SYNERGISTIC NUTRIENT RECOVERY FROM SPENT COFFEE GROUND AND ORGANIC WASTEWATER VIA AN INTERGATED BERKELEY COMPOSTING-VERMICOMPOSTING SYSTEM

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A project report submitted in partial fulfilment of the requirements for the award of the degree of Bachelor of Civil Engineering (Environmental) With Honours

Faculty of Engineering and Green Technology
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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled **"SYNERGISTIC NUTRIENT RECOVERY FROM SPENT COFFEE GROUND AND ORGANIC WASTEWATER VIA AN INTERGATED BERKELEY COMPOSTING-VERMICOMPOSTING SYSTEM" was prepared by LIM CHUAN ZHONG** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Civil Engineering (Environmental) with Honours at Universiti Tunku Abdul Rahman.

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ABSTRACT

Increasing demand in the Food and Beverages (F&B) and livestock industry has generated thousands of tonnes of spent coffee grounds (SCG) and swine wastewater (SW) annually in Malaysia, creating significant waste management challenges such as landfill leachate. This study investigates their utilization in an integrated Berkeley–vermicomposting system using *Eudrilus Eugeniae* to convert these wastes into a stabilized organic amendment that supports circular economy principles. Four treatment sets (A–D) were evaluated through a sequential Berkeley composting and vermicomposting process. Key physicochemical and biological parameters monitored including total organic carbon (TOC), total nitrogen (TN), C/N ratio, available nutrients (N, P, K), caffeine content, pH, electrical conductivity (EC), earthworm biomass, and germination index (GI). The findings showed that the system improved compost maturity, as evidenced by a reduction in C/N ratio (14.32 ± 0.36 to 19.95 ± 0.66) and enhanced nutrient enrichment. Among all treatments, Treatment D demonstrated the best overall performance, achieving the highest nitrogen concentration (3624 ± 186 ppm) and potassium concentration (7254 ± 363 ppm) contents, along with the greatest earthworm biomass gain (92.82 ± 4.75%) and germination index (118.52%), indicating low phytotoxicity and high compost stability. Low phytotoxicity was also supported by the reduction in caffeine content (-14.3% to -36.8%) throughout the integrated system. Overall, the integrated system effectively produced a nutrient-enriched final product with potential for agricultural application while promoting sustainable waste valorisation.

Keywords: Vermicomposting; Berkeley Composting; Spent Coffee Grounds; Swine Wastewater; Nutrient Recovery

Subject Area: TD783–812.5 Municipal refuse; Solid waste (Environmental Engineering)

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INTRODUCTION

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1.1.1 Organic Waste (OW) Overview

Organic Waste refers to biodegradable; carbon-based materials derived from living organisms that can be decomposed through aerobic or anaerobic microbial processes. Organic waste can be generally classified into several groups based on its origin and composition, including Agro-Industrial Residues (AIR), Animal Manure (AM), Green Municipal Waste (GMW), Municipal Organic Waste (MOW), Sewage Sludge (SS) and other biodegradable residuals. The physical, chemical, and biological properties of organic waste vary greatly depending on its origin and content (Campuzano and González-Martínez, 2016; Dadrasnia et al., 2021; Fermoso et al., 2018; Kacprzak et al., 2017; Reyes-Torres et al., 2018).

Generally, organic waste streams have high moisture content which play a key role in shaping the microbial community involved in the breakdown dynamics. A common behaviour across most OW is their high fermentability, which allows for nutrient recycling and microbial degradation. During this process, organic carbon is mineralised to carbon dioxide or methane depending on prevailing aerobic or anaerobic condition; while associated nutrients are transformed into more bioavailable form. The rate and efficiency of degradation are strongly influenced by the key process-relevant parameters include carbon-to-nitrogen ratio, moisture content, pH, and its biodegradability (Reyes-Torres et al., 2018).

As summarised in Table 1.1, different organic waste streams exhibit distinct combinations of organic matter content, nutrient availability, moisture level and biodegradability. MOW and AM are generally high putrescible and nutrient-rich, making them suitable for rapid biological treatment, although they are often prone to seasonal variability and contamination concern. In contrast, GMW and many AIR are characterised by high organic matter content and relatively higher C/N ratios, which provide structural stability but result in slower biodegradation. SS is typically nutrient-rich and highly biodegradable; However, its application is constrained by presence of pathogen and inhibitory contaminants (such as antibiotics and microplastics) and therefore require material stabilization to maximize its agronomic valorisation (Buta et al., 2021).

Table 1.1: Types of Organic Waste and their characteristics

(Cucina, 2023; Sandra Ropero Portilo, 2025)

Organic Waste	*Key Characteristics*	*Limiting Features*	*Example*
AIR	• High content of organic matter • Variable moisture content	• Seasonal production variability • Unbalanced nutrients ratio	• Olive mill waste • Wheat straw • Corn stover • Sugarcane bagasse
AM	• High putrescible • High content of nutrients • Variable moisture content	• High seasonal variability • Presence of emerging contaminants & pathogen	• Pig manure • Cattle manure • Poultry manure
GMW	• High content of organic matter • Low moisture content	• Less putrescible	• Yard waste • Leaves • Grass clippings
MOW	• High putrescible • High content of nutrients • High moisture content	• High seasonal variability • Presence of inert materials	• Food waste • Kitchen scraps
SS	• High putrescible • High nutrient content • High moisture content	• Unbalanced nutrient ratios • Presence of emerging contaminants & pathogen	• Wastewater treatment sludge

While high content of nutrients and high content of organic matter are intrinsically related, they play distinct roles in organic waste management and nutrient recycling processes, including anaerobic digestion, composting, vermicomposting and other approach. Organic matter content typically refers to carbon-based substances obtained from plant wastes, paper waste, or other lignocellulosic materials that serve as energy and structural substrates for microbial and earthworm activities. High organic matter levels are often linked with feedstocks with a high C/N ratio (for example, coffee ground, straw), which stimulate microbial proliferation but may necessitate nitrogen supplementation for balanced breakdown (Zhang and Sun, 2017a). In contrast, nutritional content refers to the concentration of key plant-available elements, mainly nitrogen(N), phosphorus(P), and potassium(K), which are mostly obtained from nitrogen-rich materials such as manure, sludge, swine wastewater w ith relatively lower C/N ratio. While both factors influence compost quality, organic matter determines bulk substrate availability and stability, and nutrient content influences the fertilisation potential of the final vermicompost (Dai et al., 2016; Zhang and Sun, 2017b).

Recognizing this distinction is important, as a substrate may possess high organic matter content but low nutrient availability, or vice versa. This variation can affect the rate of vermicompost stabilization and its agronomic value. However, certain organic waste contains undesirable elements such as heavy metals, pathogenic bacteria, or organic contaminants, which associated with environmental or health risk if not adequately managed. In our focus, Spent Coffee Ground (SCG) and Swine Wastewater (SW) are our main feedstock utilised in vermicomposting approach. SCG may be classified either as MOW or a AIR, depending on its source and scale of generation. When it derived from households and café during coffee brewing, SCG is commonly regarded as food waste under MOW, representing post-consumer solid organic residue. Conversely, when generated in large quantities from coffee processing plant and instant coffee manufacturing, it is more appropriately categorised as an AIR due to its industrial origin and relatively homogeneous composition. Whereas SW falls under animal manure-derived waste, representing the treated liquid fraction of farm effluent including pig manure, urine, and wash water. Orfanoudaki et al. (2020) reported that SCG are a carbon-rich material with a C/N ratio of approximately 28:1, characterized by high proportions of lignin, cellulose, and other carbonaceous compounds. In contrast, pig manure has a much lower C/N ratio of around 10:1. This reflecting its substantial nitrogen content from animal waste, making it a nutrient-rich but less carbon-dense substrate.

1.1.2 Organic Waste Management Challenges

In this era of rapid urbanization and industrialization, the generation of organic waste has been escalating globally driven by accelerated population growth, agricultural and livestock sector expansion, organic effluents from food processing and manufacturing industries, as well as increasing prevalence of short-lifecycle consumer products. Nowadays, organic waste management is one of the most pressing priorities to address secondary environmental issues, including the depletion of groundwater, the loss of essential genotypes, the deforestation of tropical regions, the decline of biodiversity, soil erosion, desertification, and climate change (Bhattacharjya et al., 2021). In many developing countries, an estimated 64% of generated waste in low-income regions is organic, representing potential underutilized resource that could be leveraged in circular economy initiatives (Mishra et al., 2023). According to the Waste Management Association of Malaysia (WMAM), our nation generates over 40,000 tonnes of Municipal Solid Waste (MSW) per day, which is equivalent to about 1.2 kg per person per day. At current rate, national waste generation is expected to rise from 14 million tonnes in 2021 to 19 million tonnes by 2050. Based on data from the Solid Waste Management and Public Cleansing Corporation (SW Corp), an appointed agency under the Local Government Development Ministry, food waste is the biggest organic component of the waste stream, accounting for around 36% of total MSW (K. Harinderan, 2023).

In Malaysia, landfill disposal remains the predominant practices of waste management, with 82% of MSW directed to landfill sites annually (K. Harinderan, 2023). However, this approach is increasingly discouraged due to pressing challenges such as leachate generation, dwindling landfill capacity, and greenhouse gas emission. Landfills pose long-term risk on human health and the environment due to the leaching and accumulation of hazardous heavy metals. Heavy metals such as arsenic (As), hexavalent chromium (Cr)(VI), cadmium (Cd), mercury (Hg), and lead (Pb) are non-threshold pollutants that can cause adverse effects even at levels below the critical value (Hussein et al., 2021).In 2021, there are only 21 operating landfill facilities out of 137 registered meeting sanitary landfill standards (K. Harinderan, 2023).A proper landfill facility must include engineered liners, leachate management systems, and environmental containment measures (Yaashikaa et al., 2022). Improper landfill facility will always associate with landfill leachate issues. Leachate is one kind of wastewater formed when rainwater or other fluid percolate through solid waste in a landfill and extract dissolved or suspended hazardous substances. It typically contains high concentration of organic matter, ammoniacal nitrogen, heavy metals that result in soil contamination and groundwater pollution if not properly collected and treated. Coagulation-flocculation, advanced oxidation processes, membrane filtration are engineering technique employed in conventional leachate management (Kumar et al., 2025).

The Waste Management Association of Malaysia (WMAM) has warned that the nation risks exhausted existing landfill capacity by 2050. On average, a landfill site in Malaysia lasts just two decades until the underlying soil structure deteriorates and becomes chemically contaminated, making it unfit for further use. When a site is decommissioned, it cannot be immediately restored. Instead, an estimate of 20-year recovery period is required to allow for soil stabilisation and a gradual reduction of residual contamination to safe levels. Since 2015, Malaysia has recorded closure of 174 landfill sites, with all require continuous post-closure care. The maintenance cost was estimated RM1.9 billion annually to prevent the contaminants seeping into surrounding environment (K. Harinderan, 2023).

Instead, landfill possess significant environmental challenges due to their role as largest contributor of methane emission in Malaysia. Organic waste decomposition in landfills emits huge amounts of methane, a greenhouse gas with a global warming potential more than 25 times that of carbon dioxide over a 100-year period (Climate Change, 2023). According to International Energy Agency (IEA), the waste sector accounts for 56% (894 kilo tonnes) of Malaysia 's total methane emissions in 2021, surpassing the energy sector's 30% contribution (K. Harinderan, 2023). Organic waste dumped in landfills undergoes anaerobic decomposition in the absence of oxygen. Thus, releasing methane, a strong greenhouse gas that contributes significantly to anthropogenic climate change (Kharola et al., 2022). Reliance on disposal-based waste management is not aligned with Malaysia 's carbon neutrality commitments under the Paris Agreement-Net Zero Carbon 2025.

Therefore, the urgent need of environmentally sustainable approach to organic waste is necessary. Alternatives organic waste management methods, including anaerobic digestion, composting, and incineration with energy recovery are recommended (Gao et al., 2025). The biological approach such as vermicomposting is ideal for nutrient-rich waste because they stabilise organic materials, reduce pathogens, and recycle nutrients into soil amendments. Vermicomposting is proved to create a higher-quality final product with increased nutrient availability and beneficial microbial communities (Madhubala et al., 2025).

1.1.3 Role of Vermicompost in Agriculture

The excessive reliance on chemical fertilisers in modern agriculture has prompted serious concerns about soil deterioration, environmental contamination, and long-term food security. Therefore, the adoption of sustainable nutrient recycling organic fertilisers is necessary. Vermicompost emerged as particularly promising alternative due to their capacity to replenish soil nutrients while minimising adverse environmental effects. Vermicompost is a stabilized, humus-rich organic amendment produced by the bio-oxidative decomposition of organic waste by earthworms and other microorganisms. Unlike traditional composting that dependent on thermophilic microbial activity, vermicomposting is a mesophilic process. Earthworms mechanically breakdown organic wastes, increase surface area exposure, and drive microbial proliferation through the activities of their gut-associated microflora. Generally, Vermicompost improves agricultural productivity by improving soil fertility and increasing plant tolerance to abiotic and biotic stresses, stimulating physiological and biochemical processes, and promoting long-term crop growth through its high nutrient content, beneficial microorganisms, and bioactive compounds (Soobhany, 2019a).

Abiotic and biotic stresses pose significant challenges to plant growth and productivity. Abiotic stresses can be physical or chemical in nature, such as drought and salinity, while biotic stresses involve exposure to biological agents including insects, bacteria, viruses, and fungi (Verma et al., 2013). Saline soils, characterized by elevated concentrations of soluble salts, hinder plant development by reducing nutrient uptake and affecting photosynthetic pigments such as chlorophylls and carotenoids (Akoto-Danso et al., 2019). Vermicompost application has been shown to alleviate the toxic effects of salinity in crops such as maize by enhancing photosynthetic pigment levels. The mechanism involves stimulation of enzymatic activities, promotion of protein synthesis, and enhancement of physiological processes. In addition, vermicompost inoculated with beneficial microorganisms such as Azotobacter chroococcum and Bacillus subtilis has been demonstrated to improve tolerance to Sodium Chlorine (NaCl) stress in crops including maize, barley, rice, wheat, and soybean (Shilev, 2020).

Drought stress is regarded as one of the most detrimental abiotic stresses due to its negative effects on plant–water relations at both cellular and systemic levels. This will result in reduced CO_2 assimilation, impaired glucose synthesis, and decreased photosynthetic efficiency, Furthermore, water scarcity promotes the generation of reactive oxygen species (ROS) and accelerates chlorophyll degradation (Kaur et al., 2021). To address this issue, vermicompost contain iron (Fe) content that act as a prosthetic group in ROS-detoxifying enzymes such as catalase, peroxidase, and superoxide dismutase (Roy et al., 2010). Application of vermicompost at 10–20% has been reported to significantly increase chlorophyll concentration, intracellular CO_2 content, net photosynthetic rate, and quantum yield of PSII photochemistry. (Hosseinzadeh et al., 2016). Moreover, vermicompost enhances photosynthetic machinery and nutrient uptake, leading to improved protein content, total soluble sugars, and antioxidant enzyme activity in both leaves and roots.

Biotic stresses, particularly plant diseases caused by bacteria, viruses, and fungi, are another major constraint to crop productivity. Among these pathogens, fungi are the most prevalent. During vermicomposting, earthworms secrete coelomic fluid, which possesses antimicrobial properties that suppress pathogens present in organic waste (Gudeta et al., 2021). For example, earthworm-derived lumbricine-PG, an antifungal peptide bound to the surface of vermicompost, thereby conferring protection against fungal invasion. Vermicompost amendment has been shown to reduce bacterial vascular wilt incidence (You et al., 2019). Vermicompost enhances microbial diversity and activity in the rhizosphere, thereby protecting plants through induction of systemic resistance against phytopathogens and secretion of pathogen-suppressive metabolites.

Additionally, vermicompost facilitates hormonal and biochemical stimulation by supplying humic substances and hormone-like compounds, including auxins, cytokinin, and gibberellins (Ravindran et al., 2016). These bioactive compounds regulate plant physiology by improving photosynthetic rate, increasing chlorophyll content, and optimizing carbohydrate allocation. Vitamins and amino acids present in vermicompost, and its derivatives can modulate plant developmental pathways, leading to earlier flowering, enhanced fruit set, and improved yield quality. The combined hormonal and humic effects also promote root system development and nutrient use efficiency, explaining the consistent yield and quality improvements observed across multiple crop trials (De Castro et al., 2023).

1.1.4 Sustainable Synergistic Rationale

According to Statista Research Department, in 2022, Malaysia produces an anticipated 48,000 metric tonnes of coffee waste, primarily consisting of SCG and chaff (Suhaila et al., 2024). Concurrently, based on data from the Department of Veterinary Services (DVS), Malaysia has generated around 75,000 tonnes of SW daily in 2021 (Wong Siew Lyn, 2022). These abundant wastes pose significant challenges for disposal and environmental management. In practice, SCG are diverted to landfills, where their lignocellulosic composition promotes leachate formation and anaerobic degradation, further increase the risk of groundwater contamination and methane emissions. Whereas SW are commonly treated in anaerobic lagoon system (North Carolina State University, 2000). However, this operation is associated with environmental vulnerabilities during discharge event which may lead to subsequent eutrophication and surface water degradation. Therefore, utilising SCG and SW in a vermicomposting system may offer a sustainable waste management strategy.

Both SCG and SW present complementary opportunities and technical challenges for vermicomposting approach. SCG are rich in carbon but contain recalcitrant lignocellulosic compounds and bioactive phytotoxins (e.g., caffeine), often form compact, poorly aerated beds that inhibit microbial activity and earthworm performance (Hanc et al., 2021). Conversely, SW is nutrient-dense with readily available nitrogen but may exhibit high levels of biochemical oxygen demand, salinity, pathogen loads and contain trace metals. These unfavourable features produce ammonia toxicity, oxygen depletion, and metal accumulation in the earthworm tissues or vermicompost. (Oliveira et al., 2021; Vanotti et al., 2018).

Therefore, integrating SCG and SW could be a potential sustainable approach, as their physicochemical properties could offset one another. The fibrous and carbon-rich structure of SCG may improve porosity, moisture balance, and alkalinity buffering, while supplying labile carbon to support microbial activity. At the same time, SW may provide moisture and nitrogen. When blending with SCG, this could favourably shift the ammonium–ammonia equilibrium and promote microbial-driven nitrogen incorporation. Such interactions have the potential to alleviate phytotoxicity, reduce heavy-metal bioavailability through adsorption to lignocellulosic surfaces, optimize the C:N ratio, enhance organic matter degradation and final vermicompost quality.

1.2 Problem Statements

Spent coffee grounds (SCG) and swine wastewater (SW) are two high-volume organic wastes produced globally by the food processing and livestock sectors. The large-scale generation of these waste streams creates significant technical and environmental management challenges. Vermicomposting offers a biological route to valorise SCG and SW into a stable, nutrient-rich soil amendment. This valorisation promptly resolves Malaysia's major organic waste management concerns (landfill) and reducing reliance on synthetic fertilisers.

However, co-processing SCG and SW in Berkeley-Vermicomposting systems presents significant technical challenges. Their contrasting physicochemical properties, SCG’s bulkiness and carbon dominance versus SW’s high moisture and nitrogen load, complicate the formulation of safe, effective feedstock blends for vermicomposting. Furthermore, there is little standardized guidance or global research on optimal SCG–SW mixing ratios, earthworm tolerance thresholds, and process controls for lab-scale vermicomposting systems. Moreover, there is limited understanding of how nutrient stabilisation, pathogen attenuation or bioaccumulation are transformed during co-processing. Without addressing these knowledge gaps, reliable scale-up, comprehensive risk assessment and practical implementation of SCG-SW vermicomposting will remain constrained. Bridging these gaps will enable SCG-SW vermicomposting to function as a sustainable resource recovery system, producing marketable organic fertiliser, allowing successive use of earthworm in composting cycles, and recycling nutrients back to the soil, thereby supporting circular economy objectives in alignment with Malaysia ’s commitment to support Net Zero Carbon by 2050.

1.3 Aims and Objectives

Aim: To investigate the feasibility and performance of lab-scale integrated Berkeley-Vermicomposting systems blending SCG and (SW) to produce stable, nutrient-rich vermicompost while overcoming physicochemical and biological compatibility challenges.

Objectives:

I. To evaluate the performance of an integrated Berkeley–Vermicomposting system using different SCG- and SW-based feedstock compositions.

II. To monitor changes in physicochemical properties during the Berkeley-Vermicomposting processes.

III. To assess the maturity and quality of the final vermicompost based on nutrient content, germination index and earthworm biomass.

1.4 Scope and Limitation of Study

This study is conducted at the laboratory scale using a self-designed Berkeley-Vermicomposting bin with temperature, moisture, and aeration manually adjusted to maintain suitable condition for earthworm activity. This research focuses on locally generated organic waste SCG and SW as primary feedstocks. Scope of study includes selecting earthworm species, optimising mixture, and evaluating vermicompost quality using physicochemical and biological parameters. The study does not cover large-scale process engineering, automation and long-term field application trials. Only a single earthworm species is evaluated, which may limit the generalisability of results. Additionally, only locally sourced SCG and SW are used, so waste composition variability from other sources is not considered. Limitations include inherent seasonal or source-related variability in SCG and SW composition, species-specific tolerance levels of earthworms, insufficient prior research on relevant topics, and restricted access to advanced analytical instrumentation for microbial community analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Vermicomposting Overview

According to the Research Institute of Organic Agriculture, total land of 72.3 million hectares was cultivated under organically integrated technologies such as vermicomposting, marking a 1.6% growth compared to 2018 (FiBL, 2021). Vermicomposting is a biological approach that converts organic matter into a stable, agriculturally valuable product through the synergistic activity of earthworms and mesophilic microorganisms (Samal et al., 2019a). During vermicomposting, earthworms fragment and loosen the substrate, increasing surface area and enhancing microbial decomposition of organic matter (Soobhany et al., 2015). Under favourable moisture, temperature, pH conditions, earthworm ingest organic matter and excrete a homogeneous substances called vermicast. Vermicast is composed predominantly of pure worm casting (excrement) and contains an active microbial community, plant-available nutrients and exchangeable ionic species (Samal et al., 2019a). In contrast to conventional composting, vermicomposting commonly achieves faster stabilisation, greater humus content and lower residual phytotoxicity. In vermicomposting process, greenhouse gases emission is unavoidable. Vermicompost is abundant in plant -growth regulators (e.g. auxins and gibberellins) and active soil enzymes. Thus, it can hold nutrient for a longer period and have lesser environmental impact compared to ordinary compost (Soobhany, 2019b). Vermicompost cultivation can range from a modest, home-based system to a large-scale commercial operation, with the method varying on volume of organic waste handled.

2.2 Earthworm

2.2.1 Overview

Earthworms (taxonomic classification: phylum Annelida, class Clitellate) are segmented terrestrial oligochaetes that inhabit humid soils (Domínguez and Domínguez, 2018). It has long been recognised that earthworms are soil engineers and important detritivores. Morphologically, earthworms have a cylindrical, segmented body with a prominent clitellum signifying maturity, a prostomium that aids burrowing, and S-shaped setae on most segments that allow locomotion. Earthworms have a closed circulatory system with segmental arteries and haemoglobin-containing blood, and they breathe cutaneous through a moist epidermis by diffusion. Anatomically, earthworm 's digestive system consists of a continuous alimentary canal extending from mouth to the anus (Pushpendu Mondal, 2021). Nephridia (septal & pharyngeal forms) engage in its excretory system to expel metabolic wastes and regulate fluid balance. In nature, earthworms are hermaphrodites, which contain both male and female sex organs. Cross-fertilization and copulation are the most common methods of reproduction. They lay their eggs in a tiny spherical capsule called a cocoon, which has an incubation period of 3-6 weeks varying on species (Aira et al., 2002a; Samal et al., 2019b).

Anecic (deep-burrowing), endogeic (shallow-burrowing), and epigeic (surface-dwelling) are the three major types of earthworms characterized by distinct habitat preference, burrowing behaviour, morphological traits, life cycles and reproductive characteristics (Bottinelli et al., 2020). This ecological differentiation determines their roles in soil processes and suitability for organic waste degradation and vermicomposting applications. In vermicomposting, earthworms are key regulators of soil ecosystems, significantly influencing microbial community composition, bioconversion, soil porosity and aeration. Earthworm activities such as digestion, burrowing, mucus secretion, and casting enhance microbial interactions, thereby accelerating organic matter decomposition (Domínguez et al., 2010). The digging and burrowing activities of earthworms create holes and channels in the organic matter. This makes the composting bed more porous so that atmospheric oxygen diffused into the filter bed. Aerobic conditions dominate and encourage the growth of aerobic microorganism in composting beds. Due to the porous structure of the bed, water and nutrient disperse all around (Edwards and Arancon, 2022).

2.2.2 Types of Earthworms and its characteristics

Table 2.1 demonstrate three major types of earthworms characterized by distinct habitat preference, burrowing behaviour, morphological traits, life cycles and reproductive characteristics.

Table 2.1 Types of Earthworms and their characteristics
(Bottinelli et al., 2020; Domínguez and Edwards, 1997; Garg et al., 2012; Lavelle, 1988)

Types	Habitat & Burrowing Style	Size & Pigmentation	Life cycle & Reproduction	Examples
Anecic	-Live in vertical galleries that extend deep into soil profile -Build vertical permanent burrow -Deep burrowing (30-90cm) -Deposit excreta on opening of galleries -Loose & granular casting occurs on soil -organo-mineral cast	-Large size -Often dark head(red/brown) with paler posterior	-Long life cycle -Moderate reproduction rate -Fresh lit ter feeder	-*Lumbricus terrestris* -*Lumbricus Polyphemus* -*Lumbricus friendi*

Endogeic	-Live within mineral-organic horizons -Build extensive, highly branched horizontal burrows -Medium burrowing (10-30cm) -Galleries filled with excrement -Organo-mineral cast	-Medium size -Little or no pigmentation	-Medium life cycle -Low reproduction rate -Soil feeder	-*Aporrectodea caliginosa* -*Aporrectodea rosea* -*Allolobophora chlorotica* -*Octolasion cranium*
Epigeic	-Live in organic horizon, near surface -Active in litter layers (3-10cm deep) -Do not build burrow -Loose and granular casting occur on surface -Homorganic cast	-Small size -Rich pigmentation (dark colouring)	-Short life cycle -High reproduction rate -Surface feeder	-*Eisenia fetida* - *Eisenia Andrei* -*Lumbricus rubellus* - *Perionyx excavates* - *Eudrilus eugeniae*

2.2.3 Properties of optimal composting earthworm

Table 2.2 summarizes that optimal composting earthworms are characterized by high bioconversion efficiency, rapid consumption and decomposition capacity, and polyphagous feeding behaviour, enabling effective transformation of diverse organic wastes into stable vermicast and biomass. In addition, wide thermal tolerance, high reproductive output with short life cycle and strong resistance to disease are essential to ensure sustained population growth and composting performance (Domínguez and Edwards, 1997; Huang et al., 2014).

