 30 to 45 days, and the total number of fungi fluctuated somewhat between 45 and 90 days. The minimal microbiological number of total fungi was attained after 30 days with a 32.7% drop in population (Zhou et al., 2022b). The different variable pattern of total fungal population in the presence and absence of epigeic earthworms was attributable to fungi being the principal food source for epigeic earthworms (Villar et al., 2017).

While investigating the impact of vermicomposting on the abundance and activity of beneficial bacteria, it was shown that the number of bacteria with nitrogen-fixing bacteria (NB), phosphate-solubilizing bacteria (PB), and potassium-solubilizing bacteria (KB) increased considerably during vermicomposting. After 90 days of composting, the increased percentage of NB was 130.3%, PB was 223.0%, and KB was 111.4%. In the vermicomposting procedure, much more significant percentage increases of NB, PB, and KB were observed: 912.3%, 1227.3%, and 577.5%, respectively (Zhou et al., 2022b). The beneficial population of NB, PB, and KB was significantly increased in the vermicomposting process. These results are not surprising because earthworms usually have a significant role in boosting plant growth-promoting bacteria in soil, as described in previous research to be a result of increased mucus production and surface area for microbial development (Zhou et al., 2022b).

A team of researchers from Vigo University in Spain compared microbial diversity in raw and composted earthworm castings. They also measured the abundance of specific groups of microbes. They found that the diversity of bacteria and fungi was much higher in raw than composted earthworm castings (Domínguez et al., 2021). This is not surprising because raw worm castings contain many bacteria that cannot grow in the high temperatures needed for composting. In contrast, composted earthworm castings were dominated by species of microorganisms that had survived the composting process. However, the researchers found that many bacteria found in raw and composted earthworm castings were present within both environments. This suggests that both the castings and the soil surrounding them may play an important role in maintaining the microbial diversity of the agricultural ecosystem.

Earthworms maintained high phylogenetic bacterial diversity by increasing soil heterogeneity, and they also exerted a biotic control on the relations between bacterial diversity and function. Future research is recommended to evaluate the ecological implications of biotic controls on correlations between diversity and function on ecosystem services (such as soil detoxification) at greater scales (Monard et al., 2011).

4. Microbial structural changes during vermicomposting

Studies have highlighted how fast vermicomposting influences bacterial populations from the original substrate. They give a convincing illustration of how microbial succession is influenced by variations in the organic carbon supply throughout the vermicomposting process. During vermicomposting, the temporal variations in decomposing organic matter and microbial structure via intestinal and cast-related mechanisms still need to be fully characterized. Such modifications occur gradually as microbial succession advances (Domínguez et al., 2019). Prior research on the bacterial populations linked with vermicomposting processes has mainly examined the finished product. Notwithstanding, characterizing the temporal variations in microbial population over the course process may provide insight into the biological processes underpinning vermicomposting and, therefore, the qualities of the final product.

Proteobacteria, Actinobacteria, and *Bacteroidetes* have been identified as the three most prominent taxa initially present in the sludge and its vermicompost (Huang et al., 2020). The same phyla were reported to be dominant in the earthworm intestine (Drake and Horn, 2007; Horn et al., 2003). 53.83% of the microorganisms in vermicomposting belonged to the Proteobacteria phylum, as determined by the research conducted by Huang et al. in 2022. In addition to Proteobacteria, the end products were dominated by *Actinobacteria*, *Bacteroidetes, Chloroflexi,* and *Firmicutes*. However, compared with the original organic waste, the vermicomposting process reduced *Proteobacteria* by 11.4%. *Actinobacteria*, the primary degraders of recalcitrant organic waste, rose by 5.31% in the vermicomposting treatment.

Knapp et al. (2009) hypothesized that elevated *Actinobacteria* might be related to earthworm excrement. In addition, the vermicompost's high level of *Actinobacteria* showed that its ultimate product was well-matured. The principal decomposers of macromolecular compounds, *Bacteroidetes, Chloroflexi,* and *Firmicutes*, did not change significantly between the vermicomposting and composting treatments. This appears to contrast with previous research in which *Firmicutes* were the major bacterial population in vermicompost, followed by Proteobacteria (Vaz-Moreira et al., 2008).

The community structure mainly reflected microorganisms that had just traversed the earthworm gut and been ejected. These digested materials break down quickly and serve as a nutrient and microorganism supply that could influence decomposition within the vermicomposting system. It is anticipated that during the active stage, water-soluble nutrient pools released from casts will promote the growth of bacteria with rapid carbon turnover rates and a preference for rich and soluble substrates named copiotrophic. Copiotrohic bacteria, such as those belonging to *Proteobacteria* and *Bacteroidia*, were found in greater abundance during the active phase of grape marc vermicomposting (Brandón et al., 2019). Bacteria related to oligotrophic conditions, including those from the groups *Acidimicrobiia*, *Planctomycetacia*, and *Fimbriimonadia*, were much more prevalent in the process' later phases, which eventually replace copiotrophic bacteria, are more effective at utilizing substrates and are capable of metabolizing the last residual recalcitrant substrates in the casts during the final stage (maturation) (Chiba et al., 2021). Indeed, evidence of bacteria with lignin-decomposing ability has emerged from members of the phyla *Acidobacteria* and *Planctomycetes*, but to a smaller level than fungus (Brandón et al., 2019)

The Alpha- and Gammaproteobacteria classes are prevalent in wastes at the start of the vermicomposting period, although this decreased dramatically with time (Castillo et al., 2013). The relative abundances of the two bacterial groups were shown to be associated with one another and with overall bacterial abundance. According to DeAngelis et al. (2011), Alpha- and Gammaproteobacteria taxa are associated with lignin breakdown. Both, together with fungi, were suspected of being the cause of the drop in organic matter during the initial phases of vermicomposting. Nonetheless, the relative abundance of the *Actinobacteria* class was adversely connected to the overall bacterial population, notably the relative abundance of Gammaproteobacteria. The findings must be connected to this taxonomy's capacity to colonize the earthworm castings and gut and its antagonistic behavior toward other taxa (Kumar et al., 2017). As members of the Gammaproteobacteria class, dangerous bacteria like *Escherichia coli*, *Pseudomonas* sp., *Salmonella* sp., *and Vibrio cholerae,* as well as fungi, which belongs to *Actinobacteria*, are widely recognized for producing antibiotics that suppress or destroy (Monroy et al., 2009). When a potentially harmful substrate is subjected to vermicomposting, a substantial abundance of the *Actinobacteria* community ensures the product's quality and safety. The relative abundance of *Proteobacteria* and *Actinobacteria* decreased near the end of the maturation phase, but Gammaproteobacteria increased significantly. Under these maturation conditions, this taxon of bacteria has to be among the most resilient. Conversely, reducing the proportion of *Actinobacteria* may reduce their antagonistic impact on fungi and certain other bacteria (Kumar et al., 2017).

At the genera taxa level, *Lysinibacillus, Bacillus, Pseudomonas, Morganella, vibrio*, and *Aeromonas* were the dominating bacterial communities in vermicompost (Raimi et al., 2022; Blomström et al., 2016). However, the repartition of genera changes throughout the vermicomposting process. For example, while studying the development of microorganisms generating GH6 cellulase, the dominating microbial taxa were identified as *Cellulomonas, Janthinobacterium*, *Paraphaeosphaeria, Rhizobacter,* and *Streptomyces,* in the earliest materials (Huang et al., 2022). *Cellulomonas* and *Cellulosimicrobium* were the dominating taxa in the substrate after 15 days of precomposting. The relative abundance of *Cellulosimicrobium and Cellulomonas* enhanced significantly. In one instance, this abundance rose from 3% to 31% (Huang et al., 2022). *Streptomyces*' relative abundance fell from 27% to 3%. They discovered that *Streptomyces* was the dominating species early in the composting process, but its relative abundance dropped as it progressed (Chen et al., 2022). However, in Huang's research, the dominating genera were represented by *Verminephrobacter, Lelliottia, Flavobacterium, Chitinivorax*, *Thermomonas, Candidatus-Microthrix*, and *Aeromonas*. The prevalent bacterial species in vermicomposting shifted significantly in the end output compared to the beginning organic waste (Huang et al., 2022).

In three trials, comparing the abundances of *Cellulosimicrobium* and *Cellulomonas* revealed that the density of earthworms influenced microbial structure. However, the density of earthworms does not alter the development of the genera abundance during vermicomposting. In the presence of 60 earthworms per kilogram of the substrate, the proportion of *Cellulomonas* and *Cellulosimicrobium* peaked on day 30 (7% and 13%, respectively), then decreased and grew again after the process (21% and 37%, respectively). With an earthworm density of 120 per kg on day 30, the relative abundance was 27% for *Cellulomonas* and 37% for *Cellulosimicrobium*. However, the abundance dropped dramatically on day 45, with equivalent values of 3% and 5%. The quantity of *Cellulomonas and Cellulosimicrobium* rose, reaching 19% and 26%, respectively, after 75 days. *Cellulomonas's* abundance was 30% on day 30 in vermicomposting with 180 earthworms per kg, then fell quickly to 6% on day 45. On day 75, it jumped to 22%. *Cellulosimicrobium* relative abundance was 13% on day 30, then declined to 3% on day 45, relatively low until the vermicomposting was completed. On the exact sample time, the proportion of *Cellulosimicrobium* was considerably more significant in the vermicompost than in the control treatment, confirming that the introduction of earthworms causes the changes in bacterial structure. *Cellulosimicrobium* has been linked to the intestinal tract of the earthworm *Eisenia fetida* (Chen et al., 2022).

The microbial community changes with the same earthworm density, which implies that the substrate influences microbial populations during vermicomposting. As a result, the influence of the substrate on vermicompost microbial communities will be explored in the next section.

5. Microbial functional changes during vermicomposting

Some bacterial communities can perform a variety of plant growth-promoting (PGP) activities, including mineralization, nutrient solubilization, and disease control, in addition to managing the vermicomposting process (Raimi et al., 2022). According to predictive functional profiling, the bacterial communities in vermicompost play various roles that are important for the economy, particularly in agroecosystems. Some anticipated biological processes have an impact on biogeochemical cycles like N, P, K, C, and S cycles (Aragón et al., 2014).

A recent study identified 386 major metabolic pathway profiles, along with 1965 predicted metagenome functions. Notably, bacterial inoculum in the field produces enzymes contributing to plant growth-promotion functions like denitrification, biological nitrogen fixation, phosphate mineralization and phytohormone production, which were the expected biological functions of great importance given their positive effects on agroecosystems (Raimi et al., 2022).

A process known as biological nitrogen fixation converts atmospheric nitrogen to the plant-useable form of NH_4^+ and NO_3 by the action of N-fixation functional entity nitrogenase, which was observed to be expressed during vermicomposting (Richards, 1990). Numerous bacterial species, including *Bacillus, Citrobacter, Enterobacter, Lysinibacillus, and Pseudomonas*, have been found to produce nitrogenase (Mus et al., 2018; Yadav et al., 2018). Similarly, the findings of Thamizharasan et al. (2021) support the findings of an earlier study that revealed a high diversity of the N-fixing bacteria, all through vermicomposting. For biological nitrogen fixation, nitrogenase was predicted in all the vermicompost samples, as reported by Raimi et al. (2022). In addition, an enormous amount of nitrate reductase was also predicted in the vermicompost. Noteworthy, *Pseudomonas* was primarily responsible for the high relative abundance of nitrogenase and denitrification enzymes, while *Escherichia-Shigella* contributed to the abundance of nitrate reductase (Raimi et al., 2022).

Bacterial communities such as *Pseudomonas* and *Bacillus*, abundant in enriched vermicomposting, are well known for their capacity to solubilize nutrients (Zaheer et al., 2019). Additionally, these genera produce siderophores and phytohormones (cytokinin, IAA, and gibberellin) that promote plant growth (Naureen et al., 2017). For IAA biosynthesis, ferredoxin oxidoreductase and indole pyruvate decarboxylase predicted are critical enzymes (Lwin et al., 2012). The vermicompost's bacterial composition, which generates these enzymes, may affect plant physiological structures, including stem formation and root architecture (Bhutani et al., 2018; Lata et al., 2006; Sun et al., 2017). The indole pyruvate ferredoxin oxidoreductase was prevalent in vermicomposts, with *Paraclostridium* predicted to be primarily responsible. The high concentration of 1-aminocyclopropane-1-carboxylic acid (ACC) was attributed to *Pseudomonas* and *Aeromonas* (Raimi et al., 2022).

Likewise, bacteria with siderophore production capability were noticed during the vermicomposting (Farokh et al., 2011). Iron-chelate-transporting ATPase Arylsulfatase, and adenylyl-sulfatekinase, associated with the metabolism of iron and sulfur, were present in high concentrations in vermicompost (Raimi et al., 2022). According to the predicted ferric chelate reductase, bacterial communities of the vermicompost may improve siderophore activities in soil, enhancing iron absorption in plants and then suppressing pathogens through the indirect impacts of soil iron sequestration on microbial life (Bhattacharjee and Dey, 2014). Further, enzymes that aid in the breakdown of various carbohydrates including such galactosidase, glucosidase, and mannosidase, were frequently anticipated to be present in vermicompost in great quantity, with the exception of beta-mannosidase, which was found to be extremely rare (Raimi et al., 2022). The high quantity of these enzymes was mostly attributed to *Lysinibacillus, Bacillus,* and *Paenibacullus* (Zaheer et al., 2019).

Some bacteria found in vermicompost, including *Bacillus, Enterobacter, Lysinibacillus, Klebsiella, and Pseudomonas,* have nitrophenyl phosphatase as well as alkaline and acid phosphatases (Behera et al., 2017). Solubilization is a primary ecological role of bacterial inoculum that enhances the bioavailability of soil nutrients. Therefore, the abundance of acid phosphatase indicates that vermicompost bacterial communities under acidic pH environments can survive and solubilize P (Chandra et al., 2017; Muthukumarasamy et al., 2017; Souchie et al., 2006). In various vermicomposts, phosphatases engaged in P mineralization under acid and alkaline environments were significantly abundant among bacterial communities. In Raimi's recent work (2022), *Escherichia-Shigella* were mostly responsible for the high quantity of alkaline phosphatase and *Lysinibacillus* for the acid phosphatase abundance.

Glutamicibacter species isolated from a night-soil compost exhibit traits such as cold adaptability, effective degradation, and the capacity to stimulate plant development (Borker et al., 2021). The vermicomposting system was where the first report of another genus, *Chitinibacter*, with strong chitinase enzyme-secreting capabilities, was made. Using various carbon sources, including simple sugars and complex hemicelluloses, the *Acidobacteria* genus may help stabilize and reduce sludge (Xu et al., 2014). Moreover, it was proved that some dominant genera identified in a drying treatment vermicomposting—*Accumulibacter*, *Candidatus, Dechloromonas, Nitrosomonas,* and *Thauera*—are predominantly linked to denitrification (Du et al., 2019).

The vermicompost treatment had no significant effect on the number of soil bacteria in Xiang et al. experiments (2022), but the homogeneity increased. It was discovered that the structure of the bacterial population was affected by total phosphorus and dissolved organic matter. Functional annotation of prokaryotic taxa analysis further clarified that vermicompost improved nitrogen fixation by 46.3%, xylanolysis by 55.4% and inhibited nitrification by 59.8%. Correlation analysis revealed that these function groups highly correlate with *Actinomycetales, Clostridiales, and Nitrospirales* (Xiang et al., 2022).

Chen et al.'s (2022) study investigated the function of Glycoside hydrolase family 6 (GH6) cellulase-production of microbial communities during vermicomposting. It proved that the concentration of *Cellulosimicrobium* and *Cellulomonas* increased noticeably during the early phase and the GH6 gene increased significantly correspondingly. Therefore, it was possible to infer that *Cellulosimicrobium* and *Cellulomonas* were the dominant genera harboring GH6 gene. Exoglycanase and endoglucanase are enzymes that *Cellulomonas* produce to break down lignocellulosic materials (Bill et al., 2021; Yausheva et al., 2016). Some strains of *Cellulomonas* possess advantageous functions over other microbes concerning the production of cellulase and have a wider range of temperature and pH tolerance (Swathy et al., 2020). The *Cellulosimicrobium* is a representative bacterium that degrades plant biomass and resides the earthworm intestinal tracts (Hong et al., 2011). These variations in functional diversity of the bacterial communities throughout vermicomposting provide a plausible mechanism for improved plant performance when grown in vermicompost (Brandón et al., 2019).

Furthermore, Mohd et al. (2021) noted that adding vermicompost to the soil-crop system enhances the positive impacts of plant growth-promoting bacteria. Still, it depends on the vermicompost dose and the crop used. Otherwise, the metabolic functions of vermicompost microbiomes might also vary based on the starting substrate. In conclusion, applying earthworm species or vermicomposting can have the most remarkable consequences on their advantages when employed as a soil amendment and plant growth booster.

Through a mix of direct impacts (effects brought on by direct earthworm activity) and indirect effects, earthworms change the microbial community (effects caused by indirect earthworm activity). To better understand these effects, researchers have conducted experiments to test how indirect earthworm effects interact to affect microbial community functioning (Aira and Dominguez, 2011). They showed that adding three distinct earthworm species' worm-worked substrates to fresh organic matter caused alterations similar to those occurring when earthworms are present. The microbial biomass and enzyme activity significantly increased after being introduced to worm-worked substrates. Consequently, little is known about the interactions between earthworms and microbes. Because direct and indirect impacts cooperate to create larger changes to microbial biomass and enzyme activity, it suggests several positive feedbacks during earthworm activity.

6. Substate effects on bacterial community during vermicomposting

Variations in residues, like cow dung, food and crop waste, and sewage sludge used in farm studies, influence the bacterial diversity of the vermicomposting products (Edwards et al., 2010; Grantina-Ievina et al., 2013). Some studies using food and plant waste, cow manure, and sewage sludge as feedstocks report that *Chloroflexi and Actinobacteria* are the most dominant phyla (Meng et al., 2019). In contrast, in the presence of similar feedstock, *Chloroflexi and Actinobacteria* were not dominant in Huang's study; they were found at extremely low relative abundances. Other elements, like the density of earthworms, may be responsible for the discrepancy. Vermicompost made from cattle dung has been reported to comprise *Firmicutes, Proteobacteria* and *Bacteroidetes* (Blomström et al., 2016). Besides, during vermicomposting processes, earthworms and microbial communities sanitize the organic waste by feeding on pathogens (Swati and Hait, 2018). However, even though they are relatively rare, some organic wastes can enhance potentially harmful microorganisms. The prevalence of pathogenic bacteria in the vermicompost has previously been linked to variations in feedstock and vermicomposting methods (Edwards et al., 2010; Van der Wurff et al., 2016). Pathogenic bacteria such as *Clostridium, Escherichia-shigella, and Vibrio* have been reported in vermicompost made from animal waste, such as cow dung and sheep manure (Blomström et al., 2016). Nevertheless, there is a need to improve vermicomposting efficiency while considering the used feedstocks continually. Depending on the waste used, some bacterial phylum showed differences in the evolution during vermicompost. The Alphaproteobacteria and Gammaproteobacteria classes were observed to be more dominant in olive-oil wastes than in winery wastes. In the winery wastes substrate, the relative abundance of the two bacterial groups was correlated with each other and with the total bacterial abundance. However, in the olive-oil waste substrates, this did not occur (Castillo et al., 2022).

Raimi et al. (2022) investigated bacterial communities in vermicompost collected from different production farms. The vermicompost from farm F1 was mostly made with cattle manure, whereas farm F2 employed grass clippings, vegetable waste, sheep manure, and municipal waste (paper sludge), and farm F3 used composted grasses, vegetable waste, cattle manure, and municipal waste (paper sludge). The highest and the lowest ASVs (amplicon sequence variants) were observed in F1 and F3, respectively. The detected ASVs and Chao1 richness in F3 was three times that of F2. Moreover, the vermicompost samples with the highest Pielou's evenness and Shannon−Weiner variety were F3. The vermicompost samples in cattle manure had more ASVs compared to the mixed feedstock (F2 and F3). This can be supported by the fact that cattle manure has been cited as the greatest substrate for vermicomposting (Warman and AngLopez, 2010; Blouin et al., 2019). Additionally, several of the ASVs were exclusive to a single sample. These unique ASVs were most abundant in the cattle manure substrate. This substrate also showed a high relative abundance of *Bacteroidetes*, *Epsilonbacteraeota and Morganella*, while the genera *Advenella, Butyricicoccus, and Haloimpatiens* were unique to only F3.

The richness and diversity of microbial communities changed differently in a substrate with a 60:40 ratio of maize stover to cow dung than they did in a substrate with 100% maize stover (Chen et al., 2022). The starting materials of maize stover/cow dung had values for Chao1 and ACE that were almost identical to those of maize stover. Yet, compared to maize stover alone, the precomposting stage led to a smaller percentage rise in maize stover/cow dung. Shannon indices in maize stover/cow dung were often higher than those in 100% of maize stover on the same sample day and with the same earthworm density. It demonstrated that the substrate's composition affected the microbial community's richness and diversity during vermicomposting. Using different ratios of maize stover to cow dung in the substrates and the density of earthworms (the number of earthworms per kg of the substrates) showed to affect the microbial community of the vermicompost. The same study has highlighted that in the initial materials of maize stover/cow dung, *Cellulomonas* (92%) and *Cellulosimicrobium* (5%) were the two most common genera. The most prevalent microbial taxa in the 100% maize stover samples were *Janthinobacterium*, *Paraphaeosphaeria*, *Cellulomonas*, *Rhizobacter*, and *Streptomyces*, with relative abundances of 5%, 15%, 17%, 23%, and 27%, respectively (Chen et al., 2022). The microbial composition did not significantly alter throughout the precomposting. It differed from maize stover-based vermicomposting, where the relative abundance of *Cellulosimicrobium and Cellulomonas* rose considerably during the precomposting stage. This can be explained by the function of both bacterial groups related to plant biomass degradation due to lignocellulosic and cellulolytic properties (Kim et al., 2020).

The material used and the vermicompost age are two factors that play a crucial role in driving microbial communities' composition and activity during the process (García-Sánchez et al., 2017). The effect of *E. andrei* on basal respiration and microbial biomass carbon utilized as proxies of microbial activity, varied with the type of marc, and this impact was time-dependent. As a result, there were no discernible variations in microbial biomass carbon with earthworm presence in the reactors fed with raw marc. However, lower microbial biomass carbon levels were recorded later in reactors supplied with raw and distilled marc from a different grape variety. A decrease in basal respiration was recorded during the active phase of vermicomposting due to earthworm activity. This decrease was more significant for the raw marcs (two to three times lower than the control) than for the distilled marc (1.5 times lower). *E. andrei*'s impact on the number of bacteria and fungi differed according to the type of marc and sampling time. Despite the constant impacts on overall microbial activity and keeping with the tendency in microbial carbon biomass, *E. andrei* did not significantly influence the bacterial or the fungal abundances in the reactors supplied with Albariño raw marc variety during the vermicomposting. The contradiction is that the abundance of bacteria and fungi in the Albariño raw marc did not decrease during the vermicomposting period, despite a long-term decrease in microbial activity. It was explained by the persistence of the extracellular DNA fractions in the environment after cell death due to physical protection against enzymatic denaturation (Nagler et al., 2018).

7. Physicochemical properties affecting microbial changes during vermicomposting

Despite the fact that vermicomposting is a common practice, very few researchers were interested in analyzing the physical and chemical changes that the substrate goes through throughout a prolonged period of vermicomposting and how they affect microbial changes. Most earlier investigations evaluated alterations in nutrient availability that took place within a brief vermicomposting period. Nevertheless, understanding how the qualities of the substrate, earthworm biomass and microbial population evolve over a prolonged time of vermicomposting can help assess the efficiency of this process. Ultimately, the variation in vermicomposting time determines the quality of the vermicompost. Obviously, the microbial community composition is closely related to the organic waste properties. However, standards to control the variety of organic waste vermicompost have not been made available until now. Moreover, as the microbial composition is influenced by the physicochemical properties of the vermicompost, the initial feedstock can have a dissimilar decomposition pathway, thus altering the final maturity degree of the waste product (Huang et al., 2022).

Vermicompost has the potential to enhance the soil's physical properties, such as porosity, aeration, drainage, corrosion resistance, and infiltration, making it a better environment for root development. It was found that using vermicompost enhanced the soil's physical qualities, boosting the bean's growth, yield, and quality. According to earlier investigations, vermicompost contained a high concentration of polysaccharides. Indeed, to build and maintain the soil structure for improved aeration, water retention, drainage, and aerobic conditions, polysaccharides work as a cementing agent in the soil, providing aggregate stability. Maintaining soil structure is essential for plant nutrient availability and root development. The soil's aggregate stability is increased by the mucus production from the earthworm's stomach and the microorganisms that live there. In addition, the organic matter in vermicomposts, which is absorbent, increases the soil's ability to hold water. The increased microbial population and activity, which led to the development of aggregates and a rise in soil porosity, is mostly responsible for the decreased particle and bulk densities. Moreover, vermicompost has been shown to have a bigger rise in oxidation potential and base exchange capacity (Lim et al., 2015).

The chemical factors can affect and be affected by the vermicomposting process, with C, N, and P being especially important for composting optimization (Brown and Smith, 1998; Bernal et al., 2009). The vermicomposting decreases the C:N ratio, showing that rates of organic matter mineralization and breakdown are faster. During the first phase of vermicomposting, the ash and total nitrogen levels significantly rise, reflecting the earthworm's fast mineralization of nitrogen and breakdown of carbon compounds. CO_2 evolution decreases rapidly by 44% at the precomposting phase and continues at a lower rate throughout the process, indicating increasing organic matter stability. During the breakdown of organic materials, earthworms can speed up the conversion of ammonium-nitrogen into nitrate and improve nitrogen mineralization (Atiyeh et al., 2000). Similar statements were made by Hand et al. (1988), who discovered that *Eisenia fetida* in cow slurry boosted the substrate's nitrate-nitrogen concentration.

As previously stated, microorganisms are what fuel the vermicomposting process. These require more carbon than nitrogen, as nitrogen is solely used to construct cell components, while carbon is required as an energy source and for cell growth. Yet, if there is an excessive amount of carbon in comparison to nitrogen available during vermicomposting, decomposition is slowed down since there will be less N, which is needed for protein synthesis, leading to the progressive death of the microorganisms (Tuomela et al., 2000). Therefore, the microbial activity, abundance, and structure in the vermicompost critically depend on the proportion of different chemical elements and changes relatively. It is crucial that carbon and nitrogen are supplied in the right proportions for the healthy nourishment of both earthworms and microorganisms (Mupambwa and Mnkeni, 2018).

A correlation analysis between environmental parameters and active microorganisms was employed in the Yang et al. (2021) study. The environment's pH and ammonia nitrogen levels are strongly correlated with active microorganisms. Eukaryotes and dissolved total nitrogen had a strong correlation. Dissolved organic matter (DOM), electrical conductivity (EC), and nitrate nitrogen were all substantially associated with active bacteria and eukaryotes. Environmental variables and active bacteria were correlated in the following order: pH $>$ DOC $>$ nitrate $>$ EC $>$ ammonium $>$ dissolved total nitrogen. Environmental variables and active eukaryotes were correlated in the following order: DOC $>$ EC $>$ nitrate $>$ pH $>$ dissolved total nitrogen $>$ ammonium. But according to a prior study, pH has the greatest impact on both bacteria and fungi (Cai et al., 2020). As a result, environmental conditions have a significant impact on the population structure of active bacteria and eukaryotes in the vermicomposting system. Compared to the nitrogen supply, the microbial carbon source was substantially more linked to practically all bacteria and eukaryotes. In contrast to these results, a previous study claimed that there was only a tenuous connection between environmental conditions and the bacterial community (Xia and Huang, 2021). This is most likely related to the active microbial community or functional microorganisms found. Consequently, earthworms can directly adjust the microbial population in vermicomposting by altering environmental conditions.

It was concluded that the physicochemical factors affect the various bacterial genera differently. For example, *Dokdonella* negatively correlated with dissolved organic carbon and pH in vermicompost. However, *Ralstonia* displayed an inverse correlation. In contrast to dissolved organic carbon, *Glutamicibacter* was positively correlated with nitrate, total nitrogen, and EC. *Rhogostoma*, *Arcella*, and *Telotrochidium* for the eukaryotic genus showed favorable associations with pH and dissolved organic carbon. *Cryptomonas, Vertebrata, Haplotaxida, and Epistylis* were strongly positively linked with nitrate, ammonium, and total nitrogen (Yang et al., 2021).

Xiang et al.'s (2022) research conducted redundancy analysis to identify the major environmental variables determining microbial community structures. The dominant microbial order was *Clostridiales* and was related to total phosphorus and dissolved organic matter. The increased dissolved organic matter may lead to an increase in *Clostridiales*, which would then boost the soil's ability to break down xylan and aromatic hydrocarbons. High dissolved organic matter concentrations might facilitate the circulation of elements while promoting microbial development, water retention, and the provision of carbon and energy (Flores-Mireles et al., 2007).

It was reported that the first stage of vermicomposting is characterized by vigorous microbial activity that stimulates the reduction reactions in cattle manure by converting organic matter into CO_2 and subsequently increasing nitrogen mineralization (Ferraz Ramos et al., 2022). Nevertheless, in the last stage, all other variables (EC and P, K, Mg, Cu, and Zn concentrations) were grouped. Due to the mineralization of more easily degradable natural compounds and subsequent decrease in substrate volume, the generation of a large biomass of earthworms only happens in the last stage of vermicomposting when there is also a greater concentration of nutrients (Zziwa et al., 2021). It was proved that the vermicompost generated for 30 days has ideal levels of moisture, pH, conductivity, and nutrient content. The final stage witnessed the most physical-chemical changes as well as the greatest density of earthworms. Furthermore, during the first vermicomposting phase, the earthworms' interaction with the high microbial activity caused a rise in the level of aromaticity of the organic matter (Ferraz Ramos et al., 2022).

8. Conclusion

Although the vermicomposting process has been investigated by numerous researchers, different influencing parameters have yet to be adequately optimized in order to fully control the earthworm-microbe-driven process. Various organic sources were employed during the vermicomposting process, but relatively few researchers took into account the crucial factors that affect composting optimization. Substrate composition, earthworm species, stocking density, physicochemical factors, vermicomposting process type and duration, all those factors can modify microorganisms 'abundance, succession, activity, structure, and function during vermicomposting' (Fig. 2.2).

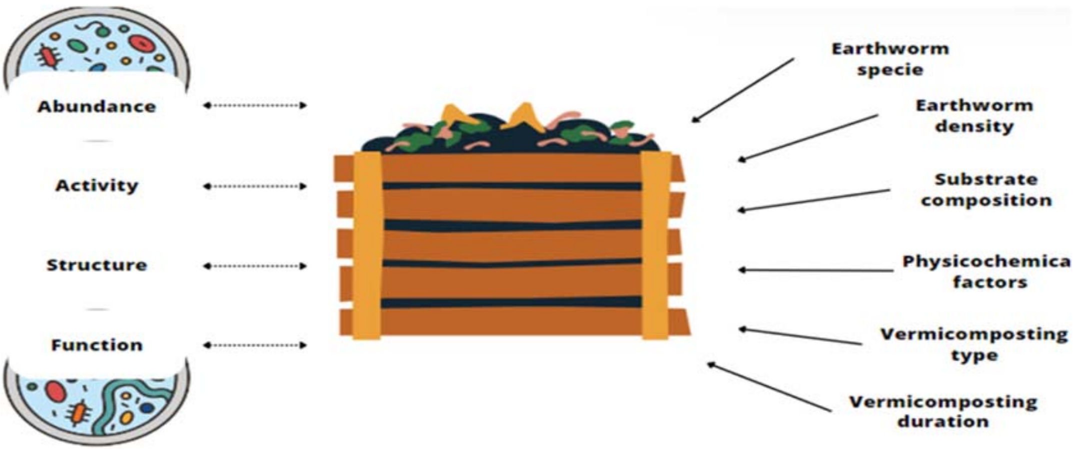

FIGURE 2.2 Vermicomposting process properties affecting microbial community.

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CHAPTER

3

Exploring the transfer and transformation of Polycyclic Aromatic Hydrocarbons in vermifiltration for domestic wastewater treatment

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1. Introduction

Because of rapid urbanization and industrialization as well as agricultural production, the pollution of thousands of anthropogenic organic chemicals have become a challenging environmental problem (Yang et al., 2021). The removal of hazardous organic matter originating from domestic and industrial wastewater has drawn increasing a attention to protect water resources. Polycyclic aromatic hydrocarbons (PAHs) are one of the myriad end-products polymerized by two or more benzene rings. In 1970, the US Environmental Protection Agency (EPA) proposed monitoring a set of 16 PAHs frequently found in environmental samples, which are potential health threats to humans. The sources of PAHs from human activities are various, including but not limited to fuel, cosmetics, disinfectants, pesticides, and refrigerants. These lead to high concentrations of PAHs in municipal wastewater (Stogiannidis and Laane, 2015). Because of their carcinogenic and teratogenic effects, PAHs have come to the forefront and become priority pollutants (Gope et al., 2018), especially for the 16 EPA

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PAHs. Therefore, the fate of the 16 PAHs has attracted extensive concerns because of their safety risk for the environment.

Biodegradation technology is regarded as a green way to remove PAHs from wastewater. In wastewater treatment, PAHs are almost quantitatively relocated into sludge efficiently as a consequence of their strongly hydrophobic properties (Man et al., 2017). However, concentrations of PAHs in residual suspended solids (SS) in treated effluents were sometimes several magnitudes higher than those in excess sludge (Zhang et al., 2019). Their bioaccumulation and biodegradation are also important factors that define the best approach to waste sludge treatment. On the other hand, the accumulation of PAHs in sewage sludge is an issue of major concern, together with potential ecological impacts related to the potential use of these types of waste as soil amendments, for example. As a result, to track the fate of these organic compounds in treatment processes is a topic of current concern.

The removal efficiency of PAHs and potential mechanisms in biological treatment processes vary significantly with factors such as the molecular weight of PAHs. In general, a decrease in low molecular weight (LMW) PAHs (33%−100%) was higher than that in high molecular weight (HMW) PAHs (18%−60%) (Ozaki et al., 2015; Liu et al., 2017; Zhang et al., 2019). The removal of LMW PAHs possibly results from biodegradation and volatilization whereas HMW PAHs are usually removed by adsorption. PAHs may undergo physical transportation and chemical conversion, but microbiological degradation remains the major degradation pathway.

Previous studies on PAHs in biodegradation technology mainly focused on conventional activated sludge treatment. Vermifiltration (VF) as a novel and promising decentralized wastewater treatment technology has not been well-studied for the removal of persistent compounds. However, it has been reported that earthworms can be a driver to accelerate the removal of contaminants. For example, earthworms were used as an effective means to remove PAHs in soil remediation and waste solid management (Rodriguez-Campos et al., 2014). As a more eco-friendly and competitive technology, VF advances organic matter decomposition and nutrients (N and P) removal and greatly reduces SS compared with conventional biofilter (BF) (Xu et al., 2013; Jiang et al., 2016). However, the removal of PAHs as target nonconventional pollutants in wastewater has not been investigated in VF.

Hence, this study evaluated the occurrence of PAHs, especially the 16 EPA PAHs, in the VF with a conventional BF (no earthworms) as a control during domestic wastewater treatment. First, the magnitude was evaluated by focusing on the total contents and solid−liquid partitioning of the 16 EPA PAHs in effluents and excess sludge. Then, the removal rates of different molecular weight PAHs were compared. Also, the bioavailability of PAHs in excess sludge is discussed using sequential solvent extraction methods.

2. Materials and methods

2.1 Experimental setup and operation

To account for the role of earthworms affecting the fate of PAHs, two sets of filters were set up with naturally ventilation. One set was a VF (Fig. 3.1) with an initial adult earthworm density of 32 g L^{-1} (fresh weight basis, with well-developed clitella), as suggested by a previous study (Zhao et al., 2010). Another parallel experiment without earthworms was set up as a control, designed as a conventional BF. Each filter (two stages of each reactor, diameter of

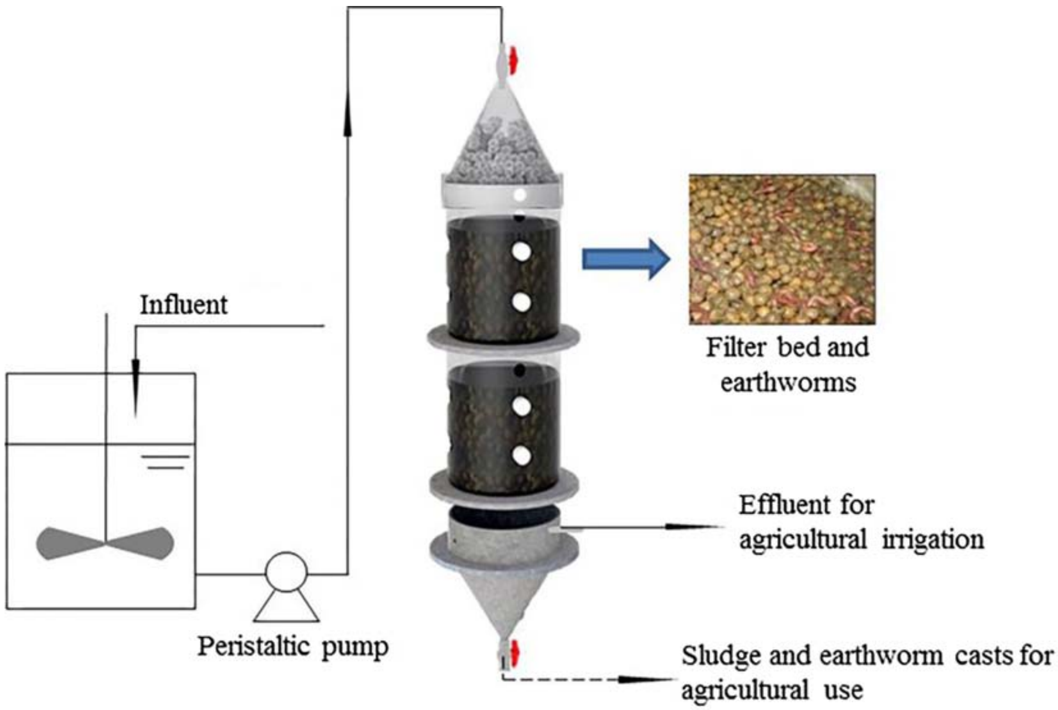

FIGURE 3.1 Schematic diagram of small-scale reactor (vermifiltration, with earthworms).

20 cm and depth of 30 cm of each stage, made of Perspex) had a working volume of 18.8 L and was packed with ceramsites (diameter: 6–9 mm; composition: SiO_2, Al_2O_3, and Fe_2O_3; packing density: 1.03×10^3 kg m^{-3}; and specific surface: 4.51×10^{-3} m^2 g^{-1}). A water distributor was placed on the top of the filter bed to avoid direct hydraulic adverse effects on the earthworms and ensured even water distribution. The influent was obtained from the aerated grit chamber of the Quyang municipal wastewater treatment plant in Shanghai, China. The hydraulic loadings of the two sets of filters were kept at 2 m^3 m^{-2} d^{-1}, and the organic loading of inflow was approximately 1.0 kg COD m^{-3} d^{-1}. Table 3.1 compares the physicochemical parameters of influent water and typical rural domestic sewage.

2.2 Chemical analysis and sludge yield coefficient calculation

The influent and effluent samples were collected and stored at 4°C for less than 24 h before analysis. Standard methods were adopted to measure chemical oxygen demand (COD), biological oxygen demand (BOD_5), total nitrogen (TN), ammonia nitrogen (NH_3—N), total phosphorus (TP), and SS. The sludge yield coefficient Y_{obs} was calculated from Eq. (3.1):

$$Y_{obs} = \frac{\Sigma SS}{\Delta BOD_5} \tag{3.1}$$

TABLE 3.1 Comparison of physicochemical parameters between influent water used during experimental period and typical rural domestic sewage.

Physicochemical parameters	Chemical oxygen $demand_{Cr}$ ($mg\ L^{-1}$)	Biological oxygen $demand_5$ ($mg\ L^{-1}$)	Suspended solids ($mg\ L^{-1}$)	Total nitrogen ($mg\ L^{-1}$)	Total phosphorus ($mg\ L^{-1}$)	NH_3–N ($mg\ L^{-1}$)
Typical rural domestic sewage	300	150	150	40	7	30
Influent water	240–320	120–200	130–160	25–40	4.5–7.5	20–30

where ΣSS represents the increased SS contents (g) during each calculating period; ΔBOD_5 represents the amount of removal BOD_5 (g) during each calculating period, and Y_{obs} represents the observed biosolids during each calculating period.

2.3 Sample pretreatment and extraction

A PAH standard solution mix, which contained 16 PAHs including naphthalene (Np), acenaphthylene (Acy), acenaphthene (Ace), fluorene (F), phenanthrene (Ph), anthracene (An), fluoranthene (Fl), pyrene (Py), benzo[a]anthracene (B[a]An), chrysene (Chry), benzo[b]fluoranthene (B[b]Fl), benzo[k]fluoranthene (B[k]Fl), benzo[a]pyrene (B[a]Py), indeno[1,2,3-cd]pyrene (I[1,2,3-cd]Py), dibenzo[a,h]anthracene (dB[a,h]An), and benzo[g,h,i]perylene (B[g,h,i]Pe), was used as a standard sample for the quantitative analysis. Sample pretreatment and extraction were as in Tian et al. (Tian et al., 2012). The influent and effluent water samples were conducted in May, Jun., Sep., Nov., and Dec. At each sampling, wastewater and sludge were collected over five consecutive days. The collected zwastewater samples, with acidification by HCl, were stored at 4°C in amber glass bottles and then extracted within 48 h for PAH extraction. The excess sludge precipitated under the collecting basins was centrifuged (8000 rpm) for 15 min at 4°C. Solid samples were freeze-dried, passed through a 0.15-mm mesh sieve, and stored at −20°C for further analysis.

To account for particular attention to the solid–liquid partitioning, water samples were filtrated using 0.45 μm filters. Solid-phase extraction with C18 cartridge was applied to separate PAHs from the water phase. After that, the elution was transferred into cylinder flasks, rotary-evaporated into a small volume, and then metered to 1 mL. Solid samples were extracted by accelerated solvent extraction with dichloromethane (DCM)-acetone. The extracts were concentrated in a rotary evaporator to 1 mL, redissolved in 10 mL n-hexane, and further concentrated to 1 mL. Finally, the extracts of solid samples were cleaned on silica gel.

During each sampling period, about 310 L sewage was treated, and the excess sludge was collected. After centrifugation and freeze-drying, the collected sludge was used to determine the PAH content. Liquid centrifuged from the collected excess sludge was not used to determine the content of PAHs, because its value could be neglected compared with the treatment of water (less than 0.1 L). The determination methods of PAHs in the sludge samples were the same as introduced earlier.

2.4 Sequential solvent extraction of polycyclic aromatic hydrocarbons

The pretreated solid samples were mixed with an extracting solution (methanol/water, 1:1 by vol) as a ratio of 1:3 (w:v) in 50-mL centrifuge tubes. The tubes were shaken for 24 h on a rotary shaker at 200 rpm at 21°C and then centrifuged at 8000 rpm for 20 min. The supernatant was carefully removed for PAH determination passing through C18 extraction cartridges. The solid-phase extraction cartridges were conditioned with 10 mL of DCM, followed by 10 mL of methanol. The PAHs were eluted from the sorbent with 10 mL of DCM. The eluted solution was concentrated in a rotary evaporator, and then the solvent was exchanged into n-hexane.

The centrifuged solid samples from this were loosened using a clean stainless-steel spatula and isopyknic n-butanol was added. The tubes were shaken for 24 h on a tumble shaker and then centrifuged at 8000 rpm for 20 min. The supernatant was carefully removed, cleaned, and determined with gas chromatograph/mass spectrometry (GC/MS). Solids remaining in the centrifuge tubes were ultrasonically extracted for 0.5 h in an ultrasonic bath with DCM twice. The extracting solution was cleaned and determined with GC/MS.

2.5 GC/MS analysis

The extracting solutions of water and solid samples were determined by GC/MS. GC/MS analyses were performed with a Shimadzu QP2010 Series system coupled with mass spectrometry. An HP-5 MS column (30 m long, 0.25 mm internal diameter, 0.25 μm film thickness) was used. The column temperature was increased from 90°C to 180°C at 25°C min^{-1} and held for 5 min at 180°C, and then further increased at 5°C min^{-1} until 280°C and held for 11 min. The inlet and transfer line temperatures were 250°C and 280°C, respectively. Data acquisition and analysis were performed in selected ion monitoring mode. Each PAH was quantified using a five-point calibration of mixed standard solutions at 1–2000 μg L^{-1}, and correlation coefficients were higher than 0.995 for all PAHs.

2.6 FT-IR spectrum analysis

The samples were freeze-dried, ground, and sieved through a 0.15-mm mesh. One milligram of subsamples was mixed thoroughly with 100 mg IR transparent KBr (FT-IR grade) and molded into pellets under high pressure (10 t cm^{-2} for 1 min). Then, thin and well-prepared polymer films were put into the sample chamber and scanned at 4000–400 cm^{-1} using a NICOLET 5700 FT-IR. The bands in an excess sludge spectrum can correlate with a defined structure (Table S.1) (Habchi et al., 2022). Mean relative absorbance as a percentage of the sum of all selected peak heights of FT-IR spectra was calculated based on Eq. (3.2):

$$rA_i = A_i / (A_{2920} + A_{2850} + A_{1650} + A_{1540} + A_{1454} + A_{1240} + A_{1052}) \times 100\% \quad (3.2)$$

where rA_i denotes the relative absorption intensity and A_i denotes the absorption intensity of the baseline correction.

TABLE S.1 Positions of characteristic bands and categories of organic matter.

Wavenumber (cm^{-1})	Band assignment	Region
2920	CH_2 asymmetric stretch	Fatty acid region
2850	CH_2 symmetric stretch	
1652–1648	Amide I, (CO) different conformation	Protein region
1550–1548	Amide II, N–H, C–N and structure of proteins	
1460–1454	C–H bend from CH_2	
1240	P=O from phosphate, C–O–P	Mixed region
1052	C–Os, C–O–C from polysaccharides	Polysaccharide region

2.7 Three-dimensional fluorescence analyses for water-extractable organic matter

Fifty-milligram freeze-dried samples were mixed with 50 mL sterile water and shaken for 15 min in an end-over-end shaker. Water-extractable organic matter was made by centrifugation at 10,000 rpm and filtration through a 0.45-μm pore polycarbonate filter (Federici et al., 2017). The extracted water was diluted using 0.1 mol L^{-1} phosphate buffered saline (PBS) until the dissolved organic carbon content was less than 10 mg L^{-1}. Fluorescence spectra were acquired by an F-4600 fluorescence spectrophotometer (Hitachi, Japan). Both the excitation and emission wavelengths were 250–600 nm.

2.8 Data analysis

All statistical analyses were performed with SPSS software (version 17.0, (SPSS, Inc., United States). Significant differences between samples were analyzed using analysis of variance.

3. Results and discussion

3.1 Determination of 16 EPAs originating in sewage

All 16 EPA PAHs except for B[g,h,i]Pe were detected in the influent, as discriminated by different ring numbers (Fig. S.1). The average concentrations of 16 EPA PAHs in the influent are listed in Tables 3.2 and 3.3. The PAHs with three and four rings accounted for 41.83% and 37.49%, respectively, among which Ph, An, and Fl were the three dominant species that accounted for 50.36% of the total PAHs in influent. The average concentration was higher in the winter months of Nov. and Dec., especially in Dec., when the temperature was below 15°C. The correlation coefficients shown in Table S.2 suggest that the increase in PAH concentrations in domestic wastewater was directly related to the temperature and TN, but not SS.

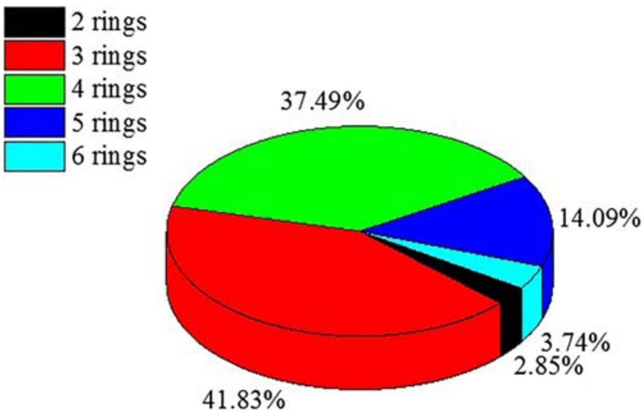

FIGURE S.1 Polycyclic aromatic hydrocarbon composition according to different ring numbers.

TABLE 3.2 Average concentrations of 16 PAHs and other pollutants in influent during experimental period.

	Temperature	PAHs	NH_3–N	Chemical oxygen demand	Total nitrogen	Total phosphorus	Suspended solids
	°C	μg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
May	21.8	15.74	21.80	293.00	26.96	18.11	4.63
Jun.	22.9	31.03	22.90	272.50	29.50	19.97	4.27
Sep.	24.4	35.84	24.40	310.75	29.95	16.62	4.68
Nov.	15.5	67.65	15.50	285.60	36.00	28.43	4.01
Dec.	12.6	119.39	12.60	295.00	38.98	27.28	4.29

Isomer ratios could be used to deduce the sources of PAHs in samples of environmental systems (Yang et al., 2017). Four isomer ratios were calculated for this study (Fig. 3.2); 178 and 202 g mol^{-1} of PAH molecular mass were commonly used to distinguish between combustion and petroleum sources (Budzinski et al., 1997; Bahrami et al., 2021). In this part, the average ratio of An to An plus Ph (An/An + Ph) was 0.41. According to Yang et al. (2017), An/(An + Ph) was higher for the combustion source (>0.1) and lower for the petroleum source (<0.1). Fl/(Fl + Py) indicated petroleum sources below 0.4, petroleum combustion sources of 0.4–0.5, and biomass or coal combustion sources above 0.5. Accordingly, PAHs in the municipal wastewater obtained in this study were generated from burned diesel oil, shale oil, coal, and some crude oil.

TABLE 3.3 Influent concentrations of 16 PAHs recommended by US Environmental Protection Agency.

Parameter ($\mu g\ L^{-1}$)	May	Jun.	Sep.	Nov.	Dec.	Average ± standard deviation ($\mu g\ L^{-1}$)
Naphthalene	1.40	2.37	1.44	1.37	1.11	1.54 ± 0.43
Acenaphthylene	0.24	0.16	0.04	0.15	1.74	0.47 ± 0.64
Acenaphthene	0.08	0.18	0.09	1.17	0.45	0.39 ± 0.41
Fluorene	0.87	0.97	1.49	2.16	3.94	1.89 ± 1.12
Phenanthrene	2.70	3.64	6.77	8.83	28.69	10.13 ± 9.54
Anthracene	2.86	0.24	7.42	7.40	30.53	9.69 ± 10.78
Fluoranthene	1.64	3.56	5.48	7.45	18.58	7.34 ± 5.94
Pyrene	0.03	2.54	0.52	6.74	10.72	4.11 ± 4.06
Benzo[a]anthracene	1.46	3.40	2.72	7.04	6.28	4.18 ± 2.13
Chrysene	1.38	3.12	2.72	9.29	6.42	4.59 ± 2.88
Benzo(b)fluoranthene	1.29	2.69	2.49	4.09	4.84	3.08 ± 1.25
Benzo(k)fluoranthene	0.37	2.28	1.78	3.58	3.73	2.35 ± 1.24
Benzo(a)pyrene	0.65	3.06	1.76	4.78	0.60	2.17 ± 1.58
Indeno(1,2,3-cd) pyrene	0.33	1.34	0.25	2.09	0.05	0.81 ± 0.78
Dibenz(a,h) anthracene	0.44	1.23	0.58	1.54	1.69	1.10 ± 0.50
Benzo(g,h,i)perylene	nd	0.26	0.30	nd	nd	0.11 ± 0.14

TABLE S.2 Correlation coefficients (R-values) of polycyclic aromatic hydrocarbons (PAHs) and other physicochemical parameters in influent.

	PAHs	Temperature	COD	TN	NH_3-N	TP	SS
PAHs	1						
Temperature	−0.904	1					
COD	0.077	0.114	1				
TN	0.969	−0.921	0.005	1			
NH_3-N	0.814	−0.942	−0.298	0.905	1		
TP	−0.504	0.659	0.656	−0.658	−0.862	1	
SS	0.664	−0.420	0.520	0.708	0.428	−0.209	1

COD, chemical oxygen demand; *SS*, suspended solids; *TN*, total nitrogen; *TP*, total phosphorus.

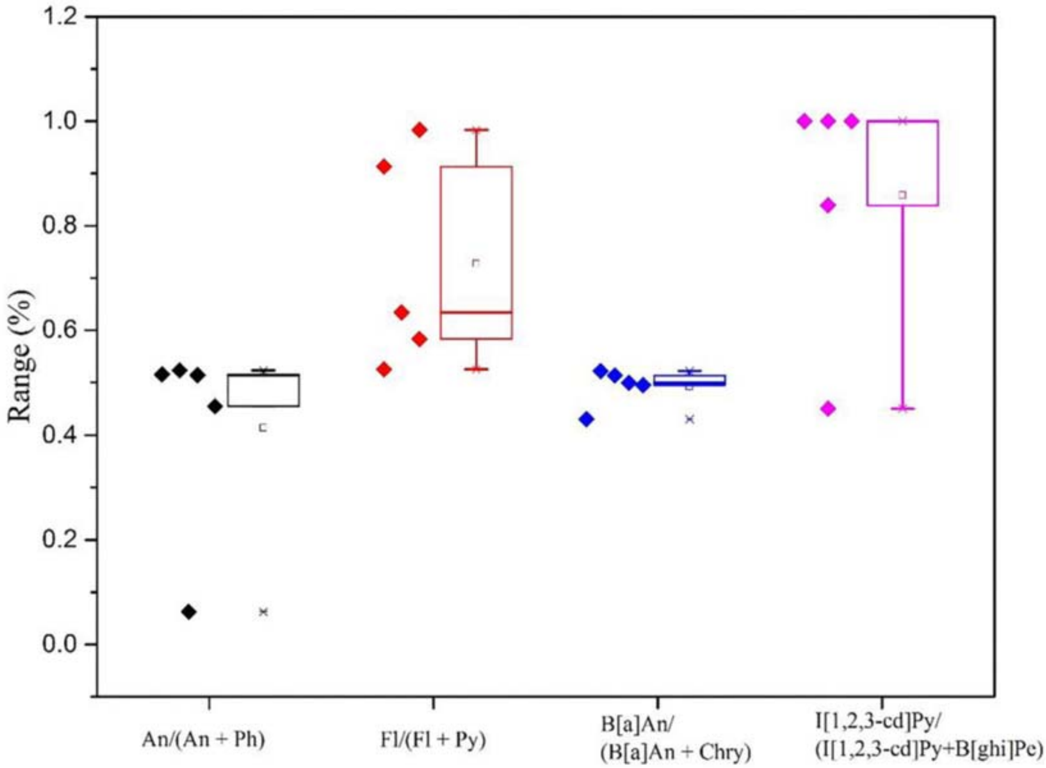

FIGURE 3.2 Box-and-whisker plots of isomer ratio in influent. *An*, anthracene; *B[a]An*, benzo[a]anthracene; *B[ghi] Pe*, benzo[g,h,i]perylene; *Chry*, chrysene; *Fl*, fluoranthene; *I[1,2,3-cd]*, indeno[1,2,3-cd]pyrene; *Ph*, phenanthrene; *Py*, pyrene.

3.2 Total removal performance of 16 PAHs by vermifiltration

Table 3.4 lists the average concentrations of the 16 PAHs after the VF and BF treatment. The VF had a higher PAH removal efficiency with the synergy of earthworms, as shown in Eq. (3.3):

$$\frac{\mathrm{Inf}(P+D)-\mathrm{Eff}(P+D)}{\mathrm{Inf}\ (P+D)}=\begin{cases}67.06\%\ (\mathrm{VF})\\63.71\%\ (\mathrm{BF})\end{cases} \tag{3.3}$$

where Inf = influent; Eff = effluent; P = particulate phase; and D = dissolved phase.

The VF had an average removal efficiency of 67%, which was slightly higher than that in the BF system (63%). Compared with other biological treatment techniques in previous studies (more than 85%), the removal efficiencies were slightly lower owing to the relative short hydraulic retention time for BF systems (Mozo et al., 2011; Qiao et al., 2014; Singh et al., 2015).

TABLE 3.4 Concentration of 16 PAHs in each separated part.

			Influent	Effluent	
				Vermifiltration	Biofilter
16 PAHs	Dissolved (D)	$\mu g\ L^{-1}$	6.59 ± 4.09	3.00 ± 2.05	2.04 ± 1.19
	Particulate (P)	$\mu g\ L^{-1}$	48.70 ± 32.68	14.83 ± 10.00	17.61 ± 12.19
	Particulate	$\mu g\ kg^{-1}$	293.11 ± 184.82	1148.03 ± 495.73	1448.18 ± 638.46
	Excess sludge	$\mu g\ kg^{-1}$	–	50.12 ± 20.79	67.51 ± 28.74
	D + P	$\mu g\ L^{-1}$	54.13 ± 36.91	17.83 ± 12.03	19.64 ± 13.37
Suspended solids (SS)		$mg\ L^{-1}$	153.3 ± 17.9	11.45 ± 3.47	12.3 ± 3.70

Considering the PAH concentrations in excess sludge and the sludge yield coefficient (Eq. 3.1), the removal efficiencies of 16 PAHs of the VF and BF systems were calculated by Eq. (3.4):

$$\frac{\mathrm{Inf}(P+D)-\mathrm{Eff}(P+D)-\mathrm{ES}}{\mathrm{Inf}\ (P+D)}=\begin{cases}64.03\%\ (\mathrm{VF})\\ 56.89\%\ (\mathrm{BF})\end{cases} \tag{3.4}$$

where ES represents the PAH concentrations in excess sludge, which were calculated according to the sludge yield coefficient in Eq. (3.1) and the concentration of the unit mass in excess sludge during one calculating period.

Obviously, 64% of the total PAHs was removed biologically or vaporized in the VF, which was approximately 7% higher than that in the BF. Earthworms in the VF system grazed directly on and digested SS intercepted by bedding materials and biofilms. Earthworms can effectively facilitate decomposition by ingestion, grinding, dispersion, and digestion (Blouin et al., 2013). These activities influence the distribution balance of PAHs between the liquid and solid phases in the treatment system. Earthworms also have relatively high body-lipid content, so they accumulate PAHs through passive diffusion through the epidermis from the aqueous phase and diffuse PAHs across gastrointestinal tissues (Ma et al., 2012). Moreover, the application of earthworms affects the microbial community in biofilms and extends the food web of the VF, resulting in an increase in protozoa (Xing et al., 2014).

3.3 Transferring of polycyclic aromatic hydrocarbons during vermifiltration treatment

Because of higher hydrophobicity, poorer water solubility, and lower volatility properties than smaller compounds such as short chain hydrocarbons, many studies reported that PAHs were adsorbed onto solid organic matter (Brunk et al., 1997; Ruby et al., 2016; Ukalska-Jaruga et al., 2019). However, the distribution ratio between the dissolved phase and particulate phase in VF and BF influent and effluent was different:

$$\frac{D}{D+P+ES}=\begin{cases}12.22\%(\text{Inf})\\15.46\%(\text{Eff, VF})\\8.78\%(\text{Eff, BF})\end{cases} \tag{3.5}$$

Sorption onto particulate matter is a dominant phase transfer process affecting the movement and fate of hydrophobic pollutants in the aquatic environment. The effect of dissolved organic matter (especially amphoteric substances containing both hydrophilic and hydrophobic groups) in the aqueous phase can significantly affect the partition coefficient for PAHs in heterogeneous systems (Brunk et al., 1997). Also, the particle size, pore size, content of organic matter, and species of organic matter influence the sorption of PAHs (Ukalska-Jaruga et al., 2019).

In general, PAHs contained in the dissolved phase accounted for small proportions of the total concentrations. Compared with other studies, the obtained results were on the same order of magnitude (Ozaki et al., 2015). In this study, the dissolved phase PAHs in the BF effluent was 8.78%, lower than that in the influent (12.22%). However, the VF effluent had a high result (15.46%). Earthworms in the VF were able to transform insoluble organic materials to a soluble form and then selectively digest suspended particles of 10−200 μm to finer particles of 0−2 μm (Zhao et al., 2010), which led to a large specific surface area for adsorption. Moreover, the presence of earthworms can significantly improve the degree of humification (Yang et al., 2013) and thus increase aqueous solubility. With the role of earthworms, the polysaccharides and humic acid (HA) contents in the aqueous phase increased (data not shown). It was likely that the HA in aqueous phase resulted in the increase of PAHs in dissolved parts.

Another interesting result was that concentrations of PAHs adsorbed in the particulate phase in the effluent (Table 3.4) were more than 20 times higher than those in excess sludge in both the VF and BF systems. Density was the essential factor in separating particulate in effluent and excess sludge, so the organic matter compositions of the light and heavy fractions were entirely different.

3.4 Insights into polycyclic aromatic hydrocarbon removal based on molecular weight

Fig. 3.3 shows the distribution of the 16 PAHs, as well as the sum of LMH (two to three rings), medium molecular weight (MMW) (four rings), and HMW (five to six rings) species (LMW: sum of Np, Acy, Ace, F, Ph, and An; MMW: Fl, Py, B[a]An, and Chry; HMW: B[b]Fl, B[k]Fl, B[a]Py, I[1,2,3-cd]Py, dB[a,h]An and B[g,h,i]Pe). The transference of residuals was fairly different, especially under the actions of earthworms, which can significantly change the organic matter composition and secrete mucus. The particle phase of LMW, MMW, and HMW PAHs to the total BF effluent was similar. Conversely, owing to the interference of earthworms, the proportions of the solid−liquid allocation of different molecular

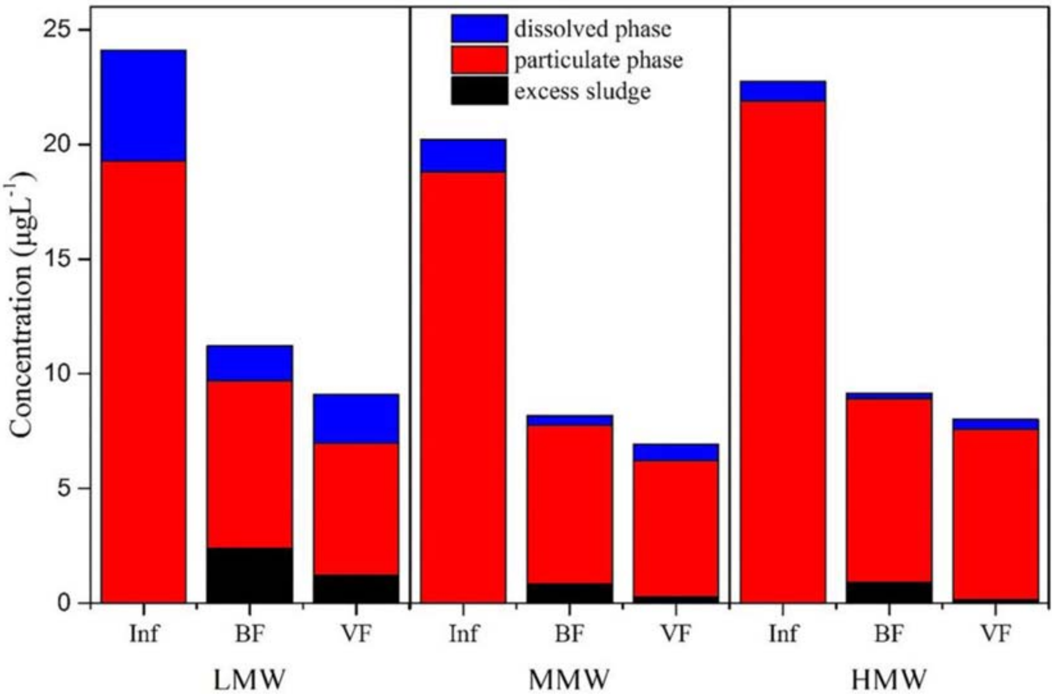

FIGURE 3.3 Sum of concentrations of low molecular weight (LMW), middle molecular weight (MMW), and high molecular weight (HMW) polycyclic aromatic hydrocarbons in influent water and vermifiltration (VF) and biofilter (BF) effluent water.

weights were markedly different. These findings suggested that most lower molecular species were biologically or chemically transformed, or vaporized, whereas most higher molecular species were absorbed in excess sludge:

$$\frac{\mathrm{Eff(P)+ES}}{\mathrm{Eff(P+D)+ES}} = \begin{cases} \mathrm{LMW}: 90.59\%\ (\mathrm{BF});\ 80.28\%\ (\mathrm{VF}) \\ \mathrm{MMW}: 92.95\%\ (\mathrm{BF});\ 94.94\%\ (\mathrm{VF}) \\ \mathrm{HMW}: 89.90\%\ (\mathrm{BF});\ 96.32\%\ (\mathrm{VF}) \end{cases} \tag{3.6}$$

The removal efficiencies of LMW, MMW, and HMW in influent and effluent are shown in Eq. (3.7):

$$\frac{\mathrm{Inf(P+D)-Eff(P+D)-ES}}{\mathrm{Inf(P+D)}} = \begin{cases} \mathrm{LMW}: 56.49\%\ (\mathrm{BF});\ 62.26\%\ (\mathrm{VF}) \\ \mathrm{MMW}: 59.58\%\ (\mathrm{BF});\ 65.75\%\ (\mathrm{VF}) \\ \mathrm{HMW}: 59.72\%\ (\mathrm{BF});\ 64.81\%\ (\mathrm{VF}) \end{cases} \tag{3.7}$$

Earthworm activity may stimulate aerobic PAH biodegradation and reduce PAH adsorption by consuming organic matter and increasing bioavailability (Rajadurai et al., 2022). The abilities of earthworms to change the structure, biomass, and functioning of microbial communities may indirectly stimulate PAH biodegradation, which predominantly depends on microbial activity (Okpashi, 2015; Martinkosky et al., 2017; Sampaio et al., 2019). Therefore, the introduction of earthworms constitutes a potential bioremediation tool for persistent organic contaminants. The influence of earthworm activity on microbial biomass, enzyme activities, fatty acids, and respiration activity in VF systems for wastewater treatment has been investigated (Zhao et al., 2014; Wang et al., 2016).

The removal ratio varied with the molecular weight of PAHs (MMW > LMW > HMW). First, the availability of the contaminant determines its degradability. For example, Np has a solubility of 32 g L^{-1}, so earthworms have a minimal effect on removing LMW PAHs. Microorganisms living in biofilms will degrade these pollutants rapidly with higher availability. Second, the availability of the contaminant is low, but its degradability is high (MMW). In this situation, the application of earthworms will accelerate the removal of the contaminant from municipal wastewater. Third, the degradability of the contaminant is low. Independent of its availability, the application of earthworms will have little impact on removing recalcitrant compounds from soil (Rodriguez-Campos et al., 2014).

3.5 Stabilization of polycyclic aromatic hydrocarbons in waste sludge

Fig. 3.4 depicts the concentration and percentage of three sequentially extracted fractions in excess sludge. Among the three extractable fractions, the dominant one was the DCM extractable fraction, whereas the nonextractable fraction was mainly humin-bound PAHs. VF excess sludge contained a large amount of worm poop (castings), which had the potential for land use directly after dewatering. The sorption of PAHs to sediment was an important process controlling their environmental fate and effects. Adsorbed compounds were less available for partitioning and leaching in groundwater and exhibit reduced bioavailability, toxicity, and genotoxicity. The percentage of nonextractable PAHs in excess VF sludge among the total PAH concentrations was 59.42%, which was higher than 53.75% in excess BF sludge. Sewage sludge has the potential for application to agricultural soil to improve the nutrient status, organic matter content, aggregate properties, and crop production. The sorption behavior of PAHs is an important consideration in determining the effects of applying sewage sludge to agricultural land.

The presence of earthworms can enhance the stability of organic matter into a humus-like product, which is a common way to reclaim organic waste for agricultural use (Schiavon et al., 2019). In particular, binding PAHs by HA can reduce PAH availability to organisms, increasing their stability in the environment and potentially reducing their toxic effect to higher organisms (Sayara and Sánchez, 2020). Similarly, aliphatic moieties in HAs have a significant role in PAH binding and transformation (Han et al., 2017). The fluorescence excitation-emission matrix (EEM) spectra of water-extractable organic matter fractions extracted from BF and VF excess sludge are shown in Fig. 3.5. The peak A region is associated with a typical aromatic protein region such as tryptophan and tyrosine, and peak B is related to soluble microbial by-product–like materials, whereas peaks C and D fall into HA-like and

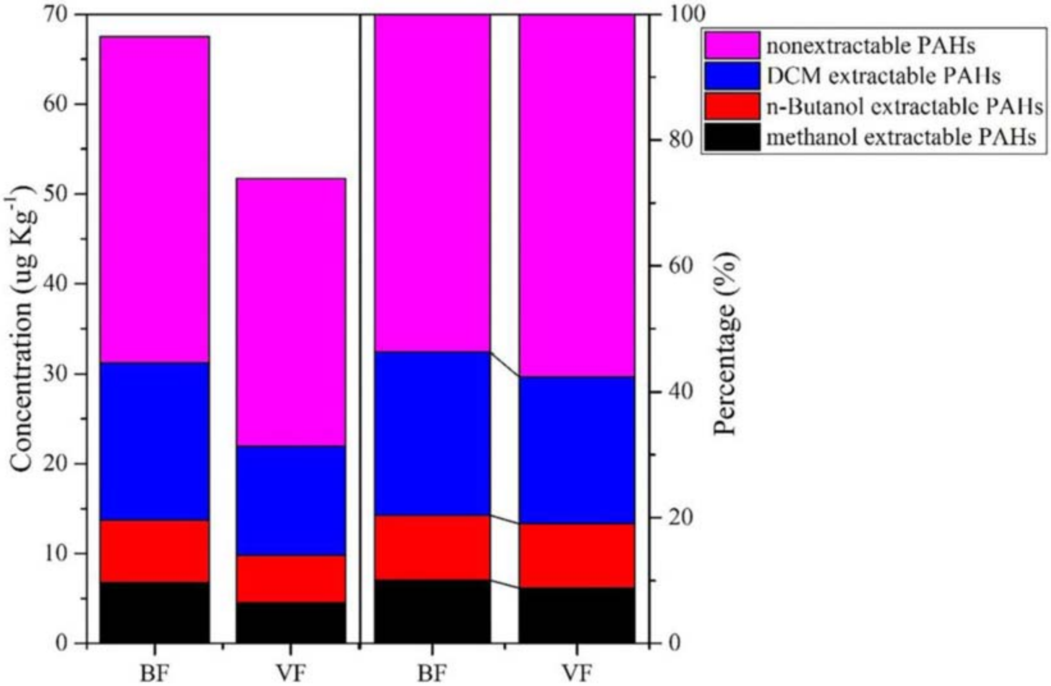

FIGURE 3.4 Total concentration of polycyclic aromatic hydrocarbons (PAHs) based on sequential extraction in the biofilter (BF) and vermifiltration (VF) excess sludge. *DCM*, dichloromethane.

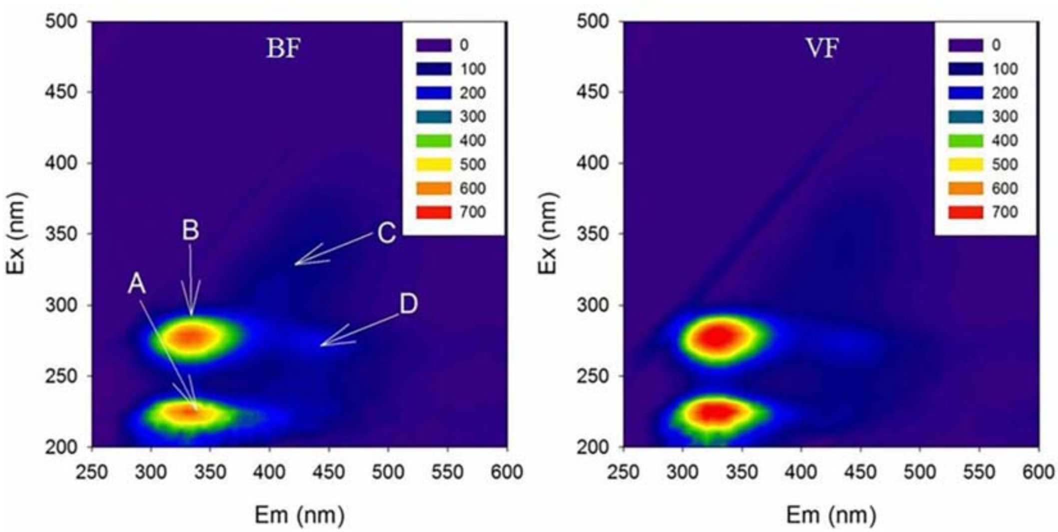

FIGURE 3.5 Fluorescence excitation-emission matrix spectra from biofilter (BF) and vermifiltration (VF) excess sludge.

fulvic acid–like regions, respectively. The fluorescence characteristic of these peaks from BF and VF excess sludge samples are dramatically different (Table S.3). The specific fluorescence intensity of peaks C and D in the VF samples were 105.2 and 190.2, which were much higher than 85.0 and 165.6, respectively, in the BF samples. The infrared spectrum is typically used to identify and characterize organic functional groups. As displayed in Fig. 3.6, the infrared spectrum peaks of these four regions revealed significantly different relative absorption intensities (Table S.4). The polysaccharide region relative absorbance of the VF samle was 24.12% higher than that of the BF sample, which was assigned as C-O and C-O-C streching vibration (Table S.1). According to results from FT-IR and EEM spectra, the addition of earthworms had an influence on the characteristics of excess sludge, which eventually determined the degree of stability and harmlessness for agricultural use.

TABLE S.3 Evolution of E_x/E_m maxima of water extracts from the excess sludge of biofilter (BF) and the vermifiltration (VF).

Sample	Peak A		Peak B		Peak C		Peak D	
	E_x/E_m [a]	SFI [b]	E_x/E_m	SFI	E_x/E_m	SFI	E_x/E_m	SFI
BF	225/325	797.6	280/330	806.9	340/440	85.0	275/430	165.6
VF	225/330	764.3	280/330	783.4	335/435	105.2	275/435	190.2

[a] *E_x/E_m are excitation/emission wavelength pairs.*
[b] *SFI, specific fluorescence intensity.*

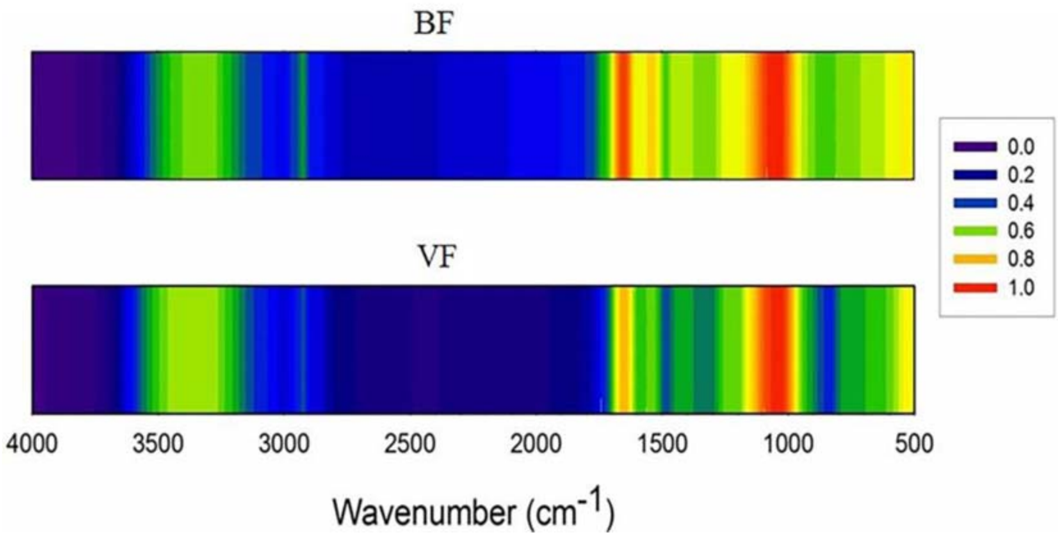

FIGURE 3.6 FT-IR spectra of excess sludge samples from biofilter (BF) and vermifiltration (VF) system.

TABLE S.4 Relative absorbance as percentage of sum of all selected peak heights of FT-IR spectra of biofilter (BF) and vermifiltration (VF) excess sludge.

Relative absorbance rA_i (%)	Fatty acid region		Protein region			Mixed region	Polysaccharide region
Wavenumber (cm^{-1})	2920	2850	1650	1540	1454	1240	1052
BF	9.55	7.21	19.75	15.68	12.91	14.03	20.87
	16.76			**48.34**		**14.03**	**20.87**
VF	9.89	7.21	18.88	14.29	11.63	13.98	24.12
	17.10			**44.80**		**13.98**	**24.12**

4. Conclusions

(I) Under the action of earthworms, VF had a higher removal efficiency for PAHs than did the BF system in wastewater treatment, especially for MMW PAHs. The excellent performance of the removal ratio was mainly attributed to earthworms causing PAH availability and degradability.

(II) Through treatment with the VF system, the distribution of PAHs between liquid and solid portions changed significantly. It was likely that the increased HA content in the aqueous phase resulted in an increase in PAHs in the dissolved parts. Moreover, the concentrations of PAHs that absorbed in the particulate phase in the effluent were more than 20 times higher than those in excess sludge. The ability to transform suspended particles of 10–200 μm to finer particles of 0–2 μm was a major cause of this effect.

(III) Sequentially extracted concentrations of PAHs in excess sludge demonstrated that PAH stabilization improved. The addition of earthworms had an influence on the characteristics of excess sludge from FT-IR and EEM spectra techniques, which eventually determined the degree of stabilization.

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Disclosure statement.
The authors report there are no competing interests to declare.

Data availability statement.
The data that support the findings of this study are available from the corresponding author, Meiyan Xing, upon reasonable request.

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CHAPTER

4

Vermiremediation of organic wastes: vermicompost as a powerful plant growth promoter

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1. Introduction

The widespread use of chemical and inorganic fertilizers in agricultural sectors has prompted concerns about environmental health. The reliance on chemical fertilizer for improved crop growth and production has been rising, and the constant application of synthetic fertilizer in agricultural practices leads to soil pollution and eventually threatens all life forms. Using chemical fertilizers to boost crop growth and productivity may have some advantages, but over the long haul, they are more harmful than beneficial. Consuming these inorganically fertilized crops may not immediately represent a threat to one's health, but over the long term, these "toxic" compounds build up in the important organs of the human body (bioaccumulation) (Quaik et al., 2012; Angmo et al., 2022). To protect humanity from these threats, research into substituting chemicals with various organic soil additives such as manure derived from the farmyard and green waste, compost, or vermicompost (VC) has increased significantly (Makkar et al., 2022). They promote environmental quality by supplying natural nutrients, decreasing the need for artificial ones, fostering biodiversity, boosting biological activity in the soil, and preserving its physical characteristics. Additionally, the population of the globe is steadily growing, massive amounts of food and beverages are

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being processed in industrial settings, and more animals are being raised for their meat and other products, all of which contribute to the generation of vast quantities of organic waste (Vijayabharathi et al., 2015). These wastes are disposed of or treated predominantly with techniques that include landfilling, incineration, and open dumping and create environmental problems such as air pollution, groundwater contamination, and the release of foul smells (Quadar et al., 2022). Therefore, it is of the utmost importance to manage these wastes with an appropriate and sustainable technology that not only alleviates the load of the waste problem but also provides nutrition to the development of plant life. Vermicomposting can efficiently handle waste with minimum economy and labor assistance while providing quality amendments essential for plant growth in the form of VC (Yadav and Garg, 2011; Bhat et al., 2018; Huang et al., 2022). Vermicomposting is a method or procedure that decomposes organic material via the cooperative activity of bacteria and earthworms under ideal conditions (Bo et al., 2021; Devi and Khwairakpam, 2022). Earthworms play a primary role in vermicomposting. Earthworms generate a colloidal substance called mucus in the environment that boosts soil's ability to retain water. Additionally, mucus can preserve moisture by absorbing it (Bhat et al., 2017; Quadar et al., 2022). With their fragmenting characteristics and the addition of casts to the soils around them, earthworms play a crucial role as facilitators of nutrient transport during biogeochemical cycles (Thakur et al., 2021). Earthworm mucus supplies nitrogen to VC, which is important for breaking seed dormancy (Hilhorst and Karssen, 2000) and shortens germination duration. Transforming waste generated from industries into nutrient-rich VC is essential to monitor and manage pollution, as VC holds potential applications in waste reduction as well as remediation (Angmo et al., 2020; Yatoo et al., 2022; Cui et al., 2022) reported that the temperature during vermicomposting is an important factor that significantly determines the types of bacterial communities, antibiotic resistance genes, and their linkage during vermicomposting. The author further stated that 20°C is favorable for vermicomposting to achieve the highest elimination efficiency of the antibiotic resistance genes and to obtain the high biostability of the finished product. Adding VC as an organic amendment regulates the phytopathogens by modulating the surrounding rhizosphere and soil environment (Sarma et al., 2010). VC adversely affects the pathogen life cycle and makes plants more resilient to attack by enhancing vigor and changing root physiology. VC serves as an organic source of nutrients essential to suppressing bacterial populations while promoting plant growth (Huang et al., 2022). This chapter explains the process for vermicomposting from organic waste, the role of VC in plant disease and pest management. Specifically, it emphasizes the role of VC as a plant growth promoter.

2. Vermicompost and its production

Organic matter is oxidized via the collaborative activity of microorganisms and earthworms to prepare VC (Makkar et al., 2022; Dutta et al., 2023). Vermicomposting has two stages—primary and secondary. In the primary phase, earthworms play a key role in degrading organic material by changing its chemical and physical makeup, gradually lowering the carbon and nitrogen ratio, and subsequently increasing the surface area available to microbes to speed up their activity and further decompose organic waste. The secondary phase entails the further decomposition of organic waste, which is mostly governed by microbes. The rate of the degradation process is directly associated with the abundance and species of earthworms, moisture, aeration, and features of organic waste. Additionally, it was determined that the output of soil and crops might be enhanced by the consumption of organic substances by certain epigeic species of earthworms (Dutta et al., 2021; Singh et al., 2020). Depending on their feeding and burrowing habits, about 4400 earthworm species are distinctly categorized into three habitats: epigeic, anecic, and endogeic (Brown, 1995; Bhatnagar and Palta, 1996). Epigeic species have pigmentation, do not dig permanent burrows, subsist on decomposing plant debris and other litter, and are considered surface litter converters (Ahmad et al., 2021). Endogenic species dwell in horizontal burrows at a depth of around 10−15 cm within the soil and consume organic materials, while anecic species that are specifically larger in their structure dwell vertically within soil tunnels to consume decaying organic matter and live beneath the surface. Epigeic species are ideal for vermicomposting because of their strong preferences for organic-based substrates, high rates of intake and digestion, adaptability to environmental changes, brief life cycles, rapid reproduction rates, and ease of cultivation. Many researchers have developed VC employing a few species, including *Eisenia fetida* (Bouché), *Eisenia andrei* (Savigny), and *Perionyx excavates* (Perrier) (Quadar et al., 2022; Sohal et al., 2021; Singh et al., 2022; Wiethan et al., 2018). *E. fetida* is utilized more often than others in much research, including the influence of VC on plant development, size of bacterial community and composition, and soil physical and chemical characteristics Table 4.1. *Perionxy excavates* is an epigeic earthworm found in tropical regions while *E. fetida* and *E. andrei* species can be found more in temperate regions.

2.1 Factors influencing vermicomposting

2.1.1 pH

Earthworms function well at neutral pH and have an optimum pH range of 4.5−9.0. Earthworm sensitivity and the physicochemical properties of organic waste are the main determinants of the best pH (Edwards and Bohlen, 1996). The pH of the vermicomposting process is primarily affected by differences in the physicochemical parameters of waste. The decomposition of organic matter results in intermediates, namely ammonium and humic acids, that affect pH shift depending on its negative and positive charged groups, resulting in neutral or acidic pH. Because various intermediate species are produced by substrate types, the pH of the vermicomposting process is likewise affected. As a result, various wastes exhibit various pH shift behaviors (Suthar, 2009). Additionally, the vermicomposting process reduces total pH from alkaline to acidic (Kaushik and Garg, 2003; Yadav and Garg, 2011).

2.1.2 Moisture

The amount of moisture present in a vermicomposting system affects the earthworm population (Rodríguez-Canché et al., 2010). An ideal moisture range of 50% to 80% is recommended. If the moisture level is low, it delays the earthworm's sexual maturation. Reinecke and Venter (1985) suggest an optimal moisture content of about 70 for *E. fetida*. Tomlin et al. (1980) observed that some earthworm species, like *Lumbricus terrestris*, thrive in arid environments, whereas *E. fetida* thrives at a moisture content of 82%.

TABLE 4.1 Vermicomposting of different waste and duration of the vermicomposting process.

S.No	Organic waste	Amendment used	Type of earthworm species	Vermicomosting duration (days)	References
1	Allopathic pharmaceutical industry sludge	Cow dung	*E. fetida*	180	Singh et al. (2022)
2	Cocounut husk	Cow dung	*E. fetida*	120	Quadar et al. (2022)
3	Thermal fly ash	Cow dung	*E. fetida*	105	Sohal et al. (2021)
4	Banana leaf waste	Cow dung	*E. fetida*	105	Magoet al. (2021)
5	Agricultural waste corn cob	Vegetable waste and eggshell	*E. fetida*	42	Castillo-González et al. (2021)
6	Water hyacinth and paddy straw	Rock phosphate, dolomite and mica, microbial inoculums	*E. fetida*	90	Das et al. (2021)
7	Plant residues (acacia litter, bamboo leaf litter, terrestrial weed/Mikania micrantha)	Cow dung	*Perionyx excavatus*	45	Debnath and Chaudhuri. (2020)
8	Food industry sewage sludge	Biochar (pyrolysis of waste woodchips)	*E. fetida*	60	Wiethan et al. (2018)
9	Sewage sludge	Cow dung and straw	*E. fetida*	21	Belmeskine et al. (2020)
10	Paper mill solid waste	Sawdust and cow dung	*E. fetida*	14	Mohapatra et al. (2019)
11	Palm oil mill effluents	Palm press fiber	*Eudrilus eugeniae*	15	Rupani et al. (2019)
12	Cattle manure	High Trichoderma doses (liquid form	*Eisenia andrei*	60	Wiethan et al. (2018)
13	Dewatered sludge (paper and pulp mill)	Saw dust	*E.fetida*, *Eudrilus eugeniae*, *Perionyx excavatus*	45	Fu et al. (2016)
14	Compostable municipal waste solid	Cow dung	*E.fetida*	56	Suthar et al. (2015)
15	Bagasse waste	Cow dung	*E.fetida*	135	Bhat et al. (2015)
16	Industrial organic waste (sugarcane bagasse, sugarcane pressmud)	Farm manure	*Lumbricus rubellus*	45	Shah et al. (2015)
17	Pressmud	Cow dung	*E.fetida*	120	Bhat et al. (2014)
18	Distillery sludge	Cow dung	*E.fetida*	150	Singh et al. (2014)

2.1.3 C:N ratio

The C:N ratio is essential for earthworm cell production, development, and metabolism. Carbon and nitrogen must be present as substrates in the optimal proportions for healthy feeding (Ndegwa et al., 2000). The C:N ratio is one of the most important waste markers employed in the compost maturity index. When the substrate's commencing C:N ratio is 25, it enhances the maturity of its compost, i.e., expressed by a C:N ratio of less than 20 (Kaushik and Garg, 2003; Tripathi and Bhardwaj, 2004). As a consequence of rapid mineralization and organic content breakdown, carbon is mislaid as carbon dioxide in microbial respiration, while simultaneously, nitrogen gets increased by earthworms in mucus forms, causing drop in the overall C:N ratio. Because nitrogen escapes as volatile ammonia when the pH is high, a drop in pH is another factor contributing significantly to oxygen retention (Deka et al., 2011; Garg and Kaushik, 2005).

2.1.4 Temperature

The ideal range of temperature for earthworms is 25^{-}–37°C. Earthworms have highly sophisticated reactions to temperature variations (Sinha et al., 2002). Chemical and microbiological actions in the substrate increase at higher temperatures (over 30°C), lowing oxygen content and harming earthworms (Domingeuz and Edwards, 2004). Various earthworm species respond to temperature in different ways. For instance, *E. fetida* develops best around 25°C and tolerates temperatures as high as 35°C, but *Dendrobaena veneta* exhibits optimal development at lower temperatures and has a poorer tolerance for high temperatures (Edwards, 1988).

2.2 Microbial community in vermicomposting

In the vermicomposting process, earthworms play a significant role in the biochemical breakdown of organic waste carried out by microorganisms—either soil bacteria or those found in the earthworm's digestive tract (Edwards and Bohlen, 1996). There is no doubt that earthworms and microbes depend on one another and that their interactions are mutually beneficial (Sinha et al., 2010). Earthworms provide aeration for substrate mixing and grinding, particularly enhancing the accessible space for microbe residence and influencing their structure, composition, activity, and growth rate (Ravindran et al., 2016; Vig et al., 2011). A vast variety of microbes, such as phosphate solubilizers, nitrogen-fixing bacteria, enzyme producers, and plant growth promoters, are concentrated in the VC's final output. Microflora found in organic waste and the earthworm's stomach, in conjunction with the enzymes found in their guts, are responsible for the disintegration of organic waste. In general, the activities of bacterial populations have an impact on soil characteristics, other organisms present in the soil, and the natural cycle of nutrients including carbon, nitrogen, and phosphorus. Parthasarathi and Ranganathan (1998) stated that the increased levels of nitrogen, phosphorus, and potassium in VC are the result of the enzymes secreted by the microbial populations that exist in the gut of earthworms. It has been shown that earthworms' fecal phosphatase activity and the bacterial activity in their digestive tracts contribute to an increase in total phosphorus throughout the process of vermicomposting. Due to the presence of microorganisms in the organic waste substrate, the phosphorus content in vermicasts has been found to increase (Sharma et al., 2009). Although the presence and contribution of microbes during the vermicomposting process are widely documented, there are only a few studies that demonstrate the temporal shift in microbial composition during the process of vermicomposting. Scotch broom (*Cytisus scoparius*) vermicomposting results in the development of three distinct bacterial communities: day (0) microbes found in a newly cut Scotch broom, day (14) microbes recently passed via earthworm intestines and discharged, and days (42 and 91) microbes linked to the aging process of casts. Their findings revealed that microbial communities were divided into the phyla Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, and Verrucomicrobia. Proteobacteria were most numerous at the start of the procedure, and after the 14th day, their numbers started to decline but continued to be substantial. On the 14th day, other phyla began to emerge, although their abundance varied according to the vermicomposting phase (Domínguez et al., 2019). Kolbe et al. (2019) stated similar findings of microbial changes during the vermicomposting of grape mac observed from day 7 to 91, with a maximum increase in Firmicutes. Microbial diversity was quite low in the initial material of Scotch broom and grape marc. Although bacterial diversity is often low in beginning materials at the initial stage of vermicomposting phase, it rises considerably as the process proceeds. Chitrapriya et al. (2013) stated that the VC developed using cattle dung as well as sawdust comprises Bacillus (Firmicutes), Streptomyces (Actinobacteria), and Pseudomonas sp. (Proteobacteria) as phosphate solubilizers and Azobacter (Proteobacteria) as nitrogen-fixing bacteria. According to (Ravindran et al., 2015), VC has higher levels of indole acetic acid, GA3, and kinetin than compost, indicating the importance of the earthworms' gut microorganisms. A study done by Anastasi et al. (2005) also indicates the differences in fungus diversity in terms of structure in compost and VC. 194 unique fungal species were identified; 66 were found in both compost and VC, while compost yielded 118 and VC, 142. This suggests that the variety of fungi in VC is greater than that of compost. The shift in the organic carbon supply, pH, and physical qualities of the substrate (which might encourage the development of aerobic bacteria) are also connected with shifts in microbial communities.

3. Vermicompost as a plant growth promoter

VC influences the systems that produce plant growth regulators in addition to improving nutrient cycling and the availability of vital microorganisms. During vermicomposting, earthworms and a variety of microorganisms add compounds similar to plant growth hormones to organic debris (such as fulvic or humic acid) Fig. 4.1. It has been proposed that key components influencing improvements in the growth of plants and production are the employability of plant growth regulators in VC. Plant growth regulators are known to be created by microbes and are already present in VC (Vijayabharathi et al., 2015). Nielson (1965) was the first to identify plant growth-promoting compounds in earthworms. He discovered indole-like compounds in the tissue extracts of *A. caliginosa*, *L. rubellus*, and *E. fetida* and noticed an enhancement in the growth rate of a garden pea. Several works have been documented on VC comprising plant growth promoters including auxins, gibberellins (Grappelli et al. (1985); Krishnamoorthy and Vajranabhaiah (1986)), cytokinins, and humic acid (Masciandaro et al., 1997; Atiyeh et al., 2002). Tomati et al. (1988) observed that after the inclusion

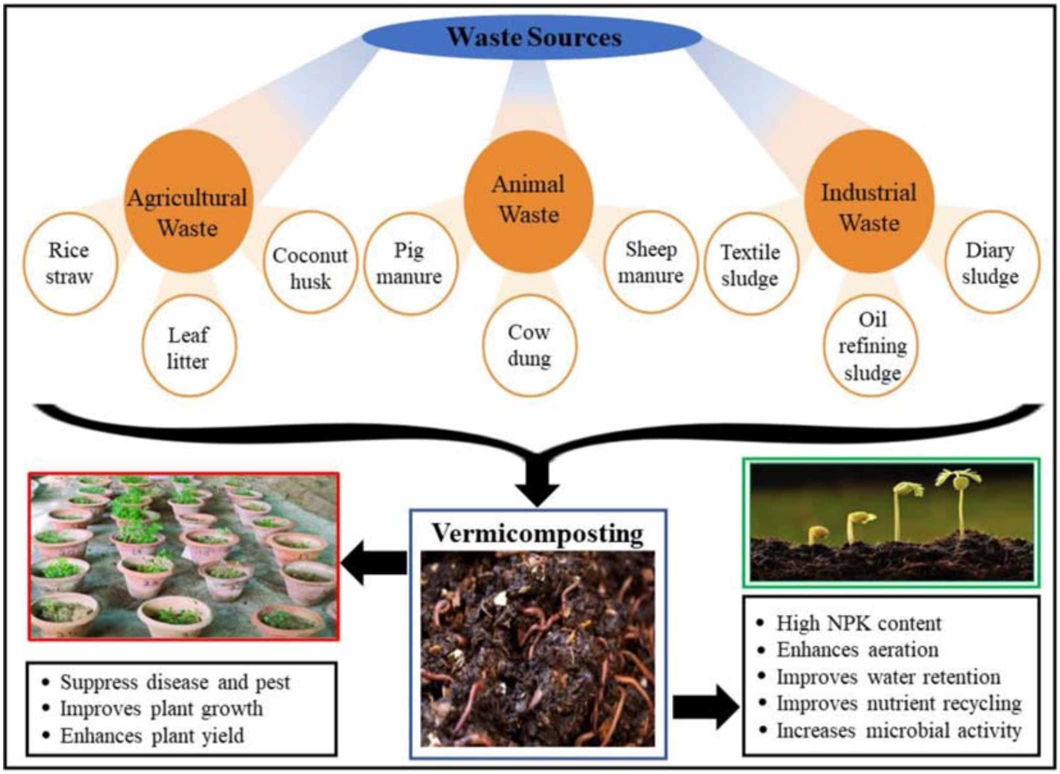

FIGURE 4.1 Different types of waste for vermicomposting.

of aqueous extracts of VC, the development of selective ornamental plants exhibited a growth pattern comparable to those produced by naturally derived plant hormones, such as auxins, gibberellins, and cytokinins. VC application improved plant spread (by 10.7%), leaf area (by 23.1%), dry matter (by 20.7%), and total strawberry fruit output (by 32.7%) (Singh et al., 2008). Warman and Anglopez (2010) demonstrated that the addition of 10% VC generated from various feedstocks boosted the leaf area and biomass of radish, marigold, and upland cress, despite the fact that the VC extract inhibited seed germination in all three species. According to Sallaku et al. (2009), VC favorably affected plant development even under saline circumstances.Young cucumber seedlings cultivated in VC had a much greater relative growth rate during the nursery stage relative to one grown in commercial peat compost, and this trend remained throughout the stand-establishing phase. There have been several research on the variety of fungi in VC and earthworms in addition to bacterial diversity. The primary phyla in the initial substrate of VC were Saccharomycetes, Lecanoromycetes, and Tremellomycetes (Huang et al., 2013). Red clover and cucumber plants planted in soil supplemented with VC contained higher mineral levels in their shoot tissues, including Ca, Mg, Cu, Mn, and Zn (Sainz et al., 1998). The seed germination and shoot and root lengths of cluster bean (*Cyamopsis tetragonoloba*) treated with vermiextract and urea solution revealed that the growth was enhanced in vermiextract, which was attributed to hormone-like compounds (Suthar, 2010). Vermiwash made from cow manure was subjected to HPLC and GC-MS tests, which revealed the availability of considerable levels of indole acetic acid, gibberellins, and cytokinins. Likewise, Zhang et al. (2014) observed the presence of cytokinins like trans -Zeatin (tZ), N6 -Isopentenyladenine (iP) and N6 -Isopentenyladenosine (iPR) in VC developed using water hyacinth (*Eichhornia crassipes*) and chicken manure at a ratio of 4:1 (w/w) and further stated the beneficial role of VC in plant yield. The presence of hormones in VC not only improved the yield and development of plants but also provide resistance to withstand stress induced by biotic and abiotic conditions (Aremu et al., 2015).

3.1 Stimulation of plant growth using vermicompost infused with beneficial microbes

The plant growth stimulating characteristics of VC may be improved by using helpful microbes. By combining VC with fertilizer N and biofertilizers, Jeyabal and Kuppuswamy (2001) demonstrated that the rice-legume cropping system might benefit from integrated nutrition. They discovered that using VC, nitrogen fertilizer, and biofertilizers such as *Azospirillum* and phosphobacteria have together boosted rice output by over 15% as compared with only applying N fertilizer. Overall, the integrated application enhanced the absorption of N, P, and K, while a similar result was reported by Hameeda et al. (2007) in the application of rice straw VC that significantly improved the lengths of shoot, root, and leaf areas as well as mycorrhiza colonize (*Glomus* species). An increase in rice straw VC led to reduced plant biomass even after mycorrhization compared with using rice straw VC singly. This further demonstrates that using microbial inoculants in combination with increased compost concentrations may not be beneficial to plant development. Gutiérrez-Miceli et al. (2008) also documented the influence of sheep manure VC on mycorrhization and the development of maize plants. VC enhanced the phosphorus content of the plants but not the nitrogen content, accounting for most of the variance reported in leaf number, moist weight, stem height, and diameter. Additionally, the addition of diazotrophic bacteria with VC resulted in a rise in mycorrhizal colonization. A Similar was reported that maize plants grown in Peat moss are treated with VC and are augmented with *Glomus fasciatum* and diazotrophic bacteria.

3.2 Stimulation of plant growth by humic substances

The growing market for humic compounds has gained massive attention in composting as a potentially cost-effective method of extracting them, hence decreasing dependence on costly fossil matrices, primarily mined lignite (e.g., leonardite) (Martinez-Balmori et al., 2014). The end product of vermicomposting (VC) is abundantly loaded with humic acids that are considered well-known for their ability to stimulate development of plant, particularly root systems (Muscolo et al., 1999; Canellas et al., 2002; Arancon et al., 2003; Eyheraguibel et al., 2008). Additionally, humic compounds extracted from VC successfully promoted the production of the plasma membrane (PM)H + -ATPase in a typical auxin-like manner, hence promoting development of lateral root (Canellas et al., 2002). The efficiency of VC-derived humic extracts as plant growth stimulants is significantly dependent on the physicochemical properties of the initial organic material as well as the phase of composting dramatically influences the final makeup of the humified organic compound (Dobbss et al., 2010; Canellas and Olivares, 2014). Previous studies documented the benefit of HAs for plant development. Martinez-Balmori et al. (2014) observed a 36%−135% increase in the number of lateral roots in maize plants grown with humic acids from mature VC relative to the control plant. Humic acids (HAs) components extracted from VC also affect plant development. When maize and *Arabidopsis* seedlings were exposed to various concentrations of size fractions (SFs) of HAs extracted from VC, it was shown that every SF enhanced root development in seedlings of *Arabidopsis* and maize. However, the effects varied based on the molecular size and plant species. Arabidopsis seedlings exposed a typical significant auxin-like exogenous response, with a shortened primary root axis and stimulated lateral root formation, when exposed to high content of HA and its SF. In maize, the impact was associated with low auxin-like levels, as shown by increased primary axis length and lateral root induction (Canellas et al., 2010). Arancon et al. (2003) also derived HAs from cow, food, and paper-waste VC following an alkali/acid fractionation technique and applied them at varied ratios to immature marigold, pepper, tomato, and strawberry plants grown on a soil-less growth medium MM360 and adding humic acids derived from VCs enhanced the root dry weight of *Tagetes*, *Capsicum annum*, *Solanum lycopersicum*, and *Fragaria ananassa*.

Similarly, humic acid prepared during the composting accelerate the primary root length of the maize plant by 30%−40% compared to the control. Additionally, compared to control seedlings, the dry weight of seedlings roots treated with HA extracted from composted material typically increased by 25%−30%. Furthermore, HA stimulated vanadate-sensitive ATPase activity in maize seedlings (Jindo et al., 2012). According to Ievinsh et al. (2017), the effective humic acid dose for plant germination, specifically hemp (*Cannabis sativa* L) is 0.1 mg mL^{-1} and has a stimulant effect on radicle mass at 0.05 mg mL^{-1} (1.25 mL equivalent dose). The mass of hemp seedlings decreased in the presence of high humic substance concentrations. HAs are not only associated with the enhancement in the emergence of lateral roots but are also found to decrease the intensity of bacterial spots on plants exposed to HAs. HA-treated plants displayed three times more defense enzyme activity (Faccin and Di Piero, 2022). Humic acid treatment decreased carotenoid concentration and increased chlorophyll *a* and *b* and suggested that a concentration of 150 mg L^{-1} is optimal for plant absorption of the nutrient and hence encouraged its use in the cultivation of mangosteen seedlings. If applied to mangosteen seedlings in the nursery, VC humic acids may improve plants' nutritional status (Gomes et al., 2019). Humic extracts are an important supplementary biological product for a variety of agricultural practices.

4. Vermicompost as a plant disease suppression and pest control

There has been an increase in the number of studies done in the past 2 decades that have demonstrated the efficiency of VC-derived products in protecting plants from different diseases and pest infestations (Edwards, 1988). The effectiveness of VC in preventing disease and controlling pests depends on several different factors, including maturity and quality of compost, brewing, aeration, temperature, pH, microbial inoculants, dietary supplements, and compost-to-water ratio are all significant factors. The two major mechanisms involved in disease suppression are the general and specific suppression mechanisms (Fig. 4.2)

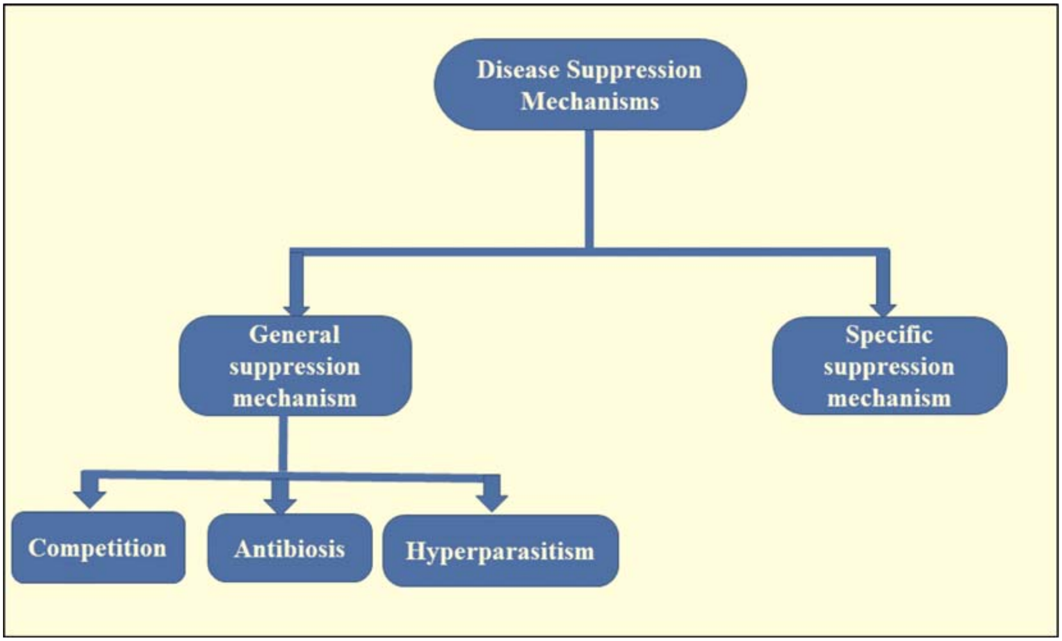

FIGURE 4.2 Disease suppression mechanisms by vermicompost.

(Pieterse et al., 2014; Baum et al., 2015). VC prepared in the lab, greenhouse, and field has a high potential for disease suppression induced by pathogens like *Pythium*, *Phytophthora*, *Fusarium*, *Rhizoctonia*, and *Verticillium*. The potential of VC developed from wide waste sources, including animals manure (Szczech and Smolinska, 2001), dairy sludge (Kannangara et al., 2000), sludge from sewage treatment process (Szczech and Smolinska, 2001), and a blend of vegetable discards, bark (*Salix* spp.), and cattle manure (Simsek-Ersahin, 2010) was tested on *Phytophthora nicotianae, Fusarium oxysporum,* and *Rhizoctonia solani*. Furthermore, organic amendments increase the number and variety of microorganisms; one of the likely causes of disease suppression may be microbial antagonism. Traditional composts promote only specific microorganisms growth, whereas VCs promote microbial diversity and activity and contain a variety of antagonistic bacteria that function as efficient biocontrol agents in suppressing diseases induced by soil-borne phytopathogenic fungi (Scheuerell et al., 2005; Singh et al., 2008). According to Doube et al. (1994), earthworm activities prevent root infections caused by Rhizoctonia. Incorporating VC into the soil substantially inhibited *R. solani* in wheat (Stephens et al., 1993), *Phytophthora nicotianae* (Nakamura, 1996; Szczech, 1999; Szczech and Smolinska, 2001), and *Fusarium* in tomatoes (Nakamura, 1996). Application of VC significantly decreased the occurrence of Powdery Mildew, Color Rot, and Yellow Vein Mosaic in Lady's finger (*Abelmoschus esculentus*) (Agarwal et al., 2010). Pharand et al. (2002) determined the efficiency of paper and pulp mill wastes to prevent crown and root rot of tomatoes induced by *Fusarium oxysporum f.* sp. *radicis-lycopersici* Table 4.2. *Fusarium* inoculated plants' roots were examined histologically and cytologically, and the results showed that the plants were more resistant to fungal colonization. In extensively injured hyphae, the pathogen's

TABLE 4.2 Disease and pest control by vermicompost tea.

S.No	Source of vermicompost	Duration of vermicomposting (days)	Pest/disease	Crop	Growth/yield characteristics	References
1	Herbal plants waste	60	*Fusarium wilt*	Chickpea	4%–19% reduction of Fusarium wilt disease incidence was observed	Gopalakrishnan et al. (2011)
2	NA	NA	Helicoverpa Zea	Corn	Vermicompost was effective in inducing antixenosis (non-preference) and antibiosis (lower performance) resistance to H. zea in corn, which is effective against both adult (reduced oviposition) and immature insect stages (lower immature weight gain and survival)	Cardoza and Buhler. (2012)
3	Mixture of animal dung and agro waste	60	Aphid *(Lipaphis erysimi)*	Mustard	Enhanced overall growth, induced early flowering, and improved mustard yields by 3.5 fold	Nath and Singh. (2012)
4	Cattle dung	NA	Root Knot nematode	Tomato	Vermicompost enhanced soil quality, raised root defense metabolite concentrations, and defense-related gene expression.	Xiao et al. (2016)
5	NA	NA	Root Knot nematode	Cucumber	Vermicompost has a significant effect on larvae mortality anincreasesed the plant growth	Rostami et al. (2014)
6	Food waste	NA	*Fusarium oxysporum*	Tomato	Tomato plants treated with vermicompost showed substantial differences in disease incidence reduction, improved plant growth and production, and better antioxidant stimulation.	Basco et al. (2017)
7	Cattle dung	NA	*Fusarium oxysporum*	Tomato	*Fusarium oxysporum* is inhibited by Nocardioides, Ilumatobacter, and Gaiella bacteria introduced by vermicompost.	Zhao et al. (2019)

(*Continued*)

TABLE 4.2 Disease and pest control by vermicompost tea.—cont'd

S.No	Source of vermicompost	Duration of vermicomposting (days)	Pest/disease	Crop	Growth/yield characteristics	References
8	Agro waste	NA	*Polyphagotarsonemus latus*	Chili	*P. latus* population was decreased the number of eggs laid by *P. latus* significantly (5.05 eggs/leaf) in comparison to control (no fertilizer; 6.18 eggs/leaf).	Jangra et al. (2019)
	Farm yard manure	NA	Late blight diseas	Potato	FYM resulted in the lowest leaf blight incidence and intensity of (42.21% and 44.44%) and (23.61% and 25.90%) at dehaulming stage.	Peerzada et al. (2020)
	NA	NA	*Fusarium wilt*	Cucumber	Vermicompost treatments greatly reduced the occurrence of Fusarium wilt and the amount of nitrate in the cucumber fruit, but they also markedly enhanced plant heights, stem diameters, and leaf areas, as well as the amount of soluble sugar, soluble protein, and fruit produced per plant.	Zhang et al. (2020)
9	Agro waste	60	*Pythium* sp	Tomato, Bell pepper, Eggplant	enhanced plant growth, decreased leaf disease incidence, and better disease resistance of the plants' seeds	Alshehrei et al. (2021)
10	Cattle dung	45	*Meloidogyne incognita*	Ashwagandha	the combined application of vermicompost (60% or 100%)+vermicompost extract, a significant decrease in gall development and an increase in seedling growth parameters were observed, as shown by a rise in seedling biomass and chlorophyll and protein contents.	Kaur et al. (2022)

wall-bound chitin was also changed. Scientific studies demonstrate that vermicomposting effectively reduces the *disease P. nicotianae* var. *F. oxysporum* sp. *lycopersici* (Szczech et al., 1993). The nutrients in earthworm castings are abundant (Lunt and Jacobson, 1944); and calcium humate, a binding substance (Edwards, 1988), prevents individual castings from drying out and promote beneficial microbes like *Trichoderma* species (Tiunov and Scheu, 2000), *Pseudomonas* species (Schmidt et al., 1997). Istifadah et al. (2021) reported that the fungal isolated from VC water extract were antagonistic to *A. solani* in vitro and could potentially be biological control agents for early tomato blight disease (Istifadah et al., 2021). Moreover, According to Mondal et al. (2021) research, adding 1.2% biochar and 5% VC to rice might help reduce the stress caused by RRKN. Therefore, the use of biochar and VC might be an efficient substitute for hazardous chemical nematicides and advised as environmentally sound management methods against *M. graminicola* in rice. Similarly, Öztürkci and Akköprü. (2021) observed disease development was decreased by 48% when 10% solid VC was added to the peat growth medium. In soil growth medium, 10% and 20% application levels inhibited disease development by 62% and 54%, respectively. Were et al. (2021) reported that VC application impacted plant emergence favorably. The prevalence of root rot disease decreased by upto 40% and 50% respectively during the every season. Also, biochar and VC treatments helped in reducing the number of fungal pathigens while affecting the population of beneficial microorganisms like *Trichoderma* and *Paecilomyces lilacinus* (Subashini et al., 2021). Tikoria et al. (2022) observed that VC prepared with neem exhibited lethality to juveniles in their second stage of development; 82% of them died after exposure to the maximum dosage. Exposure of eggs to 100% VC inhibited hatching by 33.8%, suggesting that VC had an antagonistic impact on nematode egg hatching.

5. Conclusions and future perspectives

The sustainable use of these effective and safe substitutes can guarantee food security for a growing global population. VC has demonstrated benefits for crop development and productivity. Still, there are some reasons why it is not as frequently employed as an inorganic chemical, including the fact that it is new and emerging, less accessible on the market, and farmers are unaware of its beneficial impacts. More farmers should be educated about them and trained to enhance their utilization rate. Vermicast and inorganic chemicals must undergo a comprehensive cost-benefit analysis to determine whether or not they are productive for farmers and how, in the future, these amendments can be made more effective so that farmers in both poor and wealthy countries can benefit from them without any doubt. VC applications have been studied for plant protection purposes, and research on their effectiveness in suppressing disease and pest attacks in solid and liquid forms has been conducted. Further research is needed to achieve a deeper understanding of mechanisms and the factors affecting disease prevention and pest infestation and the possibility of integrating them into sustainable and environmentally friendly crop production systems. Improving crop quality also requires optimizing the production and exploitation of vermicast products without compromising food safety. Plant disease management needs to be supplemented or replaced by these groundbreaking, environmentally friendly disease control technologies in the future rather than the current form that completely depends on synthetic pesticides.

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CHAPTER

5

Vermiremediation of plant agro waste to recover residual nutrients and improve crop productivity

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1. Introduction

With the ever-increasing amount of solid organic waste like plant agro waste, the world faces serious problems with sustainable waste management. Apart from agricultural crop residues from crop fields, the Agricultural Produce Marketing Committee and agro-industries contribute huge quantities of solid organic waste from pulses, cereals, fruits, vegetables, and herbs (Mane and Raskar Smita, 2012). Brazil was the third-largest fruit and vegetable waste producer in 2011, with an estimated 1728 million tonnes produced globally (Edwiges et al., 2018). Reports state that dumping such waste into landfills causes havoc for the environment by emitting significant quantities of methane into the air, producing air pollution and leachates that contribute to water and soil pollution. As a result, effective methods must be used to transform this wasteful green biomass into a resource that can be used indefinitely by integrating it into a circular system of reduce, reuse, recycle, and regenerate rather than moving it along the linear path of take, make, and dispose of (Rhodes, 2017). Since prehistoric times, people have recycled organic biomass to manage waste, enhance the quality of the soil, and produce nutrients for better crops. Composting presents a convenient way of reducing and recycling organic waste in an eco-friendly manner. To meet the growing demand for phytochemicals, compost made from agricultural waste can act as a biofertilizer for crop improvement. This is a safer, more environmentally friendly, and less expensive alternative to intensive farming methods, which overfeed the soil with synthetic fertilizers to meet the rising demand for food supply. In contrast to compost, which is a stable and

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wholesome byproduct of organic waste produced via aerobic degradation, vermicompost is a composted peat-like substance produced by the breakdown and digestion of organic matter using particular earthworms that incorporate, grind, and digest. It assimilates the organic waste in their gut in conjunction with endogenous aerobic and anaerobic microbial consortia, thus transforming the unstable waste into a dark, uniform amorphous matter called humus (Gupta and Garg, 2009; Raza et al., 2022). The biological process of vermicomposting offers a good alternative and is a very economical and environmentally friendly solution for waste bioconversion (Bhat et al., 2017a). According to some research, epigeic earthworms are ideal for vermicomposting because of their ravenous appetite, increased tolerance to consuming organic waste, short lifespan, rapid reproductive rate, and possessing a robust metabolic system comprising of earthworm gut microorganisms and chloragocyte cells that allows them to eliminate toxic pollutants from industrial wastes. Sohal et al. (2021) studied the use of vermicomposting in the management of hazardous waste like biomedical waste ash (BA) using the earthworm *Eisenia fetida* and cow dung (CD) as a nutrient medium. They observed that the decrease of heavy metal content below the legal limits and the best growth and reproduction of the earthworms in the final product indicate the vermicomposting's ability to deal with hazardous solid wastes like BA. In the process of vermicomposting, earthworms emit coelomic fluids (CFs), which kill the parasites and bacteria in the waste and produce pathogen- and odor-free final vermicompost (Wang et al., 2021; Bhat et al., 2018; Kale and Krishnamoorthy, 1981). Vermiwash is a vital vermicompost byproduct that is readily accessible to plant roots. It is a solution that's been gathered after draining earthworm-rich vermicompost. It is rich in CF and other bioactive substances, including enzymes, hormones, vitamins, proteins, mucous, micro- and macronutrients, and decomposer microorganisms, forming a symbiotic interaction with earthworms. Important metabolites are released by the decomposing microbes in vermicompost and vermiwash to guard against plant diseases (Gudeta et al., 2022). A research conducted by Yatoo et al. (2022), nutrient enhancement of vermicompost was examined for the first time by adding CD and organic nutrient supplements (eggshell, bone meal, banana peel, and tea waste) to free-floating macrophyte (*Azolla*, *Lemna*, and *Salvinia*) biomass for a wide range of environmental advantages. During vermicomposting, the earthworm gut secretes various enzymes like lipases, cellulases, proteases, chitinases, and amylases, transforming inaccessible minerals such as calcium, potassium, phosphorus, and phosphorus nitrogen in organic substances into forms that plants can use (Hand, 1988). Large volumes of plant agro wastes, such as toxic jute mill waste, mushroom waste substrates, rice Straw, toxic weeds, fruit and vegetable waste, etc., have been effectively converted into vermicompost through vermiremediation technology to recover residual nutrients from crops to increase their growth and yield. Recent research has concentrated on managing fruit and vegetable wastes (FVW) by processing them using various valorization processes to extract value-added products, such as biofuels, biopolymers, enzymes, bioplastics, and many bioactive chemicals (Esparza et al., 2020). However, such extraction processes require a pre-treatment process involving the previous separation of the liquid phase along with huge consumption of energy (Rubio-Senent et al., 2013), while some other waste management technologies like anaerobic digestion present a disadvantage of instability of the processed waste, that would require an organic amendment to be added after the treatment (Bustamante et al., 2013). FVW have a lot of potential for reuse, recycling, and recovery due to their high water content and abundance of biodegradable organic substances (i.e., carbohydrates, lipids, and organic acids). However, a few studies showed that earthworms could not survive in fresh FVW, probably due to their high water content and electrical conductivity (Gunadi and Edwards, 2003). According to Li et al. (2020), using excess activated sludge in the vermicomposting process accelerated the nitrification and mineralization process, increasing the nitrogen and phosphorous contents in the finished product. This favorably contributed to the decomposition of FVW (Fig. 5.1).

Soil supplemented with humus-rich composts has great advantages in terms of organic matter content, microbial biomass, fertility, and phytotoxicity, which is not normally fulfilled through conventional thermophilic systems of composting (Solaiman et al., 2019; Singh and Suthar, 2012). The humus-like product being dense in macro- and micronutrients, vitamins, enzymes, and several growth hormones should be stable but also mature since it is one of the ways to improve horticultural output and lessen soil contamination. Compared to the original raw waste, the final vermicompost typically has a grainy and permeable texture. Fourier transform infrared (FT-IR) spectroscopy, scanner electron microscopy, ultraviolet-visible

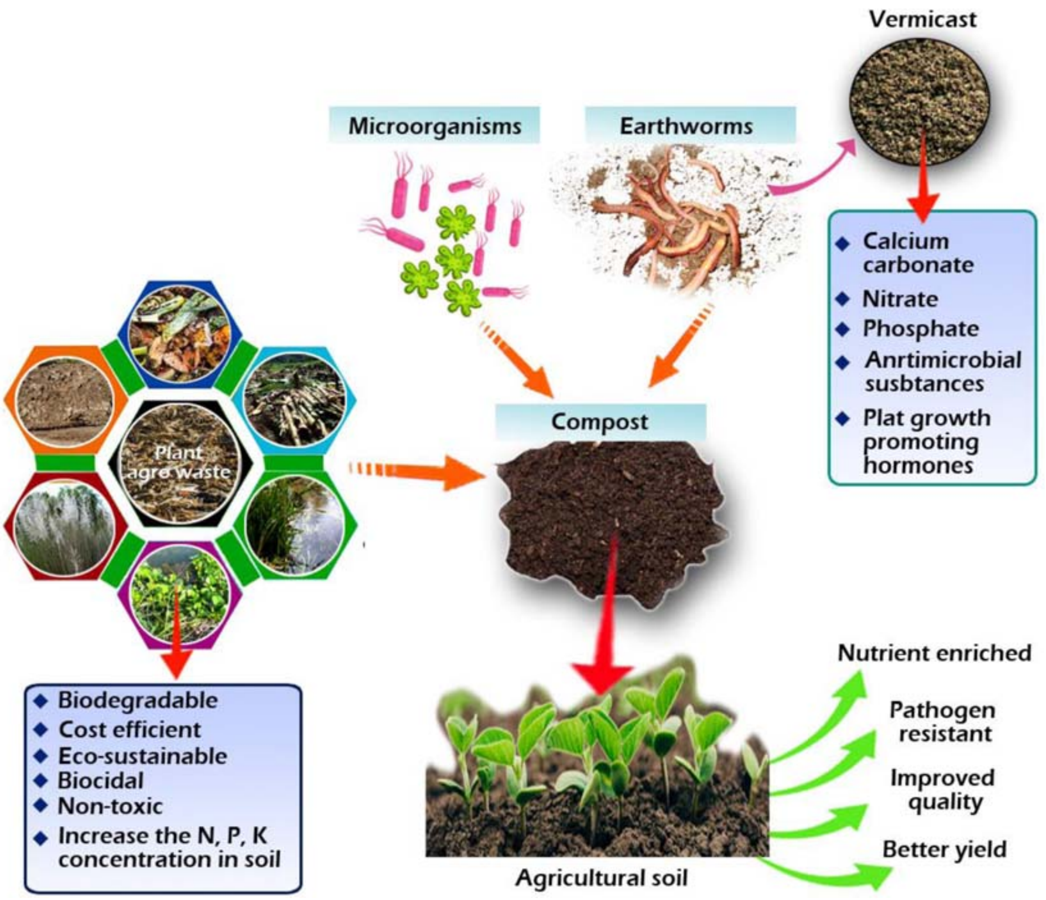

FIGURE 5.1 The soil profile is improved by the plant waste after being treated with earthworms and bacteria.

spectroscopy, and thermogravimetry techniques can be used to detect changes in texture and structure that occur as vermicompost matures (Bhat et al., 2017a,b). This chapter primarily addresses some promising technologies that utilize the biological activity of earthworms to accelerate the vermicomposting activity while comparing the procedures used in vermicomposting various plant agro wastes and how they contribute to increasing crop quality, that will assist in providing deeper insights into plant waste management systems and sustainable farming.

2. Vermiremediation technology

The term "vermiremediation" is a fusion of the Latin terms: vermis, meaning worm, and remedium, which implies rectifying or getting rid of anything wrong (Shi et al., 2020). Vermitechnology uses the biological activity of chemical-tolerant earthworm species to handle a variety of biodegradable materials. The soil's pollutants can be treated by the earthworms that are already there, without removing the top profile. Pollutants such as crude oil, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chemical fertilizers can also be eliminated with the aid of earthworms (Rodriguez-Campos et al., 2014). Earthworms are therefore utilized in the remedial process. Vermiremediation takes place throughout the earthworm's whole life cycle, as the worm feeds on contaminants, burrows them, and goes through metabolic processes to change the organic molecules before degrading the contaminants in the environment. Nutrient recovery from organic wastes like municipal, agricultural, and food waste is crucial for waste management and environmental protection. However, when these organic wastes are poorly disposed into the environment, a large amount of the nutrients they contain are lost or reduced. Through the use of vermitechnology, these vital nutrients could be recovered and employed as nutrient-rich fertilizers in agricultural fields to increase soil fertility (Yatoo et al., 2020; Soobhany, 2019). Vermiremediation can be improved by employing surfactants, nutrient additions, management techniques, or a combination of these with other remediation treatments (Shi et al., 2020).

2.1 Basic process

Vermiaccumulation, vermiextraction, vermitransformation, and drilodegradation are the four fundamental processes that makeup vermiremediation (Fig. 5.2)

2.1.1 Vermiaccumulation and vermiextraction

Earthworms retain toxins they have ingested through vermiaccumulation, specifically through vermiextraction from the soil and water sources. This technique lessens organic pollutants while planning a healthy environment for the microbial flora. Vermiaccumulation is related to the physiochemical behavior of the earthworm, based on the biochemical content in the cell, the concentration of aqueous solubility of organic compounds, and pollutants like PAHs, PCBs, pesticides, etc., present in the soil profile. Earthworm follows two pathways to absorb the organic compound: dietary uptake and passive epidermal uptake.

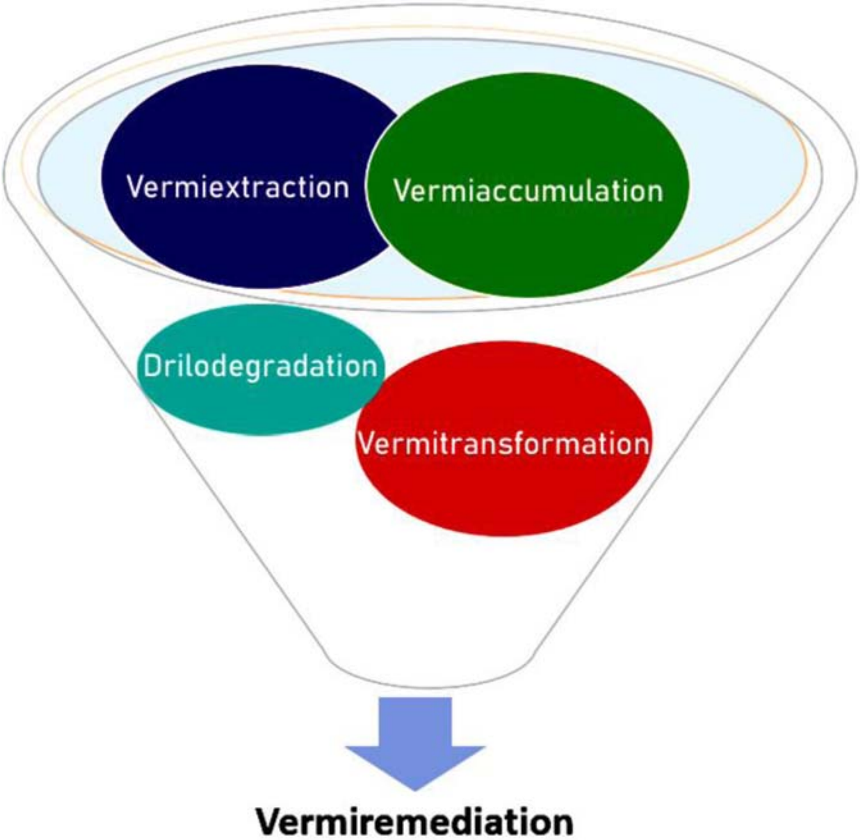

FIGURE 5.2 Various processes involved in vermiremediation of organic waste.

The epidermal pathway for passive absorption involves passive uptake that moves toward declining chemical potential. Organic molecules weakly bind to the soil substrate during the passive uptake phase, and the earthworm's body walls then absorb these particles. However, during the process of dietary uptake, earthworms consume soil that contains organic-based compounds that can be broken, digested, and put through various processes that are part of an earthworm's existence, causing the digestive tract to absorb the organic substances in the soil (Shi et al., 2020). According to Jager et al. (2003), the main route for the intake of organic chemicals by earthworms is through their passive epidermal pathway. To analyze the organic molecules that have fractionated in the suborganisms, tissues, and subcellular sections of the earthworm's body, researchers have used a hierarchical strategy to study worms (Zhi-Ming et al., 2014; Jager et al., 2003). The act of vermiaccumulation is associated with the physiological traits of earthworms, including the lipid content of their tissue, the concentrations of contaminants present in the soil or sewage, and the physicochemical traits of organic-like water solubility and bioavailability (Rodriguez-Campos et al., 2014). Although there has been extensive research on vermiaccumulation and vermiextraction, little is known about their function in vermiremediation, perhaps because it is unclear whether earthworms can accumulate significant levels of pollutants (Shi et al., 2018).

2.1.2 Vermitransformation

With the aid of their gut microorganisms and enzymes, earthworms undertake the fundamental process known as vermitransformation, which allows them to break down organic contaminants. The biodegradable waste can be converted into fertilized cast using enzymes like peroxidase and cytochrome P450 (CYP450). Vermitransformation or vermidegradation is one of the processes involved in "vermicomposting" and "vermiconversion." Vermiconversion involves quickly transforming valuable fertilizer compounds from poorly biodegradable solid wastes. Vermicomposting also involves the natural breakdown of organic waste to produce stable and mature organic fertilizer, called vermicompost. However, vermiconversion and vermicomposting are focused on biodegradable solid wastes, whereas vermitransformation and vermidegradation are specifically targeted at the ecotoxicological effects of organic pollutants on earthworms (Shi et al., 2020). Studies have revealed that earthworms have chemical metabolites that can detoxify organic xenobiotics, such as herbicides, 2, 4, 6-trinitrotoluene (TNT), PAHs, etc. (Zhang et al., 2016; Schmidt et al., 2017). According to Zhao and Zhu (2017), earthworms can convert fluorotelomer alcohol into perfluorodecanoate, perfluorononanate, and perfluorocarboxylic acids.

Earthworms' biochemical metabolic digestion of organic contaminants has been defined as a succession of enzyme-catalyzed reactions by Saint-Denis et al. (1999). Enzymatic degradation occurs in two major biochemical processes: transformation and conjugation (Shi et al., 2020). Phase I is the process of transformation in which nonpolar pollutants or hydrophobic contaminants are transformed into more polar active molecules and water-soluble compounds to prepare them for the phase II reaction or conjugation (Brown et al., 2004). Although reactions like epoxidation, N-, O-, S-dealkylation, sulphoxidation, peroxidation, aromatic and aliphatic hydroxylation, and oxidative desulphurization may take place, it appears that the majority of focused reactions are oxidations that are catalyzed by cytochrome P450 (CYP450) in Phase I pathways (Li et al., 2018). Phase II conversion comprises the direct conjugation of organic pollutants or the conjugation of phase I reaction metabolites using sugars, amino acids, or glutathione (Brown et al., 2004). Any of these endogenous compounds can be conjugated with, forming hydrophilic conjugates as a result of covalent bonding (Stroomberg et al., 2004). Although vermitransformation is a valuable and significant mechanism of vermiremediation, there are still many things that need to be clarified: (i) it is unknown whether organic contaminants in earthworms are broken down by the worms themselves or by their gut microorganisms; (ii) it is unknown how well organic contaminants can be transformed by earthworms; (iii) it is also unknown how much vermitransformation contributes to the process of vermiremediation (Shi et al., 2020).

2.1.3 Drilodegradation

The microbial community is concentrated in drilospheric soil. It is the 2 mm thick zone built along the walls of the burrow that is inhabited by earthworms. The air-channeling tunnel created by the earthworm burrow speeds up the metabolism of aerobic bacteria by supplying these microbes with improved aeration (Liu et al., 2011b; Kuzyakov and Blagodatskaya, 2015). Additionally, the earthworms excreted cast, residues, and mucus that enhanced this drilosphere with carbon and nitrogen, thus promoting the sustainability of microbial growth and eukaryotic grazers like nematodes and protozoa (Stromberger et al., 2012). The carbon-containing substances, such as amino acids, low-molecular organic acids, carbohydrates, nucleic acid derivatives, enzymes, etc., secreted from the earthworm's mucus, as well as the reliable source of organic carbon from the earthworm's excreta, are responsible for increasing the microbial load inhabiting the drilospheric soil (Zhang et al., 2009). The multitude of microbial processes occurring in this zone triggers the breakdown of carbon-based contaminants, and this process is called drilostimulation or drilodegradation. Another strategy to encourage drilodegradation besides increased contaminant degrader number and agility is probably increased bioavailability. The glutinous mucus excreted from the earthworm's body wall may contain some complicated components with surfactant properties that can boost the perceived solubility of hydrophobic organic molecules, as well (Pan et al., 2010). An important reason for driloremediation success is better desorption in the drilosphere since hydrophobic organic compound bioavailability restricts their uptake and transformation (Katayama et al., 2010). Liu et al. (2011a) found that the drilospheric soil contains more mineralized 2-methyl-4-chlorophenoxyacetic acid, a phenoxyalkanoic acid herbicide, than the surrounding soil.

2.2 Vermiremediation for a cleaner environment and sustainable agriculture (nutrient amendment and degradation of toxins through vermiremediation)

Earthworms are utilized in vermiremediation, which has the capacity to mineralize nonrecyclable chemicals to remediate soil. According to studies, earthworms have the tendency to remove metal ions from contaminated soil (Das et al., 2016). The soil's texture has changed recently as a result of increased industrialization, urbanization, and pollutant discharge. The harmful surfactants have a high probability of destroying the soil's microbial diversity and harming the crop fields' sustainability. In India, where the average person generates about 0.67 kg of municipal solid waste (MSW) per day, a growth of almost 5% annually creates disposal and environmental problems (Ghatak, 2016). Moreover, a sizable percentage of MSW being made up of organic wastes that are "biodegradable" can be vermicomposted to produce a very nutritious bio-fertilizer. The waste conversion method uses earthworms as biocatalytic and detoxifying agents, which reduces waste and produces useful vermicompost. However, the biochemical transformation process known as vermicomposting greatly facilitates the recovery of nutrients from a range of organic substrates, which is beneficial for crop development and yield (Biruntha et al., 2020). Regarding crop growth and food safety, vermicompost outperforms traditional composts by a factor of four to five and even outperforms artificial fertilizers (Rajiv et al., 2010). The environment and people are in danger due to potential ecological hazards caused by organic chemicals such as volatile compounds, herbicides, pesticides, polychlorinated biphenyls, polycyclic hydrocarbons, petroleum hydrocarbons, and many other carbon-based compounds. Chemical treatments, soil vapor extraction, soil washing, electrokinetic remediation, and many more common technologies are utilized for the remediation or removal of contaminants. However, these technologies incur high capital costs and substantial labor demand and have a primary impact on the microbial ecology of the soil (Li et al., 2019). The ability of earthworm bioremediation to treat a wide range of environmental pollutants can be the most suitable and practical method for restoring the soil's texture and preserving healthy microbial diversity. According to Rodriguez-Campos et al. (2014), earthworms can help remove herbicides, pesticides, PCBs, PAHs, and crude oil from contaminated soil. Since vermiaccumulation for organic pollutants may contribute significantly, as opposed to phytoaccumulation, it is a comparatively efficient approach in comparison to phytoremediation strategies (Wu et al., 2018). It is an environmentally sound in situ method for cleaning up PAH-contaminated sites in a little over a year throughout multiple growth cycles (Kuppusamy et al., 2017). Vermiremediation has the added benefit of strengthening soil quality by increasing organic matter, nutrient concentrations, and biological activity (Sinha et al., 2008).

3. Activity of suitable earthworm species and their associated microbes in composting and remediation

The subsurface communities of organisms play the most significant functions in maintaining a stable and healthy soil food web system, which in turn determines how long soil can be sustained. Both prokaryotic and eukaryotic organisms efficiently work together to maintain the soil's fertility, especially the earthworm population, which eats the soil's organic particles and leaves behind rich, partially digested organic casts with the aid of microorganisms (Edwards and Bohlen, 1996). The process by which these terrestrial oligochaetes accelerate the soil's physiological, biochemical, and ecological structure is called vermicomposting. Taking into account the various structural and functional stoichiometric techniques employed by the different earthworm species, Bouche' (1977) grouped them into three major groupings. These groups included endogeic species (species that feed on soil and litter), anecic species (compact species that produce tiny-granular casts), and epigeic species (litter dwelling and transformers earthworms). In addition, a few species of earthworm are further divided into epi-anecic, endo-epigeic, and endo-anecic groups based on the vertical distribution of soil layers, morpho-physiological traits, and ecological categories. Epigeic species of earthworm are independent of environmental factors like weather, temperature, soil pH, etc. They, therefore, have a stronger capacity for ingesting and digesting organic material than the other earthworm types. The vermicomposting process and the partial digestion of organic waste are both aided by the active microbial flora in the gizzard of epigeic earthworms. The composition of these microbial communities is significantly altered by intestinal transit (Romero-Tepal et al., 2014). The earthworms in the vermicomposting process aerate, break up the substrate, increasing the surface area for microorganisms, and change the activity of microbes in organic waste leftovers to speed up the decomposition process (Lavelle et al., 2006). Therefore, it clearly says that adopting an epigeic earthworm for vermicomposting will be more advantageous.

As they migrate, these mesofauna create what is known as "tilled" tunnels. These tunnels later serve as a conduit for water absorption. The field with these tunnels will absorb more water than fields without earthworms, and this fact contributes to the situation by reducing water runoff and preserving groundwater even during dry times. Environmental factors including pH, temperature, the level of soil moisture, and the availability of food sources assist in determining the quantity and variety of earthworms in the soil profile. Under abiotic stress, such as drought, earthworms dig deeper into the soil or even go into diapause; when there is an excess of water during the rainy season, they tend to lean toward the upper horizon of the soil profile to avoid drowning. The temperature of the soil also affects the seasonal activity of the earthworm (Peijnenburg and Vijver, 2009).

Earthworms and microbes work together in vermitechnology to break down a variety of solid wastes quickly. Different species of earthworms have been effectively used to stabilize anthropogenic wastes and slurries produced by a variety of industries (Bhattacharya and Kim, 2016). The complicated feed materials are pulverized to extremely fine sizes as they move through the powerful, thick-walled earthworm gizzards, expanding the surface area to a large extent (Bhattacharya et al., 2012). However, despite their enormous intake, earthworms only use a very small part of the materials they consume for their own needs and excrete 90%−95% of them as vermicast. Because earthworms' intestines contain a variety of microorganisms in large quantities, these vermicasts frequently display a rich population of helpful microbes (Goswami et al., 2013; Sahariah et al., 2014). This process helps with the aerobic breakdown of the substances to produce suitable vermicompost. For the earthworms to continue vermicomposting and multiply fast, they need optimal conditions. Dark, humid environments are ideal for earthworm survival. Although they can survive in a temperature range of 5°C−29°C, to function properly, they require a temperature between 20°C and 25°C with between 60% and 75% moisture (Sinha et al., 2010). The rate of earthworms' metabolism and productivity begins to slow down if the soil temperature exceeds 35°C. Kaushik and Garg (2004) determined that the optimal soil pH range should be between 5.5 and 8.5. The growth and development of aerobic earthworms are directly correlated with the C/N ratio. Studies have shown that the soil and litter's C/N ratio is less than 25:1 because of the presence of earthworms (Ndegwa et al., 2000). Because earthworms are photophobic species, even brief contact with sunlight can result in fatal symptoms and the organism's eventual death.

3.1 Earthworm species (*Perionyx ceylanensis*, *Metaphire posthuma*, *Perionyx excavatus*, *Polypheretima elongata*, *Eudrilus eugeniae*, *and Eisenia fetida*) involved in composting

According to Julka (1983), there must be over 3000 species of earthworms found throughout the world, 384 of which can be found in India. Each of them varied greatly in terms of size, length, and habitat. The majority of earthworms are omnivorous, although there are a few species that are carnivorous, including the genus *Agastrodrilus*, a member of the Eudrilidae family that feed on other earthworms (Lavelle, 1983). The capacity of the earthworms to consume diverse organic leftovers varies substantially among species and between ecological divisions (Lattaud et al., 1998). In Indian agriculture, earthworms like *Perionyx ceylanensis*, *Metaphire posthuma*, *Perionyx excavatus*, *Polypheretima elongata*, *Eudrilus eugeniae*, and *Eisenia fetida* are extensively employed for vermicomposting. Appelhof et al. (1996) stated that in order to perform specific vermiculture activity, the selection of a particular earthworm is the most important step.

To stabilize the anthropogenic pollutants and slurries produced by a variety of sectors, there is a need for efficient earthworm utilization. *P. ceylanensis*, an epigeic earthworm, is responsible for degrading surface litter and organic matter even in the absence of soil (Paul et al., 2011). The trash treatment of sludge wastes dumped by the sugar companies was vermicompost using *P. ceylanensis* (Prakash and Karmegam, 2010). Paul et al. (2011) used *P. ceylanensis* in their experiment on MSW to vermicompost the waste material and break it down into a nutrient-rich product. Moreover, *P. ceylanensis*, in the presence of *P. excavatus*, helped in degrading the agricultural and urban trash to recover its nutrients (Biruntha et al., 2020). *P. excavatus* is well known for being more resilient than other species to a wide range of moisture and temperature fluctuations; hence this species sees dominant use in tropical regions for vermicomposting (Soto et al., 2022). Suthar (2006) used *P. excavatus* to vermicompost the wastes deposited by the guar gum industry using three different proportions of CD and sawdust. Pattnaik and Reddy (2009) concluded that vermicompost produced by *E. eugeniae* is 45% more effective at reducing the dry mass of municipal organic solid waste than traditional compost. Endogeic earthworms like *Polypheretima elongata* produce a thick, sticky substance that adheres to the soil's surface. *P. elongata* is dominant in the tropical region that helps in vermicomposting the sludge produced by the distillery industry (Suthar and Singh, 2008), waste produced from the agricultural sector (Suthar, 2007), waste of Java citronella biomass (Deka et al., 2011b) and sludges produced from the paper mills (Yuvaraj et al., 2018).

In accordance with Viljoen and Reinecke (1992), *E. fetida* exhibits more resistivity and demonstrates proper functioning than *E. eugeniae* and *P. excavatus*, with soil temperatures ranging from 42°C to below 5°C. *E. fetida* has been used in the bioconversion of various waste materials. Sludges waste from olive mills (Moreno et al., 2000), paper mill sludges (Sahariah et al., 2014), leather processing sludges (Ravindran et al., 2008), and biowaste including sago industrial waste (Subramanian et al., 2010), waste from sugar factory (Sangwan et., 2010) and grape marc waste (Gómez-Brandón et al., 2011), are transformed into nutrient-rich products by using *E. fetida* species. *E. fetida,* in combination with *E. eugeniae,* helped degrade vinasse biowaste (Pramanik and Chung, 2011). Additionally, both these species fall into the epigeic group and are thus located in the soil's top horizon. *Metaphire posthuma*, on the other hand, is a type of geophagous worm. These species have a large capacity for digesting organic material. They are endogeic in nature because they exist below the soil's surface, in the mineral layer. In his 80-day experiment, Das et al. (2016) came to the conclusion that the number of heavy metals in the waste from the jute mill industry significantly decreased when it was bulked up with cow manure and vegetable waste vermicompost using *M. posthuma*.

3.2 Structural and functional profiling of microbial diversity in the compost

Vermicomposting is accomplished by earthworms ingesting waste materials and microorganisms. These earthworm species improve the soil's structural and functional texture by excreting the mineral after enzymatically digesting organic substances in their intestines (Aira et al., 2006). Earthworm produces organic matter, and as it gets decomposed, the soil surface area increases, enabling microorganism development and having an impact on the soil fauna (Edwards and Bohlen, 1996). Soil environments are thereby gets enhanced by earthworm feces supporting microbial growth and development more readily. On the other hand, the biochemical processes of these microorganisms maintain the food web of the soil environment together with other living beings. The earthworm boosts nitrogen mineralization productivity together with microbial interaction. They participate in the nitrification process, which produces nitrates from ammonium-nitrogen (Atiyeh et al., 2000b). Microbes multiply as a result of the favorable conditions created by the mesofauna, which aids in the mineralization of carbon. Earthworm-amended soil showed negative development of microbial biomass in an experiment conducted by Zelles (1999). The viable microbial biomass was measured using phospholipid fatty acid (PLFA) analysis. The rates of bacterial and fungal development have a significant impact on how efficiently microbial communities' function. To estimate the rate of their growth, radioactive leucine, which is specific to bacteria, was incorporated into proteins, and radioactive acetate, which is exclusive to fungi, was incorporated into lipids (Domínguez et al., 2010). The earthworm can reduce bacterial development; however, it has little effect on fungi. Domínguez et al. (2010), in their findings, concluded that while the earthworms' production of the organic cast initially intensifies the microbiota community and promotes the growth of microbes, there will eventually be a dramatic decrease in the resources available, which will inhibit the growth of bacteria. Estimates suggest that the microorganisms and earthworms in the compost ecosystem may face competition for carbon resources (Tiunov and Scheu, 2004).

4. Vermiremediation of different plant agro waste

The applications of vermiremediation technologies in conversion of different plant waste into value added products are discussed in the succeeding sections (Fig. 5.3)

4.1 Green manure amended pressmud

After the separation of the sugarcane juice, sugar mills release pressmud, a compacted, nutrient-rich waste. For every 100 tonnes of crushed sugarcane, a pressmud cake byproduct of about 3 tonnes is created (Gupta et al., 2011). According to Bhat and Vig (2019), India alone generates over 40% of the 30 million tonnes of industrial sugar sludge produced globally, which causes disposal and pollution issues that need to be remedied using sustainable approaches (Katakojwala et al., 2019). Balachandar et al. (2020) vermicomposted pressmud with cow dung and nitrogen-rich green manures (*Gliricidia sepium* and *Leucaena leucocephala*) for 50 days employing *Eudrilus eugeniae*. The vermicompost has been shown to have a notable rise in microbial population and NPK levels, as well as a noticeable decrease in total organic carbon (TOC), C/N ratio, C/P ratio, water-soluble organic carbon (Cws)/Norg, and pH levels (Table 5.1).

4.2 Patchouli bagasse mixed with cow dung

Patchouli (*Pogostemom cablin*) is an aromatic herb that produces essential oils, and the by-product generated during the extraction of the oils in many agro-industries is called patchouli bagasse (PB). Leading nations, including Indonesia, China, and Malaysia, are thought to produce 1200–1300 metric tonnes of patchouli oil each year on a global basis (van Beek and Joulain, 2018). Additionally, India provides more than six million tonnes annually to the 20,000 million tonnes of worldwide outlets. Open disposal of such waste biomass has resulted in

FIGURE 5.3 Bioconversion of various agro waste in value added material by vermeremediation technology.

many environmental side effects; vermicomposting using earthworms can negate these effects by altering the waste's composition by reducing its toxic effects, TOC, C/N ratio, as well as by enhancing the amounts of macro and micronutrients in the final product. Vermicomposting of PB has been carried out using CD as the bulking agent and epigeic earthworm *Eisenia fetida* under a series of treatments. The findings showed that the proportion of CD administered to PB in the treatments has an inverse relationship with the formation of vermicompost. After 50 days of the testing period, it was discovered that both the control and vermicompost samples had less TOC, lower pH, and increased ash contents. Variations were observed in the macronutrient profiles (Total potassium; K, Total Kjeldahl nitrogen; TKN, total calcium; TCa, and total magnesium; TMg) across a range of vermicompost assays due to the various metabolic processes that the earthworms and microbes engage in (Ahmed and Deka, 2022).

4.3 Jute mill waste

Jute is a natural fiber that naturally possesses several benefits, including luster, structural rigidity, low flexibility, mild heat resistance, and extended staple lengths. However, the jute industry generates a significant amount of cadies, also known as jute mill waste (JMW), which is a processing waste. India has massive jute-producing industries that generate a great amount of hazardous JMW that poses an environmental risk when discarded in public spaces or close to water sources as it is filled with several chemicals, oils, and colors (Das et al., 2016).

TABLE 5.1 The table displays the sugarcane vermicompost's physiochemical characteristics and the presence of macro and micronutrients (Conceptualized from Shah et al., 2015).

	Plant extract: Sugarcane					
	Sugarcane baggase		Pressmud		Sugarcane baggase + pressmud + effluent	
Nutrient present	**Precomposted**	**Postvermicompost**	**Precomposted**	**Postvermicompost**	**Precomposted**	**Postvermicompost**
pH	7.7 ± 0.10	7.3 ± 0.10	7.5 ± 0.09	7.1 ± 0.09	7.6 ± 0.08	7.1 ± 0.08
Organic carbon (%)	15.43 ± 0.89	18.34 ± 1.93	12.1 ± 1.00	16.92 ± 2.06	13.72 ± 1.53	16.41 ± 1.89
Nitrogen (g kg^{-1})	1.29 ± 0.032	1.34 ± 0.032	1.53 ± 0.012	1.6 ± 0.252	1.39 ± 0.015	1.44 ± 0.044
Phosphorus (g kg^{-1})	0.64 ± 0.05	0.77 ± 0.20	2.61 ± 0.67	2.79 ± 0.34	1.97 ± 0.33	2.07 ± 0.05
Potassium (g kg^{-1})	1.84 ± 0.10	1.96 ± 0.13	1.95 ± 0.20	2.27 ± 0.30	3.11 ± 0.19	4.27 ± 0.22
Ca^{2+} (g kg^{-1})	1.77 ± 0.10	1.89 ± 0.16	2.19 ± 0.05	2.71 ± 0.22	4.14 ± 0.17	4.79 ± 0.12
Sodium (%)	0.43 ± 0.11	0.49 ± 0.08	0.50 ± 0.07	0.58 ± 0.08	1.39 ± 0.12	1.62 ± 0.04
Sulfate (mg kg^{-1})	1.27 ± 0.14	1.41 ± 0.02	1.92 ± 0.17	2.23 ± 0.09	1.99 ± 0.12	2.37 ± 0.06
Boron contents (g kg^{-1})	11.13 ± 0.12	15.82 ± 0.13	13.47 ± 0.03	17.62 ± 0.12	11.26 ± 0.11	15.34 ± 0.11

Das et al. (2016) displayed that bioconverting JMW was successful for agricultural usage using vermicomposting technology. *Metaphire posthuma* was fed various blends of vegetable waste, CD, and JMW in vermireactors. Evidence suggested a rapid increase in earthworm population, body weight, and cocoon formation. Although a notable decrease in TOC and pH was found, vermicomposting led to a significant rise in NPK availability, fulvic acid production, stable humic acid, as well as the carbon content of microbial biomass. Vermicomposted JMW has greater stability than composted JMW, according to FT-IR spectroscopy. The sharp reduction in the concentration of the heavy metals (i.e., Cr, Fe, Pb, and Zn) in the vermicomposted JMW also supports the effectiveness of *M. posthuma* in the successful transformation of the poisonous JMW into a beneficial product.

4.4 *Lantana camara* biomass

Considered one of the most destructive weeds in the terrestrial ecosystem, *Lantana camara* has a high level of environmental tolerance. Its resilience and rapid growth have made it one of the most aggressive biological invaders (Hussain et al., 2015). Patel (2011) found that while *L. camara* cannot be completely controlled by physical, chemical, or biological means, an integrated approach can at least partially manage it. To control soil fertility over time and recover nutrients from this weed's massive biomass, composting or vermicomposting may be a useful choice (Rajiv et al., 2010; Ngo et al., 2011). Suthar and Sharma (2013) employed *E. fetida* to demonstrate the outcomes of vermicomposting tests using leaf litter from the noxious weed *L. camara* (LL) spiked with CD in varying ratios (i.e., 0%, 20%, 40%, 60%, and 80%). Variations in both chemical and microbiological characteristics of vermibeds have been documented for 60 days following the establishment of five different treatments. A reduction in pH (19.5%–30.7%), TOC (12%–23%), and the C/N ratio (25%–35%) was observed among all treatments, whereas a rise in ash content (16%–40%), total N (11%–32%), available phosphorous (445%–629%), exchangeable potassium (K_{exch}) (63%–156%), exchangeable calcium (Ca_{exch}) (67%–94%), and $N-NO_3^-$ (164%–499%) was documented. After the vermicomposting process, the numbers of bacteria, fungi, and actinomycetes increased 0.33–1.67 times, 0.72–2.33 times, and 2.03–2.99 times, respectively. The seed bioassay test revealed that all vermicomposts had germination indices ranging from 47% to 83%. Hussain et al. (2015) demonstrated that vermicomposting eradicated the harmful effects of *L. camara* and caused increased germination success in three plant species (ladies' finger, green gram, and cucumber) when used with a concentration of 1.5% in soil (w/w). Fourier transform infrared spectrometry indicated that phenols and sesquiterpene lactones that give *L. camara* its allelopathic effect were largely eliminated during the vermicomposting process.

4.5 Vegetable waste and tree leaves

Both vegetable waste and tree leaves are produced in large quantities in the environment. Waste from both vegetables and their by-products is rich in bioactive compounds and biodegradable organic matter like carbohydrates, proteins, and lipids and some poorly biodegradable compounds like lignocellulosic biopolymers (Edwiges et al., 2018). Kalamdhad et al. (2009) studied the biological as well as the physicochemical properties of vegetable waste combined with dry tree leaves by vermicomposting them in a rotary drum for 7 days in an aerobic environment maintaining a temperature of 60°C–70°C. The total nitrogen and phosphorous content increased with a reduction in the net content of organic carbon and CO_2 emission as a metabolic end product.

4.6 Pineapple waste

In Uganda, large amounts of pineapple waste (culled pineapple, peels, crown, and core) are generated both through monocropping at most farms and at the dried fruit processing facility and are being disposed of in the gardens or processing yards, causing numerous environmental challenges (Baidhe et al., 2021). Pineapple waste can be transformed into numerous significant products like bioenergy, biochemicals, animal feed, and vermicompost for a circular bio-economy (Sarangi et al., 2022). Zziwa et al. (2021) performed both batch (B) and continuous (C) vermicomposting systems for 60 days to recover nutrients from the waste of pineapple peels and pulp (PW), mixed with cattle manure in a ratio of 4:1 (w/w). It was observed that the waste degraded was at 60% and 54%, whereas the increase in earthworm biomass was 57% and 129% for BPW and CPW respectively. The continuous system has been recommended as the better option for vermicomposting pineapple waste, as more nutrients were preserved in the vermicompost compared to the batch, along with the increase in earthworm biomass (Table 5.2).

4.7 Waste biomass of medicinal herbs mixed with cow dung

Medicinal herbs like ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) are extensively grown during the monsoon season in India, and the annual cultivation covers

TABLE 5.2 The table shows physiochemical properties and the presence of macronutrients in the pineapple compost (mean ± SD, n = 3) (Conceptualized from Zziwa et al., 2021).

	Plant extract: Pineapple peel		
Nutrient present	**Pre-composted**	**Batch pineapple peel vermicomposting**	**Continues pineapple peel vermicomposting**
pH	4.47 ± 0.08	6.05 ± 0.03	6.03 ± 0.02
Organic carbon	41.43 ± 0.64	21.41 ± 0.78	21.60 ± 1.04
Kjeldhal Nitrogen	0.95 ± 0.03	0.38 ± 0.01	0.44 ± 0.01
C/N Ratio	43.46 ± 0.47	55.86 ± 0.17	49.13 ± 0.37
Phosphorus	0.43 ± 0.03	0.79 ± 0.01	0.75 ± 0.02
Potassium	1.80 ± 0.06	0.79 ± 0.01	0.86 ± 0.02
Sodium	1.53 ± 0.05	0.54 ± 0.01	0.58 ± 0.01

approximately 63−147 1000 hectares of land (Prasad and Vijay, 2005). After harvesting, only the rhizomes of ginger and turmeric are used for food and medicine; however, the biomass that grows above the earth serves no useful purpose and is afterward discarded in the form of waste. Das et al. (2022) employed *E. fetida* for vermicomposting such waste with cow dung to observe the changes in the nutrient profiles, biological and physicochemical structure after 60 days and observed a net increase in total K, total Ca, and total Mg, along with an elevated ash content, which indicated rapid decomposition of the substrate materials. A net decrease in TOC was found, which has been attributed to carbon assimilation and respiration by the earthworms and microbes. Herbal industries also contribute a considerable amount of spent waste (which contributes to local pollution, as it has no direct utilization) while taking their herbal biomass through the process of pre-processing, oil extraction, and distillation. Vermiremediation has presented as a useful technology to address these wastes for developing sustainable industries.

4.8 Coir pith

Coir pith comprises the outer fibrous lignocellulosic cementing material of coconut, and roughly 7.5 million tonnes of output are generated annually in India (Nattudurai et al., 2014). Due to the high levels of lignocellulosic, phenolic, and C/N ratio, coir pith has a persistent character and accumulates in enormous amounts along roadsides and wastelands, causing contamination of air, water, and soil (Awasthi et al., 2019; Swarnam et al., 2016). Coir pith, along with nitrogenous legume plant *Gliricidia sepium* and cow dung in a ratio of 2:3:1, is a nutrient-rich substrate for vermicompost production. After being subjected to a 50-days vermicomposting period, a rise in total NPK, calcium, and electrical conductivity and a reduction in total organic carbon, organic matter, C/P ratio, C/N ratio, and total phenolic content was observed in the resultant vermicompost (Jayakumar et al., 2022). To create an effective vermifertilizer, coir pith was mixed with *Sesbania sesban*, cow dung, and various combinations of them for 28 days with *Pleurotus sajorcaju*, then for 50 days with *Eisenia fetida* and *Eudrilus eugeniae*. It was found that the results significantly differed from the control in terms of cellulose, lignin, organic carbon, C/N ratio, C/P ratio, and an enhancement in plant nutrients (Karmegam et al., 2021).

4.9 Spent mushroom substrate combined with agro-residues

The world's mushroom production is expanding rapidly as a result of shifting dietary choices and growing demand for organic and nutritious foods. However, along with the increased production of mushrooms, massive amounts of the by-products such as stipes, caps, and spent mushroom substrate (SMS) are being produced (Antunes et al., 2020). For every kilo of mushrooms produced, around 5 kg of the discarded mushroom substrate is generated (Hřebečková et al., 2020). This residual substrate does have the potential to be used as a source of energy, animal feed, soilless growing media, adsorbent, biofertilizer, or organic amendment due to its abundance in fungal mycelium, residual enzymes (cellulase, hemicellulase, protease, laccase, manganese peroxidase, and lignin peroxidase), organic compounds (such as chitosan, chitin, cellulose, lignin, proteins, and fats) and inorganic nutrients that include nitrogen, potassium, and phosphorus. The symbiotic interaction of microorganisms and earthworms during vermicomposting effectively transforms SMS into a nutrient-rich, humus-like substance (Ruangjanda et al., 2022). Hřebečková et al. (2020) used *Eisenia andrei* to vermicompost SMS for 7 months and analyzed the biological and agrochemical parameters in both the control and vermireactor. The C/N ratio and the number of earthworms were observed to decrease during the process of vermicomposting. Higher values of total P, K, and Mg were found in the vermicomposter without earthworms. The microbial phospholipid fatty acid level was also lower, in comparison to the earthworm-containing vermicomposter. However, the vermicomposter devoid of earthworms had a two-fold higher amount of fungal phospholipid fatty acids. Except for arylsulphatase, most hydrolytic enzymes were more active in the vermicomposter devoid of earthworms. Although the amount of laccase activity was below the detection threshold, the amount of Mn-peroxidase activity was higher in the vermicomposter not having earthworms. According to Tajbakhsh et al. (2008), during a 90 days investigation to examine the capacity of the epigeic earthworms *Eisenia fetida* and *Eisenia andrei* to turn a variety of agricultural residues and leftover mushroom compost into vermicompost, they noticed a sharp decrease in the C/N ratio, pH, electrical conductivity, TOC, TK, and a rise in TKN, TP, as well (Table 5.3).

4.10 Leafy waste of cauliflower and cabbage

Both cauliflower and cabbage belong to the Cruciferae family, which constitutes one-fifth of the vegetable market in the United States. The outer and damaged leaves, the core, the stalk, and the base of the root are the principal by-products of such leafy vegetables, and they are frequently underutilised even though they have a large potential for producing nutraceuticals, chemicals, energy, and fertilisers. These vegetables and their by-products

TABLE 5.3 The table displays the macronutrient content and physiochemical characteristics of the mushroom vermicompost that has been combined with cow and pig manure, respectively (Song et al., 2014).

	Plant extract: Mushroom			
	Cow manure + mushroom		Pig manure + mushroom	
Nutrient present	**Pre-compost**	**Post-vermicompost**	**Pre-compost**	**Post-vermicompost**
pH	8.42	7.57	8.66	7.35
Organic carbon (mg g^{-1})	369.77	257.6	388.57	273.67
Kjeldhal nitrogen (mg g^{-1})	10.53	23.03	11.54	26.21
C/N Ratio	35.36	11.32	33.83	10.43
Phosphorus (mg g^{-1})	8.20	15.51	10.21	19.79
Potassium (mg g^{-1})	12.41	17.33	9.91	15.33

are rich in numerous phytochemical compounds, including carbohydrates, proteins, dietary fibers, minerals (Ca, Mg, K, P, Fe, Zn, and Mn), vitamins (vitamins A, B complex, and C, and carotenoids), and pigments (Aramrueang et al., 2019). Mago et al. (2022) performed a study with 60% CD for 90 days to assess the vermicomposting capacity of the residual biomasses of two cruciferous vegetables. After vermicomposting, the findings revealed a drop in pH (5.3%−9.8%), a rise in electrical conductivity (33%−99.4%), and a rise in ash material (144.7%−187.8%). The ratios of C/N (49.5%−76.4%) and C/P (62.8%−66.04%) were significantly reduced, and the ratios of total Kjeldahl nitrogen (49.3%−85.3%), total available phosphorous (68.2%−98.1%), and total potassium (91.8%−120.3%) increased. It was concluded that compared to residual biomass from cabbage, residual biomass from cauliflower had a higher breakdown efficiency.

4.11 Distillation waste of *Citronella* plant

The citronella plant is primarily grown to separate valuable essential oils and aromatic components. To isolate citronella oil, the species of *Cymbopogon winterianus* Jowitt is grown in cultivation, India being one of the top producers (Sharma et al., 2018). *Eudrilus eugeniae* and *P. excavatus* were used in two seasons, summer and winter, to vermicompost the distillation waste from java citronella (*C. winterianus* Jowitt). When employed with *E. eugeniae*, the vermicompost experienced a lower C/N ratio (83.5%−87.7%), increased content of ash, and contained more macro and micronutrients. After 105 days of stabilization, the vermicompost's FT-IR spectroscopy showed a decrease in aliphatic and aromatic compounds and an increase in amide groups. While using *P. excavates*, the vermicompost demonstrated a 5.8-fold drop in the C/N ratio and a 5.6-fold improvement in ash content. Also, the vermicompost's nutritional content (N, P, K, Ca, and Mg) had increased by 1.2−4.1 times from their original level, and in comparison, with the initial level of the biowaste materials, the vermicompost's FT-IR spectra showed a rise in nitrogen-rich chemicals and a reduction in aliphatic/aromatic compounds (Deka et al., 2011a,b).

4.12 Lignocellulosic green waste of *Saccharum spontaenum*

Lignocellulosic green waste constitutes variable quantities of cellulose, hemicellulose, and lignin as their major components and small amounts of extraneous components like protein, fats, oils, pectin, ash, wax, inorganic compounds, and some extractive substances like phenols, terpenes, and resins (Himmel et al., 1994). One of the lignocellulosic organic substrates that are regarded as troublesome terrestrial weed is *Saccharum spontaenum* which has spread throughout the world. *S. spontaenum* was found to contain 45.10% cellulose and 22.75% hemicelluloses (Chandel et al., 2009). On-farm vermicomposting holds a good example for managing such lignocellulosic waste in the context of green waste management. Devi and Khwairakpam (2020b) conducted various studies on the effectiveness of vermicomposting of *S. spontaenum* mixed with CD to find an optimal ratio for producing nutrient-rich vermicompost. Vermicompost trials were carried out in vermireactors referred to as Ref1, Ref2, Ref3, Ref4, and Ref5 in five different mixing ratios of *S. spontaenum* amended with CD in ratios 3:7, 4:6, 5:5, 6:4, and 7:3. Vermicomposting was done for 45 days with only one earthworm feeding. The physio-chemical characteristics observed in the result were as follows: The final C/N ratio ranged from 10 to 16, with Ref1 showing the biggest drop. The greatest rate of earthworm growth was noted in Ref2, where the net biomass of earthworms changed by 34.25% in percentage. The final vermicompost of Ref3 had a maximum TKN of 2.95% and the highest TOC loss of 31.4%. The study also revealed that *Eisenia fetida* is the ideal choice for biodegrading lignocellulosic weed material.

4.13 Cassava peel waste

Despite being nutrient-rich, the skins of the bitter cassava (*Manihot utilissima*) root, which is a significant source of carbohydrates for people living in the tropics, generate toxic wastes that can kill soil invertebrates and hinder root development. However, studies have demonstrated that earthworms, *Eudrilus eugeniae* (Eug), have the ability to partially detoxify hazardous waste, multiply on them, and transform the peels into beneficial vermicompost biofertilizers. Mba (1996) examined the effects of three agricultural wastes—CD, guava leaves, and poultry droppings—on *E. eugeniae* fecundity and biomass production during cassava peel vermicomposting (Cas), as well as the effectiveness of the resultant vermicomposts as bio-fertilizer in cowpea is grown field sites, in order to maximize the output of cassava peel biofertilizer. It was found that Cas vermicompost increased the production of cowpea aerial biomass but increased the acidity of the soil. The other added components, such as Cag (guava leaves), Caco (cow dung), and Capo (poultry droppings), had different contributions to this optimization. Capo enhanced cowpea aerial biomass and soil P availability. Caco successfully maximized Cas vermicomposting by dramatically increasing Eug fecundity and biomass production, while Cag increased Eug fecundity and reversed the negative effects of Cas on cowpea seed output. In addition, Cag improved soil buffering ability, neutralized Cas's acidifying effect, and encouraged earthworm diversity and activity in cowpea plots. Thus, it can be concluded that the treatment with guava leaves optimized the resourcefulness of cassava peel wastes.

4.14 Banana crop waste

Bananas are the second most produced crop after citrus and are grown worldwide in subtropical and tropical regions. Around 120 countries worldwide cultivate bananas, which produce 86 tonnes of crop waste per hectare (Mayadevi et al., 2017). The entire banana plant, including the leaves, stems, and rhizome, is packed with several macronutrients, micronutrients, and essential amino acids. After the fruits are harvested, the waste biomass is either burned on-site or dumped in agricultural fields, where it might take several months to degrade, causing negative environmental effects. Vermicomposting comes as an effective mechanism to rescue the vital nutrients that can be lost during such processes. Khatua et al. (2018) employed *E. fetida* to analyze the physical and chemical modifications that occurred as a result of the vermicomposting period of banana stem waste (BS) spiked with various quantities of CD. After 60 days of vermicomposting, all treatments showed a progressive increase in the plant nutrients (P, Ca, K, Mg, and Fe). The final compost contained nitrate, as indicated by the FT-IR's strong N—O stretching vibration and rising BS content. Achsah and Prabha (2013) determined the effectiveness of vermicompost employing waste from Banana peel and *Eudrilus eugeniae* earthworms. All of the macronutrients, including NPK, enzymes (amylase, cellulase, and invertase), and micronutrients like Fe and Cu, revealed higher amounts in vermicompost than the control (raw waste). Following 30 days of growth, the vermicompost treatment on tomato plants showed the greatest root, shoot, and leaf lengths when compared to the control and the plant treated with chemical fertilizer (NPK 19:19:19). While Mago et al. (2021) documented biomass from vermicomposted banana crop residue using earthworm *E. fetida* and cow dung as the bulking agent. Six vermireactors with varying proportions of CD and waste banana leaf biomass (BL) were used for the experiment, which lasted 105 days. Earthworm activity dramatically decreased the wastes' pH, TOC, C/N, and C/P ratios. However, following vermicomposting, the amount of both macronutrients and micronutrients increased. Thus, vermicomposting can be a part of the entire waste management strategy for the banana crop.

4.15 Sugarcane trash

The most abundant agricultural residue generated worldwide is sugarcane trash and bagasse, which represents the primary solid waste products from the manufacturing of sugar and ethanol. The trash is made up of dried and green sugarcane tops and leaves, while the fibrous residue left over from recovering sugar juice through crushing and distilling is known as sugarcane bagasse. In tropical and subtropical nations like India, sugarcane is an important cash crop. India produces 270 million tonnes of sugarcane annually on average, which generates numerous by-products like pressmud, bagasse, and sugarcane residue. Although some of these by-products can be used to make molasses and alcohol, there is still a sizable volume of garbage that needs to be taken care of (Kumar et al., 2010). A bulk of this garbage is either typically burned in the fields after harvest, or is not recovered from the field. The garbage comprises typical lignocellulosic substances including 18%–20% lignin, 25% hemicellulose, and 40% cellulose (Singh et al., 2008) which can be effectively converted into humus- and plant-nutrient-rich organic manure. Vermicompost of sugarcane trash combined with leaf litter and cow dung, using epigeic earthworms *E. fetida*, *Drawida willsi*, *P. ceylanensis*, and *P. encavatus*, and microbial treatment, has been studied. The results in the worm and microbes worked substrates indicated a rise in electrical conductivity, total Kjeldahl Nitrogen, Ca, Mg, Fe, Cu content, and microbial activity, and a marginal decrease in phosphorous and potassium, while a substantial drop in C/N ratio and TOC over worm and microbes un-worked substrates, over 60 days (Karmegam et al., 2012; Kumar et al., 2010).

4.16 Wetland plant waste

Wetland plants have experienced significant growth, which has caused environmental issues like hypoxia and the extinction of aquatic life. Biomass from these plants, especially growing in heavy metal contaminated areas poses a huge threat to the ecosystem, as they constitute an accumulation of hazardous organic chemicals. By acting as organic amendments, vermicomposting these nutrient-dense plants will increase soil fertility and crop yield while lowering environmental concerns. Raza et al. (2022) have studied the varying effects of vermicomposts made from various wetland plants like *Hydrocotyle vulgaris*, *Canna indica*, *Acorus caalmus*, and *Cyperus alternifollius*; combined with 40% pig manure and 60% waste residues, on the fertility of the soil and plant growth for 3 months, and found that *Canna indica* vermicompost (CiV) application led to the highest soil total nitrogen (one-fold percentage increase) and soil organic matter (two-fold percentage increase) values for the four species of wetland plants after growing maize for 2 months, suggesting that the organic remediation of CiV may enhance soil fertility.

4.17 Crop residues

Organic wastes produced by agricultural practices include crop residues and animal waste. Recycling these wastes can provide plant nutrients and enhance the soil's physical properties and the environment's quality (Mishra et al., 1989; Bhardwaj, 1995). During a 90 days composting trial, Bansal and Kapoor (2000) studied vermicomposting of mustard residues and sugarcane waste combined with cattle manure using the earthworm *E. foetida*. Dehydrogenase assay results showed that microbial activity peaked after 60 days and then began to decline. The compost created by earthworm inoculation contained a higher total N. The variations, nevertheless, were not significantly different. Compost made using earthworm inoculation did not contain more total P, K, or Cu than compost made using uninoculated treatments.

To address the issue of decomposition of lignocellulosic waste, particularly over the winter, Singh and Sharma (2002) conducted preliminary tests using a wheat straw to investigate the technical feasibility of a composting system that includes bioinoculants and subsequent vermicomposting. By inoculating the straw of wheat along with various combinations of *Aspergillus niger, Trichoderma harzianum*, *Pleurotus sajor-caju*, and *Azotobacter chroococcum*, the substance underwent pre-decomposition for 40 days. Vermicomposting was done following this for 30 days. The samples' chemical examination revealed that cellulose, hemicellulose, and lignin concentrations reduced significantly after vermicomposting and pre-decomposition. N, P, and K concentrations significantly rose during pre-decomposition using bioinoculants. Chemical analysis revealed that the substrate was processed with all four bioinoculants simultaneously, accompanied by vermicomposting, to create the best compost possible.

4.18 Coffee pulp

One of the primary concerns with conventional coffee processing plants, or "beneficos," is waste disposal. One tonne of dry de-hulled coffee requires the use of four tonnes of water and about three tonnes of byproducts. The largest of these by-products, coffee pulp, is created during the "wet process" when coffee beans are removed using "depulpers" from fresh fruit. A good source of humus and organic carbon is coffee pulp solids. If you turn coffee pulp into a pile of regular compost every few days, it will decompose in 3 weeks. Earthworms can speed up the process of turning coffee pulp and many other materials into excellent vermicompost for use in farming and urban applications (Thakur et al., 2021; Aranda and Barois, 2000). Orozco et al. (1996) assessed the ability of the earthworm *Eisenia fetida* to transform coffee pulp to produce beneficial compost. Investigations were done on how bed depth and length affect different C fractions, N content, and nutritional availability. The findings demonstrated that while time altered both C and N contents, bed depth did not affect either. During vermicomposting, low levels of humic-like compounds were observed, as well as a higher fractionation ratio, calculated by dividing the amount of carbon in the fraction less than 100 m by the total amount of carbon in the samples. After the earthworms consumed the pulp, it was found that P, Ca, and Mg availability increased, but K availability decreased.

4.19 Oil palm empty fruit bunch

For the past few decades, the Malaysian palm oil industry has been a major source of lignocellulosic waste. The oil palm plantations covering 5.87 million hectares of land that make up its entire agricultural biomass waste account for more than 90% of the total. To produce 1.0 t crude palm oil, a palm oil mill requires roughly 5.5t of fresh fruit bunch (FFB). 28% of the trash produced is empty fruit bunch (EFB), 24% is fibers, 6% is the shell, 3% is decanter cake, and 3% is palm oil mill effluent. These EFBs separated from the FFBs contain lignin, cellulose, and hemicelluloses, and are usually burned, contaminating the air. The volume of this EFB biomass can be effectively reduced by converting it into organic fertilizer through composting. An excellent example of a compost-based fertilizer is vermicomposting, which is purely organic, and comprises phosphorous, potassium, organic nitrogen, organic carbon, sulfur, vitamins, hormones, enzymes, and antibiotics to help enhance crop quality and yield. Sabrina et al. (2009) ran an experiment to find out how vermicomposting of EFB from oil palm affected the nutrition availability for crops. EFB and oil palm frond were employed for vermicomposting using cow dung as the feeding source for the earthworms. Results revealed that vermicompost had higher TN, TP, and TCa content at its highest earthworm density (Mahmud and Chong, 2021; Gandahi and Hanafi, 2014).

4.20 Water hyacinth and *Salvinia* sp

One of the world's most challenging weeds, water hyacinth primarily threatens the basis of sustainability. It originated in the South American Amazon basin and has since spread worldwide. In their study, Goswami et al. (2017) used staggered blocks with five different types of treatments—farmyard manure (FYM), inorganic fertilizer (NPK), FYM + NPK, vermicompost (VC + NPK), and water hyacinth drum compost (WHDC + NPK)—to evaluate the growth of the tomato and cabbage crops and the quality of the soil. It was observed that the best biofertilizer combinations for tomatoes and cabbage were WHDC + NPK and VC + NPK; however, the water hyacinth compost exhibited a considerable buildup of metal in the plants. Earthworms (*E. fetida*) have various enzymes and gut microorganisms that help with metal biotransformation and absorption that protects the metals in the organism's tissues rather than bringing them back to the biofertilizer as worm castings, which causes a considerable decrease in the metal content when earthworms are present in vermicompost.

Efficient vermicomposting of *Salvinia natans* is a promising approach for preserving wetlands affected by the weed, since the *Salvinia* species is highly invasive. However, the prevalence of harmful metals in the weeds might deter use. Singh and Kalamdhad (2016) studied the physico-chemical, biological, and bioavailability and leachability of nutrients and heavy metals (i.e., Zn, Cu, Mn, Fe, Ni, Pb, Cd, and Cr) while vermicomposting five different combinations of *S. natans* mixed with cattle manure and sawdust. Trial one involved (eight *S. natans*: one cattle manure: one sawdust), trial two comprised (seven *S. natans*: two cattle manure: one sawdust), trial three constituted (six *S. natans*: three cattle manure: one sawdust), trial four involved (five *S. natans*: four cattle manure: one sawdust) and trial five comprising of (10 *S. natans*: 0 cattle manure: 0 sawdust), using earthworm *E. fetida* for 45 days. Results showed that trial four developed the greatest population of earthworms, having the largest percentage of cattle dung (40%). Trial four also showed the greatest decrease in soluble biochemical oxygen demand (BOD, 82.3%) and volatile solids (38.6%). Additionally, there was a considerable rise in all nutrients' water-soluble forms. After the procedure, there was a favorable reduction in the DTPA-extractable and highly accessible water-soluble forms of heavy metals. The toxicity characteristic leaching procedure test revealed that the amount of leachable heavy metals in the vermicomposts had decreased and was now below the acceptable range for agricultural purposes.