Table 2.2: Properties of optimal composting earthworm

Properties	Description
Efficient bioconversion	• Earthworm must be able to effectively transform organic waste into body protein and stable vermicast
Wide thermal tolerance	• Earthworms must be able to withstand ambient temperature fluctuating between 18 ° C to 30 ° C
High consumption and decomposition rate	• Earthworms able to ingest organic substrates rapidly, digest efficiently and accumulate biomass effectively
Polyphagous feeding habit	• Earthworms must be able to accept wide range of organic wastes rather than only one specific substrate
High reproductive output & short life cycle	• Cocoon production should be prolific, with short incubation periods to allow for rapid population growth. • Furthermore, the species' life cycle should be brief, allowing earthworms to attain sexual maturity fast
Strong Disease and Parasite Resilience	• Earthworms must be able to withstand common pathogens and environmental stressors

2.2.4 African Nightcrawlers (Eudrilus Eugeniae)

Earthworms are commonly used for the bioconversion of organic solid waste, but only a few species have been considered. The common species used are Eisenia fetida, Lumbricus Rubellus, Perionyx Excavatus, and Eudrilus Eugeniae. Amongst these, Eudrilus Eugeniae (E. Eugeniae), an epigeic earthworm native to West Africa, is one of the most prevalent composting species due to its extraordinary tolerance to a wide range of substrates, quick reproductive capability, big and high consumption rate (Balachandar et al., 2020; Blakemore, 2015; TOURE et al., 2024). Comparative studies of earthworm species for vermicomposting of agricultural waste have demonstrated that E. Eugeniae surpasses Eisenia Fetida in term of reproductive performance and nutrient enrichment (N, P, K) in vermicompost (Thorat, 2022).

E. Eugeniae 's body is reddish brown in colour, spherical-shaped, and metameric ally segmented. Its body consists of 145-203 segments separated by ring-like grooves called speta. They have chemoreceptors and photoreceptors to sense their surroundings. The dorsal surface of their body is reddish brown, while the ventral surface is light brown. The reddish-brown colour is caused by the presence of porphyrin. (K. Subbulexmi, n.d.). This species is characterized by a prominent clitellum when matured, and morphological features such as the prostomium for burrowing, gizzard for mechanical grinding, and calciferous glands for digestion. Figure 2 illustrate the anatomy of E. Eugeniae in internal and external view.

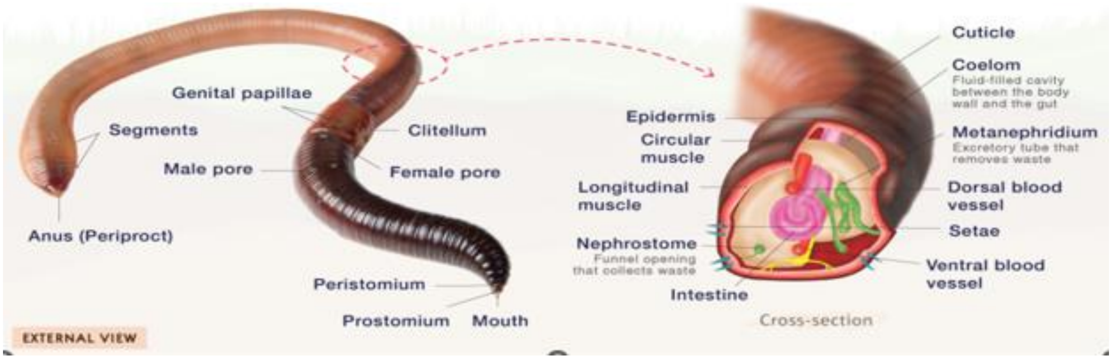

Figure 2.1 Anatomy of E. Eugeniae

(Anushka Chatterjee, 2025)

E. Eugeniae reproduce hermaphroditically, exchanging sperm during copulation to produce lemon-shaped cocoons that hatch in around 2 to 3 weeks. Development occurs directly without a larval stage, and 2-7 hatchlings emerge per cocoon. Juveniles attain sexual maturity within 40-45 days, marked by the formation of the clitellum. As demonstrated in Figure 2.2, its typical life cycle ranges from 55-70 days, and a mature worm can produce about one cocoon every three days, continuing reproduction for up to 250 days within a lifespan of estimated 300 days. African nightcrawlers thriving in decaying organic waste like leaf litter, manure, and vegetative residues, rather than mineral soil. They exhibit dietary generalist, consuming a wide range of substrates. Several earthworm studies revealed that E. Egeniae exhibits reduced growth and poor reproductive performance at temperatures below 20°C. In terms of thermal tolerance, they can survive and reproduce optimally in temperatures ranging from 22 to 28 °C, with cocoons capable of freezing and remaining dormant until favourable conditions return (U M Shagoti et al., 2001; Viljoen and Reinecke, 1992). Given this thermal profile, E. Eugeniae is more suitable for vermiculture in tropical or worm-moderate regions rather than in colder climates. Therefore, its temperature adaptability aligns well with the climatic tropical country such as Malysia, making it a suitable species for vermicomposting application in such regions. Economically, their rapid reproduction results in lower maintenance costs make them cost-effective and practical.

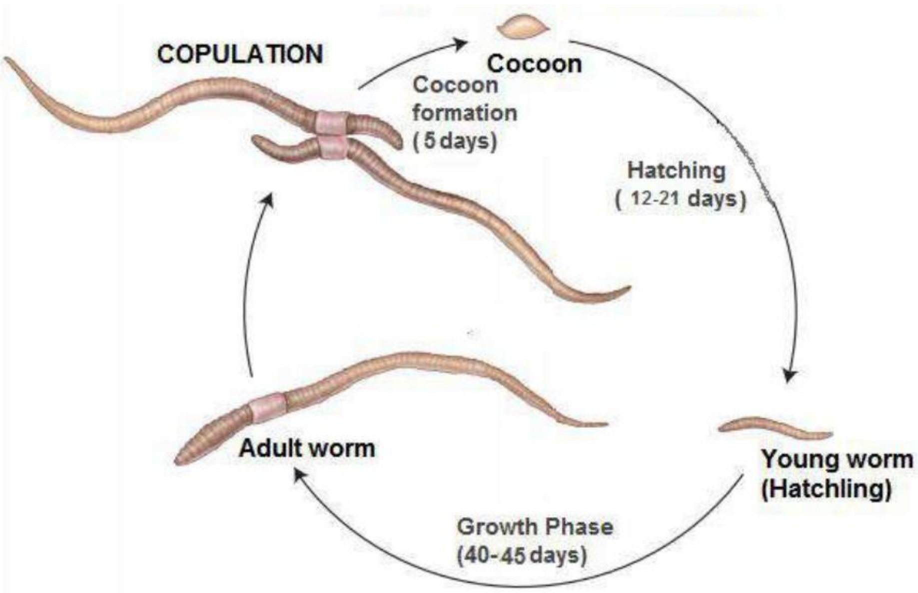

Figure 2.2 : Lifecycle of African Nightcrawlers

(S.Viswanathan, n.d.)

2.3 Vermicomposting Mechanisms

As shown in Figure 2.3,contemporary studies describe vermicomposting as a two-phase process: (1) Active phase (earthworm-dominated) where gut-associated processes (GAPs) modify the physical state and microbial composition of the substrate, and (2) Maturation phase (microbe-dominated) where cast-associated processes (CAPs) age, mineralize and humify the worm-processed material into stable, black humus fertilizer (final product of vermicompost). This conceptual partition is useful because it identifies distinct drivers (earthworm physiology vs. free-living microbes) and explains observed patterns in nutrient dynamics, enzyme activity, and microbial succession.

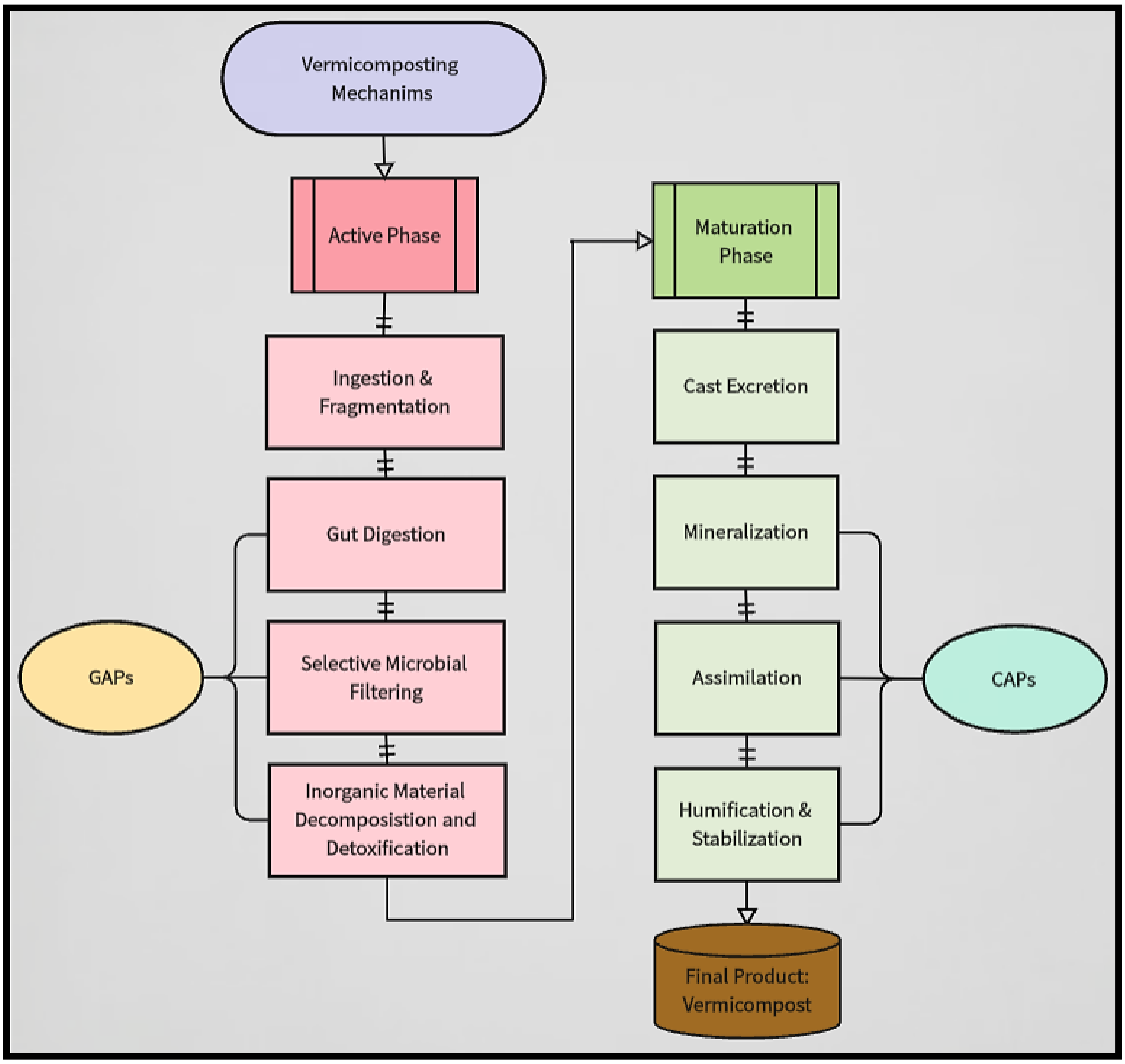

Figure 2.3: Flow chart of vermicomposting mechanism

2.3.1 Active Phase (Earthworm)

The active phase of vermicomposting is governed by the biological and physicochemical activities occurring within the earthworm digestive system. During this phase, earthworms function as bio-reactive processors that fragment, biochemically transform, and selectively regulate the organic substrate. This section discusses the key mechanism involved, including (I.) Ingestion and Fragmentation (II.) Gut Digestion (III.) Selective Microbial Filtering and pathogen suppression, and (IV.) Inorganic Material Decomposition and Detoxification. Collectively, these processes enhance substrate stabilization, nutrient mineralisation, microbial restructuring, and partial sanitisation, thereby contributing to the production of nutrient-enriched and environmentally safer vermicompost (Fornes et al., 2012; Thorat, 2022; U M Shagoti et al., 2001).

I.) Ingestion and Fragmentation

Epigeic earthworms ingest surface organic materials, such as litter, manure, or waste. The ingested organic substrate is initially moistened and softened by salivary secretion(mucus) in the buccal cavity. The pharyngeal glands contain chromophil cells that secrete saliva and enzymes, including proteases, phosphatases, and mucin, to aid in the digestion of organic matter. The mucus of earthworms contains a mixture of carbohydrates and protein-like compounds that have many ecological functions in soil drilosphere (Zhang et al., 2016). The drilosphere refers to the approximately 2 mm thick zone of soil surrounding an earthworm burrow. It is the key biologically active zone in the soil, governing the decomposition, the cycling of nutrient, and the regulation of microbial processes.

Earthworm 's mucus has significant effect on stimulation of microbial activity (Marichal et al., 2011). Within the oesophageal region, calcium ion excreted from the epithelial lining act to neutralize acidic compounds and further condition the substrate (Al-Maamori, 2024). The partially processed material is subsequently transferred to the muscular gizzard, where it undergoes mechanical grinding into finer particle. This grinding is an inherent physiological mechanism of earthworms that reduces particle size and increases the substrate's specific surface area, thereby enhancing microbial colonisation and accelerating subsequent enzymatic hydrolysis. Sufficient fragmentation improves mass transfer (oxygen and solutes) and promote microbial succession (Aira et al., 2002b).

II.) Gut Digestion

After mechanical grinding in gizzard, the organic material is transferred to intestine for further microbial decomposition. Earthworms 's digestive tract acts as a bioreactor, transforming the microbial communities through gut-associated processes (GAPs). The gut is a vital organ in earthworms, with oxygen depletion condition favouring the dominance of anaerobic and facultative anaerobic bacterial community. These generally include members of the phyla Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria (Drake and Horn, 2007a; Horn et al., 2003a). The amount of aerobic and anaerobic bacteria in the worm gut is 12-20 and 10-4000 times higher than that in soil (Horn et al., 2003b). The strongest enzyme activity is observed in the fore and mid-gut of earthworm (Brown et al., 2000). During gut transit, the substrate undergoes a variety of metabolic shifts, including the incorporation of sugar derivatives, low-molecular-weight mucus, ammonia, nitrogen-containing excretory products, and soluble carbon molecules (Samal et al., 2019a).

Endosymbiotic bacteria in the gut hosts a diverse microbial community and secretes respective extracellular and intracellular enzymes that convert macromolecules into soluble oligomers and monomers (amino acids and fatty acids) (Al-Maamori, 2024). Specifically, certain genera play important roles in organic matter decomposition and nutrient cycling. For instance, Bacillus species (Firmicutes) produce hydrolytic enzymes such as amylases, proteases, and cellulases, which break down starch, proteins, and cellulose, respectively. Pseudomonas (Proteobacteria) helps degrade lignin and aromatic chemicals, and certain strains have antimicrobial properties. Similarly, Enterobacter (Proteobacteria) participates in nitrogen transformations, including ammonification. These microbes make up a crucial component of the earthworm gut microbiome, promoting breakdown processes. Some of which are absorbed by the intestinal epithelium are used for worm growth and cocoon development (assimilation). The remaining material is compact and microbially enriched since it is excreted as casts (Budroni et al., 2020).

III.) Selective Microbial Filtering

The gut is a selective microenvironment that suppresses some microbial taxa (including many pathogens and labile bacteria) while allowing others to survive or emerge. Metabarcoding and culture studies consistently show a drop in microbial diversity soon after GAPs, followed by a transition in community composition in casts. For instance, Srivastava et al. (2021) reported that Proteobacteria were the dominant phylum at the initial vermicomposting stage with a relative abundance (RA) of 47.87%. Subsequently their RA declined to 31.81% in intermediate phase and 16.84% in final phase as vermicomposting progressed, reflecting microbial restructuring effects of earthworms GAPs.

Harmful microbes like E. coli and Salmonella often cannot survive the gut passage because of enzymes, digestive juices, and competition with gut flora. The resident gut flora produces antimicrobial compounds, such as short-chain fatty acids and other metabolites, that directly inhibit the growth of foreign or potentially hazardous microbes (Gorbach, 1996). Study on sewage - sludge vermicomposting revealed that most native bacteria (96%) and fungi (91%) are eliminated through the gut process. As a result, the surviving species shape new microbial community that exists in worm casts shifts towards beneficial, fast-growing decomposers (Domínguez et al., 2021). It able to achieve partial sanitization (pathogen suppression) by killing pathogenic microbes. Classic syntheses show that earthworm gut conditions (low oxygen and high organic substrate concentrations) act as a selective filter during gut passage, stimulating denitrifying and fermentative/anaerobic bacteria while suppressing other taxa. As a by-product of this microbial activity, nitrous oxide (N2O) may be emitted, reflecting the biochemical transformation occurring during anaerobic gut passage (Drake and Horn, 2007b).

IV.) Inorganic Material Decomposition and Detoxification

Enzymatic activity in the earthworm digestive system immobilises harmful metals, suggesting the potency of vermicomposting approach for removing heavy metal from organic wastewater. Earthworms generate intracellular metal-binding molecules that play an important role in detoxification and metal homeostasis during vermicomposting. These molecules can be classified into two types: phytochelatins and metallothionein.

Phytochelatins (PCs) are thiol-rich oligopeptides synthesized from glutathione (GSH), a tripeptide containing glutamate, cysteine, and glycine. They are produced in reaction to exposure to soft metals and metalloids like cadmium (Cd) and arsenic (As). PCs chelate metal(loid) species (MEs), resulting in PC-ME complexes which cooperate with metallothionein to stabilize and neutralize heavy metals. Metallothionein (MTs) are cysteine-rich, low-molecular-weight proteins that can bind divalent transition metals such as copper (Cu), nickel (Ni), and zinc (Zn) (Hussain et al., 2021).As demonstrated in Figure 2.4, heavy-metal pollutants are first processed in the extracellular system into MEs. Subsequently, detoxification proceeds by rapid chelation and sequestration, which GSH induce PCs synthesis and MTs bind metal ions to form stable PC-metal and MT-metal complexes that are stored in intestinal/chloragogenous tissues, thereby lowering metal bioavailability. In the context of vermicomposting, these pathways enable earthworms to tolerate metal-contaminated substrates and enhancing the remediation potential of earthworm-based treatment systems (Al-Maamori, 2024; Hussain et al., 2021)

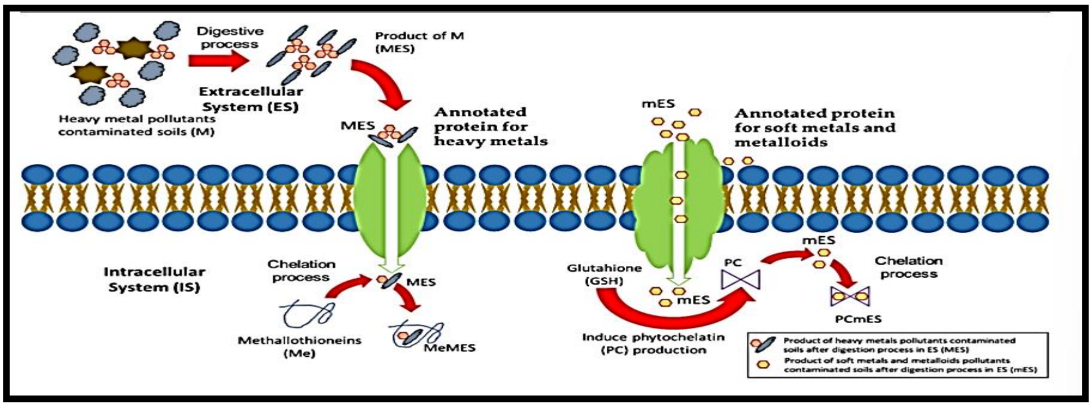

Figure 2.4: Schematic diagram of inorganic material decomposition (Ratnasari et al., 2021)

Figure 2.5 demonstrated that the earthworm gut also facilitates the transformation of organic, polymeric contaminants and antibiotics (Bhat et al., 2018). For instance, high toxic organochlorine pesticides (e.g., DDT) undergo reductive dichlorination and dehydrochlorination by gut-associated dehalogenases in reducing micro zones (Mudziwapasi et al., 2016). This yield less-chlorinated metabolites (e.g., DDE). Low density polymeric microplastics (e.g., polypropylene) are mechanically fragmented in the gizzard and exposed to microbial depolymerases that can be metabolized by bacteria such as Brevibacillus borstelensis in worm 's gut (Auta et al., 2017; Huerta Lwanga et al., 2018).This result in production of oligomers and shorter-chain hydrocarbons (e.g., Docosane & Eicosane) that are more tolerant to biodegradation. Besides, the abundance of microorganisms in the worm gut makes it an ideal micro-zone for antibiotic degradation (Lin et al., 2015). For instance, study reported that antibiotics (e.g., oxytetracycline) was able to be transformed to metabolites such as 4-epi-oxytetracycline with reduced antimicrobial potency.

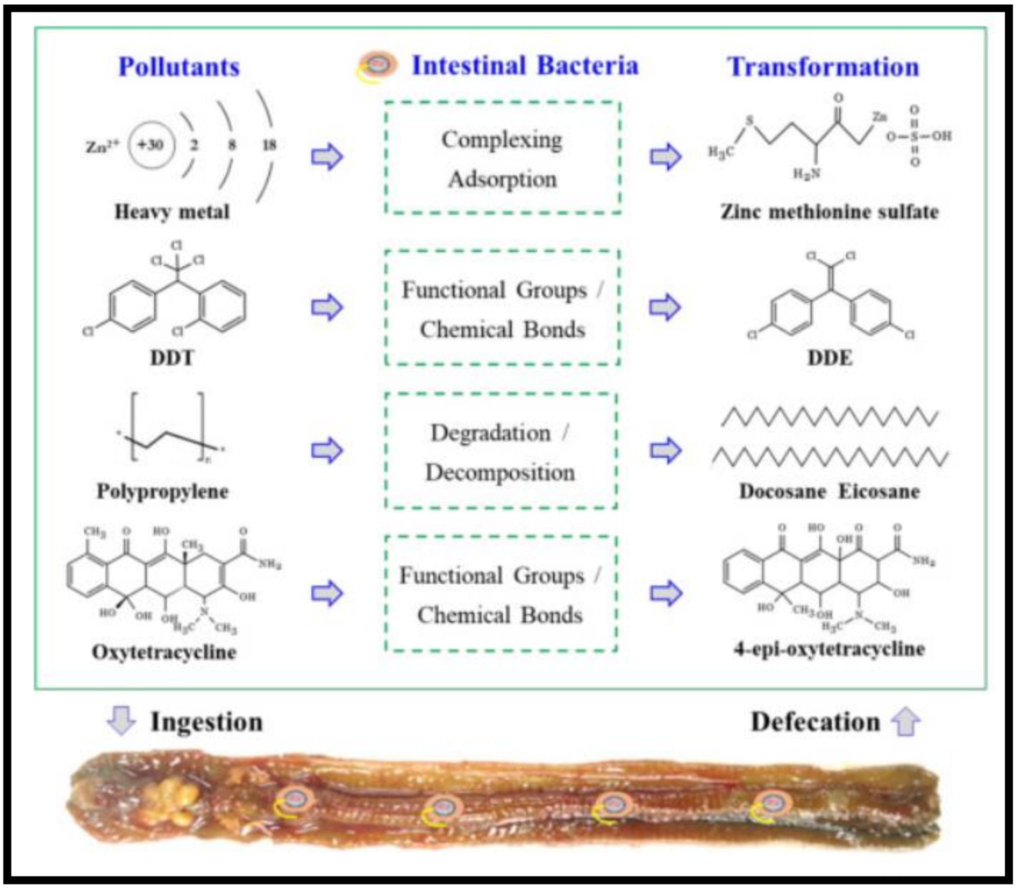

Figure 2.5: Pollutants Detoxification Transformation

(Sun et al., 2020)

2.3.2 Maturation Phase (Microbial)

The maturation phase of vermicomposting is governed by microbial and biochemical processes that occur after GAPs. During this phase, freshly excreted casts serve as biologically active microenvironments. This section outlines the key mechanisms involved, including (I.) Cast Excretion (II.) Mineralization (III.) Assimilation. Collectively, these processes drive nutrient release, organic matter stabilisation, microbial succession, and the formation of mature, agronomically valuable vermicompost.

I. Cast Excretion (entry point to CAPs)

After GAPs, worm excrete partially digested material as small pellets called worm casts. Fresh casts are morphologically fine, chemically enriched (higher levels of NH_4^+ & exchangeable nutrients), and biologically active with high enzyme activity and dense populations of surviving or gut-introduced bacteria (Eastman et al., 2001). Excreted casts form on and within the soil profile. When deposited on the surface, rainfall can redistribute or erode fresh casts. As the casts dry over time, microbial mucilage and fungal hyphae bind the particles together, which increases their structural stability (Samal et al., 2017; Wang et al., 2014).

Due to their nutrient enrichment and microbial inoculation, casts stimulate the growth of organic degrading and nitrifying/denitrifying bacteria. Fungal spores or hyphal fragments that survive the gut transit proliferate rapidly in earthworm excreta, while protozoa also increase in relative abundance by feeding on surviving microbial populations (Bhatia et al., 2013). Hence, casts provide a temporally concentrated supply that stimulates rapid microbial colonisation and enzymatic turnover in further CAPs (Singh et al., 2017). Table 4 summarises representative bacterial taxa detected in earthworm casts, including members of Bacteroidetes, β-proteobacteria, μ-proteobacteria, y-proteobacteria, and firmicutes. Collectively, these microorganisms participate in key biochemical processes such as enzymatic degradation, protein hydrolysis, biofilm formation, nitrogen transformation etc.