5. Different properties of plant agro waste compost

Farmers suffer a tremendous loss as agricultural trash makes up almost 16% of the industrial sector's annual total waste production. Through the combined efforts of earthworms and microorganisms, organic farming recycles agricultural waste by turning unavailable plant components into ones that increase soil nutrition (Edwards and Fletcher, 1988). The range of nutrients found in vermicompost made from agricultural wastes relies on the number of lignocellulosic components in the waste, and the pace of mineralization is influenced by the C/N ratio. According to a study by Hadas et al. (2004), the biochemical composition, particularly the amount of soluble carbon, controls the early pace of residue degradation. Still, the amount of more resistant components, such as lignin, controls the medium to the prolonged breakdown of additional carbon. According to previous research, the overall N concentration or the plant waste C/N ratio will decide whether N mineralization or immobilization predominates throughout its breakdown (Santos et al., 2021), and residues commonly exhibit rapid rates of mineralization and breakdown when they have high N content and low lignin/N and C/N ratios (Raiesi, 2006). There have been examples of agro-industrial waste being decomposed using vermicompost, such as treating palm oil with the earthworm *E. eugeniae*, which significantly decreased the C/N ratio (0.69%–79%) (Gupta et al., 2019). Vermicomposting fruit and vegetable wastes will enable sustainable organic farming to maintain a healthy soil profile while fostering plant growth.

5.1 Biocidal properties of plant compost

Plants consist of many biocidal components, mainly secondary components, responsible for providing resistivity against biotic stress. Secondary metabolites with biocontrollable qualities include saponin and terpene-rich plant components (D'Addabbo et al., 2014). Compost from such green waste can lessen pathogenic infection (Milinković et al., 2019). Composted olive waste has been shown to have antibacterial activities in response to certain soil pathogens (*Pythium ultimum*, *Fusarium oxysporum*, *Verticillium dahliae,* and *Sclerotinia sclerotiorum*) by Alfano et al. (2011). In a recent study, Milinković et al. (2019) reported that the effectiveness of compost tea made from green waste manure against harmful fungal species (*Rhizoctonia* sp., *Fusarium oxysporum,* and *Pythium debaryanum*) was primarily due to some biochemical substances like protease, chitinase, lipase, and β-1,3 glucanase. Rai and Suthar (2020) conducted an antimicrobial test on composted *Parthenium* biomass. They found that nonsterilized compost extract has excellent biocidal action against the plant diseases *Xanthomonas campestris*, *Xanthomonas citrus*, and *Erwinia carotovora*. Additionally, the humic substance produced by plant compost enhances the texture and quality of the soil, making it appropriate for crop production (Bertoncini et al., 2008).

5.1.1 *Bacterial pathogen inhibition by* **Lantana** *compost*

Lantana camara is considered one of the 100 most invasive alien hazardous weeds (Sharma et al., 1988). It can damage a region's biological dynamics by reducing the diversity of the local species and evading cultivation (Gerber et al., 2008). Recent research has found that compost weeds have the potential to supply enough nutrients for plants to grow more successfully, while their phytochemical qualities make them resistant to pathogens (Rai and Suthar, 2020). Because of the presence of essential oils and other bioactive chemicals as well as their chemical makeup, the entire plant, but particularly the leaves of *L. camara*, has been utilized as compost. Ganjewala et al. (2009) stated that secondary metabolites and oil contents of the plant vary between the plant and its parts based on genetic and regional differences as well as the different stages of inflorescence and leaf maturity. *L. camara* releases allelochemicals or the secondary metabolites secreted by nearby plant parts and the rhizosphere due to the breakdown of plant wastes, leachates, rainfall, root exudation, etc. According to Rajbanshi and Inubushi's (1997) report, *L. camara* compost leaves have a high nutritional content. Devi and Khwairakpam's (2020a) study found that *L. camara* leaf biomass could also be used to make high-nutrient manure that is acceptable for agronomic uses. This was accomplished by employing earthworms to create manure with high levels of plant nutrients. A survey report revealed that *Lantana* biomass could be used to create biofertilizers for eco-friendly farming practices. Agar well diffusion bioassay of an experiment demonstrates the potency of lantana-based compost against several pathogenic microorganisms, including *Xanthomonas campestris*, *Salmonella* sp., *E. carotovora*, *Pseudomonas aeruginosa*, and *Xanthomonas citri* (Rai et al., 2021). In their experiment, Rai et al. (2021) also concluded that *L. camara*, when combined with fresh cow dung in a ratio of 2:1, exhibited biocidal capabilities and provided nutrients to the plant without impairing crop seed germination.

5.1.2 *Tea-based compost inhibits the growth of* **Rhizoctonia solani** *in potato plants*

Compost is a biocontrol product that demonstrates resistance to diseases caused by many pathogens. According to experimental data, several water-based compost solutions are used to treat plants to suppress and prevent the spread of plant diseases. The composting of tea leaves typically involves one of two methods: either introducing oxygen via a pore-filled sack to the water compost hanging over an exposed tank, known as aerated compost teas (ACTs), or fermenting the water and other elements in the composted tea to create nonaerated compost teas (NCTs). The NCT approach is more cost-effective and energy-efficient and has greater evidence of demonstrating antagonistic qualities against the pathogen than the ACT method, which is more focused on reducing manufacturing time. The antagonistic property of compost tea is used mainly in suppressing soil-born fungal disease (Scheuerell and Mahaffee, 2002). Black scurf and Rhizoctonia canker, both caused by *Rhizoctonia solani*, are fungal infections that have a serious impact on potato output. Mengesha et al. (2017) explained the physio-chemical characteristics of NCTs and the method to prevent the growth of the phytopathogen in an experiment where they employed the NCT against this pathogenic fungus that was born in the soil. He noted that in his experiment, the compost from vineyards had a 12.8% C/N ratio, with 29.8% carbon and 2.32% nitrogen, and a pH value of roughly 7.6, which is regarded as neutral. The C/N ratio in the commercially generated organic compost was 13.2%, and the pH was kept at 7.5. Due to the nearly identical pH of both parent forms, the microbial content in both types of compost was almost similar. Various microorganisms can be found in compost tea, and their antagonistic traits prevent the spread of disease. They concluded that the composition of biotic elements is essential in suppressing the phytopathogen. *R. solani*'s mycelial development in potato plants has been inhibited by the NCTs in both commercially generated organic compost and compost made from vineyard waste. Therefore, the water-based compost promotes greater plant growth, and the phytochemicals offer resistance to the targeted disease.

5.2 Vermicompost's impact on various crop yields

Vermicompost is a unique, environmentally friendly soil amendment that modifies the nutrient profile of the soil. The coelomic fluid, which the earthworm secretes, is antipathogenic and guards against phytopathogens. The earthworm and microbial communities are combined during this non-thermophilic vermicomposting process. The earthworm's castings promote the plant's growth and development and increase agricultural productivity. Although the usage of chemical fertilizers rose following the green revolution (the 1960–1980s), the resultant soil, water, and air pollution have harmed the ecosystem.

Vermicompost encourages the quantity of macro- and micronutrients like N, C, P, and K. In an experiment, crop seeds with a greater germination rate were discovered when incorporated with vermicompost matter (Atiyeh et al., 2000a). In an experiment, Mohammad et al. (2011) discovered that utilizing vermicompost organic matter caused *Matricaria chamomilla* to grow taller. Compared with the control, the potato's height increased when the soil was amended using vermicompost. Although the growth of plants varies with the different vermicompost compositions, it is mainly due to the variation in the ratio of growth-promoting nutrients obtained by the plant from the soil (Azarmi et al., 2008). Compared to the controlled sample, the phosphorus-rich vermicompost aids in boosting the root volume of plants like *Setaria* grass (Sabrina et al., 2013). It is estimated that adding vermicompost and soil in a 4:1 ratio lengthens plant roots. When it comes to the development of shoots, a mixture of 60% vermicompost, 30% sand, and 10% soil increases the weight of the shoot in *Tagetes* (Shadanpour et al., 2011). Compost with nitrogen promotes plant development and increases the leaf area index, which increases light absorption (Ravi et al., 2008). After being treated with vermicompost, the cucumber plant produced more leaves, increased chlorophyll content, and greater dry leaf weight (Azarmi et al., 2008). After treatment with vermicompost, crops like *Brassica rapa*, *Sorghum bicolor*, *Lilium* plant, and *Lycopersicum esculentum* had higher leaf counts, leaf area indices, and chlorophyll contents than the control plant. The root length increases due to a rise in the positive activity of microbes and decreased infection in the compost soil. This increases total leaf area, photosynthetic rate, nutrient uptake, and water uptake. This general improvement in the plant aided in healthy fruit growth and proper inflorescence development. The marketable yield of commercially significant crops increased because of this change in plant behavior.

6. Conclusion

Earthworms and bacteria are used in vermiremediation to inadvertently remove toxic substances from waste materials. To improve soil health and crop output, it also supports sustainable crop recycling due to the higher concentration of growth hormones and key soil enzymes. Vermicomposting and vermiremediation, which utilize the four composting mechanisms of the earthworm species—vermiaccumulation, vermiextraction, vermitransformation, and drilodegradation—to break down organic waste, are the two fastest-growing remediation methods. The enzymes inside the earthworm gut partially digest the cast that earthworms excrete into the soil, enhancing soil texture and quality. Additionally, the microbial community nitrifies the soil. The remediation of plant waste aids in increasing the population of microbes and contains an enormous amount of high biocidal components in plant compost. Proper soil aeration has demonstrated higher soil chlorophyll content, fostering the growth and development of roots and shoots and increasing the production of secondary metabolites in agricultural plants. Vermiremediation can be enhanced further using several techniques, such as including surfactants, soil amendments, applying agronomic methods, and developing biomass.

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CHAPTER

6

Biochemical alterations of vermicompost produced from *Eichhornia crassipes* (water hyacinth) and cattle dung

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1. Introduction

Composting is becoming an essential component of agricultural productivity for safe and eco-friendly technology. Compost has a positive impact on soil properties with an enhancement of water percolation, nutrient availability and soil aeration facilitating plant growth (Domínguez et al., 2019; Domínguez and Gómez-Brandón, 2012; Mckenzie et al., 2022). Composting is a process performed mainly by microorganisms (bacteria, fungi) and invertebrates (earthworms, beetles) (Edwards et al., 2013; Arjune and Ansari, 2022). The organic material is reduced into smaller components by composting with a slow process of degradation resulting in a reduction of carbon to nitrogen ratio. There is a slow release of nutrients with the use of compost in soil (Curry and Boyle, 1987; Ansari et al., 2022).

Different types of organic waste that can be degraded (farm waste, kitchen waste, vegetable waste from the market, agro-waste from industries, waste generated from the livestock industry, etc.) are the feedstock for recycling through vermicomposting (Ansari et al., 2022; Yatoo et al., 2022). The earthworms consume these types of wastes and hereby reduce the volume by 40%–60%. Each earthworm consumes waste equivalent to its body weight (0.5–0.6 g), and the cast produced is equivalent to about 50% of the waste consumed in a

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day. The castings have a moisture content of about 32%–66% and a pH of around natural (7.0) (Adhikary, 2012).

Several researchers have reported that the nutrient contents of vermicompost varies between: organic carbon (9.15%–17.98%), nitrogen (1.5%–2.2%), phosphorus (1.8%–2.2%), and potassium (1.0%–1.5%) with added availability of micronutrients like Sodium (Na), Calcium (Ca), Zinc (Zn), Sulfur (S), Magnesium (Mg) and Iron (Fe) (Adhikary, 2012; Ansari and Kumar, 2010). It is also reported to be rich in plant growth promoters (auxins and cytokinins), enzymes, vitamins, essential microbes (*Actinomycetes, Nitrosomonas, Azotobacter*), protozoans and fungi. These are important in conversion of organic matter to final product that is completely composted to fine humus like material (Jaikishun et al., 2014; Arjune and Ansari, 2022).

Large quantity of organic waste generated in agricultural fields by farmers can be recycled through vermicomposting and can offer solution to waste disposal and generation of useful product that can be sued for crop production (Arjune and Ansari, 2022; Singh et al., 2022). Such ecofriendly technologies can be economically and ecologically sustainable and viable (Domínguez et al., 2019). Major industries that contribute to environmental pollution are sugar and rice mills thereby causing air, water and soil pollution. There is increase in carbon dioxide in atmosphere which is the critical in contributing to climate change and global warming along with particulate pollution that affects human health. These have negative impact on soil organic matter, physico-chemical properties of soil and reduction on microbial biomass (Livan and Thompson, 1997; Alves et al., 2022). With all the reasons on effects on climate change, organic farming has become important toward soil health, sustainable food security and human health (Diver, 1999; Ansari et al., 2022).

The advantages of using vermicompost over natural composting are that the latter takes much longer (nearly 1 year) while using worms takes about 45–50 days. Natural composting produces lots of heat with a high number of microorganisms together with an unpleasant odor (Ismail, 1997; Ansari et al., 2022).

Organic waste is recycled and converted to nutrient-rich vermicompost by the process of vermicomposting using composting earthworms. There are many techniques of composting and vermicomposting used by gardeners where they utilize organic waste generated from garden and kitchen waste (Domínguez and Gómez-Brandón, 2012). Other practitioners make use of boxes containing kitchen waste for composting which helps to reduce the organic waste by one-third and the final product (vermicompost) generated is used for the cultivation of plants in pots and roof gardens for the production of healthy food. This vermicompost is enriched with earthworm casts (Addison, 2009; Ismail, 1997; Ansari et al., 2022; Joshi et al., 2022).

Vermicomposting has many benefits. The detritus material passes through the gut of the earthworm, which has microflora responsible for degrading the organic material. It is also mixed with gut mucous. The earthworm caste excreted contains highly enriched microbiota compared to unprocessed organic waste. It is devoid of any harmful pathogens. The microorganisms promote plant growth. This is one of the critical roles of the vermicomposting process (Addison, 2009; Domínguez et al., 2019; Alves et al., 2022). The nature of organic material determines vermicompost quality and nutrient content (Ismail, 1997; Ramnarain et al., 2019).

Earthworm cast is highly enriched with nutrients like nitrogen, phosphorous, and potassium, along with essential micronutrients and beneficial soil microorganisms. These facilitate the growth of plants in soil amended with worm caste. It is also rich in humic acid that neutralizes soil pH and is a highly effective soil conditioner. It is also known to contain plant growth promoters, as in seaweed (Ansari et al., 2020; Domínguez et al., 2019; Ramnarain et al., 2019; Ramos et al., 2022).

Earthworms are oligochaetes belonging to the phylum Annelida. They are classified in the order Opisthopora based on the presence of male pore opening outside of the body posterior to the female pore (Edwards and Bohlen, 1996; Ismail, 1997; Ansari and Ismail, 2012; Alves et al., 2022). Earthworm species are known to exist in a wide range of environmental conditions across the globe, with the species numbers recorded to be approximately 4400 (Rajendran and Thivyatharsan, 2014). The ecological classification of earthworms is based on feeding and burrowing behavior. There are categorized into epigeic (surface earthworms that feed on surface litter and detritus organic material), anecic (subsurface earthworms feed on organic matter and organic matter in soil within the burrows) and endogeic (earthworms deep within the soil layers feeding on organic matter in soil). Epigeics and anecics are known for vermicomposting (Ansari and Ismail, 2012; Nair et al., 2019; Alves et al., 2022; Ansari et al., 2022).

Local species of earthworms are advantageous in the light of competing behavior between indigenous species and exotic species (Kale and Krishnamoorthy, 1982; MacKenzie, 1991). They do not coexist comfortably in mixed cultures, indicating competition among the species of earthworm food (Abbott, 1980), which is not desirable and disturbs natural biodiversity (Ismail, 1995). The earthworms have been known to be decomposers, referred to as the "intestines of the earth" by Aristotle (4th century BCE) and Darwin 200 years ago (Ramsay and Hill, 1978; Siddique et al., 2022).

Impact of earthworms on soil physical structure:

➢ Turnover: a large quantity of soil from deep layers is brought to the surface and deposited as casts which will assist in modifying the overall structural properties of soil on a long-term basis (Kale and Krishnamoorthy, 1982; MacKenzie, 1991; Siddique et al., 2022).
➢ Soil particle breakdown: vermicast consists of fine humic soil along with some sand particles mixed with mucus, facilitates the soil to be enriched for easy uptake by plants (Ismail, 1997; Moonilall et al., 2020)
➢ Soil aggregation: the burrowing activity and cast production of earthworms contribute to soil aggregation contributing to soil stability. The burrows are lined with earthworm cast containing humic matter and clay components (Ansari and Ismail, 2001; Ansari et al., 2022; Siddique et al., 2022).
➢ Aeration, porosity, and drainage: aeration is improved by burrowing activity but also influences the soil's porosity, while water filtration is faster (4–10 times) in soils with earthworms than barren soils (Kale and Krishnamoorthy, 1982; MacKenzie, 1991; Siddique et al., 2022).

Effect of earthworms on soil chemical processes:

➢ Nutrient distribution: earthworms consume large amounts of surface litter and detritus material. They move the organic matter into the subsurface layer of the soil, and while processing these materials, they are ingested, macerated, and excreted (Garag and Ramesh, 2006; Alves et al., 2022).
➢ Base exchange capacity: the soil in earthworm burrows lined by vermicast has a higher base-exchange capacity and is highly enriched with organic matter, total exchangeable bases, phosphorous, exchangeable K, Mn, and Ca (Garag and Ramesh, 2006; Alves et al., 2022).
➢ Microbial activity: earthworms do not ingest all of the materials that they usually take into the subsoil; a small amount is taken into the digestive system (Lee, 1985). The organic matter consumed is broken down, mixed with mucus and inorganic minerals while passing through the gut and excreted as vermicast. Microbial biomass and activity are facilitated in vermicast that makes nitrogen available in soluble form without the loss of any protein-leaching process (Ismail, 1997; Alves et al., 2022; Ansari et al., 2022).
➢ Worm species can neutralize soil pH with special calciferous glands to create a more favorable environment for themselves (Dunn, 2004; Ansari et al., 2020).

Eisenia fetida and *Lumbricus rubellas* are two common species of earthworms used in vermicomposting. Normally the earthworm population is doubled in 30 days. The reproduction rate is faster in conducive environment (0.5 kg of worms can increase to 454 kg in a year) but in working conditions of vermicoposting process there is loss of cocoons at the time of harvest (Ansari et al., 2016, 2020; Edwards et al., 2013). Each mature composting earthworms produce 2–3 cocoons in a week that transforms into hatchlings after 3 weeks. These are half inch in size but grow very fast and attain sexual maturity in 4–6 weeks. The reproductive cycle continues (Addison, 2009; Ansari et al., 2016; Ismail, 1997). The rapid rate of earthworm's population is related to the conditions in vermicomposting units and supply of feed (Addison, 2009; Domínguez et al., 2019; Edwards et al., 2013; Ansari et al., 2020).

Worms are sensitive to variations in climate. The process of vermicomposting is facilitated between the range of 12–25°C. When the temperature is high, the vermicomposting bins should be kept in a shady area or can be done indoors that will avoid effects due to temperature changes (Domínguez and Gómez-Brandón, 2012; Ismail, 1997; Ansari et al., 2020). Earthworm breeding is promoted by optimal conditions and required feed material (organic waste). The earthworms would be alive and healthy. They enhance the soil properties through their activity of burrowing and depositing earthworm casts along burrows. The porosity of the soil is aided, which is helpful for water percolation, aeration, and plant root development (Kladivko, 2001; Ansari et al., 2020). The organic matter is broken down in the earthworm gut while feeding on detritus material and soil. The different fractions are well mixed and are excreted as cast. The burrowing activity facilitates the movement of soil from the subsurface to the surface (Edwards et al., 2013; Edwards and Bohlen, 1996; Ramnarain et al., 2019; Ramos et al., 2022). One of the important earthworms used in organic waste management is *E. fetida*, followed by *E. andrea*, due to their tolerance for wide fluctuations in temperatures (Edwards et al., 2013; Edwards and Bohlen, 1996; Sheppard, 1988). The reproductive and composting capacity of these composting earthworms is similar (Reinecke and Viljoen, 1991).

The experiments conducted on the effect of vermicompost as an amendment on the growth parameters of paddy by Ansari and Ismail (2008) indicated a positive effect on soil quality (physicochemical parameters) and yield compared with chemically treated and control experimental plots (Ansari and Ismail, 2008; Ansari et al., 2016). Vermitech was beneficial as technology in terms of cost-effectiveness for the cultivation of paddy. Organic amendments (vermicompost) resulted in an increase in soil organic matter with a positive impact on soil amelioration. These amendments are sustainable in the long term for soil and plant growth (Ansari and Ismail, 2008; Ansari et al., 2016, 2020; Domingo et al., 2012; Joshi et al., 2022).

The vermicast is highly rich in macro and micronutrients when compared to the soil due to the processing of detritus material in earthworm gut by the gut microflora and its mixing with mucus, thereby resulting in conversion into available nutrients (Kladivko, 2001). The presence of plant growth regulators in vermicompost adds value to plant growth (Arancon et al., 2003; Ansari et al., 2020). Several researchers have reported on the role of earthworms in soil improvement related to various properties that positively affect plant growth (Joshi et al., 2022; Makkar et al., 2022). But there are still gaps to be filled in terms of knowledge about the effect of earthworms on soil structural changes (Shipitalo and Le Bayon, 2004). The optimum temperature was determined to be 25°C with a tolerance range of 0–30°C and a pH between 5.0 and 9.0 (Edwards et al., 2013).

Many researchers suggest that vermicompost can promote and stimulate plant growth, root development and yield. Vermicompost is enriched with nitrogen (N), phosphorus (P), potassium (K) and micronutrients, with rich microbial biomass and enzyme activities (Manyuchi et al., 2018; Zaefarian and Rezvani, 2016; Joshi et al., 2022). They also contain plant growth promotors like auxins, cytokinins, gibberellins, and humic acids (Bhardwaj and Sharma, 2016; Gopal et al., 2012; Ansari et al., 2020). Humic acids are known to enhance root growth and increase root absorption of nutrients due to an increase in root cell permeability (Makkar et al., 2017; Joshi et al., 2022).

Research with brinjal (Sundararasu and Jeyasankar, 2014), pepper (Luján-Hidalgo et al., 2016), tomato (Kaur et al., 2015), and gladiolus (Karagöz et al., 2019) found that vermicompost significantly enhances the plant growth parameters and yield. They also result in early flowering and fruiting (Makkar et al., 2017), which is beneficial for farmers. The production is also uniform, and the fruits ripen uniformly (Makkar et al., 2017). In contrast, it is also reported that high doses of vermicompost result in poor plant growth due to the toxic effect of excessive nutrients and humic acids absorbed (Makkar et al., 2022). Research comparing chemical fertilizers showed that the best plant growth and production were reported for the chemical fertilizer, but the organic fertilizer (vermicompost) had significant results (Bhardwaj and Sharma, 2016; Joshi et al., 2022).

The plants with vermicompost had also less pest and disease incidence in comparison to the chemical fertilizer. Some research has reported no pests and insects observed, which means that the organic fertilizers (depending on the feed used) have a biopesticide effect (Samadhiya et al., 2013; Verma et al., 2019; Joshi et al., 2022). There is also suggested that the fruits or crops obtained from the organic fertilizers (vermicompost) have a better quality and nutritional value with a longer shelf life (Verma et al., 2019).

2. Materials and methods

2.1 Work site

The vermicomposting units were set up (in a shady area) at the University of Guyana, Turkeyen Campus, Georgetown, Guyana (South America). All experiments were carried out on campus. The collected samples were analyzed at the Guyana Sugar Corporation, La Bonne Intention, East Coast Demerara.

2.2 Setting up units

Plastic buckets were used to set up six (6) units. Each unit was set up with a layer of broken bricks at the bottom. A layer of sand was placed above the bricks, and this was followed by a layer of garden soil (Figs. 6.1–6.4). This experiment was performed three times (three cycles).

- **Cycle 1**: Leaves of *Eichhornia crassipies* were picked and left to air dry for a day. Leaves moisture content was determined, then weighed and separated into two 200 g parcels and two 100 g parcels. Dried cattle dung was collected and prepared the same way as the leaves.
- **Cycle 2**: The experiment was repeated a second time with few changes to the method. The total amount of material added was the same as the first cycle (400 g). This amount was added in three proportions (unlike cycle 1, where there were two additions). This was done during the first week of the experiment. The first addition was on Monday of the first week, in which 114 g of material was added. Two days later, there was a 200 g addition, followed by an 86 g addition at the end of the week. The number of worms added to the experimental units (2, 4, and 6) increased from 80 in the first cycle to 125 in cycle 2. Units 1 (Fig. 6.6), 3, and 5 were the control in the experiment. Fifty earthworms of the local species *E. fetida* (Fig. 6.5) were added to units 2 (Fig. 6.7), 4 (Fig. 6.8), and 6 (Fig. 6.9). Each unit was sprinkled with approximately 200 mL of water three

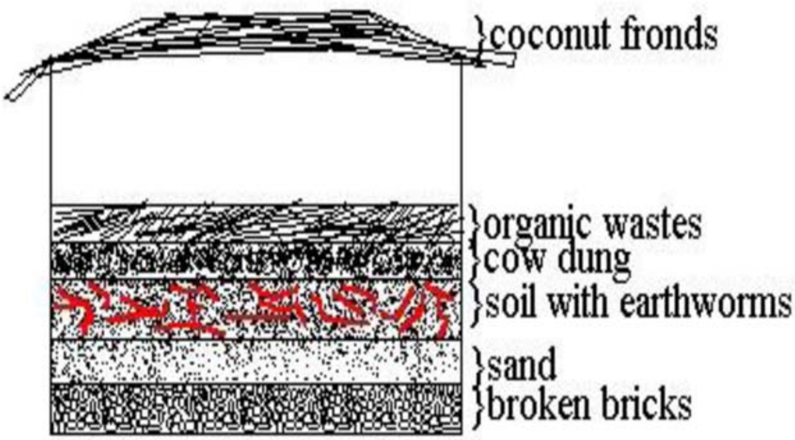

FIGURE 6.1 Setting up vermicompost units.

FIGURE 6.2 Bricks at the bottom of units.

FIGURE 6.3 Sand over the bricks.

FIGURE 6.4 Garden soil over the sand.

FIGURE 6.5 *Eisenia fetida*.

FIGURE 6.6 Unit 1.

FIGURE 6.7 Unit 2.

FIGURE 6.8 Unit 4.

FIGURE 6.9 Unit 6.

times weekly. After the first month, another 30 earthworms were added to the experimental units (2, 4, and 6), amounting to 80 worms each. After 10 days, another 200 g of material was added to each unit. The material was added in the same amount and proportion as the first (1st) that was added.
- **Cycle 2**: The experiment was repeated a second time. However, there were a few changes to the method. The total amount of material added was the same as the first cycle (400 g). This amount was added in three proportions (unlike cycle 1, where there were two additions). This was done during the first week of the experiment. The first addition was 114 g of material, and 2 days later, there was a 200 g addition, followed by an 86 g addition at the end of the week. The number of worms added to the experimental units (2, 4, and 6) increased from 80 in the first cycle to 125 in cycle 2.

2.3 Data collection and analyses

The number of worms was counted at the end of each cycle. The temperature was checked and recorded at 10-day intervals. Samples were collected every 30 days starting from the beginning of the experiment. A sample was collected from each unit. The last sample was collected 120 days into the experiment. Samples were left to air dry after collection. After drying, they were stored in polythene bags.

The samples were analyzed for the following—pH, electrical conductivity, organic carbon, *TKN* (*total Kjeldahl nitrogen*), nitrogen, phosphorus, potassium, magnesium, and calcium—following the protocol by Homer (2003).

3. Results and discussion

The number of worms collected from cycle 1 was less than the amount that was originally introduced. During the second cycle, the researcher noted that worms were coming out from the bottom of the units. It was assumed that the holes were probably too big at the bottom of these units. However, it was soon realized that the reason for this was that the water content was too high. It was immediately reduced. During the third cycle, the researcher sprinkled a small amount of water, enough to keep the soil moist and keep the worms in the units. After looking at the experiment and making observations, it was noted that the researcher sprinkled a lot of water the first time. A mesh was used to cover the unit in cycles 1 and 2. The materials were at the top of the units and got dry very fast due to air exposure. The soil was moist, and in trying to keep the materials moist, the environment became too wet for the worms. Already into the third cycle and not yet finished with the second, the researcher tried to find a solution to these problems. Thus, the units in cycle 3 were covered in such a way that little air was allowed to enter. It was noted days later that the leaves did not dry out so fast. The units with different treatments were kept moist (Table 6.1).

The highest temperatures were seen in units 2, 4, and 6. These three units had earthworms present, and thus, there was increased microbial activity, which led to higher temperatures (Edwards, 2004; Ansari et al., 2022). The lowest temperature was seen at the beginning of units 1 and 3. The greatest deviation in temperature was seen in unit 5. There was

TABLE 6.1 Treatments.

Units	Treatment
U1	200 g water hyacinth
U2	200 g water hyacinth + 50 earthworms
U3	200 g cattle dung
U4	200 g cattle dung + 50 earthworms
U5	100 g water hyacinth +100 g cattle dung
U6	100 g water hyacinth +100 g cattle dung + 50 earthworms

temperature fluctuation in all the units. There were mostly decreases and small increases in temperature. This was because of environmental conditions. The units were small; thus, their temperature reading was affected by the outside temperature. The optimum temperature is between 25 and 27°C (Table 6.2). The initial sample of water hyacinth had a higher Mg, K and N content than the initial sample of cattle dung. Its electrical conductivity was also higher (Table 6.3).

TABLE 6.2 Temperature change in each unit.

	Temperature (°C)					
Days	**U1**	**U2**	**U3**	**U4**	**U5**	**U6**
1	25.5 ± 0.68	26 ± 0.89	25.5 ± 1	25.6 ± 1.04	26.5 ± 2.33	25.9 ± 1.38
10	27.1 ± 2.46	27 ± 1.76	26 ± 1.73	26 ± 1.52	26 ± 1.73	26.3 ± 1.44
20	27 ± 2.59	27 ± 2.03	26 ± 2.21	26 ± 2.47	26 ± 2.64	26 ± 2.17
30	26.8 ± 1.25	27 ± 1.06	25.8 ± 1.07	25.9 ± 0.90	25.7 ± 0.93	25.9 ± 0.96
40	27 ± 1.80	27.5 ± 0.86	27 ± 0.86	26 ± 1.58	26.7 ± 0.92	27 ± 1.32
50	27.6 ± 0.17	27 ± 1.30	26 ± 1.22	26.5 ± 1	26.5 ± 1.21	27.3 ± 2.02
60	27.1 ± 1.89	27 ± 1.58	27 ± 0.79	27 ± 1.06	26.8 ± 1.25	27 ± 0.97
70	27 ± 0.51	27 ± 0.72	26.9 ± 0.12	27.5 ± 0.50	26.7 ± 0.26	27.3 ± 0.29
80	26.6 ± 0.76	26 ± 0.5	25.7 ± 0.64	26.3 ± 0.46	26 ± 0.31	26.6 ± 0.3
90	26 ± 0.79	27 ± 1.27	26 ± 0.83	26.6 ± 0.63	26.4 ± 0.92	26.6 ± 1.04
100	26.8 ± 0.29	26.8 ± 0.29	26.5 ± 0.36	27 ± 0.38	26.8 ± 0.29	27.6 ± 0.58
110	26.3 ± 1.15	26.5 ± 1.17	26.5 ± 0.86	26.5 ± 0.86	26.6 ± 1.15	27 ± 1.15

TABLE 6.3 The nutrient concentration of the initial samples of raw materials used.

Parameters	Cattle dung	Water hyacinth
EC	3.36 ± 0.03	6.9 ± 0.01
pH	7.00 ± 0	7.0 ± 0.13
OC (%)	2.90 ± 0.26	2.9 ± 0.13
N (%)	1.46 ± 0.12	1.62 ± 0.17
C:N	1.99	1.22
P (ppm)	381.60 ± 6.41	311.2 ± 2.70
K (ppm)	22.01 ± 0.36	36.04 ± 0.73
Ca (ppm)	10.69 ± 0.45	10.01 ± 0.79
Mg (ppm)	19.64 ± 1.75	23.02 ± 0.34

3.1 Electrical conductivity

Unit 2 had the greatest change in electrical conductivity, followed by U1, then U5 and U6. There was a decrease in these units (Tables 6.4 and 6.6). These were the units containing water hyacinth. This was followed by an increase in the EC of U3 and a lesser increase in U4.

TABLE 6.4 The nutrient content after first (M1) and final months (MF) and the percentage increase or decrease over time.

	Water hyacinth			Water hyacinth + earthworms		
	Nutrient content			Nutrient content		
Parameters	Initial	Final	% change	Initial	Final	% change
EC	6.21 ± 1.42	3.33 ± 1.88	−46.4	6.62 ± 2.88	28 ± 0.57	−46.4
pH	8.0 ± 00	7.62 ± 0.53	−4.6	8.5 ± 0.70	6.57 ± 0.6	−22.6
OC (%)	2.30 ± 1.55	1.7 ± 0.28	−26	5.45 ± 0.21	1.51 ± 00	−72.2
N (%)	1.51 ± 1.44	1.48 ± 0.58	−2	1.96 ± 1.61	0.67 ± 0.58	−65.6
C:N	1.52	1.14	−25	2.78	2.3	−17.2
P (ppm)	296.1 ± 36.76	255.1 ± 43.98	−10.4	344.1 ± 19.14	290.8 ± 59.11	−15.4
K (ppm)	39.97 ± 23.23	16.32 ± 8.13	−59	41.28 ± 14.14	18.8 ± 12.02	−54.4
Ca (ppm)	11.03 ± 1.48	7.751 ± 5.58	−29.7	10.53 ± 0.82	2.83 ± 5.23	−38
Mg (ppm)	1.04 ± 4.30	15.83 ± 9.64	−31.2	40.05 ± 33.02	27.09 ± 18.10	−32.3

TABLE 6.5 The nutrient content after first (M1) and final months (MF) and the percentage increase or decrease over time in cattle dung and cattle dung with earthworms.

	Cattle dung			Cattle dung + earthworm		
	Nutrient content			Nutrient content		
Parameters	Initial	Final	% change	Initial	Final	% change
EC	2.54 ± 0.14	3.07 ± 1.49	20.6	1.94 ± 0.12	2.0 ± 0.13	2.8
pH	7.5 ± 0.70	6.65 ± 0.49	−11.3	7.0 ± 0	6.55 ± 0.64	−6.4
OC (%)	3.05 ± 2.75	7.5 ± 0.36	−39.4	6.65 ± 4.03	7.5 ± 4.8	12.7
N (%)	0.61 ± 0.19	0.60 ± 0.08	−1.39	0.46 ± 0.10	0.80 ± 0.22	73.9
C:N	5	3.06	−32	14.46	7.5	−45.4
P (ppm)	343.1 ± 11.31	242.8 ± 91.64	−29.2	333.4 ± 25.10	387 ± 7.91	4.6
K (ppm)	14.41 ± 4.18	10.0 ± 0.2	−30	9.34 ± 3.15	8.65 ± 0.64	−7.8
Ca (ppm)	10.38 ± 0.29	7.75 ± 3.43	−42.5	9.12 ± 0.42	5.63 ± 4.48	−39.5
Mg (ppm)	12.55 ± 3.24	16.74 ± 2.66	33.3	10.15 ± 1.29	8.39 ± 7.61	−17.3

TABLE 6.6 Nutrient content after first (M1) and final months (MF) and the percentage increase or decrease over time.

	Water hyacinth + cattle dung			Water hyacinth + cattle dung + earthworm		
	Nutrient content			Nutrient content		
Parameters	Initial	Final	% change	Initial	Final	% change
EC	4.39 ± 0.45	2.86 ± 0.86	−34.8	2.86 ± 0.24	1.99 ± 1.46	−30.4
pH	7.5 ± 0.70	6.33 ± 0.94	−15.6	7.5 ± 0.70	6.6 ± 0.55	−12
OC (%)	1.25 ± 0.35	1.01 ± 0.01	−76.5	4.3 ± 3.37	4 ± 2.8	220
N (%)	1.21 ± 0.22	1.06 ± 0.49	−12.3	1.075 ± 0.98	1.33 ± 0.79	24.2
C:N	1.03	0.95	−7.7	4	2.99	−25.25
P (ppm)	308.6 ± 28.10	322.8 ± 93.05	4.6	409.3 ± 11.73	529.3 ± 197.5	29.3
K (ppm)	29.61 ± 1.13	11.25 ± 1.2	−61	47.32 ± 7.29	44.99 ± 49.5	−4.9
Ca (ppm)	11.53 ± 0.61	6.05 ± 5.11	−47.4	11.04 ± 0.39	9.292 ± 1.89	−15.8
Mg (ppm)	18.27 ± 4.43	17.94 ± 0.06	−1.8	15.73 ± 0.30	16.28 ± 1.03	3.4

Therefore, the plant material has a higher EC than animal waste (cattle dung). It is more stable in terms of conductivity than either U2 or U4. Electrical conductivity decreased in all units with water hyacinth. However, it increased in U3 and U4 (cattle dung) (Table 6.5). Water hyacinth has less electrical conductivity than cattle dung, and vermicompost has less EC than compost (Wang et al., 2021).

3.2 pH

The greatest change in pH was seen in U2, followed by U5, U6, U3, U4 and U1. The vermicomposted plant material and plant material + cattle dung had a higher pH change. These changes were brought about to achieve a more stable pH (Table 6.6). Their calciferous glands reduced all the pH in an effort to stabilize (Kale and Krishnamoorthy, 1982; Singh et al., 2020; Sinha et al., 2009; Alves et al., 2022).

3.3 Organic carbon

Organic carbon content was highest in U4 and U6. However, there was a greater increase in U6 than in U4. The vermicompost of the mixture of water hyacinth and cattle dung had the most organic carbon. The vermicompost of water hyacinth had less nitrogen than the compost. However, the vermicompost of cattle dung and also cattle dung + water hyacinth had more nitrogen than their respective composts. This is because the worms released the nitrogen faster, so there was less nitrogen in the vermicompost (Ansari et al., 2020; Domínguez and Gómez-Brandón, 2012; Pisaheba et al., 2013). The vermicompost in unit 6 and compost in U1 had the highest nitrogen (Table 6.4). There was a great change in U6, U5, followed by the U2, U3, U1, U4. Organic content, however, was increased in U6 and U4 (Tables 6.5 and 6.6). The organic content was lowered in the other units due to bacterial decomposition (Edwards, 2004; Ramnarain et al., 2019). Earthworms accelerate the decomposition of organic matter. Therefore U2, U4, and U6 should have had a greater decrease. However, earthworm activity may have been inhibited by one or more factors.

3.4 Nitrogen

Nitrogen was reduced greatly in U2, followed by U5, U3, and U1. It was increased in U4 and U6. Nitrogen was reduced due to NH_3 volatilization incorporation into the earthworm tissue (Domínguez and Gómez-Brandón, 2012; Edwards, 2004; Ji and Brune, 2006). In U4 and U6, the increase was probably caused by the release of NH_3 gas and other nitrates released (Tables 6.5 and 6.6).

3.5 Phosphate

There was a great change in U2, then U5, followed by U6, U4, U3, U1. The phosphate increased in U1, U2, and U6 due to mineralization and mobilization by bacteria (Ji and Brune, 2006; Krishnamoorthy, 1990). The decrease in U3, U4, and U5 was due to leaching and incorporation into the soil. A great increase was seen in unit 2, then unit 1 (Table 6.4). These are plant materials, and the vermicompost unit had greater phosphate. Moreover, in U6, the vermicompost with cattle dung and water hyacinth had a greater increase than the vermicompost with only leaves (Table 6.6).

3.6 Potassium

Potassium decreased in all the units. It was incorporated into the soil or leached (Krishnamoorthy, 1990). The greatest change was in U5 followed by U1, U2, U3, U4, U6. Potassium content was highest in U6, followed by U2 (Tables 6.4 and 6.6). Therefore, the vermicompost of water hyacinth + cattle dung has the most potassium. Moreover, potassium is more readily released in cattle dung; therefore, the compost of cattle dung has a higher nutrient content than the vermicompost (Ansari et al., 2020; Domingo et al., 2012; Pisaheba et al., 2013). The vermicompost of water hyacinth had the most magnesium. This was followed by the compost and vermicompost of (cattle dung + water hyacinth) respectively. Vermicompost of cattle dung had the lowest magnesium content. The C:N ratio fell progressively. This decreased due to the combustion of carbon substances during respiration. The greatest decrease was in U4, followed by U3 (Table 6.5). These were the vermicompost and compost of cattle dung, respectively. This was followed by U6, U1, U2, and U5. It was also due to the incorporation of derived plant organic matter (water hyacinth) and its transmission throughout the gut of the earthworm (Chaudhuri et al., 2000; Alves et al., 2022).

3.7 Calcium

Calcium decreased in each unit. The greatest decrease was in U5, followed by U3, U4, U2, U1 and U6. The calcium is released during composting and distributed or leached into the soil, where it forms other compounds. There was less change in the units containing water hyacinth (Krishnamoorthy, 1990).

3.8 Magnesium

The greatest change was in U3, followed by U2, U1, U4, U6, U5. The decrease in U1, U2, U4, and U5 was due to the release of Magnesium into the soil (Krishnamoorthy, 1990). The vermicompost had higher phosphate content than the compost (U2, U4, U6) (Tables 6.4–6.6). The vermicompost of U6 had higher phosphate than U2 or U4. However, U4 had more than U2. The vermicompost had less calcium than compost in all two cases, U2 or U4 (Tables 6.4 and 6.5). This is because the worms broke down the calcium and released it into the soil faster. It could have been leached off from the surface. Unit 6 (vermicompost) had the highest calcium content because of its combined contents (Table 6.6) (Ansari et al., 2020; Domingo et al., 2012; Pisaheba et al., 2013). The pH decreased in all units. The vermicompost all had between 6.55 and 6.6 pH range. The worms stabilized their environment (Johnson, 1971; Singh et al., 2020). The compost had a wider range, 7.62 to 6.33. Earthworms maintain a stable pH (Wang et al., 2021; Wen et al., 2004). Their metabolic activities produce carbon dioxide, which decreases the pH of their body fluid. Their calciferous glands produce carbohydrate anhydrase, which catalyzes carbon dioxide into calcium carbonate to counteract the reaction (Kale and Krishnamoorthy, 1982; Singh et al., 2020; Sinha et al., 2009; Alves et al., 2022).

3.9 Economic analysis

The cost of setting up the vermicomposting units along with the organic input was 250 Guyana dollars (1.2 USD) for 60 days. The production of vermicompost was to the tune of 4.8 kg per cycle. The cost of production was 52 Guyana dollars per kg (0.25 USD kg^{-1}). The calculation is based on Unit 6 treatment. The economic cost analysis showed that vermicompost production is cost-effective.

4. Conclusion

Vermitechnology is an effective method of recycling organic waste material. The study expounded that water hyacinth can be recycled by vermicomposting technique and can be converted to useful organic product-vermicompost that is rich in essential nutrients needed for plants. The vermicompost of cattle dung had the lowest C:N ratio, followed by water hyacinth and cattle dung together. The vermicompost of cattle dung and water hyacinth was rich in nutrients and hence can be suitable organic fertilizers to enhance plant growth and productivity. Such cost-effective technology can be useful for large-scale implementation that would enhance the recycling of the organic waste causing environmental pollution, thereby resulting in useful products like vermicompost for sustainable organic agricultural production.

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CHAPTER

7

Use of vermicompost and vermiwash for the growth and production of tomatoes (*Lycopersicon esculentum* Mill.): A case study in Suriname

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1. Introduction

Urbanization and industrialization have resulted in the generation of huge quantities of waste material that pose serious issues for disposal and contribute to climate change. This organic waste from a wide variety of sources can be recycled using effective waste management technologies that could resolve problems by producing useful products such as vermicompost and vermiwash. The use of vermiwash and vermicompost can facilitate crop cultivation, reducing dependence on agricultural chemicals and transforming the agriculture sector toward sustainability and an organic revolution (Bhat et al., 2018a,b; Alves et al., 2022; Ansari et al., 2022; Bhagat et al., 2022; Siddiqui et al., 2022; Singh et al., 2022; Yatoo et al., 2022).

Ansari and Ismail (2012) classified earthworms under the phylum Annelida and subclass Oligochaeta. Ecologically, earthworms can be divided into three categories, depending on how they eat and dig their burrows. Epigeics eat decaying organic matter and live on the surface litter. These decomposers are used in vermicomposting. Anecics move detritus material and enriched soil in the tunnels; they are used for feeding. Endogeics feed on soil with decaying material lining the tunnels (Ansari and Ismail, 2012; Nair, 2019; Alves et al., 2022; Ansari et al., 2022; Siddiqui et al., 2022).

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Vermicomposting employs a variety of earthworms in varying quantities and with a different types of organic waste. *Eisenia foetida* earthworms from the Lumbricidae family are frequently used in vermitechnology (Manyuchi, 2016; Bhat et al., 2018a,b; Alves et al., 2022; Ansari et al., 2022; Bhagat et al., 2022; Singh et al., 2022; Yatoo et al., 2022). This earthworm (Nair, 2019) is referred to as the red wiggler worm. It is segmented, and groups of setae on each segment assist the earthworm in locomotion. It has an anus at the end of its body and a sensory lobe (prostomium) in front of the mouth (Jim, 2017). Red wigglers are hermaphrodites (Shahnawaz et al., 2011). They reproduce by joining clitella, which are broad bands that are visible during fertilization. The two worms then release casings, which contain a few eggs. According to Ansari and Ismail (2012), the cocoons are lemon-shaped and initially pale yellow. As the earthworm matures, the color darkens to brown. *E. foetida* is most commonly used for vermicomposting owing to its widespread distribution, short life cycle, natural organic material colonization, wide tolerance range for temperature and moisture, and ease of handling (Ansari and Ismail, 2012; Alves et al., 2022; Ansari et al., 2022; Bhat et al., 2018a; Singh et al., 2022; Yatoo et al., 2022).

1.1 Vermicompost

Earthworms decompose natural waste to supplement-enriched earthworm casts through the combined activity of microorganisms, referred to as gut microbiota (Manyuchi, 2016; Bhat et al., 2018a,b; Alves et al., 2022; Ansari et al., 2022; Bhagat et al., 2022; Singh et al., 2022; Yatoo et al., 2022). Earthworms are critical to decomposition because they increase amelioration and break up detritus, which facilitates microbial activity. Earthworms change the physicochemical state of organic matter by lowering the carbon to nitrogen ratio, increasing the amount of surface area available for biochemical degradation by microorganisms (Manyuchi, 2016; Bhat et al., 2018a,b; Alves et al., 2022; Ansari et al., 2022; Bhagat et al., 2022; Singh et al., 2022; Yatoo et al., 2022). According to Dominguez and Edwards (2011), this has two phases:

a) Also known as the active phase, this phase occurs when earthworms process waste and alter its physical state and microbiological composition.
b) A mature stage occurs during which earthworms move into the new detritus material. This is followed by microbial decomposition.

All biodegradable waste from farms, kitchens, markets, agriculture-based industries biowaste, and livestock waste can be used to produce vermicompost. Earthworms consume this kind of waste, reducing its volume by 40%–60%. The waste that each earthworm consumes (0.5–0.6 g) is the same as its body weight, and the cast that is produced is about half of the waste that is consumed each day. According to Adhikary (2012), the castings have a pH of around neutral (7.0) and a moisture content of approximately 32%–66%.

According to the findings of several researchers, the organic carbon content of vermicompost ranges from 9.15 to 17.98, with an average of 1.5%–2.2% nitrogen, 1.8%–2.2% phosphorus, and 1%–1.5% potassium. According to Adhikary (2012) and Ansari and Sukhraj (2010), it also contains micronutrients such as calcium, iron, magnesium, sodium, sulfur, and zinc. Other than these supplements, it contains phytohormones including auxins and cytokinins, compounds, nutrients, and valuable microbes. *Actinomycetes*, *Azotobacter*, *Nitrosomonas*, and protozoans significantly changing organic detritus material to humus as a sweet-smelling fine soil amendment (Jaikisun et al., 2014; Bhat et al., 2018a,b; Alves et al., 2022; Ansari et al., 2022; Bhagat et al., 2022; Joshi et al., 2022; Yatoo et al., 2022).

1.2 Vermiwash

The movement of water through earthworm burrows in vermicomposting units results in the formation of brownish liquid known as vermiwash. When the liquid turns pale or brownish compared with the initial collection, the vermiwash is ready for harvest (Prabina et al., 2018; Arjune and Ansari, 2022). Vermiwash contains earthworm mucus and excretory products enriched with essential nutrients and phytohormones. It also contains essential enzymes, micronutrients, plant growth hormones, and NPK components in abundance (Manyuchi et al., 2013; Prabina et al., 2018; Verma et al., 2018; Arjune and Ansari, 2022; Bhagat et al., 2022). It has a variety of enzymes, including phosphatase, amylase, unease, and protease, which help the plant grow and develop and increase crop yield and productivity. According to Kaur and coworkers, microbial studies of vermiwash indicated that it is rich in phosphate-solubilizing bacteria as well as nitrogen-fixing bacteria such as *Acetobacter*, *Agrobacterium*, and *Rhizobium*, which improve soil health (Kaur et al., 2015; Arjune and Ansari, 2022; Bhagat et al., 2022).

1.3 Soil properties and impact of vermicompost and vermiwash

The biochemical properties of soil are significantly affected by the application of vermicompost and vermiwash. According to Ansari and Sukhraj (2010), using vermicompost and vermiwash as organic amendments significantly improves the physicochemical properties of soil (Tharmaraj et al., 2011; Bhat et al., 2018b; Ansari et al., 2022; Joshi et al., 2022). Available nutrients in the organic amendments slowly facilitate uptake by plants. Amylase, lipase, cellulase, and chitinase are enzymes found in vermicompost that can convert organic material into an available form for their slow release and uptake by the roots of the plant (Adhikary, 2012). Soil fertility is enhanced by applying vermicompost, which affects the water-holding capacity and makes the soil biologically and biochemically rich with nutrients (Bhat et al., 2018a,b; Prabina et al., 2018; Alves et al., 2022; Ansari et al., 2022). The foliar application of vermiwash and the use of vermicompost as a soil amendment improve soil nutrients and microbial biomass (Arjune and Ansari, 2022; Bhagat et al., 2022).

1.4 Impact of vermicompost and vermiwash on plant growth parameters and productivity

According to a number of studies, vermiwash and vermicompost can improve plant growth, root development, and yield. According to Manyuchi (2016), vermicompost and vermiwash are rich in NPK and other micronutrients and are enriched with effective microbes and enzymes (Zaefarian and Rezvani, 2016; Arjune and Ansari, 2022; Bhagat et al., 2022; Yatoo et al., 2022). In addition, they contain humic acids and phytohormones such as auxins, cytokinins, and gibberellins (Gopal et al., 2012; Bhardwaj and Sharma, 2016). According to Makkar et al., 2017, humic acids improve root growth and the absorption of nutrients. Pepper (Luján-Hidalgo et al., 2017), eggplant (Sundararasu and Jeyasankar, 2014), tomato (Kaur et al., 2015), and gladiolus (Tamrakan et al., 2018) have been cultivated using vermiwash and vermicompost with significant effect on plant growth parameters. Vermiwash and vermicompost increased the crop yield and had a positive impact on plant growth. Moreover, the early development of flowers and fruits is promoted (Makkar et al., 2017; Arjune and Ansari, 2022), which serves farmers' interests. The production and ripening of the fruits are consistent (Makkar et al., 2017). On the other hand, the excessive use of vermicompost has a negative impact on plant growth because of toxic humic acids and excessive nutrient absorption (Makkar et al., 2017). According to Bhardwaj and Sharma (2016), studies comparing organic fertilizers with chemical fertilizers revealed that organic fertilizers (vermicompost, vermiwash, or their combination) produced significant results whereas chemical fertilizers had the best plant growth and production.

In addition, the incidence of pest and disease was lower on plants that received vermicompost and vermiwash compared with plants that received chemical fertilizer. According to some studies, no pests or insects were observed, which indicated that organic fertilizers have bio-pesticide effects (depending on the feed used) (Samadhiya et al., 2013; Verma et al., 2018). Verma and coworkers suggested that organic fertilizer-grown fruits and vegetables (vermiwash and vermicompost) had a higher nutritional value and a longer shelf life (Verma et al., 2018; Arjune and Ansari, 2022; Joshi et al., 2022; Bhagat et al., 2022; Yatoo et al., 2022).

1.5 Cultivation of tomato (*Lycopersicon esculentum* Mill.)

Tomatoes ares a widely consumed fruit and are a member of the Solanaceae family of nightshades. The round, oval, and cherry varieties of this crop are well-known, and they share the same nutritional qualities. Iron, phosphorus, vitamin A, and vitamin C are just a few of the minerals and vitamins tomatoes contain (Bhowmik et al., 2012). Hybrid tomato varieties have been developed to produce more tomatoes of higher quality and quantity, with resistance to diseases and pests. Compared with openly pollinated varieties, hybrid tomato varieties have numerous advantages. According to Opena et al. (2011), they typically mature earlier and more uniformly. There are two main types of tomato hybrids: determinant and indeterminate. Production is consistent and the tomatoes are larger in determinant tomato plants compared with indeterminate plants, and the size of the tomato is small with a lower yield. After the first harvest of fruits, the plants are less productive with little or no fruit because they do not continue to grow. This kind of tomato plant is used in factories that make tomato paste, and it requires a lot of tomatoes at once. In contrast, indeterminate plants continue to produce flowers and fruits throughout the growing season until they die. According to Ibsen and Dagma (2019), these kinds of tomato varieties should be suckled to produce fruits of higher quality and a greater size. The exact days of each stage may vary, depending on the variety and environmental factors such as temperature, humidity, soil type, and nutrient availability (Shamshiri et al., 2018). The number of days from cultivation to harvest for the main organic products differs from 45 to 100 days, depending on the developmental level of the cultivar (Shamshiri et al., 2018). Tomatoes can be grown under different soil conditions, provided that the soil is enriched with nutrients and has the proper structure. The pH of a tomato plant's ideal soil is 6.0–6.6, but most tomato plants are grown in low-pH soil.

Organic agriculture has become significant owing to the excessive use of chemical fertilizers and pesticides in the agricultural sector, which has resulted in poor soil health, reduced productivity, and an increase in the incidence of pests and diseases, contributing to environmental pollution. This research was carried out in the developing country of Suriname to explore the effect of organic inputs (vermiwash and vermicompost) produced from organic waste on the growth, development, and yield of tomatoes under greenhouse and field conditions.

2. Materials and methods

This research was carried out at the Anton de Kom University of Suriname from Mar. to Aug. 2018. There were two phases to the experiment. Using three different kinds of feed-dry grass clippings and neem leaves, the first phase consisted of producing vermiwash and vermicompost. The vermiwash was collected and analyzed after 60 days. Tomato plants (*Lycopersicon esculentum* Mill.) were grown in the second phase using vermiwash from the first phase, which contained the most nutrients in the field and greenhouse (Fig. 7.1).

FIGURE 7.1 Greenhouse and field experiments at Anton de Kom University of Suriname.

2.1 Vermiwash production

2.1.1 Earthworm collection

Earthworms (*E. foetida*) were obtained from the Anton de Kom University of Suriname's vermicomposting station (Fig. 7.2). A total of 450 earthworms, including juvenile, nonclittelate, and clittelate earthworms, were collected for the experiment (Fig. 7.3).

2.1.2 Establishment of vermiwash units

A hole was drilled in the bottom of 20-L plastic barrels to fix the tap to collect vermiwash (Fig. 7.4). The culture bed was set up according to the following steps:

Layer I: Broken bricks or blue metal stones (4.5 cm), followed by coarse sand (4.5 cm) were added on top to regulate water effectively.

Layer II: Moistened loamy soil (8 cm) was set on top of the basal layer. Each bucket contained 50 earthworms for this layer.

Layer III: Dry material (grass clippings, neem leaves, or a combination of them), as well as fresh and dry cattle dung, was added (4.5 cm), which made up the feed.

The unit was kept moist and the tap was left open for 60 days. On day 60, the tap was shut off and a bottle was hung from the barrel to act as a water sprinkler (Fig. 7.5). The bottle was filled with approximately 1 L of water to drip overnight into the vermiwash unit. The following day, vermiwash was collected by opening the tap. Subsequently, the process was repeated to collect vermiwash.

FIGURE 7.2 *Eisenia foetida* earthworm.

FIGURE 7.3 Collected earthworms of different age groups.

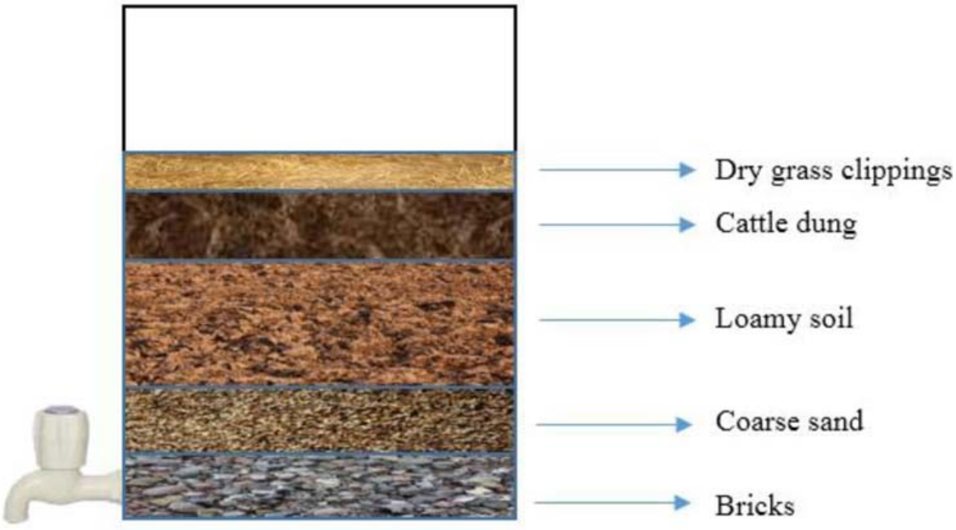

FIGURE 7.4 Culture bed of vermiwash bucket.

FIGURE 7.5 Vermiwash bucket with sprinkler.

2.1.3 Experimental design

The vermicomposting unit of Anton de Kom University of Suriname, which is located behind Building 7 in Leysweg, Paramaribo (Suriname), served as the setting for the experiment. There were three treatments and three replications in the nine-bucket experiment, in the order:

- T1: grass clippings (dry)
- T2: neem leaves (dry)
- T3: grass clippings and neem leaves (dry)

The units were added with the respective treatment feed two times each week into each of the experimental buckets. Every 2 days, the units were sprinkled with water.

2.1.4 Observation and measurements

Throughout the vermicomposting process, the temperature, moisture content, and pH of the units were measured every week up to day 90. The color changes of the vermiwash were observed and recorded until day 90 after 30 days.

2.1.5 Physicochemical analysis

At 60 and 90 days, chemical analyses were carried out on the vermiwash, the vermicompost from the various treatments, and the vermicompost (rice straw and cattle dung as feed). Using the procedures outlined in the soil laboratory's laboratory prescription, parameters were examined at the Anton de Kom University of Suriname: electrical conductivity (EC), cation exchange capacity (CEC), pH H_2O, organic carbon, NPK, and micronutrients (Ca, Na, and Mg).

2.2 Crop cultivation (tomatoes)

2.2.1 Experimental design

From Apr. to Jul. 2018, research involved crop cultivation (tomatoes). This was done as two separate experiments: potted greenhouse experiment (G) and field (pot) experiment (F). Trials were carried out using an Randomized block design (RBD) (in triplicate) in addition to four treatments. There were seven plants in each of the four rows that made up each block. Treatments involved plants receiving 100 g of fertilizer as the control (C); vermicompost (V): 100 mL of fertilizer applied to plants; and vermiwash (W): 50 g of fertilizer applied to plants vermicompost and 50 mL of vermiwash.

2.2.2 Sowing to transplanting

The Delhi 501 tomato variety's seeds were sown in potting soil in seed trays. The rate of germination was 96%. On May 1, 2018, 3 weeks after germination, the seedlings (Fig. 7.6) were transplanted to carry out the experiment. Shells and compost were used as the experiment's medium (1:1). At the beginning and end of the experiment, this mixture was tested for pH, CEC, OC, NPK, Na, Mg, and Ca. Vermicompost (rice straw and cattle dung as feed) from the vermicompost unit was used. The vermiwash used in the experiment with grass clippings and neem treatment had the highest nutrient content based on an analysis of vermiwash; this was used in the crop trials.

The following was done during the experiment:

1. The soil mixture was used to transplant the control treatment plants.
2. 100 g of vermicompost was added to the vermicompost treatment plant pots, and the tomato plants were transplanted subsequently.
3. 100 mL of vermiwash (Fig. 7.7) treatment was added to plant pots, and the tomato plants were transplanted subsequently.
4. 50 g of vermicompost and 50 mL of vermiwash was added to the third combined treatment, and the tomato plants were transplanted subsequently.

2.2.3 Fertilization

At transplantation, the tomato plants received fertilizer. Then, tomato plants received fertilizer every 2 weeks. Treatment involved fertilizing the plants with either vermicompost (100 g/plant), vermiwash (100 mL/plant), or a 50/50 mix of vermicompost and vermiwash. Vermiwash (100 mL) was added three times over 2 weeks. The vermiwash was applied to the leaves as a spray. The fertilizer was added on a total of four occasions. Table 7.1 displays the total amount of fertilizer applied to each plant.

FIGURE 7.6 Seedlings.

FIGURE 7.7 Vermiwash.

TABLE 7.1 Total amount of fertilizer added per plant.

Symbols	Treatment	Total amount added per plant
Control	Control (soil)	0
Vermicompost	Vermicompost	400 g
Vermiwash	Vermiwash	400 ml
Vermicompost–vermiwash	Vermicompost and vermiwash	200 g + 200 ml

2.2.4 *Data collection*

Using a temperature and relative humidity data logger, the greenhouse's temperature and humidity were measured. Climatic parameters for the field experiment were recorded from the Suriname meteorologic station. Up to the second harvest, measurements were taken once per week during the tomato plants' cultivation. We recorded the height of the plant (the distance from the plant's highest point to the ground), the stem thickness(the distance between the ground and the first branch of the stem), and the root length at the end of the crop trial. The biomass (wet and dry weights of the shoots and roots) were measured at the end of the trial. Data gathered to determine whether the vermiwash had an effect on production included the number of fruits per plant and weight of fruit per plant. The fruits were harvested when the color changed from green to yellow.

3. Results and discussion

3.1 Vermicompost: physicochemical properties

As depicted in Figs. 7.8 and 7.9, the vermicompost that was produced had excellent properties with humus-like fine composted material with soil-affecting properties (Ansari and Ismail, 2012; Maheswari et al., 2016; Bhat et al., 2018a,b; Alves et al., 2022; Ansari et al., 2022). The vermicompost had a fine, smooth texture, a desirable soil odor, and a dark color. Cocoons were also present in the bins for composting. The physicochemical properties of the vermicompost produced by vermiwash units and the vermicomposting unit (rice straw and cattle dung as feed) are listed in Table 7.2.

The highest and lowest pH values were 6.50 and 5.90, respectively, according to the results of vermicompost based on neem and rice straw as feed. According to Jaikisun et al. (2014) and Ramnarain et al. (2019), there was a decrease in pH during vermicomposting. Good compost had a pH that is slightly acidic (Jaikisun et al., 2014; Ansari et al., 2022). The production of carbon dioxide and organic acids that regulate the pH and buffer compost to neutrality is influenced by earthworm activity and microbial interaction during the vermicomposting (Kaushik and Garg, 2004; Alves et al., 2022; Siddiqui et al., 2022). The intensive mineralization of organic nitrogen into nitrate, nitrite, and ortho-phosphates, as well as the biotic conversion of organic matter into various intermediate materials, can also cause the

FIGURE 7.8 Vermicompost of bins fed with neem.

FIGURE 7.9 Vermicompost of bins fed with grass.

TABLE 7.2 Physicochemical properties of vermicompost.

	Treatment			
Parameters	**Grass**	**Neem**	**Grass + neem**	**Rice straw**
pH H_2O	6.30	6.50	6.30	5.90
Electrical conductivity (mS)	4.76	5.34	5.36	9.63
Cation exchange capacity (mEq/100 g)	37.67	47.19	42.82	40.51
Organic C (%)	16.46	24.19	28.70	16.85
Organic matter (%)	32.92	48.39	57.40	33.71
Total N (%)	1.41	1.77	1.61	1.35
Total P (%)	0.61	0.71	0.68	0.49
P Bray (%)	0.13	0.14	0.14	0.11
Total K (%)	0.26	0.29	0.25	0.22
Exchange K (ppm)	9.94	11.82	11.04	6.44
Total Ca (%)	0.57	0.66	0.56	0.78

TABLE 7.2 Physicochemical properties of vermicompost.—cont'd

	Treatment			
Parameters	Grass	Neem	Grass + neem	Rice straw
Exchange Ca (ppm)	11.70	24.58	19.94	31.69
Total Mg (%)	0.31	0.27	0.26	0.18
Exchange Mg (ppm)	5.94	11.12	9.48	7.60
Total Na (%)	0.04	0.03	0.03	0.04
Exchange Na (ppm)	0.35	0.74	1.38	0.86
C/N ratio	11.67	13.67	17.83	12.48

pH to drop. Because the nutrient volatilizes into ammoniac gas at an alkaline pH, a reduction in pH is critical to maintain nitrogen (Zarei et al., 2018). According to the findings, vermicompost made from rice straw had the highest EC (9.63 mS) and grass vermicompost had the lowest EC (4.76 mS). According to Zarei et al. (2018), EC depends on mineral salts and free ions. These are facilitated by gut activity and excretion by earthworms.

Vermicompost from grass and neem had the highest levels of organic matter and carbon (57.40% and 28.00%, respectively), whereas vermicompost from grass had the lowest levels (32.92% and 16.46%, respectively). Also, vermicompost from grass and neem had the highest C/N ratio (17.83), but vermicompost from grass had the lowest ratio. The degradation of organic waste resulted in a reduction in the C/N ratio, which is critical to the maturity of vermicompost compared with the initial organic waste material (Dominguez and Edwards, 2011). During humification, there is a loss of carbon dioxide owing to microbial activity and an increase in nitrogen because of excretion by earthworms. This results in a decrease in the C/N ratio, which is important for the maturity of vermicompost (Zarei et al., 2018; Alves et al., 2022; Ansari et al., 2022). The use of neem as feed in vermicomposting had significantly high levels of N (1.77%), P (0.71%), and K (0.14%) in vermicompost, whereas paddy straw—based vermicompost contained N (1.35%), P (0.49%), and K (0.11%). The nutrient content and quality of vermicompost depend on the type of organic matter used in vermicomposting (Kaur et al., 2015; Zarei et al., 2018). It has a pleasant smell, maintains a healthy pH, is low in electrical conductivity, has a high capacity for cation exchange, and has concentrations of readily available nutrients (Zarei et al., 2018; Alves et al., 2022; Ansari et al., 2022).

3.2 Vermiwash: physicochemical properties

Fig. 7.10 depicts the color change of vermiwash that was obtained from the vermiwash production units. According to Prabina et al. (2018) the maximum nutrient value of vermiwash was found when there was a change in color from transparent to pale yellow to brownish. The physicochemical properties of vermiwash collected from vermiwash units after 60 and 90 days are listed in Table 7.3. Vermiwash (grass + neem treatment, 70 ppm) had

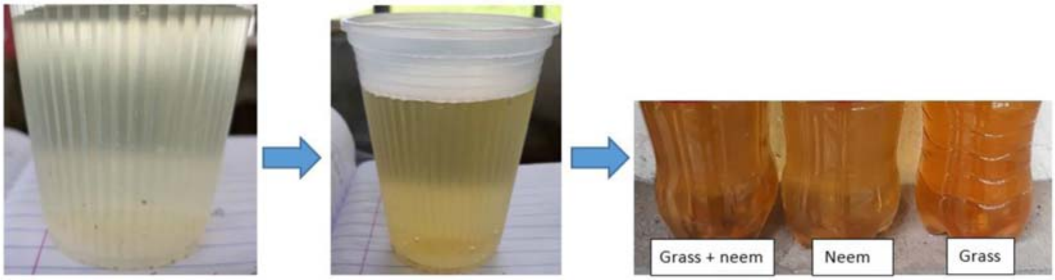

FIGURE 7.10 Color change in obtained vermiwash.

TABLE 7.3 Physicochemical properties of vermiwash harvested at 60 and 90 days of composting.

	Vermiwash					
	60 days			90 days		
	Treatment			Treatment		
Parameters	**Grass**	**Neem**	**Grass + neem**	**Grass**	**Neem**	**Grass + neem**
pH H_2O	7.2	6.9	7.3	7.4	7.5	7.3
Electrical conductivity (mS)	9.09	9.61	8.93	8.69	8.84	8.85
Total N (ppm)	212	256	216	246	303	236
Total P (ppm)	35	63	70	34.1	64.1	71.8
Total K (ppm)	1274.5	1148.9	1327.6	1278.6	1055.0	1056.0
Total Ca (ppm)	330.8	271.8	258.2	254.7	292.1	233.0
Total Mg (ppm)	362.1	313.0	210.5	307.0	280.2	239.4
Total Na (ppm)	376.9	373.6	245.2	343.5	355.4	294.6

higher phosphates, followed by vermiwash (neem treatment, 63 ppm). The nutrient contents were similar in vermiwash harvest at 60 and 90 days, which suggests that nutrients did not increase after 60 and 60 days and remained stable. Based on the findings, vermiwash (grass + neem treatment) was used in trials conducted on the cultivation of tomatoes.

Analyses of the vermiwash revealed the significant presence of nutrients, which agrees with other researchers (Ansari and Sukhraj, 2010; Kaur et al., 2015; Arjune and Ansari, 2022). The quality and nutritional status of vermiwash depend on the type of organic material used in vermicomposting. However, the nutrient content varied in vermiwash produced by different researchers owing to differences in the starting organic material (Kaur et al., 2015; Zarei et al., 2018; Arjune and Ansari, 2022).

3.3 Cultivation of tomato plants

3.3.1 Climatic conditions

The greenhouse data showed that the average temperature during the day was mostly between 29.50°C and 30.61°C, whereas during nights it was between 24.32°C and less than 24.89°C. Average humidity during the day was 77.81%, whereas it was 94.68% at night. The daytime temperature in the field was a maximum of 27.81°C and a minimum of 26.87°C, respectively, with a maximum humidity of 84% and a minimum humidity of 74.14%. The greenhouse had a higher average daytime temperature than the field, and the field had a higher average daytime humidity than the greenhouse. According to Jones, the daytime temperature should be 21−29.5°C, and the nighttime temperature should be 18.5-21°C (Shamshiri et al., 2018). This indicates that the greenhouse's daytime and nighttime temperatures were above the ranges, whereas the field's daytime temperatures were within the ranges. Throughout the entire growth period of tomato plants, the range of humidity was 50%−70%. Tomato pollination was boosted by around 60% relative humidity, according to some studies. However, it is typical for greenhouses to have a relative humidity range of 60%−90% (Shamshiri et al., 2018). Suriname has a relative humidity above 70%, which accounts for the deviation in relative humidity from the ideal requirement.

3.4 Soil: physicochemical properties

Table 7.4 lists the physicochemical properties of the soil initially and after the conclusion of the experiment. At the beginning of the experiment, the soil analysis revealed that the pH was slightly alkaline. The physical and chemical characteristics of the soil made it suitable for tomato cultivation.

Soil nutrients at the beginning and end of the experiment were not significantly different from one another. Soil amended with different treatments had slightly higher nutrient values for P, K, Ca, and Mg in the greenhouse at the conclusion of the experiment compared with the nutrient value of the initial soil. The GVW treatment had higher values in terms of all physicochemical properties. The field experiment showed roughly the same results, but the experiments were affected by rain, which leached nutrients from the soil. According to this research, the combined use of vermicompost and vermiwash was highly effective in improving soil quality, including enhancing microbiota (Ansari and Sukhraj, 2010; Tharmaraj et al., 2011). Vermicompost added to the soil contributed in terms of enzymes that degraded organic material and facilitated the release of nutrients from the soil, slowly increasing soil fertility and enhancing soil structural sustainability (Adhikary, 2012; Prabina et al., 2018).

3.5 Greenhouse experiment

3.5.1 Plant height

At harvest, the greenhouse's results indicated that the maximum height was 112.62 ± 4.33 cm and the minimum was 85.38 ± 7.37 cm. During treatment, VW had a maximum increase (98.69) and C showed a minimum increase (98.69 cm) (Table 7.5). Treated and control plants were significantly different ($P = .05$). In addition, V and VW plants

TABLE 7.4 Physicochemical properties of soil at beginning and end of experiment.

		End							
		Treatments							
Parameters	**Beginning**	**GC**	**GV**	**GW**	**GVW**	**FC**	**FV**	**FW**	**FVW**
pH H_2O	8.1	8.3	8	7.9	7.9	8.3	8.1	8.2	8
Electrical conductivity (mS)	2.4	2.13	3.02	2.63	3.08	2.34	2.98	2.72	3.11
Cation exchange capacity (mEq/100 g)	8.48	10.4	8.89	9.3	9.45	9.65	9.49	9.23	9.9
Organic C (%)	4.29	4.21	3.66	4.46	3.87	3.89	3.99	3.76	4.1
Organic matter (%)	8.57	8.42	7.32	8.92	7.74	7.78	7.99	7.53	8.2
Total N (%)	0.24	0.18	0.22	0.19	0.21	0.22	0.22	0.21	0.24
Total P (%)	0.01	0.02	0.03	0.04	0.04	0.02	0.04	0.03	0.05
P Bray C (ppm)	6.5	2	38	64	83	5	32	52	88
Total K (%)	0.05	0.07	0.07	0.09	0.09	0.07	0.09	0.09	0.07
Exchange K (ppm)	0.13	0.10	0.26	0.30	0.34	0.08	0.11	0.11	0.26
Total Ca (%)	6.21	7.55	7.38	8.82	9.00	6.26	8.97	7.84	6.37
Exchange Ca (ppm)	11.66	10.41	13.14	11.92	13.50	9.42	9.84	9.82	8.39
Total Me (%)	0.18	0.18	0.17	0.19	0.21	0.15	0.20	0.16	0.15
Exchange Fe (ppm)	3.66	2.80	4.20	4.25	5.23	2.57	2.96	3.03	2.60
Total Na (%)	0.36	0.31	0.38	0.41	0.42	0.31	0.33	0.27	0.27

F, field experiment; *G*, greenhouse experiment.

TABLE 7.5 Plant height (cm) and percent increase in greenhouse.

		Plant height (mean ± SEM)			
	Treatment	**Week 1**	**Week 10**	**Increase (cm)**	**Increase (%)**
Greenhouse	**Control**	11.40 ± 1.87	85.38 ± 7.37	73.98	87%
	Vermicompost	12.71 ± 1.76	100.90 ± 11.69	88.19	87%
	Vermiwash	12.79 ± 1.70	108.81 ± 11.16	96.02	88%
	Vermicompost—vermiwash	13.93 ± 1.33	112.62 ± 4.33	98.69	88%

TABLE 7.6 Stem thickness (cm) and percent increase in greenhouse.

	Treatment	Stem thickness (mean ± SEM)			
		Week 1	Week 10	Increase (cm)	Increase (%)
Greenhouse	Control	0.30 ± 0.03	0.77 ± 0.07	0.47	61%
	Vermicompost	0.34 ± 0.06	1.02 ± 0.06	0.68	66%
	Vermiwash	0.31 ± 0.04	0.97 ± 0.08	0.67	68%
	Vermicompost−vermiwash	0.34 ± 0.04	1.03 ± 0.09	0.68	66%

differed significantly ($P = .05$). W and VW plants did not differ significantly ($P = .148$). This was also true for V and W plants ($P = .175$). The W plants increased by 96.02 cm from weeks 1 to 10, whereas V plants increased by 88.19 cm (Table 7.5).

3.5.2 *Stem thickness*

At harvest, the results of the greenhouse experiment revealed that VW stem plants were the thickest (1.03 ± 0.09 cm) and C stem plants were the thinnest (0.77 ± 0.07 cm) (Table 7.6), each of which also saw a greater increase (0.68 cm) and minimal increase (0.47 cm) (Table 7.6). Significant differences were seen between treated and control plants ($P = .000$) based on the least significant difference (LSD) statistical test. In addition, W and V plants ($P = .021$) and VW and W plants ($P = .003$) differed significantly. VW and V plants ($P = .520$) did not differ significantly ($P = .520$). The VW and V plants were about the same thickness, but the W plants were thicker.

3.5.3 *Biomass and root length*

a. Fresh and dry weight of shoots

Table 7.7 show that the fresh and dry weights for different treatments differed significantly ($P = .000$). There was a greater average for fresh and dry weight of shoots (1107 ± 0.45 and 320 ± 0.40 g, respectively) for treatment W and lower for C plants (160 ± 4.04 and 83 ± 0.21 g, respectively) (Table 7.7). However, the greenhouse's moisture content was lowest for C plants (77 g) and highest for V plants (810 g) (Table 7.7).

TABLE 7.7 Fresh and dry weight of shoots (mean ± SEM) (g) and moisture content (%) in greenhouse.

		Shoot fresh weight (mean ± SEM)	Shoot dry weight (mean ± SEM)	Moisture content
Greenhouse	Control	160 ± 4.04 a	83 ± 0.21 a	77
	Vermicompost	1030 ± 0.80 b	220 ± 0.26 b	810
	Vermiwash	1107 ± 0.45 c	320 ± 0.40 c	787
	Vermicompost −vermiwash	1070 ± 0.70 d	286 ± 0.25 d	784

Values followed by different letters are significantly different at $P \leq .05$ according to least significant difference multiple range test.

TABLE 7.8 Fresh and dry weights of roots (mean ± SEM) (g) and moisture content (%) in greenhouse.

		Root fresh weight (mean ± SEM)	Root dry weight (mean ± SEM)	Moisture content
Greenhouse	**Control**	8.00 ± 1.73 a	4.67 ± 1.53 a	3.33
	Vermicompost	110 ± 17.32 b	46.67 ± 7.63 b	63.33
	Vermiwash	84.33 ± 3.79 b	19.33 ± 1.15 c	65.00
	Vermicompost −vermiwash	213.33 ± 77.67 c	90.00 ± 10.00 d	123.33

Values followed by different letters are significantly different at $P \leq .05$ according to least significant difference multiple range test.

b. Fresh and dry weight of roots

Control and treatment plants differed significantly ($P = .05$). The LSD test indicated that the fresh weight of roots differed significantly for V and VW plants ($P = .013$) and W and VW plants ($P = .004$) (Table 7.8). V and W plants did not differ significantly ($P = .453$). V plants had greater fresh weight compared with W plants. All treatments differed significantly in terms of root dry weight ($P = .05$) (Table 7.8). The fresh and dry weights were greater for VW plants, and C plants had the least weights. The moisture content was higher for VW plants (123.33) and the least for C (3.33) (Table 7.8). The root extension was longer in VW plants compared with V and W plants (Fig. 7.11).

c. Root length

The LSD test revealed that VW and V plants differed significantly ($P = .025$). Treated and control plants differed significantly ($P = .000$). There was a significant difference between W and VW plants ($P = .392$) compared with V and W plants (Table 7.9). VW plants had the longest average root length (97.67 ± 5.51 cm), followed by W plants (91.33 ± 8.08 cm), V plants (78.33 ± 14.01 cm), and C plants (38.67 ± 1.53 cm).

3.5.4 Production

After 3 weeks of transplantation, all VW plants showed flower initiation, followed by V plants (60% initiation) and W plants (40% initiation), caused by the application of amendments such as vermicompost and vermiwash. The C plants did not produce fruit during the experiment. All treatments differed from each other ($P = .05$) in terms of harvest and fruit weight, as determined by the LSD test (Table 7.10). In the greenhouse experiment, the VW plants had a greater yield per plant (16.52 ± 1.01), whereas the V plants had a lower yield per plant (9.38 ± 0.44). In addition, the V plants had the highest average fruit weight per plant (646.71 ± 68.09 g) and the lowest average fruit weight per plant (380.52 ± 31.88 g) (Table 7.10). Fig. 7.12 shows that the VW plants likewise had the greatest natural products.

3.6 Field trials

3.6.1 Plant height

The field trials revealed that the maximum plant height measured was 95.71 ± 9.32 cm for treatment VW and the minimum plant height measured was 80 ± 12.49 cm for Control

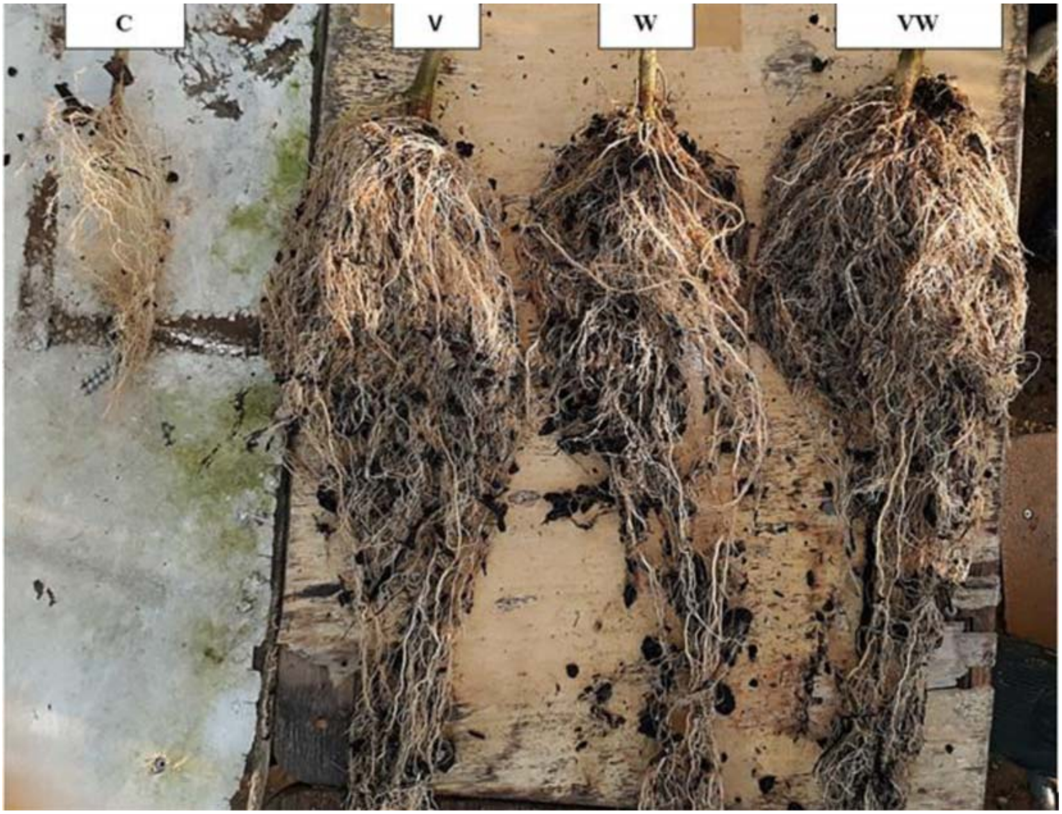

FIGURE 7.11 Root development of different treatments in greenhouse. *C*, control; *V*, vermicompost; *VW*, vermicompost–vermiwash; *W*, vermiwash.

TABLE 7.9 Root length (mean ± SEM) in greenhouse (cm).

	Root length (mean ± SEM)
	Greenhouse
Control	38.67 ± 1.53 a
Vermicompost	78.33 ± 14.01 b
Vermiwash	91.33 ± 8.08 be
Vermicompost–vermiwash	97.67 ± 5.51 c

Values followed by different letters are significantly different at $P \leq .05$ according to least significant difference multiple range test.

(Table 7.11), with W plants experiencing the greatest increase (78.45 cm) and C plants experiencing the smallest (65.48 cm) (Table 7.11). Treated and control plants differed significantly ($P = .05$). There was no major contrast between the treated plants ($P > .05$). However, the cultivation period was greater for VW plants followed by W and V plants.

TABLE 7.10 Harvest and fruit weight per plant (mean ± SEM) in greenhouse (g).

		Harvest per plant (mean ± SEM)	Fruit weight per plant (mean ± SEM)
Greenhouse	**Control**	0.00 ± 0.00 a	0.00 ± 0.00 a
	Vermicompost	9.38 ± 0.44 b	380.52 ± 31.88 b
	Vermiwash	13.38 ± 0.58 c	466.05 ± 17.41 c
	Vermicompost—vermiwash	16.52 ± 1.01 d	646.71 ± 68.09 d

Values followed by different letters are significantly different at $P \leq .05$ according to least significant difference multiple range test.

FIGURE 7.12 Difference in fruit diameters among treatments in greenhouse.

TABLE 7.11 Plant height (cm) and percent increase in field.

		Plant height (mean ± SEM)			
	Treatment	**Week 1**	**Week 10**	**Increase (cm)**	**Increase (%)**
Field	**Control**	14.52 ± 1.63	80.00 ± 12.49	65.48	82%
	Vermicompost	16.02 ± 2.10	89.43 ± 4.66	73.40	82%
	Vermiwash	15.17 ± 2.05	93.62 ± 9.33	78.45	84%
	Vermicompost—vermiwash	17.95 ± 1.87	95.71 ± 9.32	77.76	81%

TABLE 7.12 Stem thickness (cm) and percent increase in field.

	Treatment	Stem thickness (mean ± SEM)			
		Week 1	Week 10	Increase (cm)	Increase (%)
Field	Control	0.31 ± 0.03	0.75 ± 0.09	0.44	59%
	Vermicompost	0.321 ± 0.03	1.13 ± 0.09	0.81	72%
	Vermiwash	0.30 ± 0.02	1.15 ± 0.10	0.85	74%
	Vermicompost–vermiwash	0.35 ± 0.04	1.19 ± 0.10	0.83	70%

3.6.2 *Stem thickness*

The stem thickness of plants was 1.19 ± 0.10 and 0.75 ± 0.09, cm, respectively for C (maximum) and VW (minimum) (Table 7.12), but W had the largest increase (0.83 cm) and C had the smallest one (0.44 cm) (Tables 7.4–7.12). The LSD test revealed a significant difference ($P = .000$) between treated and control plants. The treated plants did not differ significantly ($P = .05$). The VW plants were the thickest during the cultivation period, followed by the W and V plants, with the W plants gaining the most (0.85 cm) (Table 7.12).

3.7 Biomass and root length

a. Weight of shoot (fresh and dry)

Various treatments differed significantly ($P = .000$) in terms of shoot fresh and dry weight (Table 7.13). The V plants had the highest average shoot fresh weight (1246 ± 0.20 g) and the C plants had the lowest (179 ± 0.4 g) (Table 7.13), and VW plants had the highest average dry weight (365 ± 0.26 g), and C plants the lowest (62 ± 0.42 g) (Table 7.13). However, the moisture content was maximal for the V plants (930 g) and minimal for the C plants (117 g) in the field experiment (Table 7.13).

b. Fresh and dry weight of roots

W and VW plants differed significantly ($P = .05$) for the fresh weight of roots. Treated and control plants also differed significantly ($P = .05$) for the fresh weight of roots. These inferences were drawn from the LSD test. V and W plants ($P = .643$) and

TABLE 7.13 Shoot fresh and dry weight (mean ± SEM) (g) and moisture content (%) in field.

Treatment	Shoot fresh weight (mean ± SEM)	Shoot dry weight (mean ± SEM)	Moisture content
Field Control	179 ± 0.40 a	62 ± 0.42 a	117
Vermicompost	1246 ± 0.20 b	316 ± 0.21 b	930
Vermiwash	1142 ± 0.40 c	320 ± 0.42 c	822
Vermicompost –vermiwash	1172 ± 0.36 d	365 ± 0.26 d	807

Values followed by different letters are significantly different at $P \leq .05$ according to least significant difference multiple range test.

TABLE 7.14 Root fresh and dry weight (mean ± SEM) (g) and moisture content (%) in field.

	Treatment	Root fresh weight (mean ± SEM)	Root dry weight (mean ± SEM)	Moisture content
	Control	10.00 ± 2.00 a	3.00 ± 1.00 a	7.00
Field	**Vermicompost**	115.33 ± 21.78 b	73.33 ± 7.37 b	42.00
	Vermiwash	97.33 ± 11.68 b	71.00 ± 5.20 b	26.33
	Vermicompost −vermiwash	240.67 ± 88.10 c	150.67 ± 71.04 c	90.00

Values followed by different letters are significantly different at $P \leq .05$ according to least significant difference multiple range test.

the V and VW plants ($P = .10$) did not differ significantly in the dry weight of roots (Table 7.14). The treated plants and the control plants had significantly different root dry weights ($P = .05$). The treated plants differed significantly (0.05), except for the V and W plants (Table 7.14). There was better root development in VW plants (Fig. 7.13).

c. Root length

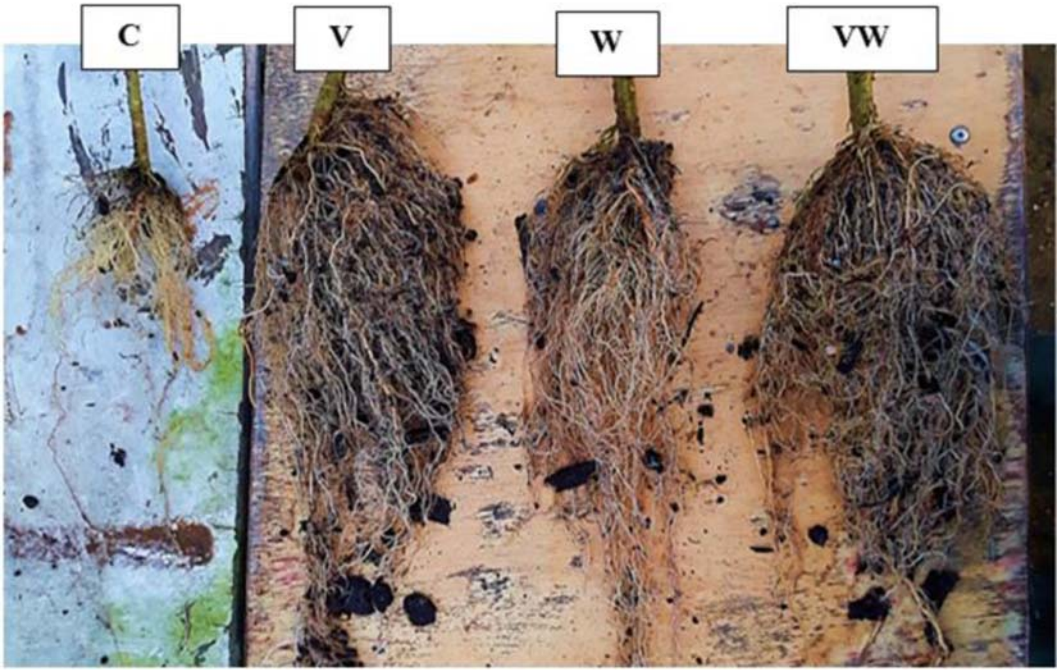

FIGURE 7.13 Root development of different treatments in field. *C*, control; *V*, vermicompost; *VW*, vermicompost−vermiwash; *W*, vermiwash.

TABLE 7.15 Root length (mean ± SEM) in field (g).

	Root length (mean ± SEM)
	Field
Control	30.33 ± 2.52 a
Vermicompost	56.67 ± 5.69 b
Vermiwash	54.33 ± 9.07 bc
Vermicompost–vermiwash	45.00 ± 7.21 c

Values followed by different letters are significantly different at $P \leq .05$ according to least significant difference multiple range test.

Table 7.15 depicts the average length of roots. V plants had the longest roots (56.67 ± 5.69 cm), followed by W plants (54.33 ± 9.07 cm), VW plants (45.01 ± 7.21 cm), and C plants (30.33 ± 2.52 cm). The LSD test revealed that the V and VW treated plants were significantly different from the other treated plants ($P = .000$) and the control plants ($P = .062$). The W and VW plants did not differ significantly ($P = .675$) from the V- and W-treated plants (Table 7.15).

3.7.1 Production

In the field trials, the beginning of blooms was observed 3 weeks after transplantation for all VW-treated plants, followed by V-treated plants (60%) and W-treated plants (40%). This indicates that the application of vermicompost caused early flower initiation. The C plants produced no fruit during the experiment. Different treatments were significantly different from each other ($P = .05$) at harvest in terms of fruit weight, based on the LSD test (Table 7.16). VW treatment produced the highest yield per plant (1919.88 ± 20.40 g) and the number of fruits (38.81 ± 0.41), whereas the lowest results were recorded for V-treated plants (yield per plant: 1295.34 ± 183.67 g; number of fruits: 25.43 ± 3.61) (Table 7.16). The larger fruits were produced from VW-treated plants (5.41 cm), followed by W-treated plants (5.13 cm) and V-treated plants (4.92 cm) (Table 7.16 and Fig. 7.14).

TABLE 7.16 Harvest and fruit weight (g) per plant (mean ± SEM) in field.

	Treatment	Harvest per plant (mean ± SEM)	Fruit weight per plant (mean ± SEM)
Field	**Control**	0.00 ± 0.00 a	0.00 ± 0.00 a
	Vermicompost	25.43 ± 3.61 b	1295.34 ± 183.67 b
	Vermiwash	32.86 ± 2.86 c	1673.51 ± 145.52 c
	Vermicompost–vermiwash	38.81 ± 0.41 d	1919.88 ± 20.40 d

Values followed by different letters are significantly different at $P \leq .05$ according to least significant difference multiple range test.

FIGURE 7.14 Differences in fruit diameters among treatments in field.

4. Overall discussion

The production of vermiwash and vermicompost in the experiment was successful, as evidenced by production results in the first phase. The vermicompost and vermiwash produced in the experiments were according to the standard requirement in terms of quality, with an appropriate C:N ratio and optimum nutrients that had a significant influence on the plant growth parameters of tomatoes and affected the soil physicochemical properties. Analytical reports confirmed the works of several researchers (Ansari and Sukhraj, 2010; Ansari and Ismail, 2012; Maheswari et al., 2016; Bhat et al., 2018a,b; Bhagat et al., 2022). The vermiwash had a brown color. The differential quality and nutrient availability of vermiwash depend on the initial organic raw material used in vermicomposting (Kaur et al., 2015; Zarei et al., 2018). As a result, vermiwash contains fewer nutrients than vermicompost. The plants are able to access nutrients directly when vermiwash is applied as a foliar spray (Makkar et al., 2017). In the experiment conducted (second phase) in the greenhouse as well as under field conditions, the combined treatment of vermicompost and vermiwash had a significant impact on plant growth parameters (plant height, stem thickness, number of leaves, total branches, fresh and dry shoots, root weight, root density, yield, number of flowers, and fruit size and weight). This is in accordance with previous research (Jaybhaye and Bhalerao, 2015; Kaur et al., 2015; Maheswari et al., 2016; Makkar et al., 2017; Alves et al., 2022; Ansari et al., 2022; Arjune and Ansari, 2022; Joshi et al., 2022; Siddiqui et al., 2022). In agreement with research conducted by Makkar and coworkers (2017), it was also noted that vermiwash used as a spray on plants significantly enhanced flowering and fruit-setting. In addition, findings demonstrated that the use of vermicompost and vermiwash in isolation was significantly effective for plant growth parameters. According to Makkar et al. (2017), the use of 50% vermicompost and foliar application of vermiwash had a maximum yield with a positive impact on plant growth parameters (number of branches, capsules, dry weight, and number of seeds). Vermiwash is enriched with metabolites, vitamin B, and provitamin D and facilitates plant growth (Jaikisun et al., 2014; Luján-Hidalgo et al., 2017). A study on strawberry plant cultivation using vermicompost application had an impact on increasing the dry weight of the plant (Joshi and Vig, 2010). Vermiwash is effective in promoting the growth of plants in terms of plant height, the internodal length and diameter, the number and surface area of leaves, and the dry and wet shoot weight, according to several researchers (Samadhiya et al., 2013; Kaur et al., 2015; Arjune and Ansari, 2022). According to Jaybhaye and Bhalerao (2015), vermiwash application was more effective than using vermicompost on the growth parameters of eggplants. Vermiwash and vermicompost had the highest plant dry weight in another study (Makkar et al., 2017; Arjune and Ansari, 2022).

According to Makkar et al. (2017), the foliar application of vermiwash promoted fruit ripening and consistent production, which is in line with the current research, in which the combination of vermiwash and vermicompost was used. According to studies (Makkar et al., 2017), applying vermiwash to the leaves of flowering and fruiting plants shortens their life cycles (Tamrakan et al., 2018). The ratio of flowers to fruits also increased, according to researchers (Sundararasu and Jeyasankar, 2014; Maheswari et al., 2016; Ansari et al., 2022). According to Bhardwaj and Sharma (2016), when vermicompost is applied to crops, the initial growth rate is slower; however, as the nutrients slowly release, the plant experiences rapid growth. Compared with vermicompost, vermiwash has a higher nutritional value because of the direct availability of micronutrients and macronutrients for plants. According to Ansari (2008), the gradual availability of nutrients and the presence of phytohormones (auxins, cytokines, and gibberellin) promoted plant growth and higher yields when vermicompost and vermiwash were combined. This could explain why the vermicompost plants appeared to be shorter and smaller than the VW and W plants. Moreover, the combination of vermiwash and vermicompost (VW plants compared with W and V alone) produced plants with a bushier appearance and multiple branches. This is because VW plants are more efficient at photosynthesis when a foliar application is used, which leads to a greater yield and fruit weight. This work agrees with the work of Makkar et al. (2017).

Vermicompost and vermicast (the excretory product of earthworms) have a beneficial impact on plant growth with specific reference to root and shoot biomass (Tomati et al., 1988; Jaikisun et al., 2014; Bhat et al., 2018a,b; Bhagat et al., 2022).

The combined use of vermiwash and vermicompost yields superior results compared with using vermicompost and vermiwash in isolation (Samadhiya et al., 2013; Sundararasu and Jeyasankar, 2014; Kaur et al., 2015; Makkar et al., 2017). The presence of humic and fulvic acids observed in vermiwash and vermicompost promotes root growth parameters by enhancing the uptake of nutrients through the root cell membrane owing to an increase in permeability, and it affects the plant height and biomass (Wright and Lenssen, 2013; Makkar et al., 2017). Vermiwash was applied to the leaves as a spray, and not to the roots. The abundance of nutrients, hormones, and enzymes in vermiwash resulted in a root system that was larger and longer than that of the control plants. There was an improvement in the root structure in VW-treated plants. The application of vermicompost in the research had a positive impact on soil fertility at the completion of the research trials because of the presence of essential microbes and enzymes (amylase, lipase, cellulase, and chitinase) that degrade organic material, resulting in the gradual availability of nutrients to plant roots (Adhikary, 2012). Vermicompost is also beneficial in soil amelioration, with an impact on the physical, chemical, and biological properties (Bhat et al., 2018a,b; Prabina et al., 2018).

There was a contrast in the results between greenhouse and field conditions for cultivating tomatoes using different soil amendments. Production (fruit size and yield) was higher in field trials compared with greenhouse experiments because the greenhouse's daytime average temperature was higher than that of the field (26.87−27.81°C). According to observations, the greenhouse's inadequate ventilation of air resulted in high air temperatures and persistent heat stress to the plants. According to Harel et al. (2014), the average daily temperature is critical in pollen and flower development. Relative humidity is an additional important factor. According to Shamshiri et al. (2018), the ideal relative humidity for tomato pollution is 50%−70%. According to Harel et al. (2014), higher pollen quality and fruit set can be achieved by increasing humidity at the ideal temperature. The increased yield and fruit weight observed in the field experiment may be attributable to this. According to Harel et al. (2014), pollen's susceptibility to heat stress may rise by 90% at temperatures above 29°C. The temperature in the greenhouse increased to 29°C and the relative humidity dropped below 77.81%, which affected fertilization and reduced productivity. Pesticides and fungicides were not used during the cultivation phase of the experiment because neither was thought necessary. Vermiwash and vermicompost probably act as pesticides, as evidenced by the absence of disease or pests in the crop (Verma et al., 2018; Bhat et al., 2018b; Bhagat et al., 2022).

5. Conclusion

The use of dried matter (grass clippings and neem leaves) with cattle dung in vermicomposting was successful using epigeic earthworms (*E. foetida*). The produced vermicompost was of high quality because it had a dark color, looked like finely divided peat, had the pleasant smell of soil, was fine and smooth, and had enough nutrients. A liquid with a brownish color was produced by the various vermicomposting bins as vermiwash that was standardized with the presence of essential nutrients (N, P, K, Ca, Mg, and Na) necessary for plant growth. The results indicated that such eco-friendly technologies used to produce vermiwash and vermicompost as biofertilizers can be a sustainable approach to agriculture. The critical analysis suggests that cultivating tomatoes using a combination of vermiwash and vermicompost improved the plant growth parameters and production. The field conditions were better in terms of production (higher yield and larger fruit) and economic viability compared with experiments conducted in the greenhouse.

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CHAPTER

8

Earthworm mediated amelioration of heavy metals from solid organic waste: an ecotechnological approach toward valorization

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1. Introduction

Industrial solid wastes contain a variety of heavy metals that impart considerable toxic effects on topsoil and the food chain (Sahariah et al., 2014). Major contributor to solid organic waste is the industrial wastes from a range of industrial sectors which include distilleries, textiles, tanneries, pulp and paper, and petroleum processing and production units. Different types of heavy metals like Manganese (Mn), Lead (Pb), Copper (Cu), Iron (Fe), Chromium (Cr), Zinc (Zn) and Nickel (Ni) are present in different primary effluents often termed as spent wash (Ravikumar et al., 2008). The occurrence of such heavy metals makes it a potential source of harmful compounds imposing problems not only for disposal but also for water-terrestrial-atmospheric compartments of the surrounding environment. For instance, metals in tannery waste generate a broad range of complex chemical compounds which render bioavailability to plants. Recent studies have highlighted the metal buildup in plants inhabiting in tannery waste-contaminated soil (Barman et al., 2000; Sinha et al., 2006).

Globally among different industrial sectors, the leather sector produces more than 600,000 tonnes of solid waste annually. These pollutants damage aquatic ecosystems when they are thrown into bodies of water because they hinder sunlight by enhancing the turbidity of surface water, and thereby lowers photosynthetic efficiency and the quantity of dissolved

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oxygen in the water. They diminish soil alkalinity and manganese availability when they are put into agricultural soils, which prevents seed germination and depletes vegetation. The eco-assessment of high quantity of phenol, sulfate, and heavy metals, as well as high levels of total dissolve solids (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), and other complex organic pollutants, can be used to identify the range of harmful effects of this complex waste (Chandra and Kumar, 2017). Henceforth, the disposal of organic industrial wastes in an environment friendly manner is highly challenging for developing nations globally.

Through the alteration of the physicochemical composition of sludge and bioconcentration of different heavy metals, soft-bodied earthworms act as environmental engineers. Worms affirm the probable danger of transferring different heavy metals to a wide variety of predators residing in higher trophic levels in a terrestrial environment (Ganguly and Chakraborty, 2021a). Despite the existence of several cutting-edge technologies, vermicomposting has a substantial effect on lowering the bioavailability of heavy metals in accordance with various regulatory criteria. According to earlier studies, earthworms can take up heavy metals through feedstuff or dermal contact (Kilpi-koski et al., 2019). The metal-contaminated samples are ingested, undergo intestinal fragmentation, and release toxic metals that concentrate in the soft tissues of earthworms (Ganguly and Chakraborty, 2020).

Many different applications on contaminated environments have shown the efficiency of epigeic worms for the productive degradation of organic sludge, including the dairy business (Bhat et al., 2018), paper and pulp mill industry (Kaur et al., 2010; Ganguly and Chakraborty, 2018), wood chips wastes (Miller et al., 2020), winery wastes (Moorthi, 2016), and tannery waste (Ravindran et al., 2013; Karmegam et al., 2021).

Earthworms ingest organic materials that have been crushed up by their gizzards as part of the vermicomposting process. The surface area for microbial adhesion is increased by this process, making it simpler to supplement nutrients (Lin et al., 2020). With the aid of enzymes, earthworms quickens the process of biodegradation and generate value added products (Barthod et al., 2018). This alteration speeds up the putrefaction process and brings about modifications in the chemical and physical characteristics that allow the correct stabilisation of carbon-rich materials. The technique is separated into three crucial stages: (a) Stage of Degradation (b) Stage of Transformation (c) Stage of Maturation (Aira et al., 2006; Ganguly and Chakraborty, 2020).

Heavy metals are frequently used to describe elements with densities of more than $4\ g\ cm^{-3}$. For a number of enzymes, some of the metals served as coactivators, prosthetic groups, cofactors, etc., and can be benificial to living being if consumed below toxic concentration. Molybdenum, zinc, copper, and other such elements are regarded as vital elements. However, nonessential metals like cadmium, lead, and others have no impact on human metabolism. Even at modest concentrations, these metals are very hazardous. As the heavy metals are lipid soluble, they show amplification in content across food chains. There are several bioremediation techniques that have been used to reduce harmful effects and maintain ecosystem health (Ganguly and Chakraborty, 2020).

In this context, vermitechnology has proven successful in degrading complex organic compounds like industrial effluents. Earthworms typically bioaccumulate persistent heavy metals from the organic substrate, in addition to transforming organic waste from cities into useable nutrients (Lanno et al., 2019; Sharma et al., 2005; Paul et al., 2018). Additionally, studies have shown that earthworms use a process called vermiremediation to lower the amounts of harmful chemicals in the environment (Aira et al., 2006; Wu et al., 2018).

The earthworm has been suggested as a very efficient bio-indicator species of the terrestrial environment, with the capacity to influence the transition of toxic inorganic and organic chemicals, either directly or indirectly, leading to a decrease of heavy metals in the final vermicompost (Bhat et al., 2018; Ganguly and Chakraborty, 2018).

It is especially important to have a thorough knowledge of the accumulation of heavy metals in earthworm bodies for the assessment of ecological hazards since the accumulation of nonessential metals in earthworm tissue offers a high risk of secondary toxicity to vertebrate predators due to the bio-magnification and bio-accumulation of toxic pollutants (Maáková et al., 2014; Mohee and Soobhany, 2014). Therefore, the current experiment has demonstrated the efficiency of several earthworm species in the removal of heavy metals from vermicompost samples.

2. Sources of heavy metals in organic waste

Among the anthropogenic sources of metal-bearing solids at contaminated sites are metal mine tailings, coal combustion residues, biosolids (sewage sludge), high metal wastes dumped into unprotected landfills, petrochemicals, leaded gasoline, compost, pesticides, lead-based paints and atmospheric deposition (Khan et al., 2008; Zhang et al., 2010; Basta et al., 2005).

2.1 Agricultural sources

2.1.1 Fertilizer

Plants require not only the macronutrients like nitrogen, phosphorus, potassium, magnesium, and calcium, but also the critical micronutrients in order to grow and complete their life cycles (Scragg, 2006). Essential metals (such as nickel, cobalt, manganese, copper, iron, molybdenum and zinc) are required for growth of plants (Lasat, 2000), and therefore, farmers may need to supplement their crops with these elements by spraying large volumes of fertilizers on the plants, although heavy metals like Cd and Pb have no such significant effects on physiology. As the fertilizers contain impurities in the form of trace levels of heavy metals (such as Pb and Cd), the soil may become significantly more concentrated with these metals if fertilizer is applied regularly (Jones and Jarvis, 1981). Owing to the widespread use of phosphate fertilizer, heavy metals have accumulated to higher concentrations in the agricultural soil (Verkleji, 1993; Carnelo et al., 1997).

2.1.2 Pesticides

Significant quantities of metals were found in a variety of conventional pesticides that were utilized to a large extent in agricultural and horticultural practices in the past. For fact, nearly 10% of the chemicals in the United Kingdom (with licenses for use) are used as fungicides and insecticides which are found to release heavy metals like manganese, copper, zinc, mercury, or lead (Jones and Jarvis, 1981). Other sources have contributed various amounts of Zn, Cd, Ni, Cr, Pb, and particularly fungicides, inorganic fertilizers and phosphate fertilizers (Kelepertzis, 2014; Tóth et al., 2016). Arsenic-containing substances are often used in the banana business to get rid of pests like cow ticks. The potential of plants to bioaccumulate Cd is extremely alarming as Cd shows a high rate of bioaccumulation inside mesophyll tissue of leaves.

2.2 Biosolids

The majority of the solid materials produced during the wastewater treatment process are known as biosolids, commonly referred to as sewage sludge, and they can potentially be repurposed for useful uses (USEPA, 1994). In the soil, after being applied to land by the dumping of different biosolids (such as cattle manures, composts, and municipal sewage sludge), heavy metals including As, Cr, Se, Hg, Cu Ni, Pb, Zn, Mo, Cd, Tl, and Sb can accumulate to different soil horizon (Basta et al., 2005). On their fields and pastures, farmers frequently apply solid or liquid manures generated from animal wastes like chicken, cow, and pig dung (Sumner, 2000).

Although most manures are regarded as beneficial fertilizers, arsenic in chicken health products has the potential to result in metal contamination of the soil (Sumner, 2000; Chaney and Oliver, 1996). The technique of reusing biosolids generated by urban populations and using them to spread organic waste to land during the landfilling process is common in many countries (Weggler et al., 2004). Using biosolids in agricultural techniques has drawn concern since it might contaminate soil with heavy metals as Ni, Pb, Zn Cd, Cu, and Cr (Canet et al., 1998). The application of biosolids to soils has the potential to introduce metals, which may then leach downward through the soil profile and pollute groundwater (McLaren et al., 2005).

2.3 Industrial sources

Mining and refining are two of the many important industrial processes that contribute significantly to the pollution of heavy metals. The types of heavy metals emitted by mining activities vary widely according to the specific mining procedures that are carried out. For instance, the use of mercury in gold mining has developed into one of the most significant contributors of this element to the environment (Clemens and Ma, 2016; Pavilonis et al., 2017). In a similar vein, coal mines are the primary source of arsenic, cadmium, and iron, which can mutilate the soil in the surrounding area. Aerosols are formed when vapourized heavy metals such as copper, zinc, lead, tin, and cadmium mix with water and then undergo condensation (Nagajyoti et al., 2010). These can either be dry deposited (in which case they are carried by the winds) or wet deposited (in which case they are precipitated in the form of rainfall), both of which result in the contamination of water and soil. Several procedures used in refineries can contribute to the contamination of the soil with heavy metals.

Heavy metals such as Se, Cu, Zn, Cd, and Ni are released into the environment by industries like petroleum, power stations that burn coal, and high-tension cables (Verkleji, 1993; Ahmed and Ahmaruzzaman, 2016; Zhu et al., 2016). The process of manufacturing done by several industrial sectors like plastic, paper and pulp, textiles, leather etc. contribute majorly in terms of heavy metal pollution. Antiwear protectants used in automobiles are responsible for the leakage of lead, cadmium, nickel, mercury, chromium, and zinc, particularly in inefficient engines. Incinerators for municipal solid waste produce a significant amount of zinc, lead, aluminum, tin, iron, and copper, and the burning of lead-containing gasoline releases lead into the atmosphere (Srivastava et al., 2017).

3. Different methods applied for heavy metal removal from solid organic waste: a review of phytoremediation

Plants have the ability to eliminate organic and inorganic contaminants from soil and water through a variety of methods, which vary according to the species of plant and on the nature of surrounding environment (Anton and Mathe-Gaspar, 2005; Antoniadis et al., 2017). The interplay of plants with their surroundings (the soil, water, and air) and the microorganisms in that environment show an essential part in the detoxification process (Dary et al., 2010). The contaminant, the plant type, and the soil all contribute in the success of the remediation process. Numerous factors affect remedial efficacy, including plant biomass and metabolism, soil pH, electric conductivity, organic matter in the soil, microbial processes, and different soil additives (Anton and Mathe-Gaspar, 2005; Nissim et al., 2018). The potential phytoremediation methods used to remove heavy metals from solid wastes are described below (Fig. 8.1).

FIGURE 8.1 Schematic view of vermiremediation of organic waste.

3.1 Phytoextraction

Phytoextraction, also called phytoaccumulation, starts when plant roots absorb harmful metals that are subsequently transported to the plant's shoots and deposited in metabolically dormant plant components such as vacuoles, cell walls, and cell membranes (Chatterjee et al., 2013). The root and shoot tissues of hyperaccumulator plants store a larger quantity of toxic metals than nonaccumulators. The plant cell absorbs metal cations and forms a metal-phytochelatin complex (M-PC) or metal-ligand complex to accumulate the toxic heavy metals (Asgari Lajayer et al., 2019) and then translocated to the plants' vacuole and deposited (Yadav, 2010). Plant biomass and the concentration of heavy metals in aboveground plant tissues are the primary factors determining the plant's extraction potential (Li et al., 2010). Henceforth, a potential plant species for phytoremediation must not only endure and successfully absorb heavy metals but also grow quickly, produce a massive biomass, and have tangible economic profits (Bian et al., 2020). Some plants with a potential role in phytoextraction are described in Table 8.1.

TABLE 8.1 Plant methods of phytoremediation to remove heavy metals.

Plant species	Heavy metal (HM)	References
Plants known to remove HMs by phytoextraction		
Cannabis sativa L. *Thlaspi caerulescens*	Cd	 Koptsik (2014)
Alyssum markgrafii *Thlaspi caerulescens*	Ni	Salihaj et al. (2018) Koptsik (2014)
Tagetes minuta	As	Salazar and Pignata (2014)
Betula occidentalis *Helianthus annuus* *Brassica nigra* *Medicago sativa*	Pb	Koptsik (2014)
Plants known to remove HMs by phytostabilization		
Eupatorium cannabinum	As	González et al. (2019)
Kosteletzkya pentacarpos *Willow Wax*	Cd, Zn Cd	Zhou et al. (2019) Yang et al. (2019)
Helianthus annuus	Cu, Zn, Pb, Hg, As, Cd, Ni	Jadia and Fulekar (2008)
Plants known to remove HMs by phytovolatilization		
Polypogonmon speliensis	As	Ruppert et al. (2013)
Brassica oleracea	Se	Raskin et al. (1997)
Scirpus robustus	Se	Arthur et al. (2005)
Typha latifolia	Se	Pilon-Smits et al. (1999)
Plants known to remove HMs by rhizodegradtion		
Sesbania cannabina	Pb	Maqbool et al. (2012)

3.2 Phytostabilization/phytoimmobilization

The toxicants or pollutants surrounding the rhizospheric environment can be inactivated or immobilized in order to accomplish the removal process. The immobilization is carried out with the assistance of phytochemical effluences, cornered on the root exterior by transporter protein or confined within the vacuole of the root cell by cellular process (Mitra et al., 2020). Conversion of hazardous metals into nonhazardous compounds is possible by amalgamation with sugars, proteins, and amino acid derivatives or through the formation of complexes in the rhizosphere. For example, arsenic (As) was immobilized by plant root epidermis by binding arsenic to ferric sulfate inside the vacuoles and producing a trivalent complex of arsenic tris-thiolate in the rhizosphere (Hammond et al., 2018). Some plants with a potential role in phytostabilization are described in Table 8.1.

3.3 Phytovolatilization

Phytovolatilization is a multi-step process involving plants' absorption of toxic metals from the soil, modifying slightly volatile compounds into more volatile forms followed by the releasing of contaminants into the atmosphere. This strategy works when volatile contaminants from soil to the atmosphere have fewer adverse consequences (Mitra et al., 2021). The volatilization approach is predominantly effective for organic contaminants (Limmer and Burken, 2016). Some molecules volatilize from the stem and leaves, while others from the root-soil interplay. The hydrophobic organic molecules depart the stem and leaves through hydrophobic plant barriers, such as cuts, epidermis, suberin, and other dermal layers. Additionally, the transpiration stream also releases many compounds that are lost into the atmosphere. For mercury (Hg) and selenium (Se), phytovolatilization's potential is considerable since it converts metals into volatile forms for release and dilution into the atmosphere (Bhargava et al., 2012). By eliminating the requirement to harvest and predispose contaminated plants, this technique has the potential to be more cost-effective than conventional phytoremediation approaches for metal(-loid) removal. Some plants with a potential role in phytovolatilization are described in Table 8.1.

3.4 Phytodegradation

Phytodegradation, or phytotransformation, is the process by which organic contaminants that have been taken up are degraded through metabolic processes by plant enzymes (Spaczynski et al., 2012). Therefore, organic pollutants are debilitated into more simple molecules through processes that are referred to as "ex planta" metabolic processes. These simpler molecular forms are absorbed into plant tissues to assist growth (McGrath and Zhao, 2003). As a result, the earth's plant life might be thought of as a "green liver" for the entire biosphere (Kafle et al., 2022). The direct absorption of chemicals into plant tissue is governed by several parameters, including uptake efficiency, transpiration rate, and the concentration of the chemical in the medium. Furthermore, the uptake efficiency is dependent on the chemical speciation, physico-chemical properties, and characteristics of plants, whereas the rate of transpiration is dependent on the type of plant, the area of its leaves, the nutrient content, the soil moisture, the temperature, the wind conditions, and the relative humidity (Chandra and Kumar, 2017). For this technology to be successfully implemented, the altered molecules that collect within the plant must either be harmless or significantly less hazardous than the original compound(s). However, this method is successfully applied for biodegradtion of organic pollutants such as synthetic herbicides, phenanthrene, trinitrotoluene, trichloroethene, atrazine, insecticides, and chlorinated solvents (chlorodinitrobenzene).

3.5 Rhizodegradation

Rhizodegradation, also known as phytostimulation, is a process in which plant-provided substrates drive the formation and succession of microbial communities in the rhizosphere for the break down of organic contaminants in the soil (Vishnoi and Srivastava, 2008). The rhizosphere of a plant offers a unique habitat for the growth of microorganisms proficient in converting potentially harmful contaminants into nontoxic and safe compounds. Rhizodegradation occurs when root exudates release plant nutrients into the environment, which increases the population and activity of microorganisms in the rhizosphere and speeds up breakdown of soil contaminants. Rhizospheric microorganisms encourage plant growth in various ways, including oxidation of nitrogen, mobilization of nutrients (such as phosphorus), formulation of plant growth regulators, a reduction in the levels of stress hormones produced by the plant, defense against plant pathogens, and downgrading the pollutants before the plant is impaired by them (Chandra and Kumar, 2017). The metabolic action of microorganisms are able to degarade organic pollutants alongside support plant growth. Such nutritional benifits takes place due to mutualistic association of microorganisms with the roots and surrounding rhizospheric soil. However, such interactions are often controlled by multifarious environmental factors alongwith some restricting factors like abundance of weeds, infestation of plant pathogens etc (Chandra and Kumar, 2017). In addition, rhizoremediation is only applicable to the soil near plant roots and is not appropriate for more deeply buried areas. In addition, plants can bioaccumulate harmful pollutant metabolites, necessitating restrictive standards for the disposal of plant matter. Rhizodegradation has certain advantages over other phytoremediation methods because of its ability to destroy contaminants at the source and its propensity to completely mineralize organic pollutants. Plants that have a potential role in rhizodegradation are described in Table 8.1.

4. Role of vermitechnology in reduction of heavy metal load: a case study using paper mill wastes

Vermitechnology is an evergreen technology that uses earthworms under appropriate environmental conditions. The study has taken into account a case study including various paper mill wastes in order to comprehend the role of earthworms in the reduction of metal load. The current study has considered the participation of two different earthworms, *Eisenia fetida* and *Perionyx excavates*, in the composting of paper mill sludge to understand the role of earthworms in lessening heavy metal loads in waste samples (Fig. 8.2).

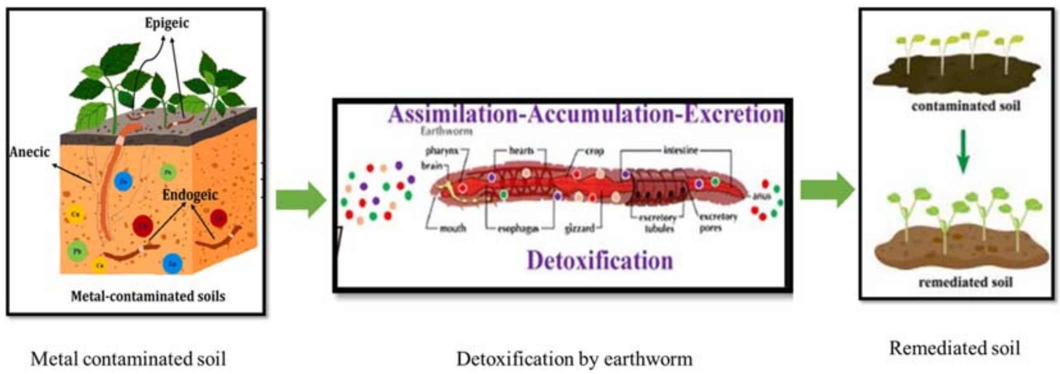

FIGURE 8.2 Uptake of heavy metals by earthworms and vermiremediation.

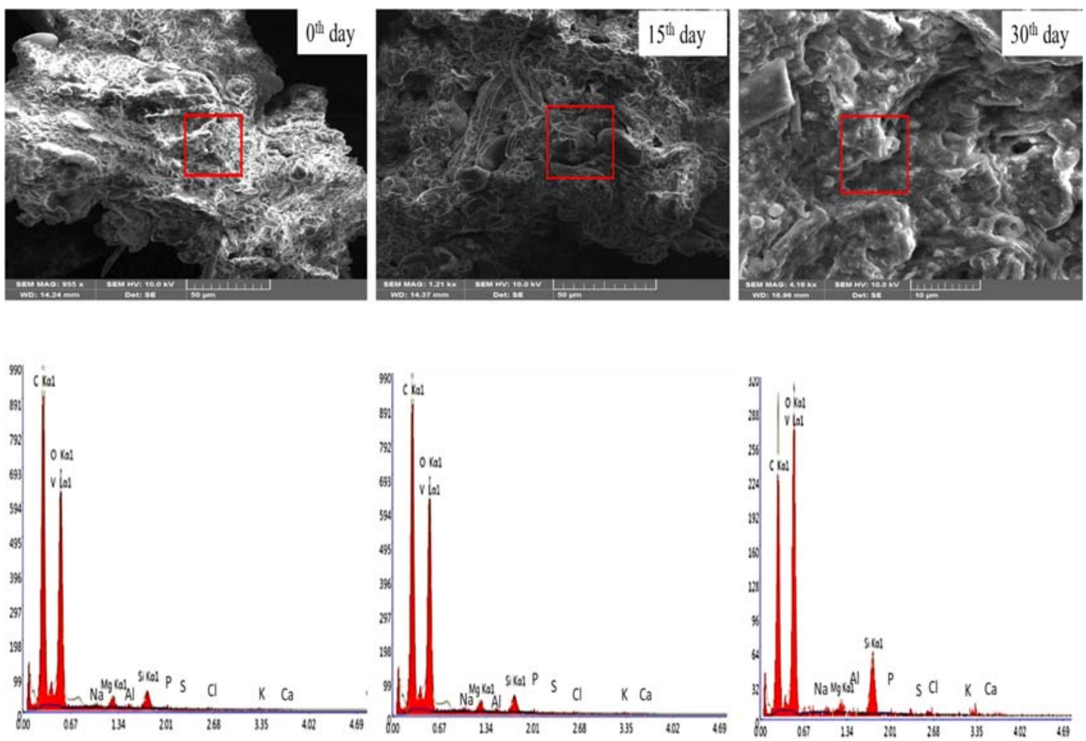

FIGURE 8.3 SEM-EDX analysis of the vermicompost weed samples at 0th day, 15th day and 30th day respectively. Spectroscopical data represents the distribution of elements among the samples.

Earthworms are crucial in reducing the harmful metal load in the final vermicompost material, despite the fact that varied pre- and posttreatment procedures used by the paper mill sector caused metallic content to decrease or show variances across various types of waste.

- Vermicomposting trial sets using *Eisenia fetida* (VEP2 and VES2) (Table 8.4)
  For primary sludge (VEP2), a significant reduction of toxic heavy metals could be graded as Zn ($F_{value} = 103.12$, $R^2_{value} = 0.61$) > Pb ($F_{value} = 54.23$, $R^2_{value} = 0.53$) > Cr ($F_{value} = 113.48$, $R^2_{value} = 0.63$) > Cu ($F_{value} = 41.20$, $R^2_{value} = 0.85$) (Table 8.4). For secondary sludge (VES2), decrease in heavy metals were recorded as Zn ($F_{value} = 71.12$, $R^2_{value} = 0.70$) > Pb ($F_{value} = 84.11$, $R^2_{value} = 0.70$) > Cr ($F_{value} = 110.34$, $R^2_{value} = 0.32$) > Cu ($F_{value} = 45.30$, $R^2_{value} = 0.70$). Furthermore, the prevailing research has depicted the correlation between reduction of heavy metals and C/N ratio as VEP1 ($F_{value} = 40.13$, $R^2_{value} = 0.62$, $r = 0.69$) and VES1 ($F_{value} = 72.23$, $R^2_{value} = 0.73$, $r = 0.56$).
- Vermicomposting trial set using *Perionyx excavatus* (VPP2 and VPS2) (Table 8.5)
  For primary sludge (VPP2), a significant reduction of toxic heavy metals could be ranked as Zn ($F_{value} = 91.56$, $R^2_{value} = 0.67$) > Pb ($F_{value} = 31.33$, $R^2_{value} = 0.50$) >Cr

TABLE 8.2 Removal of heavy metals from contaminated soil by microorganisms.

Microorganism	Sources	Heavy metals removed	References
Conglomerate of *Serratia marcescens, Pseudomonas pyogenes, Erwnia amylovora, and Enterobacter cloacae*	Contaminated soil and effluent of paper mill	Pb, Cd, Zn, As, Cr, Cu, Ni	Nwaehiri et al. (2020)
Association of *Bacillus subtilis and Staphylococcus aureus*	Contaminated soil and effluent of paper mill	Pb, Cd, Zn, As, Cr, Cu, Ni	Purwanti et al. (2019)
Sphingobium SA2	Mercury contaminated soil	Hg	Mahbub et al. (2017)
Pannonibacter phragmitetus BB	Chromium containing slag	Cr	Wang et al. (2015)

TABLE 8.3 Plant growth promoting bacteria-assisted phytoremediation in heavy metal remediation.

Microorganism	Host species	Heavy metal removed	References
Arthrobacter sp.	*Glycine max*	Cd	Rojjanateeranaj et al. (2017)
Bacillus cereus	*Vetiveria zizanioides*	Cr	Nayak et al. (2018)
Cupriavidus basilensis	*Pteris vittata*	As	Yang et al. (2020)
Paxillus involutus	*Populus canescens*	Pb	Szuba et al. (2017)
Pseudomonas libanensis	*Helianthus annuus*	Ni	Ma et al. (2019)
Microbacterium arborescens	*Leptochloa fusca*	U/Pb	Ahsan et al. (2017)
Pseudomonas fluorescens	*Trifolium repens*	Sb	Daryabeigi et al. (2020)

TABLE 8.4 Trend of change in heavy metal load (mg kg^{-1}) in the process of vermicomposting (V) of PMS and respective bioaccumulation factor (BAF) of heavy metals in earthworm tissues of *Eisenia fetida* in VPP2 and VPS2 set of trials (Mean ± S.E., $n = 3$).

Trial sets			Zn	Cr	Pb	Cu
VEP2		**0th day**	250.57 ± 0.09	88.76 ± 0.04	51.24 ± 0.14	110.81 ± 0.12
		60th day	150.20 ± 0.10[a]	63.40 ± 0.14[a]	32.66 ± 0.06[a]	82.09 ± 0.25[a]
VES2		**0th day**	72.16 ± 0.08	17.61 ± 0.15	5.34 ± 0.11	24.13 ± 0.05
		60th day	39.85 ± 0.06[a]	15.32 ± 0.25[a]	3.10 ± 0.03[a]	19.31 ± 0.08[a]
PS		**0th day**	255.11 ± 0.14	92.37 ± 0.09	57.24 ± 0.05	114.13 ± 0.11
		60th day	251.08 ± 0.11[a]	87.06 ± 0.03[a]	50.08 ± 0.09[a]	109.27 ± 0.10[a]
SS		**0th day**	76.13 ± 0.09	23.13 ± 0.12	8.13 ± 0.05	29.46 ± 0.15
		60th day	71.24 ± 0.20[a]	20.86 ± 0.13[a]	7.02 ± 0.09[a]	27.24 ± 0.05[a]
Earthworm tissue	VEP	**60th day**	60.44 ± 1.52	11.68 ± 0.15	3.57 ± 0.14	19.51 ± 2.26
	VES	**60th day**	18.95 ± 1.14	3.30 ± 0.11	0.43 ± 0.03	4.83 ± 0.26
Reduction of metal	VEP	**60th day**	40%	29%	36%	26%
	VES	**60th day**	45%	13%	42%	19%
	PS	**60th day**	2%	5%	13%	4%
	SS	**60th day**	6%	10%	14%	8%
BAF	VEP	**60th day**	0.4	0.18	0.11	0.24
	VES	**60th day**	0.48	0.22	0.14	0.25
	CD		95 ± 1.03	0.47 ± 0.30	0.31 ± 0.05	19.69 ± 1.44
Permissible standards (For Vermicompost)						
EU range (mg kg^{-1})[b]			210–4000	70–200	20–200	1750
Brinton limit (mg kg^{-1})[b]			2800	1200	420	1500
Indian limit (mg kg^{-1})[c]			1000	50	50	300

[a] *Represent the significance of the test at P < .05 validated through Tukey HSD (honestly significance difference) test as post hoc analysis.*
[b] *Brinton (2000).*
[c] *Central Pollution Control Board (CPCB), India (2006).*
Adapted from Ganguly, R.K., Chakraborty, S.K., 2021a. Cleaner Engineering and Technology 2, 1000706.

($F_{value} = 103.65$, $R^2_{value} = 0.45$) >Cu ($F_{value} = 24.17$, $R^2_{value} = 0.89$). For secondary sludge (VPS2), heavy metals can be ranked as Zn ($F_{value} = 64.11$, $R^2_{value} = 0.47$) > Pb ($F_{value} = 51.23$, $R^2_{value} = 0.62$) > Cu ($F_{value} = 40.30$, $R^2_{value} = 0.57$) > Cr ($F_{value} = 70.37$, $R^2_{value} = 0.35$). Such trend of reduction has been positively correlated with the C/N ratio as VPP2 ($F_{value} = 33.12$, $R^2_{value} = 0.55$, $r_{value} = 0.32$) and VPS2 ($F_{value} = 43.11$, $R^2_{value} = 0.46$, $r_{value} = 0.61$) (Ganguly and Chakraborty, 2021a).

TABLE 8.5 Trend of change in heavy metal load (mg kg^{-1}) in the process of vermicomposting (V) of PMS and respective bioaccumulation factor (BAF) of heavy metals in earthworm tissues of *Perionyx excavatus* in VPP2 and VPS2 set of trials (Mean ± S.E., *n* = 3).

Trial sets			Zn	Cr	Pb	Cu
VPP2		**0th day**	250.57 ± 0.09	88.76 ± 0.04	51.24 ± 0.14	110.81 ± 0.12
		60th day	145.20 ± 0.09[a]	60.40 ± 0.10[a]	30.56 ± 0.05[a]	80.09 ± 0.25[a]
VPS2		**0th day**	72.16 ± 0.08	17.61 ± 0.15	5.34 ± 0.11	24.13 ± 0.05
		60th day	36.45 ± 0.05[a]	13.12 ± 0.05[a]	2.81 ± 0.06[a]	17.12 ± 0.04[a]
PS		**0th day**	255.11 ± 0.14	92.37 ± 0.09	57.24 ± 0.05	114.13 ± 0.11
		60th day	250.05 ± 0.08[a]	85.67 ± 0.06[a]	49.12 ± 0.05[a]	105.07 ± 0.08[a]
SS		**0th day**	76.13 ± 0.09	23.13 ± 0.12	8.13 ± 0.05	29.46 ± 0.15
		60th day	70.56 ± 0.10[a]	18.26 ± 0.13[a]	7.38 ± 0.09[a]	28.12 ± 0.08[a]
Earthworm tissue	VPP	**60th day**	64.13 ± 0.16	12.15 ± 0.05	4.83 ± 0.04	20.15 ± 1.2
	VPS	**60th day**	20.13 ± 0.07	4.05 ± 0.05	0.40 ± 0.05	5.15 ± 0.06
Reduction of metal	VPP	**60th day**	42%	31%	40%	27%
	VPS	**60th day**	49%	25%	47%	29%
	PS	**60th day**	2%	7%	14%	8%
	SS	**60th day**	7%	21%	9%	5%
BAF	VPP	**60th day**	0.44	0.20	0.16	0.25
	VPS	**60th day**	0.55	0.31	0.14	0.30
	CD		95 ± 1.03	0.47 ± 0.30	0.31 ± 0.05	19.69 ± 1.44
Permissible standards (For Vermicompost)						
EU range (mg kg^{-1})[b]			210–4000	70–200	20–200	1750
Brinton limit (mg kg^{-1})[b]			2800	1200	420	1500
Indian limit (mg kg^{-1})[c]			1000	50	50	300

[a] *Represent the significance of the test at P < .05 validated through Tukey HSD (honestly significance difference) test as post hoc analysis.*
[b] *Brinton (2000).*
[c] *Central Pollution Control Board (CPCB), India (2006).*
Adapted from Ganguly, R.K., Chakraborty, S.K., 2021a. Cleaner Engineering and Technology 2, 1000706.

High R^2 and correlation values indicate an earthworm's competence at removing harmful heavy metals from various forms of sludge and their impact on the generation of decreased metal-intoxicated vermicompost. These research findings also showed that the presence of complex organic carbon sequesters metallic components, resulting in a decrease in the quantity of heavy metals in vermicompost samples (Fig. 8.3).

Earthworms are the main proponents of the approach, therefore evaluation of an annelid's behavior in a stressful environment was restricted by the metallic content of vermicompost and earthworm tissue. Earlier studies have shown a link between the bioavailability of heavy metals and the amount of each metal had accumulated in earthworms' soft tissues. Current study shows a strong association between the amount of bioaccumulation and the availability of metallic content (Li et al., 2010). Either by skin contact or eating on organic substrate, metallic components enter the earthworm tissue. Earthworm bioaccumulation accelerated the process of mineralization, according to ecotoxicological research.

- Bioaccumulation of heavy metals among earthworms

  The bioaccumulation of respective heavy metals among earthworms (*Eisenia fetida*) in a trial set using primary paper mill sludge (VEP2) as Zinc (0.41) > Copper (0.24) > Chromium (0.19) > Lead (0.12) and secondary paper mill sludge (VES2) as Zinc (0.48) > Copper (0.24) > Chromium (0.22) > Lead (0.15) (Ganguly and Chakraborty, 2021a).

Due to its role in metabolism, reproduction, and earthworm maturity, zinc has demonstrated the highest level of heavy metal clearance and bioaccumulation in earthworm tissue among other heavy metals (Fig. 8.4). However, there has been less bioaccumulation of copper and chromium.

Even though copper is a necessary macronutrient because it is required to participate as a cofactor in the mitochondrial enzyme, there is less accumulation of both metal ions in tissues (Fig. 8.5) for the reasons listed below: (1) Both chromium and copper act like Lewis acids and are captured by the organic material of vermicompost. Reduced bioavailability as a result of this sequestration encourages less bioaccumulation. (2) A higher excretion rate (Kilpi-Koski et al., 2019).

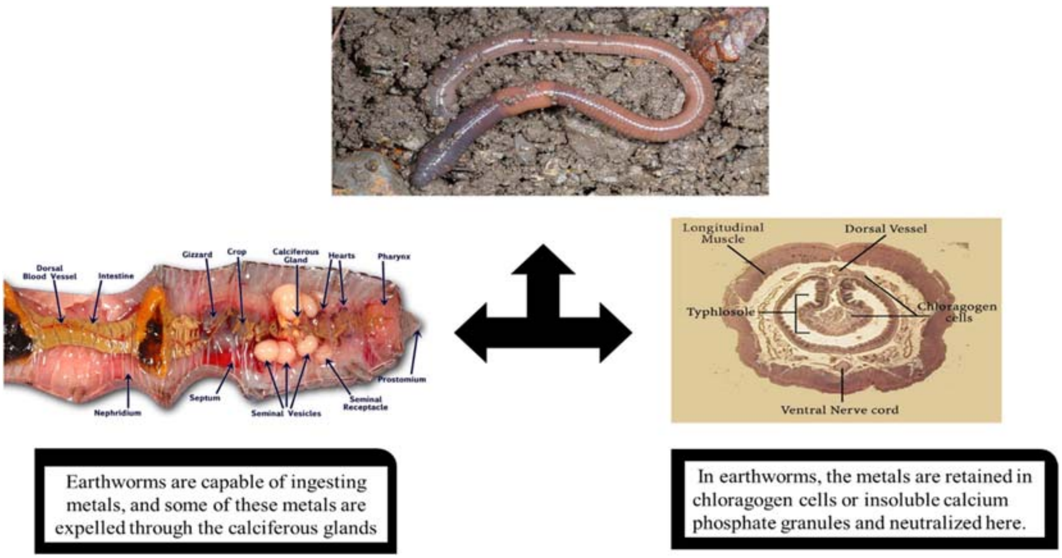

FIGURE 8.4 Role of chloragogen cells in earthworms.

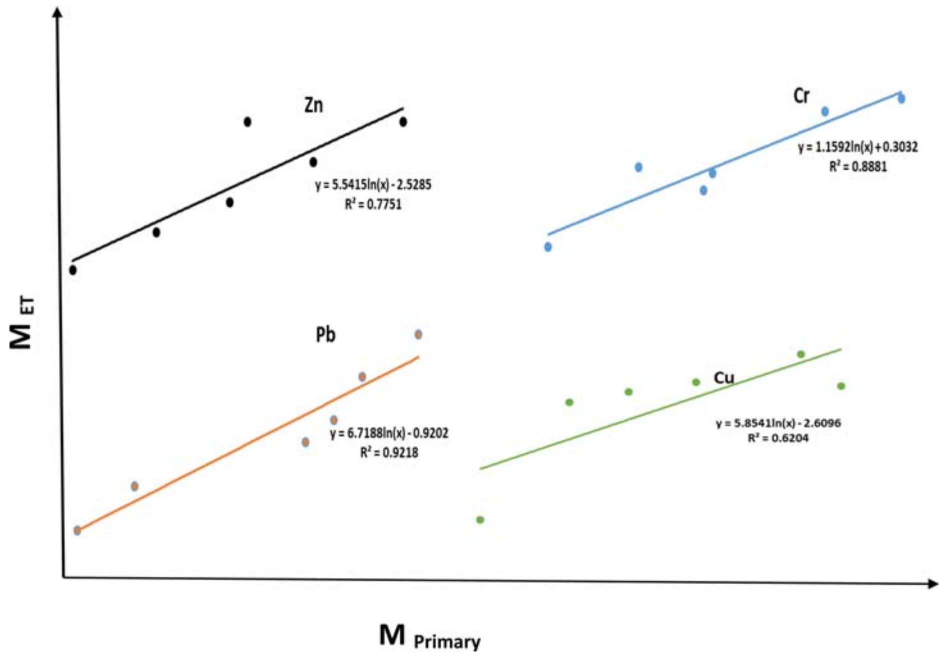

FIGURE 8.5 Log linear regression relationship between the heavy metal content present in earthworm tissues (M_{ET}) and primary paper mill sludge ($M_{Primary}$). *Adapted with copyright permission from Ganguly, R.K., Chakraborty, S.K., 2021a. Valorisation of toxic paper mill waste through vermicomposting: an insight towards cleaner engineering through alleviation of wastes. Cleaner Engineering and Technology 2, 100070, Elsevier.*

Due to the formation of various lead macromolecular complexes, the amount of lead (Pb) bioaccumulation was lower, which resulted in reduced bioavailability, mobility, etc (Lanno et al., 2019). Darling and Thomas (2005) found in earlier investigations that the pH of organic samples served as a deciding factor in the adsorption of macromolecular lead molecules by biological substrate (Darling and Thomas, 2005).

5. Role of microbes in remediation of heavy metals

Bacterial adaptive mechanisms for removing toxic metals in particular, can create defense mechanisms to deal with toxins in their habitats (Kapahi and Sachdeva, 2019; Ganguly and Chakraborty, 2021b). Natural selection plays a role in this process by driving the bacteria to adapt to their surroundings by changing their phenotype and genotype (Lenski, 2017). In particular, bacteria defend themselves by proliferating by developing resistance to environmental toxins like heavy metals. Bioaccumulation, biosorption, bioreduction/bio-oxidation, and Biotransformation (Ahemad, 2019; Fernández et al., 2018; Juwarkar and Yadav, 2010) are some of the ways that resistant bacteria can get rid of harmful heavy metals. With the biosorption technique, heavy metals may be attached to both active and inactive bacteria cells through the interaction of the two without the need for ATP hydrolysis (Kurniawan et al., 2019; Mohapatra et al., 2017; Timkova et al., 2018).

The concentration of metals inside cells rises as a result of the simultaneous processes of biosorption and bioaccumulation (Titah et al., 2018). However, bioaccumulation allows for ion exchange or physical adsorption for metal attachment and transport in an ATP-dependent way. Bioaccumulation starts off quickly and proceeds gradually (Srinath et al., 2002). Metals generally build up inside of cells as complex metal complexes due to their resistance mechanisms and various enzymatic activities (Imron et al., 2021). Biooxidation and bioreduction are the two routes through which biotransformation occurs. Either extracellularly or intracellularly, several activities can take place. Harmful heavy metal oxidation states are changed into harmless or less toxic oxidation states via bio-oxidation pathways, which mainly take place extracellularly (Ahemad, 2019). For example, As(III) is changed into a less toxic As(V) (Titah et al., 2018).

Intracellular bioreduction takes place, for example, when Cr (VI) becomes Cr (III) (Pradhan et al., 2017). Both methods frequently require the use of enzymes to reduce or transform the toxic heavy metal before releasing the less damaging heavy metal into the biosphere (Jobby et al., 2018). The linkages between chemical or functional groups in the bacterial cell wall may serve as heavy metal resistance mechanisms (Imron et al., 2021). Table 8.2 lists some soil bacteria that have been suggested to have heavy metal bioremediation capability.

The effectiveness of heavy metal phytoremediation is significantly influenced by the metals' bioavailability for plant uptake (Shah and Daverey, 2020). Plant growth-promoting bacteria (PGPB), which are microscopic organisms found in the rhizosphere, control the environment to make it favorable for phytoremediation. Through a variety of biological mechanisms, such as accumulation, transformation, leaching, chelating, degradation, sorption, volatilization, and PGPB actively work to increase the bioavailability of metals and convert them into less toxic forms so that they can be readily absorbed and extracted by plants (Manoj et al., 2020). Heavy metal pollution in soil results in stunted plant development, which can be lessened by adding these microbes to the soil.

Therefore, PGPB can be categorized as biofertilizers, phytostimulators, rhizomediators, or biopesticides, all of which contribute to improved plant development and, in turn, more effective phytoremediation (Purwanti et al., 2020). Examples of some PGPB mediated phytoremediation are enlisted in Table 8.3.

6. Mechanisms involved in combating heavy metal stress in earthworms

Metals are often not biodegradable; however, earthworms may remove them from the soil environment by primarily absorbing them through their skin and intestines, which causes the metals to collect in their bodies. Due to the toxicity of many metals, prospective plant and animal metal accumulators must have the requisite defensive mechanisms to deal with large metal loads in order to prevent detrimental interactions with biomolecules. When metals are ingested by earthworms, some of them are excreted by calciferous glands, while the rest collect inside the worm's body (Fig. 8.6).

Two unique metal-binding processes in earthworms have been identified:

- Firstly, accumulation of metals in chloragosomes or insoluble calcium phosphate granules without affecting the metabolic processes (Morgan and Morgan, 1999).

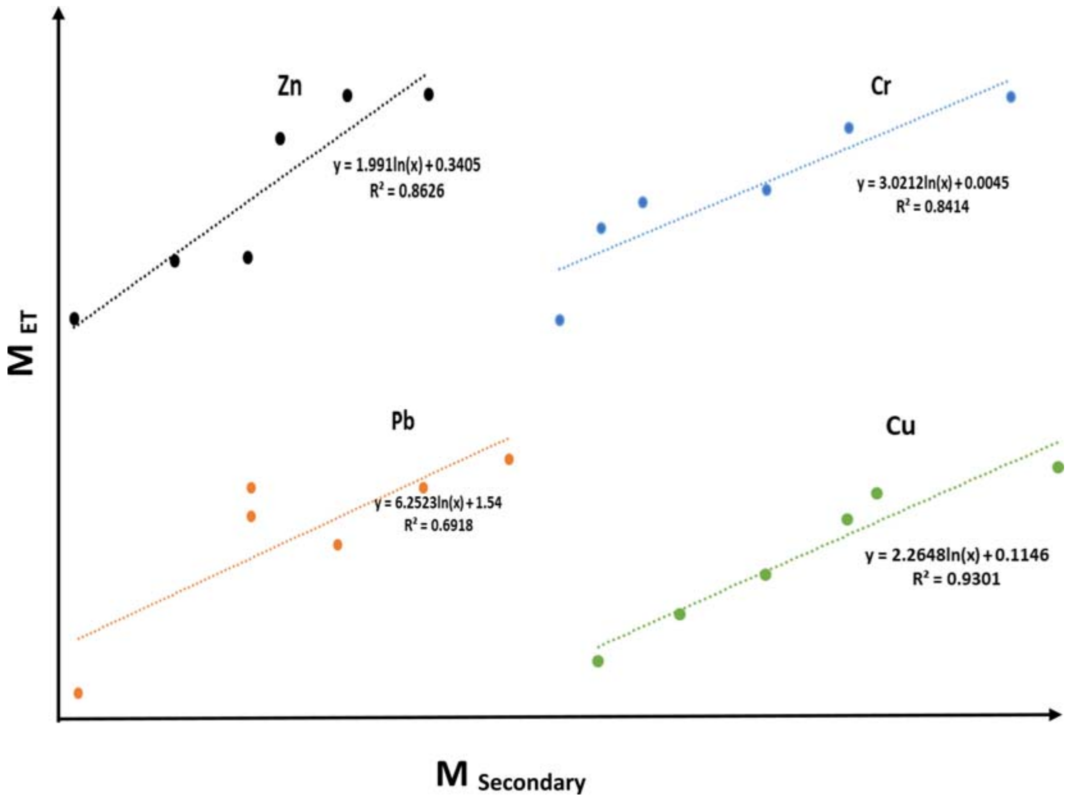

FIGURE 8.6 Log-linear regression relationship between the heavy metal content present in earthworm tissues (M_{ET}) and secondary paper mill sludge ($M_{Secondary}$). *Adapted with copyright permission from Ganguly, R.K., Chakraborty, S.K., 2021a. Valorisation of toxic paper mill waste through vermicomposting: an insight towards cleaner engineering through alleviation of wastes. Cleaner Engineering and Technology, 2, 100070. Elsevier.*

- Secondly, chelation of insoluble metals by the metallothionein's sulfur-donating ligands followed by neutralization inside chloragogenous tissue (Asensio et al., 2007).

Coelomocytes, which are present in earthworms, have a substantial impact on the movement of heavy metals inside the body. As a response of sensitivity to heavy metals, the expression of several genes that are implicated in metal elimination and/or lesion get increased. For instance, the expression of heat shock proteins and metallothioneins (MTs) are increased in the presence of heavy metals (HSPs) (Calisi et al., 2009).

In fact, metallothioneins are required for essential and nonessential metal metabolism, transport, homeostasis, and detoxification (Calisi et al., 2013). Out of the two isoforms of MT, MT1 is more involved in physiological processes than MT2, which binds nonessential metals like cadmium and aids in detoxification (Morgan et al., 2004). Hence, they are particularly well recognized for shielding cells against oxidative stress and Cd toxicity (Zhang et al., 2017). Aside from its significance in the bioremediation of metals, metallothionein is crucial for earthworm survival in polluted soils.

Heavy metals can stimulate the expression of cytoprotective heat shock proteins (HSPs), which serve as molecular chaperons to control protein-protein interactions and prevent recurrent protein aggregations. The class of 70-kDa heat shock proteins, in particular HSP-70 and HSP-72, blocks the caspase-3 pathway and shields earthworm cells from cellular stress (Nadeau et al., 2001).

The outer wall of the intestine is encircled by a sheath of modified peritoneal cells called the chloragogenous tissue. Earthworms have microscopic spheroidal chloragosomes, which are responsible for heavy metal uptake and immobilization (Sinha et al., 2010).

Morgan and Morgan (1999) has demonstrated the precipitation of insoluble mass of Cd, Pb, Zn and Ca in the chloragosomal matrix (a collection of intracellular vesicles throughout the alimentary canal). Since it appears to prevent the diffusion of significant amounts of hazardous metals into other earthworm tissues, they suggested that this compartmentation may function as a detoxifying mechanism based on accumulative immobilization.

In addition to their function in encapsulation and the formation of brown bodies, chloragocytes have also been associated to immune defense because they emit bacteriostatic chemicals (Valembois et al., 1982).

Glutathione, the main nonenzymatic radical scavenger in animal cells, provides a secondary line of protection against oxidative damage. GSH can catalyze the removal of reactive electrophilic xenobiotics from the body. This enzyme aids in cell differentiation in addition to its role in oxidative stress defense (Yang et al., 2012). In earthworm cells, glutathione is reduced and oxidized, providing antioxidant protection against the heavy metal Cd. The expression of GSH appears to be sensitive to cadmium since it significantly decreased even at low cadmium concentrations. This depletion may be caused by the extensive oxidation of two GSH molecules into a molecule of GSSG. Additionally, GSH scavenges reactive oxygen species (ROS), and it has the ability to sequester Cd to block it from adversely interacting with biomolecules.

Metallothionein induction was found to play an important role in the survivability of earthworms in Pb- and Zn-contaminated soils through heavy metal sequestration. In Nigeria, three tropical earthworms (*Alma millsoni*, *Eudrilus eugeniae*, and *L. violaceous*) collected from three slaughterhouse soils had their metallothionein production examined by Dedeke et al. (2016). The levels of heavy metals (Co, Zn, Cd, Cu, Ni, Pb, Mn, and Cr) and MTs were assessed in earthworm tissues and slaughterhouse soils. Compared with the control, it was discovered that earthworms and soil from abattoirs usually had greater amounts of Cu, Zn, Pb, Cd, and Mn (undisturbed soil). Earthworms from metal-contaminated abattoir soil had greater levels of metallothioneins. Thus, one of the main mechanisms by which earthworms are able to accumulate, detoxify, and sequester large quantities of metals in their bodies is the induction of metallothioneins. The concentrations of metals in the soil decrease as more metals are absorbed by earthworms. The induction of metallothioneins is suggested to be the cause of many species' physiological tolerance to heavy metals.

The quantity of metals present in the organic materials that earthworms ingest directly correlates with the volume of heavy metals that accumulate in their tissues. During vermicomposting, organic waste is transformed into shorter-chain or simpler organic acids, which bind the metal ions to form stable metal complexes (Ganguly and Chakraborty, 2019).

7. Conclusion

Waste management systems are significantly disposed to diverse ecological and socioeconomic factors. Henceforth, several nations have removed or reconstructed their age-old methods of waste disposal and have established innovative layouts of ecotechnology for the proper salvaging of organic sludges. Different concepts of green chemistry have been accentuated to construct proper management of different hazardous and nonhazardous wastes for the development of a "zero waste" environment for future generations. In such context, vermitechnology has proved to be an efficient technique in the biodegradation of biological wastes by the utilization of earthworms and the microbiota associated with them. It can therefore be anticipated that the implementation of the core concepts of environmental management, together with the application of various biotechnological resources, will raise sufficient challenges in meeting the economic burden and building greener environments in growing nations such as India.

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CHAPTER

9

Vermicomposting as a tool for removal of heavy metal contaminants from soil and water environment

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1. Introduction

The rapidly increasing global population has led to a huge increase in waste generation and pollution. To reduce the number of landfills, the growing population needs improved food production as well as effective waste management. The growing urban population, especially in developing countries, has increased concerns about waste management and the need to find disposal alternatives, especially for household waste and waste from produce markets. With excessive waste damaging the ecology and especially the health of the soil, waste management is an urgent concern (Das et al., 2022). Increasing waste production has led to various environmental issues, including soil pollution, water pollution, air pollution, loss of biodiversity, and so on. Solid waste disposal releases a variety of greenhouse gases that contribute to global warming. According to reports, solid waste produced by cities is more than 1300 million tonnes (MT) each year, and this number is predicted to increase to 2200 MT by 2025 (Malav et al., 2020). Over the next 15 years, this amount characterizes a rise in the waste generation rate from 1.2 to 1.42 kg per inhabitant per day (David et al., 2019). In India today, about 90 MT of solid waste is produced per year, with a waste

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generation rate of 0.34 kg per person per day (Sharma and Jain, 2019). Even though establishing appropriate recycling processes to improve proper treatment is a difficult task, vermicomposting has the distinct advantage that its operating and maintenance costs are lower than those of some other waste management systems, and its use for organic waste management has grown significantly over time.

Vermicomposting has gained popularity worldwide in recent decades as it is nutrient-smart, readily available, and highly effective (Hussain and Abbasi, 2018; Ray et al., 2020; Tripathi et al., 2020). Vermicomposting is a mesophilic, bio-oxidative, naturally occurring breakdown process where earthworms and microbes work together to mineralize and convert organic waste into organic fertilizer that is rich in nutrients (Pramanik and Chung, 2021; Bordoloi et al., 2015, 2018). Vermicomposting can use a range of wastes as source materials, including industrial wastes (Lee et al., 2017), municipal wastes (Sharma and Garg, 2018), agricultural wastes (Chatterjee et al., 2016; Soobhany et al., 2017), and animal wastes (Sharma and Garg, 2017; Chatterjee et al., 2021). In this way, the vermicomposting method enables the remediation of such waste materials, and the compost can then be used to produce food for humans without the risk of heavy metal accumulation in plants. Earthworms, during the composting process, not only transform the organic substances of urban waste into bioavailable nutrients, but they also remediate the residual heavy metals of the waste by accumulating them in their bodies (Rajiv et al., 2010). Accumulation of Cd, As, Pb, Zn and Cu in earthworms is influenced by a variety of environmental conditions. Metal concentrations in earthworm tissue were closely correlated with heavy metal contamination of soil and substrate (Lapinski and Rosciszewska, 2008). Earthworms have therefore been recognized as important bioindicators and bio accumulators of resistant environmental pollutants, such as heavy metal contamination (Hamidian et al., 2016).

Therefore, it is essential to understand the metals that accumulate in earthworm tissues and their fate in the ecosystem. Although several experiments have demonstrated high levels of heavy metals in sewage sludge and municipal waste (Rorat et al., 2017), the issues of vermiremediation (Huang et al., 2022) and bioaccumulation have received far less attention and are poorly defined (Javed and Hashmi, 2021), particularly in India. In addition, there is insufficient knowledge on the reduction and bioaccumulation of metal ions in earthworm body tissues, especially in tropical climates (Mohee and Soobhany, 2014). Therefore, it was found that vermicompost from different wastes not only had a high content of plant nutrients but also reduced the danger of environmental pollution due to a lower concentration of heavy metals.

2. Vermicomposting process and raw materials used

A cheap way to convert organic waste into nutrient-rich humus is by vermicomposting. Vermicomposting includes pre-composting, composting, and separation (Chatterjee, 2014) (Fig. 9.1).

Pre-composting of bedding material: Vermicomposting should not be done with fresh wastes as the raw material; raw bedding materials should be pre-composted instead.

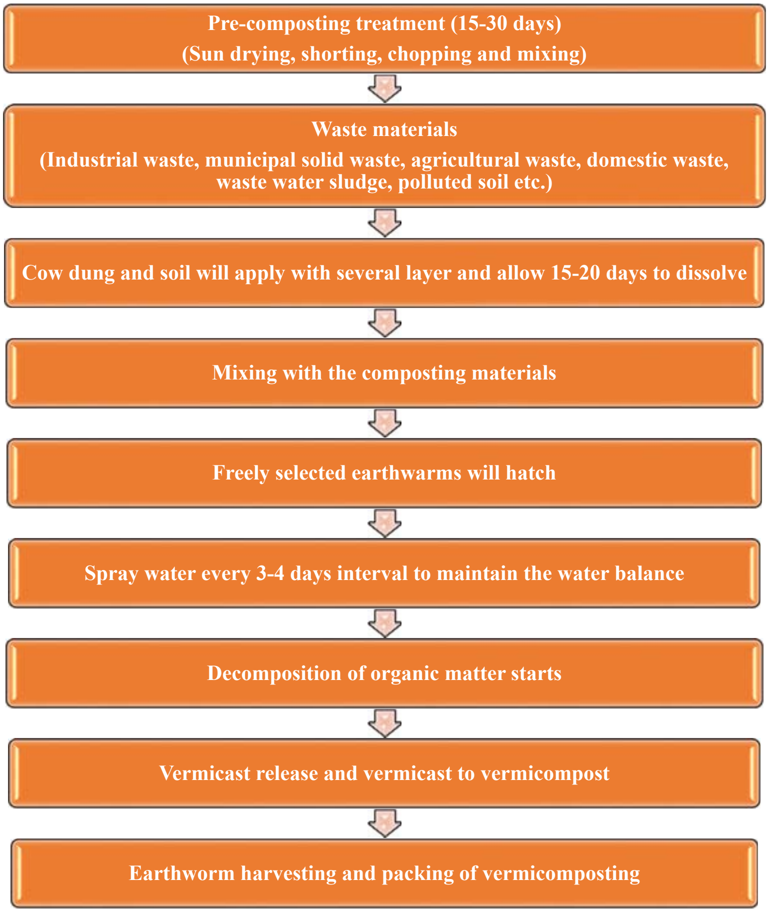

FIGURE 9.1 Schematic diagram of vermicomposting from organic waste.

2.1 Composting

After pre-composting, the raw materials are placed in the pit to start the composting process (Fig. 9.2). The first layer is often sand and has a thickness of 5–6 cm. The layer below is made of rice straw or other agricultural waste and is 5–7.5 cm thick. Above the layer of rice straw is the third layer, which consists of 5–7.5 cm of evenly distributed cow dung. Continue with these layers until the material is 60 cm high. The pit must be watered frequently to maintain the proper moisture level. Earthworms are then poured into the pit at a rate of 1000 worms per square meter or 10 kg per 100 kg of organic material. It is never advisable to let the pit dry out. As a result, water is sprayed on a regular basis.

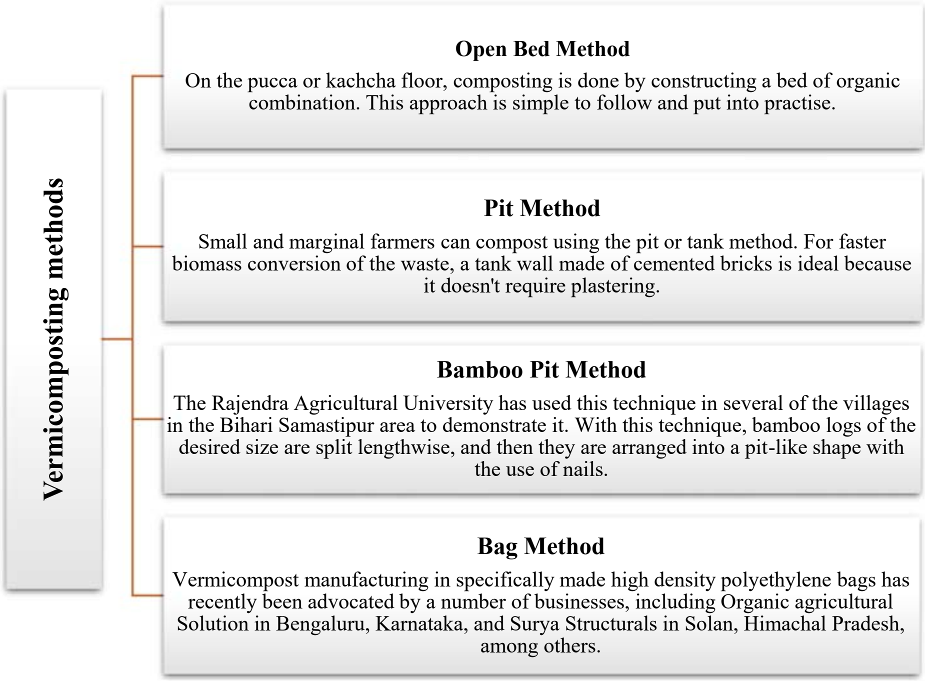

FIGURE 9.2 Types of vermicomposting

2.2 Harvesting of the product

When raw material has entirely broken down, it looks grainy and black. Water needs to be discontinued while compost is preparing. So that earthworms might move from composted to partially digested cow manure, the compost should be kept on top of the cow dung pile. Compost may be sorted and sieved for use after 2 days.

3. Importance of vermicomposting

➢ Earthworms improve soil fertility, and by which a important amount of mineralized N that is more readily available for plant growth (Table 9.1). Also, earthworms ingest a large quantum of plant organic matters containing significant amounts of N and in the form of their stashing, ample of this are returned to the soil (Thakur et al., 2021).

➢ The body of an earthworm has 3% ash, 14% lipids, 14% carbs, and 65% protein (Govindan, 1988). When an earthworm dies, approximately 0.01 g of nitrate is released into the soil, with protein accounting for 72% of its dry weight (Ronald and Donald, 1977a,b).

➢ The fact that compost made by earthworms is entirely organic. Earthworms may quickly break down and fragment the waste, producing a stable, nontoxic product with good structure that has the potential to be highly valuable economically and serves as a soil conditioner for plant growth (Domfnguez, 2004).

TABLE 9.1 Physico-chemical and biological properties of vermicompost.

Sl. No.	Properties of vermicompost	References
Physical properties		
1	Vermicompost is a homogeneous substance that resembles peat and has no odor. Applying, handling, and storing it are simple.	Edwards and Bohlen (1996)
2	Vermicompost enhances soil structure, aeration, texture, water retention, and erosion prevention.	Lazcano and Dominguez (2011)
3	Vermicompost has better physical characteristics to conventional manures, including porosity, bulk density, wetness, and water retention ability.	Moradi et al. (2014)
Chemical properties		
1	Vermicompost contains humic substances that speed up the humification process and keep the level of organic matter optimum.	Arancon et al. (2005)
2	The optimum C/N ratio is between 15–20 in vermicompost.	Hait and Tare (2012)
3	All the major plant nutrients may be found in abundance in vermicompost. Vermicompost has greater levels of both macro and micronutrients than the other farmyard manure.	Kiyasudeen et al. (2015)
4	Vermicompost is a potent stimulator of plant development because it has a greater concentration of vital minerals like N, P, and K and releases those elements gradually.	Atiyeh et al. (2000)
Biological properties		
1	Vermicomposts have a higher population of actinomycetes, fungus and bacteria than other inorganic and organic additives, which makes them preferable.	Huang et al. (2016)
2	Vermicompost is devoid of viruses and harmful substances.	Soobhany et al. (2017)
3	Urease, dehydrogenase, phosphatase, arylsulfatase, and b-glucosidase activity are all increased in soils supplemented with vermicompost.	Yang et al. (2015)
4	Utilizing vermicompost significantly enhanced the N, C content, respiratory rate, and enzymatic activity.	Manivannan et al. (2009)

➢ Vermicomposting creates a product that is naturally crafted to provide plants with numerous advantages. The nutrients are very readily accumulated by plant roots in earthworm compost. As it contains worm mucus, vermicompost is more difficult to remove from the soil than chemical fertilisers are (Lim et al., 2015).
➢ The compost is enhanced with bacteria and microorganisms when it passes through the worms' body. These aid in the disease resistance of plants and deter some plant pests. Increased microbial activity makes the environment much more appealing to birds, assisting in eradication plant pests (Awasthi et al., 2014).
➢ Vermicompost contains hormones promoting plant growth, encourages seed germination, enhances plant growth, and increases crop yield (Ndegwa and Thompson, 2001).

➢ Vermicompost is a colloid which stores nine times water than its weight. When dry spell occurs, presence of vermicompost make a tremendous difference. Because the water is retained at an organic level, it evaporates slowly while remaining available to the plants (Reinecke and Venter, 1987).
➢ Because it can give the economic advantage and is impoverished a second source of income, vermicomposting can be a helpful cottage enterprise (Samal et al., 2019).

4. Vermicomposting for removal of metal ions from-

4.1 Detoxification of industrial wastes/sludges using earthworms

Earthworms are able to physiologically transform and remove most heavy metals in industrial sludge. This is due to their robust metabolism and the involvement of a diversified gut microbiota, enzymes, and their chloragocyte cells that convert toxic to nontoxic forms (Bhat et al., 2018). Vermiremediation, as evidenced by the joint activity of bacteria and earthworms, is an efficient strategy for reducing the genotoxicity of industrial pollutants. Vermicomposting alone makes the waste difficult to treat, but it can be combined with other organic fertilizers, such as cattle manure, to stabilize various types of hazardous and toxic industrial wastes in safe housing. Chemical analysis exhibited that metal (Cr, Ni, Zn, Cu, and Pb) concentrations in industrial wastes decreased by 10−50% in the finished vermicompost (Kizilkaya et al., 2021). The genotoxicity and elimination of metals in the finished vermicompost show that earthworm application has the ability to clean hazardous industrial sludge. Bioassay of Allium cepa has been used to evaluate the toxicity of municipal sewage sludge and vermicomposting for sewage sludge bioremediation (Sohal et al., 2021). The study also found that after vermicomposting by the earthworm *Eisenia fetida*, all elements (Ni, Pb, Cr, and Cu) decreased. During vermicomposting of sewage sludge, the amount of water-soluble metals such as Cr, Zn, and Cu was significantly reduced (Hait and Tare, 2012). As organic material passed through the earthworm's gut, some of it was decomposed and gut microbial activity and pH improved, increasing the ability of metals to bind to carbonates and ions in the ingested material. As a result, the bioaccumulation rate of metals in the water-soluble portion in the stomach of the worm could be increased (Suthar et al., 2014). Due to metal bioaccumulation and formation of metal-organic complexes, the availability of heavy metals reduced (Table 9.2) during the vermicomposting (Singh and Kalamdhad, 2013).

4.2 Removal of metals by vermicomposting from municipal solid waste

Population, urbanisation, and affluence all have an impact on waste generation. Municipalities in most developing countries continue to face significant challenges in collecting, recycling, treating, and disposing of growing amounts of municipal solid waste (Sharholy et al., 2008). In terms of municipal waste management, vermicompost is becoming increasingly popular as a less harmful alternative to chemical fertilisers for maintaining and improving soil quality. Additionally, the use of vermicomposting in the management of biodegradable solid waste is being encouraged by the global recognition of the necessity to recover organic materials and return them to the soil (Sim and Wu, 2010). Heavy metals

TABLE 9.2 Various waste materials removed from soil and water by earthworms.

Type of waste	Species of earthworm used	Results	References
Industrial waste	*Eisenia foetida*	The pollutant load was lowered, and heavy metals were bioaccumulated by earthworms effectively. Earthworms readily bioaccumulated copper oxychloride.	Maboeta et al. (2004), Mondal et al. (2020)
Municipal solid waste	*Eisenia foetida*	Due to the metal bioaccumulation in the guts of earthworms, the volume of metals (Zn, Fe, Mn, and Cu) in earthworm-treated sludge was significantly reduced.	Suthar (2008), Cui et al. (2018)
Industrial sewage and sludge	*Hyperiodrilus africanus*	Ethylbenzene, benzene, xylene and toluene were biodegraded or bioaccumulated by the earthworms.	Ekperusi and Aigbodion (2015)
Agricultural waste	*Aporrectodea caliginosa*	The breakdown of pesticide in soil was assisted by earthworms, which also accumulated the chemicals inside their guts.	Schreck et al. (2008)
Polluted soil	*Aporrectodea Caliginosa, Lumbricus terrestris*	In typical soil microhabitats where earthworm activity is prevalent, atrazine mineralization was shown to be reduced. Earthworms had a great impact on how the bacterial populations in the soil were organized.	Kersanté et al., 2006

are common components of municipal and industrial wastes and have the ability to affect plant growth and microbial activity in soil. In addition, from an environmental risk and ecotoxicology perspective, it is becoming increasingly clear that the bioavailable (mobile) fractions, which are often a tiny fraction of the total heavy metal concentration, are of critical importance to the environment (Amir et al., 2005). Geogenic sources, mining, livestock, agricultural practises, industrial effluents, and domestic sewage are the primary causes of heavy metals contamination in the environment. The vermicomposting method successfully stabilises, removes, and recycles heavy metals contained in wastes by utilising the ecological function of earthworms and microbial diversity (Singh et al., 2011). In case of vermicomposting of the textile mill wastewater in combination with cow dung utilizing the *E. fetida*, a decrease in the total concentration of Zn (11.5%−38.2%), Fe (3.1%−12.3%), and Cr (0%−25%) was reported in the final products (Kaushik and Garg, 2003). Similarly, a considerable decrease in the content of Fe (3.8%−38.1%), Cd (33.3%−60.9%), Pb (15.8%−45.8%) and Zn (28.6%−53.8%) was observed after 77 days of vermicomposting of textile wastes mixed with cow and poultry manure using *E. fetida* (Garg and Kaushik, 2005).

4.3 Vermicomposting to remove metal ions from polluted soil

Soil heavy metal pollution is largely due to human activities such as smelting, painting, industrial emissions, mining and agricultural pesticide application (Vareda et al., 2019). Earthworms have shown promise in cleaning heavy metal contaminated soils (Ahadi et al., 2020). The intestinal tissue of earthworms, known as chloragogenus tissue (Liang et al., 2011), can absorb significant amounts of heavy metals and is believed to serve as a cation exchange system that can absorb and store the heavy metals easily (Sivakumar et al., 2003). The bioaccumulation of Cd, Zn, and Hg by earthworms is significantly higher in polluted soil because their bioaccumulation factor is above 1.0 (Richardson et al., 2005; Li et al., 2020). According to a recent study, 17.60% of Cd from contaminated soil can be removed by earthworm (Wu et al., 2020). Vermicomposting can hasten phytoextraction of the metals present in soil (Ma et al., 2003) by affecting microbial activities and fungal communities (Jusselme et al., 2015).

4.4 Vermicomposting for wastewater sludge treatment

Vermicomposting has been employed as a low-cost wastewater sludge treatment process. Vermicompost has efficiency in reducing heavy metal content (Ni^{2+}, Cu^{2+}, Al^{3+}, Zn^{+}, Fe^{2+}) from galvanoplastic wastewater (Jordão et al., 2010). This might be due to the experiment's high pH and the presence of phosphates in vermicompost. Vermicompost is used as an adsorbent to remove heavy metals as well as cationic dyes, for example, crystal violet (de Godoi Pereira et al., 2009) and methylene blue. Dried vermicompost can be used as an adsorbent to remove lead, copper, manganese, cadmium, zinc and nickel from laboratory wastewater. According to the study, the pH of the solution increased due to the application of vermicompost and effectively adsorbed all heavy elements (Barbosa et al., 2018). Manyuchi et al. (2018) used vermifiltration to decontaminate distillery wastewater and discovered nutrient-rich vermicompost and a 90% decrease in various pollutants. Inoculation of *Eisenia foetida* during composting of textile sludge resulted in a reduction of Zn (11.5%−38%) and Cr (25%) in the final product of vermicomposting (Kaushik and Garg, 2003). A significant reduction was observed for Cu (26.9%−49.1%), Cd (20.8%−58.1%) and Pb (42.7%−72.4%) in the final product of vermicomposting of aquatic plants using cow dung as a filler (Badhwar and Singh, 2021).

5. Vermicomposting for breaking down of heavy metal in organic pollutants

The vermicomposting process successfully stabilizes, recycles, and reduces heavy metals in waste using the ecological activities of earthworms and microbial diversity (Singh et al., 2020a,b). During vermicomposting, enzymes are used to mineralize and humify organic wastes, resulting in short-chain organic acids. Freshly formed organic acids (humic acid) also bind to metals in the environment and form stable metal silicate complexes (Swati and Hait, 2017). Meanwhile, certain metal fractions readily accumulate in earworm tissues, leading to a decrease in total metal concentrations. Heavy metals need to be removed by applying organic modifications in four steps.

5.1 Immobilization

The first step in the immobilization process is the application of vermicompost to increase the retention of ionic chemicals (Kamari et al., 2011). There are two possible explanations for such phenomena. First, the biodegradation process mediated by the earthworm increases the amounts of humic substances that can effectively immobilize metals by forming stable humic metal complexes due to negative charges on the surface (Bolan et al., 2014). Secondly, earthworm chloragosomes, which consist of modified epithelial cells and intestinal electrocytes and contain components of ion exchangers such as phenolic hydroxyl, phosphoric acid, carboxylic acid, and sulfonic acid groups, act as an ion exchange complex that can absorb heavy metals (Cooper, 1996). During vermicomposting, hydrolytic enzymes are produced by earthworms, and due to the increased enzymatic activity, the mobile metal content in the vermicompost increases. These mobile components are rapidly absorbed and deposited into the cutaneous tissues of earthworms by the epidermal tissues. Earthworms accumulate metals in their guts as a stress response when they come into contact with waste containing heavy metals. Most earthworm species have survived in this situation by maintaining a balanced rate of intake and excretion during metabolism. To cope with the stress caused by high metal concentrations, the metal excretion rate of earthworms rapidly increases and exceeds tissue absorption (Li et al., 2009). The metals are taken up by earthworm epidermal tissues and accumulate in the earthworm body. According to some researchers, metals can also bind to cysteine-rich metal-binding proteins such as metallothioneins, rendering them physiologically inert. These metals, in which proteins are bound, are released to the soil ecosystem and immobilized in the humus components of the soil when earthworms die. The amount of metal contamination in substrates and soil was closely related to metal concentrations in earthworm tissues (Hobbelen et al., 2006). As a result, earthworms have been identified as important bioaccumulators and indicators of persistent pollutants such as heavy metal contamination in the environment (Suthar et al., 2008). Metals with a higher affinity for protein binding include Zn, Co, Cu, and Cd. Heavy metal accumulation is influenced by a number of parameters, including earthworm species, environmental conditions, degree of metal contamination, and duration of exposure (Bhat et al., 2018).

5.2 Reduction

Second, vermicompost serves as an important source of electrons and carbon for microorganisms, leading to a reduction process. The microbial activity of vermicompost has resulted in lower concentrations of several heavy metals in soils. In tropical soils, the application of vermicompost reduced concentrations of copper ions (Cu^{+}) and cadmium ions (Cd^{+}) (Jadia and Fulekar, 2008). Vermicompost was used to reduce the concentrations of Ni^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, and chromium ion ($Cr_2O_7^{2-}$) in synthetic wastewater (Pereira et al., 2014).

5.3 Volatilization

The third phase, volatilization, is enabled by the use of vermicompost, which has a microbial activity that methylates certain elements such as selenium (Se), mercury (Hg), and arsenic (As) (Park et al., 2011). The microbial activity of vermicompost contributes to bioremediation by creating a favorable environment for the reduction and methylation processes.

5.4 Modification of the rhizosphere

Finally, vermicompost releases weak acids (oxalic acid, maleic acid, butyric acid, lactic acid, citric acid, and propanoic acid) into the soil that contribute to rhizosphere modification and regulate soil pH (Singh and Singh, 2017). Excess hydronium ions (H_3O^+) released into the soil by weak acids assist the bioremediation process in this case (Dokuchayeva, 2021). On their way through the digestive tract, the waste substrates consumed by earthworms were chemically and microbiologically altered, with a large part of the organic fraction being converted into labile forms more accessible to the organisms.