Table 2.2: Earthworm Casts Bacteria and their activity

Taxon	Bacteria	Activity
Bacteroidetes	• *Saprospiraceae*	-Protein hydrolysis and uses amino acids as energy and carbon sources.
β-proteobacteria	• *Azospira* sp. • *Fluviicola*	-Perchlorate-reducing bacteria degradation process. -Gram negative and aerobic bacteria which may help in the sludge digestion process.
δ-proteobacteria	• *Aeromonas caviae*	-Degrade organic matter, reduce nitrate to nitrite, and show strong biofilm-forming capacity.
y-proteobacteria	• *Aeromonas hydrophila*	-Enhance the degradation of organic matter by secreting enzymes.
Firmicutes	• *Bacillus subtilis*	-Induce beneficial biofilm production and produce a variety of proteases

II. Mineralization

Following microbial colonisation, the microbial community within casts becomes more diverse, with taxa specialised in mineralisation processes such as proteolysis, ammonification, nitrification and polysaccharide decomposition (cellulolytic bacteria and fungi). The key processes of mineralization include conversion of organic nutrients into plant-available inorganics forms through microbial activity. Nitrogen mineralization occurs via ammonification, where organic nitrogen is transformed into ammonium (NH4+), followed by nitrification hat converts ammonium into nitrate (NO3-). Similarly, phosphorus mineralization release inorganic orthophosphate (PO43-) from organic phosphorus compounds, while potassium is liberated from decomposing plant tissue.

During this stage, shifts in microbial community composition may occur as different taxa dominate in response to changing substrate availability throughout the mineralisation process. Srivastava et al. (2021) used metabarcoding analysis to investigate fungal succession during vermicomposting and reported a shift from early opportunistic fungi to later-stage decomposer fungi. For instance, early-stage fungi, often opportunistic taxa such as Cladosporium, were observed to decrease over time, whereas later stages showed enrichment of decomposer fungi such as Trichoderma. Similarly, study revealed that bacterial communities undergo succession where early increase in Bacteroidetes right after GAPs, followed by a more diverse community enriched in Actinobacteria and genera which contribute to plant growth promotion (Domínguez et al., 2019). Ultimately, the mature vermicompost is typically dominated by bacterial phyla including Actinobacteriota, Proteobacteria, Bacteroidetes, Chloroflexi, Firmicutes and (Lei et al., 2024; Xing et al., 2024)

III. Assimilation

During GAPs, a portion of the substrate carbon and nitrogen is absorbed into worm biomass (tissue and cocoons). This assimilation reduces the total mass of the substrate while concentrating nutrients inside the worms, which can be reused (seeded into new batches) or valorised as protein-rich biomass. Microbes in CAPs digest the soluble nutrient pool into microbial biomass, which becomes part of stable soil organic matter after transition. Plants assimilate the nutrients that were effectively mineralized in soil surrounding.

III. Humification and Stabilization

Fungal and actinomycete extracellular oxidases (e.g., laccases, lignin- and manganese-peroxidases) oxidise phenolic and lignin-derived breakdown products, resulting in radical-mediated coupling processes. These oxidative polymerisation and condensation events generate larger, recalcitrant humic-and fulvic-like macromolecules, which aid in humification and long-term soil organic matter stabilisation as cast aging. This black, humus-rich macromolecules are the final product of vermicomposting, knowns as mature vermicompost. Mature vermicompost exhibits low labile carbon (a lower C:N ratio), a steady humic fraction, a neutral pH, an odourless condition, better aggregate structure, and an enrichment in plant-available nutrients. These characteristics emerge from the combined effects of GAPs and CAPs and are accountable for the agronomic benefits

2.4 Factors Affecting Vermicomposting Process

Vermicomposting efficiency is influenced by a variety of environmental, chemical, and biological parameters. These factors determine the metabolic activity of earthworms, microbial succession, and ultimately the quality of the vermicompost produced. Proper regulation of these parameters ensures optimal decomposition and nutrient recovery

2.4.1 Aeration

Adequate oxygen supply is essential for maintaining aerobic microbial activity and preventing the emergence of anaerobic conditions. Earthworms actively enhance aeration through their burrowing, which increases substrate porosity and facilitates oxygen diffusion. Aeration is equally important for earthworm physiology, as respiration occurs through their vascularized moist skin. Oxygen diffuses into the bloodstream, where it is circulated to body tissues to support metabolic oxidation of food. This process produces carbon dioxide as a by-product, which diffuses back into the blood and is released through the skin, ensuring continuous respiratory gas exchange (Chowdhury et al., 2014). Incorporating bulking agents like straw, husk, or shredded paper improves the substrate's physical structure, increasing porosity and supporting the restoration of aerobic conditions (Ren et al., 2023).

2.4.2 Light

Earthworms are extremely sensitive to sunlight because they rely on moist, permeable skin for respiration. Prolonged exposure raises body temperature above safe levels and can induce paralysis or even mortality due to thermal and oxidative damage (Enviroliteracy, 2025). Instead, exposure to ultraviolet (UV) radiation such as UV-A or UV-B induces strong muscle contractions, disrupts epithelial integrity, reduces oxygen consumption, and is lethal in many species (Chuang et al., 2006). Besides, artificial light (AL) at night significantly disturbs earthworm ecology. Experiments with Lumbricus terrestris, Mittmannsgruber et al. (2024) revealed a 76% decrease in surface activity and a 37% decrease in casting under light pollution. Furthermore, 85% of mating occur in absence of AL, demonstrating that AL inhibits reproductive behaviour.

2.4.3 C:N ratio

The initial C:N ratio of feedstock is a principal control on microbial dynamics during decomposition. Carbon provides energy and structural substrate, whereas nitrogen is required for microbial protein synthesis. A balanced C:N ratio therefore supports steady microbial growth and efficient organic-matter breakdown. When the C:N ratio is excessively high, decomposition slows due to nitrogen limitation, resulting in incomplete mineralization. Conversely, low C:N ratios promote rapid ammonification and may liberate toxic concentrations of free ammonia that are harmful to both earthworms and microbial communities (Roohollah Rostami, 2011). Managing feedstock mixtures by adding high-carbon bulking agents (e.g., dry leaves, straw, and rice husk) or nitrogen-rich materials (e.g., manure, fruit scraps, and tea waste), helps steer the system toward nutrient balances. As summarised in Table 2.3, studies have reported that an initial C:N ratio between 18 and 30 generally produces favourable vermicomposting outcomes, including improved earthworm growth, higher nutrient yield and better compost quality.

Table 2.3: Recommended C:N ratio for different substrate and earthworm species

Substrate/ Feedstock	Earthworm Species	Initial C/N Ratio	Key Outcomes	Reference
Pineapple Peel, Rice Straw, + Cattle Manure	Eisenia Fetida	24-30	Optimal initial C/N for efficient vermicomposting	(Lin et al., 2024)
Sewage Sludge + Wheat Straw	*Eisenia Andrei*	18	Highest earthworm population and maximum compost quality	(Dume et al., 2023)

Sheep Dung + Rice Straw	*Eudrilus Eugeniae*	30	Increase nutrient enrichment	(Hidayati et al., 2021)
Spent Mushroom + Pig Manure	*Eisenia Fetida*	20-25	Highest earthworm biomass and substrate degradation	(Shi et al., 2020)
Farm Wastewater + Rice straw	*Eisenia Fetida*	15-27	Enhanced nutrient transformation	(Liu et al., 2021)
Biosolids + Paper mulch	*Eisenia Fetida*	25	Highest product stability	(Ndegwa and Thompson, 2000)
Chicken Manure + Paper Waste	*Eisenia Fetida*	30	Improved nutrient availability	(Ravindran and Mnkeni, 2016)
Seaweed + Cow dung	*Eudrilus Eugeniae*	18	Highest earthworm growth rate	(Biruntha et al., 2020b)

2.4.4 Earthworm Stocking Density

Stocking density has a significant impact on the efficiency of vermicomposting. Uvarov and Scheu, (2004) discovered that increased density causes higher mortality, slower growth, and poorer cocoon output. Besides, Domínguez and Edwards (1997) reported that overcrowded conditions resulted in slower development and reduced biomass accumulation of earthworm, whereas underpopulated condition produced lower casting and digestion rates. Hence, maintaining an optimal stocking density is thus critical for optimising population growth and ensuring quick conversion of organic materials into casts, while preventing resource competition and stress (Ndegwa et al., 2000a). Table 2.4 summarizes the recommended stocking densities for different substrate and earthworm species, reporting both feed-based and space-based metrics (e.g., worms.kg^{-1} feed, g-worms.kg^{-1} feed, kg-worms.m^{-2}, and mg-worms.cm^{-3}) alongside the reported outcome including bioconversion efficiency, nutrient enrichment, reproductive rate and degree of humification.

Table 2.4: Recommended Stocking Density by Substrate and Species

Substrate/ Feedstock	Earthworm Species	Stocking Density	Key Outcomes	Reference
Green Waste	Eisenia Fetida	30 worms kg^{-1} feed	Highest earthworm growth	(Ma et al., 2024)
Paper waste + cow manure	Eisenia Fetida	12.5g worms kg^{-1} feed	Highly humified vermicompost	(Unuofin and Mnkeni, 2014)
Biosolid + Paper Mulch	Eisenia Fetida	1.60 kg worms m^{-2} (vermibed surface area)	Highest bioconversion rate	(Ndegwa et al., 2000b)
Cattle Dung	Perionyx Excavatus	0.068-0.342mg worms cm^{-3} (vermibed volume)	Maximum reproductive rate	(Suthar, 2012)

2.4.5 Moisture Content

Moisture content is a critical parameter in vermicomposting, as it influences oxygen diffusion, microbial activity, and the physiological functions of earthworms. Reinecke and Venter (1987) reported that insufficient moisture adversely affects the sexual development of earthworms, delaying clitellum formation. Water also serves as the medium for numerous biochemical reactions and facilitates nutrient transport during the vermicomposting process. In contrast, excessive moisture can be detrimental. When water accumulates and seeps through the vermicompost, it displaces air from pore spaces. Microorganisms subsequently consume the limited oxygen available in these waterlogged conditions, leading to an anaerobic environment that restricts aerobic microbial activity and reduces overall process efficiency. Table 2.5 summarize the recommended moisture ranges for various substrates and earthworm's species in vermicomposting, with most studies indicating an optimal moisture range of 65-85% promoting efficient earthworm activity, nutrient mineralization and stable vermicompost production.

Table 2.5: Recommended Moisture Ranges by Substrate and Species

Substrate/ Feedstock	Earthworm Species	Moisture Range	Key Outcomes	Reference
General	Eisenia Fetida an	80-85%	Highest biomass growth and maturation	(Singh et al., 2018)
Municipal Sludge	Eisenia Fetida	72%	Highest yield of nitrogen (2.76%), phosphorus (1.80%), potassium (1.88%)	(Banda et al., 2023)
		78%	Highest nitrogen mineralization rate	(Qin et al., 2021)

Cattle Manure	Eisenia Fetida	70%	Improved crude fibre degradation	(Hossen et al., 2022)
Food Waste	Eisenia Fetida	65-75%	Optimal worm growth and process performance	(Roohollah Rostami et al., n.d.)
Pig Manure	Eisenia Andrei	85%	Increased earthworm biomass	(Domíngue z and Edwards, 1997)
Oat Straw + **Cow Manure**	Eisenia Fetida	70%	Highest compost stability and earthworm production	(Contreras-Ramos et al., 2005)
Bio-Methanation Sludge + **Fruits Vegetables Waste**	Eudrilus Eugeniae	70-80%	Maximum compost yield and Highest NPK content	(Mulla and Pathade, 2020a)

2.4.6 pH

The pH of the vermicomposting substrate has a considerable influence on microbial enzymatic activity and earthworm performance. Neutral to slightly alkaline conditions are generally most favourable for mesophilic decomposer microbes and commonly used epigeic earthworms, which exhibit optimal growth and reproduction near neutrality. Acidic substrates inhibit bacterial activity and stunt earthworm reproduction and survival, hindering biological processes responsible for decomposition (Wu et al., 2020). In contrast, excessively alkaline environments stimulate ammonia volatilisation, which are hazardous to worms and destabilise nitrogen retention in the substrate. Early in the decomposition process, pH often fluctuates due to the production of organic acids and the transformation of nitrogen compounds. However, the pH gradually converges toward neutrality as humification progresses. This stabilization occurs through a balance between organic acid formation and microbial ammonium assimilation, both of which are enhanced by earthworm activity, thereby improving system resilience. This observation is supported by N.B Singh and his group (2006). They reported that pH values initially range widely from approximately 4.3 to 8.2 but typically stabilizes at around 7.0 by 30 days under worm activity as vermicomposting prolonged. Such stabilization contributes to the production of a mature vermicompost that suitable for soil application. Table 2.6 summarizes the reported pH ranges for various substrates and earthworm species in vermicomposting with most studies indicating that near-neutral pH (6-7.5) promote optimal earthworm activity and stable vermicompost formation.

Table 2.6: Recommended pH by Substrate and Species

Substrate/ Feedstock	Earthworm Species	pH Range	Key Outcomes	Reference
General	Multiple	6.4-7.6 (effective)	Improved nutrient enrichment in mature vermicompost	(Meera T. Sose, 2017)

Apple pomace + Straw	Eisenia Fetida	5.9-6.9 (final)	Production of stable and mature vermicompost	(Hidayati et al., 2021)
Cow dung + Kitchen residue	Perionyx Excavatus	6.5-7.5 (initial)	Optimal starting pH of feed and Stabilization occurs faster	(N.B.Singh et al., 2006)
Bio-methanation sludge + Vegetable waste	Eudrilus Eugeniae	7 (optimal)	Highest earthworm growth and Highest compost yield	(Mulla and Pathade, 2020b)
Digested biogas slurry + Kitchen waste + Palash Leaf litter	Eudrilus Eugeniae & Eisenia Fetida	5-8 (effective)	High earthworm survival rate	(Katiyar et al., 2023)

2.4.7 Temperature

Temperature plays a critical role in regulating earthworm activity. At lower temperatures, food consumption decreases markedly, accompanied by a reduction in reproduction rate and metabolic activity (Zhou et al., 2021). The tolerance of temperature varies among species, as earthworms are poikilothermic organisms whose body temperature is strongly influenced by the ambient temperature (Nick A.Romero, 2025). Studies revealed that optimal temperature for soil nitrification and denitrification ranges between 20–30 °C. Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria which serve as key functional groups in the nitrification process, can stay metabolically active up to temperatures of around 30-35 °C (H.Shang et al., 2009; Lai et al., 2019). Below 20°C, microbial activity is substantially reduced, whereas temperatures that highly elevated from optimum range (e.g. 40-45°C) may cause enzyme denaturation (Zhang et al., 2020). Sui et al. (2024) reported that removal efficiency of NH_4^+-N reach 90% at temperatures ranging from 25 °C to 30 °C, highlighting its effective ammonia removal capability under mesophilic conditions. Table 2.7 summarizes the recommended temperature ranges for different earthworm species, indicating that mesophilic condition (20-28 °C) generally supports optimal earthworm metabolic activity.

Table 2.7: Recommended temperature by Species

Earthworm Species	Temperature Range	Key Outcomes	Reference
Perionyx Excavatus	20-25°C	Highest rate of reproduction	(Edwards et al., 1998)
Eudrilus Eugeniae	25-28°C	Optimal metabolic rate	(Karmegam and Daniel, 2009)
Eisenia Fetida	13-32°C	Able to survive within this range	(Steve Churchill, 2020) (Zhang et al., 2020)
	25°C	Optimal temperature	

2.5 Spent Coffee Grounds (SCG)

2.5.1 Production and Processing

Coffee, a globally traded commodity, belongs to the family Rubiaceous and is primarily represented by two commercially dominant species, Coffea arabica (Arabica) and Coffea canephore (Robusta). Industrial coffee processing produces residues, including husks, parchment, pulp, mucilage, and spent coffee grounds (SCG), with total volumes exceeding millions of tons annually (Andrade et al., 2022). SCG is the major solid waste by-product, accounting for approximately 45% of the coffee mass used in brewing. It is formed during beverage preparation, where green beans are first roasted, then milled to a specific particle size, and subsequently subjected to extraction methods such as espresso, drip, immersion, or cold brew (Mussatto et al., 2011). Throughout the process, soluble compounds are removed by water, while the remaining fibrous matrix constitutes the SCG.

2.5.2 Factors Influencing the Composition

The composition of SCG is influenced by intrinsic factors such as coffee species and cultivar (coffee plant), which determine baseline levels of lipids, carbohydrates, nitrogen, phenolic compounds and secondary metabolites (e.g., caffeine). Roasting is a very important process when discussing SCG. Melanoidins are produced by roasting via a non-enzymatic Maillard reaction, which contain polysaccharides such as mannans, morphogenetic proteins, chlorogenic acids, and other compounds (Cavanagh et al., 2023). Processing conditions such as roast degree modifies chemical composition through the degradation of chlorogenic acids and the formation of melanoidins, thereby altering the balance between soluble and insoluble constituents (Trugo and Macrae, 1984). Furthermore, operational variables including grind size, extraction temperature, and time, may affect the residual concentrations of cellulose, hemicellulose, lignin, lipids, and bioactive compounds in SCG. (Genovese et al., 2025; Tarigan et al., 2022)

Figure 4 illustrates the anatomical structure of the coffee fruit and the processing pathways involved in coffee production. During dry and wet processing, various by-products such as coffee husks, mucilage, parchment, and silver skin are generated, while subsequent roasting, grinding, and brewing stages further produce secondary residues, particularly spent coffee ground.

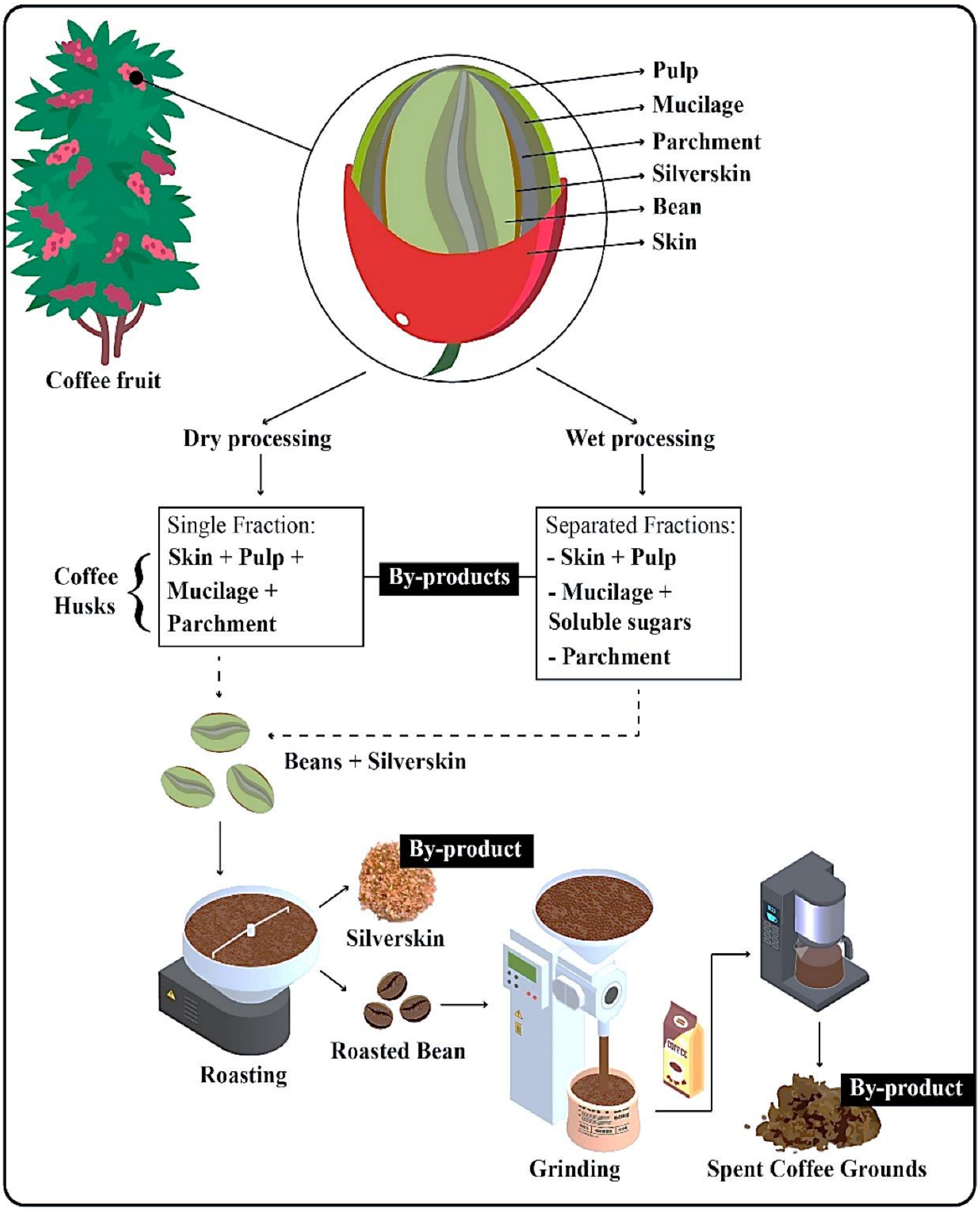

Figure 2.6 : Pollutants Detoxification Transformation

(Oliveira et al., 2022a)

2.5.3 Physical Properties and Chemical Composition

2.5.3.1 Physical Properties

SCG exhibit physical properties of fine particle size (100-500 μm), high moisture retention, high bulk density, and strong water-holding capacity (WHC) of 3-4 g H_2O g^{-1} dry SCG. High WHC supplies moisture but raise risk of waterlogging (Bejenari et al., 2022). These characteristics, small particle size with high WHC, enable microbial colonisation and adsorption of soluble compounds, although excessive accumulation may lead to substrate compaction and reduced aeration

2.5.3.2 Chemical Composition

I. Carbohydrates

SCG are rich in carbohydrate polymers, which about half of the material is made of cellulose and hemicellulose, and around 70% of these carbohydrates stay in the SCG (Zabaniotou and Kamaterou, 2019). Hemicelluloses in SCG are heterogeneous which comprising mannans, galactose, arabinose, and glucose. The amount and relative proportions of these sugars vary with coffee variety, roasting degree, and brewing procedure, resulting in compositional variances across SCG from various sources (Campos-Vega et al., 2015) . Extracted polysaccharides from SCG have showed functional capabilities such as antibacterial, antioxidant, and thermal stabilisation capability (Ballesteros et al., 2014).

II. Nitrogenous Compounds

Total nitrogenous material in SCG has been reported to range between 8.5% and 13.6%, and this fraction appears to be relatively stable among SCG (Werner Grosch et al., 2009). These nitrogenous components include proteins, free amino acids, peptides, and alkaloids. Thermal condition during coffee processing denatures protein fractions into smaller polypeptides. Afterward, subsequent reactions between reducing sugars and polypeptides can create condensed nitrogenous pigments (Cruz et al., 2012c). Protein hydrolysates derived from SCG exhibit antioxidant activity and in vitro angiotensin-converting enzyme inhibitory properties. Non-protein nitrogenous compounds present in SCG possess agricultural potential. While direct application of SCG can induce phytotoxic effects, biological treatments such as vermicomposting may convert them into nitrogen-enriched fertilizers (Jin Cho et al., 2022).

III. Lipids

In coffee, lipids are mainly present as oils and fats. However, their concentration in SCG is comparatively low, as a substantial portion of the lipophilic fraction is extracted into the beverage or lost during filtration. The fat content of SCG has been reported to range from approximately 10% to 15%, with glycerides accounting for the majority fraction (80%–90%), while minor components include terpenes, sterols, and tocopherols (Zhao et al., 2024).

IV. Phenolic Compounds

SCG contain a high polyphenolic content, which influences coffee quality while additionally improving the residue's nutritional and functional value. Major phenolics found in SCG include chlorogenic acid, protocatechuic acid, and different flavonoids. These compounds have antioxidant, hypoglycaemics, and antibacterial properties, making SCG a viable source of natural additives (Castaldo et al., 2021). Phenolic compounds are main determinants of antioxidant capacity and have the potential for photoprotective applications, helping to minimise the detrimental effects of UV radiation on biological tissues – a feature that may benefit species like earthworms (Saewan and Jimtaisong, 2013).

V. Micronutrients/Minerals

The inorganic residue (ash) of SCG is relatively minor (1.3-2.77%) but contains several essential minerals as determined by ICP-AES analysis (Ballesteros et al., 2014). The most abundant element is potassium, followed by phosphorus and magnesium, indicating that SCG fit for soil amendment. Figure 5 illustrates the major chemical composition of SCG, including carbohydrates, lipids, minerals, phenolics, caffeine and nitrogenous compounds.

2.5.3.3 Summary

Table 9 summarizes the key physical parameters and chemical composition of SCG. Overall, SCG is physically characterized by fine particle size, moderate acidity, and high water-holding capacity, while chemically it contains high organic carbon content, significant lignocellulosic compounds, moderate nitrogen levels, phenolic compounds and the presence of bioactive compounds such as caffeine and tannins.