6. Safe disposal of metal-enriched compost

Microorganisms degrade organic material, absorb essential nutrients, and partially accumulate metals in tissues through the production of hydrolytic enzymes, while earthworms expedite the process by processing the waste substrate. In vermicomposting, earthworms aerate, mix, shred, fragment, and digest the substrate enzymatically, transforming it into a much more humic, fine, and microbially active substrate (Samal et al., 2019). As a result, vermicomposting worms (*E. eugeniae, E. fetida, L. rubellus, and E. andrei*) are not only an important biological indicator but also a good collector of heavy metals (Singh et al., 2020a,b). It is generally believed that the detoxification of heavy metals in soil is mainly due to the complex metabolic system and active intestinal flora of the earthworm. Bacteria are known to be able to detoxify and are resistant to heavy metal contamination. *Bacillus licheniformia* strain KX 657,843, obtained from the stomach of *Metaphire posthuma*, was found to be resistant to Cu^{2+} and Zn^{2+} (Biswas et al., 2018a). Further investigation revealed that the strain not only tolerated high Zn^{2+} and Cu^{2+} concentrations but also inhibited their accumulation by complex formation via the extracellular polymeric components of KX 657,843. Similarly, other heavy metal detoxifying bacteria, *Bacillus licheniformia* strain MF589720, *Bacillus megaterium* strain MF589715, and *Staphylococcus haemolyticus* strain MF589716, were isolated from the stomach of *M. posthuma* (Biswas et al., 2018b). All strains observed as PSB were able to reduce Zn^{2+} and Cu^{2+} toxicity by forming a complex between the phosphate ion and the heavy metal. Similarly, Pseudomonas sp., which is found in large numbers in the worm gut, can fix Pb^{2+} by increasing phosphate solubility and thus reducing Pb^{2+} toxicity (Teng et al., 2019). Different research examined how microorganisms in the gut of *Lampito mauritii* and *E. feotida* responded to exposure to Mn^{2+}, As^{2+}, and Zn^{2+}. The intestinal bacteria released metallothionine, which reduced the toxicity of metal ions through uptake and complex formation (Goswami et al., 2014). Consequently, the existence of these variants in the worm gut apparently led to a reduction in metal toxicity through bioaccumulation, biotranslocation, biodegradation, and other mechanisms under mesophilic and aerobic conditions (Bhat et al., 2017; Cui et al., 2018).

6.1 Vermiaccumulation

Vermiaccumulation is the process by which pollutants are absorbed by earthworms and stored in their tissues (Fig. 9.3) (Javed and Hashmi, 2021; Singh et al., 2022). Pollutants can be taken up by earthworms through food or epidermal absorption (Wang et al., 2019). *E. fetida* feeds on soils containing various pollutants and can carry out specific processes such as crushing and digestion, leading to the uptake of these pollutants through the digestive tract and subsequent translocation into the bodies of other earthworms (Park et al., 2011). On the other hand, the chemical potential of pollutants may decrease during epidermal

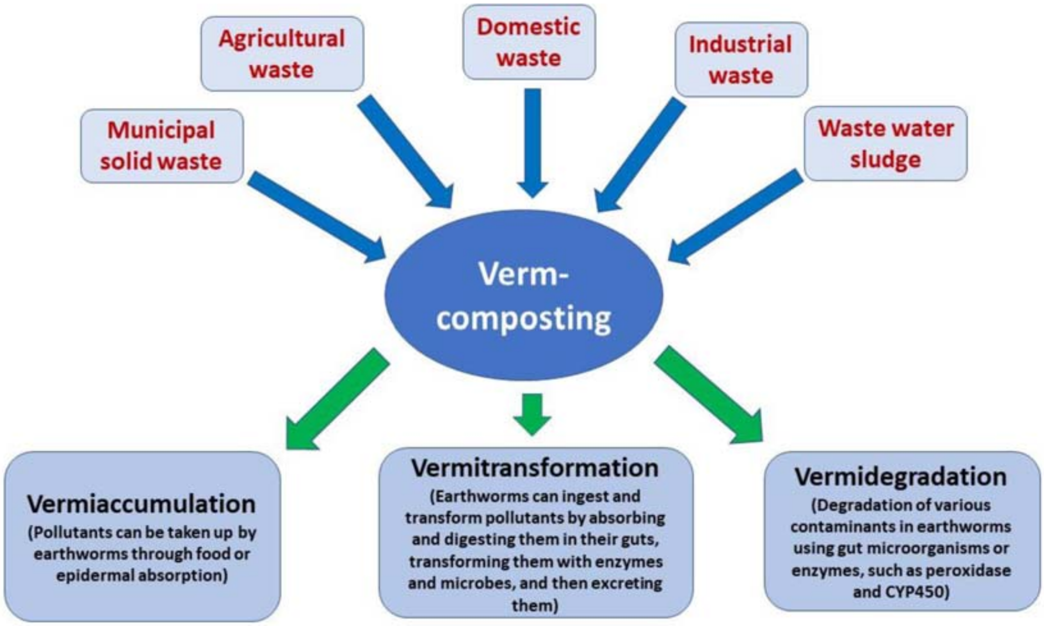

FIGURE 9.3 Safe disposal of metal-enriched compost through vermicomposting

absorption, resulting in their absorption by body membranes and translocation into the body of *E. fetida* (Zeb et al., 2020).

6.2 Vermitransformation

Vermitransformation, which is also a key process in vermiremediation, is the rapid conversion of gradually decomposing waste materials into useful fertilizer components through the combined activity of earthworms and soil microorganisms (Goswami et al., 2013). By absorbing and digesting pollutants in their intestines, transforming them with enzymes and bacteria, and then excreting them, earthworms can ingest and modify pollutants (Katagi and Ose, 2015). Metals are accumulated in the gut of *Eisenia fetida, Nicodrilus caliginosus, Lampito mauritii*, and *Allolobophora rosea* by inducing metallothionein (Goswami et al., 2014). The bioavailability and detoxification cycles of heavy metals are controlled in the earthworm stomach by metallothionein (Amiard et al., 2006; Maity et al., 2011).

6.3 Vermidegradation

Vermidegradation is the process of degradation of various contaminants in earthworms using various microorganisms or enzymes present in the gut, such as peroxidase and CYP450 (Christyraj et al., 2022). Earthworms contribute to the microdegradation of organic matter by increasing the surface area for microbial growth, degrading substrates, and aerating the environment (Zeb et al., 2020). Earthworms can enhance the interaction between microorganisms and a contaminant, making it easier to remove the contaminant from the soil.

7. Strategies for improving vermiremediation

Several strategies can help boost vermicomposting. First, adding surfactants to the bioremediation process has been found to improve the process. Surfactants are a class of naturally occurring and man-made compounds that can desorb, dissolve, or emulsify substrates that are difficult to dissolve (Megharaj et al., 2011). They can help remediate soil contamination by mobilizing and desorbing contaminants, making them more accessible to earthworms, which can take up more of them (Paria, 2008). Although their use in vermiremediation has not been adequately studied, surfactants such as cetyltrimethylammonium bromide, sodium dodecyl sulfate, TX100, and Brig30 (Schneider and Bahnemann, 2013) are active bioremediation accelerators.

Secondly, the growth of earthworms had to be promoted by providing sufficient food and improving the conditions for inoculation to support this process. The absorption rate and elimination of pollutants from the soil are improved by enhancing earthworm development (Rodriguez-Campos et al., 2014).

Finally, combining vermiremediation with other remediation methods (biochar, phytoremediation, and microremediation) will greatly improve the process (Yuvaraj et al., 2021). The combined use of earthworms and biochar can help improve soil quality and biodiversity. Earthworms have recently been widely applied in biochar studies, either as biological targets for biochar toxicity tests or as pollution indicators in biochar-enriched soils (Verheijen et al., 2010). In terms of microbial and extracellular enzyme activation, earthworms can have a significant impact on biochar.

Interactions between earthworms and microbes can help improve vermicomposting. Microbe-assisted remediation combines earthworms and microbes to increase the effectiveness of remediation by improving the detoxification and degradation of contaminants, as well as the uptake, transport, and binding of contaminants. Soil microorganisms are essential to the soil environment because they play an important role in maintaining nutrient cycling, regulating energy transfer, restoring equilibrium in the terrestrial environment, and degrading organic materials (Prasad et al., 2021).

Phytoremediation and vermiremediation could be used in combination. The use of earthworms increased the biomass and Cd content in the shoot of Solanum nigrum by 61.71% and 35.8%, respectively (Ji et al., 2020). *Eisenia fetida* increased Se uptake and translocation in plants, resulting in higher Se contents in shoots (Huang et al., 2020). In addition, earthworm (*Metaphire posthuma*) gut bacteria can promote plant development while eliminating hazardous heavy metals from the ecology (Biswas et al., 2018a,b).

8. Precaution to be taken during vermiremediation

Vermiremediation, a unique tool for bioremediation technology, remediates contaminants but possesses some limitations or precautions. Moderately contaminated soils are more suitable for this method of remediation. Earthworms cannot survive in adverse conditions like higher soil pH, salt concentrations, organic or heavy metallic contaminants, pesticide residues, crude oils, as well as emerging toxic substances that transmute their community structure and pose a threat to their survival (Zhang et al., 2020). The susceptibility of earthworms to hazardous pollutants, such as heavy metals, pharmaceutical wastes, biomedical wastes, saline wastes, and so on, necessitates additional research. These wastes are rarely vermicomposted because of their toxicity. Vermicomposting can be done on a regular basis if the chemical and physical qualities of the waste are altered.

Vermiremediation is only effective in earthworm-active strata, which vary depending on the biological groupings of the earthworms utilized. A combination of earthworm species (epigeic, endogeic, and anecic) could be utilized instead of a single species to optimize pollution removal during vermifiltration. Before mixing multiple earthworm species in a system, the suitability of each species must be assessed.

Lack of enough food to survive and provide high-level performance will be a limiting problem because the capacity of worm species to break down pollutants depends mostly on their dietary patterns (Johnston et al., 2014). Furthermore, toxins accumulated in earthworms could infiltrate the food chain if they are not adequately handled or relinquished (Shi et al., 2020).

Vermiremediation techniques are subjected to strict guidelines. The amount and kind of carbon are crucial factors in vermicomposting. The regular addition of new garbage to the bed leads the soil to become anaerobic, which is very detrimental for both aerobic bacteria and earthworms. The trash must be mixed at regular intervals to allow oxygen to circulate. The focus of the research should be on creating a composting bed that can automatically supply garbage with ambient oxygen.

Earthworms are delicate creatures, and temperature (Cui et al., 2022), seasonal weather, or other environmental factors may have an impact on their existence and subsequent vermiremediation activities (Sanchez-Hernandez et al., 2019). Extreme heat or cold can have an impact on earthworm survival, and soil moisture content must be enough (8%–57%) for earthworms to dig through it (Richardson et al., 2009). If pollutants gathered in earthworms are not adequately cleaned or disposed of, they can infiltrate the food chain (Shi et al., 2014).

The procedure of removing earthworms from composted bedding takes a long time. There are no effective methods for it. Earthworms should be carefully separated from bed materials; if not managed properly, they risk death or injury. The separation procedure needs to be handled in an efficient manner.

9. Conclusions

Vermicomposting is a method that uses earthworms and microorganisms to break down, accumulate and detoxify various waste materials and convert them into a product that can be used agriculturally. This cost-effective waste management method is environmentally friendly and preferable to other waste disposal methods. Enzymatic activities in the earthworm's gut lead to the immobilization of toxic metals, which means that heavy metals can be removed from industrial wastes/sludges, sewage sludges, laboratory effluents and polluted soils by vermiremediation. According to the information in this article, vermicomposting can be defined as an integrated waste management strategy that can efficiently interact with polluted soil, municipal solid waste, industrial waste, and wastewater to reduce heavy metal pollution from various waste materials, improve crop productivity and soil health, and generate energy. Heavy metal removal can reduce groundwater pollution and soil and plant toxicity. However, further studies are needed to overcome the few limitations of the technology to achieve the goal of sustainable development.

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CHAPTER

10

Earthworms and microplastics: Transport from sewage sludge to soil, antibiotic-resistant genes, and soil remediation

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Abbreviations

ARB Antibiotic-resistant bacteria
ARG Antibiotic resistance gene
EU European Union
HGT Horizontal gene transfer
MGE Mobile genetic element
MP Microplastic
NP Nanoplastic
OM Organic matter
PA Polyamide
PBS Polybutylene succinate
PE Polyethylene
PET Polyethylene terephthalate
PLA Polylactic acid
PP Polypropylene
PS Polystyrene
PVC Polyvinylchloride

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TC Tetracycline
WWTP Wastewater treatment plant

1. Introduction

Human activities have had a huge impact on the environment since the start of the Industrial Revolution. Since the 1960s/70s, the mass production of plastic polymers has resulted in the accumulation of plastics of different sizes and shapes across different soil ecosystems (Okoffo et al., 2021). Although apparently unrelated, the indiscriminate use of antibiotics has led to biologic adaptations in bacteria, with an increase in the abundance of antibiotic resistance genes (ARGs) in rivers and other water bodies that feed into soil systems (Seyoum et al., 2022). ARGs can reach soil systems from diverse sources (which will be addressed in Section 1.2), mainly via the agricultural use of animal manure and sewage sludge generated at wastewater treatment plants (WWTPs). In addition, the presence of ARGs on the surface of microplastics (MPs; plastic fragments of longest dimension <5 mm) and the capacity of the MPs to act as vectors, and thus as a new route of entry for ARGs in soil ecosystems, have been reported in recent years (Liu et al., 2021).

Numerous recent studies have focused on the effects of MPs and their fate in soil (Mendes, 2021; Tian et al., 2022; Ya et al., 2021). There has also been growing interest in the consequences of the presence of ARGs in soil organisms (Delgado-Baquerizo et al., 2022). However, little is known about the combined effects of MPs and ARGs on soil ecosystems, despite these being two of the main emerging and prevalent contaminants in terrestrial environments (Lu and Chen, 2022).

Bioremediation techniques have been developed over the years, and their potential to provide solutions to anthropogenic problems has been explored. In particular, the use of earthworms in the vermicomposting process can be considered a nature-based solution or strategy for minimizing or ameliorating the risks associated with hazardous pollutants (Sanchez-Hernandez and Domínguez, 2019). Therefore, this chapter aims to provide important up-to-date knowledge on the combined effects of MPs and ARGs on soil systems, as well as on the use of vermicomposting as a bioremediation and environmentally friendly approach to counteract this issue.

1.1 Microplastics in sewage sludge and soil

Increased levels of urbanization and industrialization have gone hand in hand with an increase in wastewater generation. This, in turn, has led to the release of larger amounts of sewage sludge residues, mainly due to inefficient operational treatments in WWTPs. Although sewage sludge production accounts for nearly 1%–2% of the total effluent volume, management costs can make up to 20%–60% of the total operating costs (Rostami et al., 2020). The main producers of sewage sludge worldwide are Europe, North America, and East Asia (Shaddel et al., 2019). Increasing awareness about the high nutrient value of sewage sludge has changed the perception of this type of waste, which is now considered a valuable product. In addition, recent changes to European Union (EU) legislation now accept the use of certain types of waste, including sewage sludge, for their application as fertilizers in line with EU waste reuse and recovery strategies (European Commission, 2018). Overall, the best-known methods of use for sewage sludge worldwide include the application to land (as fertilizer), composting, and bioremediation (Sobik-Szołtysek and Wystalska, 2019). Moreover, sewage sludge has been used to generate energy via aerobic and anaerobic digestion (Li and Feng, 2018).

A main concern is that plastic particles can accumulate in sewage sludge derived from WWTPs owing to the massive plastic consumption associated with human activities. Although different treatments can be applied to reduce the amounts of plastic in sewage sludge, these processes are not 100% effective (Mahon et al., 2017). For instance, accumulation of on average $22.7 \pm 12.1 \times 10^3$ plastic particles/kg dry sewage sludge have been reported for different WWTPs across China (Li et al., 2018b). In Europe, values of $170 \pm 29 \times 10^3$ plastic particles/kg dry weight and $133 \pm 29 \times 10^3$ p/kg have been recorded for WWTPS in Finland (Lares et al., 2018) and Spain (Edo et al., 2020), respectively. In Canada, average values of 14.9 ± 6.3 and 4.4 ± 2.8 p/kg have been found for primary sewage sludge (Gies et al., 2018) and secondary sludge (Hatinoğlu and Sanin, 2021) respectively.

Plastics are not only detected in wastewater and the associated sewage sludge but also in soil systems. It has been shown that agricultural practices such as mulching can act as a source of MPs in soils (Beriot et al., 2021; van Schothorst et al., 2021). In addition, the use of sewage sludge as a fertilizer has doubled the concentration of MPs in agricultural soils (Corradini et al., 2019; van den Berg et al., 2020). MPs are globally present in horticultural and agricultural soils, at concentrations of around 4.5 mg kg^{-1} dry soil. Furthermore, soils in urban areas may contain 10 times more MPs, and extreme values between two to four orders of magnitude higher have been reported in industrial zones (Büks and Kaupenjohann, 2020). The accumulation of MPs in the soil system is also highly important due to the ability of these contaminants to act as vectors for others such as organic pollutants, ARGs, or heavy metals (Abbasi et al., 2020; Fajardo et al., 2022). This can lead to modulation of the proper MPs themselves and/or the associated toxicity, by altering the biologic identity of these compounds (Reilly et al., 2023). Indeed, there is evidence for the transfer of plastic debris across terrestrial trophic food chains (Huerta Lwanga et al., 2017).

1.2 Presence of antibiotic resistance genes in soil

Antibiotic resistance is recognized as one of the top global threats to public health, according to the World Health Organization (World Health Organization, 2020). ARGs are involved in the resistance of bacteria to antibiotics (White and Hughes, 2019) and can also occur naturally in soil microorganisms (Dcosta et al., 2011). Although natural selection restricts the flow of bacteria and genes between ecosystems, a wide range of ARGs can be mobilized and horizontally transferred by conjugation, transformation, or transduction, via mobile genetic elements (MGEs) to many bacterial species, particularly those causing disease (Larsson and Flach, 2022). The spread of ARGs is likely to favor their accumulation and prevalence in the environment, with significant consequences for both human and animal health through uptake by plants, by fertilization, or by release to water via runoff (Chen et al., 2019; Zhang et al., 2021). This represents a threat for the overall ecosystem health as considered in the "One Health framework[1]" (Li et al., 2022a; McEwen and Collignon, 2018; Zhu et al., 2019). Taken together, ARGs have been recognized as emerging pollutants of concern, with diverse effects on the environment (Zhu et al., 2021c). In this regard, ARGs are known to disrupt soil systems by modulating the soil microbiome, with the application of organic fertilizers being a major entrance of ARGs into soil (Han et al., 2022; Huygens et al., 2022; Shekhawat et al., 2022).

Existing studies have focused on determining ARG contents within microbial genomes; however, genomic information cannot always be translated into functional capacities. To fully understand the underlying mechanisms of resistance in natural environments, it is essential to consider which ARGs are expressed and do not remain "silent" in the host bacteria (Li et al., 2022a; Xu et al., 2020). Soil is one of the major natural reservoirs of ARGs on Earth, containing the so-called soil antibiotic resistome or intrinsic resistome (Nesme et al., 2014), which harbors an immense diversity of microorganisms exceeding that of the human and domestic animal microbiota (Blum et al., 2019). Furthermore, the resistome can be enriched by stress or exogenous contaminants (Pal et al., 2015), particularly in soils fertilized with manures (Zhang et al., 2022d). Soil fauna also plays an essential role in generating and disseminating ARGs, but its role has not been fully explored yet. Of note is the synthesis and release of ARGs associated with alterations to the gut microbiota in collembolans (Zhu et al., 2018) and earthworms (Li et al., 2021a). Apart from soil biota, the incipient presence of ARGs in soils is determined by other multiple sources (Fig. 10.1), such as WWTPs, animal manure, and air pollution (Zhu et al., 2019).

WWTPs represent one of the main sources of antibiotics and ARGs, mainly due to their inefficient removal after water treatment (An et al., 2018; Congilosi and Aga, 2021). Sewage sludge and animal manure are widely used as organic fertilizers as they are among the most abundant types of organic waste generated in WWTPs and farms, respectively (Sanz et al., 2022). Sewage sludge is a primary source of MPs and has been found to play a critical role in transporting these contaminants to soil systems. The capacity of MPs to act as vectors for surrounding pollutants is determined by their average size and abundance (Ky et al., 2022; Wang et al., 2023). Moreover, the use of water reclaimed from WWTPs to irrigate parks and gardens can stimulate the expression of ARGs that are ultimately incorporated into the soils (Wang et al., 2014).

Both sewage sludge and manure are considered important sources of ARGs (Huygens et al., 2022; Xu et al., 2020; Zhang et al., 2022a; Zhang et al., 2022d; Zhao et al., 2021), and their use as fertilizers may facilitate the horizontal transfer of ARGs between sludge/manure-borne bacteria and indigenous soil microbiota via the presence of MGEs (Xu et al., 2020). Their application into agricultural land can potentially promote the transfer of ARGs accumulated in soils to the food chain via transmission to different plant tissues (Chen et al., 2017; Li et al., 2022a; Sanz et al., 2022). In addition, the soil itself contains native antibiotic-resistant bacteria (ARB) that can be enriched by inputs of fertilizer (Yang et al., 2022a, 2022b, 2022c). It is therefore of utmost importance that effective management measures are considered prior to applying manure and sewage sludge to land. The excessive

[1] One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems. https://www.who.int/health-topics/one-health#tab=tab_1.

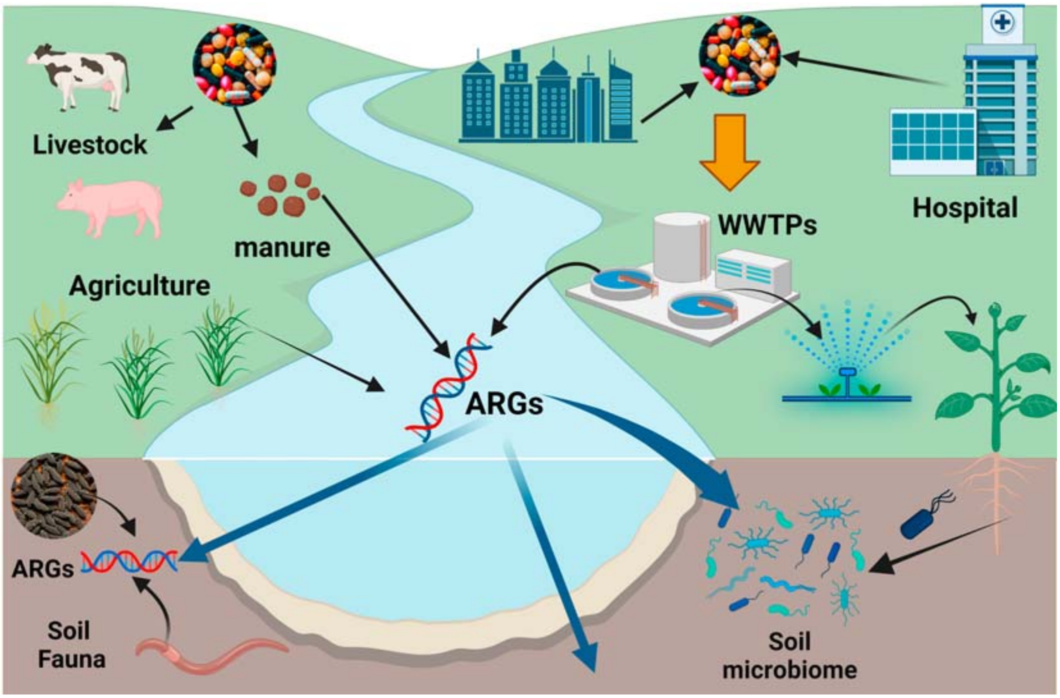

FIGURE 10.1 Main sources of ARGs and their interactions in soil systems.

input of poorly managed organic wastes to soil may significantly impact the environment we rely on, bearing in mind the risk of resistome transfer from sludge and manure to agricultural soils. Furthermore, the increasing incidence of air pollution, particularly in highly industrialized countries, is promoting the entry of ARGs into soil mainly by wet and dry deposition (Li et al., 2018a), with ARGs being able to be transported through physical barriers and even reaching soils in remote regions.

The extent of the presence of ARGs in the soil compartment is determined by several factors, including soil physical and chemical properties such as pH, organic matter (OM) content, texture, and total nitrogen and phosphorus contents; in addition, the microbial community composition and diversity are considered key drivers of the presence and dissemination of ARGs (Wang et al., 2020). The time of exposure to manure and sewage sludge may also determine the quantity of ARGs transferred and the expression of the indigenous soil ARGs. For instance, He et al. (2021) demonstrated a time-dependent decrease in the abundance of ARGs in manure-amended soils over a period of 2 years (He et al., 2021).

1.3 Earthworms as targets of exposure to contamination and as tools for soil remediation

Earthworms (phylum Annelida) are terrestrial invertebrates that ingest and feed on OM. They contribute to the turnover of soil OM by breaking down large carbon chains, through the gut microbiome and the activity of secreted enzymes, e.g., carboxylesterases (Aira et al., 2006; Sanchez-Hernandez et al., 2019). Earthworms can also alter the soil microbiome by releasing gut bacteria in the egested material known as casts (Aira and Domínguez, 2011). In addition, earthworm burrowing activity can modify the soil structure and contribute to the transport of nutrients, minerals, and soil particles to different depths. Earthworms are thus commonly known as soil engineers and play a key role in the functioning and the dynamics within soil ecosystems (Blouin et al., 2013).

Due to their soil-dwelling nature, earthworms are in close contact with the soil matrix through their dermis and/or by ingestion of soil particles. Consequently, they are exposed to any contaminants present in the surrounding environment, both particulate matter and solutions (Jager, 2004), since they require habitats with high humidity (Perreault and Whalen, 2006). On the one hand, earthworms are valuable model organisms for standard ecotoxicologic tests (OECD, 2004, 1984); and they can be used as bioindicators of soil quality in response to organic and inorganic pollutants (Fusaro et al., 2018). On the other hand, they are also able to accumulate toxicants in their tissues and remove or reduce the concentration of contaminants, such as metals, from the soil (Sanchez-Hernandez and Domínguez, 2019). As such, earthworm species, such as *Eisenia andrei* have been widely used in soil and waste remediation (Domínguez et al., 2019; Gómez-Brandón et al., 2020). In this regard, vermicomposting is considered an environmentally friendly alternative for waste bioconversion and fertilizer production under mesophilic conditions (Bhat et al., 2013; Domínguez et al., 2010; Gómez-Brandón and Domínguez, 2014). In this bio-oxidative process, detritivorous earthworms (e.g., *E. andrei*, *E. fetida*, *Eudrilus eugeniae*, or *Perionyx excavatus*) trigger biologic processes by stimulating the proliferation and dispersion of microorganisms through the feedstock by casting and mucus secretion (Domínguez et al., 2010). The end-product of the vermicomposting process, known as vermicompost, is a nutrient-rich and biologically active organic amendment with various beneficial effects when used as a soil conditioner and/or as a plant growth promoter. The bioconversion of biomass waste through vermicomposting thus contributes to avoiding reliance on the linear "take−make−dispose" model by generating value-added products with the potential to improve soil health and crop yields (Domínguez et al., 2018; Gómez-Brandón et al., 2020). The quality of the final vermicompost largely depends on the type of feedstock and the processing time. For safer use of vermicompost as a soil amendment, it is crucial to shed light onto how earthworms and the vermicomposting process in general can be used to deal with emerging contaminants such as MPs and ARGs, as described below in Sections 3.1 and 3.2.

2. Microplastics and antibiotic resistance genes

2.1 Co-transport from sewage sludge to and within the soil

Recent evidence has confirmed that expression of ARGs has increased as a result of the presence of antibiotics in sewage sludge and soil (Lu et al., 2020; Seyoum et al., 2022; Su et al., 2021). The role of MPs as vectors for ARGs was also found to increase the presence of ARGs in soils, even to a greater extent than the irrigation of crops with wastewater, despite this leading to the unintentional introduction of antibiotics (e.g., amoxicillin) (Seyoum et al., 2022). MPs can act as vectors for both organic and inorganic pollutants (Abbasi et al., 2020; Fajardo et al., 2022). Interactions between MPs and ARGs are likely to promote the co-transport between one system (sewage sludge) to another (soil). The use of plastic mulch was accompanied by an increase in the abundance of ofloxacin and oxytetracycline in soil (Seyoum et al., 2022), indicating that plastic-mediated enrichment of antibiotics occurs, which may play a role in the dissemination of ARGs from WWTPs to sewage sludge and to soil.

The development of a plastisphere, consisting of MP particles with a large surface area, has been the object of recent research (Amaral-Zettler et al., 2020). The plastisphere is of particular interest in regard to ARG transport, as the plastic material can promote the development of bacterial biofilms (Zhu et al., 2021b). In particular, the plastisphere found in sludge and soil due to the release and degradation of macroplastics has been found to act as a niche for the development of some bacteria, namely members of the phylum Proteobacteria, which can capture ARGs and disseminate them via horizontal gene transfer (HGT) (Xu and Yu, 2021; Yang et al., 2020, 2022b).

Furthermore, the nature of the MPs is an important factor concerning the transport of ARGs within sewage sludge. Polyvinylchloride (PVC)-based MPs, the type of MPs most commonly found in WWTPs, together with polyethylene (PE)-based MPs favored the incidence of ARGs in sewage sludge (Dai et al., 2020; Shi et al., 2020). The use of polyethylene terephthalate (PET) also favored the release of ARGs from sewage sludge during anaerobic digestion (Zhang et al., 2022b). In addition, the release of MPs in WWTPs effluents has led to bacterial colonization of MPs, including ARB in a polymer-dependent manner (Martínez-Campos et al., 2021). Other factors are also involved in the transport of ARGs via MPs. For instance, it has been reported that the presence of MPs in soil treated with manure and sewage sludge and rich in heavy metals can promote the spread of ARGs in soil systems (Wang et al., 2021). This is supported by the fact that the land application of activated sewage sludge increased the abundance of ARGs relative to non-MPs and single MP plastispheres (Li et al., 2022c).

Altogether, these findings provide evidence that MPs can potentially act as vectors for the transport of ARGs from sewage sludge to soil, thereby affecting different components of soil systems, as described in the following section.

2.2 Effects on soil systems

There is a growing body of literature regarding the impact of MPs on soils organisms, mainly earthworms, showing adverse effects on the growth, reproductive rate, and antioxidant systems by alteration of DNA and proteins (Jiang et al., 2020; Rodríguez-Seijo et al., 2018; Shi et al., 2020). The toxicity of other pollutants was also enhanced due to the action of MPs as vectors (Li et al., 2021b; Xu et al., 2021a). However, few studies have focused on the interactions between MPs and ARGs and on the essential role that antibiotics may have in these interactions. The use of antibiotics in animal husbandry to improve growth and prevent diseases in livestock has increased greatly over the past few decades. As 30%–90% of the antibiotics used cannot be assimilated by animals, they enter the environment via feces or urine, thus polluting soils (Fang et al., 2015) and affecting the soil fauna and microbial communities (Sazykin et al., 2021).

2.2.1 Effects on earthworms and other soil invertebrates

The presence of antibiotics in the soil negatively impacts earthworm growth. For instance, exposure of earthworms to 0–51 mg kg^{-1} of ciprofloxacin was accompanied by oxidative protein damage (Wang et al., 2018). Excess amounts of antibiotics may also impact the microbial diversity in the earthworm gut. For example, oxytetracycline (10 μg g^{-1}) has been found to significantly decrease the abundance of Proteobacteria in the earthworm gut (Wang et al., 2018). Interestingly, earthworms can generally adapt the gut micro-zone in response to different conditions and develop microbial communities capable of efficiently degrading contaminants (Aira et al., 2016; Liu et al., 2018). A recent study has shown that polystyrene (PS) has a size and concentration-dependent effect on the presence of ARGs in the gut of the earthworm species *E. fetida*; higher concentrations of MPs (10 g kg^{-1} of 10-μm-sized PS) were found to alter the gut microenvironment, thus affecting the gut tissue and the immune system and promoting the dissemination of ARGs (Xu and Yu, 2021).

Few studies have considered the combined effects of MPs and ARGs on other invertebrates, such as enchytraeids and collembolans. A clear increase in the abundance of the bacterial phylum *Planctomycetes* was observed in the intestine of *Enchytraeus crypticus*, which was stimulated by the degradation of ciprofloxacin in soil (Zhang et al., 2019). Exposure to nanoplastics (NPs) and MPs together with tetracycline (TC) enriched the presence of ARGs in the gut microbiome of *E. crypticus*, and a shift in the bacterial taxa present in the gut was observed by the combined exposure to both contaminants (Ma et al., 2020a, 2020b; Yang et al., 2022c). The combination of polyamide (PA) or PVC and TC modulated the gut microbiome in *E. crypticus*, increasing the presence of *Proteobacteria* in the case of PA + TC, which could influence the health of *E. crypticus* (Ma et al., 2020b). In this line, a similar response was observed in the gut resistome of *Folsomia candida* after combined exposure to PS and the antibiotic sulfamethoxazole (Xiang et al., 2019). Moreover, the combined action of MPs and antibiotics can modify the diversity and abundance of ARGs, with PVC having a notable role in this effect (Ma et al., 2020a,b), which is of vital importance and may be key in the future response to bacterial infections.

2.2.2 Effects on plants

Early references to the potential effects of MPs on soils date from the early 2010s (Rillig, 2012), but the specific effects of these pollutants on plants were not addressed until 2018 (Li et al., 2022b; Qi et al., 2018). The diverse studies published to date indicate that the impacts of MPs on plants can be related to both direct (due to interaction between MPs and plant organs) and indirect processes (due to modifications of soil system that subsequently affect the plants) (Li et al., 2022b; Rillig et al., 2019; Zhou et al., 2021). Moreover, these impacts can be positive (i.e., increase of plant biomass or other physiologic parameters), neutral, or negative, depending on the soil physico-chemical properties, the culture technique (hydroponic or pot experiments), the plant species under study, and the shape, type, concentration, and size of MPs (Huang et al., 2022; Khalid et al., 2020; Li et al., 2022b). Few studies have considered the combined effects of MPs and other pollutants, and the findings are also diverse (He et al., 2022; Zhang et al., 2022b). In particular, co-contamination with MPs and the antibiotic oxytetracycline alleviated the stress caused by MPs on cherry radish shoots but not in its roots (Cui et al., 2022c).

To our knowledge, no research has been conducted to test the joint effect of MPs and ARGs on plant performance. By contrast, most of the research on ARGs and plants has been conducted from the point of view of crop plants as intermediates in the transmission of ARGs from the soil system into humans via diet (Tien et al., 2017; Wang et al., 2015; Zhu et al., 2017). As MPs mainly accumulate on the surface of roots and other underground plant parts, the composition of rhizosphere bacterial communities will probably be affected. In this regard, Sun et al. observed that the presence of MPs induced an increase in ARGs in the root endophytic community, with potentially negative effects on plants (Sun et al., 2023). Moreover, there is evidence that the effect of MPs and ARGs on plants may not be restricted to roots. Thus, several studies have reported that NPs can enter roots and be transported into the aerial parts of plants (Li et al., 2021d; Lian et al., 2020), enabling the spread of sorbed ARGs into the plant body, which would be a consequence of MP degradation or further fragmentation in soil prior to contact with plants. In addition, soil bacteria carrying ARGs as plasmids have been shown to be able to enter plants and transfer ARGs to bacterial endophytes (Xu et al., 2021b). Altogether, these findings indicate that MPs and ARGs can have potentially severe disruptive effects on the plant microbiome and plant function.

2.2.3 *Effects on the soil microbiome*

MPs have a dual role regarding the presence of ARGs in soils, acting as a vector for bacteria and ARGs and also enhancing the concentrations of ARGs that occur naturally in the soil (Lu and Chen, 2022; Wang et al., 2020). Soils amended with high-density PE (HDPE) MPs showed different responses in the presence and the absence of phthalate. The relative abundance of ARGs was decreased after exposure to HDPE without phthalates and increased when phthalates were present, emphasizing the importance of the composition of MPs and the release of these components in soils. In addition, soils amended with MPs + phthalates were richer in certain bacteria species capable of acting as hosts for ARG (e.g., *Calditrichaeota*, *Candidatus Spechtbacteria*, and *Ignavibacteriae*), and the time of exposure determined the types of ARGs that were present (Lu and Chen, 2022). In recent years, there has been growing interest in the development and use of biodegradable plastics within the concept of sustainability (Blaise et al., 2021; Touchaleaume et al., 2016). However, a recent study comparing conventional (PS, PE) and biodegradable MPs (polybutylene succinate, PBS; polylactic acid, PLA) reported a greater abundance of ARGs, primarily those related to multidrug resistance and bacitracin, in the presence of PBS, which was accompanied by an increase in the number of virulence factors (Song et al., 2022).

3. Impact of earthworms on microplastics and antibiotic resistance

The sustainability of the soil environment in the face of multiple stressors brought about by human activities is far from acceptable. Nonetheless, the use of ecosystem resources such as earthworms may provide support for the mitigation of the impact of hazards such as MPs accumulation and ARGs dispersion across the terrestrial system due to sludge application. As summarized in Fig. 10.2 and detailed in Sections 3.1 and 3.2, earthworm species can mediate the degradation of MPs and lead to a decrease in ARG abundance in matrices

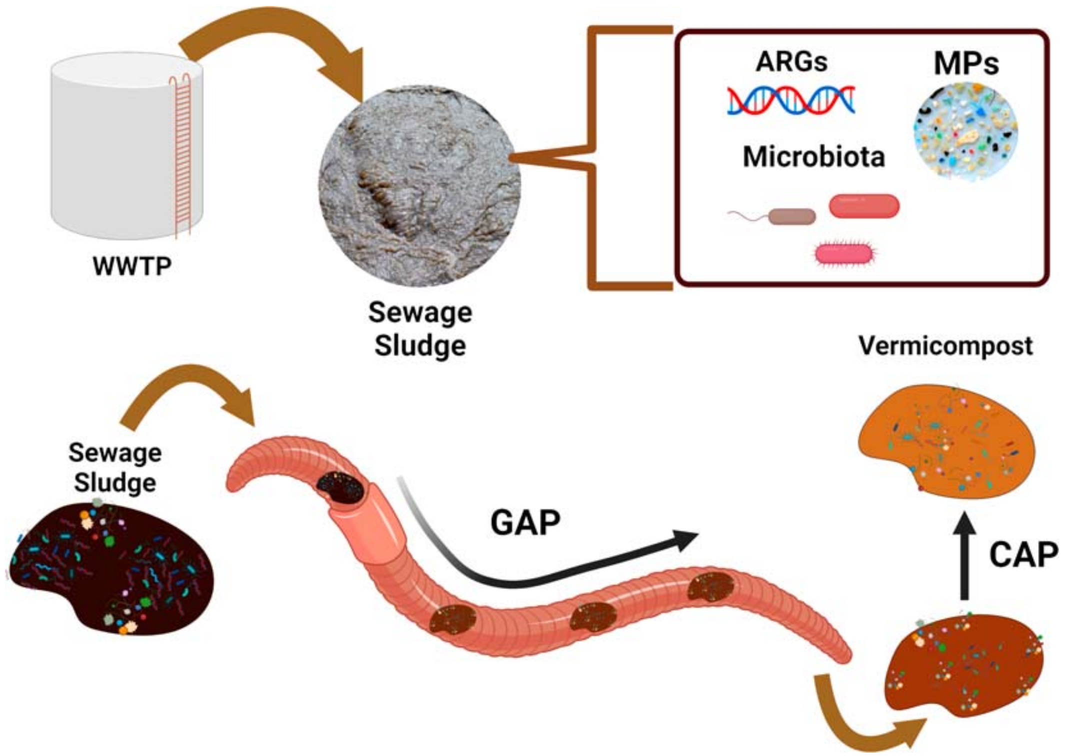

FIGURE 10.2 The impact of vermicomposting action in the decrease in ARG abundance and MP degradation from sludge to vermicompost.

with high content of OM. In turn, earthworms have the potential to be used as bioremediation tools for contaminated soils and also as a further treatment step for wastes prior to their release and application in the environment, acting as an environmentally friendly solution.

3.1 Earthworm-mediated microplastic degradation

Earthworms can survive in environments containing large amounts of organic carbon including matrices contaminated with plastic polymers, which are generally characterized by large carbon polymers (Rillig, 2018). In recent years, some studies have highlighted the ability of earthworms to ingest (Lahive et al., 2022), fragment, and even degrade polymeric structures present in micro- and nanoplastics (Kwak and An, 2021).

Several reports have shown that sewage sludge can retain >99% of the MPs, even accumulating up to 17,000 MPs per kg of sewage sludge, thus acting as one of the main sources of MPs in the soil environment (Edo et al., 2020; Hernández-Arenas et al., 2021; Lares et al., 2018). The earthworm species *E. fetida* is capable of fragmenting and degrading MPs, with a decrease in average particle size of PLA and PET in earthworm casts after 45 days (Wang et al., 2022). In addition, *E. fetida* was found to degrade MPs derived from tires, as reflected by alteration of carbon functional groups and surface modification in the egested materials (Sheng et al., 2021). Degradation of MPs after ingestion by earthworms can be mediated by the combination of enzymes as well as the bacterial consortia existing in the gut (Sanchez-Hernandez et al., 2020). This has been demonstrated by the degradation of low-density PE (LDPE) by bacteria extracted from the gut of the earthworm *Lumbricus terrestris* (Huerta Lwanga et al., 2018).

Recent research has shown an increase in MP abundance in a vermicomposting system with *E. fetida*, accompanied by a large decrease in average particle size and the incidence of surface damage, across different polymers including polypropylene (PP) and PE (Cui et al., 2022b). While a decrease in size indicated earthworm-mediated activity, it is not clear whether it was in the form of fragmentation, associated with mechanical activity, or via degradation mediated by enzymes and bacteria. Moreover, a trial with *Eudrilus eugeniae* showed a notable decrease in the abundance of MPs (PP and HDPE) after 14 weeks, reaching 78% due to the biodegrading activity of the earthworms (Ragoobur et al., 2022).

The earthworm gut is considered an enriched microenvironment for the degradation of MPs. The mechanism behind the degradation process relies on the fact that low-weight MPs such as LDPE, PP, and PVC act as carbon sources that favor the degradation within the earthworm gut (Sun et al., 2020). However, high concentrations of MPs can negatively affect the earthworm growth rate. For example, exposure to 60% polyethylene has been shown to significantly inhibit the growth of *L. terrestris* (Huerta Lwanga et al., 2016).

3.2 Impact of vermicomposting on antibiotic resistance genes

Vermicomposting has been found to reduce the presence of tetracycline resistance genes, as well as those conferring resistance to quinolones and fluoroquinolones in sewage sludge (Cui et al., 2018; Huang et al., 2018). Besides, earthworm activity has been shown to affect the bacterial and fungal diversity during vermicomposting of complex matrices (Cui et al., 2019; Domínguez et al., 2021; Gómez-Brandón and Domínguez, 2014), and the predominant hosts of ARGs can thus be altered over the course of the process. Furthermore, a decrease in the abundance of the integrase gene *intI1* in the presence of earthworms may affect the HGT-mediated transmission of ARGs during vermicomposting (Li et al., 2021c; Yang et al., 2021). Although the vermicomposting process affects ARG-encoding plasmids and integrons, specific new ARG subtypes can be identified in vermicompost, indicating that HGT could still occur (Huang et al., 2020). The efficacy of the process has also been shown to be species dependent; thus, the endogeic earthworm *Metaphire guillelmi* reduced the abundance of ARGs to a greater extent than the epigeic *E. fetida* in field fluvo-aquic soil used for corn production (Yang et al., 2021). Considering the aforementioned findings, some possible mechanisms have been postulated as being the main drivers for ARG removal over the course of vermicomposting as a result of earthworm activity: (i) changes in microbial community succession; (ii) modifications in the physico-chemical properties of the initial feedstock, such as pH and OM content and quality; and (iii) reduction in the pathogenic bacteria (Cui et al., 2019, 2020). Together these factors may affect the possible host bacteria of ARGs and/or the abundance of MGEs during vermicomposting, which could influence the horizontal transfer of ARGs (Huang et al., 2018). Further research has shown that a temperature of

20°C is optimal for achieving a decrease in the abundance of ARGs in sludge throughout the process of vermicomposting using *E. fetida* (Cui et al., 2022a). In addition, the presence of antibiotics such as tetracycline in sewage sludge promotes the presence of the related resistance genes in the final vermicompost (Xia et al., 2019). The latter authors suggested that although there is a clear decrease in the abundance of ARGs in vermicomposted sludge, it is still considered enriched and, as such, unsuitable for some agricultural crops (Huang et al., 2020).

The earthworm-mediated elimination of ARGs has also been observed in soil systems treated with organic manure, which is reflected in the gut microbiome of soil fauna, e.g., collembolans and enchytraeids, with a significant decrease in the number and abundance of ARGs (Zhu et al., 2021a). Furthermore, different genera of bacteria (*Microvirga, Sphingomonas, Methylobacterium*, and *Bacillus)* present in the earthworm gut were found to enhance the degradation of sulfamethoxazole (SMX) by 35.7% during 30 days of vermicomposting (Zhang et al., 2022c). In the same study, it was also observed that the bacterial community (except for the genus *Bacillus*) entered and colonized the soil with earthworm casts, resulting in further degradation of SMX. In addition, the level of *sul1*, *sul2*, and *intI* resident genes increased in the SMX-contaminated soil during vermicomposting by *E. fetida*, potentiating the HGT of antibiotic resistance in soil microbiome (Zhang et al., 2022c). Nonetheless, the enrichment of ARG bacterial hosts in the earthworm gut (Yang et al., 2023) may represent a risk for dissemination of ARGs in soil (Li et al., 2021e). Therefore, additional steps for treating vermicomposted sewage sludge have been suggested prior to its application to soil (Kui et al., 2020).

4. Discussion

The widespread and often indiscriminate use of plastics has increased in recent decades, particularly in the last 2 years due to the impact of the COVID-19 pandemic (owing to the use of single-use plastics, such as masks and gloves) (Anastopoulos and Pashalidis, 2021). MPs can exert diverse effects on soil organisms at different levels, with the subsequent impact on the surrounding ecosystems, which has enhanced awareness about these pollutants (de Souza Machado et al., 2018; Rodriguez-Seijo et al., 2017; Rodríguez-Seijo et al., 2019). In addition, the continued use of antibiotics has favored the presence of both MPs and ARGs in aquatic systems and, therefore, its potential use as a source and vector of further transmission to soil systems (Sazykin et al., 2021). The increasing presence of antibiotics in sewage sludge and soils can stimulate the release and transmission of ARGs and can also modulate the microbiome composition (Huygens et al., 2022; Seyoum et al., 2022). ARGs are considered among the newest emerging contaminants in soils and able to affect the natural functioning of these complex systems and their microbial and faunal communities (Kampouris et al., 2021; Sanz et al., 2022; Shawver et al., 2021). This chapter has emphasized the lack of information on the impact of the combined effect of MPs and ARGs on a system as fundamental as the soil. There is scant evidence regarding the potential effects on soil organisms (Xu and Yu, 2021), including earthworms and other invertebrates (Ma et al., 2020a; Zhang et al., 2019). More research is also needed on plant fitness and transmission of ARGs and ARBs to edible plant parts.

Manure and sewage sludge are among the main reservoirs of both MPs and ARGs, and as such, their use as soil amendments will likely stimulate the subsequent release of these emerging contaminants into soil. The degree of such effects varies depending on the time of exposure and the initial composition of the sewage sludge/manure. The type and composition of the soil (e.g., particle size, pH, conductivity) on which these amendments are applied are also important factors. A marked alteration of the composition of ARGs was observed in three different soils in China, and the major differences in the ARGs composition in the soil was explained by the MGEs and bacterial profile, rather than by the concentrations of heavy metals or antibiotics (Zhang et al., 2022e). In line with the United Nation's Sustainable Development Goals and current EU strategies, biodegradable plastics are increasingly being produced by the industry with the aim of reducing the impact of conventional plastics in our daily lives. However, biodegradable plastics (e.g., PLA) may actually generate more microplastic particles in natural environments than conventional plastics, due to their relatively lower physical strength (Song et al., 2022). This can lead to a higher risk of contamination as well as increased availability to act as vectors in these systems. Moreover, a greater impact on soil microbiome has been observed in response to the combined effects of MPs and antibiotics, with potential implications for the dissemination of ARGs within soil systems (Song et al., 2022).

Different studies have shown that the impact of manure and antibiotics/ARGs on soil microbial communities can persist for a long time, and appropriate soil management strategies should therefore be used to counteract the incipient antibiotic resistance (Shawver et al., 2021). Some questions remain to be addressed, considering the importance of MPs in transporting and disseminating ARGs. As described in Sections 1.2 and 2.1, ARG-related bacteria may colonize MP surfaces, while on the other hand, MPs also promote the presence of ARG bacterial hosts in sewage sludge and soil.

Vermicomposting has been demonstrated to be a valuable tool for bioremediation of organic wastes. To our knowledge, the impact of vermicomposting on MP−ARG interactions and the related toxicity has not yet been investigated in-depth. However, there is evidence that earthworm activity may lead to an increased abundance of smaller MPs (Cui et al., 2022b), which could act as vectors for ARG dissemination in soil. In parallel, earthworms themselves could act as vectors for ARG dissemination, e.g., due to an increase in ARGs in the gut once exposed to contaminated environments (Li et al., 2021e), with the subsequent release in the egested casts.

Vermicomposting may also promote the presence of particular ARGs through the changes in the structural and physico-chemical conditions of the feedstock; and, new ARG subtypes may appear regardless of the initial ARG content (Huang et al., 2020). Additional steps such as mixing vermicomposted sewage sludge with biochar have been tested, considering its potential benefits as a bulking material (Kui et al., 2020). Biochar promotes the stabilization of nutrients, extracellular enzymes, and microbial communities in the resultant vermicompost, owing to its high specific surface area and open porosity. Biochar may also contribute to adsorbing pollutants, thereby reducing their potential toxicity to earthworms. However, further studies are needed to shed light into the potential use of biochar for mitigating the effects of ARGs during vermicomposting.

While vermicomposting has been shown to have potential to not only degrade MPs into particles with smaller size, on par with the selection of bacterial and ARG content in both soil and sludge, the exact mechanisms by which any of these processes occur are still unknown, as they can occur either as part of the gut-associated processes (GAP) and cast-associated processes (CAP) (Domínguez et al., 2010). While there are hints, such as the presence of carboxylesterases in earthworm gut than may breakdown polymeric carbon chains (Sanchez-Hernandez et al., 2009, 2020), there are still some question marks, namely their actual activity and effectiveness to different types of MPs.

In parallel, the factors driving the bacterial selection within the gut are still knowledge gaps that require further studies.

5. Conclusions and perspectives

The occurrence, fate, and behavior of MPs and ARGs as emerging pollutants was summarized and discussed. The joint action of earthworms with their gut bacteria appears as an effective strategy to reduce the ARGs and MGEs present in the organic wastes over the course of vermicomposting. Further research merits toward this direction to achieve a safe use of sewage sludge and manure as organic fertilizers in agricultural fields. However, the persistence of ARBs, ARGs, and MGEs in the final products (vermicompost) can increase the likelihood of mobility of ARGs to the soil environment with potential implications for soil fauna and plants, ultimately entering the food chain. Therefore, while promising, further investigation should be carried out to improve and optimize the vermicomposting process for a safer applicability of such treatment technologies across different types of sludge waste, toward providing a circular economy model.

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CHAPTER

11

Instrumental characterization of matured vermicompost produced from organic waste

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1. Introduction

Fertilization is one of the most effective ways to increase the efficiency of obtaining better return products in the agricultural sector (Klimczyk et al., 2021). However, the rampant utilization of chemical fertilizers to meet food demands in recent decades has raised serious concern due to its association with pollution in water, air, and soil, increased emission of greenhouse gases, and reduction of soil fertility in the distant future (Nadarajan and Sukumaran, 2021; Kumar et al., 2019; Pahalvi et al., 2021; Srivastav, 2020). With the advent of science and an increase in understanding of the environment, there is a rising demand for adopting organic fertilizer as an alternative to replace or minimize the usage of chemical fertilizers (Nosheen et al., 2021). Organic fertilizers are derived from the residues of plants, vegetables, industrial waste, animal matter, and excreta (Diacono et al., 2019). They improve soil texture, nutrient profile, water-holding capacity, aeration, and beneficial microbial population, resulting in a higher output of agricultural crops (Lim and Wu, 2015). Besides, organic fertilizers are superior in the context of environmental management, quality of the product, and recycling of bio-waste (Verma et al., 2020). The population explosion in recent decades has resulted in sizable growth of bio-waste originating from agriculture, households, and industries (Bhat et al., 2018a,b; Mirabella et al., 2014). Without adequate treatment, the disposal of these wastes might have harmful repercussions on human health and the surrounding environment (Bhat et al., 2017a). Therefore, the conversion of this waste to a useful resource through nature-friendly methods is a rising interest in the issue of waste management (Taiwo, 2011).

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Vermicompost, also known as black gold, is a golden organic fertilizer that has garnered worldwide attention due to its environmental friendliness, organic nutrient richness, and growth-promoting potential for plants (Ali et al., 2015; Aynehband et al., 2017). Vermicomposting is an easy and affordable technique for biomanagement of a wide variety of substrates originating from industries, municipalities, animal farms, and agricultural fields (Karmegam et al., 2019; Yang et al., 2017). It is a mesophilic and bio-oxidative approach where complex organic materials are transformed into nutrient-rich stabilized vermicompost through the synergistic effect of earthworms and related microorganisms, as seen in Fig. 11.1. The beneficial effect of vermicompost on improving the soil environment in terms of physical, chemical, and biologic aspects, as well as agricultural product yield, is well established (Srivastava et al., 2020; Singh et al., 2022). Vermicompost contains various hormones and enzymes that not only aid the plant in its growth and development but also reduce the occurrence of diseases (Bhat et al., 2018b). However, the beneficial effect of vermicompost applications on field depends upon its maturity and stability status, which otherwise may cause toxicity to the plants and soil microbes. The microbe in immature vermicompost continues its decomposition process resulting in the continuous release of acetic acid, methane, ammonia, or other substances (Hannet et al., 2021). Besides, it creates an anaerobic environment and competes for available nutrients, resulting in nutrient deficiency symptoms in plants (Warman and Anglopez, 2010; Alidadi et al., 2016). Thus, the application of immature vermicompost may limit seed germination and cause retardation in plant growth and development, disease, and root destruction (Lim and Wu, 2015). Therefore, a proper characterization technique and method are necessary to assess the stability and maturity status of the final vermicompost before field application.

The traditional techniques used to assess vermicompost's stability and maturity include changes to its physical, chemical, as well as biologic profiles. Despite having different

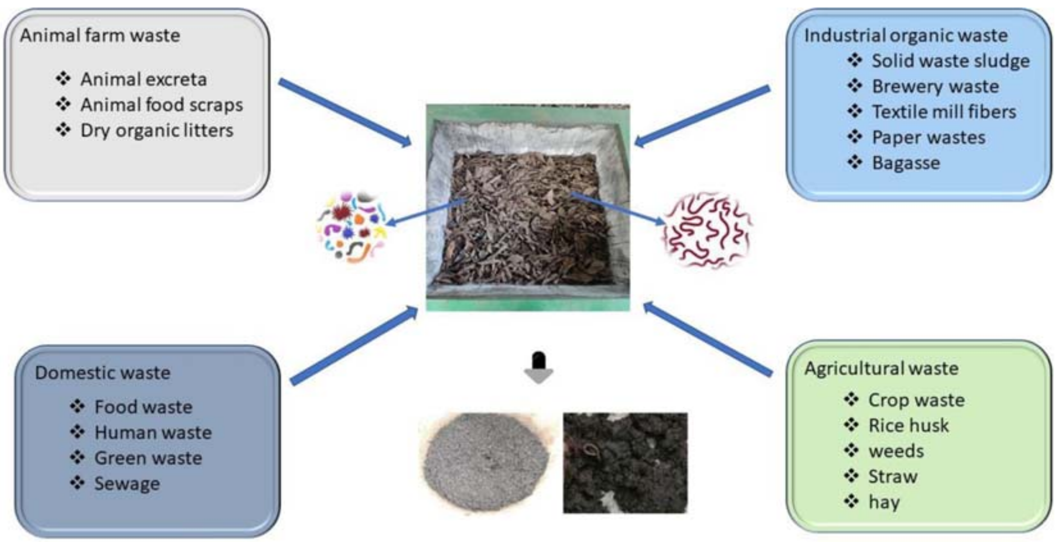

FIGURE 11.1 Vermicomposting of organic wastes from various sources.

conceptual meanings, stability and maturity are often used synonymously while describing vermicompost quality. The stability of the vermicompost is directly linked with the biologic activities and extent of biodegradation of organic substrate, while its maturity is defined by the absence of phytotoxins (Srivastava et al., 2020). There is not a single indicator that best describes the maturity and stability of the final vermicompost. For this reason, it is best appraised with multiple parameters that are companionable to the soil environment and its dependent biota. The physical methods for determining the maturity of final vermicompost are changes in its odor, color, bulk density, aeration, texture, porosity, water retention, and organic matter (Lv et al., 2013; Sinha et al., 2010; Sharma and Garg, 2019). Likewise, the chemical methods include changes in its pH, carbon:nitrogen (C:N), total organic carbon (TOC), carbon:phosphorous (C:P), NPK (nitrogen, phosphorous, potassium) concentration, humic acids, and many others (Angelova et al., 2013; Maji et al., 2017; Sarma et al., 2018). Furthermore, the biologic profiles assessed to establish the maturity status of the final vermicompost are changes in its beneficial microbial population (N_2 fixer, P, and K solubilizer bacteria), seed germination index, and microbial enzymatic activities (Bhat et al., 2014; Hussain et al., 2018; Bargaz et al., 2018). All these characteristic changes are brought about by earthworms and the associated microbes, which enhance the pace of degradation and decomposition of the organic waste substrate (Lakshmi et al., 2014).

However, recent studies have employed several sophisticated instruments to understand the characteristic features of the mature vermicompost. The instrumentation techniques are used to reveal the exemplary transformation and changes in properties of the organic wastes that occur during the vermicomposting process (Lohri et al., 2017). They are fast, reliable, and accurate for recording structural, compositional, and functional changes in mature vermicompost (Ravindran et al., 2021). The skeletal changes in the substrate or raw materials that occur during the vermicomposting process due to earthworm activity can be viewed and confirmed by scanning electron microscopy (SEM) (Quadar et al., 2022). Likewise, Fourier transform infrared spectroscopy (FTIR), liquid chromatography−mass spectrometry (LC-MS), atomic absorption spectroscopy (AAS), thermogravimetric analysis (TGA), and gas chromatography−mass spectrometry (GC-MS) can be used to evaluate the quality of mature vermicompost in terms of the extent of organic complexes degradation, changes in macro- and micronutrients composition, heavy metals concentration, presence of plant growth−promoting substances and degree of humification (Bento et al., 2021; Boruah et al., 2019). These sophisticated instruments help in overcoming the limitations of traditional methods (results heavily depend upon the nature of the substrates and/or substrate mixtures) while establishing the maturity of the vermicomposting end product (Lim and Wu, 2015). In this chapter, the use of sophisticated instruments in characterizing the mature vermicompost will be discussed along with the modifications that take place in the organic substrate throughout the vermicomposting process.

2. Characteristic of mature vermicompost: a brief overview

A mature vermicompost is the ultimate product that has undergone the final phase of non-thermophilic degradation of organic substrates via the collaborative efforts of earthworms and microorganisms (He et al., 2017). A mature and stable vermicompost should be rich in nutrients and have the capability to augment soil physical and chemical features in addition to the biologic qualities (Srivastava et al., 2020). From a physical point of view, the vermicompost is usually a fine peat-like particulate structure that is usually dark in color with a homogenous organic material, finely levigated, and granulated with an earthy scent (Kibatu and Mamo, 2014; Saha et al., 2022). It has a high porosity that results in better oxygenation, drainage, and water retention capacity. The finely divided structure of finished vermicomposted materials also possesses adsorption and nutrient retention properties (Kiyasudeen et al., 2015).

The vermicompost contains growth hormones, enzymes, vitamins, and the major and minor elements required in plant metabolism and growth (Lim and Wu, 2016; Olle, 2019; Ahmad et al., 2021). The pH level in final vermicompost samples usually ranges within 6–7 irrespective of the highly alkaline and/or acidic condition of the initial feedstock or substrate (Quadar et al., 2022). The adjustment of the pH toward the neutral side in the final vermicompost makes the nutrients ideally accessible to plants and is also compatible to plant root development (Wang et al., 2010). Similarly, electrical conductivity (EC) is a key measure for nutrient availability, and a high range of EC hampers the uptake of available nutrients by plants. Earthworms and accompanying bacteria work together to lower the EC level in the substrate to an admissible level throughout the vermicomposting process (Ramnarain et al., 2019). Additionally, the organic carbon level and C/P and C/N ratios usually reduce by the end of the vermicomposting process. The reduction in the C/N and C/P ratios is caused by the increase in the decomposition of the organic substrate resulting in the release of higher phosphorous and nitrogen content. Similarly, TOC decreases due to an increase in microbial respiration resulting in the loss of carbon in the form of CO_2 (Pandit et al., 2020; Swarnam et al., 2016). Moreover, the mature vermicompost shows high activities of β-glucosidase, urease, and dehydrogenase, which further confirm the flourishing of functional microbial populations. High urease activity in vermicompost shows the potential of using external or internal urea as a source of nitrogen (Sudkolai and Nourbakhsh, 2017). An endoenzyme, dehydrogenase reflects the availability of physiologically active microbes and is a marker for overall microbial activity in the mature vermicompost (Singha et al., 2022; Boruah and Deka, 2021). Similarly, β-glucosidase is a broadly distributed sensitive enzyme engaged in the carbon cycle and hence is regarded as a quality indicator of the end vermicompost (Hanc et al., 2022).

The quality and nutrient contents of the mature vermicompost depend upon the nature of the substrate used during vermicomposting (Lim and Wu, 2015). Besides, the diverse substrates used in vermicomposting may yield products of varying qualities (Jayakumar et al., 2011; Borang et al., 2016). The characteristics changes that occur in the waste organic substrate are brought about by the earthworm and its companionate gut microorganisms. Initially, the organic wastes are processed through the grinding of their muscular gizzard followed by the enzymatic and microbe-mediated decomposition that occurs in the earthworm gut (Ravindran and Mnkeni, 2016). Earthworms utilize only a minuscule amount of the consumed substrate for their body synthesis and release a vast amount of it in a semidigested form. This semidigested product quickly decomposes and becomes the final and stable mature vermicompost. This is only possible due to the various enzymes, hormones, and microorganisms present in the earthworm gut, which play an active role in the processing of the waste substrate (Tajbakhsh et al., 2011). Additionally, the high levels of amylases, cellulases, chitinases, lipases, and proteases activity amassed during the microbe's associated digestion within the earthworm's metabolic pathway are also retained in the vermicompost (Pathma and Sakthivel, 2012). All these key features make vermicompost an outstanding soil conditioner and organic fertilizer.

3. Traditional methods for understanding vermicompost maturity

The conventional methodology for determining the maturity and stability of the final vermicompost incorporates the physical, chemical, and biologic alterations that occur during vermicomposting. Fig. 11.2 provides an overview of the important physical, chemical, and biologic parameters commonly used for determining vermicompost maturity. It informs the numerous changes that take place during vermicomposting in terms of color, texture, nutrients composition, and microbial profiles. The following sections go through the most often used physical, chemical, and biologic parameters.

3.1 Physical methods

The physical method is the most basic and fundamental step in characterizing the maturity and stability of the vermicompost. The important parameters used in characterizing the physical property of the final vermicompost are changes in its bulk density, structure, water-holding capacity, texture, porosity, and ash contents (Wako, 2021; Belda et al., 2013). It also includes the visual appearance of the vermicompost like color, homogeneity, and shape. The mature vermicompost is dark in color, has an earthy odor, and is finely fragmented like

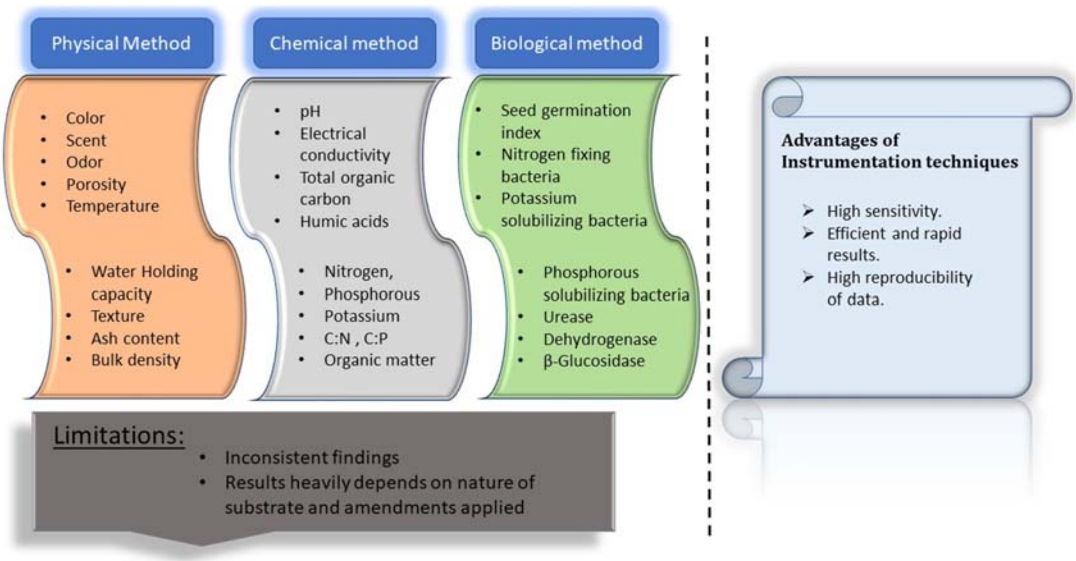

FIGURE 11.2 Traditional methods used to determine the vermicompost maturity.

peat (Kumar et al., 2013). The breakdown and stabilization of the organic matter that happens throughout the vermicomposting process causes the substrate, which is typically light colored, to take on a black coloration (Ali et al., 2015). Besides, the alteration in the texture and structure of the final vermicompost provide information on the degradation and decomposition of the substrate that takes place in the earthworm's gut during the process of vermicomposting (Banerjee et al., 2018). As the worms and their accompanying bacteria break down the substrate into tiny pieces, the bulk density of the produced vermicompost decreases. Porosity also rises when organic matter degrades, allowing more room for air and water to flow. As a result, more air can infiltrate the substrate, i.e., aeration, or the quantity of oxygen present in the substance also increases. The bulk density, porosity, and aeration give an idea on the suitability of the final vermicompost for agricultural applications (Gong et al., 2017). Furthermore, mature vermicompost has a substantially greater ash content and water retention capacity than its starting substrate. The information on water retention capacity conveys the total volume of water absorbed by the vermicompost, while the ash content shows the mineralization of the substrate (Chatterjee et al., 2017). Hence, these physical approaches are a key signal for evaluating the maturity of the vermicompost and identifying its quality.

3.2 Chemical methods

The tracking of changes in the chemical parameters is the most common and widely used method for determining the maturity of vermicompost. It includes the determination of pH, total organic matter, EC, TOC, N, P, K, humic acid profiles, and C/N and C/P ratios (Morales-Corts et al., 2014; Srivastava et al., 2020; Lim and Wu, 2016). Among these, evaluation of pH, EC, TOC, NPK contents, and C/N and C/P ratios are the most commonly studied parameters for understanding the maturity and stability of the vermicompost. Regardless of the starting substrate, the pH level in end vermicompost normally fluctuates between 6 and 7. Besides, as the earthworm and bacteria work together to disintegrate the beginning substrate, EC decreases to an admissible level. The nutrient absorption rate is dependent on the pH of the vermicompost, while the EC measures the level of salinity in the final vermicompost (Khare et al., 2005; Bhat et al., 2017a,b). The C/N and C/P ratios, which are closely related to the NPK and TOC profiles, are significant indicators for determining the rate of organic matter decomposition throughout the vermicomposting process. For this reason, it is vital to analyze the NPK concentration and TOC content of the final vermicompost to establish its maturity and stability (López et al., 2021; Prashija and Parthasarathi, 2016). NPK (nitrogen, phosphorus, and potassium) are vital plant nutrients that play an indispensable role in determining the maturity of vermicompost. Worms and microbes feed on and decompose the organic starting materials during the vermicomposting process, releasing important plant nutrients such as nitrogen, phosphorus, and potassium (Bhat et al., 2018b; Quadar et al., 2022). Furthermore, the organic matter content is tightly linked to the activity of earthworms and the extent of mineralization in the final vermicompost (Chatterjee et al., 2017). Overall, these chemical methods provide a comprehensive understanding of the final vermicompost maturity and quality.

3.3 Biologic methods

Apart from the physical and chemical methods, the finished vermicompost biologic profiles are also screened to establish its maturity. The primary biologic methods used for determining the vermicompost maturity and toxicity are seed germination assay and enzyme assay (Pandit et al., 2020; Boruah and Deka, 2021). The seed germination assay reveals the phytotoxicity, while the enzyme assay reflects the total microbial activities in the mature vermicompost (Khatua et al., 2018). A germination index value greater than 100% indicates stable and mature vermicompost, while a value below 50% is an indication of immature vermicompost with high phytotoxicity (Boruah and Deka, 2021). The three main enzymatic activities that are typically used to understand the maturity of the finished vermicompost are dehydrogenase, urease, and β-glucosidase. The β-glucosidase is an important biomarker for assessing worm biomass during the vermicomposting process, while dehydrogenase and urease are important indicators for microbial biomass and activities (Castillo et al., 2013). The stabilization point of the dehydrogenase activities can be regarded as the maturity time of the vermicompost (Alidadi et al., 2016). The activity of the enzymes cellulase, urease, amylase, invertase, and protease are additional significant enzyme profiles that are regularly assessed (Lakshmi et al., 2014). Besides, the presence of and significant improvement in N_2 fixers, P, and K solubilizer bacteria are key indications for establishing the quality of finished vermicompost. These beneficial bacteria facilitate the conversion of inaccessible nitrogen, phosphorus, and potassium into easily obtainable forms for the dependent plants (Ahmed and Deka, 2022). As a result, biologic parameters are a valuable resource not only for assessing the maturity of the completed vermicompost, but also for testing its toxicity.

4. Limitations of traditional methods

The physical, chemical, and biologic approaches are the basic and most extensively used methodologies for the characterization of mature vermicompost. However, these approaches have their own significant drawbacks. For example, the physical parameters such as odor, color, porosity, texture, and temperature do not provide adequate data on the vermicompost stability and maturity state (Boruah and Deka, 2021). In addition, the chemical features of the mature vermicompost vary with the kind of substrate and amendments added. For this reason, inconsistent findings have been reported in several investigations. Many studies have revealed the vermicompost to be relatively acidic in nature (Bisen et al., 2011; Hanc and Chadimova, 2014). However, contradictory findings have also been reported in multiple studies with a mature vermicompost showing alkaline nature with $pH > 7$ (Alidadi et al., 2016; Lim and Wu, 2016; Ravindran and Mnkeni, 2016). Besides, the EC, C/N, and C/P ratios exhibit substantial fluctuation in mature vermicompost and are directly dependent on the kinds of substrates utilized (Ali et al., 2015; Sudkolai and Nourbakhsh, 2017). Similarly, the microbial population growth pattern is directly dependent on the source of the substrate used during vermicomposting. For example, a reduction in bacterial population was reported in a study when paddy straw substrate was applied as a substrate, but in maize stover and leaf litter, a higher bacterial population was observed after vermicomposting (Pandit et al., 2020). Moreover, biologic factors like the germination index simply verify the toxicity of the vermicompost but do not quantify the types of contaminants and amounts of pollutants present in it. Furthermore, both reduction and increase in urease activities have been reported in the mature vermicompost. This can be attributed to the sort of substrate used and the microenvironmental setting of the vermicomposting unit (Pramanik et al., 2007; Wang et al., 2021). As a consequence, reliable and efficient alternative methods are required for the determination of the stability as well as maturity status of the vermicomposted materials.

5. Emergence of instrumentation techniques

Instrumental characterization offers such services with better sensitivity and reproducibility of the data. The modern instruments such as SEM, LC-MS, FTIR, AAS, GC-MS, and TGA are incredibly sensitive and efficient and offer speedy and precise results about the compost maturity and stability. Accordingly, advanced instrumentation technologies are gaining popularity to alleviate the shortcomings of traditional procedures while defining the maturity and stability status of the finished vermicompost. Fig. 11.3 displays some of the advanced, sophisticated instruments applied routinely for characterization of the finished vermicompost.

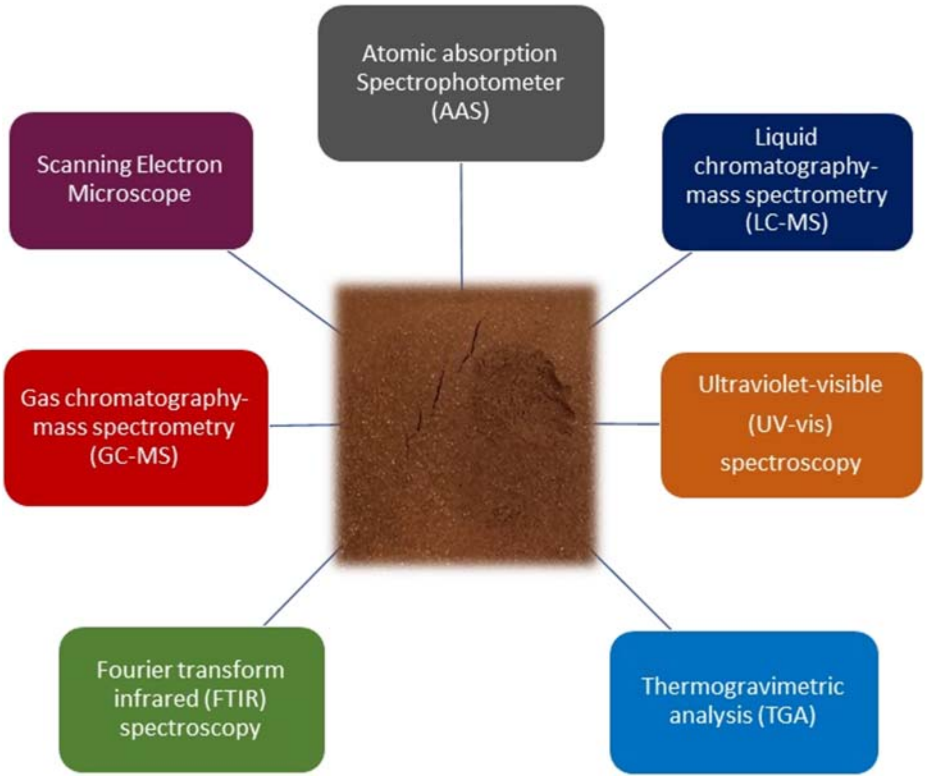

FIGURE 11.3 Instrumental technique used in characterizing mature vermicompost.

5.1 Atomic absorption spectroscopy

AAS technique is used to determine the amount of a metallic element in a different material based on energy absorbed from a certain wavelength of light. It is an extremely sensitive equipment that generates high-quality and reproducible data. The system is fast, simple working, and does not require metal separation (García and Báez, 2012). For these reasons, it is used to evaluate the level of heavy metals in the final vermicompost. Furthermore, it is used in analyzing the micronutrient composition of the mature vermicompost (Mupondi et al., 2018; Jamaludin and Mahmood, 2010; Lv et al., 2013).

The characterization of heavy metal content and its concentrations in vermicompost is required as it is directly applied to the agricultural field. Excessive levels of heavy metals may promote bioaccumulation in agricultural products resulting in food chain contamination (Zuo et al., 2019). As a consequence, the heavy metals concentration of the final vermicompost must be checked and brought down to acceptable levels before agricultural application. Multiple studies have reported a reduction in metal concentration by the end of vermicomposting. For instance, a study on water hyacinth vermicomposting revealed a substantial reduction in heavy metal leachability and bioavailability (Singh and Kalamdhad, 2013). Besides, a decrease in the bioavailability of Cr, Pb, and Cd was observed in mature vermicompost (Wang et al., 2017). The heavy metals reduction is caused by the immobilization and deposition of mobile metals in the worms' bodies (Wuana and Okieimen, 2011). Moreover, the earthworms and their related microorganisms work together to enhance the enzyme activities within the gut and the cast-linked processes. As a consequence, the accessibility of heavy metals is constrained, lowering the bioavailability of heavy metals in the final vermicompost samples (Swati and Hait, 2017). Furthermore, the applicability of AAS to confirm vermicompost maturity by exploring its micronutrient status has been extensively reported in the literature. Micronutrients are those elements that are required in minute quantity by plants to develop into a full-fledged body. They include elements such as iron, zinc, copper, boron, manganese, and molybdenum (Belda et al., 2013). A study conducted by Kumar et al. (2015) reported significant improvement in the micronutrient level (Zn, Cu, Fe, and Mn) of the mature *Eichhornia crassipes* vermicompost. Similarly, Rini et al. (2020), Pandit et al. (2020), and Rajiv et al., 2013b utilized AAS to report the micronutrients status of the mature vermicompost derived from cow dung, lignocellulosic organic wastes, and *Parthenium hysterophorus*. The studies reported significant improvement of micronutrient (Cu, Zn, Mn, and Fe) conditions in the mature vermicompost when amended with different manures. The increased micronutrient content indicates the earthworm's active participation in breaking down the organic waste and mineralizing the substrate materials (Pandit et al., 2020). Hence, AAS is a vital instrument for assessing the safety and usability of the finished vermicompost by reporting on its micronutrient and heavy metal levels.

5.2 Liquid chromatography−mass spectrometry

The LC-MS method is a technique of choice for detecting analytes in microgram to nanogram concentration due to its high sensitivity, selectivity, and capability to produce accurate results. It incorporates both the physical separation of the target analytes and their mass measurement in a sample employing liquid chromatography and mass spectrometry capabilities (Niessen and Tinke, 1995). In recent years, LC-MS has emerged as the most widely used method for detecting and identifying phytohormones in a sample. Besides, this method is viable for determining the presence of a broad range of analytes in a single assay (Zhang et al., 2015). The use of LC-MS to quantify and evaluate phytohormones in finished vermicompost is a relatively new approach. It is critical to describe the phytohormones present in vermicompost since they not only promote plant development but also help plants respond quickly to abiotic and biotic stress (Moore, 2012). The applicability of vermicompost in enhancing the development of plants has been observed in several pieces of literature (Karagöz et al., 2019; Befrozfar et al., 2013; Moghadam et al., 2012; Blouin et al., 2019). The vermicompost-mediated plants show a growth pattern similar to that of plants treated with exogenous cytokinin, gibberellin, and auxin through the soil (Ruangjanda et al., 2022). Zhang et al. (2015) reported the presence of cytokinin (tZ, iP and iPR) and auxin such as IAA in the final vermicompost derived from vegetables, fruit peels, and water hyacinth. The amount and type of phytohormones present in the final vermicompost are also determined by the supplements used. A vermicomposting of the spent mushroom substrate when augmented with fruit peel waste boosted the amounts of cytokinin, GA3, and AA phytohormones, whereas cassava pulp only enhanced the amounts of cytokinin phytohormones (Zhang et al., 2014). The phytohormones in the final vermicompost are also assumed to be of microbial and earthworm origin. The presence of plant growth−promoting microbes in finished vermicompost is covered in many reports. These microbes secrete the various phytohormones responsible for enhancing the plant growth (Matteoli et al., 2018; Gopalakrishnan et al., 2014). Additionally, the coelomic fluid of earthworms has also been shown to have excellent capacity for boosting plant growth. Seed treated with cold coelomic fluid exhibited a higher rate of germination than gibberellin or sterile distilled water−treated seed (Nadana et al., 2020). Indole-3-acetyl-L-valine (IAVal), one of the major derivatives of auxin metabolism in rice plants, was detected in cold coelomic fluid of earthworms through LC-MS analysis (Cao et al., 2016). Besides, squalene, a brassinosteroid precursor, and heneicosane compounds have also been reported in coelomic fluid of earthworms extracted using the cold extrusion method. The IAVal metabolites cannot only enhance the growth of the root length in rice seedlings but can also defend them against phytopathogenic fungus (Nadana et al., 2020). Hence the actions of microbes and earthworms resulted in the appearance of plant growth promoter hormones in the mature vermicompost. For this reason, the quantification and analysis of the phytohormones in mature vermicompost as a quality appraisal is critical prior to on-field applications.

5.3 Gas chromatography−mass spectrometry

GC-MS is used to identify the molecular weight, elemental constitution, and molecular structure of the compounds present in the sample. It comprises two very distinct analytical instruments: gas chromatography and mass spectrometry. The gas chromatography section is used to split up and detect the chemical components of samples and quantify their amounts, while mass spectrometry measures the mass-to-charge ratio (m/z) of the charged particles (Hübschmann, 2015). For these reasons, GC-MS analysis has been widely utilized to offer a full description of the chemical composition and compounds present in the end vermicompost. Besides, it is an important technique to track the immediate compounds formed during the process of vermicomposting from the raw materials (Rajiv et al., 2013a). GC-MS spectrum analysis on vermicompost prepared from *Parthenium* weed shows 41 peaks, while its substrate only shows 20 peaks. This indicates the presence of a higher number of alkanes, alkenes, alcohol, fatty acids, and nitrogenous compounds in the final vermicompost. Besides, the study also reported formation of new humins and polysaccharides through structural modifications of the cellulose, hemicellulose, and lignin present in the organic substrate. Furthermore, the disappearance of 4-pyridinol-1-oxide compounds in the final vermicompost, which had the highest peak area (58.32%), confirms the intense disintegration, molecular reconfiguration, and biooxidation process that occur during the vermicomposting (Hussain et al., 2016). Ganguly and Chakraborty (2019) studied vermicomposting of primary and secondary sludge of paper mills and reported about both gain and reduction in the frequency of spectrum peaks after GC-MS analysis. These changes in the number of peaks signify biodegradation of the larger molecular mass and concentration of compounds equivalent to humic acids following mineralization. Similarly, Rajiv et al., 2013b reported a decreased in peak numbers in the vermicompost samples obtained from *Parthenium* weed and cow dung mixtures. The significant findings from their study were the emergence of humic acid compound such as hexadecanoic acids in the finished vermicompost. Besides, high amounts of esters, alkanes, and aliphatic compounds were detected in both the final product and initial feed. These compounds are responsible for maintaining the nutritional level during the vermicomposting process. In addition, GC-MS techniques can also be used to detect the plant growth−promoting hormones. High concentrations of indole acetic acids (IAAs), cytokinin, and gibberellins were detected in the vermicompost derived from cattle dung (Edwards and Arancon, 2004). Likewise, a significant reduction of GC-MS spectrum peaks from 21 to 12 was reported in *Eichhornia* and cow dung vermicompost samples (Kumar et al., 2015). According to them, appearance of intermediate compounds with low peak intensity indicates the presence of various humic acid compounds at different concentration in the final vermicompost samples. Saravanan et al. (2022) also utilized GC-MS instruments to assess the vermicompost maturity of sugarcane bagasse mediated with cow dung and elephant dung. The analysis revealed production of high humic acids and intermediate compounds like benzene propanoic acid 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methylester, dodecanoic acid methyl ester, hexadecanoic acid methyl ester, and many others. Additionally, no toxic compounds were detected in the finished vermicompost, indicating the substrate maturity.

Hence, the emergence of numerous humic acid−like compounds in vermicompost is an essential indication of the maturity and the mineralization process that occur during the process of vermicomposting. Furthermore, this high level of humification can be associated with the low humification index results obtained through UV-vis spectrophotometer (Ganguly and Chakraborty, 2019). Therefore, GC-MS analysis is a crucial technique in defining the vermicompost maturity by tracking the emergence of intermediate chemical compounds, humic acids, and plant growth−promoting hormones.

5.4 Fourier transform infrared spectroscopy

This technology is used to acquire an infrared spectrum of absorption or emission of a solid, liquid, and gas. It is typically measured in between the range of 400 and 4000 cm^{-1}. It helps in the detection and identification of the different functional groups present in the sample (Sindhu et al., 2015; Nikolic, 2011). The major advantage of using FTIR is the lack of chemical treatment of the sample, which may likely cause any unsuitable reaction before analyzing the sample. Samples can be analyzed in any state (liquid, gaseous, powders, pastes, fibers, films) and are more environmentally friendly due to the production of less waste after analysis. Furthermore, the various compounds available in the samples can be detected and differentiated into organic and inorganic in a single analysis (Grube et al., 2006).

The use of the FTIR technique as a novel approach to characterize the maturity of the final vermicompost is widely available across the literature (Mago et al., 2022; Firdous et al., 2019; El Ouaqoudi et al., 2015). It is used to ascertain the breakdown of polypeptides, lignin, polysaccharides, phenolic groups, aliphatic, carboxylic, and aromatic compounds that occur throughout the vermicomposting process (Bhat et al., 2017b). Besides, the mineralization of the organic matter present in the organic waste substrate can be tracked through FTIR (Soobhany et al., 2017). The different functional group that arises after the decomposition process in the finished vermicompost can be identified by analyzing the changes in the intensity of the peaks. The reduction or increase in the intensity of the peak signifies the organic matter mineralization and complex aromatic compounds degradation into simpler compounds such as carbohydrates and lipids (Paul et al., 2020). Table 11.1 shows the absorbance bands allocated to the FTIR spectra during the vermicomposting study. The presence or absence of peaks for the beneficial and important functional groups during the study helps in confirming the stabilization or degradation of the organic waste. Multiple studies have reported a significant reduction of the peaks formed by the end of the experiment. For instance, Boruah et al. (2019) show the absence of peaks in the range 1660–1600 cm^{-1} for the final vermicompost, suggesting the reduction of the carboxylic group and aromatic structure. This diminution in carboxylic groups and aromatic compounds indicates intense disintegration of the substrate throughout the vermicomposting process. Besides, the study also observed deformation around the peaks of 2925 cm^{-1} and 1100 cm^{-1} revealing the breakdown of fats, cellulose, hemicelluloses, and lipids. Khatuaet al. (2016) observed continuous elevation of 1380 cm^{-1} peak intensity with composting time and indicated N–O bending of nitrate complexes. This finding was supported further by an increase in accessible nitrogen in the finished vermicompost. In addition, the study also reported a reduction in band intensity at 3600–3100 cm^{-1} signifying the biodegradation of amines and lignins during vermicomposting. Similarly, Kumar et al. (2015) used the FTIR approach to confirm the elevation of nitrogen-rich compounds with a significant reduction in aliphatic and aromatic compounds. Hussain et al. (2016) reported flattening of 3398 cm^{-1} and the absence of 1523 and 1724 cm^{-1} peaks in the finished vermicompost. The study concluded that there was a significant reduction in phenolic content and breakdown of carbohydrates, lignins, and lignocelluloses present in the salvinia during the process of vermicomposting. Furthermore, Rajiv et al., 2013a revealed a reduction of toxin compounds like sesquiterpene, lactones, and phenols during the vermicomposting process. Hence, FTIR technique is a golden approach that can be effectively incorporated during the confirmation assessment of vermicompost maturity.

5.5 Thermogravimetric analysis

TGA is one of the most common instrumentation techniques used in determining the maturity and stability of the vermicompost. It is an analytical technique used for monitoring the changes in mass subjected to a gradual increase in temperature over time (Lothenbach et al., 2016). It is used to determine the thermal stability of a mature vermicompost by continuously measuring its weight under a controlled ascending heating rate and atmosphere.

TABLE 11.1 FTIR interpretations for vermicomposted materials obtained from various substrates.