Table 2.8: Physical Parameters and Chemical Composition of SCG

Components	Range	Reference
Physical Parameters		
Calorific value (MJ/kg)	20-26.9	(Kang et al., 2017)
Moisture content (% DW)	7.5-11.7	(Ballesteros et al., 2014.Hussein et al.,2019)
Particle size(μm)	100-500	(Kang et al., 2017)
pH (raw SCG)	5.2-5.9	(Cruz et al., 2012a)
	6	(Hassan and Al-Yaqoobi, 2024a)
Water holding capacity (g $H_2O.g^{-1}$ DW)	3.57-3.97	(Kang et al., 2017)
Chemical Composition		
Total Carbon (% DW)	47.8-69.5	(Cruz et al., 2012c)
Total Nitrogen (% DW)	1.9-2.3	(Cruz et al., 2012c; Jeguirim et al., 2014)
C/N ratio	19.8-21	(Cruz et al., 2012c; Jeguirim et al., 2014; Kondamudi et al., 2008)
Carbohydrates (%DW)	66.9-82	(Hussein et al., 2019)
Cellulose (%DW)	11.6-13.19	(Hussein et al., 2019)
Hemicellulose (% DW)	37.16-41.04	(Ballesteros et al., 2014)
Galactose (%)	14.77-18.09	(Ballesteros et al., 2014)
Mannose (%)	18.22-19.92	(Ballesteros et al., 2014)
Lignin (% DW)	22.2-25.6	(Ballesteros et al., 2014)
Insoluble (%)	16.03-19.15	(Ballesteros et al., 2014)
Soluble (%)	5.94-6.68	(Ballesteros et al., 2014)

Total dietary fibre	24.1-60.5	(Ballesteros et al., 2014; Jiménez-Zamora et al., 2015a; Johnson et al., 2022)
Insoluble (%)	17.8-50.8	
Soluble (%)	6.31-12.38	
Nitrogenous Compound		
Protein (%)	13.6-17.4	(Campos-Vega et al., 2015; Hussein et al., 2019; Jiménez-Zamora et al., 2015b)
Protein-energy value (%)	11.1	
Lipids	10-29	(Campos-Vega et al., 2015; Carruthers-Taylor et al., 2020; Jiménez-Zamora et al., 2015a; Pujol et al., 2013)
Fatty acid (%)	60-80	
Lipophilic (%)	24	
Phenolic Compounds		
Phenols (%)	23	(Carruthers-Taylor et al., 2020; Murthy and Madhava Naidu, 2012; Pujol et al., 2013)
Polyphenols (%)	0.5-2.5	
Micronutrients/Minerals		
Ash (% DW)	1.3-2.77	(Campos-Vega et al., 2015; Hussein et al., 2019; Jiménez-Zamora et al., 2015b)
Calcium (mg/100g)	34.9-120	(Ballesteros et al., 2014; Campos-Vega et al., 2015)
Iron (mg/100g)	4.6-5.2	
Magnesium (mg/100g)	190-220	(Ballesteros et al., 2014; Campos-Vega et al., 2015)
Phosphorus (mg/100g)	180-200	
Potassium (mg/100g)	882.4-1170	(Ballesteros et al., 2014; Campos-Vega et al., 2015)
Sodium (mg/100g)	3.37-20.1	
Bioactive Compounds		
Caffeine (% DW)	0.2-0.8	(Cruz et al., 2012c; Iriondo-DeHond et al., 2021)
Chlorogenic acid (% DW)	0.2-0.8	
Melanoidins (% DW)	13-25	(Cruz et al., 2012c; Iriondo-DeHond et al., 2021)
Tannins (% DW)	0.02	

(DW: Dry Weight)

2.5.4 Agronomic Potential

Spent coffee ground (SCG) are increasingly recognised as a valuable biowaste for sustainable agronomy. Its nutrient-rich composition (e.g. fatty acids, polyphenols, minerals and polysaccharides) provides both nutrients and labile carbon substrates for soil biota, including earthworms (Oliveira et al., 2022b). Study revealed that SCG as amendment in lettuce cultivation are associated with higher photosynthetic pigment concentrations and enhanced levels of total ascorbic acid and tocopherols, which yield greater crop quality (Cruz et al., 2015) Instead, SCG can be utilised as organic amendments to reduce substrate toxicity through biological treatment such as vermicomposting (Cervera-Mata et al., 2020).

Additionally, SCG possess an inherently porous structure that confers useful functional properties for both composting and soil amendment. During composting, SCG can adsorb soluble salts and other dissolved constituents, thereby lower the salinization risk of the final compost and contribute to soil restoration when the compost is applied as a soil conditioner (Cruz et al., 2014). Furthermore, SCG may function as an excellent adsorbent for soluble salts and certain heavy metals (Campbell et al., 2024; Futalan et al., 2019). Although unmodified SCG have a significantly lower specific surface area than engineered activated carbons, their diverse organic functional groups and intrinsic porosity provide measurable adsorption capacity.

Several studies have demonstrated the agronomic potential of SCG through soil amendment and biological treatment. In a vermicomposting experiment, a cow dung: SCG mixture (30:70) produced significantly greater earthworm multiplication and higher nutrient concentrations in vermicompost after 49 days, indicating improved fertilizer quality compared with other substrate combinations (Adi and Noor, 2009). Similarly, Lorenzo et al. (2022) reported that surface-applied SCG reduced naturally emerging weed biomass while promoting crop growth under warm and relatively dry conditions. In a pot experiment with lettuce (Lactuca Sativa), incorporation of 2.5-10% (v/v) SCG increased plant biomass and significantly enhanced photosynthetic pigments, including up to 90% higher lutein, 72% higher β-carotene, and 61% higher chlorophyll, although higher rates (Cruz et al., 2012b).

2.6 Organic wastewater

Organic wastewaters refer to aqueous effluent which pollutant load dominated by organic compounds composed mainly of carbon-based molecules present in dissolved, colloidal or particulate forms. These compounds may be biodegradable or persistent, encompassing natural biomolecules (carbohydrates, amino acid, lipids etc.) as well as synthetic organic pollutants (phenols, hydrocarbons, pesticides etc.) (Wen et al., 2023). Organic compounds in wastewater are typically quantified and monitored through the chemical oxygen demand (COD) and biological oxygen demand (BOD). Characteristics of organic wastewater is necessary to be studied because it serves as a principal feedstock in this study.

Organic wastewater contains a wide variety of organic compound that mainly originating from domestic sewage and industrial process such as food and beverage, pulp and paper textile dyeing etc. Polychlorinated biphenyls (PCBs), pesticides, polycyclic aromatic hydrocarbons (PAHs), and polybrominated diphenyl ethers (PBDEs) are among the most serious organic contaminants in wastewater (Zheng et al., 2013). Pesticides are most found in agricultural runoff while PAHs are typically found in effluents from coke manufacturing and petrochemical processes. Chemical industrial operations emit PCBs and PBDEs while food-processing wastewater often contains high biochemical oxygen demand (BOD). Municipal sewage contains oils, surfactants, and other dissolved organic compounds (Zheng et al., 2013).

Organic contaminants in wastewater can be categorised based on their biodegradability and environmental persistence. Readily biodegradable and hydrophilic substances such volatile fatty acid, polysaccharides and short-chain alcohols are typically mineralized by microbes, thus accelerate removal of organic pollutant from wastewater. This quick degradation reduces oxygen demand in receiving water and minimize the risk of eutrophication. However, readily biodegradable compounds such as methanol and acetone will produce acute toxicity at high concentrations and worsen water quality. On the other hands, persistent organic pollutants (POPs) such as PAHs and PCBs are impervious to biological breakdown. POPs are generally lipophilic and easily confined in sediments and biological tissues. This behaviour contributes to bioaccumulation in food chains. The long residence time and chronic toxic effects (carcinogenicity and neurotoxicity) of POPs, make them priorities for environmental monitoring and for the design of treatment and reuse strategies (Shen et al., 2024; Wang et al., 2014; Zheng et al., 2013).

2.6.1 Swine Wastewater (SW)

2.6.1.1 Generation

Swine wastewater (SW) is primarily derived from animal excreta, consisting of faeces and urine, together with process water used for cleaning and cooling within housing facilities Additional inputs include wash water from routine sanitation, feed residues originating from spillage or wastage, and trace quantities of detergents or disinfectants applied during hygiene practices. In intensive production systems, manure and urine are commonly conveyed through slotted floors into underground collection pits or open lagoons. Subsequent flushing with wash water mobilizes these wastes and increases the degree of dilution. Consequently, SW generated from piggery operations can be characterized as a dilute slurry composed of animal excreta, feed residues, and cleaning or cooling water. Owing to its elevated organic content, nutrient load, and potential pollutant capacity, this effluent necessitates appropriate management to mitigate odour emissions and reduce risks of soil and water contamination. Common management strategies include storage in pits, anaerobic lagoons, or settling basins, followed by subsequent treatment, beneficial reuse, or controlled disposal (Aneja et al., 2024). Figure 2.7 illustrates the management pathways of piggery manure and effluent under conventional and deep litter housing systems, including collection, solids separation, storage, composting, energy generation, and final reuse or disposal.

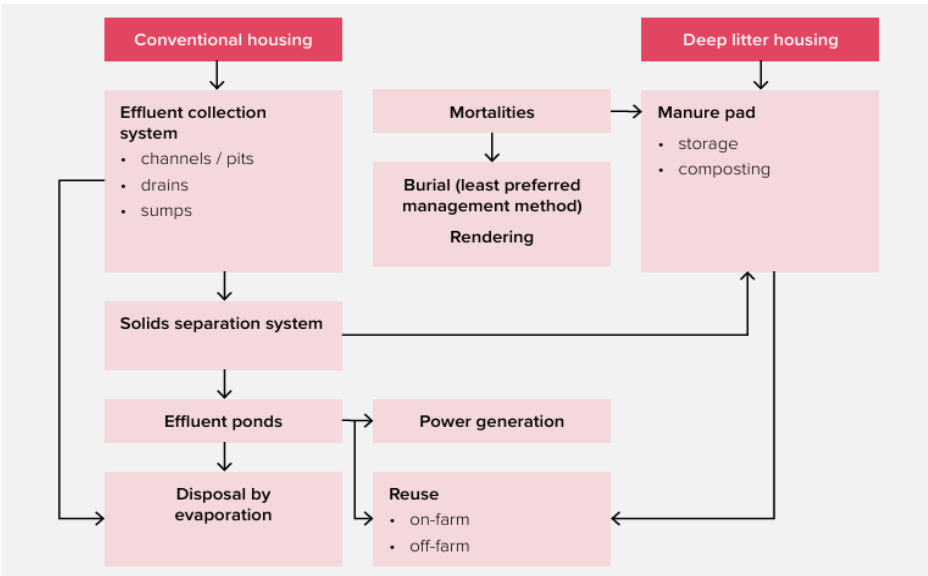

Figure 2.7: Piggery manure and effluent flow diagram
(Tucker et al., 2025)

2.6.1.2 Sources of Contaminants

Swine wastewater (SW) is a complex effluent composed of organic matter, nutrients, chemical pollutants and trace metals. With the extremely complex and homogeneous characteristics, the composition and concentration of swine wastewater can vary considerably, primarily influenced by the volume of flushing water, herd size, dietary inputs, and surrounding environmental conditions such as temperature and humidity (Cheng et al., 2020). SW may harbour more than 150 potential zoonotic agents (including pathogens such as Escherichia coli and Salmonella), which can cause gastrointestinal disease and systemic infections in humans and animals (Giácoman-Vallejos et al., 2015). The periodic addition of growth-hormones into swine feed during farm operation exacerbates the problem. Zhang et al. (2021) reported that, 22 steroid hormones were still detectable in the effluent even after treatment al. (g. anaerobic digester), underscore the persistence of steroid contaminants. The commonly found steroid hormones are 17β-oestradiol, 4-nonylphenol, progestins, and other oestrogens. Furthermore, the excessive application of antibiotics in pig farming, including sulphonamides, tetracyclines, macrolides, and β-lactams, can lead to an accumulation of antibiotic residues and resistance genes (ARGs) in manure and wastewater (Sui et al., 2016). Figure 2.8 illustrates how pig farming activities pollute surrounding environments through discharge, leaks, and wastewater overflow. Thereby releasing contaminants into soil and water system that trigger algal bloom, ecosystem degradation, and human health risks.

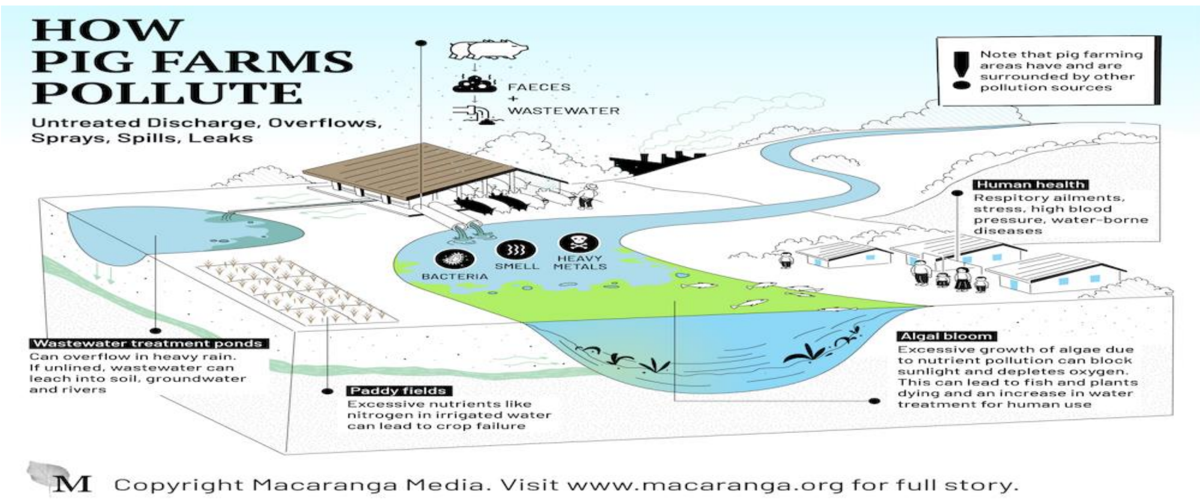

Figure 2.8: How Pig Farms Pollute

(Wong Siew Lyn, 2022)

2.6.1.3 Physical Properties and Chemical Composition

Swine wastewater (SW) is a light brown, water-dominated matrix with high moisture content and a relatively low particulate fraction. SW typically exhibits slightly alkaline conditions and elevated alkalinity. Its temperature commonly falls within the mesophilic range in common, which may support microbial activity in biological treatment systems. Chemically, SW contains substantial organic load reflected by total COD concentrations in the range of 19,000-21000 mg O_2/L. The low biodegradability index (≈0.18) suggest the presence of a significant refractory organic fraction, which may limit the efficiency of biodegradation. Furthermore, SW contain high concentrations of nutrients particularly nitrogen predominantly in ammoniacal form (NH4+-N) and phosphorus, both of which can contribute to toxicity and eutrophication if discharged without proper treatment.

Table 2.9: Physical Parameters and Chemical Composition of SW

Components	Range	Reference
Physical Parameters		
Alkalinity(mg/L)	2069-2254	(Lamssali et al., 2024)
Colour (CN)	2.24-2.418	(Díaz et al., 2022)
Moisture content (%)	70.39-80.53	(Zhu, 2006)
pH	8.55-8.6	(Díaz et al., 2022)
Total Solid (%)	1.0-2.1	(Schievano et al., 2016; Yang et al., 2019)
Suspended Solid (%)	0.9-1.2	(Schievano et al., 2016; Zhang et al., 2011)
Chemical Composition		
Biodegradability Index (BI)	0.18	(Díaz et al., 2022)
Soluble COD (mg O_2/L)	3638-3916	(Díaz et al., 2022)
Soluble BOD_5 (mg O_2/L)	672-724	(Díaz et al., 2022)
Total COD (mg O_2/L)	19442-21632	(Díaz et al., 2022)
Total Nitrogen(g-N/L)	1.6-7.3	(Yang et al., 2016; Zhang et al., 2011)
NH_4^+-N (g-N/L)	1.4-4.9	(Yang et al., 2016; Zhang et al., 2011)
NO_3^- (mg-/L)	82	(Oliveira et al., 2021)
Total Phosphorus(g-P/L)	0.2-0.5	(Yang et al., 2016; Zhang et al., 2011)
C/N ratio	10.7-11.88	(Huang et al., 2004)

2.7 Synergistic Effect of SCG and SW

Based on experimental results supported by the Ministry of Agriculture of the Czech Republic, SCG alone are generally ineffective for vermicomposting because they significantly restrict earthworm survival and development (Hanc et al., 2021). From the experiment, 100% SCG without any amendments exhibited low earthworm biomass (≤13 g/kg) and no worms are detected in the oldest layer (180 days), accounting for only 10% of total earthworm biomass across all treatments. This suppression is believed associated with SCG's inherent phytotoxicity (caffeine, tannins), high lignin content (~25–30%), and high bulk density, which can reduce aeration within the substrate. A high proportion of SCG in feedstock can lead to excessive lignocellulosic content, thereby increasing the fraction of slowly degradable carbon and reducing overall decomposition rate (Pandit et al., 2020). Earthworm-mediated fragmentation and enzymatic depolymerization are required to break down lignocellulosic polymers before complete mineralization can occurs. However, study revealed that the specialist microbes that secrete enzyme to break lignocellulose are often low in abundance, which impose additional metabolic stress on microbial communities (Meng et al., 2022). Consequently, SCG can prolong the active phase unless co-processed with more labile substrates or pretreated to improve substrate accessibility. Furthermore, untreated SCGs might contain phytotoxic or bioactive substances that hinder germination or seedling growth (Hechmi et al., 2023). The decline in relative abundance of microorganisms in earthworm digestive tracts resulted in a strong indirect association between overall microbial biomass and earthworm population (Hanc et al., 2021).

Swine wastewater (SW) alone frequently exhibits physical and chemical properties that are incompatible with the aerobic, mesophilic, earthworm-driven process of vermicomposting. A laboratory mesocosm test reported that high ammoniacal loads in SW will inhibit biodegradation of vermicompost. This is supported by the ammonium-induced worm mortality thresholds (LC_{50}=1.49gNH_4^+ kg^{-1} for NH_4CL) computed. Under typical vermicomposting condition, NH_3 content exceed 1 mg/L will causes 100% earthworm mortality (Edwards and Arancon, 2022). Furthermore, SW with high BOD level drives rapid microbial oxygen consumption when the slurry is introduced into a porous bed. Oxygen depletion creates anaerobic micro zones, promoting the proliferation of anaerobic microorganisms instead of aerobic decomposer within earthworms (Vanotti et al., 2018). Instead, Oliveria et al. (2021) reported that SW contains detectable levels of heavy metals such as copper (Cu=0.01 mg/L) and zinc (Zn=0.05 mg/L). These heavy metals can accumulate in the solid fractions of waste and within earthworm tissues during vermicomposting. At elevated concentrations, they can impair enzymatic activity, reduce reproduction rates, and increase mortality in earthworms (Sharma and Satyanarayan, 2011). Therefore, SW alone particularly at higher concentrations may be unsuitable for vermicomposting due to increased earthworm mortality, decreased reproduction. This observation is supported by Iordache et al. (2014), where they reported that earthworm mortality increases with SW concentration, reaching 10% and 20% of mortality at application rate of 12.5 mL/kg and 25 mL/kg respectively. After 14 days of exposure, reproductive performance showed an inverse relationship with SW concentrations, where low doses (3.12-6.25 mL/kg) stimulated cocoon productive relative to the control, whereas higher concentrations (25mL/kg) reduced cocoon numbers by approximately 15.39%

In short, integration of SCG and SW in vermicomposting may represent a potential strategy for optimizing physical and chemical characteristics of the substrates. Raw SCG is typically slightly acidic by nature (Sinclair et al., 2024). Therefore, when mixed with SW a high alkalinity, this will likely help to buffer substrate towards a more neutral, creating a more favourable environment for earthworms' activity. Such pH adjustment may shift the ammonium-ammonia equilibrium towards less volatile NH_4^+ form, thereby reducing the concentration of free NH_3. This mechanism may contribute to lower ammonia volatilization issues and enhance microbial activity during vermicomposting (Fangueiro et al., 2015). Therefore, blending SCG with SW is hypothesized to keep ammonia levels below lethal thresholds for the earthworm that sensitive to salinity, while added carbon from SCG support microbial assimilation of NH_4^+ into biomass (Zhang and Sun, 2017b).

Furthermore, SCG may act as an effective bulking agent, helping to reduce excessive moisture content in SW and create aerated macropores. The porous and fibrous structures of SCG, together with its strong water holding capacity may result in a well-structured, better draining vermicompost bed that reduce risk of waterlogged (Zhang and Sun, 2017b). Moreover, studies revealed that blending SCG with municipal wastewater treatment sludge (MWTS) can enhance microbial respiration as SCG provide readily degradable carbon sources and bioactive compounds, including polyphenols. lipids. and chlorogenic acids. Vítězová et al. (2019) reported that addition of SCG to MWTS resulted in nearly twofold higher microbial respiration rate compared to control samples without SCG. This increase in respiration activity indicates intensified microbial metabolism, which was accompanied by a significant reduction ($p < 0.05$)

in the concentrations of caffeine and tannins. This observation suggests that increase in microbial respiration activity may facilitate the more efficient degradation of inhibitory compounds naturally present in SCG (Vítězová et al., 2019). Thereby, the increased degradation of inhibitory compound (caffeine, tannins) in SCG assure shorter phytotoxins exposure time to earthworm. Consequently, such stimulation suggests that blending SCG with SW could accelerate organic matter decomposition in vermicomposting by providing accessible energy source for the native microbial community.

Additionally, studies revealed that SCG can serve as bio-absorbent and possess potential removal efficiency for heavy metals including copper (Cu), zinc (Zn), lead (Pb) and Chromium (Cr) through adsorption and ion exchange mechanism. Notably, SCG-derived adsorbents demonstrated excellent effectiveness in removing chromium (VI) from water, with removal efficiency ranging from 75% to 80% (Campbell et al., 2024; Futalan et al., 2019; Hu et al., 2022; Jeon, 2017). This adsorption capacity of SCG is primarily attributed to its porous lignocellulosic structure and the presence of abundant surface functional groups, including carboxyl (-COOH), hydroxyl (-OH). These functional groups may provide active binding sites that can interact with dissolved metal ion in wastewater. Therefore, integration of SCG and SW in vermicomposting has potential to immobilize heavy metals such as Cu and Zn that commonly found in livestock effluents, thereby potentially reducing metal toxicity and creating more favourable conditions for earthworm survival and activity.

Moreover, co-substrate mixtures can shift microbial community composition and functional diversity, favouring consortia that enhance organic matter transformation, humification and nutrient stabilization. Enhanced microbial activity generates stabilized organic fractions that can serve as a readily assimilable food source for earthworm, thereby improving their digestion efficiency, cast production, and the overall quality of resulting vermicompost (da Silva et al., 2023; Hachicha et al., 2012; Saba et al., 2019). Blending SCG with SW is believed to help achieve a more optimal initial C/N ratio (18-30), although the C/N ratio will gradually decrease as vermicomposting prolonged. This balance may arise as SCG provide a high-carbon substrate (C/N typically ranging from 20-24) that offsets excessive nitrogen content of SW (C/N typically ranging from 10-12)

CHAPTER 3

METHODOLOGY

This chapter describes the materials, experimental setup, and analytical methods employed in this study. The overall experimental workflow, including raw material characterisation, Berkeley composting, vermicomposting, and analytical evaluation is illustrated in Figure 3.1.

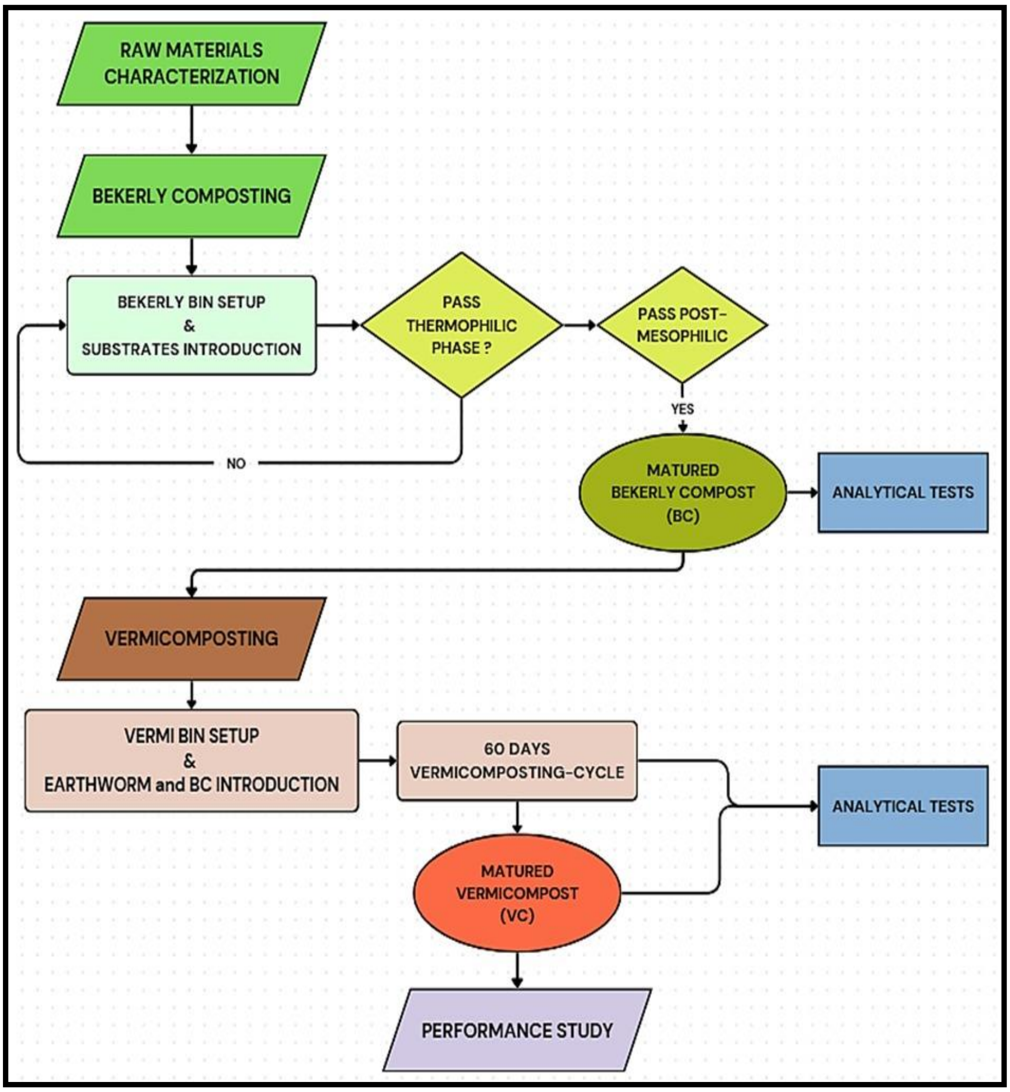

Figure 3.1: Flow Chart of Experimental Methodologies

3.1 Raw Material Characterization

Raw material characterization was conducted prior to establish baseline physicochemical properties of SCG and SW. Establishing these baseline parameters was essential for evaluating substrate quality and identifying potential constraints for subsequent experimental planning and works.