Substrate	Bands and peaks (cm^{-1})	Assignment	Functional groups	References
Citronella bagasse and paper mill sludge	3100–3000	C–H stretch	alkenes	Boruah et al. (2019)
	2900–800	C–H stretch	alkynes	
	2550	S–H stretch	mercaptans	
	1810–1760	C=O stretch	anhydride groups	
Java citronella bagasse	2915 and 2846	C–H stretch	aliphatic carbon compounds	Deka et al. (2011a, b)
	2670	N–H stretch	tertiary amine groups	
	1620	C=O stretch	amide groups	
	1556	N–H bending	amide and amine groups	
	1382	C–N stretch	amine groups	
Patchouli bagasse	2925–2850	C–H stretch	lipids, aliphatic methylene, and fats	Ahmed & Deka (2022)
	1674–1637	C–C stretch	aliphatic alkenes and aromatic groups	
	1388–1382	N–O stretch	(nitrate band)	
	1100–1000	C–O stretch	polysaccharides	
	790–760	N–H bending	amide and amine group	
Mikania micrantha kunth	3685 and 3306	O–H stretch	phenolic compounds	Kauser & Khwairakpam (2022)
	2992	C–H stretch	fatty acids and lipids	
	1490	C=C stretch	aromatic compounds	
	1034	C–O stretch	polysaccharides	
Banana stem waste	3444–3419	O–H and N–H (minor) stretch	hydrogen bonded O–H group	Khatua et al. (2018)
	2925 and 2854	C–H stretch	$-CH_2$ group	
	1410–1380	N–O stretch	nitrate band	
	1043–1034	C–O stretch	polysaccharides	
	800–780	N–H stretch	primary and secondary amine group	
Salvinia molesta	3377	O–H stretch	alcoholic and phenolic hydroxyl group	Hussain et al. (2016)
	1724	C=O stretch	lignin	
	1629	C=C stretch	aromatic compounds	

(*Continued*)

TABLE 11.1 FTIR interpretations for vermicomposted materials obtained from various substrates.—cont'd

Substrate	Bands and peaks (cm^{-1})	Assignment	Functional groups	References
Animal fleshing	3500–3300	O–H stretch	hydrogen bonded compounds	Ravindran et al. (2013)
	2925–2850	C–H stretch	lipids and fats	
	1654	C=O stretch	amide I	
	1540	C=O stretch	amide II	
	1380	N–O stretch	nitrate band	
	1230	C=O stretch	amide III	
	1100–1000	C–O stretch	cellulose, hemicellulose, and polysaccharides,	
Lantana camara	3448	O–H stretch	alcohol and phenol	Hussain et al. (2015)
	2925 and 2852	C–H stretch	fatty acids and lipids	
	1733	C=O stretch	sesquiterpene lactones	
	1661	C=C stretch	aromatic compounds	
	1447	$-OCH_3$ stretch	lignin	
	1256	C–O and O–H stretch	phenolic and –COOH group compounds	
	1015	C–O stretch	polysaccharides, cellulose, or hemicellulose	
	836	N–H wag vibrations	amine group	
Municipal solid wastes	3299 and 3269	O–H stretch	phenolic component and alcohols	Sindhu et al. (2015)
	3058–2898	O–H stretch	carboxylic acids	
	2941–2892	C–H stretch	alkanes	
	2357–2189	C≡N stretch	alkyne compounds	
	1707 and 1639	C=O stretch	aldehydes, ketones, and carboxylic acids	
	1472 and 1442	C=C stretch	aromatic compounds	
	1054––1003	C–O stretch	primary alcohols, polysaccharides, aromatic, ethers, and esters	

The benefits of utilizing TGA come from its ability to provide very precise and reproducible results with a small amount of sample (10−100 mg). Besides, it is cheap and rapid, and no pretreatment of the samples is required prior to analysis. Furthermore, these data may be utilized to supplement the chemical parameters acquired by the traditional approach, such as moisture contents (Bhat et al., 2017b). The curves obtained during TGA can be used to study the mass loss in each different stage of temperature. The dehydration of the sample typically occurs at temperatures ranging from 60°C−150°C, while the decomposition reaction usually occurs within the range of 200°C−800°C (Ravindran et al., 2013). The temperature required to achieve the same mass loss of substrate is much higher for the mature vermicompost. This reflects the vermicompost's maturity and stability, as well as the existence of less volatile solid ingredients (Bhat et al., 2017b). Several studies have employed thermogravimetry to confirm the stability of the finished vermicompost. Hussain et al. (2016) observed higher mass losses in *Salvinia* raw substrate (75.4%) than its vermicompost product (66.25%). Similarly, Lim and Wu (2015) revealed higher weight percentage in the finished vermicompost of effluent waste derived from palm oil mill. At 1000°C, the study reported mass loss of 82% for the initial stage substrate, while 67% mass loss was achieved by the end of vermicomposting. Soobhany et al. (2017) also revealed a lower mass loss in the entire final vermicompost product derived from municipal solid waste. Likewise, Srivastava et al. (2020) reported higher mass loss (47.5%) in the initial substrate (municipal waste mixtures of vegetables, flowers, papers, etc.) compared with its end vermicompost (38%) at 800°C. The presence of highly volatile chemicals accounts for the increased mass loss in the initial waste. In addition, the development of heat-resistant aromatic compounds and complex carbohydrates during the vermicomposting process also aids in the stability of the finished vermicompost. Furthermore, it is also used to determine the degradation of various compounds formed during the process of vermicomposting. It has been reported that in oil palm empty fruit bunch vermicompost, cellulose and hemicellulose decomposed between 150°C and 380°C, lignin between 380°C and 700°C, and fixed carbon between 700°C and 800°C (Yahaya et al., 2017). TGA can also help in assessing the moisture content, behavior of the sample in response to fluctuating temperatures and the evolution of organic compounds during the heating process. In addition to providing thorough information on the end vermicompost maturity, it also describes the procedures and phases involved in vermicomposting, such as the humification and degradation of complex compounds like hemicellulose, lignin, and cellulose (Díaz et al., 2021). Overall, TGA is an effective tool in vermicompost studies as it provides detailed information on the thermal stability of the finished vermicompost along with the degradation of organic matter that occurs during vermicomposting, which is an important indicator of vermicompost maturity.

5.6 Scanning electron microscopy

A scanning electron microscope is a complex and sensitive device that provides details on the crystalline structure, morphologic texture, chemical composition, and orientation of the sample materials (Akhtar et al., 2018; Inkson, 2016). A scanning electron microscope generates surface pictures of a material with the help of a focused beam of high-energy electrons (Echlin, 2011). An advantage of SEM lies in its ability to produce high-resolution 3D images. Its resolution may reach up to 0.2 nm, which is 1000x more than traditional light microscopy

(Bhat et al., 2017b). The morphologic, topological, and compositional information obtained through SEM is an invaluable resource for determining the vermicompost maturity (Monda et al., 2018). It is used in the vermicomposting study to observe the changes in morphologic structure of the finished vermicompost that occurs inside an earthworm's gut. Reports regarding morphologic surface changes in pre- and post-vermicomposted organic wastes depicted in Fig. 11.4 are abundantly available across the literature (Sharma and Garg, 2018, 2019; Das and Deka, 2021; Quadar et al., 2022).

Initially, the substrate, which is degraded in the earthworm's gizzard, goes through the gut of the earthworm. During this movement, a further degradation process takes place that may lead to changes in substrate structure and morphology. Additionally, the enzyme- and bacteria-mediated biochemical changes take place after the mechanical breakdown of the organic waste/substrate via the earthworm's gizzard (Domínguez and Gómez-Brandón, 2012). This is accomplished by the activity of several earthworm-associated enzymes and

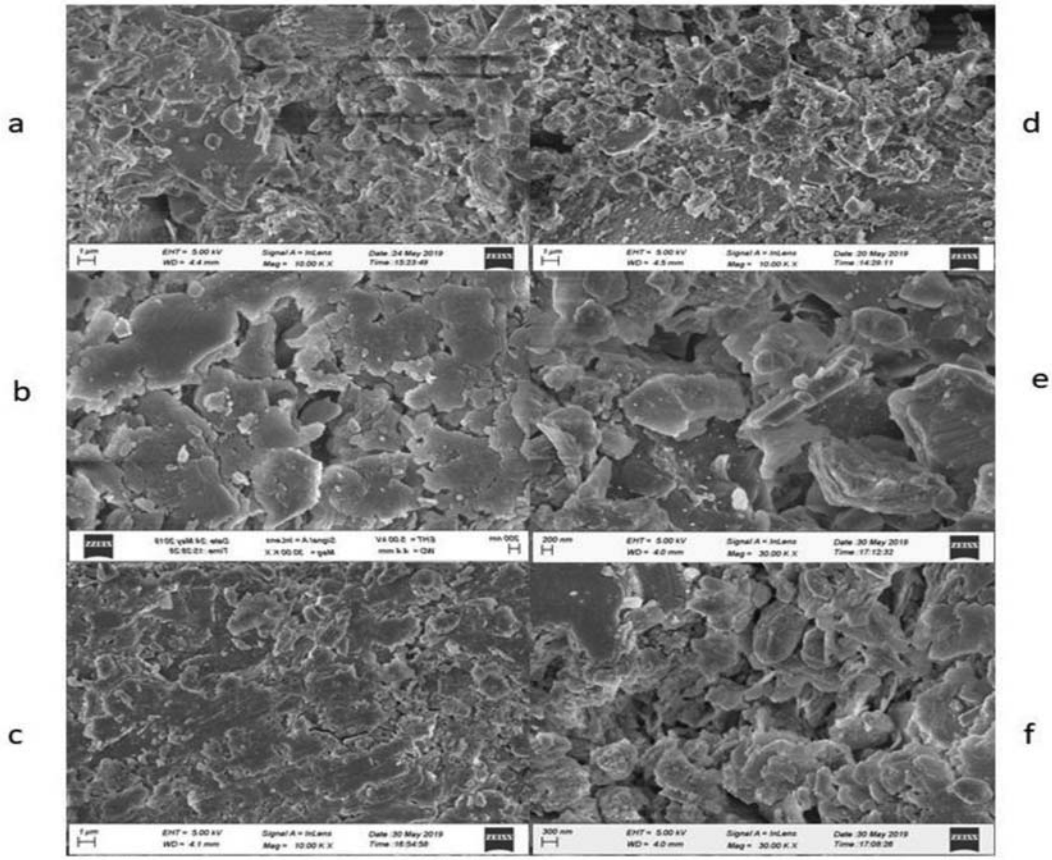

FIGURE 11.4 SEM images showing precomposted samples of (A) potato crop waste biomass, (B) citronella bagasse waste, (C) rice bagasse waste, (D) vermicompost of potato crop waste biomass, (E) vermicompost citronella bagasse waste, and (F) vermicompost of rice bagasse waste.

microorganisms. The degraded substrate and changes that occur in the surface while passing through the earthworm guts can be viewed and confirmed through the SEM analysis (Arumugam et al., 2018). Furthermore, SEM analysis can also be effectively utilized to figure out the surface area of mature vermicompost (Kumar et al., 2013). The end vermicompost derived from biomass *Curcuma longa* and *Zingiber officinale* shows a fragmented, porous, and granular structure with a higher surface area that was not observed initially. The vermicomposted sample also displayed a considerable number of grooves or pit-like shapes and a greater surface area (Das et al., 2022). Higher fragmentation and porosity in finished vermicompost coupled with granular structure is due to the deterioration of complex proteins and lignin materials during the disintegration of the organic waste substrate (Amritha, 2020). The innumerable surface irregularities of the final vermicompost in presence of earthworms and microbes confirm its maturity status. The study conducted by Ravindran and Mnkeni (2016) correlated the earthworm biomass to the intense decomposition pattern of the organic substrate. The results confirmed the earthworm's ability in decomposing the intricate organic substrate. Further, the SEM analysis revealed that as the composting process advanced, the fiber elements gradually vanished. Therefore, SEM analysis that reveals morphologic changes in pre- and post-vermicomposted organic substrates is extremely important for confirming the stability and maturity of the final vermicompost samples.

5.7 Ultraviolet−visible spectroscopy

One of the methods that is most often used to assess the stability and maturity of finished vermicompost is ultraviolet−visible (UV-vis) spectroscopy. It is an analytical technique that measures the absorbance spectra of a given sample. Humic acids have a high level of UV absorption; henceforth, the humification degree of the mature vermicompost may be assessed using UV-vis spectroscopy (Förster, 2004). High humification is an indicator of mature vermicompost. Besides, UV-vis spectroscopy is a relatively easy-to-use technique. It requires less training time prior to analysis and is inexpensive. The requirement of minimal processing of samples before data analysis and quick measurements make it accessible for integration in experimental analysis (Bhat et al., 2017b).

During vermicomposting, aliphatic molecules are quickly transformed into aromatic compounds, resulting in the formation of high molecular weight and aromatic humic acids (Wang et al., 2022). As a result, a high degree of UV absorption occurs in mature vermicompost. The three important spectrum regions where absorbance is usually measured are as follows (Sellami et al., 2008).

I. The absorbance at wavelength $\lambda = 280$ nm shows the initial step of the transformation of aliphatic and lignin compounds
II. The absorbance at wavelength $\lambda = 472$ nm designates the commencement of depolymerization and humification of the organic molecules
III. The absorbance at wavelength $\lambda = 664$ indicates highly humified material rich in oxygen and aromatic compounds.

Based on these wavelengths, the degree of vermicompost maturity can be assessed using the absorbance ratios $Q_{2/6} = A_{280}/A_{664}$, $Q_{4/6} = A_{472}/A_{664}$, and $Q_{2/4} = A_{280}/A_{472}$ (Lim and Wu, 2015). In addition, the ratios $Q_{2/4}$, $Q_{2/6}$, and $Q_{4/6}$ are also employed as humification indexes to evaluate the degree of humification in final vermicompost (Azim et al., 2018). A study conducted on effluent waste that originated the from palm oil industry by Lim and Wu (2015) observed that the final vermicompost's humification index fell in $Q_{2/6}$ and $Q_{2/4}$, while that of $Q_{4/6}$ rises. The decrease in humification index of $Q_{2/6}$ and $Q_{2/4}$ reflects the decomposition and mechanical breakdown of the organic compounds mediated by microbes and earthworm, while the rise in $Q_{4/6}$ is due to production of benzene-carboxylic and phenolic compounds in the alkali soluble humic substances. Likewise, Khatua et al. (2018) reported lower humification index in the finished vermicompost when compared with its initial substrate. The study observed a lower $Q_{2/6}$ ratio in the finished vermicompost, showing the progress in vermicomposting stages. Similarly, the decreased $Q_{2/4}$ ratios in the final vermicompost proved the effective conversion of the waste into the final humified material. In addition, the lowered $Q_{4/6}$ ratios in the final compost revealed the high amount of organic material humification. The degree of humification in organic material is inversely linked to the humification index; i.e., the higher the humification level is, lower is its humification index. Humification index values lower than five indicate a high level of humification. This high level of humification is achieved through the synergistic action of earthworms and microbes, which accelerates the degradation and decomposition of the organic substrate and transforms it into useful biofertilizer (Bhat et al., 2017b). Hence, UV-vis spectroscopy is an economical and reliable instrument that can be relentlessly utilized for determining the maturity of the substrate during vermicomposting.

6. Limitation of instrumentation techniques

The competence and effectiveness of the sophisticated instruments for determining the finished vermicompost maturity and stability are unequivocal and indisputable. However, there is also a certain limitation as these instruments are not only expensive but also require trained personnel for operating them. Sophisticated instruments like AAS require frequent calibration for maintaining the efficiency of producing quality and reproducible data. Certain instruments like a scanning electron microscope can be obstructed by the presence of any possible electric or magnetic substance that can influence the result and generate errors while computing results (Boruah and Deka, 2021). Besides, prior knowledge of the sample is required before performing the SEM, TGA, and FTIR analysis. Additionally, methods like AAS, SEM, and GC-MS require the processing of the samples before analysis. Any error during sample preparation may provide erroneous findings (Bhat et al., 2017b).

7. Conclusion and future prospects

Vermicompost is a suitable alternative when the world is transitioning toward the use of organic and environmentally friendly fertilizers. However, to obtain the maximum benefits, it is absolutely essential to assess the maturity and stability of the final vermicompost before agricultural application. The traditional approaches (physical, chemical, and biologic properties) ordinarily used express the finished vermicompost maturity state in bits and pieces. Besides, the methods have certain limitations that include dependency of the result on the nature of the substrate and amendments used. For this reason, sophisticated instrumentation techniques have been employed to overcome the challenges. The instrumentation techniques provide a clearer and more accurate picture of the major beneficial changes and transformations of organic wastes that occur during vermicomposting. Additionally, quality assurance of the finished vermicompost that arises during commercialization can be solved using these sensitive and sophisticated instruments.

A combination of the traditional method with instrumental characterization is the most ideal strategy as no such single methodology can generate an accurate picture of the vermicompost maturity and stability. Previous studies have focused on the physical, chemical, and biologic elements of transformation in vermicomposting. However, the qualitative description of the mature vermicompost employing sensitive equipment is still limited throughout the literature. Moreover, the combination of traditional technique coupled with the contemporary advanced technologies in assessing vermicompost stability and maturity is still at an infant stage. For this reason, adoption and integration of this instrumentation approach is crucial to better understand and establish the maturity of the finished vermicompost.

As evident from the above discussion, a single criterion is not enough for describing the maturity of the finished vermicompost. Hence, the combination of the traditional approaches with the instrumentation techniques remains the gold standard for the characterization of mature vermicompost. In addition, a simpler and cost-effective way of characterizing the mature vermicompost may be explored. To achieve the fullest potential of vermicompost in contributing to the world to the greener and safer side, a readily available, simpler assessment method for vermicompost maturity may be developed for large-scale utilization.

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CHAPTER

12

Exposure to emerging contaminants: ecotoxicological effects on earthworms and the potential of gut-associated microorganisms in bioremediation

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1. Introduction

The exponential growth of the human population, as well as the development of industrial, agricultural, and healthcare activities, has resulted in an unmeasurable increase in the production and use of chemicals, which have recently been labeled as "emerging contaminants" (ECs) (Maddela et al., 2022; Petrisor, 2004). The term "emerging contaminants" can refer to both newly created chemicals produced by the industry that are being introduced to the market at a rapid rate as well as well-known chemicals that have been in use for many years in a variety of applications and have been gradually released into the environment (Gomes et al., 2017; Sauvé and Desrosiers, 2014). Several authors have used the terms

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"emerging contaminants" and "contaminants of emerging concern" interchangeably and have proposed categorizing these compounds into the following main groups: pharmaceuticals, personal care products, pesticides, microplastics, nanomaterials, flame retardants, industrial additives and agents, and gasoline additives (Bayabil et al., 2022; Maddela et al., 2022; Sauvé and Desrosiers, 2014). ECs with various uses have lately been detected in groundwater, surface water, municipal wastewater, drinking water, and food sources on a regular basis, and their environmental fate has drawn attention from all over the world (Gogoi et al., 2018; Khan et al., 2022; Rout et al., 2021; Taheran et al., 2018).

Soil is considered one of the main sinks of ECs in the environment, as anthropogenic inputs of ECs into soils have been greatly exceeded in the last decade by traffic, industrial effluents, sewage treatment plant discharges, and agricultural practices (Maddela et al., 2022). Wastewater has been identified as the principal source of ECs, owing to the ineffectiveness of wastewater treatment procedures used in treatment plants in completely removing emerging contaminants (Bolong et al., 2009; Chen et al., 2022; Li et al., 2021b). As a result, soil irrigation with treated and untreated wastewater as well as fertilization with sludge from wastewater treatment plants are contributing constantly to an ever-increasing number of ECs in the soil (Ben Mordechay et al., 2022; Mohapatra et al., 2016; Picó et al., 2019). Compared with other pollutants, the majority of ECs have a high affinity for soil particles (Biel-Maeso et al., 2021; Willemsen and Bourg, 2021), which endorses their persistence in the soil and their bioaccumulation in the terrestrial biotic components. ECs are also classified as trace pollutants because they are highly toxic and have a high potential to cause adverse effects on both humans and the environment although being present in the environment at low concentrations (Chaturvedi et al., 2021; Dey et al., 2023; Gogoi et al., 2018; Mohapatra et al., 2016; Yang et al., 2019). Despite the fact that ECs have recently become a popular and trendy research topic, they continue to pose a challenge to regulatory agencies, which lack regulatory standards due to a scarcity of information about the effects of chronic exposure (Bayabil et al., 2022; Gomes et al., 2017; Sauvé and Desrosiers, 2014). For many ECs in the soil, the receptors are the microflora (bacteria and fungi), plants, and soil fauna (protozoa and invertebrates such as nematodes and earthworms) (Billings et al., 2021; Gogoi et al., 2018; Snow et al., 2017). The danger arises from the fact that most of the environmental and human toxicity of ECs have yet to be investigated, as have the by-products they produce. As a result, the increasing release of these contaminants into the environment may endanger human health and ecosystems with a lack of effective surveillance (Kumar et al., 2022).

As living organisms and ecosystems suffer immediate and/or potential long-term harm from soil pollution, an effective risk evaluation of ECs is becoming a global challenge (Gomes et al., 2017; Gwenzi et al., 2018; Pereira et al., 2015). Due to the fact that invertebrates are abundant in terrestrial ecosystems and have direct contact with the soil, they are frequently used for assessing soil contamination (Gomes et al., 2017; Wang et al., 2022a). Earthworms, the dominant fauna in most temperate terrestrial ecosystems, are one of the terrestrial organisms potentially exposed to the presence of ECs in soil. This is due to their ecologic role in the development of soil structure, material circulation, and pollutant breakdown (Bohlen, 2017; Edwards, 2004). Furthermore, because of their feeding mode and high surface-to-volume ratio, as well as the presence of chemo-receptors on their body surface, they are sensitive to the presence of chemicals in the soil, allowing them to avoid contaminated areas where soil habitat function has been compromised, making them a sensitive bioindicator of soil health

(Shi et al., 2017; Solé, 2021; Solé et al., 2021). Some international organizations have referred to earthworms as appropriate model organisms or as the standard test organism in soil ecotoxicology research (ISO, 2014, 2012a, 2012b; OECD, 2004, 1984). The earthworm biomarker is an essential soil pollution assessment tool among ecotoxicology tests (Shi et al., 2017). *Eisenia fetida* is regarded as a model organism for environmental monitoring, and it has been utilized for early warning or the diagnosis of soil pollution, as well as both short-term and long-term assessments of the toxicity of many ECs (Gillis et al., 2017; Lahive et al., 2014; Pino et al., 2015; Wang et al., 2022c; Zhang et al., 2022a,c).

Furthermore, numerous studies have investigated in recent years earthworm gut microbiota and the crucial roles they play in digestion and nutrient utilization, pathogen defense, and resistance to abiotic factors (Cao et al., 2022; Ding et al., 2019; Li et al., 2021a; Sun et al., 2020; Wang and Kasper, 2014). Although only a portion of the earthworm gut microbiota is known, their implication in host lives, metabolism regulation, host immune regulation, and gut homeostasis has been reported by many researchers (Cao et al., 2022; Chao et al., 2019, 2020). Since earthworms are capable of ingesting and digesting soil (Drake and Horn, 2007), their gut microbiota may provide crucial information on the ecotoxicologic effects of polluted soils. Recent interest in the microbiome of the earthworm and its response to a gradient of ECs has been emphasized by the advent of high-throughput sequencing techniques, elucidating the critical role and the abnormal changes in the intestinal microbial community in adapting to adverse environments including soil pollutants (Tang et al., 2019). Indeed, many researchers have recently been reporting that numerous soil pollutants have an adverse effect on the gut microbial population and diversity, such as dysbiosis and the absence of specific symbionts disrupting the gut barrier of the host and leading to health risks by reducing the microbiota's ability to direct the maturation of the immune and the reproductive system (Krishnaswamy et al., 2021; Swart et al., 2020b; Yang et al., 2022b). However, the potential effects of the various classes of ECs on the gut microbiota of earthworms, as well as their effects on total and active microbial communities, remain poorly studied. Additionally, it was reported that the earthworm gut microbiome was largely resistant to exposure to numerous ECs, whereas some gut microbial communities were significantly activated (Cao et al., 2022; Owagboriaye et al., 2021; Swart et al., 2020b; Zhang et al., 2020a; Zhang et al., 2022d), which may elucidate their potential implication in the bioremediation and mitigation of EC toxicity.

2. Earthworms' response to emerging contaminants

For risk assessment, it is essential to take into account the transformation processes of ECs to elucidate the exposure pathways and uptake potential of soil biota (Gomes et al., 2017; Maddela et al., 2022; Snow et al., 2017). Due to their sensitivity to various soil pollutants, earthworms are commonly used as model organisms in toxicology tests to determine the pollutants' susceptibility. Their use as indicator species and sublethal biomarkers in soil pollution assessment is well-established. The first ecotoxicology assessed tests were mortality based and aimed to determine toxic chemicals' lethal concentration (LC_{50}) (Solé, 2021); subsequently, sublethal responses, such as reproductive performance, were accounted for.

Recently, it has become essential to include enzymatic, molecular (genetic and metabolomic), and even behavioral biomarkers in ecotoxicity tests as indicators of sublethal toxicity (Shi et al., 2017; Solé et al., 2021). Based on the NORMAN database (www.norman-network.net), there are 21 classes of ECs, including "pesticides and other plant protection products; antibiotics, veterinary drugs, and other pharmaceuticals; food additives; industrial chemicals; plastic additives; flame retardants; metallic nanoparticles; microplastics," etc. As chronic exposure may pose a risk to soil biota and human health via the food web, Fig. 12.1 depicts the main categories of emerging pollutants that can be found in the soil, their primary sources, and their principal receptors.

The herein-reviewed studies (Table 12.1) on the potentially toxic effects of ECs on earthworms indicated that these pollutants could have detrimental effects that might vary depending on the category of the EC and its soil prevalence. ECs can have extensive effects on earthworms, with some chemicals having the potential to inhibit growth, cause mutations, affect reproduction, or cause death. The histopathological examination has revealed

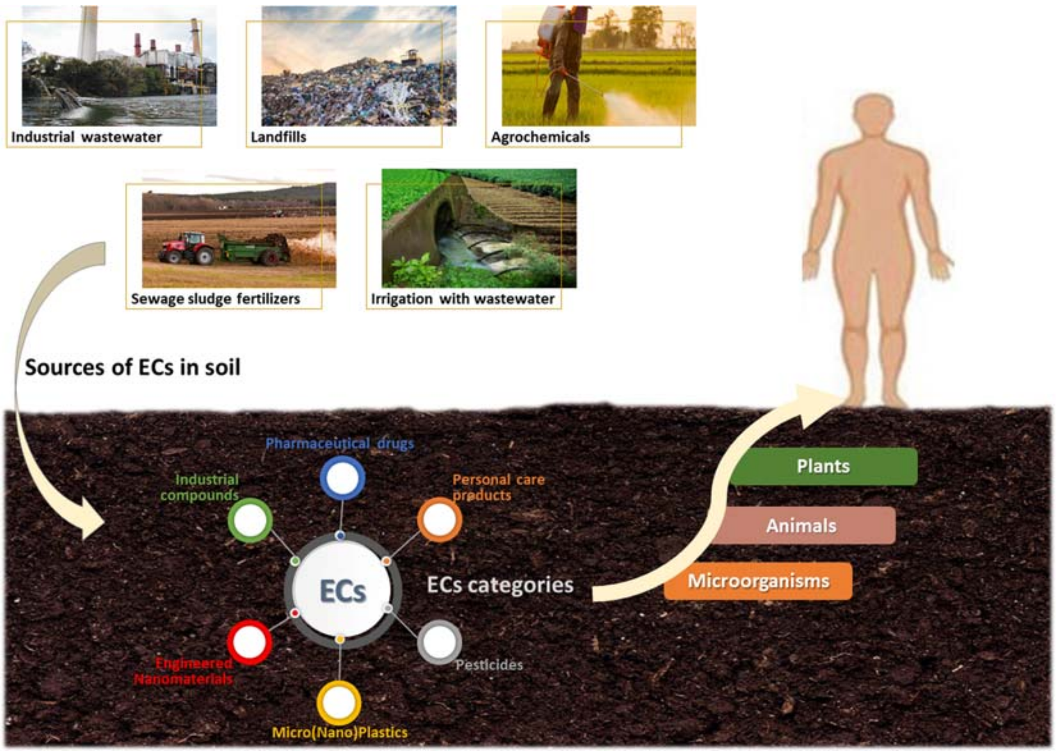

FIGURE 12.1 Sources and classes of soil emerging contaminants (ECs) that may affect soil biota and human health. *Adapted from Gomes, A.R., Justino, C., Rocha-Santos, T., Freitas, A.C., Duarte, A.C., Pereira, R., 2017. Review of the ecotoxicologic effects of emerging contaminants to soil biota. Journal of Environmental Science and Health, Part A 52, 992–1007. https://doi.org/10.1080/10934529.2017.1328946, Vasilachi, I.C., Asiminicesei, D.M., Fertu, D.I., Gavrilescu, M., 2021. Occurrence and fate of emerging pollutants in water environment and options for their removal. Water, Switzerland. https://doi.org/10.3390/w13020181.*

TABLE 12.1 Ecotoxicological impacts of emerging contaminants on earthworms: review of recent studies.

Emerging contaminant		Concentration	Earthworm species	Toxicity	References
Pharmaceutical drugs and personal care products	Monensin Roxarsone	30–60–200–350 –550 mg kg^{-1} dry soil 1.25–2.5–4–10 –20 g kg^{-1} dry soil	*Eisenia fetida*	**Mortality (LC_{50})** 346.0 mg kg^{-1} dry soil 5.74 g kg^{-1} dry soil	Li et al. (2019)
	Chlortetracycline	0.3–3–30–100 –300 mg kg^{-1} dry soil	*E. fetida*	**Reproduction (EC_{50})** Juvenile counts↓: 96.1 mg kg^{-1} dry soil Cocoon counts↓: 120.3 mg kg^{-1} dry soil **DNA damage** >30 mg kg^{-1} dry soil **Oxidative stress** >3 mg kg^{-1} dry soil **Lipid peroxidation** >30 mg kg^{-1} dry soil	Lin et al. (2012)
	Tetracycline Chlortetracycline	0.3–3–30–100 –300 mg kg^{-1} dry soil	*E. fetida*	**DNA damage** 0.3 mg kg^{-1} dry soil **Oxidative stress** 3 and 30 mg kg^{-1} dry soil	Dong et al. (2012)
	Ciprofloxacin Azithromycin	0.015 to 0.36 –1.8 mg kg^{-1} dry soil 0.0089 to 0.03 –0.16 mg kg^{-1} dry soil	*E. fetida*	**Toxicity** No effect **Bioaccumulation** 20% of ciprofloxacin 40% of azithromycin	Sidhu et al. (2019)
	Triclosan	1, 10, 50, 100, and 300 mg kg^{-1} dry soil	*E. fetida*	**Oxidative stress** > 50 mg kg^{-1} **Lipid peroxidation** >50 mg kg^{-1} dry soil **DNA damage** >1 mg kg^{-1} dry soil	Lin et al. (2010)
	Triclocarban	Up to 80.0 mg kg^{-1} dry soil	*E. andrei*	**Mortality (LC_{50})** 3.3 mg cm^{-2} **Avoidance test** >15 mg kg^{-1} **DNA damage** >1 mg kg^{-1} dry soil **Cytotoxicity** >1 mg kg^{-1} **Oxidative stress** > 10 mg kg^{-1}	Sales Junior et al. (2020)

(*Continued*)

TABLE 12.1 Ecotoxicological impacts of emerging contaminants on earthworms: review of recent studies.—cont'd

Emerging contaminant		Concentration	Earthworm species	Toxicity	References
	Oxybenzone (Benzophenone-3)	Up to 1000 mg kg^{-1} dry soil	*E. fetida*	**Reduction in the biomass** >384.16 mg kg^{-1} dry soil **Mortality (LC_{50})** 364.06 mg kg^{-1} dry soil **Reproduction** Juvenile counts↓: 7.28–36.4 mg kg^{-1} dry soil Cocoon counts↓: 36.4 mg kg^{-1} dry soil **Oxidative stress** 7.28–36.4 mg kg^{-1} dry soil **Lipid peroxidation** >3.64 mg kg^{-1} dry soil **Neurotoxicity** >3.64 mg kg^{-1} dry soil **Histopathology** >7.28 mg kg^{-1} dry soil	Gautam et al. (2022)
	Methyl-, propyl-, butyl Parabens	Up to 1000 mg kg^{-1} dry soil	*E. fetida*	**Mortality (LC_{50})** No effect **Reduction in the biomass** No effect **Reproduction** No effect	Arachchige Chamila Samarasinghe et al. (2021)
Pesticides	Sulcotrione Penoxsulam	100–250–500–750–1000 mg kg^{-1} dry soil 3–15–30–60–100 mg kg^{-1} dry soil	*E. andrei*	**Avoidance test (EC_{50})** 1263.3 mg kg^{-1} dry soil 80.6 mg kg^{-1} dry soil	Marques et al. (2009)
	Carfentrazone-ethyl	0.05–0.5–5 mg kg^{-1} dry soil	*E. fetida*	**Histopathology** No effect **DNA damage** No effect **Oxidative stress** >5 mg kg^{-1} dry soil	Li et al. (2022)
	Acetamiprid Imidacloprid Nitenpyram clothianidin Thiacloprid	0.1–1–10–100–1000 mg kg^{-1} dry soil	*E. fetida*	**Mortality (LC_{50})** 1.52 mg kg^{-1} dry soil 2.82 mg kg^{-1} dry soil 3.91 mg kg^{-1} dry soil 6.06 mg kg^{-1} dry soil 10.96 mg kg^{-1} dry soil	Wang et al. (2012)
	Cyantraniliprole	0.1–1–5–10 mg kg^{-1} dry soil	*E. fetida*	**Reproduction** Juvenile counts ↓: 5–10 mg kg^{-1} dry soil Cocoon counts ↓: 5–10 mg kg^{-1} dry soil	Qiao et al. (2021)

TABLE 12.1 Ecotoxicological impacts of emerging contaminants on earthworms: review of recent studies.—cont'd

Emerging contaminant		Concentration	Earthworm species	Toxicity	References
				Oxidative stress >5 mg kg^{-1} dry soil **Intestinal tissue functioning damage** >1 mg kg^{-1} dry soil **Functional genes expression dysregulation** >1 mg kg^{-1} dry soil	
	Imazalil	0.01–0.1–1–10 mg kg^{-1} dry soil	*E. andrei*	**Avoidance test (EC_{50})/mortality (LC_{50})/ reduction in the biomass** No effect **Reproduction** Juvenile counts↓: >1 mg kg^{-1} dry soil **Oxidative stress** >0.1 mg kg^{-1} dry soil	Pereira et al. (2020)
Micro(nano) plastics	Polystyrene microplastics (MPs) + phenanthrene	10 mg kg^{-1} dry soil (MPs size: 100 nm, 1, 10, and 100 μm) 5 mg kg^{-1} dry soil	*E. fetida*	**DNA damage** Micron-size MPs + phenanthrene **Oxidative stress** Micron-size MPs + phenanthrene **Functional genes expression dysregulation** All MPs sizes + phenanthrene	Xu et al. (2021a)
	Polystyrene MPs	100 and 1000 μg kg^{-1} dry soil MPs size: 100 and 1300 nm	*E. fetida*	**Oxidative stress** All treatments **Histopathology** 1300 nm (1000 μg kg^{-1}) MPs **DNA damage** 100 and 1300 nm (1000 μg kg^{-1}) MPs	Jiang et al. (2020)
	Polystyrene MPs (≤250 μm) Polyethylene MPs (≤300 μm)	0%–1%–5%–10%–20% dry-weight soil	*E. fetida*	**Oxidative stress** >20% dry-weight soil	Wang et al. (2019b)
Metallic Nanoparticles	TiO_2 nanoparticles (NPs, 32 nm) ZnO NPs (40–100 nm)	0.1–1–10–100–1000 mg kg^{-1} dry soil	*E. fetida*	**Mortality** 100% at 100 and 1000 mg kg^{-1} dry soil **Reduction in the biomass** ZnO -NPs at 100–1000 mg kg^{-1} dry soil **Reproduction** Cocoon counts↓	Cañas et al. (2011)

(*Continued*)

TABLE 12.1 Ecotoxicological impacts of emerging contaminants on earthworms: review of recent studies.—cont'd

Emerging contaminant		Concentration	Earthworm species	Toxicity	References
	Ag NPs	0–100–1000 mg Ag kg^{-1} dry soil	*E. fetida*	**Reproduction** Cocoon counts↓: 801 mg 10-nm Ag NPs kg^{-1} 773.3 mg 30- to 50-nm Ag NPs kg^{-1}	Shoults-Wilson et al. (2011)
	Al_2O_3-NPs (54 nm)	50–100–300–3000 mg Al kg^{-1} dry soil	*E. fetida*	**Mortality** 20% at 3000 mg Al kg^{-1} dry soil **Oxidative stress** >300 mg Al kg^{-1} dry soil	Yausheva et al. (2017)
	Ag NPs (1–10 nm) Co NPs (28 nm)	10 mg of contaminant kg^{-1} dry horse manure	*Lumbricus rubellus*	**Fatty acids of earthworm tissues** ↓ for Co NPs **Histopathology** Ag NPs and Co NPs	Vittori Antisari et al. (2016)
Carbon-based nano-objects	Multiwalled carbon nanotubes (MWCNTs)	10–50–100 mg kg^{-1} dry soil	*E. fetida*	**Mortality (LC_{50})/reduction in the biomass/reproduction** No effect **Cytochrome P450 isoenzymes activities** Inhibition at 100 mg kg^{-1} dry soil **Oxidative stress** 50 and 100 mg kg^{-1} dry soil **Changes in earthworm metabolomics** 100 mg kg^{-1} dry soil	Yang et al. (2022a)
	PdCu/MWCNT and PdNi/MWCNT NPs	1–10–100–1000–2000 mg L^{-1} injected into the coelomic space of earthworms	*E. fetida*	**Survival rate** 84% at 2000 mg PdCu/MWCNT NPs L^{-1} **Malformations of earthworm** 1000 and 2000 mg PdCu/MWCNT NPs L^{-1} **Histopathology** 2000 mg PdCu/MWCNT NPs L^{-1} **Oxidative stress** >10 mg L^{-1}	Köktürk et al. (2022)
	MWCNT Nonylphenol (NP)	0.1–1 g MWCNTs kg^{-1} dry soil 1 g kg^{-1} MWCNTs absorbed 5 mg kg^{-1} NP 10 mg NP kg^{-1} dry soil	*E. fetida*	**Mortality (LC_{50})** No effect **Oxidative stress** 1 g MWCNTs kg^{-1} dry soil 1 g kg^{-1} MWCNTs absorbed 5 mg kg^{-1} NP **Cellulase content** ↓ for 1 g kg^{-1} MWCNTs absorbed 5 mg kg^{-1} NP **Na^+,K^+ -ATPase activity** ↓ for 1 g kg^{-1} MWCNTs absorbed 5 mg kg^{-1} NP **DNA damage** All treatments	Hu et al. (2013)

TABLE 12.1 Ecotoxicological impacts of emerging contaminants on earthworms: review of recent studies.—cont'd

Emerging contaminant		Concentration	Earthworm species	Toxicity	References
	Graphene nanoparticles	30–300–3000 mg kg^{-1} dry soil	*E. fetida*	**Changes in earthworm metabolomics** Alanine, phenylalanine, glutamate, proline, histidine, arginine, maltose, glucose, malate, succinate, myoinositol, and spermidine >30 mg kg^{-1} dry soil	Zhang et al. (2020b)
	Polyaniline nanorods	125–250–500–1000 mg kg^{-1} dry soil	*E. fetida*	**Growth inhibition** All concentrations **Oxidative stress** >250 mg kg^{-1} dry soil	Shu et al. (2022)
Flame retardants	Dechlorane Plus	0.1–0.5–6.25–12.5 mg kg^{-1} dry soil	*E. fetida*	**Mortality (LC_{50})/reduction in the biomass** No effect **Oxidative stress** >0.1 mg kg^{-1} dry soil **Oxidative damage** >0.5 mg kg^{-1} dry soil **Neurotoxicity** >0.1 mg kg^{-1} dry soil **Cellulase content ↓** >0.5 mg kg^{-1} dry soil **DNA damage** >0.1 mg kg^{-1} dry soil	Yang et al. (2016)
	Dechlorane Plus	0.1–1–10–50 mg kg^{-1} dry soil	*E. fetida*	**Mortality (LC_{50})/reduction in the biomass** No effect **Oxidative damage** >10 mg kg^{-1} dry soil **Neurotoxicity** >0.1 mg kg^{-1} dry soil **DNA damage** >50 mg kg^{-1} dry soil **Alteration of transcriptomic profiles** Changes in neurotoxicity-related transcriptomic profiles	Zhang et al. (2014a)
	Brominated decabromodiphenyl ether (BDE-209)	1–10–100 mg kg^{-1} dry soil	*E. fetida*	**Oxidative stress** >1 mg kg^{-1} dry soil	Zhang et al. (2014b)
	BDE-209	0.1–1–5–10–50–100–500–1000 mg kg^{-1} dry soil	*E. fetida*	**Avoidance test** No effect **Mortality/growth rate** No effect	Xie et al. (2013)

(*Continued*)

TABLE 12.1 Ecotoxicological impacts of emerging contaminants on earthworms: review of recent studies.—cont'd

Emerging contaminant		Concentration	Earthworm species	Toxicity	References
	Triphenyl phosphate	20–40–60–80 mg kg^{-1} dry soil	*E. fetida*	**Reproduction** Juvenile counts ↓ by 15% at 1000 mg kg^{-1} **Oxidative stress** >20 mg kg^{-1} dry soil **Oxidative damage** >20 mg kg^{-1} dry soil **DNA damage** >20 mg kg^{-1} dry soil	Zhang et al. (2022a)
	DOPO-HQ	1000 mg kg^{-1} dry soil	*E. fetida*	**Mortality** No effect	Liu et al. (2018)
Plasticizers	Diisononyl phthalate (DINP)	300–600–1200–2400 mg kg^{-1} dry soil	*E. fetida*	**Oxidative stress** >300 mg kg^{-1} dry soil **Gene expression dysregulation** >300 mg kg^{-1} dry soil	Zhang et al. (2022e)
	Tris (2-chloroethyl) phosphate (TCEP) and tricresyl phosphate (TCP)	0.1–1–10 mg kg^{-1} dry soil	*E. fetida*	**Mortality/growth rate** No effect **Histopathology** Dose-effect intestine damage **DNA damage** >1 mg kg^{-1} dry soil **Oxidative damage** All concentrations **Neurotoxicity** >0.1 mg kg^{-1} dry soil	Yang et al. (2018)
	Bisphenol A (BPA) and Di-isobutyl phthalate (DIBP)	1–2.5–5–10 mg kg^{-1} dry soil	*Hyperiodrilus africanus*	**Oxidative stress** >5 mg kg^{-1} dry soil **DNA damage** >2.5 mg kg^{-1} dry soil	Olujimi et al. (2020)

abnormalities at the organ level, especially at the intestine level. Furthermore, ECs can induce changes in various stress pathways, disrupt hormone signaling, and even cause DNA damage. These overall responses (Fig. 12.2) may initiate signaling cascades that modulate important genetic markers. Such genetic modulation could then result in changes in gene expression and cellular activity, potentially resulting in alterations in the behavior of the organism–earthworms. This implies that the concentration of ECs in soil, as well as their chemical and physical properties, may influence the physiologic processes of earthworms, thereby altering their role in soil ecology. Understanding the effects of ECs on earthworms is thereby crucial for determining the environmental risk associated with their application and occurrence.

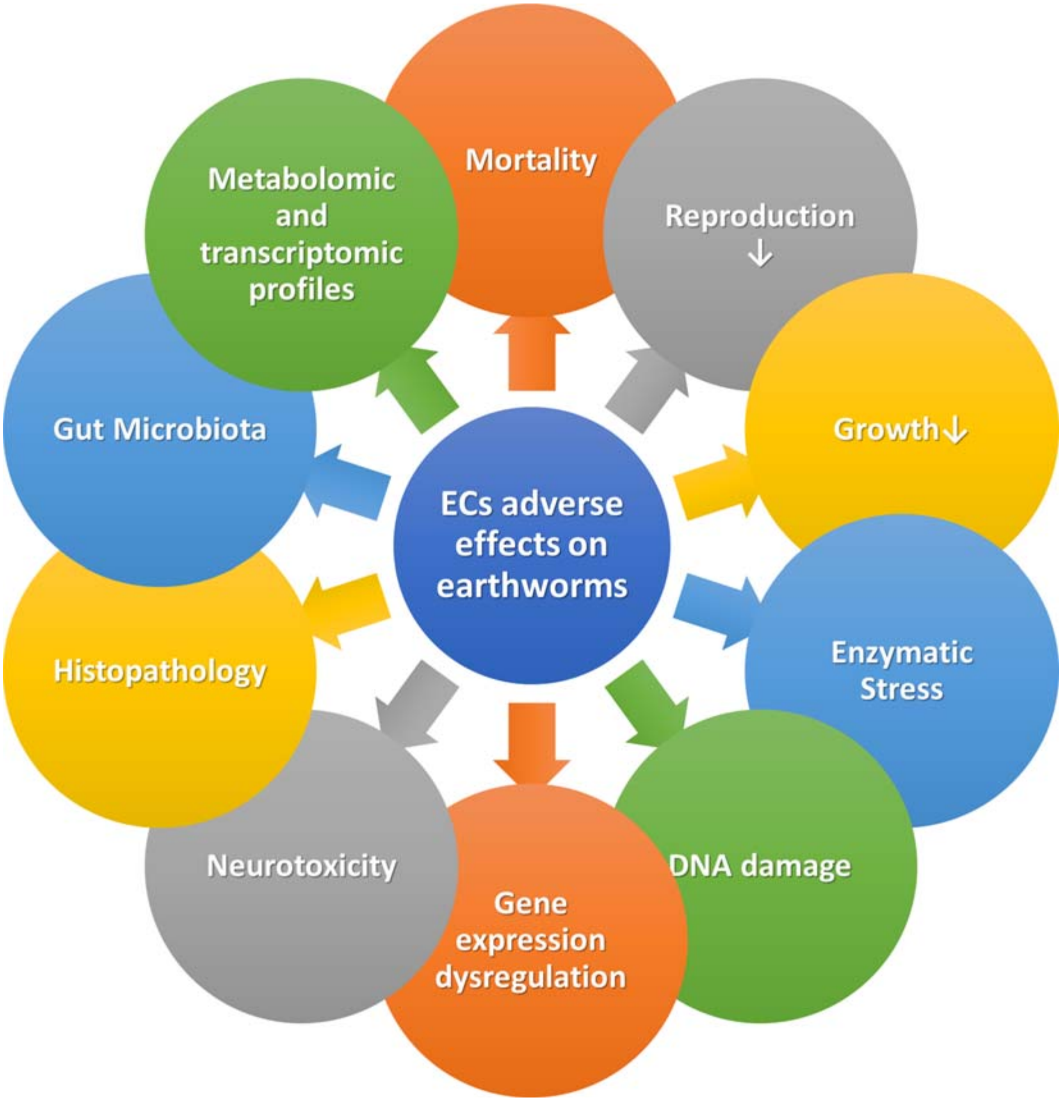

FIGURE 12.2 The reported adverse effects of many studied emerging contaminants on earthworms at the organism, organ, cellular, biochemical, and genetic levels. *Adapted from Adeel, M., Shakoor, N., Shafiq, M., Pavlicek, A., Part, F., Zafiu, C., Raza, A., Ahmad, M.A., Jilani, G., White, J.C., Ehmoser, E.-K., Lynch, I., Ming, X., Rui, Y., 2021. A critical review of the environmental impacts of manufactured nano-objects on earthworm species. Environmental Pollution 290, 118041. https://doi.org/10.1016/j.envpol.2021.118041, Cui, W., Gao, P., Zhang, M., Wang, L., Sun, H., Liu, C., 2022. Adverse effects of microplastics on earthworms: a critical review. Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2022.158041.*

2.1 Pharmaceutical drugs and personal care products

Pharmaceutical and personal care products (PPCPs) include toothpaste, skincare products, fragrances, antibiotics, pharmaceutical medicines, and veterinary drugs utilized by the agro-industry (Bayabil et al., 2022; Rosenfeld and Feng, 2011). These compounds are introduced into agricultural soils through irrigation with treated effluents or fertilization with biosolids, which contain complex mixtures of these chemicals. Due to the continuous introduction of PPCPs into the environment via various pathways and their bioactivity and known modes of action, they are ECs with potential ecotoxicologic effects on terrestrial organisms (Liu et al., 2020).

Large quantities of veterinary pharmaceuticals are released into the soil along with animal feces due to the intensification and modernization of livestock farming (Lal, 2021). In a study conducted by Parelho et al. (2018), it was determined that earthworm (*Amynthas gracilis*) exposure to soils contaminated by livestock pollutants was associated with sublethal toxicity affecting key processes, including neurotransmission, oxidative stress, cytotoxicity, and DNA damage (Parelho et al., 2018). Li et al. (2019) examined the toxic effects of commonly used veterinary drugs monensin and roxarsone on the earthworm (*Eisenia fetida*). They demonstrated that although they had low toxicity for *E. fetida*, the potential toxicity of these veterinary drug residues should not be neglected due to their mobility in the environment. Lin et al. (2012) evaluated the ecologic risk of the veterinary pharmaceutical chlortetracycline on *E. fetida* in agricultural soil contaminated with concentrations up to 300 mg kg^{-1} and confirmed that chlortetracycline might induce physiologic responses and genotoxic potentials in *E. fetida* (Lin et al., 2012). Chlortetracycline and tetracycline were also found to cause substantial effects in *E. fetida*. Dong et al. (2012) tested both antibiotic concentrations up to 300 mg kg^{-1} with exposure periods of 7, 14, and 28 days and found no mortality, but even at the lowest concentration tested, both antibiotics induced oxidative stress and caused significant genotoxicity in earthworms, with severe DNA damage on coelomocytes at a concentration of only 0.3 mg kg^{-1} (Dong et al., 2012). Nonetheless, earthworms exhibited no apparent response to the toxicity of two biosolids-borne antibiotic TOrCs, ciprofloxacin, and azithromycin, at concentrations that are environmentally relevant and much higher. Although, earthworms had high bioaccumulation factor values for both compounds (Sidhu et al., 2019).

Recent evidence suggests that oxybenzone and other chemicals in personal care products threaten the biota inhabiting various ecologic niches. Gautam et al. (2022) demonstrated that oxybenzone is toxic to the soil invertebrate *E. fetida* at concentrations relevant to the environment, causing oxidative stress, neurotoxicity, and an impact on earthworm reproduction. Triclocarban (TCC) is an EC that is widely used in antimicrobial personal care products. Sales Junior et al. (2020) conducted TCC toxicity assessments on *Eisenia andrei* earthworms and concluded that exposure to TCC caused both acute and chronic damage in *E. andrei* and that was able to induce cytotoxicity and genotoxicity at sublethal concentrations comparable to those found in biosolids. Triclosan (TCS) is a widely used antimicrobial compound found in various PPCPs that is effective against many bacteria and some fungi by inhibiting the bacterial fatty acid synthesis and causing cell death (Ramires et al., 2021). Previous research indicated that TCS does not pose an acute threat to the survival of earthworms; however, chronic TCS exposure reduced survival. TCS significantly impacted the other life cycle parameters, stimulated oxidative stress, and induced DNA damage (Swabna and Vasanthy, 2022; Zaltauskaite and Miskelyte, 2018). Even at the lowest tested concentration of 1 mg kg^{-1}, triclosan exposure caused extremely severe DNA damage in earthworm (*E. fetida*) coelomocytes (Lin et al., 2010). Due to their antimicrobial activity, parabens are commonly used PPCPs as preservatives; Arachchige Chamila Samarasinghe et al. (2021) demonstrated that methyl-, propyl-, and butyl parabens degrade rapidly in the terrestrial environment and, thus, are unlikely to pose a threat to species such as *E. fetida* even at concentrations of up to 1000 mg kg^{-1}.

2.2 Pesticides

In modern agriculture, using pesticides to control weeds, insects, fungi, and other pests has been essential for improving crop yield and quality (Carvalho, 2017; Li et al., 2022). Currently, two million metric tons of pesticides are used globally to increase crop production, but fewer than 1% of those pesticides reach their intended pests; the remainder contaminates the environment (Yatoo et al., 2022). As a result, pesticide residues are among the most persistent chemicals in soils. While they help keep pests and diseases at bay, they can also be toxic to humans and other organisms, including earthworms that come into direct contact with pesticides while delving into the soil (Pelosi et al., 2014; Yatoo et al., 2022). Marques et al. (2009) examined the toxicity of two different herbicides (sulcotrione and penoxsulam) and concluded that both herbicides induced avoidance behavior in *E. Andrei*; but given that the concentrations tested are higher than the recommended application rates for these pesticides, the avoidance response indicates that they would not have a negative effect under realistic conditions. Accordingly, the herbicide carfentrazone-ethyl was found to be harmless to earthworms when applied at environment-relevant concentrations (Li et al., 2022). Due to the high efficacy of pesticides against the target organisms, their use in integrated pest management programs must be carefully evaluated to prevent their severe harm to earthworms. In fact, Wang et al. (2012) compared the acute toxicity of 24 insecticides to *E. fetida*. Among the six tested chemical classes, the neonicotinoids were the most toxic to *E. fetida*, as the LC_{50} values varied between 10.96 and only 1.52 mg kg^{-1}. Furthermore, based on transcriptomic and enzymological approaches, Qiao et al. (2021) investigated the ecotoxicity of cyantraniliprole, a novel diamide insecticide, to the earthworm *E. fetida*. The exposure of earthworms to cyantraniliprole at varying concentrations resulted in reproductive toxicity, intestinal tract damage, and oxidative damage, as well as aberrant expression of essential functional genes (Qiao et al., 2021). Nonetheless, Pereira et al. (2020) concluded that imazalil exposure did not result in the death of earthworms ($LC_{50} > 166$ μg cm^{-1}). However, this pesticide may be detrimental to earthworm health, as *E. andrei* earthworms did not exhibit avoidance behavior when exposed to imazalil, resulting in more extended exposure periods and cumulative systemic damage.

2.3 Micro(nano)plastics

In recent years, micro(nano)plastics, a novel type of pollutant, have been found in all regions of the world and have garnered considerable attention (Peng et al., 2022).

The application of sewage sludge in agricultural practices has been theoretically estimated to be one of the largest sources of micro(nano)plastics in the soil (Bhat et al., 2022; Zhang et al., 2022b). However, the key sources can be divided into three categories: agricultural inputs, the influence of runoff and deposition, and the fragmentation of larger plastic debris (Hurley and Nizzetto, 2018; Zhang et al., 2022b). Recent studies have shown that nanoplastics (100 nm in size) are being introduced into the ecosystem at the same rate as microplastics (MPs; 5 mm in size). Because plastic items undergo continuous fragmentation, MPs are likely to become nanoplastics (Maddela et al., 2022). Micro(nano)plastics can alter the soil's structure, function, and biodiversity after entering the ground. They can be ingested by soil organisms, adversely affecting their health and survival (Wang et al., 2022a). In addition, MPs have a large specific surface area, hydrophobic properties, and a high capacity for absorbing other pollutants (Caruso, 2019). Consequently, they can carry other contaminants, such as organic pollutants and heavy metals, resulting in combined effects on soil and soil biota (Kwak and An, 2021; Wang et al., 2022a; Zhou et al., 2020). Xu et al. (2021a) assessed the accumulation of phenanthrene in earthworms in the presence of polystyrene MPs of varying diameters to determine their combined genotoxicity on earthworms (*E. fetida*). Although earthworms could ingest both phenanthrene and MPs from the soil, the results demonstrated that MPs enhanced the toxic effects of phenanthrene at the cellular and molecular levels and modulated the accumulation kinetics and toxicity of phenanthrene in earthworms. Similarly, Jiang et al. (2020) found that polystyrene MP exposure caused DNA damage in earthworms and that MPs were more toxic than NPs to earthworms. Recently, Zhang et al. (2022a,c) investigated the individual and combined effects of polyethylene (PE) MPs and zinc oxide nanoparticles (ZnO NPs) on earthworms (*E. fetida*) and concluded that coexposure to PE MPs and ZnO NPs resulted in greater Zn bioaccumulation and a more pronounced toxicity response in earthworms, thereby posing more significant ecologic risks. Interestingly, earthworm fragmentation of MPs to nanoplastics was confirmed by Kwak and An (2021); these NPs were introduced into soils through cast excretion. Despite these reported adverse effects of exposure to MPs and NPs, some researchers revealed that the exposure did not induce deleterious effects, as the earthworm growth rate was unaffected even at very high concentrations of MPs and NPs (Wang et al., 2019b; Zhou et al., 2020).

2.4 Engineered nanomaterials

A nanomaterial (NM) is a material that contains particles in an unbound state, as an aggregate or as an agglomerate; as their use has increased, so have concerns about their toxicologic effects on humans, animals, and the environment. NMs are widely used in medicine and have several well-known advantages, including increased water security, agricultural productivity, food security, energy storage, various applications in environmental services and remediation, electric and electronic components, fire safety, industrial and transportation applications, and others (Bayabil et al., 2022; Rosenfeld and Feng, 2011; Xu et al., 2021b). As with many emerging pollutants, most engineered NMs reach the soil through various uncontrolled emissions. Nanoparticles contaminate the soil system principally through leaching from nano-coated consumer goods dumped in landfills and through treated sewage waste (i.e., biosolids). Due to the migration of nanoparticles into the soil via water, most agricultural soils can receive nanoparticles when biosolids are applied, and the threat is alarming on a global scale (Maddela et al., 2022; Swart et al., 2020a).

2.4.1 Metallic nanoparticles

Metal nanoparticles (NPs) possess various physicochemical properties that enable them to have a vast array of industrial applications, such as nanomedicine and nano-agriculture (Kaningini et al., 2022; Landa, 2021). Numerous studies have documented their use as fertilizers and pesticides in agriculture (Saratale et al., 2018; Vargas-Hernandez et al., 2020), and different elements have been applied, including silver, zinc, copper, iron, silicon, titanium, magnesium, and manganese (Burketová et al., 2022). However, metallic NPs were reported to reduce earthworms' growth rates, reproduction, and respiration as a function of dose while also increasing avoidance response, lethality, subcellular damage, and oxidative stress (Adeel et al., 2019; Sun et al., 2021). In the case of silver, studies revealed that Ag NPs are frequently more toxic than Ag ions (typically $AgNO_3$ salts) (Courtois et al., 2021; Hu et al., 2012; Li et al., 2015). Furthermore, most incubation studies on the effects of ZnO NPs on earthworm species, describing the toxicity and the oxidative stress, confirmed a possible mechanism of toxicity involves the release of Zn^{2+} from ZnO NPs by dissolution (Alves et al., 2019; García-Gómez et al., 2019; Li et al., 2011; Lončarić et al., 2020). However, with contradictory reported lethal doses of ZnO NPs and the potential for bioaccumulation and long-term effects of ZnO NPs, assessing ZnO NPs' toxicity is still complex. Cañas et al. (2011) investigated the acute and reproductive toxicity of TiO_2 (approximately 32 nm) and ZnO (40−100 nm) to *E. fetida* in artificial soil and found no significant differences in reproduction compared with the control. Shoults-Wilson et al. (2011) also investigated the effect of particle size (10 nm and 30−50 nm) on the toxicity of silver NMs to earthworms. The authors found that $AgNO_3$ significantly reduced *E. fetida* growth and reproduction, but NM sizes had no significant effect on the NM toxicity. In addition, studies on NiO NPs indicated that these NMs reduced reproduction and were shown to affect embryo development and induce oxidative stress, which could significantly impact earthworms and other terrestrial invertebrates (Köktürk et al., 2022; Santos et al., 2017). Nevertheless, studies on cerium NPs, the widely used catalyst and fuel additive, revealed that CeO_2 NPs did not affect survival or reproduction, whereas Ce salt (ammonium cerium nitrate) impacted both reproduction and survival at high doses (Auffan et al., 2014; Vittori Antisari et al., 2016). Al_2O_3 NPs were found to be only toxic at extremely high concentrations that are unlikely to be found in the environment (Bystrzejewska-Piotrowska et al., 2012; Coleman et al., 2010; Yausheva et al., 2017).

2.4.2 Carbon-based nano-objects

Carbon nanotubes (CNTs) are a type of carbon with a cylindrical nanostructure. Single-walled carbon nanotubes (SWCNTs) with a single sheet of graphene and multiwalled carbon nanotubes (MWCNTs) with 2−50 graphene cylinders are the two main types of CNTs that can be produced (Gacem et al., 2022; Mohanta et al., 2019). Because of the widespread use of CNTs in the electronic industry and other environmental engineering applications, there has been concern about CNT exposure in the environment (Khan et al., 2021; Simon et al., 2019; Wieland et al., 2021); their concentration in soil is estimated to be 20-fold higher than in water (Abdullah et al., 2022; Nag et al., 2021). CNTs' expansion and environmental release increased soil exposure and potential risk to earthworms. Yang et al. (2022a) investigate whether MWCNTs have a negative impact on earthworms at soil concentrations of 10, 50, and 100 mg kg^{-1}, as well as possible toxicity mechanisms. According to the findings, at the highest concentration, MWCNT toxicity to *E. fetida* was associated with decreased detoxification capacity, excessive oxidative stress, and disruption of multiple metabolic pathways. All the while, under the stress of 10 or 50 mg MWCNTs per kg, earthworms exhibited an adaptive response (Yang et al., 2022a). Furthermore, Köktürk et al. (2022) investigated the ecotoxicity of bimetallic PdCu/MWCNT and PdNi/MWCNT NPs on earthworms by injecting approximately 20 μL of various concentrations of bimetallic PdCu/MWCNT and PdNi/MWCNT NPs into earthworm coelomic space. Toxicologic experiments in earthworms revealed that PdNi/MWCNT NPs and PdCu/MWCNT NPs could cause increased reactive oxygen species and oxidative DNA damage. However, because their toxicity was low at low application concentrations, neither NP poses a high risk ecotoxicologically. In an artificial soil, Hu et al. (2013) investigated the toxicologic effects of MWCNT combined with nonylphenol on *E. fetida*. The authors concluded that the combination of MWCNT/nonylphenol was much more harmful to earthworms than nonylphenol alone, indicating that MWCNT facilitated nonylphenol bioavailability to the earthworm while also increasing its toxic effects (Hu et al., 2013).

Graphene oxide (GO) is another advanced two-dimensional material made of a single sheet of carbon atoms arranged in a hexagonal network. Despite increased GO production, only two studies on the effects of GO on earthworm species have been published in the last 10 years. According to Zhang et al. (2020b,c), multilayer GO with varying morphologies and hydrophobicity has different toxic effects on other earthworm species. After 7 days of exposure to GO NPs at 300 mg kg^{-1}, the levels of alanine, phenylalanine, proline, and glutamate in juvenile *E. fetida* changed significantly.

Polyaniline nanorods (PANRs) are one-dimensional nanomaterials (1D NMs) widely used in medicine, batteries, and water treatment, among other applications. Eventually, with the increasing applications, PANRs will enter the soil environment, but their ecotoxicity has received little attention (Shu et al., 2022; Suba Lakshmi et al., 2020). A recent study by Shu et al. (2022) revealed that PANRs inhibited earthworm growth as measured by positive and increasing growth inhibition rates. In addition, it was found that PANRs activated the earthworm antioxidant system, induced stress-caused lipid damage in earthworms, Na^+-K^+-ATPase increased with an excellent dose-time relationship, and cellulase and AChE activities were promoted at low concentrations but inhibited at high concentrations, indicating that the higher the concentrations of PANRs, the greater is the ecotoxicity to earthworms (Shu et al., 2022).

2.5 Industrial compounds

In the chemical industry, thousands of compounds are used as intermediates (plasticizers, dyes, and resins) or as food additives, antioxidants, surfactants, and detergents. These industrial chemical substances are constantly released into the environment, causing severe ecosystem problems. The most frequently reported compounds are the flame retardants tris-2-chloroethyl phosphate (TCEP) and tris-2-chloroisopropyl phosphate, the endocrine disruptor bisphenol A, which is used as a plasticizer, and the detergent metabolite nonylphenol (Bayabil et al., 2022; Calvo-Flores et al., 2017).

2.5.1 Flame retardants

Flame retardants (FRs) have been used to prevent fires for many years and are used as additives in buildings, transportation, electronics, and consumer products worldwide, resulting in a wide variety of FRs being marketed in large volumes annually (Calvo-Flores et al., 2017; Ortega-Olvera et al., 2020). The toxic effects of halogenated FRs on earthworms have been previously documented. Yang et al. (2016) investigated the effects of exposure of earthworms to the organic flame retardant Dechlorane Plus (DP) (0.1−0.5 mg kg^{-1}) for 28 days and concluded that long-term exposure induced oxidative stress, which plays a vital role in toxicity. In addition, exposure to DP concentrations of 0.1, 0.5, 6.25, and 12.5 mg kg^{-1} did not affect body weight or lethality but increased oxidative stress (Zhang et al., 2014a). According to Zhang et al. (2014b), severe oxidative stress occurred in *E. fetida* due to the toxicity of brominated flame retardant-decabromodiphenyl ether (BDE-209) at concentrations up to 100 mg kg^{-1}; however, Xie et al. (2013) confirmed in their study that adult earthworms (*E. fetida*) had a strong tolerance for BDE-209 exposure in soils (0.1−1000 mg kg^{-1}). Triphenyl phosphate (TPHP) is an FR commonly used in everyday products. Because of its ease of spread, TPHP pollution has become a worldwide concern. Zhang et al. (2022a,c) investigated oxidative stress and DNA damage in earthworms exposed to TPHP-contaminated soil at concentrations of up to 80 mg kg^{-1}. The authors discovered that after TPHP exposure, the oxidative balance was disrupted, and all exposure concentrations caused DNA damage with a significant dose−effect relationship (Zhang et al., 2022a,c). As the demand for environmentally friendly FRs grows, new alternatives to halogenated flame retardants (HFRs) are being developed. DOPO-HQ has recently seen widespread use due to its high thermal stability and glass transition temperature. Liu et al. (2018) investigated the ecotoxicity of DOPO-HQ to the earthworm *E. fetida*. They found no effect even at the highest concentration of 1000 mg kg^{-1}, indicating that DOPO-HQ is far less toxic than HFRs (Liu et al., 2018).

2.5.2 Plasticizers

Plasticizers are low-molecular-weight organic compounds that are widely used in the production of plastics. The most studied, abundant, and perhaps well-known plasticizers that are also ECs are phthalate esters, bisphenol A (BPA), and its derivative bisphenol A diglycidyl ether (Bayabil et al., 2022; Qi et al., 2020). Because of their widespread use, these chemicals have accumulated in various ecosystem compartments. Plasticizers typically degrade in weeks or months, but they can persist in soil for decades and have been found in all land uses studied. Some plasticizers are genotoxic and can be absorbed by soil organisms, endangering ecosystems and human health (Billings et al., 2021). Previous studies have examined the ecotoxicity of plasticizers on earthworm species. Zhang et al. (2022e) investigated the subchronic toxicity of diisononyl phthalate (DINP) on earthworms (*E. fetida*) at various doses (0, 300, 600, 1200, and 2400 mg kg^{-1}). The results demonstrated that exposure of *E. fetida* to DINP increased the antioxidant and detoxifying enzyme levels, while the expression of their corresponding genes was unregulated (Zhang et al., 2022e). Following the recent prohibition on the use of polybrominated diphenyl ethers, organophosphate esters (OPEs) have been widely used as FRs, plasticizers, and antifoaming agents in a variety of consumer products. The toxicity of OPEs to earthworms (*E. fetida*) was investigated by choosing two common OPEs: TCEP and tricresyl phosphate (TCP). Yang et al. (2018) concluded that while the acute toxicity of TCEP and TCP and their effect on body weight were low in exposed earthworms, intestinal damage, oxidative stress, and changes in cholinergic synapses were observed, resulting in a neurotoxic effect even at low concentration of 0.1 mg kg^{-1}. Similar findings were reported in a study by Olujimi et al. (2020) investigating the effects of BPA and di-isobutyl phthalate (DIBP) and their combination on the antioxidant enzyme activities of the earthworm (*Hyperiodrilus africanus*). Earthworm exposure to BPA and DIBP was associated with oxidative stress; furthermore, DNA was damaged even at extremely low concentrations, indicating that their presence in the soil environment is a real threat to earthworms (Olujimi et al., 2020).

3. Effect of emerging contaminants on earthworms' gut microbiome

The microbiome of invertebrates plays a crucial role in a number of host life processes. Recent research has linked it to a variety of functions, such as aiding in digestion and nutrition, providing resistance to invading pathogens, maintaining intestinal homeostasis, assisting in dealing with abiotic factors, and even performing neurobehavioral functions (Cao et al., 2022; Ding et al., 2019; Kelly and Mulder, 2012; Liberti et al., 2021; Wang and Kasper, 2014). Earthworms' digestive tract is a microhabitat with significantly less oxygen but a higher carbon and nitrogen content than the surrounding soil environment, making it more favorable for microbial colonization of certain genera (Pass, 2015; Sapkota et al., 2020). It contains both transient soil microorganisms and microorganisms intrinsically associated with the host with diverse phylogenetic relationships and functions (Sapkota et al., 2020; Sun et al., 2020). Earlier studies of these microorganisms were based on classical cultivation and microscopy techniques, whereas researchers have been able to overcome the limitations of conventional techniques and profile microbial communities from macroenvironments and even microenvironments, such as the earthworm gut, with a high degree of taxonomic resolution, using metagenomic techniques (Cao et al., 2022; Hong et al., 2011; Owagboriaye et al., 2021; Procházková et al., 2013; Zhao et al., 2022). However, studies of the gut microbiome have solely focused on bacteria, ignoring the large proportion of eukaryotes, such as fungi, protists, and even small metazoans, that comprise the earthworm's diet (Sapkota et al., 2020). These microeukaryotes and bacteria occupy vastly different positions in the trophic network and are consequently affected differently by the environment within the earthworm gut when exposed to different contaminants.

In microbiome research, it is crucial to describe the patterns of diversity, how they are influenced by interkingdom interactions, and how the host influences these community patterns. Numerous studies have demonstrated that emerging pollutants can induce dysbiosis of the gut microbiota (Krishnaswamy et al., 2021; Swart et al., 2020b; Yang et al., 2022b; Zhao et al., 2022). As earthworms were reported to acquire ECs through soil ingestion and given the persistence and pervasiveness of these pollutants in the environment, concern has gradually grown regarding the effects of diverse categories of emerging pollutants on the earthworm gut microbiota, and thereby the host health. Numerous researchers have reported the effects of ECs on earthworm gut microbiota (Table 12.2), but there is still a paucity of data

TABLE 12.2 Impact of a variety of emerging contaminants on earthworms' gut microbiota.

Emerging contaminant		Concentration	Earthworm species	Gut microbial dysbiosis	References
Pharmaceuticals	Sulfamethoxazole and/or As(V)	Up to 10 mg kg^{-1} DW soil Up to 100 mg kg^{-1} DW soil	*Metaphire sieboldi*	▲Proteobacteria and Bacteroidetes (*Cytophagia*) ▼Firmicutes, Verrucomicrobia, and Aeromonadaceae	Wang et al. (2019a)
	Polymyxin B	0.01–100 mg kg^{-1} DW soil	*Metaphire guillelmi*	▲Planctomycetota and Dependentiae ▲*Escherichia-Shigella* and *Acinetobacter* ▼Pseudomonas, SH-PL14, Mesorhizobium, *Ralstonia*, *Bacillus*, *Candidatus_* Lumbricincola, *and Aeromona*	Zhao et al. (2022)
	Triclosan	Up to 1000 mg kg^{-1} DW soil	*Eisenia fetida*	▲*Pseudomonas, Stenotrophomonas, and Achromobacter* ▼*Bacillus, Balneimonas, Cupriavidus, Hylemonella, Kaistobacter, Lysobacter, Thermomonas, Flavihumibacter, Flavisolibacter, and Flamella* (at 10 mg kg^{-1}) ▼*Janthinobacterium*, *Arthrobacter*, *Phenylobacterium*, and *Streptomyces* (at 200 mg kg^{-1})	Ma et al. (2017)
Agrochemicals	Fomesafen	40 mg kg^{-1} DW soil	*Pheretima guillelmi*	▲Blastococcus, Nocardioides, Gaiella and Arthrobacter ▼*Bacillus*, *Microvirga*, and *Ralstonia*	Chang et al. (2021)
	Glyphosate-based herbicide	7.20 g L^{-1} sprayed 2 consecutive days	*Alma millsoni* *Eudrilus eugeniae* *Libyodrilus violaceus*	▲ *Enterobacter*, *Pantoea* and *Pseudomonas* ▼*Spirochetes*, *Planctomycetes*, *Actinobacteria* and *Verrucomicrobia*	Owagboriaye et al. (2021)
	Chlorpyrifos	100, 250, 500, 750, and 1000 mg kg^{-1} DW	*Eudrilus euginae*	▲ *Bacillus*, *Pseudomonas*, *Talaromyces*, *Trichoderma*, and *Rhodotrula* ▼*Clostridium*, *Clavispora*, *Penicillium*, and *Aspergillus*	Krishnaswamy et al. (2021)
Microplastics	Microplastics (MPs) and tris (2-chloroethyl) phosphate (TCEP)	MPs: 500 mg kg^{-1} DW TCEP: 1 mg kg^{-1} DW	*Eisenia fetida*	▲*Aquabacterium* and *Verminephrobacter* ▼the ratio of Firmicutes to Bacteroidetes and *Bradyrhizobium*	Cao et al. (2022)

(*Continued*)

TABLE 12.2 Impact of a variety of emerging contaminants on earthworms' gut microbiota.—cont'd

Emerging contaminant		Concentration	Earthworm species	Gut microbial dysbiosis	References
	High-density polyethylene (25 μm) or polypropylene (13 μm)	0.25% (w/w)	*Metaphire guillelmi*	No significant change	Cheng et al. (2021)
	Polyvinyl chloride particles	2000 mg kg^{-1} DW	*Metaphire californica*	No significant change	Wang et al. (2019b)
Metallic Nanoparticles	Zinc nanoparticle (ZnO)	1000 mg kg^{-1} DW soil	*Eisenia fetida*	▲*Verminephrobacter* and *Ochrobactrum*, ▼*Actinobacteria*, *Clostridium*, *Bacillus*, and *Pseudomonas*	Yausheva et al. (2016)
	Copper oxide nanoparticles	160 mg kg^{-1} DW soil	*Eisenia fetida*	▼*Candidatus* Lumbricinola and *Luteolibacter*	Swart et al. (2020b)
	Copper and silver nanoparticles	0, 10, 26, 64, 160, and 400 mg CuO-NPs or Ag NP kg^{-1} DW soil	*Eisenia fetida*	No significant change	Swart et al. (2020a)
	Silver sulfide nanoparticles	114, 3420, and 34,200 mg Ag kg^{-1} DW soil	*Eisenia fetida* *Pontoscolex corethrurus*	▼Denitrifiers ▼Nitrification- and denitrification-related genes: *nxrB*, *napA*, *nirS*, and *nosZ*	Wu et al. (2020)

because many EC categories have been poorly investigated. Wang et al., (2019a) investigated the effects of the broad-spectrum antibiotic sulfamethoxazole and arsenic on the gut microbial communities of the earthworm *Metaphire sieboldi*. In this study, both arsenic and sulfamethoxazole significantly altered the microbial communities of the earthworm gut, as the relative abundance of *Bacteroidetes* and *Proteobacteria* increased significantly. This increase in *Bacteroidetes* in the gut results from their ability to degrade sulfamethoxazole and further degrade its metabolic byproducts. Moreover, the relative abundance of the bacterial phylum *Proteobacteria*, a defining characteristic of microbial dysbiosis in the gut microbiota, was significantly increased indicating that both contaminants have disrupted the gut microbiota (Wang et al., 2019a). Polymyxin B (PMB) has received considerable attention as well for its use as a last-resort treatment for multidrug-resistant bacterial infections. Zhao et al. (2022) examined the influence of soil PMB residue on the microbiome and transcriptome of earthworms (*Metaphire guillelmi*). PMB was found to significantly alter the community structure and taxonomic composition of the earthworm gut even at concentrations as low as 0.1 mg kg^{-1}. In the active gut microbiota, the relative abundances of *Bacillus*, *Candidatus* Lumbricincola, and *Aeromonas* decreased and were found to be sensitive to PMB in the earthworm gut, while the relative abundances of *Escherichia-Shigella* and *Acinetobacter* increased significantly (Zhao et al., 2022). Furthermore, bacteria were found to be more susceptible to the negative effects of TCS than eukaryotic microbes in the microbial community of the earthworm gut (Ma et al., 2017). Intestinal populations of *Pseudomonas*, *Stenotrophomonas*, and *Achromobacter* were increased by TCS exposure, despite TCS being a broad-spectrum antimicrobial. These resistant intestinal bacteria may contain TCS-resistant genes or produce TCS-degrading enzymes; consequently, they may be able to aid in the remediation of TCS-contaminated soils (Ma et al., 2017). Lin et al. (2021) examined the effects of epigeic *E. fetida* and endogeic *Amynthas robustus* on tetracycline degradation and reported that both earthworms significantly sped up tetracycline degradation and stimulated soil-indigenous tetracycline degraders. *Pseudomonas* and *Arthrobacter* had relatively higher abundance in earthworm intestines among the gut microbial community; they are tetracycline degraders that can be released into soils and accelerate tetracycline degradation primarily via the epimerization−dehydration pathway (Lin et al., 2021).

Accordingly, the gut microbial community of earthworms has been proven to be susceptible to changes when exposed to pesticides and herbicides. These ECs of great concern may significantly alter the diversity of gut microorganisms, but the changes in bacterial phyla appear to be unique to each contaminant. Chang et al. (2021) investigated the effects of the herbicide fomesafen on the gut bacterial microbiota of the endogenous earthworm *Pheretima guillemini*. Exposure to fomesafen significantly inhibited the bacterial diversity in the earthworm guts as taxon-based analyses revealed that exposure to fomesafen altered the relative abundance of the predominant bacterial taxa in earthworms' digestive tracts to a greater degree than in soil and cast samples. Fomesafen exposure resulted in a decrease in *Bacillus* (belonging to the phylum Firmicutes), *Microvirga*, and *Ralstonia* (both belonging to Proteobacteria) and an increase in *Blastococcus*, *Nocardioides*, *Gaiella*, and *Arthrobacter*, all of which belong to the phylum Actinobacteria. Therefore, the rise in the abundance of the four genera belonging to the phylum Actinobacteria could emphasize their potential to remove pesticides among other organic pollutants, by utilizing these substances as energy resources (Mawang et al., 2021; Tarfeen et al., 2022). Owagboriaye et al. (2021) examined the effects of a glyphosate-based herbicide (GBH) on the microbiome of earthworms. Exposure to the GBH significantly increased the relative abundance of *Proteobacteria*, which became the dominant phylum in the three earthworm species studied (95% *Alma millson*, 93% *Eudrilus eugeniae*, and 88% *Libyodrilus violaceus*). In contrast, members of the phyla *Spirochetes*, *Planctomycetes*, *Actinobacteria*, and *Verrucomicrobia* were nearly absent in GBH-exposed earthworms. An increase in the abundance of four species in the gut microbiome of GBH-exposed earthworms: *Enterobacter* spp., *Pseudomonas putida*, *Pantoea agglomerans*, and *Pseudomonas taiwanensis* was also reported, which might indicate their ability to use glyphosate as an energy source (Owagboriaye et al., 2021). In addition, earthworms were found to stimulate indigenous pentachlorophenol (PCP) bacterial degraders and to increase the amount of the PCP-4-monooxygenase gene (Lin et al., 2016), thereby overcoming the high toxicity of this extensively used wood preservative, herbicide, pesticide, and broad-spectrum biocide (Fukushima and Tatsumi, 2007; Zheng et al., 2011). The effects of the pesticide chlorpyrifos (CPF) on the entire gut microbiome of *E. euginae* were examined using long amplicon nanopore sequencing (Krishnaswamy et al., 2021). Results revealed that while the fungal population increased, the gut bacterial population decreased, demonstrating gut fungi's stability or resistance to the pesticide. On exposure to CPF, there were serious alterations in the established basal microbial structure, allowing other microbial genera to take over. *Clostridium* was sensitive to CPF, but there was a significant increase in the abundance of *Bacillus* and *Pseudomonas* due to their ability to degrade organophosphorus pesticides, including CPF and its hydrolyzed products, and use them as a source of carbon and nitrogen. *Ascomycota* and *Basidiomycota* predominated the fungi before and after CPF exposure, with no discernible difference in fungal diversity. The CPF inhibited the naturally dominant genus of fungi, *Clavispora,* along with *Penicillium* and *Aspergillus*. However, the genera *Talaromyces*, *Trichoderma*, and *Rhodotrula* were found to dominate the earthworm gut after exposure to CPF, suggesting their potential role in the degradation of organophosphorus pesticides, specifically CPF (Krishnaswamy et al., 2021).

MPs have been shown to reduce the accumulation of metals (Ni and Pb) by earthworms, altering the gut bacterial communities, diversity, and correlation of different bacteria in the earthworm gut (Yang et al., 2022b). Cao et al. (2022) investigated the effects of coexposure to MPs and TCEP in agricultural soil on the gut microbiota of earthworms. Exposure to both contaminants decreased the ratio of Firmicutes to Bacteroidetes and the relative abundance of Rhizobiales genera (*Bradyrhizobium*) associated with nitrogen fixation and organic matter decomposition, which was attributable to a diminished TCEP degradation capacity (Cao et al., 2022). Notwithstanding, the exposure of earthworms *Metaphire guillelmi* to soil amended with polypropylene MPs solely did not induce gut microbiota dysbiosis, despite the significant differences between the microbiota in the *M. guillelmi* gut and the surrounding soil (Cheng et al., 2021). This was consistent with the effects of MPs on the bacterial communities in the digestive tract of earthworms (*Metaphire californica)* (Wang et al., 2019c). In fact, Wang et al. (2022b) reported that MPs mitigated the effect of arsenic on gut microbiota by adsorbing/binding As(V) and decreasing its bioavailability.

As an emerging pollutant in the terrestrial ecosystem, the effects of NMs on the gut microbiota of terrestrial organisms have been the subject of few studies. Yausheva et al. (2016) investigated the effect of zinc nanoparticles on the composition of *E. fetida*'s gut microflora. The normal gut microflora of the earthworm had a high diversity of microorganisms, the majority of which belonged to the taxa Firmicutes, Proteobacteria, and Actinobacteria and were represented by numerous species of the genera *Clostridium*, *Pseudomonas*, *Bacillus*, *Cellulomonas*, and other numerically smaller genera. However, exposure to zinc nanoparticles reduced bacterial diversity and the proportion of bacteria belonging to the taxon Firmicutes while increasing the proportion of Proteobacteria due to the abundance of the gut symbionts *Verminephrobacter* and *Ochrobactrum*. This significant increase was reported to be a protective mechanism against high doses of toxic nanoparticles that contributed to the survival of both the worm and its symbionts (Yausheva et al., 2016). Besides, the exposure to the biocidal CuO nanoparticles was shown to cause a shift in the gut microbiome of earthworms, as the earthworm-exclusive symbiont "*Candidatus* Lumbricincola" was negatively impacted by copper forms, which may affect earthworms' normal health and function (Swart et al., 2020a). Despite this, the microbiome of the earthworm gut was largely resistant to CuO NPs and Ag NPs (Swart et al., 2020b). In their study, Wu et al. (2020) sought to determine the effects of Ag NPs on the microbial community of earthworms, specifically denitrifiers, when added to soil at an environmentally relevant concentration. It was discovered that Ag_2S NPs reduce the copy number of the 16S rRNA gene and the relative abundance of denitrifiers in the gut of earthworms. In addition, two nitrification genes (bacterial *amoA* and *nxrB*) and four denitrification genes (*narG*, *napA*, *nirS*, and *nosZ*) were quantified in the earthworm's gut, and the Ag_2S NPs significantly reduced both nitrification and denitrification gene copy numbers in the earthworm's gut, with a concomitant reduction in N_2O emissions (Wu et al., 2020).

4. Bioremediation of emerging contaminants using intestinal microbial isolates

The abundance of nutrients and the diversity of microorganisms in the earthworm gut are conducive to the transformation of various soil-ingested pollutants, such as metals, organic pollutants, MPs, antibiotics, etc. In fact, the earthworm digestive tract and its microbial community may play a role in pollutant detoxification via biodegradation, absorption, transformation, and bioaccumulation (Bhat et al., 2017, 2018; Sun et al., 2020). Based on degradation characteristics, Singh et al. (2015) classified the bacterial community in the intestines of *E. fetida* and *P. excavatus* into the following functional groups: dehalogenation (22.2%), chlorophenol (3.7%), and aromatic hydrocarbon degraders (1.1%) (Sun et al., 2020). Despite the growing interest in earthworms in recent years, little is known about the diversity and function of intestinal bacteria and their correlation with pollutant detoxification.

A variety of pharmaceutical drugs have been reported to be degraded and thus detoxified by microorganisms from various ecophysiologic niches. According to the limited information available, the main species involved in this degradation were *Flavobacteria*, *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Planctomycetota*, and other β-lactamase enzyme-producing bacteria in the soil (Kayal and Mandal, 2022; Singh and Saluja, 2021; Wang and Wang, 2018). Therefore, the increased abundance of these genera in the worm gut after antibiotic exposure might well be directly related to their bioremediation potential. For instance, Chao et al. (2020) assessed the sensitivity of the intestinal bacterial community of *Metaphire guillelmi* to tetracycline exposure and found that the abundance of the phyla Firmicutes and Planctomycetes was positively correlated with tetracycline concentration. The substantial increase in

Planctomycetes bacteria is likely linked to their role in the conversion of ammonium to nitrogen in the earthworm gut; in contrast, the ubiquitous presence of the tetracycline resistance genes *tetC*, *tetM*, and *tetW* in the Firmicutes bacteria (Chao et al., 2019) may have contributed to the increase in the abundance of the Firmicutes phylum in the earthworm gut. In addition, Yin et al. (2021) revealed that after earthworms (*Metaphire guillelmi*) fed on tetracycline-contaminated soil, the relative abundance of *Paracoccus*, *Singulisphaera*, *Acinetobacter*, and *Bacillus* increased significantly and became the dominant bacteria during tetracycline degradation, indicating that these tetracycline-resistant bacteria may influence the degradation of this emerging pollutant (Yin et al., 2021). In a metagenomic study conducted by Chao et al. (2021), intestinal bacteria of earthworms (*Metaphire guillem*) were found to contain a massive number of tetracycline-degrading genes (dehydrogenase genes *adh*, *ETFDH*, and *gpr*, etc.) and to have a strong ability to degrade tetracycline via dehydrogenation (Chao et al., 2021). While examining the contribution of earthworms to tetracycline biodegradation, Zheng et al. (2022) found that the metabolic pathways associated with xenobiotic degradation in the intestines were enriched. In fact, tetracycline degradation–associated genes such as glutathione S-transferases, peroxidases, and superoxide dismutases from the intestinal bacteria of earthworms in the polluted area were linked to tetracycline biodegradation. In addition, the abundance of glutathione S-transferases in the intestines and their close association with tetracycline degradation may shed light on their essential role in intestinal tetracycline biodegradation (Zheng et al., 2022). Zhang et al. (2022d) examined earthworm (*Pheretima guillelmi*) intestinal bacteria that are responsible for the degradation of sulfamethoxazole, one of the most widely used antibiotics, and discovered that earthworms significantly increased sulfamethoxazole degradation through intestinal detoxification with potential degraders belonging to the genera *Microvirga*, *Sphingomonas*, *Methylobacterium*, *Bacillus*, and *Tumebacillus* (Zhang et al., 2022d).