3.1.1 Spent Coffee Ground

In the characterization of SCG, parameters including pH, total nitrogen (TN), total organic carbon (TOC), available nitrogen (N), phosphorus (P), potassium (K) and caffeine content were analysed with extraction and analytical methods, as summarized in Table 3.1. Available forms of N, P, K were determined as they represent the readily extractable and plant-accessible fractions, which are more indicative of nutrient bioavailability during composting and vermicomposting process, while total nitrogen was analysed for C/N ratio determination.

Table 3.1: Characterization of Spent Coffee Ground (SCG)

Characterization	Extraction Method	Analysis Method	Reference
pH	Water extraction (1:5 w/v)	Calibrated pH meter (EN13037:2011)	(Lončarić et al., 2024)
Total Nitrogen (TN)	Water extraction (1:10 w/v)	Persulfate digestion method (Method 10072)	(HACH Company, 2024)
Total Organic Carbon (TOC)	Drying (105°C), grinding	Loss on Ignition method at 550° C	(FAO, 2026)
Available Nitrogen (N), Phosphorus (P), Potassium (K)	Reagent extraction (TPY-6A protocol)	Rapid colorimetric analysis TPY-6A model soil nutrient tester	(TOP Cloud-Agri Technology Co., 2025)
Caffeine	Methanol extraction followed by acetonitrile clean-up, liquid extraction (Section 3.5.2.)	Ultraviolet–Visible (UV-Vis) spectrophotometry (quartz cuvette, 273 nm)	(Hassan and Al-Yaqoobi, 2024b)

3.1.2 Swine Wastewater

In the characterization of SCG, parameters including pH, total nitrogen (TN), total organic carbon (TOC), ammonia nitrogen (NH_3-N), available nitrogen (N), phosphorus (P), potassium (K), Chemical Oxygen Demand (COD) and heavy metal content were analysed with extraction and analytical methods, as summarized in Table 3.2.

Table 3.2: Characterization of Swine Wastewater (SW)

Characterization	Extraction Method	Analysis Method	Reference
pH	NA	pH meter (Method 4500)	(American Public Health Association, 2026)
Total Nitrogen (TN)	NA	Persulfate digestion method (Method 10072)	(HACH Company, 2024)
Total Organic Carbon (TOC)	NA	Loss on Ignition method at 550° C	(FAO, 2026)
Ammonia-Nitrogen (NH_3-N)	NA	USEPA Nessler Method (Method 8038)	(HACH Company, 2026a)
Available Nitrogen (N), Phosphorus (P), Potassium (K)	Reagent extraction (TPY-6A protocol)	Rapid colorimetric analysis TPY-6A model soil nutrient tester	(TOP Cloud-Agri Technology Co., 2025)
COD	NA	USEPA Reactor Digestion Method (Method 8000)	(HACH Company, 2026b)

3.2 Raw Material Preparation

Spent coffee grounds (SCG) and swine wastewater (SW) were selected as primary substrates for this study due to their abundance and potential as organic resources. SCG and SW were collected and prepared prior to their use in composting and vermicomposting processes. SCG was obtained from ZUS Coffee located at Student Pavilion I, Universiti Tunku Abdul Rahman (UTAR) Kampar campus, Perak, Malaysia. Meanwhile, SW that had undergone preliminary filtration prior to sampling was collected from a swine farm at Air Kuning.

3.2.1 Spent Coffee Ground

SCG were collected once a week from ZUS Coffee, UTAR to minimize variability in composition. Upon collection, the SCG were spread evenly on a metal tray and inspected for visible contaminants such as plastics and filter residues, which were manually removed. Due to the tendency of SCG to form clumps because of moisture content and fine particle characteristics, the material was gently broken down with spatula to achieve a more uniform particle size distribution. The SCG were then oven-dried at 60 °C for 3 hours to reduce moisture content and inhibit microbial activity. The dried SCG were immediately transferred into clean polyethylene bags with labelled date, source and batch identification. If immediate use is not possible, SCG were stored in dark environment at room temperature to be used within 48-72 hours.

3.2.2 Swine Wastewater

SW was collected from a swine farm at Air Kuning and transferred into clean high-density polyethylene (HDPE) containers. The samples were labelled with the date, source and batch identification. The collected SW was sieved through a mesh to remove course debris such a straw, feed residues or other suspended solids. The sieved SW was stored in laboratory fridge at at 4 ± 2 °C prior to use. Before application in the composting process, SW was allowed to settle in the container for at least 24hours. This settling step reduced total suspended solids (TSS) and minimized rapid oxygen depletion when applied to composting and vermicomposting system.

3.3 Earthworm Preparation

I. Acclimatization

Mature adult *Eudrilus Eugeniae* were obtained from a reliable supplier. Prior to the experiment, worms will be acclimated for 48 hours in a holding container on moist, loosely packed bedding (mixture of soaking wet shredded cardboard and coconut coir) at room temperature (25 ± 2 °C). During acclimation, worms may be feed on a small amount (approximately 20–30 g) of the Berkeley substrate to facilitate adaptation. This step reduced stress and mortality when worms were transferred into experimental bins with higher feeding rate.

II. Stocking Density and Feeding Rate

The stocking density and feeding rate were adjusted based on the substrate characteristics and experimental scale. A total of 60 adult *Eudrilus Eugeniae* were inoculated into a designed vermicomposting bin with a surface area of 40 cm × 30 cm. The biomass of 60 adult *Eudrilus Eugeniae* was 50–80 g, corresponding to a stocking density of 0.40–0.70 kg worms/m^2 of vermibed surface area**.**

Prior to inoculation, only healthy adult worms exhibiting active movement and normal body condition were selected. Worms of similar size with a visible clitellum were used to ensure maturity. Cocoons and juvenile worms were removed to standardize the initial population. The initial worm biomass was recorded before introduction into the system for subsequent growth analysis.

For feeding, approximately 150 g of feedstock was applied per week, corresponding to an estimated consumption rate of 180%-300% of the earthworm biomass per week. Feeding was carried out in a controlled manner to ensure complete consumption of the substrate and to avoid overfeeding. A conservative feeding regime was adopted to minimize potential stress caused by the combined spent coffee ground (SCG) and swine wastewater (SW) substrate.

3.4 Experimental Design

3.4.1 Berkeley Composting (BC)

I. Berkeley Composting Bin Set Up

a.) A cylindrical composting bin was constructed using galvanized wire mesh by cutting the mesh to approximately 1.0 m in height and 1.6 m in length.

b.) The mesh was rolled into a cylindrical shape with a diameter of approximately 0.5 m, and the edges were secured using cable ties.

c.) The external surface of the bin was covered with a gunny bag.

d.) The bin was elevated using bricks to enhance drainage and prevent water accumulation.

II. Feedstock Layering

a.) An initial base layer of dry leaves (DL) was added to provide a carbon-rich foundation.

b.) The feedstock materials were added sequentially in layers consisting of dry leaves (DL), spent coffee ground (SCG), vegetables waste (VE), and swine wastewater (SW).

c.) Each layer was maintained at approximately 6-8 cm thickness, and light manual mixing was performed between layers to improve material contact and to prevent compaction.

d.) The layering process was repeated until a total of 10 layers formed as shown in Figure 3.2.

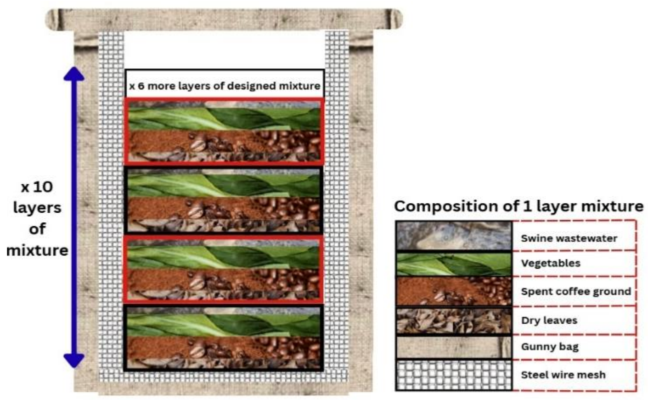

Figure 3.2: Layered feedstock arrangement in designed Berkeley composting bin

III. Treatment Matrix

Four Berkeley composting treatment sets were prepared with different feedstock compositions as demonstrated in Table 3.3. All treatments were maintained with the same layering structure (10 layers) as shown in Figure 3.2.

Table 3.3: Berkeley Composting Treatment Matrix

Treatment	Composition	Ratio
BC-A	DL: VE	2:1
BC-B	DL: SCG: VE	2:1:1
BC-C	DL: SCG: SW	4:2:1
BC-D	DL: SCG: VE: SW	5:3:2:1

IV. Moisture, Aeration and Temperature Control

a.) Moisture content was maintained within the range of 60-80% by periodic water addition (2-3 times per week) based on the substrate condition and weather.

b.) The composting materials were manually turned at intervals of 3-5 days to maintain aerobic conditions and to promote uniform decomposition.

c.) The temperature of the composting mass was recorded daily to ensure it remained within the suitable range for composting and to monitor the process progression.

V. Composting Period

a.) The Berkeley composting process was carried out for 18-24 days to achieve partial stabilization of the feedstock.

b.) The composted substrate was allowed to cool naturally and was transferred to the vermicomposting system only after the temperature decreased to the mesophilic range that suit for earthworm survival and activity.

3.4.2 Vermicomposting (VC)

A bin-based vermicomposting reactor was designed for this study, drawing on concepts from previous study (González-Moreno et al., 2020). and modified accordingly to suit the specific experimental conditions.

I. Vermicomposting bin set up

a.) A plastic bin (40 × 30 × 32 cm) was designed as the vermicomposting reactor. Ventilation and drainage holes were drilled on the lid, four side walls, and the base of the bin. The holes were evenly spaced at approximately 4 cm intervals for aeration and leachate drainage.

b.) The base of the bin was lined with fine mesh screen to prevent substrate loss and earthworm's escape.

c.) The bin was placed on a leachate tray to collect excess liquid drained from the system.

d.) The bin was fitted with a detachable lid to minimize pest intrusion.

Figure 3.3 illustrates the internal layered structure of the designed vermicomposting bin, detailing the arrangement of fine mesh base, coconut coir, bedding material, feedstock enclosed with a ventilated detachable plastic cover sitting on a leachate tray.

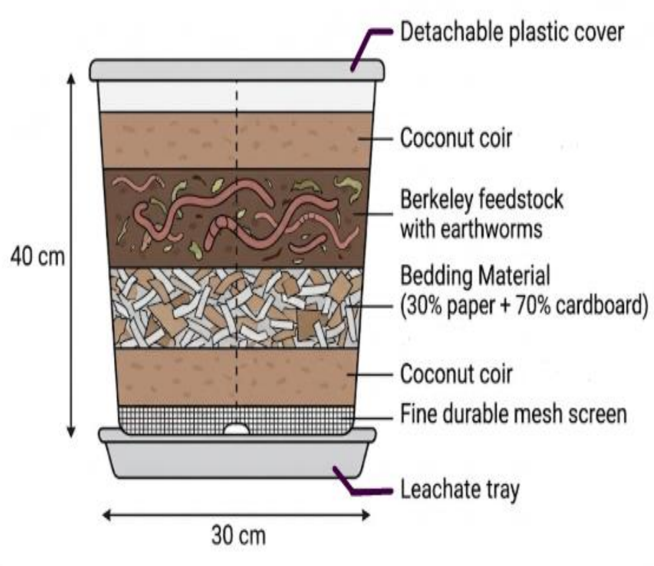

Figure 3.3: Schematic diagram of vermicomposting bin with layered vermibed structure

II Vermibed Preparation and Conditioning

a.) Bedding materials consisting of 30 % cut paper and 70 % shredded cardboard were soaked for 24h and squeezed to remove excess water, achieving a moist condition at approximately water holding capacity.

b.) A 2-3 cm layer of coconut coir was added, followed by a 3-4 cm layer of soaked bedding materials to improve aeration and structural stability.

c.) Approximately 200 g of garden soil was added to the bedding to introduce native microorganisms and facilitate initial microbial colonization.

d.) Approximately 50 g of chopped fruit waste (e.g., apple peels) was incorporated into the bedding to condition the vermibed.

III Earthworm Introduction and Feeding

a.) The prepared vermibed was allowed to stabilize for 3 days before earthworm's introduction.

b.) A total of 60 adult *Eudrilus Eugeniae* were introduced into the bin, and the initial biomass was recorded prior to inoculation.

c.) The bin was exposed to light for the first 24 h after inoculation to encourage burrowing.

d.) Matured Berkeley compost was applied as feedstock at approximately 150 g at intervals of 6-9 days depends on the earthworm consumption rate, to prevent overfeeding.

e.) The feedstock was covered with a 2–3 cm surface finishing layer of moist or coir to reduce moisture loss, odour, and light penetration, and close the bin with a detachable plastic lid.

IV. Treatment Matrix

Four vermicomposting treatment sets were prepared with different matured Berkeley composted substrates as presented in Table 3.4. A uniform stocking density of 500 worms/vermibed surface area was applied across all treatment sets.

Table 3.4: Vermicomposting Treatment Matrix

Treatment Code	Substrate	Earthworm Stock Density (worms/vermibed surface area)	Earthworm Species
VC-A	BC-A	500	Eudrilus eugeniae
VC-B	BC-B	500	Eudrilus eugeniae
VC-C	BC-C	500	Eudrilus eugeniae
VC-D	BC-D	500	Eudrilus eugeniae

IV. Moisture, Aeration and Temperature Control

a.) Moisture content was maintained within the range of 60-80% by periodic water addition (2-3 times per week) based on the substrate condition and ambient weather.

b.) Adequate aeration was maintained by placing the vermicomposting system in a well-ventilated open-air environment, supported by the bin design.

c.) The ambient temperature was maintained within range of 25-30 °C

V. Vermicomposting period

a.) The vermicomposting process was conducted for 60 days to ensure adequate decomposition and stabilization of the substrate, corresponding to the sexual maturity period of *Eudrilus Eugeniae*, which is approximately is 7-8weeks (Begum et al., 2025).

b.) At the end of the treatment period, the vermicompost was harvested and separated from earthworms for further analysis.

3.5 Physical-chemical and Biological Analyses

The physiochemical and biological characteristics of the raw material, Berkeley compost, and vermicompost were evaluated to monitor substrate stability, nutrient transformation, and compost performance throughout the experiment. The analytical methods were chosen to ensure consistency, comparability, and suitability for the selected feedstocks.

3.5.1 Physical Parameters

I. Moisture

Moisture content was determined weekly using gravimetric oven-drying method. Subsamples were dried at 105 °C for 24 h, and the moisture content was calculated based on weight loss as shown in Equation (1):

$$\text{Moisture content (\%)} = \frac{Wi - Wd}{Wi} \times 100 \qquad (1)$$

Where W_i is initial weight of sample (g), and W_d is the oven-dried weight of sample (g)

II. Temperature

The temperature of samples was monitored daily using a thermometer. Measurement was taken at consistent locations to ensure representative readings of substrate conditions.

III. pH and Electrical conductivity (EC)

For solid samples, pH was determined using a solid-liquid extraction method with distilled water at a ratio of 1:5 (w/v). The mixture was shaken for 60 minutes, and the pH of the suspension was measured using a calibrated pH meter; For liquid samples such as swine wastewater (SW), pH was measured directly without extraction using a calibrated pH meter. EC was measured to evaluate the soluble salt concertation of the samples. For solid samples, EC was determined using a water extraction method at ratio of (1:5 w/v), followed by measurement of the extract using a calibrated conductivity meter. For liquid samples such as SW, EC was measured directly using the EC meter without prior extraction.

3.5.2 Chemical Parameters

I. Total organic carbon (TOC)

For solid form samples, TOC was estimated after drying, grinding and homogenization using the loss-on-ignition method at 550 °C as demonstrated in Equation (2)- (3). For liquid samples such as SW, TOC was measured with a TOC analyser following APHA Method 5310. The analysis method then involves high-temperature combustion in the analyser, converting organic carbon to CO_2, which is then quantified by an NDIR detector to determine TOC.

$$\text{Total organic matter (TOM, \%)} = \frac{W2-W3}{W2-W1} \times 100 \quad (2)$$

where:

W1 is the weight (g) of the empty ceramic crucible

W2 is the weight (g) of the ceramic crucible with the oven-dried sample after heating at 105°C.

W3 is the weight (g) of the ceramic crucible with ignited sample after heating at 550°C.

Conversion to TOC:

$$\text{TOC (\%)} = \text{TOM (\%)} \times \frac{58}{100} \quad (3)$$

This approach allows estimation of organic carbon content in solid matrices, while instrumental analysis provides direct quantification for liquid samples.

II. Total nitrogen (TN)

TN was determined after sample preparation using the persulfate digestion method (method 10072), where all forms of nitrogen were oxidized to nitrate and measured as mg/L. As the HACH analyser measures TN concentrated in the digested solution, TN was computed by multiplying the measured concentration by the digestion volume and normalizing it to the initial sample mass (H Wien Nie et al., 2022; Purcell and King, 1996).

The C:N ratio was computed as shown in Equations (4):

$$\text{C: N ratio} = \frac{Total\ Organic\ Carbon\ (TOC)}{Total\ Nitrogen\ (TN)} \quad (4)$$

III. Ammonia-Nitrogen (NH_3-N)

Ammonia nitrogen (NH_3-N) was determined using the USEPA Nessler Method (Method 8038). In this method, the sample was treated with Nessler reagent under alkaline conditions to form a yellow to brown coloured complex. The intensity of the colour produced is proportional to ammonia concertation and further detected with HACH colorimetric system.

IV. Micronutrients [Available form of Nitrogen (N), Phosphate (P) and Potassium(K)]

Available nitrogen (N), phosphate (P) and potassium (K) were determined using a rapid colorimetric method with a TPY-6A soil nutrient tester following reagent extraction according to the TPY-6A protocol. The extracted samples were analysed directly using the instrument, which quantifies nutrient concentration based on colour intensity. These parameters were evaluated to assess nutrient availability and fertiliser potential of the matured compost.

V. Chemical Oxygen Demand (COD)

COD was determined for liquid samples with the USEPA Reactor Digestion Method (Method 8000) without additional extraction. The analysis method involves digestion of the sample with a strong oxidizing agent (potassium dichromate in concentrated sulfuric acid) under high temperature in a sealed reactor, oxidizing organic matter to CO_2 and H_2O. The residual dichromate is quantified calorimetrically to determine the COD value.

VI. Caffeine

Caffeine concentration in the soil and compost samples was determined using UV-Visible (UV-Vis) spectrophotometry following a modified solvent extraction and clean-up procedure adapted from previous studies (Belay et al., 2008). Since the analytical procedure was specifically designed for this study, the methodology is described in detail to ensure reproducibility.

Prior to extraction, the samples were air-dried at room temperature and oven-dried at 40°C until constant weight was achieved. Approximately 2.00 g of dried sample was weighed into a 50 mL centrifuge tube and extracted with 20 mL of HPLC-grade methanol. The mixture was shaken on an orbital shaker at 200 rpm for 1 hour, followed by sonication for 20 minutes at a temperature below 40°C to enhance extraction efficiency. The extract was subsequently centrifuged at 8000 rpm for 10 minutes, and the clear supernatant was carefully collected while avoiding disturbance of the solid pellet.

Approximately 18–20 mL of the methanol extract was transferred into a clean centrifuge tube for further clean-up. A volume of 10 mL acetonitrile was added, followed by vortex mixing for 1 minute. The mixture was then centrifuged at 10,000 rpm for 10 minutes, and the resulting supernatant was transferred into a separatory funnel for liquid–liquid extraction.

For phase separation, 20 mL distilled water and 20 mL ethyl acetate were added into the separatory funnel. The mixture was gently shaken with periodic venting to release internal pressure and allowed to stand until two distinct liquid layers were formed. The upper ethyl acetate layer containing the caffeine fraction was collected, while the lower aqueous layer was discarded. The collected organic phase was evaporated to dryness in a water bath at approximately 40°C inside a fume hood to avoid thermal degradation.

The dried residue was reconstituted using mobile phase consisting of distilled water, acetonitrile, and formic acid in the ratio of 80:20:0.1 (v/v/v). Approximately 1.0 mL mobile phase was added into the glass vessel, followed by gentle swirling and rinsing of the vessel walls to ensure complete dissolution of the residue. The solution was transferred into a vial, rinsed again with additional mobile phase, and vortexed until a clear and homogeneous solution was obtained.

Prior to analysis, the sample solution was filtered through a 0.22 µm syringe filter. To ensure that the absorbance values remained within the calibration range, the filtered extract was diluted five-fold using the mobile phase before UV-Vis analysis. Caffeine determination was carried out using a UV-Vis spectrophotometer at a wavelength of 273 nm with quartz cuvettes of 1 cm path length. The mobile phase was used as the blank solution. Standard caffeine solutions with concentrations of 2, 5, 10, 20, and 40 ppm were prepared to generate the calibration curve of absorbance versus concentration.

$$\text{Caffeine (mg/g)} = \frac{C \times 5 \times V}{m}$$

Where:

C = caffeine concentration determined from the calibration curve (mg/L)

5 = dilution factor

V = final extract volume (L)

m = dry sample mass (g)

3.5.3 Biological Indicators

I. Earthworm Growth, Biomass Change & Reproductive Output

Earthworm health will be monitored by recording mortality and biomass change. At the end of 60 days, worms will be carefully hand-sorted from each bin, washed, and weighed to determine total biomass gain or loss. The total biomass gain (TBG) and growth rate of the worms (GR) were calculated as shown in Equations (6)– (7), respectively.

$$\textbf{TBG} = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \tag{6}$$

$$\textbf{GR}\ \text{(mg/worm/day)} = \frac{Final\ weight - Initial\ weight}{Total\ VC\ time \times Average\ number\ of\ earthworms\ during\ VC\ period} \tag{7}$$

II. Germination Index (GI)

Compost phytotoxicity was evaluated at Day 60 of vermicompost. For each treatment, 5 g of sample was mixed with 50 mL of deionized water. The mixture was then filtered, and the filtrate was poured into a Petri dish lined with fine gauze. Deionized water was used as the control treatment. 10 bok choy (*Brassica rapa* subsp. *chinensis*) seeds were placed evenly on the filter papers in each petri dish. All samples were incubated in an incubator under dark condition at 25 °C for 3 days. After incubation period, percentage of relative seed germination (RSG) and relative root growth (RRG), and germination index (GI) were calculated by Equations (8)– (10). A seed was considered germinated when the primary root (radicle) reached a length of at least 5 mm, in accordance with USEPA guidelines (González-Moreno et al., 2020).

$$\textbf{RSG}\ (\%) = \frac{Number\ of\ germinated\ seeds\ in\ vermicompost\ extract}{Number\ of\ germinated\ seeds\ in\ control} \times 100 \tag{8}$$

$$\textbf{RRG}\ (\%) = \frac{Mean\ root\ length\ in\ vermicompost\ extract}{Mean\ root\ length\ in\ control} \times 100 \tag{9}$$

$$\textbf{GI} = \frac{RSG \times RRG}{100} \tag{10}$$

3.6 Sampling Frequency

The sampling process in this study comprised three sequential phases, including raw material characterisation (RC), Berkeley composting (BC) and vermicomposting (VC). RC phase involved analytical testing of the raw SCG and SW prior to the treatment preparation. Berkeley composting (BC) phase was conducted 21 days to achieve thermophilic decomposition and allow substrate stabilisation, followed by a 60-day VC phase.

Physicochemical parameters, including EC, pH, NH_3-N TOC, TN, available nutrients, COD and caffeine were determined at the end of the BC phase using the stabilised Berkeley compost. This action was to evaluate the degree of stabilisation prior to earthworm introduction. The final BC sample was subsequently used as the initial substrate for vermicomposting and designated as Day 0 of the VC phase. Therefore, for these parameters, the values obtained on the final day of the VC phase. Therefore, for these parameters, the values obtained on the final day (Day 21) of BC are equivalent to those at Day 0 of VC. Subsequently, these parameters analysed at Day 30 and Day 60 of VC phase.

In contrast, operational parameters such as temperature and moisture content were monitored continuously throughout both composting stages. Temperature was recorded daily whereas moisture content was measured weekly to ensure optimal conditions for decomposition and earthworm activity. These parameters were recorded along the 21-day BC period and continued throughout the 60-day VC process, resulting in continuous datasets that reflect temporal variations in environmental conditions. Last but not least, biological parameters including earthworm biomass, were recorded at the beginning and end of VC, while phytotoxicity was assessed using the GI test at Day 60. The corresponding sampling schedule was summarised in Table 3.5.

Table 3.5: Sampling Schedule for Physicochemical and Biological Parameters

Parameter Category	Specific Analysis	RC	BC	VC Day 0	VC Day 30	VC Day 60	Extraction / Method
Physical	EC	—	✓	—	✓	✓	Conductivity meter
	Moisture content	—	Once a week				Gravimetric oven-drying (105 °C, 24 h)
	Temperature	—	Once a day				Thermometer
	pH	✓	✓	—	✓	✓	pH meter
Nitrogenous	NH_3-N	✓	✓	—	✓	✓	USEPA Nessler Method (Method 8038)
C / N ratio	TOC	✓	✓	—	—	✓	I.) TOC analyser (liquid sample) II.) Loss on ignition (solid sample)
	TN	✓	✓	—	—	✓	Persulfate digestion (Method 10072)
Micronutrient	Available N, P and K	—	✓	—	✓	✓	TPY-6A rapid colorimetric method
Organic load	COD	✓	—	—	—	✓	HACH Digestion Method (Method 8000)
Contaminants	Caffeine	✓	✓	—	—	✓	Ultraviolet–Visible (UV–Vis) spectrophotometer
Biological (earthworms)	Biomass Changes	—	—	✓	—	✓	Electronic Weighing Scale

Phytotoxicity	Germination Index (GI)	—	✓	—	—	✓	Seed bioassay (*Brassica rapa subsp. chinensis)*

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Characterization of Raw Materials

4.1.1 Physical Properties

The raw materials showed clearly different physical characteristics in terms of pH, electrical conductivity (EC), and moisture content. As showed in Table 4.1, Swine wastewater (SW) recorded the highest moisture content at 99.332% and the highest EC at 6432 µS/cm, while its pH was slightly alkaline at 7.567. This indicates that SW was a highly aqueous and mineral-rich substrate. In contrast, spent coffee grounds (SCG) showed a much lower moisture content of 10.953%, an acidic pH of 5.273, and a moderate EC of 1128 µS/cm. DL exhibited a near-neutral pH of 7.034, the lowest EC (612 µS/cm), and moderate moisture content (44.505%). VE showed intermediate characteristics, with pH 6.070, EC 2347 µS/cm, and moisture content of 74.964%.