Furthermore, earthworm intestinal bacteria have been involved in the degradation of organic contaminants. Pesticide-degrading bacteria were previously isolated from the digestive tracts of various earthworms, such as *Rhodococcus* and *Bacillus* strains isolated by Verma et al. (2011) and Mudziwapasi et al. (2016) from the earthworm's gut involved in the degradation of endosulfan and dichlorodiphenyltrichloroethane (DDT), respectively. The *Rhodococcus* strain degraded up to 97.23% of endosulfan without producing any toxic metabolites within 15 days, and this degradation was mediated by genes whose expression was concentration dependent (Verma et al., 2011). Additionally, bacterial isolates *Rhodococcus* and *Bacillus* isolated from the gut of *E. fetida* exhibited 88.36% and 85.22% DDT degradation, respectively (Mudziwapasi et al., 2016). Moreover, Lin et al. (2016) found that the intestinal flora and digestive enzymes in the gut of endogeic earthworms degraded the ingested pentachlorophenol present in the contaminated soil as feeding on decayed organic matter and soil particles increased pentachlorophenol bioavailability through soil aeration and intestinal digestion (Lin et al., 2016). Besides, through the production of an extracellular nicosulfuron-degrading enzyme, the *E. fetida* gut isolate, *Bacillus velezensis* CF57, was reported to efficiently degrade a high concentration of nicosulfuron, a commonly used herbicide to protect maize crops, along with a broad spectrum of other sulfonylurea herbicides (Zhang et al., 2020a). On the other hand,Zenteno-Rojas et al. (2019) evaluated the ability of symbiotic bacteria isolated from the digestive tracts of earthworms to degrade high concentrations of the persistent organic pollutant decachlorobiphenyl and found that all of the gut isolates were able to remove decachlorobiphenyl at varying concentrations, with the highest removal recorded by the isolated strain *Pseudomonas extremaustralis* ADA-5, removing 219.7 mg L^{-1} of decachlorobiphenyl from an initial concentration of 1500 mg L^{-1}.

Nevertheless, compared with organic pollutants, the implication of earthworm gut microbiota on the removal of other EC categories such as MPs and industrial pollutants has scarcely been studied. For instance, since low-density polyethylene (LDPE) microplastic decay was found to increase after passing through the earthworm *Lumbricus terrestris*, Huerta Lwanga et al. (2018) investigated the role of earthworm gut bacteria in the degradation of MPs. A bacterial consortium, belonging to phylum Actinobacteria and Firmicutes, isolated from the gut of earthworms was reported to reduce the size of LDPE-MP within 4 weeks, resulting in the emission of volatile by-products including octadecane, eicosane, docosane, and tricosane (Huerta Lwanga et al., 2018). A recent study by Zhang et al. (2023) investigated the biologic responses of earthworms' microbiota to the coexposure to nano zero-valent iron (nZVI) and polychlorinated biphenyls (PCBs) as both earthworms and nZVI have recently been viewed as important approaches in soil remediation of PCBs. On the basis of integrated metabolomic and 16S rRNA analysis, it was determined that earthworms provided specific metabolites, including S-(2-hydroxyethyl)glutathione, 16-hydroxypalmitic acid, and formamide, which played a key role in maintaining the gut microbial community's defense against nZVI and PCBs coexposure and were beneficial to PCB-degrading microbiota (*Novosphingobium* and *Achromobacter*). Moreover, Shin et al. (2005) investigated the anaerobic biodegradation of four dinitrotoluene isomers, frequently used as solvents and as intermediates in the production of dyes, explosives, and pesticides, using the earthworm-isolated *Lactococcus lactis* subsp. *lactis* strain 27. Under anaerobic conditions, the earthworm gut isolate was found to convert four different dinitrotoluenes into their corresponding aminonitrotoluenes, most likely via hydroxylaminonitrotoluenes (Shin et al., 2005).

5. Outlook and conclusions

Soil contamination is widespread, and the rapid expansion of the chemical industry and the development of new applications every day are responsible for the introduction of a large number of new chemicals into soils around the world, thereby altering the environmental quality. In fact, only recently, as a result of the ongoing development of analytical techniques, have detectable levels of a wide variety of chemical substances begun to be detected in soils, while many others are anticipated to pose a problem for future generations. Globally, it is acknowledged that soils encounter serious threats, but until now, these threats have not been reflected in the development of effective regulations to protect soil health and sustainability.

The majority of the studies reviewed in this chapter have examined earthworm responses to a single EC, whereas, in nature, ECs typically coexist in complex mixtures. Future research should investigate the role of sublethal EC mixtures in earthworm responses in greater detail. In addition, environmental factors may play a significant role in the sensitivity of earthworms to pollutants; due to the complexity of soil characteristics, vegetation, and microbial community factors, assessments of the multiple interactions with earthworms' responses to ECs are required through field investigations. Furthermore, it is critical to investigate what happens when ECs come into contact with earthworm gut microbiota, as this can help us understand the biotoxicity of exogenous compounds and reveal new defense strategies used by earthworms, as well as overall earthworm health. However, because the ecotoxicologic effects of many categories of ECs on earthworm gut microbial communities are still poorly understood, more studies are needed to understand their negative effects, which may directly affect earthworm fitness and, as a result, their performance as a key contributor to soil health and fertility.

Even though scientists are becoming more aware of the importance of pollutant-detoxifying microorganisms in the earthworm gut for EC transformation, few studies have been published to date. Therefore, novel sequencing techniques and bioinformatics tools are essential for analyzing the whole-genome data of earthworm intestinal bacteria and other poorly characterized microorganisms. While the majority of research on earthworm bioremediation and the possible contribution of their gut microbiota is based on in vitro and petri dish experiments, more field studies are needed to understand the various interactions under natural conditions.

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CHAPTER

13

Influence of earthworm combined with bermudagrass on the content and bioavailability of heavy metal in reclaimed soil

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1. Introduction

Coal gangue filling and reclamation are essential methods to remediate the mining subsidence area. However, the coal gangue-filled reclaimed soil is so barren that it cannot support plant growth. Nevertheless, the reclaimed soil is always polluted by the heavy metals released by the coal gangue filled at the bottom. Therefore, the quantity and quality of the

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corps grown in the overlying soil were often low and poor. As a result, it is important to establish a sustainable and efficient soil bioremediation system for the reclaimed soil filled with coal gangue (Bi et al., 2021). Previous works used various restoration methods to improve soil nutrients in reclaimed soil and reduce the damage of heavy metals to soil plants. For example, Zhao et al. (2014) used six vegetation restoration modes improving soil nutrients in a coal mining area. Wu et al. (2019) combined soil conditioners with planting *Pennisetum* significantly, which improved soil pH, organic matter, available phosphorus, and available potassium content in mining soils. Mahohi and Raiesi (2021) inoculated bermudagrass with earthworm and arbuscular mycorrhiza in polluted soil, which promoted the enrichment and uptake of Pb by the plant.

Earthworms can improve physical, chemical, and biologic properties in various soils (Cao et al., 2015). They can degrade soil organic matter and increase soil fertility. In addition, earthworms can also directly enrich heavy metals in the soil through their epidermis and intestinal tract (Becquer et al., 2005). They can indirectly change the mobility and bioavailability of heavy metals by affecting the microorganisms in the soil. As a pioneer plant commonly used in ecologic restoration (Ye et al., 2021; Liu et al., 2021), bermudagrass can effectively improve the compacted soil structure in mining areas (Stumpf et al., 2018). It has evolved a dense root system that can efficiently absorb the heavy metals (Xie et al., 2021). Although the plants can improve the soil and enrich the heavy metals in the organ, the efficiency of this mechanism is relatively low, which limits the development of phytoremediation. Earthworms can indirectly affect the remediation efficiency of heavy metals by plants via improving the bioavailability of heavy metals in the arable soil (Liu et al., 2007), but little is known about the combination of earthworms and plants on the soil nutrition and heavy metals in gangue-filled reclaimed soils. Therefore, in this study, the earthworm and bermudagrass were adopted in the reclaimed soil filled with coal gangue. And the aim of this research was as follows: 1) the variation of the physical and chemical properties of the reclaimed soil inoculated with earthworms and plants; 2) the distribution and bioavailability of heavy metals modified by the combination of earthworms and plants in the reclaimed soil; and 3) the change mechanism of heavy metals behavior in the improved reclaimed soil.

2. Materials and methods

2.1 Substrate and earthworm

Coal gangue and soil were collected from a coal gangue pile and the surrounding grassland in Huainan City, Anhui Province. The sampling depth of coal gangue and soil was 0–20 cm, and the gangue and soil were air-dried and passed through a 2-mm sieve before mixing and being used for the soil column. Bermudagrass seeds were purchased from a particular agricultural company. The earthworms (*Eisenia fetida*) were collected from a breeding base. The basic properties of coal gangue and soil are shown in Table 13.1.

TABLE 13.1 The basic physical and chemical properties of soil and coal gangue.

Index	pH	OM (%)	TN ($mg \cdot kg^{-1}$)	TP ($mg \cdot kg^{-1}$)	TK ($g \cdot kg^{-1}$)	Total Cr ($mg \cdot kg^{-1}$)	Total Cu ($mg \cdot kg^{-1}$)	Total Pb ($mg \cdot kg^{-1}$)	Total Zn ($mg \cdot kg^{-1}$)
Soil	7.51 ± 0.13	3.15 ± 0.21	400.33 ± 33.21	465.71 ± 31.51	9.12 ± 0.91	70.24 ± 1.61	39.44 ± 0.38	25.24 ± 0.21	49.19 ± 1.89
Gangue	8.90 ± 0.21	6.93 ± 0.52	2169.6 ± 127.81	1106.59 ± 153.23	31.32 ± 89.02	90.94 ± 6.45	193.00 ± 9.97	29.21 ± 2.28	117.54 ± 8.12

2.2 Experimental design

According to the actual requirements of coal gangue-filled reclaimed soil (covering soil thickness > 50 cm) and previous studies (Xu et al., 2014, 2016), two types of covering soil thickness were set in this experiment: L group (low covering soil thickness of 50 cm) and H group (high covering soil thickness of 70 cm). Four treatments were arranged in each group: CK (no earthworm and plant inoculation), C (bermudagrass inoculation), E (earthworm inoculation), and CE (earthworm and plant inoculation). Each treatment was replicated three times. The soil column container is a plastic tube with an inner diameter of 10 cm. The thickness of the gangue at the bottom is 10 cm. The covering soil on the gangue deposited naturally. The edge of soil column was 10 cm higher than the covering soil to prevent the earthworms from escaping (Fig. 13.1). 30 bermudagrass seeds were planted in both the C and CE treatments in two groups, and the seedlings were thinned to 10 copies in each soil column after 7 days. The soil column was cultivated on the illumination shelf. During cultivation, the soil moisture was maintained at 65% ± 3% humidity with the addition of deionized water. The temperature was kept under the natural condition. 10 earthworms with similar weight and maturity were inoculated in the E and CE treatments when thinning the seedlings. After culturing for 40 days, the soil, earthworms, and plants were picked out from the column, and soil samples were evenly mixed for testing. The average survival rate of the earthworms was 92%.

2.3 Analytical method

Soil pH was determined by using pH meter (PHS-2C, China). OM was measured by potassium sulfate dichromate-external heating method. TN and TP were analyzed by semimicro Kjeldahl method and molybdenum-antimony anticolorimetric method, respectively. The total heavy metals were determined by the method of HF-HNO_3-$HClO_4$. The bioavailability of heavy metals was determined by using the diethylene-triaminepenta acetic acid extraction method. The heavy metal and potassium concentrations of all extracts were measured by ICP-OES (PerkinElmer, Optima 2100 DV). The national standard material (GBW07403 [GSS-3]) was adopted for quality control, and the recovery rate of each element was within a reasonable range (96.6%–109.1%).

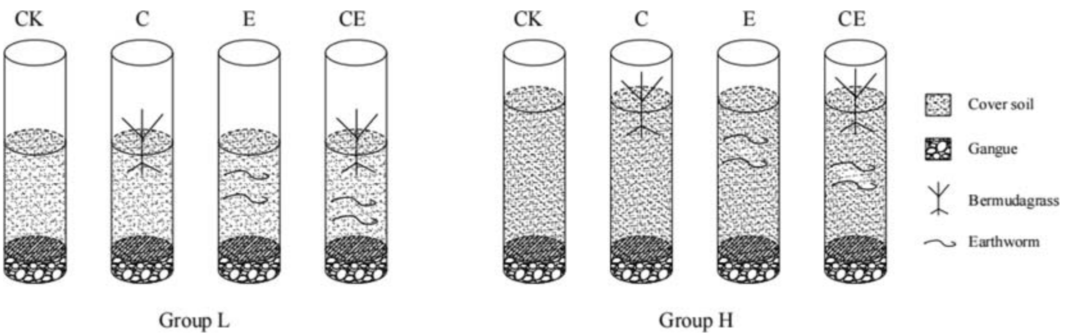

FIGURE 13.1 Schematic diagram of the soil column experiment.

2.4 Statistical analysis

All statistical analyses were conducted in triplicate. The analysis of variance, Duncan's multiple range test, and regression analysis were performed using SPSS version 18. Findings were regarded as significant at $P < .05$.

3. Results and discussion

3.1 Effects of earthworm and bermudagrass on the physicochemical properties of reclaimed soil

From Fig. 13.2, the soil pH under two covering soil thickness was all neutral to slightly alkaline. The soil pH in C, E, and CE treatments from L group was significantly lower than that of CK ($P < .05$) and following the decreasing order of CK > E > C > CE; while in H group, soil pH values in C and CE treatments were lower compared with CK. Soil OM in C, E, and CE was significantly higher than that of the CK ($P < .05$), while there was no significant difference between the treatments C, E, and CE. Peak values of soil OM in L and H group were found in CE compared with CK, and soil OM followed the order of CE > C > E > CK. TN in C, E, and CE from L group and those in C and CE from H group were higher than in CK ($P < .05$), and both TN in CE from the different groups showed the maximum values, which were 51.26% and 56.03% higher than CK. Higher TP values were observed only in CE from the two groups compared with CK, which were 46.15% and 50.71% higher than the control. TK was not significant in different treatments under different groups ($P > .05$). Therefore, inoculation of earthworm or bermudagrass or earthworm and bermudagrass can promote soil nutrition. The combination of earthworm and bermudagrass showed much better ameliorative effects in the reclaimed soil.

The restoration of soil physicochemical properties is significant for the growth of plants in reclaimed soil. Wang et al. (2020a,b) reported that inoculating earthworms with plants could reduce soil pH. Hu et al. (2021) observed that inoculating earthworms with alfalfa could significantly increase soil OM content, and Chen et al. (2020) also proved that bermudagrass could significantly increase TN and TP in soil. Similar results were also found in this study that C, E, and CE treatments decreased soil pH and increased OM, TN, and TP. The reason may be because the humic acid emitted by earthworms and the organic acids secreted by the roots of bermudagrass reduced soil pH as earthworms or bermudagrass inoculated alone (Macias-Benitez et al., 2020). The casts from earthworms and root exudates from the bermudagrass could promote the OM level (Wang, 2020). Earthworms could also increase the phosphatase activity in the soil, which indirectly affects the bioavailability of soil phosphorus, thereby enhancing the soil nutrition (Liu et al., 2003). The microorganisms in the gut of earthworms participate in soil digestion and denitrification by improving the decomposition ability of microorganisms in the soil, such as increasing the decomposition of humus, which increased the content of N and P in the soil (Cao et al., 2015; Ma et al., 2017). When earthworms and bermudagrass act in combination, in addition to the individual effects of

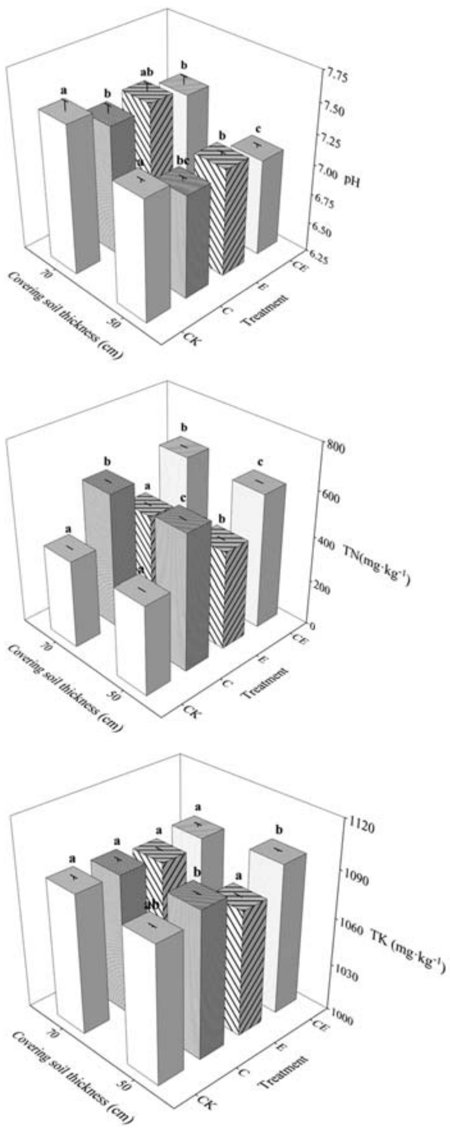

FIGURE 13.2 Effects of earthworm bermudagrass on the physicochemical properties of reclaimed soil. Different letters in the picture indicate that there are significant differences among different treatments in the same group ($P < .05$). * indicates that there are significant differences between the same treatments under different groups.

earthworms and bermudagrass, earthworms can promote the growth of plant roots, and bermudagrass can provide a suitable living environment for earthworms, and the synergistic effects of earthworms and bermudagrass are more effective in reducing soil pH and increasing soil nutrients.

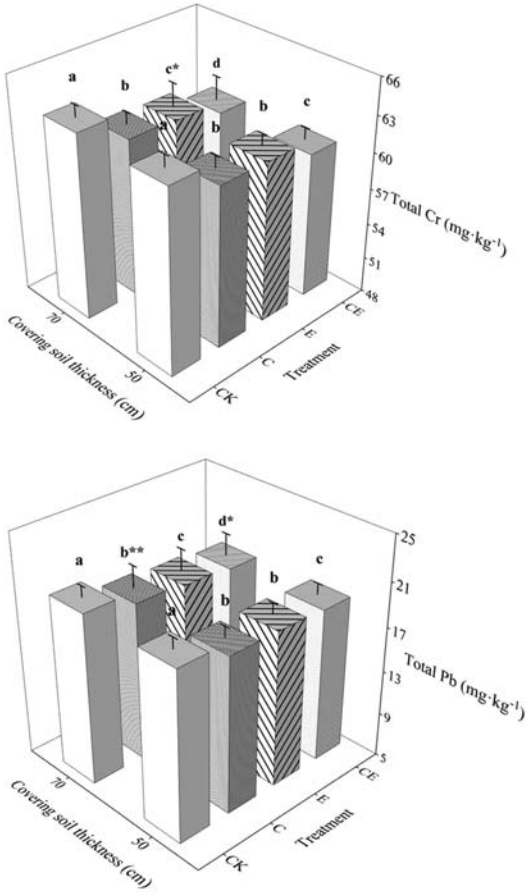

FIGURE 13.3 Effects of earthworm and bermudagrass on total heavy metals in reclaimed soil.

3.2 Effects of earthworm and bermudagrass on heavy metals in reclaimed soil

3.2.1 Effects of earthworm and bermudagrass on the total heavy metals in reclaimed soil

From Fig. 13.3, among the two types of covering soil thickness, the contents of Cr, Cu, Pb, and Zn in soil were significantly decreased in C, E, and CE treatments compared with CK ($P < .05$). The four heavy metals decreased the most in CE treatment under the different groups. Cr, Cu, Pb, and Zn content in CE treatment from the L group decreased by 4.99%, 12.82%, 6.96%, and 4.81% compared with CK, and those metals in the same treatment from H group decreased by 5.36%, 11.16%, 8.66%, and 7.04%. In addition, the contents of heavy metals in CE treatment were significantly lower than those in C and E treatments ($P < .05$), which indicated that the combination of earthworm and bermudagrass showed a more synergistic effect on reducing heavy metals than the individual effect. In comparison with different thicknesses, total Cr contents in C, E, and CE treatments from L group were higher than those values in the similar treatments in H group. Total Cu contents in CK and E treatments, total Pb contents in E and CE treatments, and total Zn contents in CK, C, E, and CE treatments from L group showed higher values than those values in the corresponding treatments in H group. These results demonstrated that total Cr, Cu, Pb, and Zn contents in E, C, and CE treatments in high soil thickness would be decreased further than those in thin soil thickness.

Heavy metals can severely interfere with the physiologic and ecologic status of the plant and hinder the usage of the reclaimed soil. Inoculation with earthworms and plants can reduce heavy metals contents in the soil. Wang et al. (2020a,b) found that the earthworm could promote the uptake of Cd and Zn by *Sedum plumbizincicola*, thus reducing the heavy metals contents in the soil. Lemtiri et al. (2016) observed that earthworms increased the enrichment of Cu, Zn, Pb, and Cd in broad beans and maize. This study also demonstrated that heavy metals in C, E, and CE treatments decreased under two soil thicknesses. The reason may be ascribed to that earthworms can absorb the heavy metals through the gut or epidermis (Lanno et al., 2004). The bermudagrass can enrich the heavy metals by its roots and can interact with interroot microorganisms via adsorption, precipitation, interroot complexation, or metal reduction (Wang et al., 2021a,b). Whereas the combination of earthworms and bermudagrass was more effective in decreasing the heavy metals contents in soil, the earthworm not only absorbed the heavy metals but also activated the metals easily taken up by the plant. Earthworms secrete signal substances, which can improve plant biomass and stress resistance and promote the absorption of heavy metals in the plant (Wang et al., 2021a,b; Bai et al., 2010). The growth of plants can also provide good living conditions for the microorganisms and create incentives for the migration and transformation of heavy metals. Therefore, the combination effect of earthworms and bermudagrass is better than that of inoculation of earthworms or bermudagrass alone in reducing the heavy metal content in reclaimed soil.

3.2.2 *Effects of earthworm and bermudagrass on the bioavailability of heavy metals in the reclaimed soil*

Available heavy metals are more easily absorbed and utilized by the plant (Li et al., 2019). As shown in Fig. 13.4, when inoculated with bermudagrass alone, the contents of available Cr, Cu, Pb, and Zn in the reclaimed soil under two coverings of soil thickness are lower than those of CK. Only available Pb decreased significantly in L group, and available Cr, Pb, and Zn decreased significantly in H group ($P < .05$). When inoculated with the earthworms alone, the available Cu and Zn increased significantly compared with CK in L group ($P < .05$), and the available Cr, Cu, and Pb increased significantly compared with CK in H group ($P < .05$). The available Cu, Pb, and Zn in CE were significantly lower than in CK from the two groups ($P < .05$), and available Cr in CE was found significantly lower only in H group ($P < .05$). Therefore, inoculation with earthworms promoted the availability of heavy metals in reclaimed soil, while inoculation with bermudagrass or bermudagrass and earthworms could decrease the heavy metals availability. Meanwhile, the available heavy metals in CE were lower than in C from the two groups, suggesting earthworms and plants could pose much more reduction effect for the heavy metals. The difference analysis demonstrated that the available Cr and Zn did not vary between the treatments in thin soil thickness, but the available Cr and Zn in the earthworm and plant treatment were

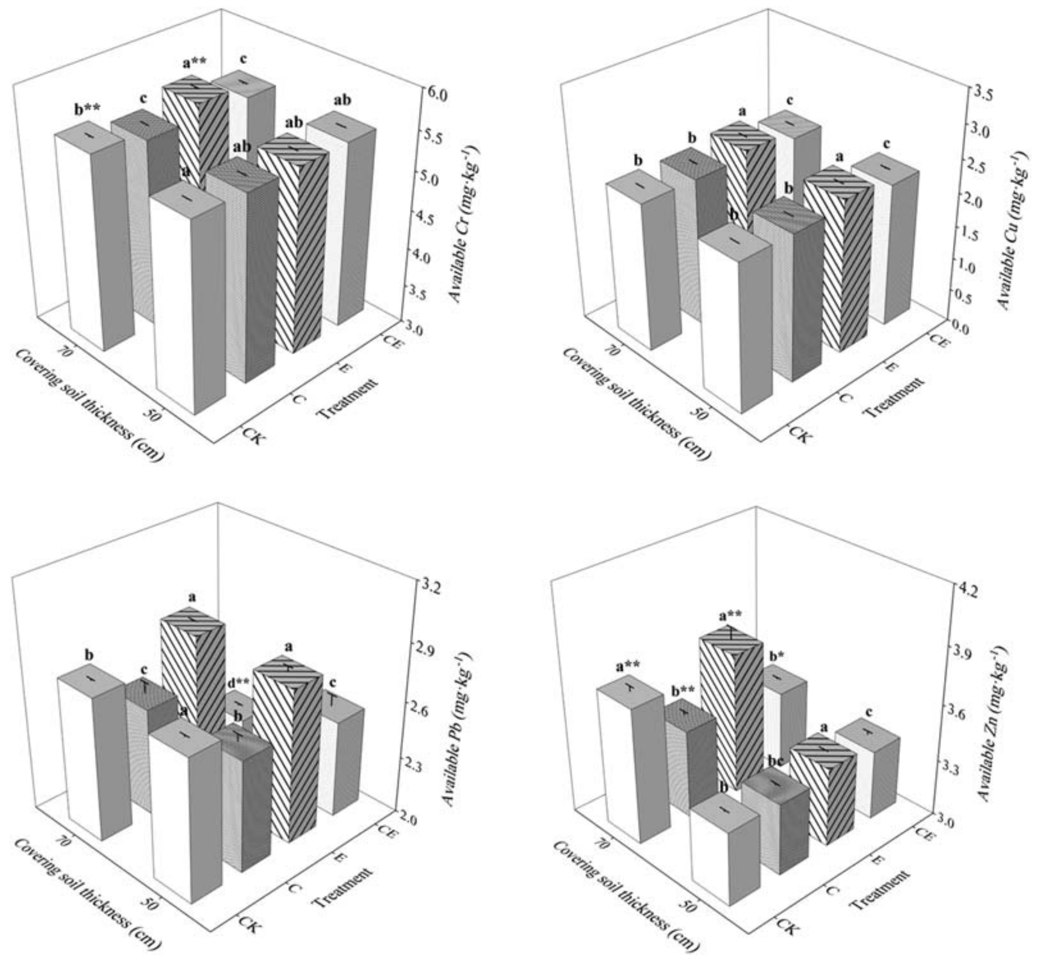

FIGURE 13.4 Effects of earthworms and bermudagrass on the availability of heavy metals in reclaimed soil.

decreased significantly compared with CK in the high soil thickness. Thereafter, the soil thickness could affect the remediation of the reclaimed soil. The application of earthworm and bermudagrass decreased the available Cr and Zn further in high soil thickness than in low soil thickness.

Inoculation with earthworms alone enhanced the heavy metals bioavailability in L and H group. This may be because heavy metals in earthworms can fully interact with OM, humus, and microorganisms through grinding, digestion, and excretion processes that facilitate the formation of heavy metal active substances, and earthworms secrete a large amount of mucus that directly or indirectly affects the properties of sludge, thus improving the bioavailability of heavy metals (Bai et al., 2010; Zhou et al., 2014). When bermudagrass is planted alone, the root can directly absorb the available heavy metals in the soil, and the plant cell wall can also combine the heavy metals (Yang et al., 2021), which reduces the availability of heavy metals.

As earthworms and bermudagrass are combined, the microorganisms discharged from earthworms promote the absorption of mineral nutrients by plants (Yang et al., 2021) and the absorption of available heavy metals by plants. The activities of plant root microorganisms also increases the enrichment of heavy metals by earthworms. Therefore, the combination of earthworm and bermudagrass is more potent in reducing the availability of heavy metals. In addition, the density of earthworms and plant roots in L group is higher than that in H group. The application of earthworms and bermudagrass was more effective in reducing the availability of heavy metals. The earthworms may selectively absorb available Cr and Zn. The combination of earthworms and bermudagrass therefore showed high potential in reducing available Cr and Zn in high-thickness soil.

3.2.3 Correlation between physical and chemical properties and heavy metals in reclaimed soil

The redundancy analysis of physical and chemical properties and heavy metals in reclaimed soil inoculated with bermudagrass or earthworm and bermudagrass is shown in Fig. 13.5. The results showed that planting bermudagrass accounted for 57.68% at one axis for physical and chemical properties and heavy metals in soil and 18.64% at a second axis. The total two axes accounted for 76.32% for heavy metals (Cr, Cu, Pb, Zn) variation. The combination of earthworms and bermudagrass accounted for 64.32% at one axis for physical and chemical properties and heavy metals in soil and 19.80% at the second axis. Total variation of 84.12% for heavy metals (Cr, Cu, Pb, Zn) was interpreted by the two axes. Therefore, two axes could reflect the effects of soil properties on the heavy metals in reclaimed soil. Actually, pH and OM affected the first axis in C and CE, which suggested that pH and OM were the main factors influencing the heavy metals in the soil. The relation between heavy metals and pH and OM in the treatments of inoculating bermudagrass or bermudagrass and earthworms was also observed in this research. When planting bermudagrass alone, pH was significantly and positively correlated with total Pb and available Zn but significantly and negatively correlated with total Zn ($P < .05$). OM was significantly and positively correlated with available Zn ($P < .05$). While inoculated with bermudagrass and earthworms, pH was significantly and positively correlated with available Zn but significantly and negatively correlated with total Pb, total Zn, and available Pb ($P < .05$). OM was significantly and positively correlated with total Zn and available Pb but significantly and negatively correlated with available Zn ($P < .05$). Meanwhile, after inoculation with earthworms, the relation between pH and total Cr, total Cu, available Pb, and available Zn increased, and the relation between OM and total Cu, total Zn, available Cr, available Pb, and available Zn increased. Therefore, inoculating with earthworms affected the pH and OM in the rhizosphere soil of bermudagrass. It also demonstrated that earthworms could affect the pH and OM, thus changing the heavy metals in the reclaimed soil.

The physical and chemical properties of soil can significantly affect the content of heavy metals. For example, Xie et al. (2019) found that pH was significantly negatively correlated with available Ni and Cd, and OM was significantly positively correlated with available Cu. Zhang et al. (2017) found that the pH of the soil in the mining area was significantly negatively correlated with total Cu, while OM was positively correlated with total Zn content. This study also observed that soil pH and OM had the most significant impacts on the contents of heavy metals in soil. Soil pH can affect the heavy metal form in soil, and OM can form

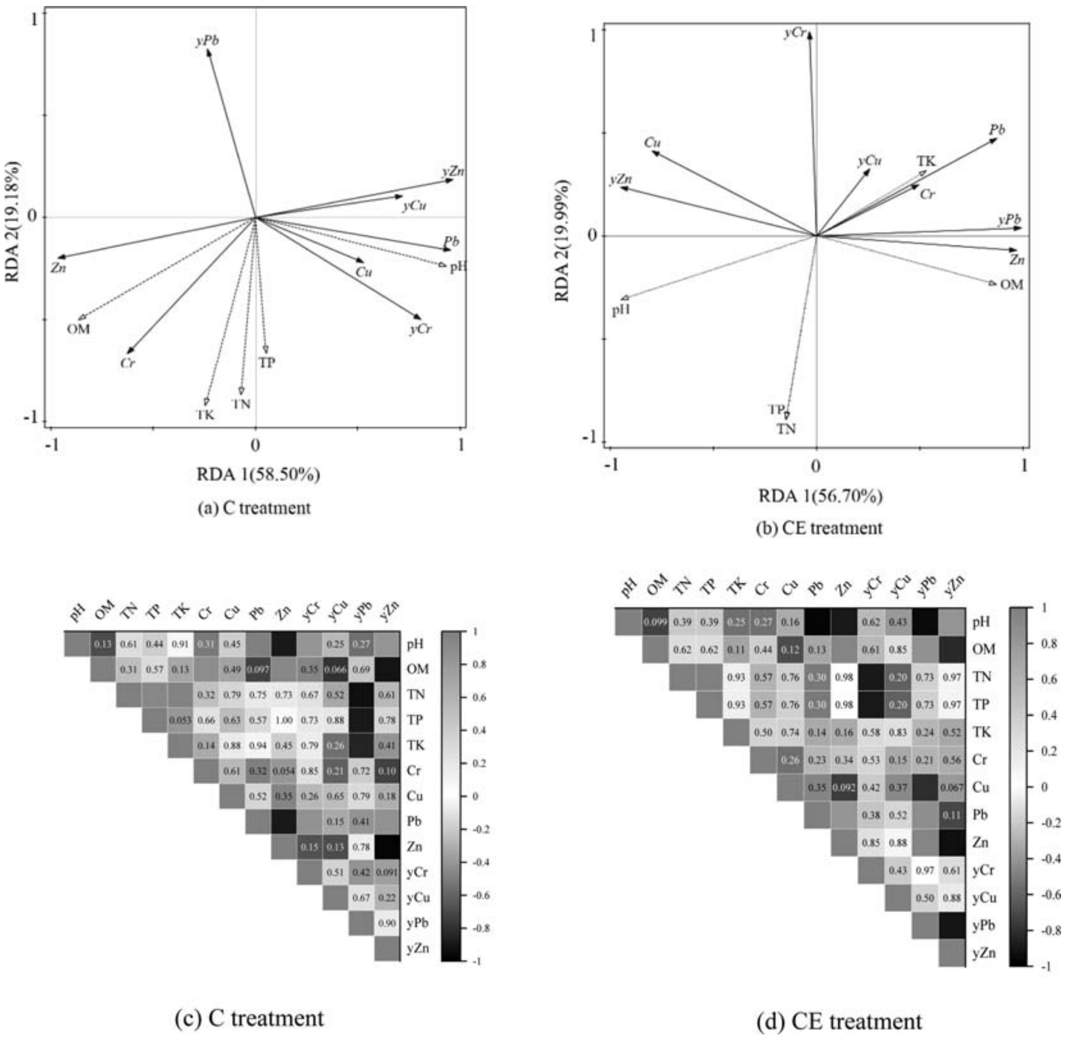

FIGURE 13.5 Redundancy analysis and correlation analysis of heavy metals and physical and chemical properties of reclaimed soil. *Note: yCr, yCu, yPb, and yZn represent available Cr, available Cu, available Pb, and available Zn, respectively. (A) and (B) are the redundancy analysis of heavy metals and physical and chemical properties of reclaimed soil when inoculated with bermudagrass or earthworms and bermudagrass. The red arrow represents environmental factors, and the black arrow represents heavy metals. (C) and (D) are the correlation analysis of heavy metals and physical and chemical properties of reclaimed soil when inoculated with bermudagrass or earthworms and bermudagrass. * represents a significant level of 0.05. The red parts in (C) and (D) represent a positive correlation, and the blue parts represent a negative one. The deeper the color is, the stronger is the trend.*

organic heavy metal complexes. Those mechanisms all reduced the heavy metals mobility in the soil (Caporale and Violante, 2016). After earthworms were inoculated with bermudagrass, heavy metals behavior in the reclaimed soil could be regulated by the modification of the soil pH and OM. Therefore, inoculation of the reclaimed soil with earthworms and bermudagrass posed positive significance for improving soil nutrients and reducing the risks of heavy metals in the coal mining areas.

4. Conclusions

(1) Inoculation of earthworms, bermudagrass, or earthworms and bermudagrass all decreased the soil pH and increased soil OM, TN, and TP, while earthworms combined with bermudagrass could significantly increase soil OM, TN, and TP in L and H groups.

(2) In the two covering soil thicknesses, the contents of Cr, Cu, Pb, and Zn in the covering soil could be reduced by inoculating with earthworms alone, planting bermudagrass alone, or inoculating with earthworms plus bermudagrass. The reduction effect from earthworms and bermudagrass was more prominent than earthworms or bermudagrass. Total Cr, total Cu, total Pb, and total Zn decreased remarkably more in group L than in group H.

(3) Inoculating earthworms alone can enhance the availability of heavy metals in soil. Planting bermudagrass alone and inoculating with earthworms and bermudagrass can reduce available heavy metals in soil. The combination of earthworms and bermudagrass posed a synergistic effect in the reduction of heavy metals availability. Earthworms and bermudagrass could significantly decrease the available Cr and Zn in high soil thickness compared with those in thin soil thickness.

(4) Redundancy and correlation analysis showed that soil pH and OM were the main factors affecting heavy metals in the reclaimed soil. Inoculation of earthworms can change the pH and OM in reclaimed soil, which altered the heavy metals uptake by the bermudagrass.

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CHAPTER

14

The power of earthworm: vermicompost drives to sustainable agriculture

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1. Introduction

The ground beneath our feet is more than just dirt; it is soil. Soil is the foundation of our agrifood system. Soil is necessary because (1) there are more living organisms in a tablespoon of soil than there are people on earth, (2) it takes over 1000 years to make 1 cm of soil, and (3) it is estimated that 95% of food is produced in soil directly or indirectly. Healthy soil is vital worldwide, particularly in urban populations and landscape regions, where soil may be lacking in organic matter and polluted with heavy metals and other contaminants and degraded (FAO et al., 2022). Soil fertility is vital to the ecosystem.

Why are plant and soil health so important? Plants provide most of the oxygen we breathe and the food we eat. Plant health is critical to ensuring sustainable agriculture and food systems and protecting ecosystems and the environment. Healthy plants lead to healthier people. However, we frequently overlook this crucial link. Healthy plants drive through being disastrous (FAO, 2020b) and can have devastating results. Plant health is increasingly under threats. Climate change and human activities have reduced biodiversity, altered ecosystems, and created new niches where pests may thrive and damage soil health (Fig. 14.2). Protecting the soil protects the plants, and (FAO, 2020b) protecting plants protects life. Sustainable agriculture in soil and agrifood, which are synergistic, avoids agricultural inflation economically (World Bank, 2022) (Table 14.1).

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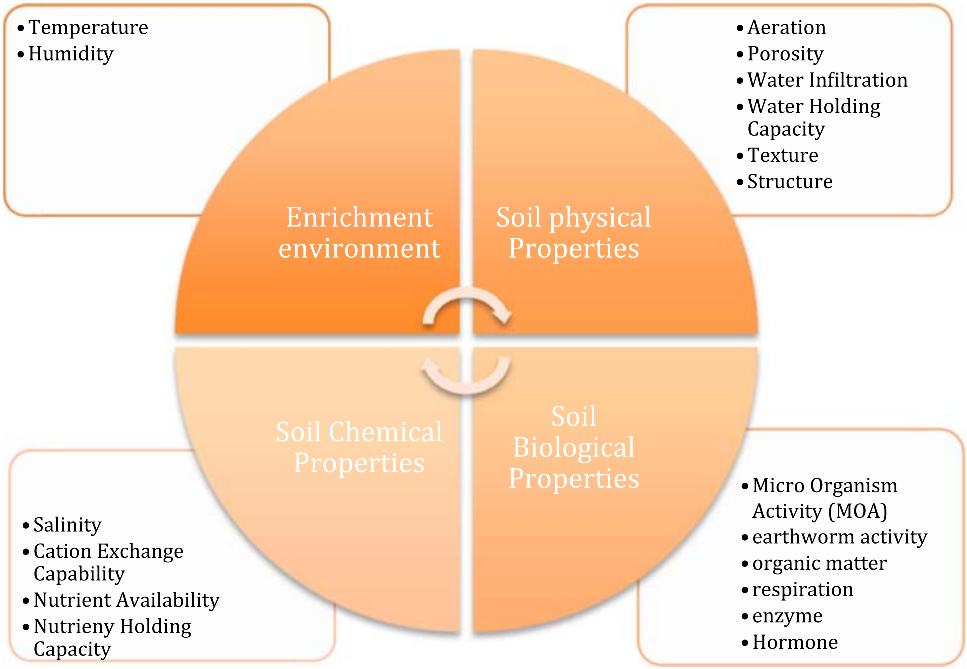

FIGURE 14.1 Properties of vermicompost.

FIGURE 14.2 Vermicompost and earthworms.

TABLE 14.1 Food inflation index, Jul. 2021 to Jun. 2022 in 16 low to upper countries (% change).

Country	7	8	9	10	11	12	1	2	3	4	5	6
East Timor	5.8	6.0	6.4	7.5	7.7	7.3	6.4	6.8	7.0	7.3	8.0	
India	4.6	3.8	1.6	1.8	2.5	4.4	5.6	6.0	7.5	8.1	7.8	7.8
Iraq	7.5	10.2	7.6	5.3	8.4	7.4	8.5	7.8	7.5	9.0	9.0	
Indonesia	2.7	3.3	3.2	3.0	3.0	3.1	3.5	2.5	3.4	5.3	5.8	9.1
Bangladesh	5.1	5.2	5.2	5.2	5.4	5.5	5.7	6.2	6.3	6.2	8.3	8.4
Iran	57.6	59.4	61.4	46.9	41.7	42.7	40.7	40.7	41.2	44.3	50.9	85.5
Pakistan	8.3	10.1	10.2	8.3	10.2	10.3	12.9	14.7	15.3	17.0	17.3	25.9
Philippines	4.0	5.6	5.1	3.8	2.3	1,5	1.6	1.1	2.8	4.0	5.2	6.4
Sri Lanka	11.0	11.0	9.9	11.7	17.1	21.6	24.3	24.4	29.5	45.1	58.0	80.1
Argentina	56.4	53.4	53.4	51.3	50.6	50.3	50.5	55.8	59.8	62.1	64.2	66.4
Brazil	13.4	14.0	12.6	11.7	8.9	7.9	8.0	9.1	11.6	13.5	13.5	13.9
China	-4.4	-4.9	-6.0	-2.7	2.0	-1.3	-3.9	-4.0	-1.6	1.7	2.2	2.9
Jordan	0.9	1.6	1.7	0.0	-0.5	2.7	3.4	2.4	4.2	4.3	5.8	4.1
Malaysia	1.3	1.2	1.8	1.9	2.6	3.1	3.6	3.8	4.2	4.2	5.3	6.1
Thailand	-0.5	-1.5	-1.1	-0.3	0.4	0.8	2.4	4.5	4.6	4.8	6.2	6.4
Turkey	25.0	29.1	29.0	27.5	27.2	43.7	55.6	64.2	71.6	90.8	60.9	94.3

Legend	Indicator
	Price increases < 2%
	Price increases 2% - 5%
	Price increases 5% - 30%
	Price increases >30%

Based on Table 14.1, inflation is increasing higher. The worst areas are in Iran, Argentina, Türkiye, and Sri Lanka, which reached 85.5%, 66.4%, 94.3%, and 80.1%, respectively, in Jun. 2022. However, the real gross domestic products and food imports were the critical determinants of food price inflation (Egwuma et al., 2017). Otherwise, the internationalization of business activities and the influence of international trends on the inflation level and effects at the national level were transferred. Given global market trends, it is reasonable to predict that agrifood prices will not fall. Nevertheless, owing to pressure exerted by population growth (i.e., on the demand side), demand inflation will be constant (Njegovan and Simin, 2020).

The grassroots to resolve food inflation is soil health resulting from food agriculture-land. Too many inorganic treatments make the plant grow quickly and need assistance against pests and diseases through the use of fertilizer and pesticides. At the same time, organic treatment has been left because of self-produced. Nevertheless, people now realize how harmful chemical and inorganic fertilizers are to soil. In addition, food security has been threatened owing to land conversion, outbreaks, pandemics, war, and population growth. Along with the food problem, farmers want to improve the soil medium. One of the best composts is vermicompost. The food inflation index indicates that the population has not fulfilled the need for food or agricultural products or to stem the failure of the food system.

Excessive increases in chemical fertilizer prices in 2022 were in a position to change this fertilizer use. Agricultural producers must use chemical fertilizers at the right amount and the right time, both economically and environmentally. Soil for a healthy life is critical for agriculture, agriculture, and the environment. For agriculture and landscaping, soil management should be given due importance, the natural structure of the soil, primarily organic matter, should be protected, and agricultural inputs should support sustainable agriculture. Chemical fertilizers only feed the plants and have a negative effect on the environment, but organic fertilizers support both the soil and the plant and make positive contributions to the environment. Vermicompost fertilizer, which has become popular to ensure sustainable agriculture, is essential for agriculture, soil, and the environment.

This chapter reveals the effect of vermicompost, an organic fertilizer, on healthy life, especially for improving soil and the environment.

2. Population and food security

Food security is described as having physical, social, and economic access to adequate and nutritious food to support a healthy and active lifestyle at all times (Egal, 2019). The Global Food Security Index examines the fundamental issues of availability, affordability, safety, and quality in 113 countries. The index is a scoring model dynamic composed of 28 distinct qualitative and quantitative indicators assessing these food security drivers in developing and developed countries. The overarching purpose of the study is to establish which nations are the most and least vulnerable to food insecurity based on the criteria of availability, affordability, safety, and quality.

Despite disasters and wars, the population of the world is growing quickly. The growing global population has led to the increased consumption of products and services, resulting in a significant rise in organic waste from homes, industry, and agriculture (Hoornweg et al., 2013). On the other hand, agricultural lands are decreasing, and the productivity and production capacity of existing lands are decreasing.

The issue of population growth has been linked to food security issues. Since the 1960s, world agricultural productivity growth has outpaced population growth. However, this success has come at a high cost. First, food systems are already surpassing planetary constraints in terms of critical resources, resulting in much food loss and waste. Second, today's diets contribute to early death and greater vulnerability to chronic and infectious illnesses. Third, food systems remain tied to massive disparities such as hunger and food insecurity and workers' battles for adequate wages across food systems. The interdependence of population, nutrition, food security, and sustainable development entails more than providing enough calories for a growing population. The world's rising population must be fed in a healthy, fair, and sustainable manner to secure a healthy future for both people and the earth.

Population patterns and trends are intertwined with the United Nations Food Systems Summit's five action tracks: access to healthy and nutritious food, sustainable consumption habits, environmentally friendly production, a fair livelihood, and resilience. Governments are invited to participate actively, especially in summit preparations and working with a diverse range of stakeholders to explore the potential for enhanced collective action at the territorial, national, regional, and global levels (FAO, 2020a; OECD-FAO Agricultural Outlook, 2022–2031, 2022).

The sustainable transformation of food and agricultural systems is essential at all levels. International collaboration, primarily regional, and interaction with stakeholders, including the commercial sector and civil society, will be essential. There are no universal. Policy must consider local circumstances, the influence of such developments on the livelihoods of those working in agriculture, and job prospects open to them (FAO, 2018).

3. Organic and chemical fertilizers

The difference between organic and chemical fertilizers is that only plants can benefit from chemical fertilizers. Organic fertilizers also benefit the soil and the plant's environment. Whereas chemical fertilizers may harm the environment, organic fertilizers do not have such a disadvantage.

Fertilizing gives some nutrients to plants to grow well. Chemical fertilizers significantly influence plant growth and increase the productivity yield (Bar-Tal et al., 2019). Chemical fertilizers nourish the soil, but they harm the soil. We cannot see this at the first application, but chemical fertilizers slowly and surely worsen soil properties. The soil becomes unhealthy. The adverse effects of chemical fertilizers are:

a. **Soil hardens:** The first thing to be attacked when chemical nutrients are applied to plants is soil. Soil is the best mediator to absorb various macroelements and microelements in the surrounding area.
b. **Increasing pests and resistance:** Not all microorganisms in the soil are plant-destroying. Some are needed to prey on (predators) various microorganisms that are destructive to plants. However, because it is difficult to control the use of fertilizers such as pesticides, species of microorganisms beneficial to plants die. The extinction of these microorganisms has resulted in an increase in populations of plant-disturbing pests because there are no more predators.
c. **Plant pest resistance:** This chemical formulation primarily aims to destroy plant pests. Nevertheless, the reality that instead of dying, the pest that had experienced a decline in number seemed to be able to read the pesticide content, so that it gains even stronger immunity (resistance) than before. This immunity causes an attacked tree to fall quickly.
d. **Becoming a natural residue material:** Residues of these chemical fertilizers are everywhere, from soil to rivers, wells, air, drinking water, and even the vegetables and fruits we eat. The bad news is that these chemical residues can last for decades because they are difficult to break down.
e. **Extinction of natural microorganisms of pest killers:** Immunity of natural enemies from several pests and plant diseases begins to decrease. This decrease in strength results in paralysis. After immunity is completely neutralized, pests and diseases caused by various microorganisms that destroy soil and trees proliferate.
f. **Threatened to break the food chain:** This situation describes the circulation of the food chain that is interrupted because certain species experience extinction. As pesticide residues infect plant rat predators, the rat population increases. This increase in particular species will break the chain formed by nature because of changes in interactions of various related species.

g. Extinction of some living animals: Some species do not have the same immune systems. Some animals can survive whereas others are vulnerable. Fertilizers also pollute them through the residues in water and air.

Chemical fertilizers have a more harmful impact on soil health than they have advantages. In contrast, organic fertilizers influence plant growth, yield, and soil health. Organic fertilizers activate microorganisms to provide nutrients to both plants and soil, improve the soil texture and structure, and increase the water holding capacity, porous, and aeration. Plant nutrient uptake is likely highest when fertilizers of the suitable composition and the right amount are applied at the right time and place. However, fertilization must be prepared and applied based on soil analysis.

4. What is vermicompost?

Food is a valuable resource cultivated in precious topsoil and purchased to feed individuals and their families, never to be thrown away uneaten. Nonetheless, food waste remains a big problem. More food in their home can nourish people as it was meant by giving compelling information and practical tools that help people shop smarter and keep food better. Similarly, by educating local citizens about how to maintain compost in their gardens and backyard, or with an internal earthworm bin, they can make organic fertilizer in their homes and farms, minimizing the quantity of waste to be collected. In short, the first stage of waste management may be to convert organic waste easily into vermicompost.

The term vermicompost refers to the ultimate result (humus-like substance) of earthworms composting organic waste products. Numerous organic food sources for earthworms may result in vermicompost production. These includes animal feces (buffalo, sheep, cow, horse, rabbit, etc.), kitchen waste materials, pruning and mowing lawn waste, fruit and vegetable garden waste, shredded papers, and other organically sourced materials (Ahmad et al., 2021; Aslam et al., 2021). This organic waste may be digested in earthworm digestive systems by a slew of microorganisms and reformed into an odorless substance so rich in organic that it is known as vermicompost (Bellitürk, 2016, 2018). Vermicomposting is an innovative way to address the increase in waste by turning organic matter into nutrient-rich compost for soils instead of dumping waste into landfills.

As the world accelerates toward an urban future, the rate of waste generation has become faster than the rate of development. Waste is linked to urbanization and economic development. The inadequate management of waste disposal has degraded the quality of natural resources on a regional, state, country, and even global scale. However, with good planning and knowledge, animal and agricultural waste can be converted into organic fertilizer. Vermicomposting is the preferred form of composting, forming organic fertilizer called vermicompost.

Under controlled conditions, epigeic earthworms have effectively accelerated the decomposition of organic materials, producing a homogenous humic substance that is high in available nutrients and has pathogen-inhibiting properties. Vermicomposting is influenced by biotic and abiotic factors, among which the C/N ratio of substrates and the feed rate are the most influential (Bellitürk, 2016).

Vermicompost improves the soil's physical, biological, and chemical properties (Aslam et al., 2019, 2020, 2021), and the environment contributes to organic enrichment. Some essential properties of vermicompost are explained in Fig. 14.1 Regarding organic fertilizers, studies on vermicompost are common. Vermicompost is high-quality manure that contains a range of essential nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, and micronutrients such as iron, zinc, copper, and manganese) in sufficient amounts to improve crop quality and yield. It enhances the biological, chemical, and physical properties of the soil (Ahmad et al., 2022; Kılbacak et al., 2021).

5. Promoting vermicompost and its effect on soil properties, plants, and environmental conservation

Some physical properties of the soil that are improved by earthworm activity are (1) the formation of macropores as a result of the formation of worm burrows, (2) the creation of a crumbly soil structure, (3) a decrease in soil bulk density, and (4) an increase in water retention. The formation of earthworm burrows creates sustainable and stable macropores. These holes generate water infiltration and air exchange. Given the input of earthworms, the infiltration rate and accumulation in the soil are higher than without earthworms.

Water accumulation will be even more significant when mulch is provided. Through the movement of earthworms, there is an overhaul of the soil structure that was initially compact and massive, to become a crumbly structured soil. The improved soil by vermicompost can be seen by comparing the structure in the soil not inhabited by worms that has them. Worm-free soil has (1) a colossal soil structure, (2) limited water retention, and (3) a high soil density.

In addition to earthworm movement, the excreta have a positive effect on several physical properties of the soil: increasing water retention and decreasing soil bulk. The relatively high clay content causes an increase in water retention and large total pore in earthworm droppings distinguished from the surrounding soil. Worm droppings contain higher amounts of water than the surrounding soil at the same water stress level.

The addition of worm manure reduces the weight of soil by about 7% of that without it. Earthworms also cooperate with microbes in forming aggregates. This aggregate is related to organic residues that cannot be digested entirely by worms and will be further degraded by soil microorganisms. The products of microorganism degradation and organic chemicals generated by microorganisms increases worms' production of crumb structures. The entry of earthworms into soil causes changes in several chemical characteristics of the soil, including (1) increasing the content of organic matter, (2) increasing the content of available nutrients, and (3) increasing the cation exchange capacity. These chemical properties occur because earthworm droppings have more nutrients and organic C than the original soil. A comparison of vermicompost and soil shows: pH 5.3 vs. 5.7; cation exchange capacity (me/100 g) 17.70 vs. 4.5; Ca^{2+} (me/100 g) 12.20 vs. 2.70; Mg^{2+} (me/100 g) 4.30 vs. 1.30; K^{+} (me/100 g) 0.70 vs. 0.20; Na^{+} (me/100 g) 0.16 vs. 0.07; Bray P (ppm) 12.60 vs. 4.50; N total (%) 0.38 vs. 0.15; C organic 3.10 vs. 1.08. In addition, vermicompost has a water holding capacity of 56%; humic acid (g%) of 5; bulk density (g/cc) of 0.5; organic carbon of 9.0; particle density (g/cc) of 1.01; available nitrogen (g%) of 1.11; available phosphorus (g%) of 1.0; and available potassium (g%) of 1.0.

Some advantages of vermicompost: Composting is a biological process in an aerobic environment (presence of oxygen). The sanitary transformation of organic waste into a homogeneous and plant-available substance is possible with proper moisture and temperature (Barker et al., 2021; Karaca, 2019). Composting is an essential component of organic gardening and farming. Biological decomposition converts food waste and plant debris into a functional soil additive while reducing landfill loads. With the aid of certain earthworms, vermicomposting may reduce the advantages of regular composting. The primary benefits of vermicompost are soil enrichment, increased harvest yields, and plant disease suppression. Vermicompost also has many advantages in terms of organic waste management. Vermicomposting species such as *Eisenia fetida*, *Perionyx excavates*, and *Eudrilus eugeniae* are highly adapted to controlled conditions and have demonstrated tolerance for a broad cross-section of substrates (Bellitürk, 2016). Furthermore, some advantages of vermicompost (Tania, 2021):

1. Vermicompost contains various nutrients that plants need, such as P, N, K, Mg, Ca, S, Mn, Fe, Al, Cu, Na, Zn, Mo, and Bo, depending on the material used.
2. Vermicompost can retain water by 40%–60% to retain moisture.
3. Vermicompost can improve soil structure and neutralize soil pH.
4. Vermicompost is a source of soil microbial nutrition that helps destroy organic waste and increase fertility.
5. Plants can consume nutrients only in dissolved form. Earthworms have a role in converting insoluble nutrients into dissolved forms.
6. Vermicompost is more accessible, cheaper, takes a shorter time, and is environmentally friendly

Some disadvantages of vermicompost: Earthworms may seem tiny and insignificant, but they can have an essential role in the ecosystem. Unfortunately, vermicompost and earthworms have disadvantages, which are that they take time, they have a noticeable odor, they require high maintenance, they have pest and pathogen problems, and they have a specific harvesting time. However, there are solutions for these. Therefore, the advantages of vermicompost are more important than the disadvantages.

5.1 Vermicompost economic advantage

Compost and vermicompost programs should be important both as a state and individually. Municipalities should also do important work in this regard. A community's composting–vermicomposting program should be planned to use and enhance existing infrastructure based on vermicomposting or zero-waste composting findings. This method enhances cost-effectiveness by lowering capital expenditures and allowing for scale economies. It has a lower environmental effect because it reduces the number of vehicles used, the quantity of processing equipment needed, and labor expenses. Various advantages and economic gains obtained in this way are used for other needs of people, and the environment is protected (Fig. 14.3).

Fertilizer prices are rapidly increasing. However, agricultural production also must continue. It does not seem right to complain about increases in mineral fertilizer prices. What needs to be done is to search for alternative fertilizer sources. Thus, composting and

FIGURE 14.3 Cultivating carrots using vermicompost.

vermicomposting are convenient, especially in Türkiye. The government must find more attractive methods to encourage producers to use this fertilizer. Training activities, incentives, public service announcements, and other supports should be increased to produce and use organic fertilizers, which also allows an evaluation of waste for agricultural and landscaping purposes. It would not make sense to stop agricultural production because of increases in fertilizer prices. The needs of the growing population are also increasing, and thus, waste is also increasing. Therefore, more academic studies should be carried out on vermicompost production in waste management, and the results should be applicable. The chemical fertilizer needs of soil improved using vermicompost decrease from year to year. Vermicompost is an environmental as well as economic benefit. It is effective in reducing the costs of production. It also has many benefits for protecting the environment.

6. How to feed earthworms

Earthworms used to obtain vermicompost are epigeic species and feed on rot. In this case, the waste is obtained from academically proven vermicompost; olive, vineyard, walnut, almond, and nut tree pruning waste; corn, wheat, sunflower, paddy, barley, and oat stubble waste; various hard fruit shells; cow, sheep, goat, and horse manure; household fruit and vegetable kitchen waste; and ground paper and sawdust waste. Apart from these, there are plans to carry out projects and preliminary research studies on the possibility of making vermicompost from by-products of food factories (Belliturk, 2016; Bellitürk, 2016; Büyükfiliz, 2016). Earthworms should not eat dairy products, meat, oily or processed food and their waste, allium plants, foods and wastes that are acidic, some animal feces (high amounts of bird and poultry feces), rotten food, and spicy foods. An essential components in establishing a flourishing worm bin to provide vermicomposting earthworms with access to varied meals they can safely ingest. Citrus fruits and their peels and rinds, for example, are unsuitable for feeding epigeic earthworms. Citrus tree fruit is acidic, and acid is unpleasant to worms when it breaks down the soil. Earthworms do not have a nose, but they are sensitive creatures.

It is not easy to acquire academic knowledge about earthworms and their feeding. It is difficult to explain this information clearly, especially regarding vermicomposting, but it may become possible with study and time. All of our knowledge of earthworm feeding ecology (Curry and Schmidt, 2007) is based on thorough observations made by early naturalists such as Charles Darwin and Gilbert White. They were further modified and expanded by several workers using a variety of methodologies such as choice chamber/arena testing, gut content analysis, and palatability studies. These include indirect food evaluations in growth studies, litter bag investigations, and, more recently, the increased use of innovative isotopic, molecular, and related approaches and techniques. Earthworms feed primarily on organic material in various states of degradation in nature and vermicomposting media (Barlas et al., 2018; Bremner, 1965; Özenç & Senlikoğlu, 2017).

Generally, earthworms eat our garbage. They can consume coffee grounds, banana peels, table scraps, and other food trash in our kitchen wastebasket. We get rid of the odor in the kitchen by learning to compost food waste outside with grass clippings, leaves, manure, and dirt. Vermicomposting converts organic waste into black, earthy smelling, nutrient-rich humus using earthworms and microbes. Apart from enhancing garden yields, the entire vermicomposting procedure is straightforward. Earthworms generate no noise and require little attention (Appelhof et al., 1993).

The price of fertilizers seems to increase continuously. However, agricultural production also has to continue. It does not seem right to complain about increases in mineral fertilizer prices. What needs to be done is to search for alternative fertilizer sources. It would not make sense to stop agricultural production because of increases in fertilizer prices. The needs of the growing population are also increasing; thus, waste is also increasing. Therefore, more academic and scientific studies should be carried out on producing organic fertilizers in waste management, and the results should be applicable (Cuaresnma et al., 2010; FAO, 2017). Of course, all living things must consume to live. However, consumed food, water, and habitable environments can be counted. As long as consumption does not reach the level of waste, it can be considered normal. However, during consumption, the generated waste must be returned to nature as valuable materials.

The evaluation of organic (animal, animal, and vegetable) waste as compost or vermicompost is a popular topic (Adiloğlu et al., 2020; Bitki et al., 2016; Karaca, 2019). Owing to environmentally friendly production, vermicompost must be produced. Thus, developed and developing countries have introduced regulations and laws regarding agriculture.

Rules for producing commercial and individual vermicompost have been determined by the relevant governments and are updated daily. We must produce and apply vermicompost (solid or liquid) for agricultural and landscaping purposes under these rules without harming environmental health. We need to increase awareness of this issue with academic studies. High-quality vermicompost is organic fertilizers that are environmentally friendly and economical compared with chemical fertilizers (Kováčik et al., 2018; Malik and Baba, 2020; Voltr et al., 2021).

7. The importance of vermicompost in sustainable agriculture

Vermicompost is a source of nutrients for soil microbes, so soil biological activity increases. Earthworms can also convert insoluble nutrients into soluble forms with the help of enzymes in the digestive tract of worms so that plants absorb them more quickly (Kusumawati, 2011). Vermicompost contains a lot of humus, increasing water-holding capacity and improving soil structure. Vermicompost also has more excellent cumulative microbial activity than ordinary compost. It contains hormones such as auxin, gibberellins, and cytokinins that stimulate root, shoot, and leaf growth.

Several types of worms can be selected for cultivation, including *E. fetida*, *P. excavates*, *Lumbricus rubellus*, *E. eugeniae*, and *P. excavate*. It is a widely cultivated earthworm because it breeds easily and quickly. Earthworm products can be used in agriculture, animal husbandry, fisheries, and pharmaceuticals. Vermicompost is a by-product that has many benefits in worm cultivation, especially in agriculture (Hoornweg et al., 2013).

Developing organic farming systems provides opportunities for vermicompost development on a broader scale. Vermicompost has a positive effect on improving soil fertility and offers profitable business opportunities (Yadav et al., 2017). Research on using vermicompost for agricultural commodities needs to be developed to produce a technology package for farmers.

7.1 How to make simple vermicompost on a household scale

Earthworm media may use manure, coco peat, sawdust, dry leaf litter, waste mushroom, vegetable, fruit, and cereal. Adding EM4 synergism to microbial activities is preferable. Earthworms love to eat banana peels. The media composition of 50% manure, 50% mushroom log waste, and vegetable waste has advantages in that it is cheaper and practical in its application and meets a minimum C content of 15% and a C/N ratio of 15–25 (minimum requirements for solid organic fertilizer) (Hidayati, 2022). The suitable media and feed can support the breeding of worms so that the more worms there are, the more feces will be produced. The tools and materials needed to make vermicompost fertilizer (Pertanian, 2018; Tania, 2021) are as:

a. A container where the worms will live, also known as a worm bin. The container can be made of rubber, wood, or plastic, if not metal. Used tires or wooden boxes can also be used as worm containers.

b. Media for the development of earthworms, such as organic materials of rice straw, livestock manure (cow, buffalo, goat, sheep, chicken, horse, and rumen contents), and household waste/organic waste. Ensure the waste contains only vegetable and fruit waste, not animal waste such as fishbones, chicken bones, milk, cheese, and eggshells.
c. Banana stem midrib for earthworms to lay eggs.
d. Earthworms can be obtained by buying them at stores or outlets that provide agricultural or livestock equipment. Several earthworms are commonly used to make vermicompost fertilizer: *E. fetida* (tiger worm) and *L. rubellus.*

Steps for making vermicompost fertilizer are:

1. The container used for the worms must be perforated at the bottom and sides to ensure the worms have sufficient airflow and circulation,
2. Organic matter for worm development media is fermented or allowed to stand for at least 2 weeks.
3. Next, put some worms in the media. If the worms leave the media, the media conditions are unsuitable for worms. A good medium for making vermicompost fertilizer is humidity of 50%–55%, temperature of 30–55°C, and pH of 5–8. There must be good aeration. Prepare organic media until the worms settle and do not leave the media.
4. Then, put a handful of worms in the organic material of worm development media.
5. At the bottom of the container or place for making vermicompost, a banana peel is given as a place for worms to lay eggs.
6. After 3 days, open the container and see if the compost has started to form and the organic waste has disappeared. If so, add the waste again and repeat the process until the amount of fertilizer produced is large. Fertilizer that is ready to use is black and odorless.
7. After a few days or weeks of the process, vermicompost will form, characterized by changes in organic matter to crumbs or crumbles, and there are small oval grains, which are worm droppings.
8. Earthworms can be separated with vermicompost fertilizer, which is then dried and ready to use.
9. Harvested worms can be used again for further composting. The time of composting depends on the number of worms. The more worms, the faster the composting process.

Vermicompost is not costly. It is an efficient, efficient, and environmentally friendly waste management method. It is an excellent source of biofertilizers to improve biological and physiochemical properties. Vermicomposting increases the population and diversity of beneficial microbial niche communities. Studies also conclude that earthworm-fed fish tremendously affect nutrients and can be complemented and substituted for animal and human diets (Edwards and Arancon, 2022).

Vermicompost products have become relevant to plant nutrition. They promote plant growth in horticulture, field crops, and agriculture, even though landscape plants. Furthermore, landscape plants, even though in a soilless culture that much developed in urban farming activities (Sundari et al., 2022).

8. Conclusion

With chemical fertilizers, pesticides, and similar substances in agriculture, soil fertility decreases daily, and adverse environmental and human health problems occur. Therefore, the need for materials and biological preparations without side effects, such as organic (Ahmad et al., 2021, 2022; Appelhof et al., 1993; Aslam et al., 2019, 2020, 2021; Barker et al., 2021; Barlas et al., 2018; Bar-Tal et al., 2019; Belliturk, 2016; Bellitürk, 2016, 2018; Bitki et al., 2016; Bremner, 1965; Büyükfiliz, 2016; Curry and Schmidt, 2007; Edwards and Arancon, 2022; Egal, 2019; Egwuma et al., 2017; FAO, 2017, 2018, 2020a, 2020b; FAO et al., 2022; Hidayati, 2022; Hoornweg et al., 2013; Karaca, 2019; Kılbacak et al., 2021; Kováčik et al., 2018; Kusumawati, 2011; Malik and Baba, 2020; Njegovan and Simin, 2020; Özenç and Senlikoğlu, 2017; Sundari et al., 2022; Tania, 2021; Voltr et al., 2021; World Bank, 2022; Yadav et al., 2017; OECD-FAO, 2022) fertilizers and vermicompost, has increased. The most crucial chemical fertilizer has yet to improve soil properties physically, chemically, or biologically in the long term. The term vermicompost refers to the ultimate result of the composting of organic waste by earthworms. Several organic food sources are available for earthworms, which produce vermicompost. Food consumption has increased globally but waste continues to be a significant problem. Vermicompost production is becoming increasingly popular in agrofood and animal waste management. Correct fertilization is vital to meet the needs of the increasing population. Agricultural production has a large share of food production. The thoughtless use of agricultural amendments, land degradation (such as the reduction of organic matter, especially in agricultural soil), decreases in agricultural land, the growing population, deforestation, and water scarcity are among the most visible manifestations of this unsustainable competition. These and similar problems urgently need to be solved, and the number of academic studies on these issues should be increased.

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CHAPTER

15

Utilization of vermicompost and vermileachate on plant growth and development: aspects to consider

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1. Introduction

Unlike hunting and gathering for food, agriculture is the natural process of producing or growing food in an environment that has been manipulated by humans to favor the growth and development of plants or animals of choice. Since the inception of agriculture, humans have appreciated that plants require nutrients for them to grow, although photosynthesis requires only sunlight, water, and carbon dioxide. In addition to carbon and oxygen, plants need at least 14 mineral elements for optimized growth, most of which come from the soil (White and Brown, 2010). These nutrients are critical to the development of the plant structure, driving plant physiologic function and the final development of the plant fruit, bulb, and tuber, among others, which are the yield parts that humans harvest. Deficiency in any of these nutrients required by plants results in reduced plant growth and yield. For plants to grow, they require some major and minor elements (i.e., nitrogen, potassium, phosphorus, calcium, magnesium, and sulfur as major elements; and boron, iron, manganese, copper, zinc, nickel, molybdenum, and chlorine as minor nutrients). All these elements exist in most soils, but their concentrations are not always adequate to allow for efficient plant

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growth and yield development, especially for the macroelements. This has been true since the beginning of agriculture, when farmers began using supplements in the form of animal manure and compost, among others, as nutrient amendments to improve soil quality and crop yields.

In the 1970s, the green revolution introduced the use of industrially manufactured synthetic nutrients such as ammonium nitrate and urea to improve crop yields in response to the depletion of soil fertility and increases in world demand for food (Mupambwa et al., 2022a). These synthetic fertilizers saw a huge increase in food production. However, over time, it was observed that these synthetic fertilizers feed only the crop while ignoring to feed the soil, which is a critical resource in sustainable food production (Mupambwa et al., 2022a). Most research on fertilizers minimizes resilience in favor of agricultural output, focusing mainly on an immediate increase in crop yield. One can argue that the major cause of this inward-looking approach, which focuses on yield instead on long-term sustainability of the soil, was influenced by the emergence of the green revolution. This green revolution, which promoted a vision of modernizing agriculture by using machines, synthetic fertilizers, pesticides, and systematically improving crop types, generalized the use of these fertilizers. The goal was to boost food production significantly and reduce hunger on a worldwide scale; thus, the emphasis on immediate crop growth is unsurprising. The green revolution's simplistic view of agriculture as matching other industrial processes, in which success was evaluated in production units, was also very much a part of an industrial worldview. Today, however, we have realized that this oversimplification has a negative impact.

Thus, there is a growing realization that to increase crop yields sustainably in agriculture, the soil also needs to be fed as much as does the crop (Mupambwa et al., 2022a). This has resulted in organic agriculture or organic soil fertility management. The concept of organic agriculture was started by Rudolf Steiner and became established among farmers, as opposed to using synthetic fertilizers. Furthermore, farmers are facing increasing weather variability, a greater number of extreme events, and a greater extent of uncertainty with respect to future developments, all of which are induced by rapid climate change (Menghistu et al., 2020).

Vermicompost, unlike traditional compost, has gained momentum as an effective and optimized organic nutrient source (Bhat et al., 2018). Vermicomposting is a biological process that uses earthworms to accelerate and optimize the biodegradation of organic waste materials, which results in the production of nutrient-rich fertilizers (Yatoo et al., 2022; Mupambwa et al., 2022b). Therefore, vermicompost has higher concentrations of elements such as nitrogen and phosphorus, and beneficial microbes as well as growth hormones (Yatoo et al., 2022) Another product of vermicomposting is leachate, which drains from compost. This liquid organic fertilizer has potential in crop fertilization, particularly in hydroponic crop production. However, the use of organic fertilizers alone is insufficient to address these challenges of soil fertility. Thus, there is a need for the optimized simultaneous use of organic fertilizers with synthetic fertilizers (Menghistu et al., 2020). This chapter highlights the potential of vermicompost and vermileachate in the production of organic crops, standard methods of vermicompost production and leachate production, and its benefits for crop and soil fertility.

2. Organics in fertilization of degraded soil

Some indicators used to characterize degraded soil include susceptibility to soil crusting, sealing, soil erosion, loss of vegetation cover, loss of nutrients, and poor water holding capacity (Lal, 2001). Recovery of these types of soil is driven by increased and sustained inputs of soil organic matter and improved nutrient cycling in the soil. Soil organic matter, which is composed of about 50% soil organic carbon (SOC), has several key functions in soil, as summarized by Lal (2016) and Lomax (2016):

- **i.** Maintains soil structure and aggregation, which aid water movement and retention and resistance to erosion.
- **ii.** Influences nutrient retention and use efficiency through increased ion exchange capacity.
- **iii.** Drives rhizospheric processes, which influence elemental transformations.
- **iv.** Moderates gaseous emissions.
- **v.** Is a natural store of key plant nutrients.
- **vi.** Improves retention of nutrient, addition, and availability to plants.
- **vii.** Improves water infiltration and retention capacity, reducing flooding, erosion, and nutrient leaching during heavy rainfall.
- **viii.** Provides an energy source for beneficial soil biota.
- **ix.** Provides a buffer against soil pH changes.

Therefore, the use of soil management practices that improve SOM have a crucial role in improving soil health and the productivity of degraded soil. Several innovative technologies are used by farmers across the world to improve SOC in soil. These technologies include conservation agriculture, integrated and diverse cropping/farming systems such as regenerative agriculture and ecological agriculture, and the use of organic amendments (e.g., vermicomposting, biochar, compost, animal manure). Other technologies include practices that restore soil and ecosystem functions, such as agroforestry and regenerative agriculture (Lal, 2016).

Of these technologies, organic options such as vermicomposting and the use of cattle manure have become popular among researchers and farmers owing to their immense benefits. They improve the availability of nutrients and also feed the soil by improving soil quality (Mupambwa et al., 2022a,b), unlike inorganic fertilizers, which only improve the elemental composition. Most important, the use of organic fertilizers improves soil microbial indices (e.g., microbial biomass carbon), unlike chemical fertilizers. Improving the soil microbial status of soils is important because the availability of nutrients in soil is driven by soil microbial processes. However, the slow mineralization of nutrients from organic manure and unknown nutrient compositions are some of the drawbacks that limit the applicability of manure in agricultures (Mupambwa et al., 2019). This has prompted researchers to advocate for combining organic and inorganic fertilizers, which was shown to have soil fertility and yield benefits. For instance, in a study to determine the influence of organic and inorganic fertilizers on maize yield and soil fertility, Sigaye et al. (2020) showed that applying an appropriate proportion of organic fertilizer with inorganic fertilizer resulted in a higher maize yield and also improved soil fertility. Similarly, a review by Adane et al. (2020)

concluded that there was a need to develop an integrated soil fertility management that enhanced soil productivity through the balanced use of all sources of nutrients, including organic and inorganic fertilizers.

Practices such as vermicomposting, through which raw organic nutrient sources can be efficiently transformed into nutrient-rich organic fertilizer, are practical solutions for improving the nutrient availability of organic manure that can easily be adopted by farmers. Other improved organic sources such as biochar have also gained in popularity among researchers and farmers owing to the benefits of carbon sequestration in addition to improvements in soil physical properties (Nyambo et al., 2021).

3. Vermicomposting and vermileachate production

The term *vermi* in vermicomposting originates from the Latin word *vermis*, which means a worm. Vermicomposting is a biological degradation process that is nonthermophilic, in which organic materials are decomposed with the aid of earthworms and mesophilic microbes to create a nutrient-rich compost as the final product (Bhat et al., 2018; Das et al., 2016). Gomez-Brandon and Dominguez (2014) described vermicomposting in more detail as "bio-oxidative process in which detrivorous earthworms interact with microorganisms and other fauna within the decomposer community, thus accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties." The definition of vermicomposting describes composting by earthworms, but biodegradation involves the action of primary, secondary, and tertiary consumers (Goswami et al., 2021). In this whole process, earthworms are critical drivers because they are involved in the mechanical breakdown of organic materials, which increases the surface area of organic materials. The increased surface area results in enhanced microbial activity, which further drives biodegradation in both the earthworm gut and compost cast–associated processes.

During vermicomposting, a liquid is produced that comes from excess moisture that drains under gravity from compost, called the vermileachate. Vermileachate has been recognized as a potential nutrient source because some macronutrients and micronutrients, complex microbiota, plant growth regulators, and humic acid in the vermicompost also tend to leach with excess water (Yatoo et al., 2022; Sanadi et al., 2021; Churilova and Midmore, 2019). Recycling this vermileachate from vermicompost enhances the process as a sustainable closed loop nutrient production technology (Sanadi et al., 2021). However, there is limited research on variations in the nutrient composition of the vermileachate based on the materials used and the range of dilution during vermicomposting (Bhatt et al., 2023). Furthermore, a detailed strategy on the use of vermileachate in fertigation or as nutrient sources in hydroponics is still limited in the literature (Bhatt et al., 2023). Several researchers have presented varying results on the chemical composition of vermicompost and vermileachate, so there is a need to develop optimized methods that allow the replicable production of compost with predictable nutrient content. Sanadi et al. (2021) suggested that from a mass balance point of view, if the nutrient range of the original materials used for vermicomposting is characterized, it might be possible to predict the end nutrient range of vermicompost and vermileachate. Such characterizations can be important to drive uptake organic nutrient sources such as vermicompost and vermileachate because they can be marketed with labels that indicate the range of the different nutrients.

4. Parameters influencing vermicompost quality

As emphasized earlier, there is a need to be able to predict the nutrient composition in the final vermicompost and vermileachate for these organic nutrient sources to be used with a level of certainty (Mupambwa et al., 2022a,b; Mupambwa and Mnkeni, 2018). However, in addition to the work of Ahamad Sanadi et al. (2021), which suggests characterizing the original materials used, other parameters that influence the biological activity of composts influence the final nutrient composition of vermicompost and vermileachate. These properties include, among others, the ratio of carbon to nitrogen, the starting compost, the earthworm species and stocking density, and the leaching or dilution level, as discussed by Mupambwa and Mnkeni (2018).

4.1 Carbon—nitrogen ratio

For every living organism, the primary source of energy is carbon, whereas nitrogen is a critical element in the formation of proteins for cell formation and function. During vermicomposting, earthworm and other naturally occurring organisms feed on carbon-based materials, which leads to the degradation of these materials, increasing the loss of organic carbon (Bhat et al., 2018). Thus, research on vermicomposting has been reported using materials with various levels of organic matter and nitrogen composition including cow dung, pig manure, chicken manure, rabbit manure, and fish offal (Mupambwa and Mnkeni, 2018). In these studies, despite the different organic sources used during vermicomposting (Fig. 15.1), few researchers considered the C/N ratio required for optimized vermidegradation.

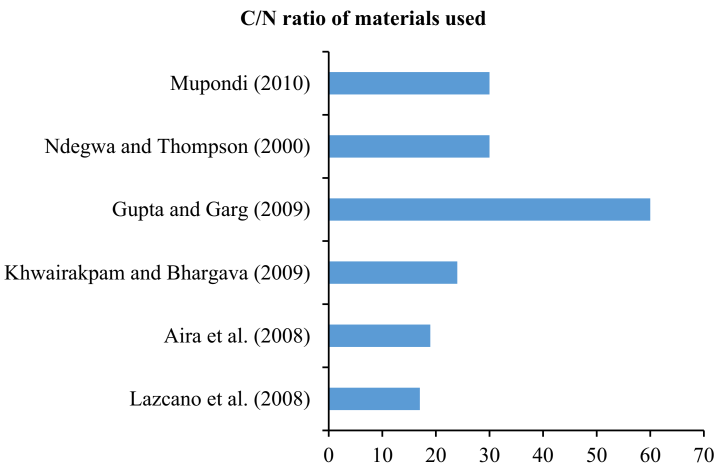

FIGURE 15.1 Data from various researchers showing the carbon to nitrogen ratio of substrates used for vermicomposting research.

For the effective development of predictable nutrients in vermicompost for use in plant nutrition, the C/N ratio of the original starting organic matter should be around 30. This information is important because it has an effect on the total time taken for biodegradation and the final C/N ratio, both of which influence the final nutrient composition in the vermicompost and vermileachate.

4.2 Earthworm species and stocking density

As alluded to earlier, vermicomposting is driven by earthworms; thus, the quantity of earthworms in the substrate influences biodegradation as well as microbial and enzyme activity and other processes. The quantity or density and species of earthworms affect biodegradation during vermicomposting, which influences the final compost nutrient composition. As indicated in a review by Mupambwa and Mnkeni (2018), several researchers used different earthworm stocking densities for vermicomposting. Those researchers reported a general trend in which the higher the stocking density, the faster the biodegradation. Furthermore, various species of epigeic (surface litter dwellers), anecic (deep soil burrowers), and endogeic (upper soil dwellers) earthworms exist with different feeding capacities. The epigeic earthworm species, *Eisenia fetida*, is recommended as ideal for vermicomposting (Mupambwa et al., 2022a,b). However, Devi et al. (2023) reported that a stocking density of five to seven worms/kg (*Eisenia fetida* and *Eudrilus eugeniae*) was more effective for increasing microbial activity and promoting macronutrient mineralization and humification whereas higher stocking densities of 15 worms kg^{-1} created a stressful environment within the compost. Research should continue to model the nutrient mineralization of different materials in different earthworm species to avoid continuing research on experience based on stocking density recommendations.

5. Qualities of vermicompost and vermileachate

The quality of vermicompost and vermileachate is mainly determined by the chemical properties of the original materials used. As indicated earlier, organic fertilizers cannot compare with inorganic fertilizers in terms of mineral nutrient composition, although vermicompost is superior in terms of improving soil quality. Table 15.1 lists various experiments on vermicomposting and the nutrient levels reported.

6. Issue of biochar in vermicompost fertility

Amending soils with biochar has been shown to improve soil fertility, promote plant growth, increase crop yield, and reduce contamination. Biochar's potential and nutritional content depend on the conditions of pyrolysis and the feedstock used during pyrolysis, whereas the availability of nutrients in biochar is element-dependent (Yao et al., 2010). Biochar feedstock can be any organic material ranging from agricultural to municipal waste (Nyambo et al., 2018, 2020a,b). Total organic carbon, fixed carbon, mineral elements of

TABLE 15.1 Research on vermicomposting and changes observed in nutrient content.

Experimental conditions	Nutrient concentrations	References
Fly ash (FA) incorporated into cow manure–waste paper (CDWP) mixture at ratio of 1:2 (FA:CDWP). The mixture was vermicomposted using *Eisenia fetida* earthworms at a stocking density of 25 g-worm kg^{-1} of biomass. Vermicomposting was done for 12 weeks.	Olsen P (764.57 mg kg^{-1}); Nitrate/nitrite (19 mg kg^{-1}); carbon/nitrogen ratio (11.1)	Mupambwa and Mnkeni (2016)
Goat manure (GM) was mixed with vegetable food waste (FW) at various ratios and vermicomposted using *E. fetida* earthworms at a stocking density of 25 g-worm kg^{-1} biomass. The mixture was allowed to vermicompost for 12 weeks.	The 75FW:25GM and 50FW:50GM treatments had the highest final concentration of Olsen phosphorus of 0.98 and 0.96 g kg^{-1} of compost. The nitrite/nitrate concentration ranged from 0.1 to 0.068 g kg^{-1} of compost.	Katakula et al. (2021)
Cow dung was used as blending feedstock and mixed at different quantities of cow dung (CD), cabbage residual biomass, and cauliflower, all mixed on a dry weight basis. Forty mature worms of an undisclosed species were used for each 2-kg mixture and vermicomposted for 90 days.	Total organic carbon (256–300 g kg^{-1}); total nitrogen (15.42–18.64 g kg^{-1}); total available phosphorus (9.38–12.56 g kg^{-1}); total potassium (11.97–15.6 g kg^{-1}).	Mago et al. (2022).
The organic fraction of municipal solid waste (containing vegetable waste, flower waste, paper waste, leaf litter, etc.) was spiked with CD at different ratios from 1:1 to 1:2 and with pure cow dung and pure municipal solid waste. Healthy, equally aged *E. fetida* was used in the experiment, which were introduced in plastic containers with the mixed substrate.	Total organic carbon (344.3–401.5 g kg^{-1}); total nitrogen (11.83–16.5 g kg^{-1}); total phosphorus (11.85–16.64 g kg^{-1}); total potassium (17.18–19.78 g kg^{-1}).	Srivastava et al. (2020)
Undecomposed pig slurry and fresh cow manure were separated by a mesh (5 mm). Fifty mature earthworms (*E. fetida*) were added to the vermireactors.	Cow manure:pig manure: nitrate/nitrite (2130 μg g^{-1}); dissolved organic carbon (1650 μg g^{-1}); ammonium (1170 μg g^{-1}). Cow manure: nitrate/nitrite (469 μg g^{-1}); dissolved organic carbon (3220 μg g^{-1}); ammonium (400 μg g^{-1}).	Aira and Dominguez (2009)
Biochar prepared from seaweed was used as an amendment to optimized goat manure–food waste mixture. Vermicomposting was done using *E. fetida* over 10 weeks.	Olsen phosphorus (0.3–0.4 g kg^{-1}); extractable Ca (15.0–18.9 g kg^{-1}); extractable K (6.4–27.4 g kg^{-1}).	Katakula et al. (2022)

biochar and ash, and the volatile matter content are among the parameters of biochar affected by feedstock properties (Zhao et al., 2013; Zhang et al., 2016).

Several studies have highlighted that biochar can potentially supply nutrients directly to plants. Nevertheless, questions remain regarding whether they are made readily available or released over time in the soil. Some studies reported that applying biochar can have negative or neutral effects on chemical properties (Hale et al., 2011), hydraulic properties (Mia et al., 2017), and crop growth and yield (Yu et al., 2019). The primary reason is that soon after application, biochar can be lost in the soil owing to its relatively high hydrophobicity, which limits its interaction with certain substrates in the soil, such as nutrients, water, and SOM. For instance, Dong et al. (2017) reported a loss of about 40% biochar in the soil and additional C loss of 36%, 35%, and 35% at application rates of 30, 60, and 90 t ha^{-1}, respectively. Also, biochar is said to localize the nutrients it contains and hardly release them during and after soil amendment (Gonzalez and Kang, 2017). Yu et al. (2019) reported a decrease of Ca, Mg, Fe, Mn, and B nutrients in lettuce tissue content, and proposed that biochar might have stored these nutrients, regulating crops' needs. Researchers agree about biochars' ability to increase soil organic carbon (Qiu et al., 2023; Nyambo et al., 2020a,b; Zhang et al., 2017); hence, it is referred to as a soil conditioner rather than a fertilizer. Biochar does not supply all required nutrients for plant growth, but it reduces the amount of fertilizer needed. Therefore, opportunities exist to enhance the nutrient content of biochar by mixing it with fertilizers or compost. Katakula et al. (2022) evaluated the potential of using seaweed-based biochar as a vermicompost amendment. They reported that adding biochar did not result in significant changes in biodegradation, although it influenced the mineral nutrient composition. It will be interesting to evaluate the influence of biochar-amended vermicompost on soil quality, an area in which research is missing.

7. Vermicompost in crop production

Researchers have looked at using vermicompost in crop production, as outlined by Mupambwa et al. (2022a,b) (Table 15.2).

8. Vermileachate in crop production

Similar to vermicompost, the concentrations of nutrients in vermileachate are different among studies that have evaluated this potentially useful liquid fertilizer. As highlighted by Ahamad Sanadi et al. (2021), a lack of research remains about the detailed use of vermileachate as a source of nutrients for crops, mainly because of high variability in the nutrient composition of vermileachates. However, the use of vermileachate cannot be considered a sole source of nutrients for crop growth, but rather as a fertilizer supplement for crops and soils. Furthermore, the application of undiluted vermileachate is not recommended, because this may result in the oversupply of potentially toxic nutrients and heavy metals. This dilution should be based on nutritional demands, which is different for different vegetable crops (Table 15.3).

9. Economic perspectives on organic nutrient sources

A holistic economic analysis of organic fertilizers must look at the production side, where the fertilizer is used as an input, and the vertical markets, in which the demand for the outputs produced using organic fertilizers are increasing. The practical goal of the farmer is to determine how much nutrient material to add and how much profit to expect after applying

TABLE 15.2 Selected studies that used vermicompost as a nutrient source in crop production.