Table 4.1: Physical Properties of Raw Materials

Raw Material	pH	EC (µS/cm)	Moisture (%)
DL	7.034	612	44.505
VE	6.070	2347	74.964
SCG	5.273	1128	10.953
SW	7.567	6432	99.332

4.1.2 Chemical Properties

4.1.2.1 C/N ratio

The chemical characterization showed large variation in total organic carbon (TOC), total nitrogen (TN), and C/N ratio among the raw materials. As presented in Table 4.2, DL recorded the highest C/N ratio at 61.00, followed by SCG at 23.64 and VE at 13.10, while SW had the lowest C/N ratio at 7.85. DL also contained relatively high TOC (50.203%) but low TN (0.823%), indicating a carbon-dominant material. SCG had a TOC of 51.264% and TN of 2.169%, giving a moderate C/N ratio that is much more balanced for biological treatment. VE contained 40.085% TOC and 3.060% TN, while SW had the lowest TOC (27.845%) but the highest TN (3.547%).

Table 4.2: TOC, TN and C/N ratio of raw materials

Raw Material	TOC (%)	TN (%)	C/N ratio
DL	50.203	0.823	61.000
VE	40.085	3.06	13.100
SCG	51.264	2.169	23.635
SW	27.845	3.547	7.850

This pattern is consistent with the feedstock roles expected from Chapter 2. SCG is described as a carbon-rich residue with substantial lignocellulosic content, while SW is nitrogen-rich and therefore likely to decompose rapidly but also to generate ammonia-related stress if not properly balanced. The very high C/N ratio of DL suggests that it is mainly a structural or bulking material, rather than a nutrient source. By contrast, the very low C/N ratio of SW indicates strong nitrogen availability, which can accelerate microbial activity but may become inhibitory if present in excess. In comparison with the literature, the measured C/N ratio of SCG is close to the range reported in Chapter 2 for carbonaceous coffee residues, which supports its suitability as a main vermicomposting substrate.

4.1.2.2 Nutrients content

The nutrient analysis in Table 4.3 showed that SCG and SW contained substantially higher available phosphorus and potassium than DL and VE. SCG recorded the highest available phosphorus at 670.5 mg/L and the highest available potassium at 1015.2 mg/L. SW also showed high nutrient content, with 497.47 mg/L phosphorus and 636.07 mg/L potassium. In comparison, DL contained only 3.67 mg/L phosphorus and 17.67 mg/L potassium, while VE contained 53.63 mg/L phosphorus and 69.4 mg/L potassium. These results show that SCG is not only carbon-rich, but also an important carrier of plant nutrients, especially phosphorus and potassium. This agrees with Chapter 2, where SCG is described as containing meaningful mineral fractions despite being a lignocellulosic waste (Hanc et al., 2021). The high nutrient content in SW is also expected because swine-derived wastes are typically rich in soluble nutrients. DL, on the other hand, appears to contribute very little in terms of available minerals, which suggests that it mainly serves a structural role in the vermicomposting mixture. In implication terms, these nutrient results are encouraging because they suggest that the final vermicompost may have strong fertiliser potential if the process is properly controlled.

Table 4.3: Available P and K of Raw Materials

Raw Material	Available Phosphorus, P (mg/L)	Available Potassium, K (mg/L)
DL	3.67	17.67
VE	53.63	69.4
SCG	670.5	1015.2
SW	497.47	636.07

4.2 Composting Process Behaviour

4.2.1 Temperature Profile During BC

The temperature profiles of all Berkeley composting treatment set as showed in Figure 4.1 present clear thermophilic pattern, with temperatures increasing rapidly during the early to middle stages of composting before declining toward the end of the process. All treatment sets started at approximately 29–33 °C and rose progressively over the first several days, reaching peak values between about 44 °C and 65 °C depending on the treatment. Among the treatments, BC-D recorded the highest peak temperature of approximately 64–65 °C, followed by BC-C at around 63 °C, BC-B at about 55 °C, while BC-A remained comparatively lower at around 44 °C. After day 15, all treatment sets showed a sharp decline in temperature, approaching ambient conditions by day 20–21. This indicates that the active microbial decomposition stage had gradually diminished by the end of the Berkeley composting period.

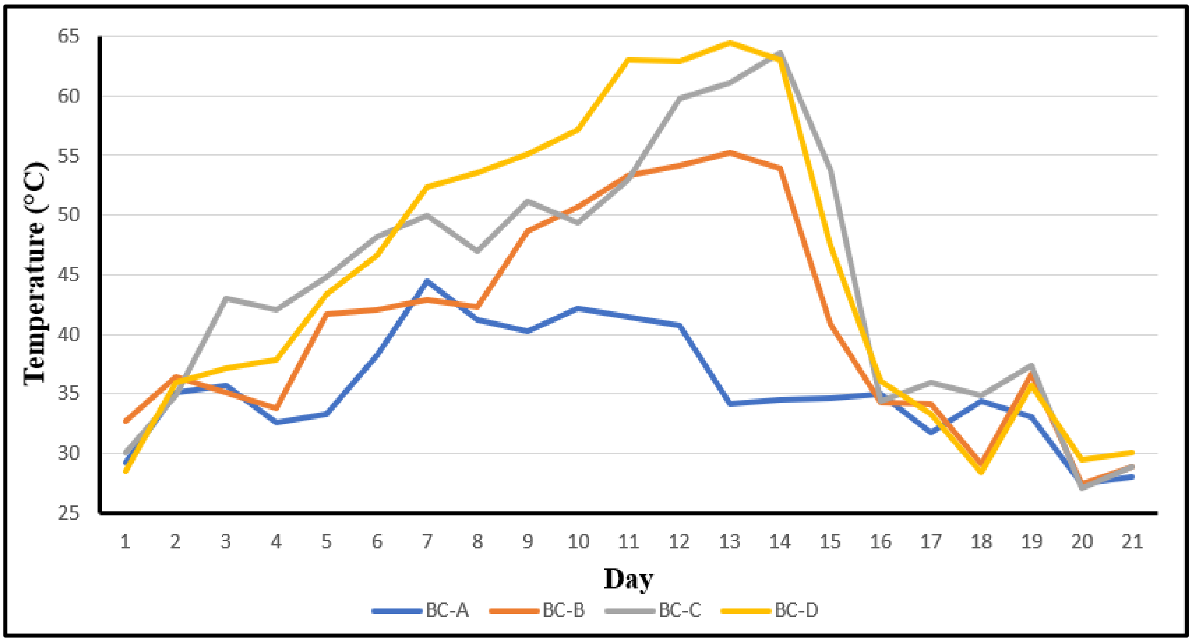

Figure 4.1:Temperature Variation of Treatment Sets During Berkeley Composting

This temperature rise is mainly driven by intense microbial oxidation of readily degradable organic matter, which releases heat as a by-product of metabolic activity. The higher peaks observed in BC-C and BC-D suggest that these treatments likely contained a greater proportion of biodegradable substrates or had a more favourable balance of moisture, carbon, and nitrogen for microbial proliferation. In contrast, BC-A showed a lower peak, implying slower decomposition or lower availability of easily degradable organic matter. The sharp cooling after day 15 indicates depletion of labile substrates and a transition from the active thermophilic phase to the cooling and stabilization phase (Waqas et al., 2023).

In comparison with composting literature, the observed temperature range is consistent with typical Berkeley composting behaviour, where temperatures commonly exceed 55 °C during the thermophilic phase and later decline as the substrate becomes stabilized (Waqas et al., 2023). The peak temperatures recorded in BC-C and BC-D are particularly important because they suggest effective microbial activity and sufficient heat generation for accelerating organic matter breakdown. Such high temperatures are also beneficial for partial sanitization, as they help reduce the viability of pathogens and unwanted microorganisms.

The implication of this result is that the Berkeley composting stage was successful in promoting rapid thermal decomposition before vermicomposting. This pre-composting phase likely improved feedstock safety and reduced the risk of ammonia toxicity or excessive heat stress when the material was later introduced into the vermicomposting stage. Overall, the temperature trend confirms that the Berkeley composting system passed through an active decomposition phase followed by stabilization, which is desirable before earthworm inoculation (Waqas et al., 2023).

4.2.2 Temperature Profile During VC

During vermicomposting, all treatment sets maintained a relatively narrow and stable temperature range, fluctuating approximately between 24.2 °C and 28.9 °C throughout the 60-day period as shown in Figure 4.2. The temperature initially started at around 28–29 °C, then gradually declined during the early days of vermicomposting. A modest rise was observed around day 21, followed by another gradual decrease and a second mild increase around day 44–46. Toward the final stage of the process, the temperature dropped further and stabilized near 24–25 °C. Across the treatment sets, the differences were small, indicating that the vermicomposting environment remained generally stable. VC-C tended to show slightly higher temperatures than the other treatments, while VC-A was often the lowest.

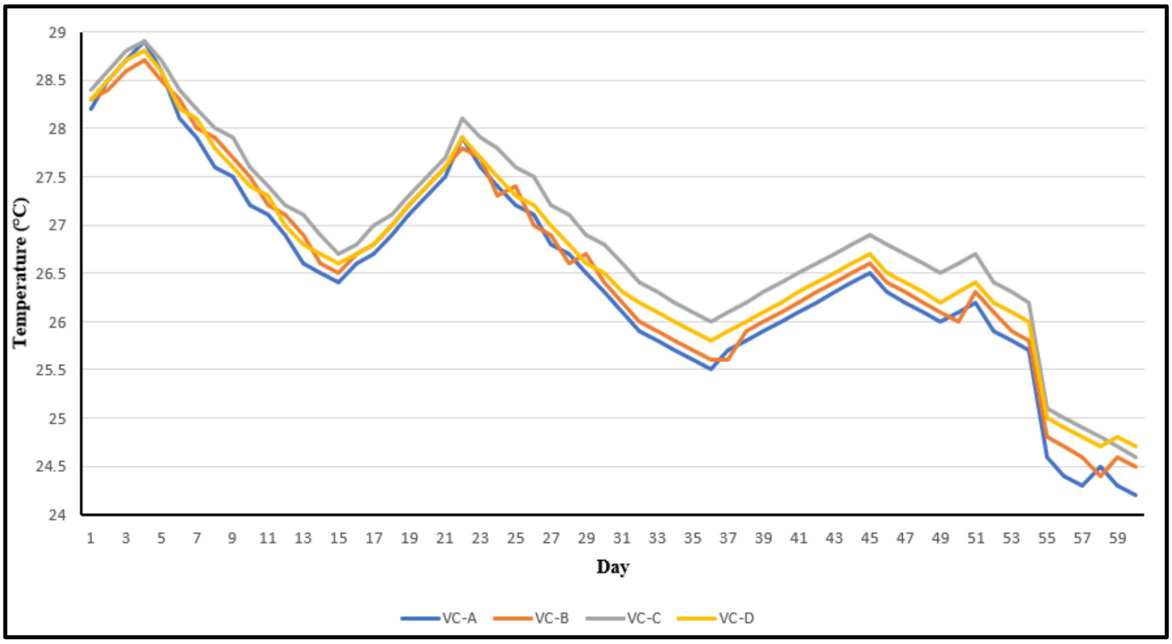

Figure 4.2: Temperature Variation of Treatment Sets During Vermicomposting

This temperature pattern reflects the mesophilic nature of vermicomposting, where the activity of earthworms and mesophilic microorganisms dominates rather than thermophilic heating. The gradual decline in temperature over time suggests progressive stabilization of the substrate and a reduction in microbial heat production as the more degradable organic matter was consumed. The small temporary increases around day 21 and day 44–46 may be associated with short bursts of microbial activity during ongoing decomposition and nutrient transformation. The lower temperature toward the end of the process indicates that the substrate was becoming more mature and biologically stable.

Compared with the ideal temperature range for composting earthworms discussed in Chapter 2, the recorded temperatures remained within a suitable mesophilic range for earthworm survival and activity. This is especially important because species such as *Eudrilus Eugeniae* perform best under warm but non-thermophilic conditions (Viljoen & Reinecke, 1992). The stable temperature profile therefore suggests that the post-Berkley composted substrate provided a favourable environment for vermicomposting without exposing the worms to heat stress.

The implication of this result is that the vermicomposting system was properly controlled and biologically suitable for earthworm-driven stabilization. The absence of large temperature fluctuations indicates that the worms and microorganisms were working in a balanced mesophilic environment, which supports cast formation, microbial succession, and final vermicompost maturation (Vyas et al., 2022). Overall, the temperature behaviour confirms that the substrate moved from active decomposition in the Berkeley phase to stable biological processing in the vermicomposting phase.

4.3 Changes in Physicochemical Properties During BC & VC

4.3.1 Changes in Total Organic Carbon (TOC)

As shown in Table 4.4, the total organic carbon (TOC) content decreased progressively across both the Berkeley composting and vermicomposting stages for all treatment sets. At the initial stage (BC, Day 0), TOC values were relatively high, ranging from 44.58 ± 1.01% to 46.82 ± 1.24%. After Berkeley composting (Day 21), a slight decline was observed in all treatments, indicating the early breakdown of organic matter. A more pronounced reduction occurred during vermicomposting, where TOC decreased further at Day 30 and Day 60. Figure 4.3 demonstrated that the overall trend was downward for all treatments, with treatment B showing the greatest reduction and treatment D retaining the highest TOC at the final stage.

This reduction in TOC is expected because organic carbon is continuously consumed during decomposition by microorganisms and earthworms. During composting, carbon is oxidized into carbon dioxide through microbial respiration, while part of it is incorporated into microbial biomass and earthworm tissue. As decomposition progresses, the more labile carbon fractions are depleted first, leaving behind more stable and resistant organic residues. The steeper decline observed during vermicomposting suggests that earthworm activity, together with microbial succession, accelerated organic matter mineralization.

Table 4.4: TOC (%) of Treatment Sets During Berkeley composting and Vermicomposting

	Treatment Set			
Stage	**A**	**B**	**C**	**D**
BC (Day 0)	46.82 ± 1.24	44.73 ± 0.85	46.15 ± 0.98	44.58 ± 1.01
BC (Day 21)	41.95 ± 0.93	41.62 ± 0.75	43.78 ± 1.33	41.4 ± 0.36
VC (Day 30)	39.37 ± 1.18	32.18 ± 0.66	35.36 ± 0.75	36.81 ± 0.54
VC (Day 60)	33.12 ± 0.61	24.97 ± 1.23	33.65 ± 1.04	34.79 ± 1.32

The trend of TOC reduction is more clearly illustrated in Figure 4.3, where all treatments exhibit a continuous decreasing pattern over time. A more pronounced decline is observed during the transition from composting to vermicomposting stages, particularly for certain treatments, whereas others show a relatively gradual reduction. Among the treatments, Treatment B demonstrated the greatest reduction in TOC, indicating the most effective degradation performance, followed by Treatment A. This suggests that the composition in Treatment B provided favourable conditions that enhanced the breakdown of organic matter during the process. By contrast, treatment D maintained a relatively higher final TOC, which may indicate slower stabilization or a greater proportion of recalcitrant carbon remaining in the material (Lashermes et al., 2012). Overall, the results reflected that the integrated composting system was biologically active and effective in transforming the raw materials into a more stabilized organic amendment.

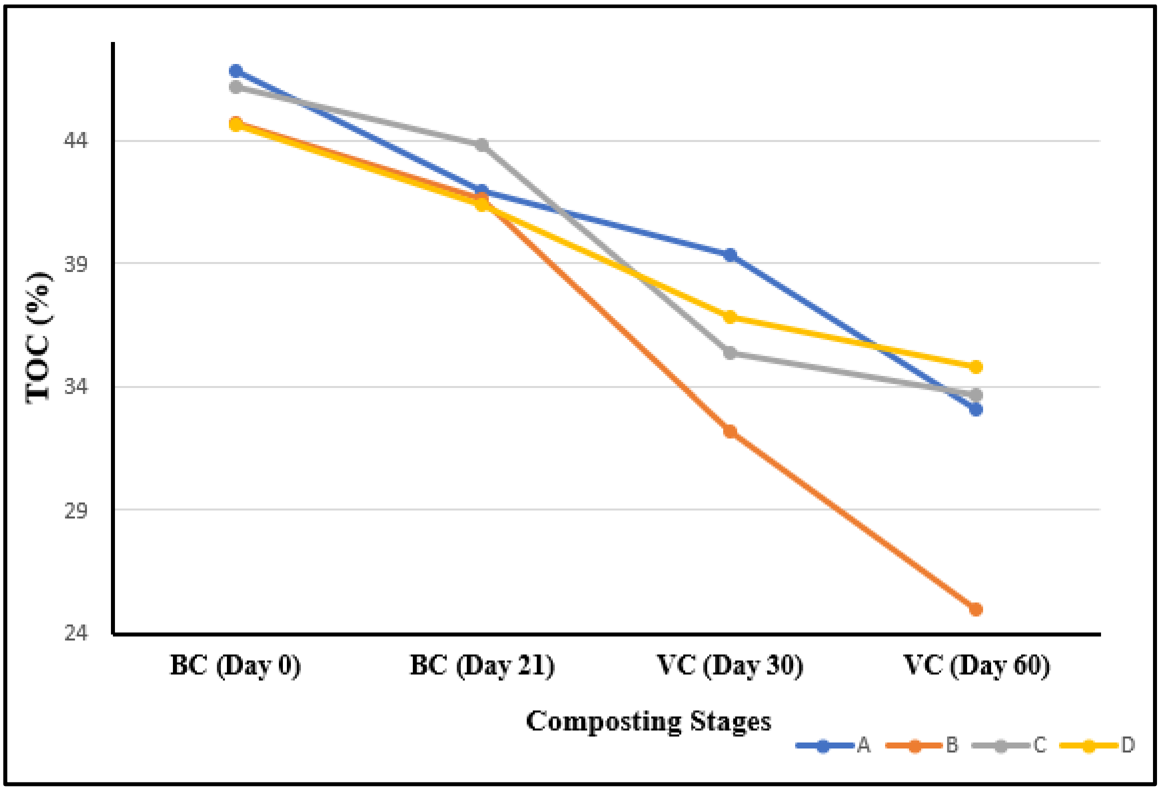

Figure 4.3: TOC (%) changes across Berkeley composting and vermicomposting stages

4.3.2 Changes in Total Nitrogen (TN)

As shown in Table 4.5, total nitrogen (TN) exhibited slight fluctuations across the composting and vermicomposting stages, with no substantial variation observed during the early stages. At BC (Day 0), TN values were relatively similar among all treatments, ranging from 1.56 ± 0.09% to 1.77 ± 0.06%, and increased slightly by Day 21. During the vermicomposting stage, Treatments A and B showed a gradual decrease to 1.66 ± 0.05% and 1.45 ± 0.08%, respectively, whereas Treatments C and D increased to 2.14 ± 0.12% and 2.43 ± 0.11% by Day 60. Overall, the changes in TN were moderate, indicating relatively stable nitrogen content throughout the process.

Table 4.5: TN (%) of Treatment Sets During **Berkeley c**omposting and Vermicomposting

	Treatment Set			
Stage	**A**	**B**	**C**	**D**
BC (Day 0)	1.57 ± 0.11	1.61 ± 0.13	1.56 ± 0.09	1.77 ± 0.06
BC (Day 21)	1.73 ± 0.08	1.76 ± 0.04	1.68 ± 0.07	1.84 ± 0.12
VC (Day 30)	1.71 ± 0.12	1.58 ± 0.10	1.85 ± 0.03	1.92 ± 0.07
VC (Day 60)	1.66± 0.05	1.45 ± 0.08	2.14 ± 0.12	2.43 ± 0.11

The trend in Figure 4.4 shows that TN did not follow the same pattern as TOC, as the treatments displayed different responses over time. Treatments A and B showed only a modest rise at Day 21 before declining during vermicomposting, whereas Treatments C and D continued to increase more consistently, especially toward the final stage. This indicates that nitrogen dynamics were more treatment-dependent and the extent of change became more evident during vermicomposting. Among the treatments, Treatment D recorded the highest final TN, followed by Treatment C, showing the strongest nitrogen retention at the end of the process. This suggests that the composition of Treatment D supported better nitrogen enrichment during composting and vermicomposting. By comparison, Treatments A and B showed lower final TN, indicating greater nitrogen loss or less effective nitrogen accumulation during the process.

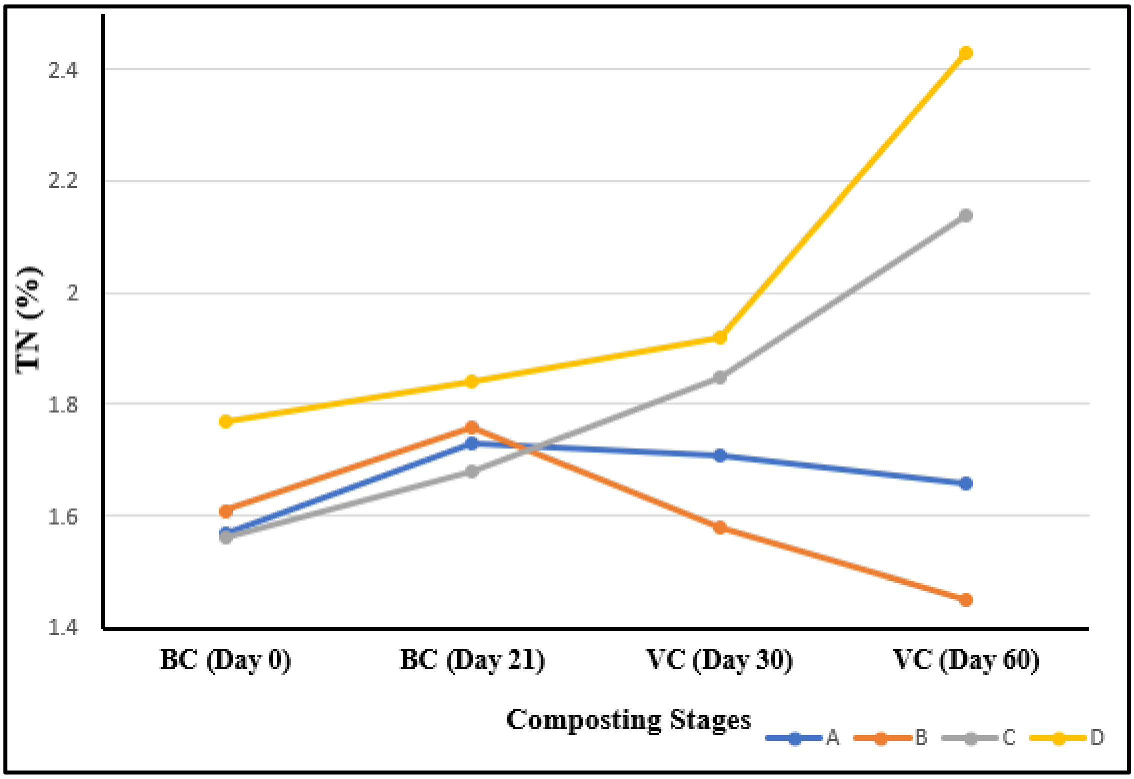

Figure 4.4: TN (%) changes across Berkeley composting and vermicomposting stages

4.3.3 Changes in C/N Ratio

As presented in Table 4.6, the C/N ratio decreased consistently across all treatments throughout the composting and vermicomposting stages. At the initial stage, the C/N ratio ranged from 25.19 ± 0.71 to 29.82 ± 1.24, indicating relatively high organic carbon content. A noticeable reduction was observed by BC (Day 21), followed by a further decline during the vermicomposting stage. By VC (Day 60), the C/N ratio decreased to 19.95 ± 0.66, 17.22 ± 0.87, 15.73 ± 0.51, and 14.32 ± 0.36 for Treatments A, B, C, and D, respectively. Overall, the continuous reduction reflects progressive stabilization of the composting materials.

Table 4.6: C/N Ratio of Treatment Sets During Berkeley composting and Vermicomposting

	Treatment Set			
Stage	**A**	**B**	**C**	**D**
BC (Day 0)	29.82 ± 1.24	27.78 ± 0.37	29.58 ± 0.86	25.19 ± 0.71
BC (Day 21)	24.25 ± 0.93	23.65 ± 0.46	26.06 ± 0.82	22.5 ± 0.29
VC (Day 30)	23.02 ± 1.05	20.37 ± 1.12	19.11 ± 0.88	19.17 ± 0.64
VC (Day 60)	19.95 ± 0.66	17.22 ± 0.87	15.73 ± 0.51	14.32 ± 0.36

The decreasing trend of the C/N ratio is more clearly illustrated in Figure 4.5, where all treatments exhibit a steady downward pattern over time. A more pronounced decline is observed during the composting stage, followed by a gradual reduction during vermicomposting. Despite slight variations in the rate of decrease among treatments, all treatments follow a similar trend, indicating continuous decomposition and maturation of the material. Among the treatments, Treatment D recorded the lowest final C/N ratio, followed by Treatment C, indicating a higher degree of stabilization by the end of the process. This suggests that the composition in these treatments supported more efficient reduction of carbon relative to nitrogen, contributing to improved compost maturity.