Vermicompost type and crop	Macronutrient composition of vermicompost	Research results	References
Commercially prepared pig manure and food wastes based vermicompost, prepared using *Eisenia fetida* Vermicompost evaluated using tomatoes and marigold flowers with METROMIX used as control	• **Pig manure vermicompost**: total N (2.36%); organic C (43.8%); total P (4.5%); total K (0.4%); nitrate (4525 μg g^{-1}); pH (5.3) • **Food waste vermicompost**: total N (1.8%); organic C (34.0%); total P (0.4%); total K (1.1%); nitrate (665 μg g^{-1}); pH (neutral)	• Minimum increase in shoot dry biomass under treatments containing 10% food waste vermicompost or 20% pig waste vermicompost compared with control. • All potting mixtures containing vermicompost had significantly greater cumulative microbial activity than METROMIX control. • Vermicomposted pig solids contained elevated levels of N, which resulted in plant growth comparable to where fertilizer had been applied.	Atiyeh et al. (2001)
Commercially produced vermicompost from dairy cow manure, supermarket food waste, and recycled paper waste applied at field rates of 10 or 20 t ha^{-1} for vegetable growth Vermicompost treatment supplemented with synthetic fertilizer	**Food waste**: N (13 g kg^{-1}) P (2.7 g kg^{-1}); K (9.2 g kg^{-1}) **Cow manure**: N (19 g kg^{-1}) P (4.7 g kg^{-1}); K (14.0 g kg^{-1}) **Paper waste**: N (10 g kg^{-1}); P (1.4 g kg^{-1}); K (6.2 g kg^{-1})	• Yield of tomatoes that could be sold in all vermicompost-treated plots were consistently greater than yields from inorganic fertilizer-treated plots. • Where vermicompost was applied, there was a significant increase in pepper growth and yield compared with where synthetic fertilizer was used. • Increased growth was attributed to increase in microbial biomass.	Arancon et al. (2003)
Vermicompost was prepared from vegetable waste amended with cattle manure while using *E. fetida*. Vermicompost was applied from 2.5 to 10 t ha^{-1} and supplemented with synthetic fertilizers	• Major nutrients (N = 0.92%; *P* = 1.21%; K = 1.45%)	• Strawberry plant spread, leaf area and dry matter, and fruit yield increased under vermicompost amendment, with fruit yield increase by up to 59%. • Supplementation with vermicompost significantly reduced physiologic disorders in strawberries as well as fungal disease such as gray mold. • Higher doses of vermicompost above 7.5 t ha^{-1} did not significantly increase growth and yield parameters.	Singh et al. (2008)

(*Continued*)

TABLE 15.2 Selected studies that used vermicompost as a nutrient source in crop production.—cont'd

Vermicompost type and crop	Macronutrient composition of vermicompost	Research results	References
Cow manure was processed using *E. fetida* to create vermicompost that was used Basil (*Ocimum basilicum*) was grown in peat medium supplemented at different vermicompost ratios	• Properties of vermicompost were organic matter content (61.4%); pH (7); EC (3.6 dS m^{-1}); total nitrogen (2.7%); total potassium (1.1%); total phosphorus (0.7%); total calcium (0.2%)	• Application of vermicompost at 10% and 20% significantly increased plant growth parameters under water stress. • Essential oil compounds estragole and eucalyptol decreased with water stress and vermicompost treatments. • Increased nutrients were reported under vermicompost-amended treatments.	Celikcan et al. (2021)
Cow manure−based vermicompost used as amendment in mine waste affected soil in which test crop being *Chloris gayana* Vermicompost was used as phosphorus amendment at 20 and 40 mg P kg^{-1} of soil	• Vermicompost properties were approximately: pH (9); total P (0.03%); total N (0.02%); nitrate (101 mg kg^{-1})	• Amendment with vermicompost to supply 40 mg P kg^{-1} resulted in the highest increase in plant growth parameters (i.e., shoot biomass, height, and root biomass) relative to control. • Vermicompost amendment had no effect on tissue trace elemental concentration (Pb, Cd, and As) • Mine waste−affected soils can be effectively corrected with 40 mg P kg^{-1} supplied as vermicompost.	Lukashe et al. (2020)

Adopted from Mupambwa et al. (2022a,b).

TABLE 15.3 Macronutrient requirements for fertilization of tomatoes, cucumbers, and peppers using leachates.

Macronutrient (mg L^{-1})	Tomatoes	Cucumbers	Peppers
Nitrogen	180	200	170
Phosphorus	50	50	60
Potassium	250	200	200

Adapted from Ahamad Sanadi et al. (2021).

extra organic or inorganic fertilizer. Nutrient costs must be weighed against crop value or even less expensive alternatives, such as investing the money in something else with a higher potential return. Technology related to fertilizers is a good example of the law of diminishing returns. Equivalent chemical inputs continue to improve yield, but less and less so at a certain threshold. The farmer's objective is to apply any form of fertilizer in the most advantageous method possible. Fertilizers can help farmers make lucrative farming adjustments. By boosting fertilizer application rates on key cash and feed crops, operators can lower production costs per unit and boost margins over total expenses. They can then invest in soil conservation and other projects.

Animal manure and litter, agricultural by-products, fresh and dried plant material, and other plant-derived substances are all part of organic fertilizers. As such, the amount of nutrients in organic fertilizers varies widely, depending on the materials used as their sources, and materials that can break down quickly are the best options. This makes an economic analysis of organic fertilizers more complex, in which a partial analysis is predominantly done by targeting specific attributes of each fertilizer. For instance, compared with chemical fertilizers, organic fertilizers often include less nitrogen and phosphorus, which makes them economically inferior per unit volume of the fertilizer. However, further economic analysis may reveal that the organic carbon content of organic fertilizer can be of equal to or greater than its nitrogen and phosphorus content. Furthermore, analyses of the nutrient actually show that what lowers or dilutes the amount of nitrogen and phosphorus in organic fertilizers is the moisture content, which is usually high in organic fertilizers. Thus, shipping high-moisture organic fertilizers across long distances may not be economically efficient or viable. Therefore, it is economically justifiable to use locally accessible sources of manure and to invest in analyzing the nutrient content of organic fertilizer to realize the full potential of the inputs.

There is also an economic argument that looks at the possible effect of low yields as a result of choosing organic over inorganic fertilizer. The question is whether the savings from using organic fertilizers compensate for the loss incurred from the low yields. On average, cost savings from fertilizer and chemicals cover 40% of the losses or extra cost incurred by lower yields and higher labor requirements. Considerable price premiums on organically produced farm products are needed to obtain renumeration of labor and capital at about the same level as in conventional agriculture.

More often, farmers select the type of farming system by considering profitability in the short run as well as the medium term. Thus, conventional farming, which depends on inorganic fertilizers, appears to be more profitable in the short run compared with organic farming, which depends on organic fertilizers. However, the long-term profitability of conventional farming systems seems questionable when hidden economic costs such as environmental and health ones are considered. Furthermore, when indirect costs such as soil erosion, pollution, and societal costs are factored into inorganic farming practices, the benefits to society from using organic fertilizers and organic farming would probably be higher.

From the production side, organic fertilizers offer numerous benefits that can be seen based on sustainability from both an economic and environmental aspect. Table 15.4 lists some economic benefits of these fertilizers to producers and the environment.

Regarding the multiple effects of using organic fertilizers, strong market demand for organic products has led to high premium prices for the products. Organic farms use organic

TABLE 15.4 Economic and environmental benefits of organic fertilizers.

Economics benefits	Environment benefits
Food products raised with natural organic fertilizers do not contain elevated levels of toxic chemicals. The food has a unique taste, which is attributed to higher mineral concentrations.	Organic fertilizers are renewable, biodegradable, and sustainable.
Natural fertilizers supply important micronutrients. These can increase the shelf life of the product after harvest.	Organics maintain the soil nutrient cycle. Organic fertilizers provide the continuous release of nutrients, resulting in a stable growth period.
It is cost-effective and less expensive.	Nutrient losses are limited to harvest.
They increase outputs, which means more income to the farmer.	Organic fertilizers build nutrients that naturally recharge the soil with high fertility
Like inorganic fertilizers, liquid organic fertilizers offer opportunities for more efficient nitrogen use when they are applied through a drip irrigation system (fertigation).	Improves soil texture, structure, and aeration. It also increases the soil's water-holding capacity.
	There is no risk of the accumulation of toxic chemicals and salts.

fertilizers intensively. Thus, their spending on fertilizers and sprays is significantly lower. Depending on the enterprise, the use of organic fertilizers often results in savings in variable costs related to fertilizers and plant-protection costs of 30%–50%. Because of the time and effort required for the use of organic-based fertilizers, some farmers may be unwilling to implement it, because it involves carefully storing the fertilizer to avoid nutrient loss; precise application, which is proactive rather than reactive; and possible further amendment with inorganic fertilizers.

10. Conclusion

Vermicompost offers an interesting opportunity to promote organic soil fertility management, because feeding the soil is considered important to organic agriculture, as opposed to inorganic soil fertility management. Various aspects that are critical to developing optimized vermicompost and vermileachate have been presented, including the chemical composition of the original materials, the earthworm species and earthworm stocking density, and the dilution aspects of vermileachates. This chapter highlighted that although research on vermitechnology has been widely done, the results of the research are variable, especially in terms of the nutritional composition. There is a need to create standard operating procedures for preparing these nutrient sources to develop nutrient-predictable organic fertilizers. There is also a need for research that focuses on combining these organic fertilizers with inorganic fertilizers so their complementary effects on crop and soil nutrition can be observed.

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CHAPTER

16

Emerging pollutants in waste: occurrence, impact, removal, and screening technologies

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1. Introduction

Increasing industrial manufacturing and consumption have generated the enormous release of heterogeneous waste each year, and thus a hazardous substance of great concern for human health and the environment, called emerging pollutants (EPs) (Marcoux et al., 2013). Many investigations have confirmed that pharmaceuticals (both human and veterinary), pesticides, textile waste, the food industry, agricultural practices, and the chemical industry are some of the main sources of EPs in nature (seawater, surface water, drinking water, wastewater, groundwater, and soil) (Gani et al., 2020; Jari et al., 2022; Necibi and Dhiba, 2021). These EPs are considered as one of the greatest challenges for scientists because of their heterogeneity, which further complicates their removal and management.

EPs are defined as a set of synthetic or natural compounds that are not monitored in the environment and that have recognized or suspected deleterious consequences for the environment and/or public health. Treated and untreated wastewater from sewage plants and industrial complex effluents, uncontrolled waste management, and the rural and urban runoff are all major generators of EPs (Alvarez, 2010). EPs include endocrine disruptors, pesticides, pharmaceuticals, nanoparticles, heavy metals, dyes, and microplastics (Gani et al., 2020; Ismail and Mokhtar, 2020; Otieno et al., 2020).

The outcome of these pollutants in waste is substantially unexplored. Nonetheless, available limited data concerning the destiny of these pollutants indicate that most of them persist in treatment and some are returned to their biologically active even after treatment (Alvarez, 2010). Despite their low concentration in nature, they create a global concern for human life and the environment. Furthermore, owing to their heterogeneity and the lack of sophisticated

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analytical techniques for detecting and disposing of these pollutants, their identification is severely lacking. With the development of technology and sophisticated analytical tools, scientists have expedited studies of the destiny and evolution of pollutants to deal with threats to public health and the ecosystem. In this respect, several technologies for treating EPs have been implemented to mitigate their effects, such as physical (adsorption), chemical (photocatalysis and ozonation), and biological techniques (wastewater activated sludge; membrane bioreactors [MBRs]; composting, especially vermicomposting; and bioremediation and/or phytoremediation) (Boshir et al., 2016; Cui et al., 2018; Fu et al., 2020; Li et al., 2021; Ismail and Mokhtar, 2020). Moreover, detection techniques such as liquid chromatography (LC), gas chromatography (GC), and inductively coupled plasma spectrometry (ICP) with mass spectrometry (MS) are widely preferred (Fu et al., 2020; Novak et al., 2014). Generally, monitoring EPs provides an overview of their threat to human health and the environment.

Ultimately, this chapter summarizes the different types of EPs and their risks to public health and the environment. Moreover, common removal technologies (biological, chemical, and physical), as well as screening methods that can be performed to handle and detect EPs such as pharmaceuticals, pesticides, hormones, chemical compounds, and plastics are discussed.

2. Emerging pollutants

EPs are synthetic or natural chemicals that are not necessarily new, but they are not regularly seen in the environment, and they can penetrate the environment and lead to known or suspected harmful effects on the environment and society (Geissen et al., 2015). These substances might be noticed in the environment but are not yet covered by surveillance plans (national and/or international), and their behavior and ecotoxicologic outcomes are not well-established (Fu et al., 2020). EPs exist in all kinds of products and applications. They may end up in various waste streams such as harmful and/or nonhazardous industrial solid waste, municipal solid waste, and municipal wastewater (Marcoux et al., 2013).

The sources and pathways of exposure of these EPs are largely related to waste and wastewater from industrial activities, agricultural practices, and municipal operations, and it can be found in a variety of environmental compartments such as atmosphere, soil, surface water and even biota (Fig. 16.1) (Gavrilescu et al., 2014; Petrie et al., 2014).

Several working groups of the "NORMAN" network identified about 970 EPs in the aquatic compartment in Europe and classified them into more than 20 categories according to their origins (http://www. norman-network.net). Major categories of EPs are pesticides, pharmaceuticals, industrial chemicals, metals, surfactants (used as detergents), and microplastics/microfibers as chemicals of high concern, which are commonly generated by decaying organic matter leading to the accumulation of persistent metabolites (Table 16.2). Likewise, they may be caused by agricultural practices leading to extensive farming and the application of manure and/or sludge on agricultural fields. Fig. 16.1 illustrates the pathways of some EPs.

Pesticides include phytosanitary products (for plant protection) on agricultural land, biocidal products (to prevent, destroy, repel, or mitigate pests such as insects or rodents,

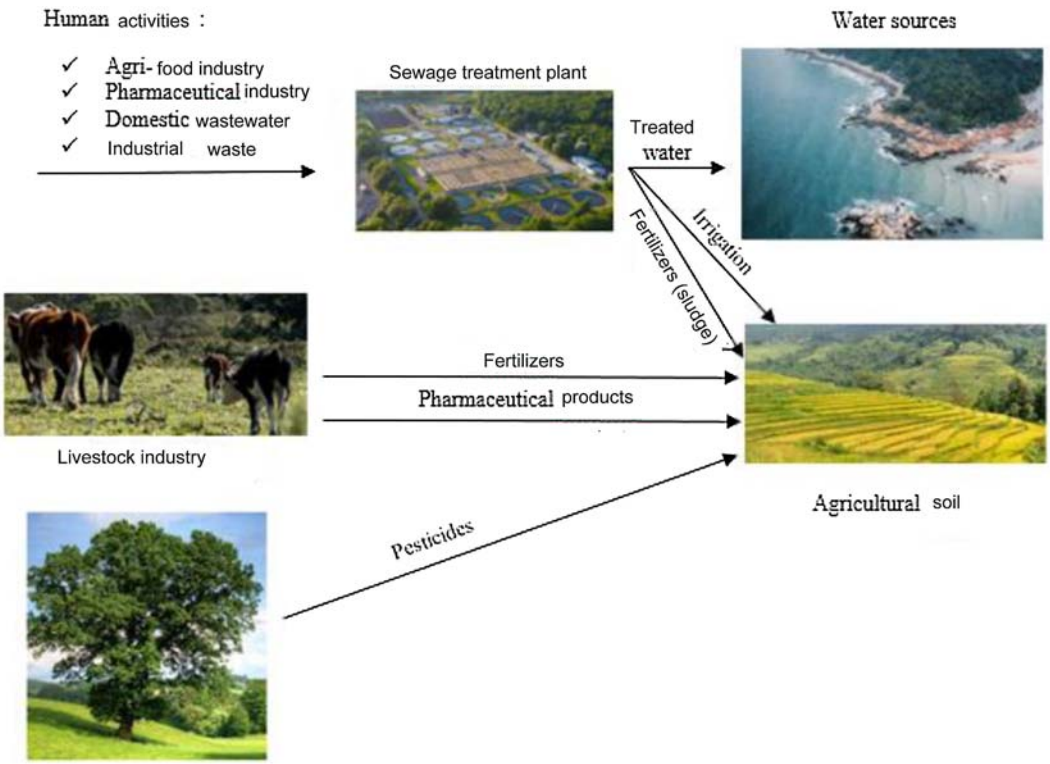

FIGURE 16.1 Pathways of several emerging pollutants, from their origins to their destinations.

or for wood production), as well as antiparasite products used on animals, such as flea control (Hassaan and Nemr, 2020; Mariane et al., 2020). These products are extensively used professional settings as well as in our daily environment throughout the world. Pesticides include more than 1000 substances that are heterogeneous in their chemical structure, properties, and mode of action on target organisms (Ahmed et al., 2018; Jari et al., 2022). They can be categorized according to their targets as herbicides, insecticides, fungicides, and bactericides.

Pharmaceuticals are chemical, natural, or synthetic compounds used for medical purposes to cure and prevent disease, to protect public health and/or for veterinary purposes (Couto et al., 2019; Jari et al., 2022). They are generally composed of one or more excipients and active substances. They might be categorized according to their therapeutic class, such as analgesics, antibiotics, antiinflammatories, lipid-lowering drugs, and antidepressants. However, in the environment, the presence of these compounds may cause harmful effects to fauna and flora. This is the case of wastewater discharged from hospitals and other medical facilities, for example (Couto et al., 2019; Geissen et al., 2015; de Oliveira et al., 2019).

Industrial activities are a potential source of organic pollutants originating from dyes, pH regulators, salts, sulfides, toxic products, and refractory organic products (Talouizte et al., 2017; Wijetunga et al., 2010). As an example, cardboard containers are typically produced

TABLE 16.1 Removal technologies performed to degrade some emerging pollutants.

	Treatment mode		Emerging pollutants	References
Biological treatment	Activated sludge		Endocrine disruptors, plasticizer bisphenol A	Boshir et al. (2016), Necibi and Dhiba (2021), Otieno et al. (2020)
	Membrane bioreactors	*Streptomyces* sp. *Sphingomonas xenophaga* *Shewanella* sp. XB	Naphthalene, bromoamine, Acid orange 7 dye	Talouizte (2014)
		–	Stigmastanol, coprostanol, and cholesterol	Jari et al. (2022), Langbehn et al. (2021)
	Sequential batch reactors		Heavy metals (Cu, Cr, Zn, Ni, Cd), recalcitrant compounds, aliphatic and aromatic compounds	Talouizte et al. (2017)
	Composting	*Bacillus*, *Pseudomonas*, *Klebsiella*, *Escherichia*, *Staphylococcus*, *Morganella*	Heavy metals (Cu, Cr, Zn, Ni, Cd), recalcitrant compounds	Biyada et al. (2020a, 2022b)
	Bioremediation (bioaugmentation)	*Aspergillus* sp., *Pseudomonas aeruginosa*, *Sphingobium chlorophenolicum*, *Burkholderia* sp., *Rhizopus* sp., *Penicillium funiculosum*, *Aspergillus sydowii*, *Stenotrophomonas maltophila*, *Pseudomonas studzeri*, *Dracaena sanderiana*	Chlorobenzene, aliphatic and aromatic hydrocarbons, 4-chloronitrobenzene, petroleum hydrocarbons, Acid orange 7 and 6, Mordant yellow 10, Methyl orange, 4-aminobenzene-4′-sulphonic acid, amaranth, tetrazine, Allura red, bisphenol A	Chaîneau et al. (2002), Mrozik and Piotrowska-seget (2010), Nzila et al. (2016)
	Phytoremediation	*Pistia stratiotes* L. (Araceae)	Bisphenol A, atrazine	Silva et al. (2020)
Chemical treatment	Photocatalysis		Acetamiprid,	Antonopoulou et al. (2020), Boshir et al. (2016), Guerra et al. (2009), Kamdem et al. (2021), Mariane et al. (2020), de Oliveira et al. (2019), Segura et al. (2013)
	Ozonation		Atrazine, paracetamol, bisphenol A	
	Electrochemical		Heavy metals (Cu, Cr, Zn, Ni, Cd)	
Physical treatment	Adsorption	Activated carbon argan nut shells, clay	Bisphenol A	Benjelloun et al. (2021), Chauhan et al. (2020),

TABLE 16.1 Removal technologies performed to degrade some emerging pollutants.—cont'd

Treatment mode		Emerging pollutants	References
	Eucalyptus bark; P-doped biochar	Atrazine	Mpatani et al. (2021), Suo et al. (2019)
	Coated natural montmorillonite clay; coffee-based biomaterial; silica gel	Paracetamol	
	Rock phosphate by-products; activated carbon from date pits	Amoxicillin	
	Corn stalk-walnut shell mix- based activated carbon; biomass compound of pine; pyrophyllite; almond shell activated carbon	Methylene blue, iodine, Malachite green, Crystal violet	Benjelloun et al. (2021)

from recycled books and can be affected by the printing ink and its constituents, such as polycyclic. This is also true for the textile industry (textile waste effluents and solid textile waste), which is known for the predominance of benzoic acid derivatives from textile dyes. These compounds are stable and persist in the environment (Biyada et al., 2022b). It is also the case for plastic waste, which has various EPs such as bisphenol A (BPA), which is prohibited in food containers and packaging (Ionela et al., 2021; Sheng et al., 2018).

3. Impact of emerging pollutants on environment and public health

The widespread presence of EPs and their potential impact on the environment and public health has attracted worldwide attention. In addition, there is growing concern owing to the lack of research, effective monitoring programs, regulation, and awareness (Malhat and Nasr, 2011). Thus, several authors proved that the EPs have an adverse effect on the growth, multiplication, and development of species in the environment (Fig. 16.2) (Ionela et al., 2021; Jari et al., 2022; Gavrilescu et al., 2014). A wide range of these compounds are used in various sectors. For example, endocrine-disrupting compounds, antibiotics, antidiabetic, antiepileptic drugs, BPA, and pesticides are considered to be toxic and threaten aquatic and terrestrial organisms. Their effects include genotoxicity, carcinogenicity in laboratory animals, and immune toxicity. EPs are frequently detected at various concentrations in groundwater, drinking water, the soil, and the food chain. The concern is that toxicity of EPs to the environment and humans has not yet been explored. In addition, the passage of these pollutants through drinking water treatment systems generates by-products whose chemical properties are still undetermined.

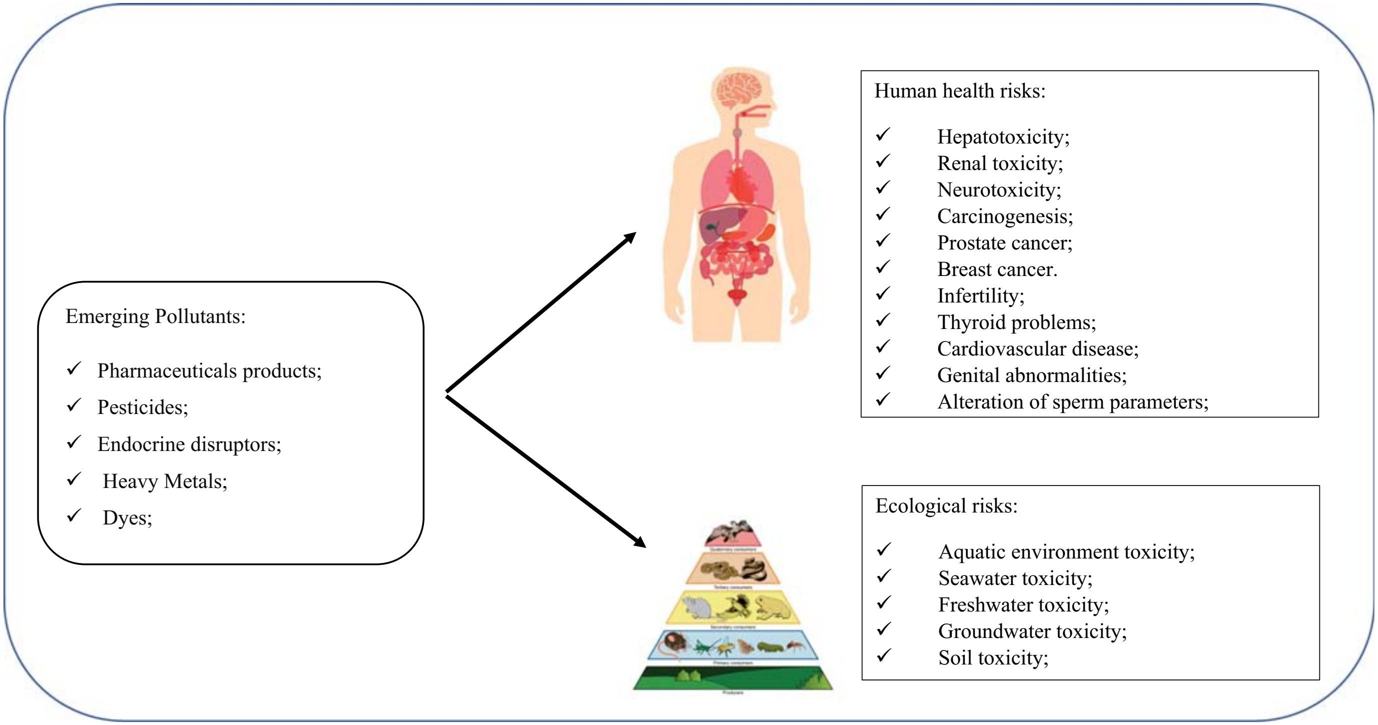

FIGURE 16.2 Exposure to emerging pollutants and outcomes for health and the environment (Ahmed et al., 2018; Cernanský et al., 2019; de Albuquerque et al., 2019; Mariane et al., 2020).

TABLE 16.2 Technologies used for to extract samples and detect emerging pollutants.

Major categories of emerging pollutants	Emerging pollutants	Extraction methods	Instrumental analysis	Samples	References
Pesticides	Fungicides Herbicides Insecticides Organochlorine pesticides, organophosphates, and carbamates Pyrethroids Neonicotinoids and heterocyclic pesticides	Ultrasound-assisted extraction SPE	LC-MS-MS GC-CG GC-MS-MS LC-HRMS (High Resolution Mass Spectrometry)-MS HPLC-MS	Surface water, soil samples, agriculture land, groundwater and stormwater runoff	Biyada (2022), Guerra et al. (2009), Hird et al. (2014), Jover and Bayona (2010)
Pharmaceuticals	Illicit drugs Preservative agents Personal care products Veterinary drugs Amoxicillin Paracetamol Antibiotics Hormones Steroids Antiinflammatory drugs Endocrine active compounds Parabens Methyl-triclosan Chlorobenzene, fluoxetine Diazepam, lipid regulators Ibuprofen Mefenamic acid	Soxhlet extraction SPE	Fourier Transform-Near InfraRed (FT-NIR) LC-MS-MS UHPLC/MS/MS GC/GC GC/MS	Wastewater, treated water, surface water, seawater, drinking water, solid waste	Albero et al. (2017), Couto et al. (2019), Jover et al. (2009), Lima et al. (2013), Novak et al. (2014), de Oliveira et al. (2019)
Microplastics	Plasticizers; Bisphenol A Polychlorinated biphenyl Polyvinyl chloride	SPE	GC-MS-MS LC-MS-MS	Seawater and water samples	Albero et al. (2017), Ismail and Mokhtar (2020), Wang et al. (2021)
Metals	Metal contaminants Mercury, Trace metals Heavy metals (Cr, Cu, Cd, Ni)	Microwave assisted digestion Liquid-liquid extraction (LLE)	ICP-MS HPLC-ICP-MS GC-ICP-MS	Wastewater, soil samples, solid waste, surface water, and seawater	Jia et al. (2019), Jover and Bayona (2010), Lu et al. (2019)

(*Continued*)

TABLE 16.2 Technologies used for to extract samples and detect emerging pollutants.—cont'd

Major categories of emerging pollutants	Emerging pollutants	Extraction methods	Instrumental analysis	Samples	References
		Microwave-assisted enzymatic extraction			
Organic waste	Recalcitrant pollutants		FT-NIR	Wastewater, soil, and solid waste	Biyada et al. (2021)
Industrial chemicals	Brominated chlorinated flame retardants Flame retardants Perfluorinated compounds, polybrominated diphenyl ethers	SPE Ultrasound-assisted extraction	.LC-HRMS-MS GC-MS-MS	Surface water and wastewater	Boshir et al. (2016), Fu et al. (2020), Novak et al. (2014)
	Artificial sweeteners	SPE	LC-HRMS-MS	Surface water	Ismail and Mokhtar (2020)
Dyes	Crystal violet Basic green Acid orange 7 and 6, Mordant yellow 10 Methyl orange 4-aminobenzene-4′-sulphonic acid, amaranth	—	GC-MS	Textile wastewater	Benjelloun et al. (2021)

GC, gas chromatography; *HPLC*, high-performance liquid chromatography; *ICP*, inductively coupled plasma spectrometry; *LC*, liquid chromatography; *MS*, mass spectrometry; *SPE*, solid-phase extraction; *UHPLC*, ultra-HPLC.

Pesticides used in agriculture, for example, are the main contaminants that threaten the health of the soil, especially its biota (Ismail and Mokhtar, 2020). Often, not just the pesticide but also its metabolites, which are detected at higher concentrations, are increasingly interesting because of their increased toxicity on soil microflora. Public concerns about pesticide exposure have been growing (Ahmed et al., 2018). Links have been made between exposure to pesticides and certain pathologies such as lymphomas, childhood cancers, Parkinson's disease, and malformations. Moreover, the endocrine-disrupting effects of certain pesticides raise questions about consequences for health (Ahmed et al., 2018; Fu et al., 2020; Otieno et al., 2020). Pesticide contamination of the environment (water, food, soil, and air) is also a public concern because it leads to decreased habitat heterogeneity and interferes with ecosystem dynamics and nutrient cycling (Otieno et al., 2020). Therefore, pesticides such as organochlorine compounds have been identified in surface water and groundwater and in the food chain. These compounds are known for high toxicity, bioaccumulation and/or biomagnification, and persistence, and represent a serious ecological risk to aquatic ecosystems, mainly for arthropod invertebrates (*Daphnia*) (Ahmed et al., 2018). Although they are administered on the ground, these compounds can be transmitted beyond the field into water bodies by surface runoff and transpiration into soil, altering water quality by threatening aquatic ecosystems and human health (Ahmed et al., 2018). Although investigations into the toxicity of chemicals alone exist, information about the concentrations and effects of pesticide on soil biota is scarce.

As an example of pesticides, atrazine is an herbicide employed in various crops to control weeds (Ali et al., 2017). Despite the positive effect, this compound may directly or indirectly influence other species. This is also true for aquatic ecosystems and plants (de Albuquerque et al., 2019; Silva et al., 2020). Atrazine is considered an endocrine-disruptor compound that may impair male reproduction tissues as a result of animals being exposed during development. Furthermore, atrazine has been found to be a DNA methylating agent in the carp brain and to induce autophagy in the liver (Vanraes et al., 2015). It may also cause mammary and vaginal cancers and may damage the human vascular system (Jari et al., 2022).

The problem of pharmaceutical residues in the environment and more particularly in potable water is becoming an increasingly prominent concern (Couto et al., 2019). These residues are detected in the environment in soil, the food chain, wastewater, surface water, groundwater, and seawater at different intensities in the form of parent molecules (identical to the ingested molecule) or metabolites (compounds formed by metabolization of the parent molecule in the body), or even in the form of transformation compounds (by ultraviolet, bacteria, and so on, in the environment) (Petrie et al., 2014). Most of these molecules are extremely soluble in water, and conventional wastewater treatment processes are not able to delete these contaminants (Jari et al., 2022). A major consequence of the availability of these molecules is that they act on the endocrine system by imitating, abolishing, or modifying the function of hormones (Cernanský et al., 2019). In the aquatic habitat, the presence of an antibiotic such as amoxicillin, which is suspected of being mutagen, carcinogen, and teratogen at a greater dose, may lead to ecosystem dysfunction by provoking toxic effects and thus to abnormalities in the anatomy of aquatic biota and altering plant growth (Meng et al., 2017; Yang et al., 2020). In addition, estrogenic pharmaceutical compounds reduce vitellogenin levels in male fish as well as vitellogenin and estradiol in the plasma of female fish. They are primarily linked to immature gonads and a weaker gonadosomatic index in adult females, and histologic changes such as germ cell degeneration (de Oliveira et al., 2019).

In terms of human health, many pharmaceutical compounds have the ability to trigger adverse outcomes such as allergies, lung disease, and cancer (Couto et al., 2019). Another major problem is the spread of resistance to antibiotics such as erythromycin, ofloxacin, sulfamethoxazole, clarithromycin, amoxicillin, tetracycline, and azithromycin in humans. This is significantly associated with a change in the bacterial ecology and thus the development of antibiotic-resistant bacteria, which have made medicine inefficient for therapy of a wide range of infectious illnesses (Couto et al., 2019). As another example, paracetamol has undesirable effects when taken at an overdose, inducing the development of mammary cancer cells. This toxicity is generally ascribed to reactive oxygen species, leading to a wide range of outcomes from protein denaturation to lipid peroxidation and DNA damage (Parolini, 2020). In addition, according to the European Union, xenobiotics can disrupt physiologic hormonal systems and affect the uterus, leading to long-term destruction and potentially severe consequences for future generations (de Oliveira et al., 2019).

4. Treatments for emerging pollutant removal

4.1 Biological treatments

Environmental threats and risks associated with the accumulation of chemically and biologically toxic micropollutants might be reduced or excluded by using various biotechnologies (Ismail and Mokhtar, 2020). Biological treatment is an extensive technology for treating and valorizing wastewater and solid waste, including a wide range of EPs. It has numerous benefits in terms of environmental friendliness and affordability, but it requires well-controlled processing conditions, which take time (Benjelloun et al., 2021; Mpatani et al., 2021). These methods mainly depend on the occurrence of aerobic and/or anaerobic microorganisms that oxidize and/or incorporate EPs into their cells (Jari et al., 2022).

Several scientists referred the biodegradation of EPs in wastewater using various systems such as activated sludge which is mainly used in wastewater treatment plants, membrane bioreactors (MBRs), sequential batch reactors (SBR) using for the treatment of leachate, tannery effluents and textile effluents …etc, and/or phytoremediation in one hand (Achak et al., 2011; Talouizte et al., 2017). On the other hand, composting and bioremediation technologies are widely used to treat EPs in solid waste (Biyada et al., 2022a). The behavior of EPs throughout biological processing is the main concern, especially for persistent compounds that may migrate through processing and have a hostile effect on the environment. Table 16.1 lists biological treatments performed to remove some EPs.

For the case of wastewater, the most commonly established biotreatment is activated sludge. Wastewater activated sludge is composed of naturally existing microorganisms that are effective in digesting several kinds of EPs (Nzila et al., 2016). Owing to the resistance of some EPs, investigations have been conducted to isolate microorganisms (bacteria and fungi) directly from activated sludge, which are known to be efficient in degrading a large variety of EPs with metabolic enzymes such as endocrine disruptors, antibiotics, and plasticizer BPA, and to reinoculate them in pure culture as well as consortia using the bioaugmentation test to treat wastewater through enzymatic activities (Langbehn et al., 2021; Mpatani et al., 2021; Wang et al., 2021). The use of these microorganisms offers great potential to remove EPs effectively.

MBRs are based on a biological treatment coupled with membrane separation (physical treatment). They retain the biomass and produce a treated effluent free of suspended solids and microorganisms (bacteria and viruses) (Talouizte, 2014). The ultrafiltration or microfiltration membrane replaces the secondary settling usually employed in conventional wastewater treatment plants. MBRs are considered a potential treatment option for conventional wastewater treatment plants, especially to remove pharmaceuticals, because they are effective for removing EPs (recalcitrant pollutants, antibiotics, etc.) and produce a better quality of treated effluent that can be neither removed nor biodegraded in activated sludge systems. They have an elimination effectiveness of over 96% for stigmastanol, coprostanol, and cholesterol, and 90% for erythromycin, tetracycline, ofloxacin, and chlortetracycline, compared with 85% with a conventional treatment facility (Jari et al., 2022; Langbehn et al., 2021). An alternative technology for environmental remediation, phytoremediation biotechnology, primarily uses plants to sequester, remove, and transfer toxic molecules. It has been implemented successfully to remove a broad variety of EPs such as BPA, atrazine, and organic contaminants (Silva et al., 2020). For this purpose, aquatic macrophytes are widely used, with the strong potential for phytoremediation in an aquatic environment, especially *Pistia stratiotes* L. (Araceae) (Mpatani et al., 2021; Silva et al., 2020).

For EPs in solid waste, studies have proven the effectiveness of biological treatment such as composting and bioremediation (bioaugmentation) in decaying EPs and removing persistent EPs in soil (Mrozik and Piotrowska-seget, 2010; Nzila et al., 2016). Composting as a sustainable biological process for organic waste has been successfully employed to remove a variety of EPs such as recalcitrant pollutants and heavy metals using selected microorganisms (Biyada et al., 2022b). Vermicomposting has been widely used to break down organic compounds, including recalcitrant pollutants. Earthworms modify the physicochemical composition of organic materials through their enzymatic systems by gut digestion, degrading organic substrates even after their secretion (Ahmad and Aslam, 2022). In addition, research has been conducted to prove the effectiveness of vermicomposting in mitigating and minimizing antibiotic-resistant genes, such as extracting tetracycline, sulfide, oxytetracycline, and quinolone from excess activated sludge. The latter is considered a store of many EPs. In addition, Cui et al. (2018, 2019) reported the effectiveness of inoculating *Eisenia fetida* to alleviate quinolone-resistant genes, and *Eudrilus eugeniae* for the bioaccumulation of heavy metals. Vermicomposting as an ecological process is effective in mitigating toxic chemicals (heavy metals and antibiotics) in organic waste (Li et al., 2020, 2021).

Bioremediation, is described a technique that improves the ability to clean areas polluted by EPs through the use of pure strains and consortia (endogenous and exogenous) of specific competent microorganisms. It is a promising technology for improving the degradability of pollutants, even recalcitrant compounds, heavy metals, phenol, and naphthalene, as well as carbazole, dibenzofuran, and dibenzothiophene. Several studies have been devoted to bioaugmentation technologies applied to treat wastewater (Mrozik and Piotrowska-seget, 2010; Nzila et al., 2016). Table 16.1 lists approaches to bioaugmentation for EP removal. Antônio et al. (2021) and Biyada et al. (2021, 2022a) indicated the effectiveness of composting and bioaugmentation for treating solid waste such as textile, agroindustrial, and sludge waste, which are rich EPs (recalcitrant molecules, heavy metals, dyes, etc.), with a removal effectiveness of greater than 98%.

The biodegradation of micropollutants relies on various requirements concerning both abiotic and biotic criteria such as the nature of the EP and its persistence, the environmental conditions of the microorganisms, and the process conditions. The entire lifecycle of EPs should also be considered, from their origin to their processing. The combination of these factors enables the mineralization, transformation, or immobilization of these contaminants.

4.2 Physical treatments

Physical treatment technologies are considered to be the most successful technologies for removing a broad range of EPs from water and wastewater owing to their adaptability, simplicity, and great effectiveness (Mpatani et al., 2021). Various physical treatments have been employed to remove EPs from water resources. The most widely used treatment is adsorption, owing to various benefits such as low cost, environmental friendliness, and ease of conception and operation (Suo et al., 2019).

Adsorption is a mass exchange treatment that requires mass shifting of the adsorbate from a liquid or gas state to the surface of the adsorbent (Suo et al., 2019). Adsorption mechanisms relate to either physical adsorption and/or ion exchange and chemical adsorption (Benjelloun et al., 2021). Owing to its simplicity, environmental friendliness, effectiveness, and low cost, adsorption is extensively performed to remove various EPs from wastewater. Furthermore, as in other techniques, adsorption may be influenced by several factors such as the nature and concentration of the adsorbate and/or adsorbent and physicochemical parameters (pH, contact time, temperature, etc.).

Several studies have explored abolishing EPs (inorganic, organic, and biological contaminants) using adsorption. Materials used and examined to study purification capacity and efficiency in EP removal include activated carbon, activated biochar, clay, zeolites, silica gel, chitosan, metal-organic frameworks, polymers, agricultural waste and by-products, biosorbents, and composite materials (Jari et al., 2022). Many studies have proven the effectiveness of activated carbon from argan nut shells for binding BPA (Mpatani et al., 2021). Suo et al. (2019), Vanraes et al. (2015) reported that 96% of atrazine was removed using P-doped biochar from corn straw. Another researcher used eucalyptus bark to remove atrazine (Mpatani et al., 2021) reported the effectiveness of agricultural waste binders for BPA uptake. Furthermore, Benjelloun et al. (2021) reported the use of adsorption as an efficacious method to remove dyes such as: methylene blue iodine; malachite green ….etc; using different types of absorbents such as activated charcoal based on mixture of corn stalks and walnut shells; pine biomass compound; pyrophyllite; almond shell activated charcoal …etc.

For pharmaceuticals, Chauhan et al. (2020) examined abolishing paracetamol, amoxicillin, imipramine, and diclofenac-sodium using coated natural montmorillonite clay, and removing amoxicillin and other organic micropollutants using rock phosphate by-products. Other investigations have used waste materials as inexpensive adsorbents. Table 16.1 lists adsorbent materials applied to remove some EPs.

4.3 Chemical treatments

Advanced oxidation methods such as Fenton reactions, photocatalytic oxidation, ozonation, and electrochemical oxidation reactions are effective in removing EPs that are not conveniently treated by standard physicochemical and biological methods (Ismail and Mokhtar, 2020). Table 16.1 lists frequently used advanced oxidation technologies.

Photocatalysis is a sustainable process for treating organic pollutants in wastewater through the implementation of photocatalysts that may be triggered by light exposure (Antonopoulou et al., 2020). This method is based on the interaction of organic pollutants with powerful oxidizing and reducing agents (h^+ and e−) emitted by a light supply, which may be solar energy, on the surface of photocatalysts (Kamdem et al., 2021). The crucial element in this procedure is the photocatalyst, which transforms solar energy into chemical energy and eliminates pollutants (Antonopoulou et al., 2020). Furthermore, the decaying efficiency of photocatalytic conversion depends on several parameters such as the exposure intensity and the type of irradiation, temperature, pH, the radiant flux and the oxygen concentration, the type and mass of the catalyst, and the relative initial content of the pollutant (Antonopoulou et al., 2020).

Many photocatalytic technologies have been used to process harmful pollutants in wastewater. Research has also been conducted to explore various photocatalytic such as oxides and perovskites (e.g., WO_3, V_2O_5, $BiVO_4$, ZnO, and $SrTiO_3$), bismuth oxyhalides (e.g., BiOCl, BiOBr, and BiOI), and sulfides (e.g., CdS, ZnS, and MoS_2) as photocatalytic materials for wastewater treatment. In the case of pesticides such as acetamiprid, several authors have proven the potential of graphene oxide–doped metal ferrites in degradation. For pharmaceutical products, photocatalysts such as graphene oxide–decorated $ZnWO^4$ are widely used (Antonopoulou et al., 2020; Kamdem et al., 2021).

Ozonation also has relatively good performance for removing EPs. This method is based on employing ozone as an oxidizing agent (i.e., the transformation of ozone into oxygen after contact with pollutants) (Huang et al., 2019; Segura et al., 2013). Ozone is a highly potent oxidant that interacts specifically with the double bonds and aromatic cycles of electron-dense EPs. Several investigations have proven its efficiency in removing EPs: by 100% for BPA, 94% for paracetamol, and 95–100% for the degradation of pesticides such as atrazine, chlorfenvinphos, diuron, and isobroturum (Boshir et al., 2016).

Electrochemical oxidation (electron transfer), is frequently used to enhance the production of hydroxyl radicals and enable the full oxidation of a wide range of organic compounds within wastewater (Guerra et al., 2009).

5. Analytical detection methods for emerging contaminants

To understand, regulate, and monitor the destiny and transport of EPs in the ecosystem, rapid, sensitive, and multiple analytical techniques are needed. Scientists consider the concentration of samples for trace EP detection to be a huge challenge. To achieve this step, traditional approaches such as Soxhlet, solid-phase extraction, pressurized liquid extraction, and ultrasound-assisted extraction as well as the combination of solid phase extraction and dispersive liquid–liquid microextraction are increasingly used to reconcentrate the different EPs. Therefore, the evaluation of these contaminants is strongly contingent on the quality of the analytical procedures applied.

Conventional methods such as GC, high-performance LC (HPLC), LC with tandem MS (LC-MS/MS) and ICP-MS enable the determination and specification of several classes of EPs. Also, multiresidue detection is becoming increasingly popular. LC-MS/MS screening is a sensitive detection method for monitoring and quantifying EPs in environmental samples at trace levels. Nonpolar, thermostable, and volatile EPs, such as pesticides are identified by GC, whereas nonvolatile, polar, and thermolabile EPs are evaluated by LC (Ismail and Mokhtar, 2020).

5.1 Mass spectrometry

MS has been widely used as an efficient method to detect, characterize, and quantify compounds or residues in the environment using mass over charge (m/z) by fragmentation (Biyada, 2022). Mass spectrometry is privileged owing to its strong analytical sensitivity, ruggedness, and selectivity for quantitation, even at trace levels in complex matrices. Its fields of application are numerous, including EP detection in pharmaceuticals and in studies of environmental pollution (drinking water, groundwater, surface water, and wastewater), pesticides, pigments, and dyes (Smith and Lansing, 2013; Montemurro et al., 2017). MS is widely coupled with other separation methods such as LC and GC, which makes it more efficient (Albero et al., 2017; Hird et al., 2014).

5.2 High-performance liquid chromatography

HPLC and LC separate and purify one or more compounds, even trace ones, from a mixture to identify and quantify them, which makes it possible to perform analyses with thin-layer or gas phase techniques (HPLC Principe, 2010). This method is widely used in many fields, including industry, pharmaceutics, veterinary, environmental, and biology. HPLC is a typical analytical technique used to evaluate a broad range of EPs that are polar and nonvolatile in assorted samples (Table 16.2) (Hernández et al., 2014; Hird et al., 2014). Ultra-HPLC (UHPLC) uses a separation approach compared with traditional HPLC across various application fields (Hernández et al., 2014), this is owing to its efficiency, high resolution, speed of analysis, robustness, reliability (Ismail and Mokhtar, 2020). To ensure proper analysis, LC or UHPLC is extensively coupled with MS, which successfully detects highly polar, thermally unstable, and high−molecular weight compounds for further improvements in selectivity, sensitivity, and high-throughput to measure complicated samples (Guerra et al., 2009; Hernández et al., 2014).

5.3 Gas chromatography

GC is an analytical technique employed to separate, detect, and identify chemical compounds (organic molecules or gases) of any sample to determine their presence, absence, or quantities (Albero et al., 2017). To obtain a satisfactory result, samples should be volatile and thermally stable to avoid degradation throughout analysis, commonly with a molecular weight below 1250 Da (Jover et al., 2009). GC is widely used across most industries to monitor the quality and safety of the final product, such as petrochemicals, pharmaceuticals, environmental samples, microplastics, and food (Jover and Bayona, 2010). To determine EPs, GC is usually coupled with MS to obtain accurate results. Several authors suggested that GC-MS/MS offers high selectivity and sensitivity compared with GC-MS (Albero et al., 2017; Jover et al., 2009; Jover and Bayona, 2010). The implementation of the MS/MS enhances analysis particularly for complex samples by reducing the interference of extracting compounds and sample matrix effects (Ismail and Mokhtar, 2020). Several investigations used GC-MS/MS to identify organic and emerging organic pollutants in seawater samples, hormones, plasticizers, personal care products, pharmaceuticals, herbicides, triazines, organochlorine biocides, and organophosphorus in wastewater and soil samples (Albero et al., 2017; Jover and Bayona, 2010; Lima et al., 2013). Scientists used two-dimensional GC (GC × GC), which is effective with a separation of 150−250 pertinent molecules with strong sensitivity in a complicated sample (Jover et al., 2009; Jover and Bayona, 2010). GC × GC increases the chromatographic resolution, enhancing analyte deployability owing to cryofocusing that occurs in the thermal modulator, and chemical ordering in the contour plots (Ismail and Mokhtar, 2020).

5.4 Inductively coupled plasma spectrometry with mass spectrometry

Heavy metals, which are part of EPs, are steadily being introduced into the environment. ICP is recognized as a suitable technique to measure the content of an inorganic element in a sample, specifically heavy metal pollutants and nanoparticles (Eesthacht et al., 2012). This method is broadly adaptable to almost all types of elemental chemicals. ICP-MS is recognized as the most accurate method for the multielemental detection of trace heavy metal in several elemental samples. It is a highly accurate and inexpensive technique with the multielemental and concurrent measurement of most elements and isotopes contained in the periodic table within minutes (Lu et al., 2019). This method exhibits exceedingly tight detection ranges from subparts per billion to trillion for most elements. Moreover, ICP can be coupled with chromatographic techniques (Gas chromatography GC and HPLC) as a great tool for determining impairment and decomposition molecules in the pharmaceutical field (Jia et al., 2019; Novak et al., 2014). Investigations have been conducted on the applying ICP-MS to the analysis of heavy metals such as Cu, Cd, Cr, Ni, and Pb in textile waste, tannery waste, wastewater, and seawater samples (Biyada et al., 2020b, 2022b). Other studies used GC-ICP-MS to recognize six polybrominated diphenyl ether condensers in water samples, and even mercury in water samples by HPLC-ICP-MS (Jia et al., 2019).

6. Conclusion

Various stakeholders, including environmental, biodiversity, and human health researchers, are increasingly concerned about the widespread presence of EPs and their impact on target and nontarget organisms. In addition, there is a dearth of data concerning regulations on the manufacture, transport, and use of these chemicals, information that is essential to assess environmental risk. There is also a scarcity of data on potential genotoxicity and mutagenicity, biochemical changes, and reproductive and endocrine effects after exposure to PEs. Predictive toxicology based on experimental data on behavioral, physiologic, and developmental responses to EPs is essential to develop and promote better management of EPs. Holistic research focusing on the impact of EPs on biodiversity is recommended to understand the deleterious effect of these contaminants. In addition, most studies have identified available concentrations of chemicals of concern, but concentrations corresponding to those found in the environment have rarely been tested. With the availability of sophisticated treatment and analytical tools, combined with faster and more accurate methods, the accumulation of these compounds in the environment, including in biota, may be more accurately assessed.

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CHAPTER

17

Analysis of carbon emissions in composting and vermicomposting of excess sludge

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1. Introduction

During the treatment of municipal wastewater, a large amount of excess sludge is produced, which it is difficult to treat and discard. It is estimated that approximately 60 million tons of sludge were produced in China in 2020 (containing 80% moisture content) (Liang et al., 2021). On the one hand, sludge is rich in nutrients such as carbon, nitrogen, and phosphorus, which makes it a solid waste with great recycling value (Zhang et al., 2022). The greenhouse gas effect caused by carbon dioxide and methane gas from carbon and nitrogen emissions is particularly severe, which poses a serious challenge to sludge treatment technologies. However, traditional sludge treatment technologies such as landfill and incineration waste resources and also emit a large amount of greenhouse gases, causing a series of ecological issues, which goes against the concept of carbon neutrality (Liang et al., 2021; Xu et al., 2021).

Previous studies reported that aerobic composting and vermicomposting consume less energy than other sludge treatment methods and effectively recover resources present in sludge (Bhat et al., 2018; Lim et al., 2015). Vermicomposting is a competitive, sustainable, and environmentally friendly approach to treating excess sludge effectively (Georgi et al., 2022). In addition, vermicomposting degrades organic matter through the joint actions of earthworms and microorganisms (Vuković et al., 2021). Compared with aerobic compost, vermicompost contains more N and P nutrients and has higher agricultural value. However, research on carbon emissions from the two sludge treatments primarily focused on aerobic composting (Xu

https://doi.org/10.1016/B978-0-443-16050-9.00009-8

et al., 2021; Piippo et al.,2017). There are only a handful of reports calculating carbon emissions during vermicomposting.

Therefore, this work calculated carbon emissions generated during the whole process of vermicomposting sludge and compared the results with those of the aerobic composting of sludge to understand the key origins of carbon emissions during sludge treatment. For this purpose, a feasible and systematic sludge vermicomposting process was proposed. The study aimed to provide a theoretical basis and reference for reducing carbon emissions in sludge treatment.

2. Experimental methods

2.1 Objectives and scope

To compare the carbon emissions of two sludge composting technologies, this study took 1 ton of excess sludge (80% water content) from a wastewater treatment plant as the research object. The two sludge composting technologies investigated were aerobic composting and vermicomposting. The process flow and carbon emission accounting pathways of the two methods are shown in Fig. 17.1. Carbon emissions from various sludge treatment paths included direct carbon emissions from a local anaerobic unit during aerobic composting and earthworms as well as indirect carbon emissions from the consumption of energy and pharmaceuticals. The CO_2 produced by sludge resulting from fermentation, earthworms, and other biological causes is not included in the carbon emissions. Other greenhouse gases from the sludge treatment process, such as methane (CH_4) and nitrous oxide (N_2O), were calculated by converting them into CO_2-equivalent emissions based on a 100-year global warming potential (GWP). According to the accounting criterion given in the Guidelines for National Greenhouse Gas Inventories published by the Intergovernmental Panel on Climate Change (IPCC) in 2019, a mass balance model was adopted to evaluate carbon emissions from sludge treatment pathways (Wang et al., 2022).

2.2 Calculation methods and parameters

2.2.1 Carbon emissions from aerobic composting

2.2.1.1 Direct emissions

Natural ventilated strip aerobic composting was adopted in the current work. As biogenic carbon, CO_2 from aerobic composting was excluded from the total carbon emissions, whereas

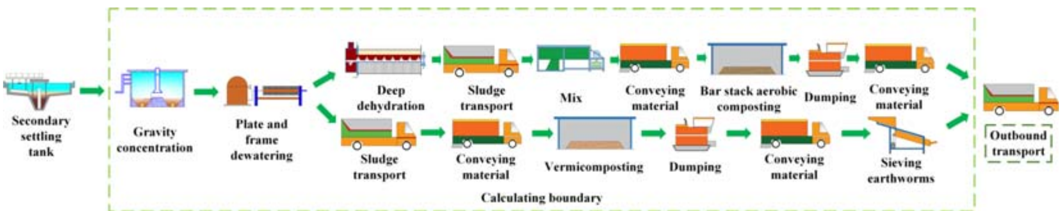

FIGURE 17.1 Process flow diagram of composting and vermicomposting.

direct emissions of CH_4 and N_2O were calculated using emission factors recommended by IPCC (Li et al., 2023):

$$E_{CH4,\ Aerobic\ composting} = m \times EF_{CH4,\ Aerobic\ composting} \times G_{CH4} \tag{17.1}$$

where $E_{CH4,\ Aerobic\ composting}$ is CH_4 emissions from the aerobic composting session of sludge (kg), m is the dry weight of sludge (tons), $EF_{CH4,\ Aerobic\ composting}$ is the CH_4 emission factor during aerobic composting of sludge (10 kg $tons^{-1}$), and G_{CH4} is a GWP of CH_4 (28).

$$E_{N2O,\ Aerobic\ composting} = m \times EF_{N2O,\ Aerobic\ composting} \times G_{N2O} \tag{17.2}$$

where $E_{N2O,\ Aerobic\ composting}$ is the N_2O emissions from the aerobic composting of sludge (kg), $EF_{N2O,\ Aerobic\ composting}$ is the N_2O emission factor during aerobic composting of sludge (0.6 kg $tons^{-1}$), and G_{N2O} is the GWP of N_2O (265).

2.2.1.2 Indirect emissions

2.2.1.2.1 Transportation Vehicles are needed to transport sludge, They cause the direct emissions of CO_2 because fossil fuels are burned. Most large trucks are diesel-based vehicles. Diesel consumption is estimated based on the transportation distance; the transportation distance from the sludge treatment facility to sludge disposal was calculated to be 50 km. A domestic truck with a capacity of 15 tons consumes about 15 L diesel oil for a 100-km distance (Wang et al., 2022):

$$RL = \frac{m}{(1-w)M} \times L \times AVG \times \rho_1 \tag{17.3}$$

where RL is the diesel consumption (kg), m is the dry weight of the sludge (tons), w is the sludge moisture content (%), M is the unit load weight (tons), L is the transport distance from the sewage plant to the disposal site (km), AVG is the fuel consumption for a 100-km distance ($L \cdot [100\ km]^{-1}$), and ρ_1 is the diesel density (0.84 kg L^{-1}):

$$E_{CO2,transport} = RL \times RZ \times C \times \alpha \times \frac{44}{12} \times 10^{-3} \tag{17.4}$$

where $E_{CO2,transport}$ represents direct carbon emissions from transportation (CO_2-equivalent; kg), RZ is the calorific value of the diesel (43.33 GJ $tons^{-1}$), C is the carbon content per unit calorific value of diesel (in elemental C) (20.2 tons TJ^{-1}), α is the rate of oxidation of the carbon of diesel (98%), and 44/12 is the ratio of the relative molecular mass of CO_2 to C.

2.2.1.2.2 Electrical energy and pharmaceuticals In general, the emission factor method is used to calculate emissions from the consumption of electrical energy and pharmaceuticals. In the method, activity consumption data are multiplied by the corresponding empirical coefficient (emission factor) that quantifies the unit of activity emissions (Li et al., 2023):

$$E = D \times EF \tag{17.5}$$

where E represents the carbon emissions (kg), D is the electricity or drug consumption, and EF corresponds to the emission factor presented in Table 17.1. The dosage of pharmaceuticals and other indirect emissions from energy consumption are listed in Table 17.2.

TABLE 17.1 Emission factors for the consumption of electricity and pharmaceuticals.

D (activity consumption)		EF (emission factors)
Electricity		$0.5839 kg \cdot (kW \cdot h)^{-1}$
Pharmacist	PAM	$25 kg \cdot kg^{-1}$
	$FeCl_3$	$8.3 kg \cdot kg^{-1}$
	CaO	$1.4 kg \cdot kg^{-1}$

TABLE 17.2 Energy consumption parameters for indirect emissions.

Processing units	Projects	Parameters	Remarks
Gravity thickening	Electricity consumption	$13 kW \cdot h ton^{-1}$	To 97% moisture content
Plate and frame dewatering	Electricity consumption $FeCl_3$ dosing CaO dosing	$40 kW \cdot h ton^{-1}$ $30 kg ton^{-1}$ $50 kg ton^{-1}$	To 80% moisture content
Deep dehydration	Electricity consumption PAM dosing $FeCl_3$ dosing CaO dosing	$125 kW \cdot h ton^{-1}$ $10 kg ton^{-1}$ 6% 10%	To 60% moisture content
Mixing	Electricity consumption	$18.5 kW \cdot h ton^{-1}$	Mixer 1 unit
Transfer material	Electricity consumption	$18 kW \cdot h ton^{-1}$	Material spreader 1 unit
Strip stacking	Electricity consumption	$28 kW \cdot h ton^{-1}$	Turner 1 unit
Sieving earthworms	Electricity consumption	$12.72 kW \cdot h ton^{-1}$	Earthworm sifter 1 unit

2.2.2 *Carbon emissions from vermicomposting*

2.2.2.1 Direct discharge

Because of the peristalsis of earthworms during composting, channels may be formed in the substrate, which increases the aeration capacity of sludge and improves local anaerobic conditions in the substrate, resulting in a reduction in carbon emissions from vermicomposting (Lubbers et al., 2013). Moreover, when the moisture was high, the NO_x and CH_4 emissions from vermicomposting were reduced by 40% and 32%, respectively, whereas when the moisture was low, corresponding values were 23% and 16%, respectively (Lv et al., 2018). In this study, typical values of 33% and 25% were used:

$$E_{CH4,\text{Aerobic composting}} = m \times EF_{CH4,\text{Aerobic composting}} \times G_{CH4} \times 0.75 + m \times C_{CH4} \times G_{CH4} \times N \div 84 \quad (17.6)$$

where $E_{CH4,\text{ aerobic composting}}$ represents CH_4 emissions from the aerobic composting of sludge (kg), m is the dry weight of sludge (tons), $EF_{CH4,\text{ aerobic composting}}$ represents the CH_4 emission factor during the aerobic composting of sludge (10 kg $tons^{-1}$), G_{CH4} is a GWP of CH_4 (28), and C_{CH4} is the equivalent CH_4 emissions from vermicomposting (Yasmin et al., 2022), for which the range of 0.6–1.43 mg g^{-1} is often reported in the literature, and therefore the value of 1.0 kg $tons^{-1}$ is used here. Moreover, N is the number of days of vermicomposting. It is taken as 15 days, and 84 is the number of equivalent test days:

$$E_{N2O,Aerobic\ composting} = m \times EF_{N2O,Aerobic\ composting} \times G_{N2O} \times 0.67 + m \times C_{N2O} \times G_{N2O} \times N \div 84 \quad (17.7)$$

where $E_{N2O,aerobic\ composting}$ represents N_2O emissions from aerobic composting of sludge (kg), $EF_{N2O,aerobic\ composting}$ represents the N_2O emission factor during the aerobic composting of sludge (0.6 kg $tons^{-1}$), G_{N2O} is the GWP of N_2O with the value of 265, C_{N2O} is the equivalent N_2O emissions from vermicomposting with a reported range of 0.3–2.1 mg g^{-1}, and the value of 1.20 kg $tons^{-1}$ is used in the current work.

2.2.2.2 Indirect emissions

Because the moisture content of the sludge required for vermicomposting is relatively high, the technology does not require deep dehydration. In addition, vermicomposting does not need to mix expansive materials such as straw, unlike aerobic composting. Therefore, there is no need for mixing machinery. The earthworms are sieved from the sludge at the end of vermicomposting; carbon emissions from such process steps are examples of indirect emissions. The indirect emissions from dosages of pharmaceuticals and other energy consumption are presented in Table 17.2, whereas the calculation method is the same as that used for aerobic compost.

3. Results and discussion

During aerobic composting, microorganisms carry out dissimilation through aerobic respiration, constantly ingesting oxygen from the external environment and oxidizing large molecules of organic nutrients such as sugars, lipids, and proteins. In this process, these large molecules are gradually degraded into smaller and simpler end-products, and energy is released along with the production of some volatile gases (Swati and Hait, 2017). The main types of gases emitted during composting are CO_2, CH_4, NH_3, and N_2O, which will harm the environment and significantly enhance the potential for global warming.

In addition to greenhouse gas emissions caused by the dissimilation of microorganisms, carbon emissions come from the gut of earthworms during vermicomposting. The earthworm gut is a unique microenvironment with a diverse microbial composition and community, and abundant anaerobic and facultative anaerobic microorganisms, which lead to the production of methane in the gut of earthworms. Moreover, the mixed activities of earthworms and the anaerobic microdomain environment of the gut strengthen the denitrification of microorganisms in the earthworms' guts and fresh vermicompost, promoting the emissions of greenhouse gases.

Carbon emissions from aerobic composting and vermicomposting of excess sludge are shown in Fig. 17.2. Total carbon emissions for each ton of sludge treated through the R1 route were 2128.35 kg ton^{-1}, among which the main carbon emissions came from the aerobic composting unit (439.00 kg ton^{-1}) and the frame dewatering unit (342.36 kg ton^{-1}). The indirect emissions from aerobic composting were caused by the consumption of electricity, which can be reduced by optimizing the process through automated control. Direct carbon emissions

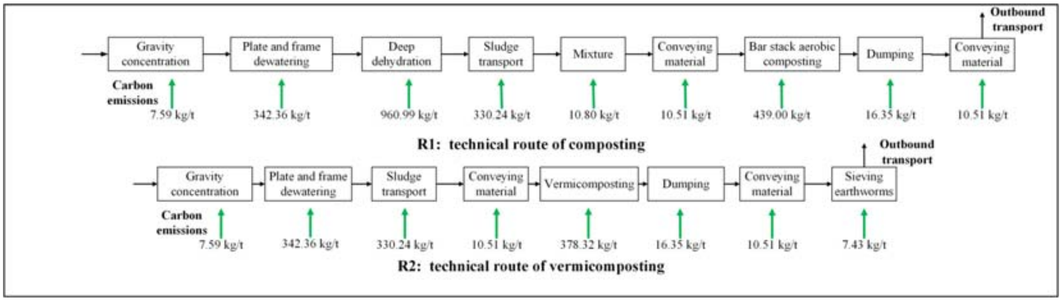

FIGURE 17.2 Carbon emissions during composting and vermicomposting.

from aerobic composting included 280.00 kg ton^{-1} CH4 emissions and of 159.00 kg ton^{-1} N_2O emissions (in terms of equivalent CO_2), whereas CO_2 from the composting of organic carbon was considered a biogenic carbon and not included in the emissions.

The total carbon emissions for each ton of sludge treated through R2 route were 1103.31 kg ton^{-1}, whereas the main carbon emissions came from the vermicomposting unit (378.32 kg ton^{-1}) and the plate and frame dewatering unit (342.36 kg ton^{-1}). Indirect emissions from vermicomposting mainly came from the consumption of electricity. Direct carbon emissions from vermicomposting included 215.00 kg ton^{-1} CH_4 emissions and 163.32 kg ton^{-1} N_2O emissions (in CO_2 equivalents). Earthworms increased the porosity of soil and disposed of a portion of the sludge. Because the addition of earthworms increased the porosity of soil, the local anaerobic condition in aerobic composting was reduced, and a part of the sludge was digested by earthworms as food. Thus, direct carbon emissions were greatly reduced compared with traditional aerobic composting technology. Therefore, using vermicomposting technology can reduce carbon emissions.

In the two composting pathways, the carbon emissions of plate and frame dewatering and deep dehydration units were higher, whereas the carbon emissions of these two process units originated from the consumption of pharmaceuticals. Therefore, the key to reducing carbon emissions was to optimize dewatering to reduce the dosage of pharmaceuticals and develop efficient and sustainable dewatering reagents. The carbon emissions of aerobic composting were mainly composed of N_2O and N_2O. In composting, inadequate aeration will lead to an increase in local anaerobic conditions, increasing the generation and emissions of N_2O and N_2O. It was reported that aeration technology with automatic controls can be adopted to detect anaerobic conditions and changes in temperature in the sludge pile in time, to adjust the aeration frequency accurately. Ultimately, both greenhouse gas emissions and the consumption of electricity were reduced (Dai et al., 2021). Although carbon emissions from vermicomposting were smaller, earthworm screening was still a process unit that could not be ignored. The existing earthworm screening machine had high carbon emissions and a poor screening effect. Therefore, improving the earthworm screening equipment is also a focus for reducing the carbon emissions of vermicomposting.

4. Conclusions

(1) The calculation model of carbon emissions can analyze the carbon emission levels of aerobic composting and vermicomposting of sludge as well as effectively manages the carbon emissions of sludge composting technology.
(2) To reduce carbon emissions from the aerobic composting of sludge, gravity thickening, plate and frame dewatering, and deep dehydration should be optimized.
(3) Compared with aerobic composting, vermicomposting greatly reduces carbon emissions, as mainly reflected in the form of indirect emissions, as well as a sharp decrease in the consumption of energy and electricity.

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CHAPTER

18

Bacterial 16s rDNA diversity in the gut of *Eisenia fetida* revealed by metagenomics and high-throughput sequencing technology

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1. Introduction

Vermicomposting is an environmentally friendly sludge resource technology that uses the synergistic action of earthworms and microorganisms including protozoan, bacteria, and fungi (Xia et al., 2019). During vermicomposting, earthworms can effectively convert organic waste into high-value products for agriculture. It is well-known that the key factor affecting vermicomposting is the intestinal digestion of earthworms (Aira et al., 2016). The gizzard, stomach, and hindgut of the earthworm constitute its complete digestive system chain. The gizzard is the front end of the intestinal digestive system of the earthworm, which mainly breaks food and trophic microorganisms through mechanical grinding (Hu et al., 2020). The stomach of earthworm can secrete amylase and protease to facilitate the further digestion of food. The hindgut of the earthworm can secrete a variety of enzymes to digest and absorb organic matter, and then transform it into nutrients. Therefore, the hindgut has been regarded as the most critical part of the whole digestive system of earthworms (Peng et al., 2022). The earthworm gut is a microenvironment close to an anaerobic state, where a large number of anaerobic or facultative anaerobic bacteria live. These bacteria have an important role in maintaining microecological stabilization in the gut of earthworm, further affecting the degradation and transformation of pollutants in vermicomposting (Chao et al., 2020). Although previous studies focused on the microorganism in the hindgut area of earthworms, few studies have been conducted on different functional segmentations of earthworms.

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The hindgut of earthworms is a site of degradation for macromolecular organic matter in sludge, where the decomposition and digestion of organic matter occur (Zhou et al., 2019). The foregut and midgut inhibit more bacterial taxa than the hindgut (Wang et al., 2021). However, most previous studies analyzing the bacterial community were based on cultural methods, which cannot well recognize noncultural microorganisms. Therefore, 16S rDNA sequencing of the earthworm gut is highly emphasized. Metagenome refers to the sum of all genetic information in the entire microbial community in a certain environment, which is one of the most important and rapid research methods for examining community and function (Zhou et al., 2022). It overcomes the defects of traditional isolation and culture methods, which are limited to culturable microorganisms, and makes it possible to understand and recognize all microorganisms in a certain environment comprehensively.

The objectives of this study were to investigate the distribution of bacterial diversity in different areas of earthworms and to reveal functional genes in the hindgut of earthworms. High through-put sequencing and metagenomics were combined to analyze the bacterial diversity of vermicomposting earthworms *Eisenia fetida*.

2. Methods

2.1 Experimental setup

To prevent anoxia inside the vermicomposting system, dehydrated sludge was first made into sludge particles 5 mm in diameter. Then, 400 healthy *E. fetida* that had cleared their guts overnight were inoculated into 3 kg fresh sludge particles. Vermicomposting experiments were carried out at a constant temperature of 20°C in a biochemical incubator. All treatments were repeated three times. All reactors were covered with plastic wrap with small holes and sprayed with a small amount of water every 3 days to keep the humidity at 60%–70%. After earthworms had digested for 5 days, 30–50 healthy earthworms were randomly selected from each reactor and quickly put into a culture dish containing anhydrous ethanol to kill them. The dead earthworms were then washed in sterile ultrapure water three times to remove the influence of surface substances of earthworms. Immediately, the earthworms were partitioned according to the number of clitellates and dissected on a sterile bench using forceps and a blade. Finally, samples of gizzard, stomach, and hindgut tissue were obtained by taking five pieces of earthworm tissues as one sample.

2.2 DNA extraction and high-throughput sequencing

Total genomic DNA was extracted from each sample using the DNeasy Power Soil Kit (Qiagen, Germany). DNA purity was assessed by 1% agarose gel, and DNA concentration (OD value) was measured by the Qubit 2.0 Fluorometer (Life Technologies, CA, USA). The V4 region of 16S rDNA gene sequence was amplified using primers 515F (5′-GTGCCAGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with barcode bases. The Phusion High-Fidelity PCR Master Mix with HF Buffer (M0531NEB) was used as amplification enzymes. The 35 cycles of PCR amplification were: denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s; with the final extension at 72°C for 10 min. The amplified product was tested by 1% agarose electrophoresis and purified by an Agencourt AMPure XP 60 mL Kit (A63881, Beckman Coulter). A Qubit dsDNA HS Assay Kit (Q32851, Life Technologies) was used to construct the library and the Illumina HiSeq platform was used for on-machine sequencing (Novogene BioInformation Technology Co., Ltd., Beijing, China) after qualification. QIIME software (version 1.8.0) was used to filter the sequence quality control, remove chimera, and get effective clean tags. Then, MEGA7.0 software (version 7.0.1001) was used to cluster operational taxonomic units (OTUs), and OTUs with 97% similarity were grouped into one class. Finally, taxonomic information for each OTU was obtained by comparison and annotated with GreenGene (version gg_13_8) and Silva (SILVA128) databases.

2.3 Metagenome sequencing

After DNA extraction, the 350-bp DNA fragment was prepared by terminal polishing by adding a tail, and the sequencing joint was purified by polymerase chain reaction (PCR) amplification. The library was sequenced on the Illumina HiSeq platform by Novogene Bioinformation Technology Co., Ltd. Sequence bases of low quality were filtered using Readfq (version 8, https://github.com/cjfields/readfq). The resulting clean reads were then assembled into scaftigs using the default parameters via MEGAHIT (version 1.1.4). Only scaftigs longer than 500 bp were successfully compared as assembled reads. Through CD-HIT software (version 4.5.8, http://www.bioinformatics.org/cd-hit), after removing redundant hits, the acquisition of open reading frames from scaftigs was predicted using MetaGeneMark (version 2.10, default settings). Unigenes were obtained by combining clean data for each sample with a unique initial gene catalog using Bowtie (version 2.2.4). Finally, DIAMOND software (version 0.9.9, https://github.com/bbuchfink/DIAMOND/) was used on the NCBI NR database (version 2018-01−02) to classify unigenes.

3. Bacterial distributions in different gut areas of earthworms

3.1 Bacterial diversity

High-throughput sequencing results showed that bacterial OTUs in the gizzard, stomach, and hindgut of *E. fetida* were 1794, 2699, and 1885, respectively. As shown in Fig. 18.1, both the Shannon index and Simpson index of the bacterial community were the highest in the stomach of *E. fetida*. The stomach of the earthworm is a site where endogenous and exogenous microorganisms interact, resulting in a large number of microbes gathered together. Both diversity indexes displayed the lowest values in the gizzard of *E. fetida*, possibly because of the bottleneck effect of the microbial quantity in the gizzard region (Gómez-Brandón et al., 2011). Median values of the beta diversity index of the gizzard, stomach, and hindgut of earthworms were 0.41, 0.55, and 1.05, respectively. A higher beta diversity index in the hindgut of *E. fetida* may be related to a gut microenvironment with rich nutrients.

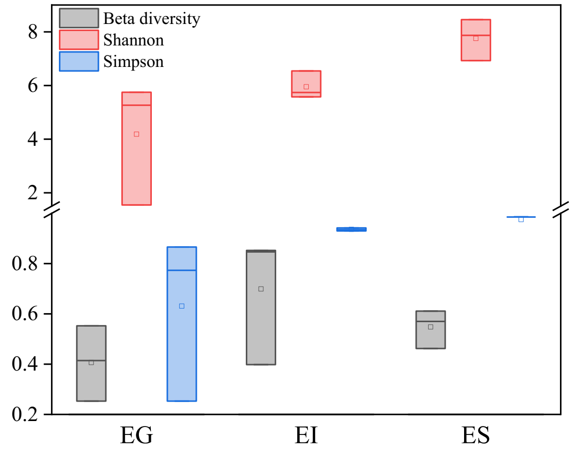

FIGURE 18.1 Box diagram of analysis of differences between shannon, simpson, and beta diversity index groups in different functional areas of earthworms. *EG*, gizzard; *ES*, stomach; *ES*, hindgut.

3.2 Dominant bacteria

The relative abundance of bacterial phyla in various functional areas of the earthworm gut is shown in Fig. 18.2A. Tenericutes (55.67%), Proteobacteria (25.4%), Bacteroidetes (5.13%), Actinobacteria (4.91%), Firmicutes (4.39%), and Chloroflexi (1.35%) were the main bacteria in the earthworm gizzard. The main bacteria in the earthworm stomach were Firmicutes (31.98%), Proteobacteria (25.35%), Bacteroidetes (16.95%), Actinobacteria (11.30%), Chloroflexi (3.01%), and Fusobacteria (1.91%). Moreover, main bacteria in the earthworm hindgut included Firmicutes (43.41%), Proteobacteria (15.20%), Actinobacteria (15.03%), Bacteroidetes (10.43%), Tenericutes (7.09%), and Chloroflexi (4.59%). Tenericutes predominated in earthworm gizzards. Previous studies demonstrated that Tenericutes had a strong ability to degrade nucleic acid (Zheng et al., 2021), which indicates that earthworm gizzards may be the main site for degrading dead cells from food. Firmicutes were the most predominant in the stomach and hindgut of earthworm, which may be related to the structure of microbial populations feeding on sludge. The abundance of Tenericutes in the gizzard, stomach, and hindgut of earthworm was reduced. Compared with the gizzard, its abundance decreased by 98.60% and 87.26% in the stomach and hindgut, respectively. The abundances of Firmicutes, Actinobacteria, and Chloroflexi increased continuously from the gizzard to the hindgut of earthworms, whereas the abundance of Proteobacteria decreased continuously. The significant decrease in Proteobacteria may be because earthworms often feed on the microorganisms of γ-Proteobacteria and δ-Proteobacteria (Hu et al., 2020; Wang et al., 2021), or they may be inhibited by some antibacterial substances secreted from the gizzard (Lund et al., 2010). Bacteroidetes increased by 2.29 times after passing through the stomach, because they are the main degraders of macromolecular organic compounds (Zheng et al., 2021; Gannes et al., 2013).

Fig. 18.2B shows the relative abundance of bacterial genus in various functional areas in the gut of *E. fetida*. *Entomoplasma*, *Verminephrobacter*, *Pseudomonas*, *Dokdonella*, *Candidatus Microthrix*, *Romboutsia*, *Thauera*, and *Aeromonas* were the dominant genera in the earthworm gizzard. Dominant genera in the earthworm's stomach were *Romboutsia*, *Clostridiales*, *Lactobacillus*, *Candidatus Arthromitus*, *Candidatus Microthrix*, *Cetobacterium*, *Mycobacterium*, and *Dokdonella*. Dominant genera in the hindgut of earthworms included *Romboutsia*, *Entomoplasma*,

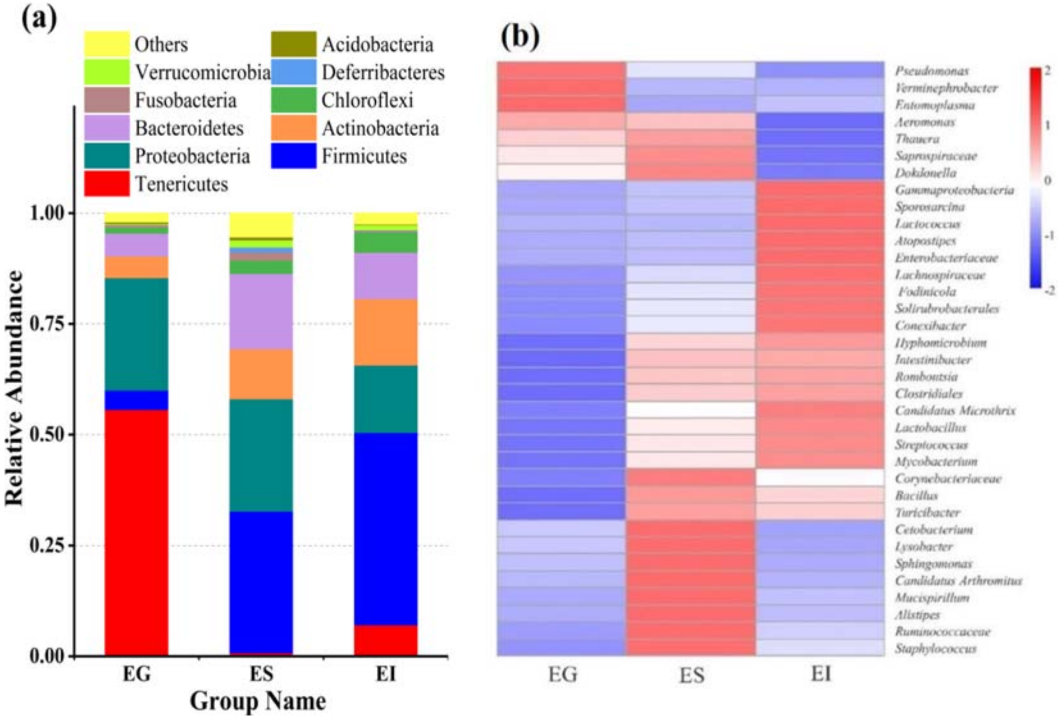

FIGURE 18.2 Changes in bacterial community structure of different functional area in earthworm gut at phylum level (A) and genus level (B).

Lactococcus, *Lactobacillus*, *Candidatus Microthrix*, *Clostridiale*s, *Enterobacteriacea*, and *Lachnospiraceae*. The genera *Entomoplasmahe* (55.65%) and *Verminephrobacter* (6.30%) were absolutely dominant in the gizzard of earthworm. Among them, *Entomoplasmahe* decreased by 87.31% from the gizzard to the hindgut. *Verminephrobacter* decreased by 98.95% from the gizzard to the hindgut. The genus *Romboutsia* was predominant in the stomach (10.11%) and in the hindgut (11.60%), which increased by 6.14 times from the gizzard to the hindgut of earthworms (Wu et al., 2019). *Verminephrobacter* is a symbiotic bacterium in earthworms belonging to the phylum β-Proteobacteria. Its high abundance can promote earlier sexual maturity of earthworms and the success rate of hatching from cocoons (Sapkota et al., 2020). A high fat diet tends to increase the abundance of *Romboutsia* in the gut (Xiao et al., 2017), and its abundance is higher in the stomach and hindgut of earthworms.

4. Functional bacteria and genes in hindgut of earthworms

4.1 Dominant bacterial genus

Metagenomics results showed that a total of 551,944 unigenes with functional annotation were obtained in the hindgut of earthworms. At the phylum level, Actinobacteria (22.10%), Proteobacteria (13.60%), Chloroflexi (7.58%), Bacteroidetes (5.32%), Planctomycetes (2.42%), Chlorobi (2.39%), Ignavibacteriae (2.27%), Acidobacteria (0.19%), Fusobacteria (0.15%), and Nitrospirae (0.09%) dominated in the hindgut of earthworms. Previous studies of high-throughput sequencing also reported that Actinobacteria, Proteobacteria, and Bacteroidetes were dominant in the hindgut of *E. fetida* (Peng et al., 2022). Actinobacteria are abundant in the intestinal wall of earthworm as core microorganisms of the earthworm gut (Sapkota et al., 2020). In addition, some studies stated that Proteobacteria were the most dominant during vermicomposting (Shin et al., 2015). It is well-known that the gut diversity of the bacterial community is associated with the type of food and earthworm species (Zhang et al., 2022). However, these high-throughput results revealed that Firmicutes was the most dominant in the hindgut of earthworms, which does not agree with metagenomic results. This may be because of the difference in two sequencing technologies. Here, it is suggested to use metagenomics to analyze the gut of earthworms.

Fig. 18.3 illustrates the taxonomic relationships of the top 20 species in abundance in the earthworm's hindgut at the genus level. Genera with high abundance were *Candidatus Microthrix* (7.88%), *Caldilinea* (3.36%), *Candidatus Nephrothrix* (1.41%), *Pseudomonas* (1.18%), *Mycobacterium* (0.84%), *Tetrasphaera* (0.81%), *Afipia* (0.63%), *Candidatus Contendobacter* (0.58%), and *Synechococcus* (0.49%). Similarly, Ding et al. (2019) reported that genera with high abundance in the earthworm gut were *Candidatus Microthrix*, *Mycobacterium*, and *Lactobacillus*. Chao et al. (2020) showed that some anaerobic or facultative anaerobic bacteria also enhance their ability to survive in the gut of earthworm. The highest abundance of *Candidatus Microthrix* in the hindgut of *E. fetida* might be related to sludge bulking that occurred during sludge treatment (Li et al., 2020). *Pseudomonas*, a bacterium in Proteobacteria, has the ability to fix metal ions (Teng et al., 2019) and degrade organic matter (Fu et al., 2019). It also has a selective sterilization effect that leads to the death of host bacteria for some antibiotic resistance genes (Khomyakov et al., 2007).

4.2 Functional genes involved nitrogen cycle

The nitrogen cycle is an important process of the mutual transformation of various forms of nitrogen in the earthworm gut. Nitrogen fixation, nitrification, and denitrification are important processes of bacteria for promoting the nitrogen cycle (Kuypers et al., 2018). Table 18.1 lists 10 types of genes involved in the denitrification module, with a total of 676 genes, among which *nar*G/*nar*Z/*nxr*A genes have the most important role in this process. Four genes were involved in the nitrification module, with a total of two genes, *amo*A and *hao* genes were not detected. There were four types of genes involved in the nitrogen fixation module, including 62 genes, among which *nif*K has the most important role in this process. In contrast, denitrification functional groups were much higher than other parts, and fermentation and methanogenic groups were more abundant in the hindgut of *E. fetida* (Hu et al., 2020).

In an anaerobic environment, nitrate reductase (nar), nitrite reductase (nir), and nitrous oxide reductase (nos) can be used gradually to reduce nitrate and nitrite and eventually generate nitrogen-containing gas (Drake and Horn, 2007). The earthworm gut is an anaerobic environment that can provide an important condition for denitrification (Kotzerke et al., 2010). In addition, the gut of earthworm enriching the mineral nitrogen, effective carbon and suitable humidity can stimulate the activity of denitrifying bacteria (Lubbers et al., 2017). The feeding habits of earthworms indirectly affect denitrification and N_2O emissions to

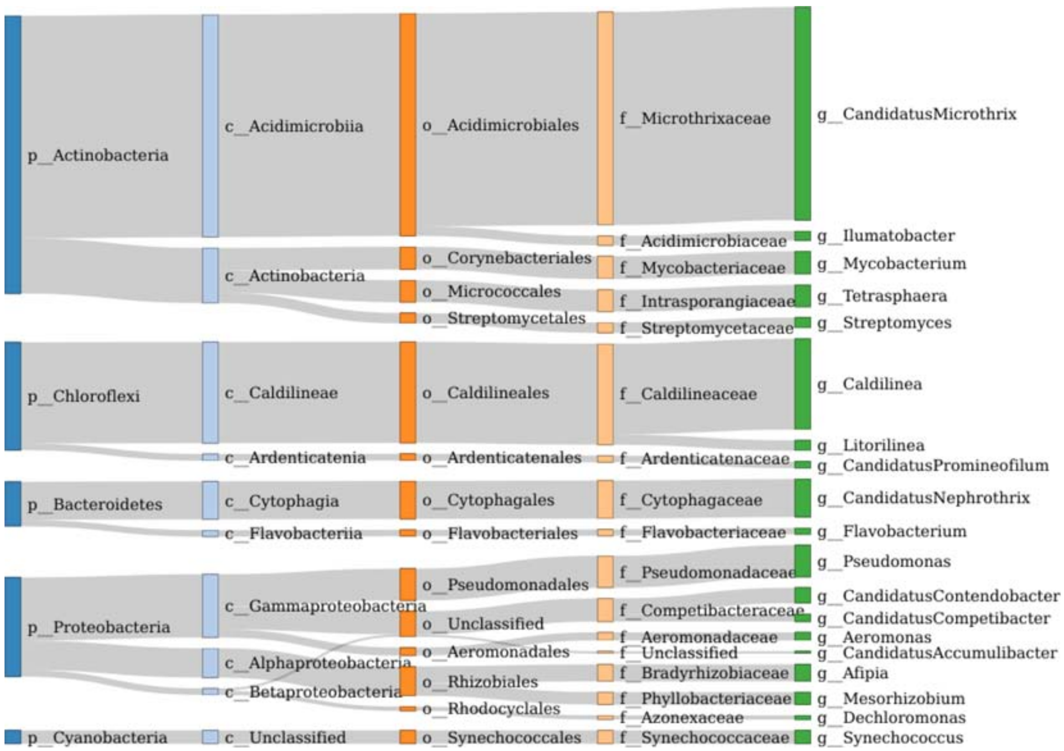

FIGURE 18.3 Taxonomic relationships of top 20 species in horizontal abundance of genus hindgut in earthworms.

TABLE 18.1 Major genes and abundance of nitrogen cycling in hindgut of earthworms.

Denitrification	*nar*G/*nar*Z/*nxr*A	*nar*H/*nar*Y/*nxr*B	*nar*I/*nar*V	*nor*B	*nir*B
	200	90	32	69	63
	*nir*K	*nos*Z	*nir*S	*nap*A	*nor*D
	63	51	42	39	27
Nitrification	*amo*B	*amo*C	*amo*A	*hao*	
	1	1	0	0	
Nitrogen fixation	*nif*D	*nif*H	*nif*K	*nif*N	
	16	11	18	17	

some extent. In situ conditions in the earthworm gut (hypoxia, and the effectiveness of carbon substrate and nitrate/nitrite) stimulate the growth and activity of uptake-denitrifying bacteria, resulting in N_2O and N_2 emissions from the earthworm (Zhang et al., 2010).

5. Conclusion

The results show that the diversity of microorganisms in the stomach were the highest, whereas those in the gizzard were the least in the earthworm gut. Firmicutes, Proteobacteria, and Tenericutes bacterial phyla are dominant in the gut. Firmicutes are concentrated in the stomach and hindgut, Proteobacteria are concentrated in the gizzard and stomach, and Tenericutes are concentrated in the gizzard. The abundance of denitrification module genes was the highest in the hindgut of earthworm during nitrogen cycling, with the dominant genes of *nar*G/*nar*Z/*nxr*A. Metagenomic sequencing is useful for analyzing the gut microorganisms of earthworms.

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