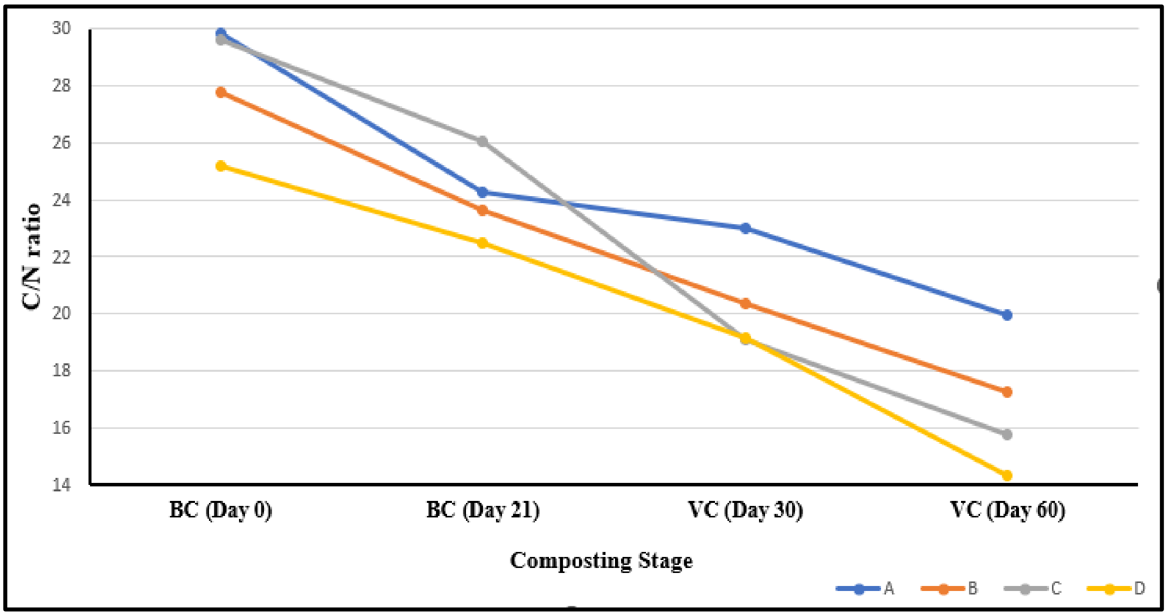

Figure 4.5: C/N ratio changes across Berkeley composting and vermicomposting stages

4.3.4 Changes in Available Nutrients (N, P, K)

I. Available Nitrogen (N)

As presented in Table 4.7, available nitrogen (N) increased overall across all treatments throughout the Berkeley composting and vermicomposting stages. At the initial stage [BC (Day 0)], the available nitrogen content ranged from 235 ± 12 mg/L to 465 ± 33 mg/L. Following Berkeley composting [BC (Day 21)], a substantial increase in available nitrogen was observed in all treatments, particularly in Treatments C and D, which recorded 1226 ± 55 mg/L and 1623 ± 78 mg/L, respectively. During vermicomposting [VC (Day 30)], slight fluctuations were observed in several treatments; however, the available nitrogen content increased again by VC (Day 60). At the final stage, Treatments A, B, C, and D recorded available nitrogen concentrations of 895 ± 40 mg/L, 1382 ± 67 mg/L, 2173 ± 191 mg/L, and 3624 ± 186 mg/L, respectively.

Compared with the initial stage, Treatment D exhibited the greatest overall increase in available nitrogen, with an increase of approximately 679.4%, followed by Treatment C (576.9%), Treatment B (488.1%), and Treatment A (161.7%). The substantial increase in available nitrogen indicates progressive mineralization of organic nitrogen into plant-available forms throughout composting and vermicomposting, with Treatment D showing the highest nitrogen enrichment and accumulation at the final stage.

Table 4.7: Available Nitrogen (N) of Treatment Sets During Berkeley composting and Vermicomposting

	Treatment Set			
Stage	**A**	**B**	**C**	**D**
BC (Day 0)	342 ± 18	235 ± 12	321 ± 26	465 ± 33
BC (Day 21)	972 ± 26	609 ± 38	1226 ± 55	1623 ± 78
VC (Day 30)	516 ± 24	688 ± 31	787 ± 36	1012 ± 42
VC (Day 60)	895 ± 40	1382 ± 67	2173 ± 191	3624 ± 186

Figure 4.6 illustrates an overall increasing trend in available nitrogen across the composting stages, with a noticeable rise toward the final stage. However, a slight decline is observed at the transition to the vermicomposting stage (VC Day 30) for Treatments A, C, and D. This temporary reduction may be associated with initial adaptation of earthworms and redistribution of nitrogen within the system before further mineralization occurs. In addition, reduction in available nitrogen observed during the transition from Berkeley composting to vermicomposting may be attributed to ammonia volatilization during material transfer and handling, as ammonia is highly volatile under composting conditions, particularly at elevated pH and temperature (de Guardia et al., 2008). Following this stage, available nitrogen increases again, indicating continued nutrient release during vermicomposting. Among the treatments, Treatment D recorded the highest final available nitrogen, followed by Treatment C, indicating the strongest nitrogen enrichment by the end of the process. This suggests that the composition in Treatment D supported more effective nitrogen availability throughout composting and vermicomposting.

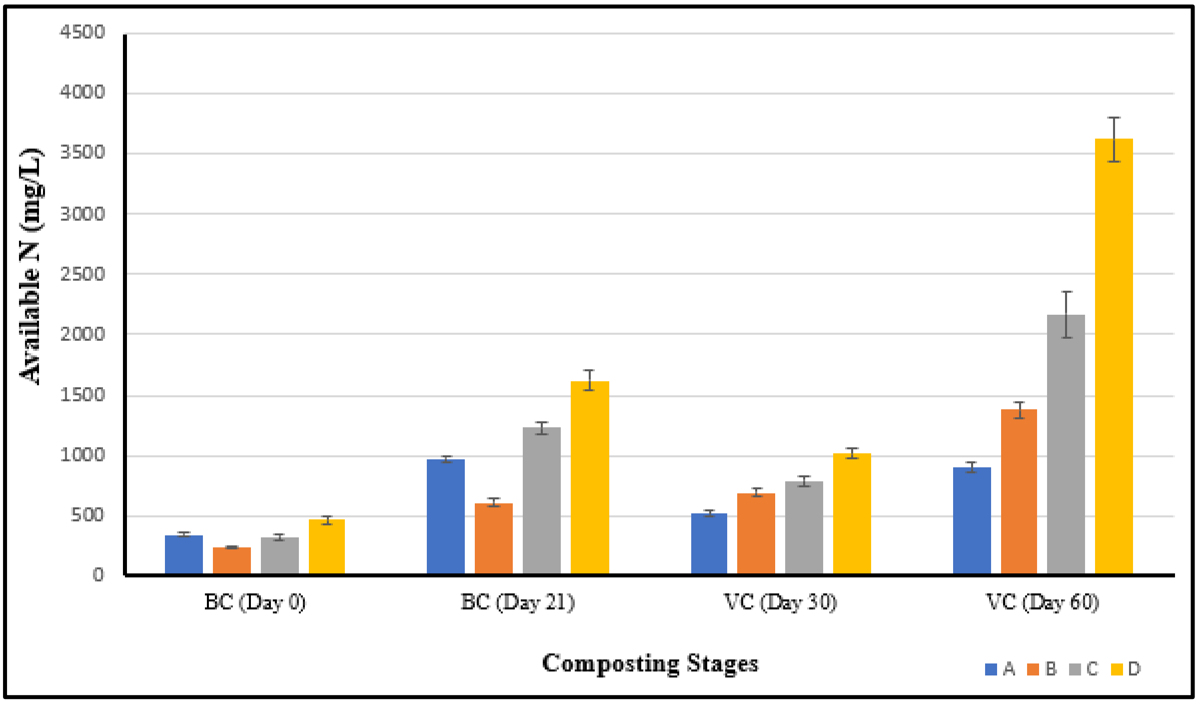

Figure 4.6: Available Nitrogen (N) changes across Berkeley composting and Vermicomposting stages

II. Available Phosphorus (P)

As presented in Table 4.8, available phosphorus (P) increased throughout the Berkeley composting and vermicomposting stages for all treatments. At the initial stage [BC (Day 0)], available phosphorus concentrations ranged from 166 ± 11 mg/L to 284 ± 16 mg/L. Following Berkeley composting [BC (Day 21)], a noticeable increase in phosphorus content was observed in all treatments, particularly in Treatments B and D, which recorded 684 ± 35 mg/L and 641 ± 37 mg/L, respectively. During vermicomposting, available phosphorus generally continued to increase, although slight fluctuations were observed in some treatments at VC (Day 30). By the final stage [VC (Day 60)], Treatments A, B, C, and D recorded phosphorus concentrations of 918 ± 48 mg/L, 1123 ± 107 mg/L, 984 ± 110 mg/L, and 884 ± 75 mg/L, respectively.

Compared with the initial stage, all treatments exhibited substantial phosphorus enrichment over time. Treatment A showed an increase of approximately 453.0%, followed by Treatment B (295.4%), Treatment C (326.0%), and Treatment D (353.3%). Overall, the increasing phosphorus content suggests progressive mineralization and release of phosphorus during composting and vermicomposting, while the relatively similar final phosphorus concentrations indicate a more uniform distribution of phosphorus among the treatment sets at the final stage.

Table 4.8: Available Phosphorus (P) of Treatment Sets During Berkeley composting and Vermicomposting

	Treatment Set			
Stage	**A**	**B**	**C**	**D**
BC (Day 0)	166 ± 11	284 ± 16	231 ± 24	195 ± 30
BC (Day 21)	412 ± 31	684 ± 35	493 ± 28	641 ± 37
VC (Day 30)	556 ± 26	709 ± 42	427 ± 29	591 ± 23
VC (Day 60)	918 ± 48	1123 ± 107	984 ± 110	884 ± 75

The trend of available phosphorus is more clearly illustrated in Figure 4.7, where all treatments exhibit a continuous increasing pattern throughout the composting and vermicomposting stages. The increase becomes more evident during the later stages, particularly from vermicomposting onward. Overall, the figure shows a consistent upward trend with slight variations in the rate of increase among treatments. Although Treatment B showed the highest value, the differences among treatments were relatively small, indicating a comparable level of phosphorus availability across the treatment sets at the end of the process.

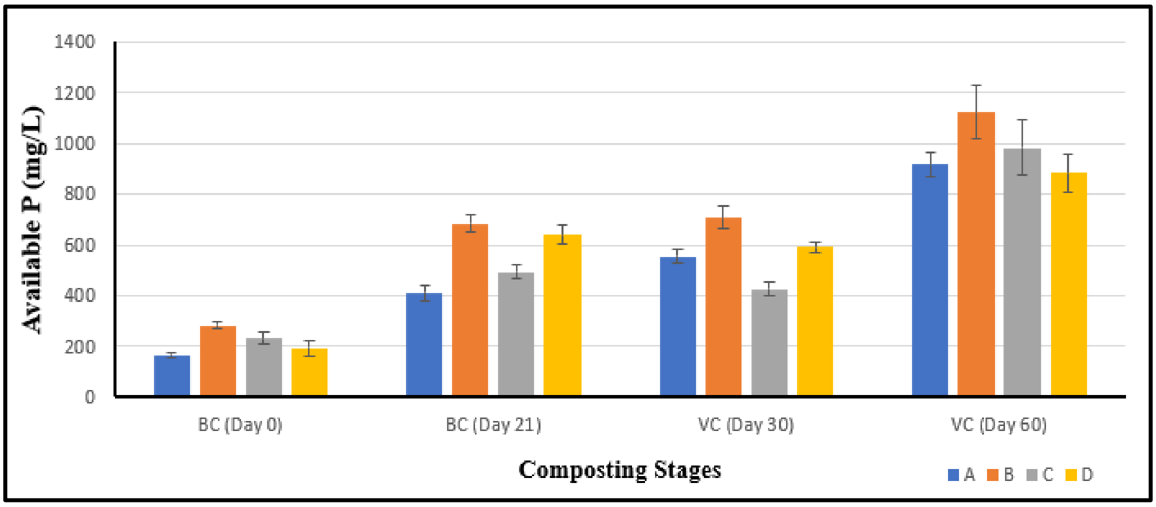

Figure 4.7: Available Phosphorus (P) changes across Berkeley composting and Vermicomposting

III. Available Potassium(K)

As presented in Table 4.9, available potassium (K) increased substantially across all treatments throughout the Berkeley composting and vermicomposting stages. At the initial stage [BC (Day 0)], available potassium concentrations ranged from 928 ± 82 mg/L to 2095 ± 104 mg/L. During end of Berkeley composting [BC (Day 21)], all treatments showed a marked increase in potassium content, with Treatments C and D recording comparatively higher values of 3638 ± 169 mg/L and 4461 ± 223 mg/L, respectively. During initial stage of vermicomposting [VC (Day 30)], slight reductions or slower increases were observed in several treatments. However, potassium concentrations increased sharply again by VC (Day 60). At the final stage, Treatments A, B, C, and D recorded available potassium concentrations of 5726 ± 286 mg/L, 5603 ± 280 mg/L, 6623 ± 331 mg/L, and 7254 ± 363 mg/L, respectively.

Compared with the initial stage, Treatment A exhibited the greatest percentage increase in potassium content at approximately 517.0%, followed by Treatment B (419.8%), Treatment C (384.1%), and Treatment D (246.3%). Overall, the substantial increase in available potassium indicates progressive mineralization and release of potassium throughout the Berkeley composting and vermicomposting processes, with Treatment D maintaining the highest potassium concentration at the final stage.

Table 4.9: Available Potassium(K) of Treatment Sets During Berkeley composting and Vermicomposting

	Treatment Set			
Stage	**A**	**B**	**C**	**D**
BC (Day 0)	928 ± 82	1078 ± 90	1368 ± 111	2095 ± 104
BC (Day 21)	3120 ± 66	2684 ± 134	3638 ± 169	4461 ± 223
VC (Day 30)	2896 ± 145	3012 ± 220	2967 ± 148	3891 ± 395
VC (Day 60)	5726 ± 286	5603 ± 280	6623 ± 331	7254 ± 363

Figure 4.8 shows a clear upward trend in available potassium across the composting stages. The increase is especially noticeable from BC (Day 0) to BC (Day 21), followed by a brief slowing or slight fluctuation at VC (Day 30) in some treatments before rising again toward VC (Day 60). The substantial increase in available potassium throughout the Berkeley composting and vermicomposting stages may be attributed to the progressive decomposition and mineralization of organic matter. During Berkeley composting, intensive microbial activity accelerated the breakdown of organic substrates, resulting in the release of potassium into more soluble and plant-available forms. The further increase observed during vermicomposting suggests continued nutrient mineralization and concentration effects associated with organic matter reduction. Treatments C and D consistently exhibited comparatively higher potassium concentrations, likely due to the incorporation of SCG and SW, which contributed additional mineral nutrients to the compost mixtures. Overall, the continuous increase in potassium content indicates progressive compost maturation and improved nutrient availability in the final vermicompost products.

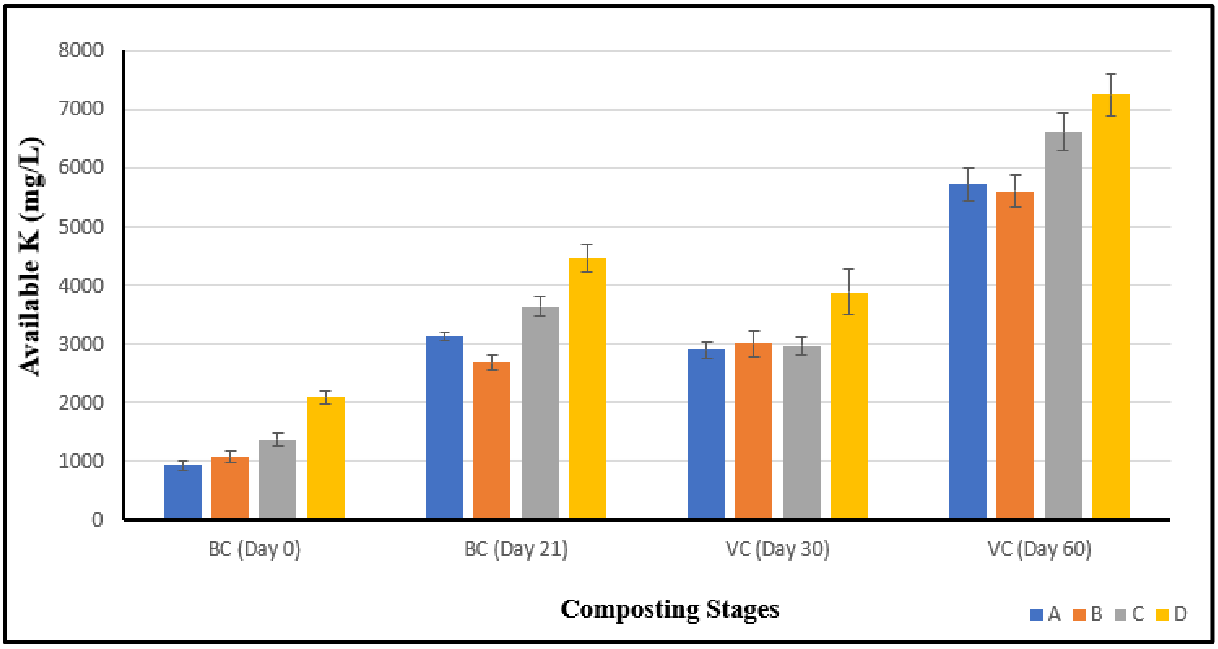

Figure 4.8: Available Potassium(K) changes across Berkeley composting and vermicomposting stages

IV. Assessment Against Market Standards for Organic Fertilizers

The nutrient composition of the final vermicompost was assessed against commonly reported ranges for commercial organic fertilizers to evaluate its agronomic suitability, as shown in Table 4.10. The measured concentrations of available nitrogen, phosphorus, and potassium in Treatments A–D were compared with typical nutrient ranges reported for organic fertilizers. The final vermicompost samples recorded available nitrogen concentrations ranging from 895–3624 mg/L, phosphorus concentrations ranging from 884–1123 mg/L, and potassium concentrations ranging from 5603–7254 mg/L. These values generally fall within the commonly reported ranges for commercial organic fertilizers (N: 1500–10,000 mg/L; P: 500–5,000 mg/L; K: 4,000–20,000 mg/L).

Although available nitrogen in Treatments A and B was at the lower end of the recommended range, phosphorus and potassium concentrations in all treatment sets remained within acceptable limits. The variation in nutrient composition among Treatments A–D may be attributed to differences in feedstock composition, particularly the incorporation of spent coffee grounds and swine wastewater, which contributed additional nutrients during the Berkeley composting and vermicomposting processes. Nevertheless, the overall nutrient composition indicates that the vermicompost products achieved acceptable agronomic quality comparable to commonly marketed organic fertilizers.

Table 4.10: Evaluation of Vermicompost Nutrient Levels Against Market Organic Fertilizer Ranges

Available Nutrient	Typical Available Range (mg/L) in Organic Fertilizers	Within Range?
N	**1500 – 10,000**	☑ **Yes**
P	**500 – 5,000**	☑ **Yes**
K	**4,000 – 20,000**	☑ **Yes**

4.3.5 Changes in pH and Electrical Conductivity (EC)

I. pH

As presented in Table 4.11, pH values across all treatments exhibited only slight fluctuations throughout the Berkeley composting and vermicomposting stages, remaining within a relatively narrow range. At the initial stage [BC (Day 0)], pH values ranged from 6.34 ± 0.04 to 7.26 ± 0.07, indicating near-neutral conditions in the compost mixtures. Following Berkeley composting [BC (Day 21)], a slight decrease in pH was observed in most treatments. During vermicomposting [VC (Day 30)], the pH values increased slightly, with Treatments C and D recording comparatively higher pH values of 7.33 ± 0.07 and 7.52 ± 0.09, respectively. By VC (Day 60), pH values stabilized between 6.58 ± 0.04 and 6.83 ± 0.05 for all treatments. Overall, the relatively small variations in pH suggest that stable composting conditions were maintained throughout the Berkeley composting and vermicomposting processes.

Table 4.11: pH of Treatment Sets During Berkeley composting and Vermicomposting

	Treatment Set			
Stage	A	B	C	D
BC (Day 0)	6.91 ± 0.05	6.34 ± 0.04	7.02 ± 0.06	7.26 ± 0.07
BC (Day 21)	6.25 ± 0.03	6.54 ± 0.05	6.87 ± 0.04	6.62 ± 0.06
VC (Day 30)	7.06 ± 0.08	6.83 ± 0.05	7.33 ± 0.07	7.52 ± 0.09
VC (Day 60)	6.58 ± 0.04	6.66 ± 0.03	6.83 ± 0.05	6.75 ± 0.04

Figure 4.9 illustrates the fluctuation of pH across the composting stages, where a slight decrease is observed during the early composting stage, followed by an increase during the transition to vermicomposting. This temporary increase may be associated with ammonification processes occurring at the initial stage of vermicomposting, which can cause a short-term rise in pH. As the process progresses, pH gradually stabilises toward the final stage. Despite minor variations among treatments, all treatments exhibit a similar pattern, indicating consistent pH behaviour throughout the process. At the final stage, pH values among the treatments were very similar, ranging from 6.58 to 6.83, with Treatment C showing the highest value, followed closely by Treatments D, B, and A. The small differences indicate that all treatments achieved comparable pH conditions by the end of the process. These near-neutral pH values suggest that the final compost is suitable for agricultural application.

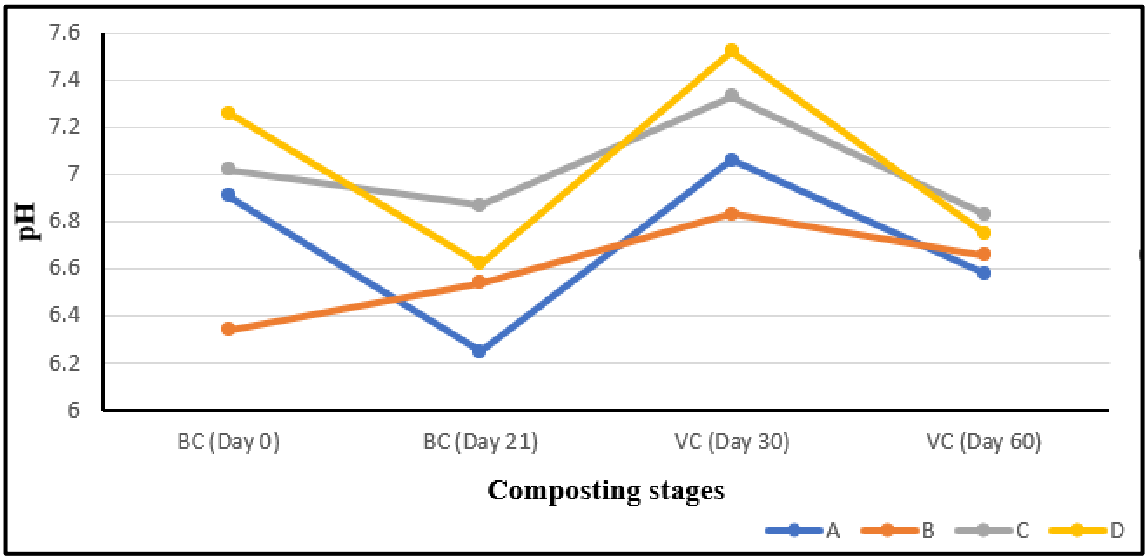

Figure 4.9: pH changes across Berkeley composting and vermicomposting stages

II.EC

As presented in Table 4.12, electrical conductivity (EC) exhibited an overall fluctuating trend throughout the Berkeley composting and vermicomposting stages. At the initial stage [BC (Day 0)], EC values ranged from 344 ± 15 µS/cm to 562 ± 27 µS/cm. Following Berkeley composting [BC (Day 21)], all treatments showed a marked increase in EC, with Treatment D recording the highest value of 1226 ± 61 µS/cm. During vermicomposting [VC (Day 30)], EC values generally decreased in most treatments before either increasing slightly or stabilizing by VC (Day 60). At the final stage, EC values ranged from 360 ± 17 µS/cm to 688 ± 34 µS/cm, with Treatment D maintaining the highest final EC value. Overall, the moderate fluctuations in EC suggest changes in soluble salt concentration during organic matter decomposition and nutrient mineralization throughout the Berkeley composting and vermicomposting processes.

Table 4.12: EC of Treatment Sets During Berkeley composting and Vermicomposting

	Treatment Set			
Stage	**A**	**B**	**C**	**D**
BC (Day 0)	412 ± 18	344 ± 15	488 ± 22	562 ± 27
BC (Day 21)	493 ± 24	602 ± 31	912 ± 46	1226 ± 61
VC (Day 30)	534 ± 26	438 ± 20	853 ± 43	812 ± 41
VC (Day 60)	360 ± 17	527 ± 26	585 ± 29	688 ± 34

Figure 4.10 shows that EC increased sharply during the early composting stage, particularly from BC (Day 0) to BC (Day 21), before declining or stabilising during the vermicomposting stage. This temporary rise may be associated with the release of soluble ions during active decomposition, while the later decrease suggests gradual stabilisation of the material. Despite minor variations among treatments, the overall pattern shows that EC became more controlled toward the final stage. Among the treatments, Treatment D recorded the highest EC at both BC (Day 21) and VC (Day 60), followed by Treatments C, B, and A at the final stage. This suggests that Treatment D retained the greatest concentration of soluble salts by the end of the process, while the other treatments showed lower final EC values. Overall, the final EC values indicate that the compost had moved toward a more stable condition for potential agricultural use

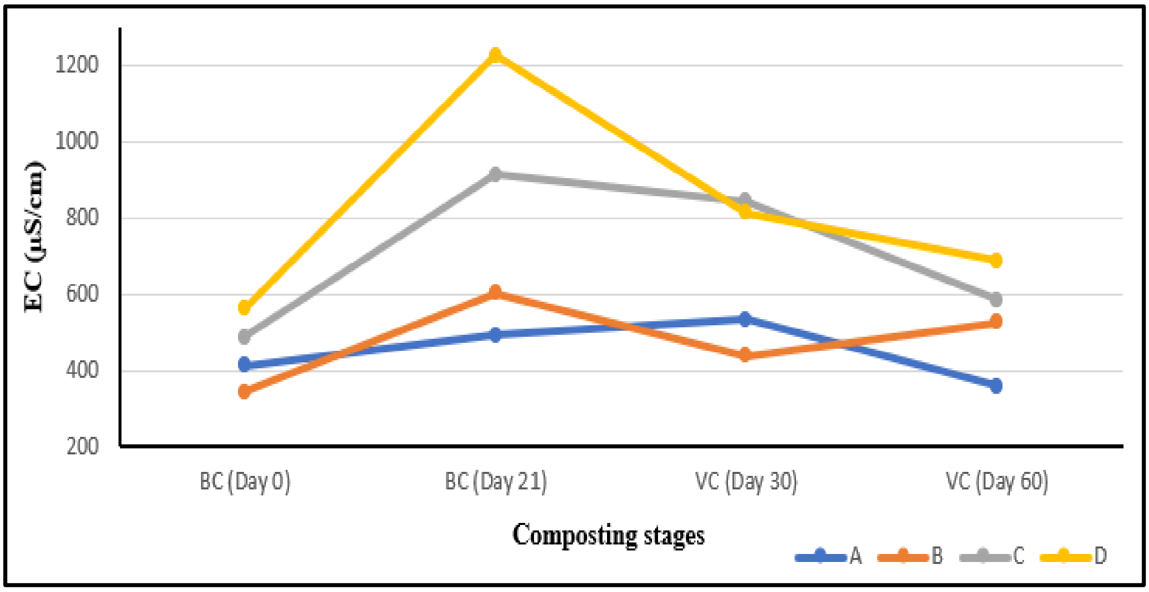

Figure 4.10: EC changes across Berkeley composting and vermicomposting stages

4.3.6 Changes in Caffeine Content

Table 4.13 presents the absorbance values of caffeine standard solutions used for calibration curve construction. The absorbance increased progressively with increasing caffeine concentration from 5 to 50 mg/L, indicating a positive correlation between caffeine concentration and UV-Vis response at 273 nm. The highest absorbance value of 0.5441 was recorded for the 50 mg/L standard solution

Table 4.13: UV-Vis Calibration Data for Caffeine Standard Solutions

Concentration (mg/L)	Absorbance
5	0.2367
10	0.2919
20	0.3721
50	0.5441

Figure 4.11 presents the calibration curve used for caffeine quantification. The standard solutions showed a clear increasing trend in absorbance with increasing concentration, confirming the expected positive relationship between concentration and UV-Vis response. The calibration equation, y = 0.0066x + 0.2209, with an R^2 value of 0.9863, indicates good linearity and supports the suitability of the curve for estimating caffeine concentration in the compost extracts. This suggests that the UV-Vis method provided a reasonably reliable basis for quantifying caffeine within the selected concentration range.

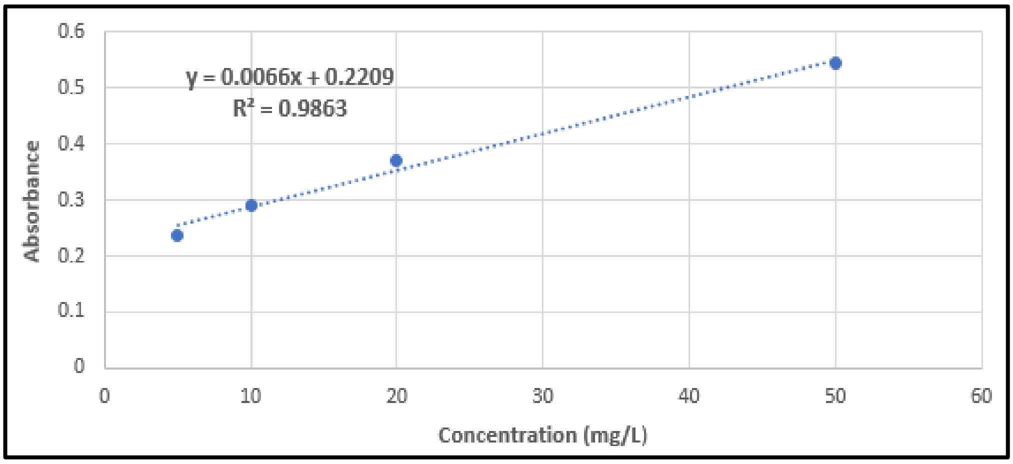

Figure 4.11: Calibration curve of caffeine standard solutions

Table 4.14 shows that the caffeine content in the Berkeley compost samples ranged from 0.037 to 0.042 mg/g, while the vermicompost samples ranged from 0.023 to 0.036 mg/g. Overall, the vermicompost samples exhibited lower caffeine content than the corresponding Berkeley compost samples, indicating a decrease after the vermicomposting stage. On average, the caffeine content decreased from 0.040 mg/g in Berkeley compost to 0.0315 mg/g in vermicompost, representing an overall reduction of about 21.3%. Among all samples, VC-A showed the lowest caffeine content at 0.023 mg/g, suggesting the most effective reduction after vermicomposting.

Table 4.14: Caffeine concentration in Berkeley compost and Vermicompost samples

Sample	Absorbance	Caffeine (mg/L)	Caffeine (mg/g)
BC-A	0.286	9.9	0.037
BC-B	0.295	11.2	0.042
BC-C	0.291	10.6	0.040
BC-D	0.293	10.9	0.041
VC-A	0.261	6.1	0.023
VC-B	0.284	9.6	0.036
VC-C	0.279	8.8	0.033
VC-D	0.281	9.1	0.034

Table 4.15 further shows the percentage reduction in caffeine content after vermicomposting. The greatest reduction was observed in BC-A → VC-A, with a decrease of 36.8%, whereas the other treatment pairs showed more moderate reductions of 14.3% to 17.5%. This pattern suggests that vermicomposting had the strongest effect in Treatment A, while the other treatments experienced smaller but still measurable decreases in caffeine content. The higher reduction in Treatment A may be attributed to the simpler feedstock composition and lower matrix complexity, which may have allowed more effective decomposition during vermicomposting. In contrast, the smaller reductions in Treatments B, C, and D may reflect the influence of SCG and other organic inputs, which could increase the organic load and make further breakdown less efficient after the initial composting stage.

Table 4.15:Percentage reduction in caffeine concentration from Berkeley compost to Vermicompost

Treatment Pair	Berkeley Compost (mg/g)	Vermicompost (mg/g)	Reduction (%)
BC-A → VC-A	0.037	0.023	36.8
BC-B → VC-B	0.042	0.036	14.3
BC-C → VC-C	0.040	0.033	17.5
BC-D → VC-D	0.041	0.034	17.1

Overall, the caffeine content decreased after vermicomposting, indicating that the process contributed to caffeine reduction in the compost mixtures. However, the SCG-amended treatments showed comparatively smaller reductions, possibly due to the greater organic loading and higher substrate complexity introduced by SCG, which may have limited further microbial degradation of residual caffeine. Furthermore, the stabilization of organic matter during composting could have reduced caffeine bioavailability, thereby slowing its subsequent degradation during vermicomposting.

4.4 Biological and Phytotoxicity Assessment

4.4.1 Earthworm Biomass Changes

As shown in Table 4.16, all treatment sets demonstrated a positive increase in earthworm biomass throughout the vermicomposting. The initial biomass ranged from 58.42 ± 2.11 g to 62.15 ± 2.63 g, increasing to final biomass values between 92.98 ± 3.57 g and 119.83 ± 4.82 g. Among the treatments, VC-D recorded the highest biomass increment (92.82 ± 4.75%), followed by VC-B, whereas VC-C exhibited the lowest biomass gain and growth rate.

The observed increase in biomass indicates that the substrates generally provided favourable conditions for earthworm activity during vermicomposting. The superior performance observed in VC-D suggests that this treatment achieved a more balance of nutrients, moisture, and physicochemical conditions, thereby enhancing feeding efficiency and biomass accumulation. In contrast, the comparatively lower growth observed in VC-C may be associated with the higher proportion of SCG and SW, which could introduce inhibitory effects such as residual phytotoxic compounds, elevated salinity, or ammonia-related stress. Such conditions may create a less favourable microenvironment for earthworm activity, thereby limiting growth performance. Overall, the results indicate that all treatments support earthworm survival and growth, confirming the suitability of the substrates for vermicomposting.

Table 4.16: Earthworm Biomass Changes of Treatment Sets During Vermicomposting

Treatment	Initial weight (g)	Final weight (g)	Biomass gain (g)	Biomass gain (%)	GR (mg/worm/day)
VC-A	58.42 ± 2.11	98.06 ± 3.84	39.64 ± 2.06	67.85 ± 3.52	11.01 ± 0.54
VC-B	61.37 ± 2.45	105.12 ± 4.16	43.75 ± 2.31	71.29 ± 3.88	12.15 ± 0.61
VC-C	59.88 ± 2.08	92.98 ± 3.57	33.11 ± 1.74	55.28 ± 2.96	9.20 ± 0.46
VC-D	62.15 ± 2.63	119.83 ± 4.82	57.68 ± 2.95	92.82 ± 4.75	16.02 ± 0.78

4.4.2 Germination Index (GI)

As shown in Table 4.17, the control recorded a GI of 100.00%, while the treatment sets ranged from 76.92% to 103.85%. Among the treatments, BC-D achieved the highest GI (103.85%), followed by BC-B (95.19%) and BC-A (84.62%), whereas BC-C showed the lowest value (76.92%). The number of germinated seeds remained similar across treatments, but the variation in mean root length and relative root growth contributed to the differences in GI. This indicates that the composting product from BC-D was the least inhibitory and showed the greatest improvement in seedling response, while BC-C appeared to retain stronger phytotoxic effects.

Table 4.17: GI of Treatment Sets During Berkeley composting

Treatment	Total seeds	Number of Germinated seeds	Mean root length (cm)	RSG (%)	RRG (%)	GI
Control	10	8	0.65	100.00	100.00	100.00
BC-A	10	8	0.55	100.00	84.62	84.62
BC-B	10	9	0.55	112.50	84.62	95.19
BC-C	10	8	0.50	100.00	76.92	76.92
BC-D	10	9	0.60	112.50	92.31	103.85

As shown in the Table 4.18, the control again recorded a GI of 100.00%, while the vermicompost treatments ranged from 86.67% to 118.52%. VC-D produced the highest GI value (118.52%), followed by VC-C (96.30%) and VC-B (93.33%), whereas VC-A recorded the lowest GI at 86.67%. Compared with the Berkeley composting stage, the vermicomposting product generally showed improved germination performance, particularly in VC-D, where both germination and root elongation were enhanced. This suggests that vermicomposting further reduced inhibitory compounds and improved the biological quality of the final material.

Table 4.18: GI of Treatment Sets During Vermicomposting

Treatment	Total seeds	Number of Germinated seeds	Mean root length (cm)	RSG (%)	RRG (%)	GI
Control	10	9	0.75	100.00	100.00	100.00
BC-A	10	9	0.65	100.00	86.67	86.67
BC-B	10	9	0.70	100.00	93.33	93.33
BC-C	10	10	0.65	111.11	86.67	96.30
BC-D	10	10	0.80	111.11	106.67	118.52

Overall, the GI results indicate that treatment performance improved from Berkeley composting to vermicomposting, with the strongest response observed in the D treatment. The higher GI values in the later stage suggest progressive detoxification, reduced phytotoxicity, and better stabilization of the organic substrate. In contrast, lower GI values in some treatments, especially BC-C and VC-A, may reflect incomplete degradation or residual inhibitory effects from the raw materials. The results also support the earlier biomass findings, where treatments with more favourable substrate balance produced better biological responses. Therefore, GI confirms that the composting process successfully improved substrate maturity, and VC-D appears to be the most suitable treatment for producing a plant-friendly final product.

4.5 Comparative Performance Assessment of Treatment Set

The comparative evaluation of the four treatment sets revealed clear differences in composting and vermicomposting performance across key maturity, nutrient, and biological indicators. In terms of organic matter stabilization, all treatments showed continuous reductions in TOC and C/N ratio, indicating progressive decomposition and compost maturation. Treatment B recorded the greatest TOC reduction, decreasing from 44.73 ± 0.85% at BC (Day 0) to 24.97 ± 1.23% at VC (Day 60), representing the lowest final TOC among all treatments. Similarly, the C/N ratio decreased substantially in all treatments, with Treatment D achieving the lowest final C/N ratio of 14.32 ± 0.36, followed by Treatment C (15.73 ± 0.51), indicating a higher degree of stabilization compared with Treatments A and B.

For nutrient enrichment, Treatments C and D generally performed better throughout the process. Treatment D recorded the highest final available nitrogen concentration at 3624 ± 186 mg/L, followed by Treatment C at 2173 ± 191 mg/L, whereas Treatments A and B recorded lower final values of 895 ± 40 mg/L and 1382 ± 67 mg/L, respectively. A similar trend was observed for available potassium, where Treatment D achieved the highest final concentration of 7254 ± 363 mg/L, followed by Treatment C (6623 ± 331 mg/L). In contrast, available phosphorus showed relatively comparable final concentrations among treatments, ranging from 884 ± 75 mg/L to 1123 ± 107 mg/L, with Treatment B recording the highest phosphorus content.

The biological assessment further highlighted differences among treatments. VC-D recorded the highest earthworm biomass gain of 57.68 ± 2.95 g, biomass increment of 92.82 ± 4.75%, and growth rate of 16.02 ± 0.78 mg/worm/day, indicating the most favourable conditions for earthworm activity and substrate utilization. In comparison, VC-C showed the lowest biomass gain (33.11 ± 1.74 g) and growth rate (9.20 ± 0.46 mg/worm/day). The germination index (GI) results also supported this trend, where VC-D achieved the highest GI value of 118.52%, indicating the lowest phytotoxicity and best seedling response among all treatments. By contrast, BC-C recorded the lowest GI value of 76.92%, suggesting comparatively higher residual phytotoxicity during the earlier composting stage.

In terms of caffeine degradation, vermicomposting reduced caffeine content in all treatments. The greatest reduction was observed in Treatment A, where caffeine concentration decreased from 0.037 mg/g in BC-A to 0.023 mg/g in VC-A, corresponding to a reduction of 36.8%. In comparison, Treatments B, C, and D showed smaller reductions ranging from 14.3% to 17.5%, possibly due to the incorporation of spent coffee grounds, which increased substrate complexity and residual caffeine content.

Although all treatment sets successfully produced stabilized compost materials, Treatment D demonstrated the strongest performance when physicochemical stability, nutrient enrichment, earthworm growth, and phytotoxicity reduction were considered collectively. The combination of a relatively low final C/N ratio, highest nutrient concentrations, greatest earthworm biomass gain, and highest GI value suggests that Treatment D produced the most mature, nutrient-rich, and biologically favourable vermicompost among all treatment sets.

Overall, the findings confirm that the integrated Berkeley composting–vermicomposting system effectively converted the initial substrates into more stable and nutrient-enriched end products. All treatments achieved a germination index above 70% at the final stage, indicating low phytotoxicity and suitability for agricultural application. In addition, the final pH values remained near-neutral and stable, supporting favourable conditions for soil amendment and crop growth. The transition from Berkeley composting to vermicomposting further improved key parameters, including continued reduction in C/N ratio and increased nutrient availability, demonstrating the effectiveness of the vermicomposting stage in enhancing compost quality.

4.6 Preliminary Commercial Viability Assessment

A preliminary commercial viability assessment was conducted to evaluate the economic potential of the proposed Berkeley composting–vermicomposting system. As shown in Tables 4.19 and 4.20, the estimated production cost of vermicompost was approximately RM35–RM50 per 10 kg, while the estimated market price ranged from RM60–RM90 per 10 kg, indicating a potential profit margin of approximately 35–60%. The relatively low production cost was mainly attributed to the utilization of readily available organic waste materials such DL, VE, SCG, and SW.

In addition, recent fluctuations in global fertilizer supply chains and increasing prices of conventional nitrogen-based fertilizers have highlighted the growing importance of locally sourced and sustainable fertilizer alternatives. The rising cost of chemical fertilizers may further increase market demand for organic fertilizers produced from renewable waste resources. In this context, the proposed integrated composting system demonstrates promising commercial potential by converting low-cost organic wastes into value-added vermicompost products while simultaneously supporting sustainable waste valorisation and resource recovery.

Table 4.19: Estimated Nominal Costs of Raw Materials and Equipment

Item	Nominal Cost
DL	RM0.05/kg
VE	RM0.10–RM0.20/kg
SCG	RM0.10/kg
SW	RM0.10/kg
BC-Bin wire mesh	RM2/treatment set
VC-Bin container	RM15/treatment set
Earthworm	RM10/ treatment set

Table 4.20:Estimated Production Cost and Market Price Range of Vermicompost

Category	Value
Total Production Cost	RM35–RM50/10 kg
Market Price Range	RM60–RM90/10 kg

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study successfully evaluated the feasibility and performance of an integrated Berkeley composting–vermicomposting system for the valorisation of spent coffee grounds (SCG) and swine wastewater (SW). The findings demonstrated that the combined system effectively enhanced organic matter stabilization, nutrient recovery, and biological quality of the final vermicompost, supporting the conversion of organic waste materials into value-added agricultural products.

Throughout the Berkeley composting and vermicomposting stages, significant improvements were observed in key physicochemical properties. Total organic carbon progressively decreased from range of 44.58 ± 1.01% to 46.82 ± 1.24% at the initial stage to range of 24.97 ± 1.23% to 34.79 ± 1.32% at the final stage. The final C/N ratio decreased to range of 14.32 ± 0.36 to 19.95 ± 0.66, confirming satisfactory compost maturity and stabilization. In addition, the final pH values remained near-neutral within the range of 6.58 ± 0.04 to 6.83 ± 0.05, indicating favourable conditions for agricultural application. Whereas available nutrients including nitrogen (N), phosphorus (P), and potassium (K) increased substantially across all treatment set from Berkeley composting to vermicomposting.

Among the treatment sets, Treatment D demonstrated the most favourable overall performance. The treatment achieved the lowest final C/N ratio (14.32 ± 0.36), highest available nitrogen (3624 ± 186 mg/L) and potassium (7254 ± 363 mg/L) concentrations, greatest earthworm biomass gain (57.68 ± 2.95 g), and highest germination index (118.52%), indicating improved decomposition efficiency, nutrient availability, and low phytotoxicity. All treatment sets achieved a germination index above 70%, confirming the suitability of the final vermicompost products for plant growth and soil application.

The transition from Berkeley composting to vermicomposting further improved compost quality by enhancing nutrient availability and promoting additional stabilization of the organic substrates. Furthermore, the nutrient composition of the final vermicompost generally fell within the commonly reported range for commercial organic fertilizers, highlighting its agronomic value and potential market applicability.

From an economic perspective, the preliminary commercial assessment indicated promising feasibility, with an estimated production cost of approximately RM35–RM50 per 10 kg compared with a market value of approximately RM60–RM90 per 10 kg, corresponding to a potential profit margin of approximately 35–60%. The utilization of readily available organic waste materials such as SCG and SW further supports the sustainability and cost-effectiveness of the proposed system.

Overall, this study confirms that the integrated Berkeley composting–vermicomposting system is an effective and sustainable approach for managing SCG and SW while simultaneously producing stable, nutrient-rich, and environmentally beneficial organic fertilizer products.

5.2 Recommendation

Based on the findings of this study, several recommendations are proposed to enhance future research and practical application of the system:

I. Further studies should explore a wider range of SCG and SW mixing ratios to identify optimal combinations that maximise nutrient recovery while ensuring earthworm health and process stability.

II. The current study was conducted at laboratory scale; therefore, pilot-scale or field-scale studies are recommended to evaluate operational feasibility, process control, and economic performance under real conditions.

III. Field trials should be conducted to assess the long-term effects of the produced vermicompost on soil properties, crop yield, and environmental impact.

IV. Further evaluation of potential contaminants such as heavy metals, caffeine, or microplastics is recommended to ensure the safety of the final product.

V. Incorporating automated control systems for temperature, moisture, and aeration could improve process efficiency and consistency, particularly for large-scale applications.

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APPENDICES

APPENDIX A: Experimental Diagrams

Figure A.1: Experimental flow of the Berkeley composting setup

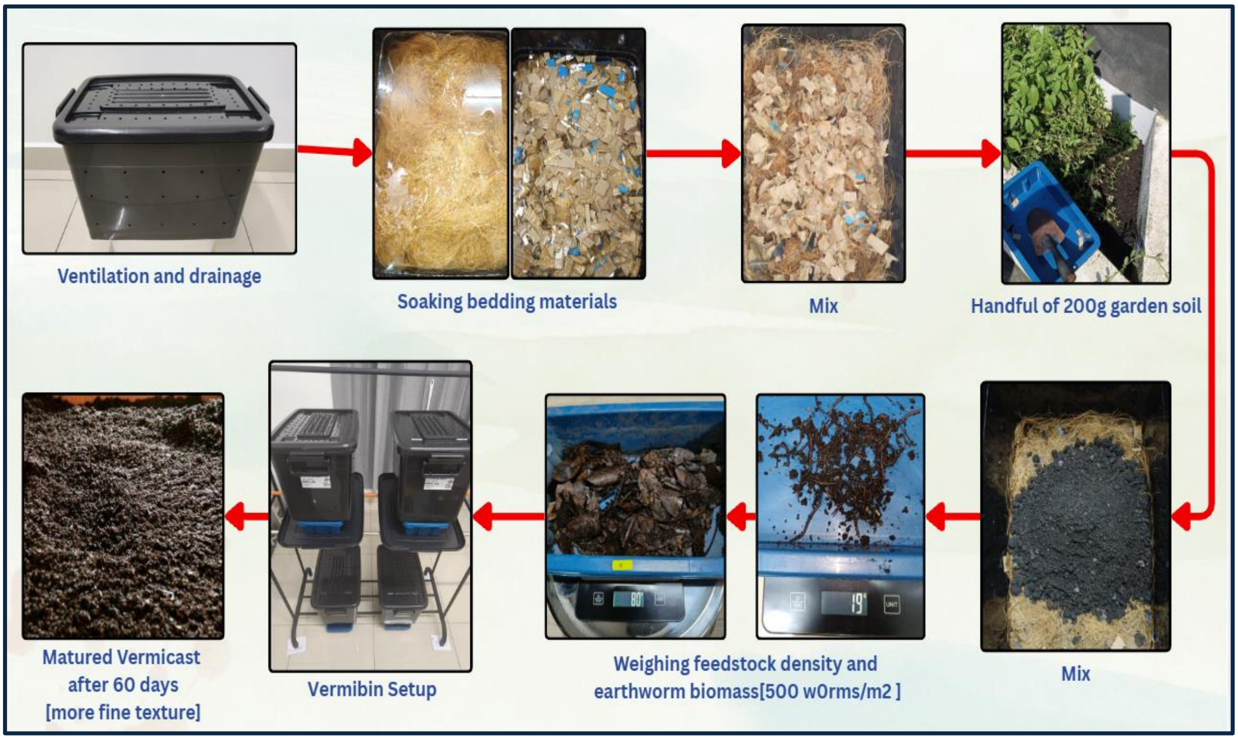

Figure A.2: Experimental flow of the Vermicomposting setup

Figure A.3: Observed earthworm growth, reproduction, and vermicast formation during vermicomposting

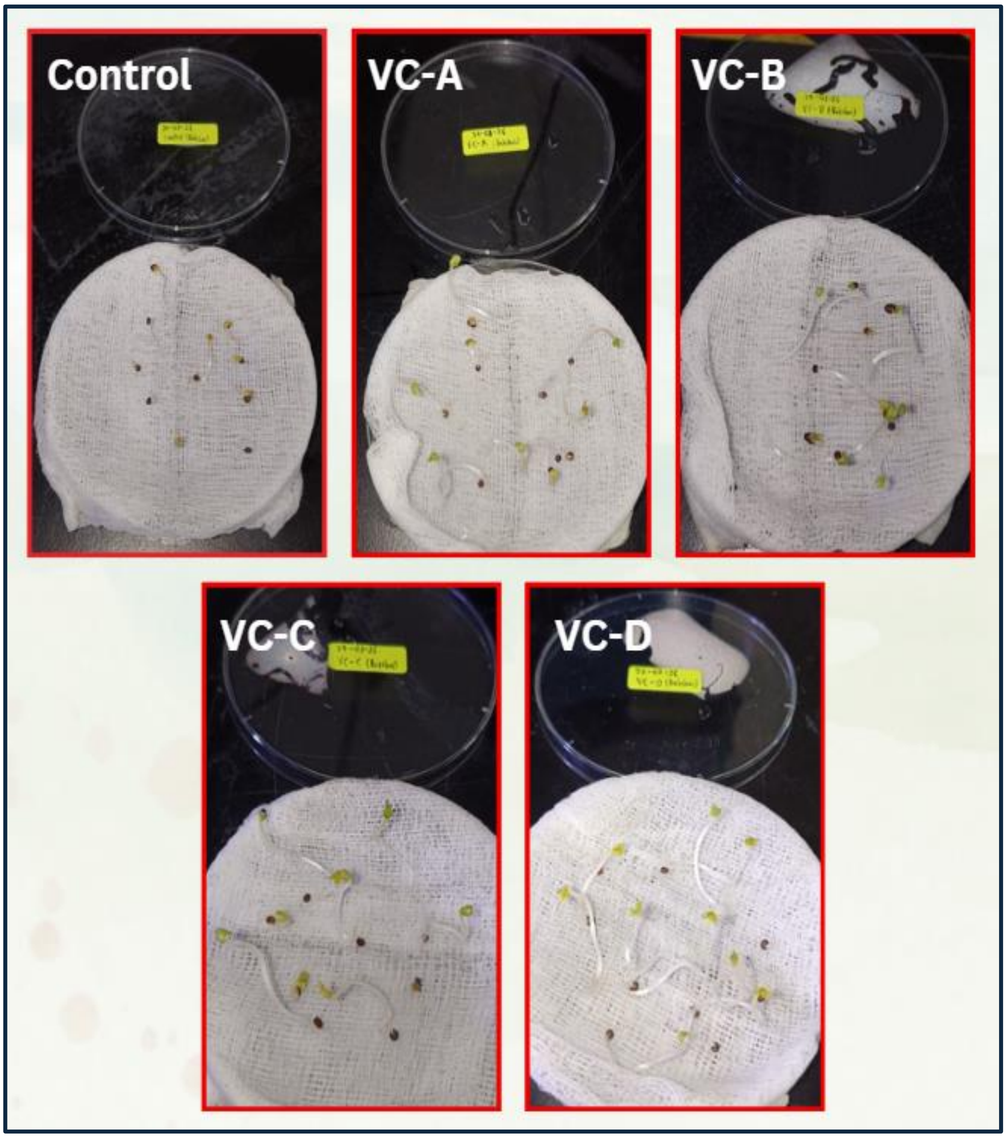

Figure A.4: Germination index (GI) testlili for control and vermicompost treatments